Archana Singh ·
Brijesh Rathi · Anita K. Verma ·
Indrakant K. Singh *Editors*

Natural Product Based Drug Discovery Against Human Parasites

Opportunities and Challenges

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ISBN 978-981-19-9604-7 ISBN 978-981-19-9605-4 (eBook)
https://doi.org/10.1007/978-981-19-9605-4

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

Parasitic infections pose a serious threat and cause a tremendous burden of disease in both the tropics and subtropics as well as in more temperate climates. The opportunities and challenges for the study and control of parasitic diseases in the twenty-first century are both exciting and daunting. Unlike most antibiotics, there is no "**general**" anti-parasitic drug available, as the selection of anti-parasitic drugs varies between different organisms. Few of the currently available drugs are synthesized chemically; however, the bulk of the drugs are derived from natural sources such as plants that have subsequently been modified chemically to warrant higher potency against human pathogens. The scientists and pharmacologists worldwide have made unprecedented efforts for finding a cure against human parasitic diseases. Parasitologists must capitalize on the neo-findings that are being generated *via* genomics, proteomics, bioinformatics, and genetic manipulations of both parasite and host. This book reviews the progress made in the discovery of natural anti-parasitic agents including schistosomiasis, trypanosomiasis, malaria, leishmaniasis, etc. which represent a major health problem, especially in impecunious areas. The chapters in this book range from enumerating basic research including plants and microbe-derived drugs, bioprospecting of marine resources for drug discovery, and semisynthetic and synthetic chemical drugs to clinical applications of nanoparticles with respect to their usage in the treatment of parasitic diseases. Furthermore, the book highlights the applications of advanced techniques in drug discovery, with a focus on computer-aided drug discovery methods, especially structure-based drug discovery and ligand-based drug design approaches, with an emphasis on virtual screening exhibiting enhanced efficacy of drugs. Additionally, the tools and techniques adopted to achieve efficient drug delivery are also discussed.

The motivation for organizing this book stems primarily from an urge to present readers with the latest research on the mechanisms associated with parasitic diseases as there are few published books, which deal with this theme at global level. Moreover, national and international experts and leading scientists and educationists from different disciplines studying parasitology, biomedical sciences, medicinal chemistry, bioinformatics, nanobiotechnology, and genetic engineering have compiled the chapters of this book. Therefore, this volume will dispense the latest information available to a wider range of researchers and professionals. This book does not claim to cover the entire field of parasitology. However, it provides a

plethora of reviews that will enable readers to have a glimpse of the basic mechanisms causing parasitic infections. Further, this book assures to deliver an overview of current knowledge on drug discovery for the management of human parasitic diseases.

In this book, we have delivered a unified summary of drug discovery against parasitic diseases of humans with special emphasis on drugs obtained from natural resources such as plants and microbes. Natural product-based drug discovery is the most suitable approach in the present scenario, since it provides an environment-friendly, cost-effective, and successful system. Modern tools and techniques are key to drug discovery, which facilitate identification, isolation, extraction, and synthesis of active compounds. The book integrates the natural medicinal sources along with the contemporary techniques of biochemistry as well as bioinformatics utilized for drug discovery against parasitic diseases.

Thanks are due to all contributors for the considerable energy, time, and effort that they spent in making this book an advancement of knowledge for understanding the mechanisms associated with parasitic diseases. We are extremely thankful to Prof. (Dr.) Rama, Principal, Hansraj College, University of Delhi; Prof. (Dr.) Rajiv Agrawal, Principal, Deshbandhu College, University of Delhi; and Prof. (Dr.) Vibha Singh Chauhan, Kirori Mal College, University of Delhi, for providing overall support for our research and academic pursuits. Dr. Anita recognizes the diligent efforts of her Ph.D. scholars—Karishma, Monika, Priyanka, Kapil, Kriti, and Mansi.

We, Dr. Archana and Dr. Indrakant, would like to appreciate our little angels Saumya and Kimaya for creating a beautiful ambiance, which allowed us to work tirelessly, and giving us all emotional support. We would like to convey our gratitude towards our Ph.D. mentors Prof. Praveen K. Verma and Prof. A. K. Singh.

Our sincere thanks go to Dr. Paula L. Mitchell, Professor Emerita of Biology at Winthrop University, USA, for her constant support in all our scientific endeavors and always being there as a troubleshooter. We also acknowledge our research scholars for their care while working on this project.

We are grateful to our parents for their constant support and blessings. Last but not least, our sincere thanks to the handling editors and publisher.

We, the IoE Fellows, acknowledge Delhi School of Public Health, Institution of Eminence.

We are optimistic that this book will be effective in broadcasting the latest knowledge about the plant-pathogen interaction.

New Delhi, India

Archana Singh
Brijesh Rathi
Anita K. Verma
Indrakant K. Singh

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About Editors

Archana Singh Associate Professor, Department of Plant Molecular Biology, University of Delhi South Campus, is a Ph.D. holder from the National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi. Dr. Singh is a recipient of "**Young Scientist Fellowship**" (2012) and "**Early Career Research Award**" (2017) from SERB, DST, Government of India; **Raman Post-Doctoral Fellowship** from the University Grant Commission; and **European Molecular Biology Organization Fellowship** from Heidelberg, Germany. Dr. Archana has worked as a visiting scientist at **Max Planck Institute of Chemical Ecology**, Jena, Germany, during 2019–2020 and at the **University of Kentucky**, Lexington, USA (2016–2017). She has been bestowed with Best Researcher Award, 2017, and Best Teacher Award, 2018. Dr. Singh has also published many research articles in journals of national and international repute such as *Scientific Reports* and others. She has also filed a patent on diagnosis of *Mycobacterium tuberculosis.* Dr. Archana has also edited two books and authored many book chapters published by Springer and Elsevier. She is a life member/member of IBS and AIMI. Besides, she has served as a reviewer for several international and national journals and book proposals. Dr. Singh has trained many postgraduate and graduate students and currently supervises two doctoral researchers and one postdoctoral researcher. Currently, she is working on "Plant Immunity and Human Health" with special emphasis on drug discovery for cancer, tuberculosis, parasitic diseases of humans, and allergens of plant origin.

Brijesh Rathi obtained Ph.D. in Chemistry from the University of Delhi and is currently working as Associate Professor of Chemistry at Hansraj College University Enclave, University of Delhi, India. He is a recipient of the prestigious "Young Scientist Fellowship" (2010) and "Early Career Research Award" (2016) from the DST, Government of India; "Excellence Awards for Teachers in Service" (2018) by the University of Delhi; and CAPES-Print Fellowship (2019) from the Ministry of Education, Brazil. Dr. Rathi has been Visiting Assistant Professor (2016–2017) at the Department of Chemistry, Massachusetts Institute of Technology (MIT), Cambridge, USA, and is now Affiliate Faculty at the University of Loyola, Chicago, and the University of Debrecen, Hungary. He has published more than 60 papers in various journals of national and international repute. Dr. Rathi holds the position of Section Editor and Associate Editor for journals. He has supervised five undergraduate research students in the past and is currently supervising various doctoral students and one master's thesis student. His main research interests are centered at the development of new anti-parasitic and antiviral agents and their target identification.

Anita K. Verma is Professor of Zoology and has over 32 years of teaching and research experience at Kirori Mal College, University of Delhi, Delhi, India. She has done postdoctoral research at the National Institute of Immunology, New Delhi (1994–1998), and worked as a Senior Scientist at the School of Pharmacy, University of Manchester (1998–2000). Her research interests include identifying predictive biomarkers and using plant products as immunomodulators to mitigate cancer, arthritis, and osteoporosis; exploiting nanotherapeutics for human health and disease; making in vivo models for quantifying nanoparticulate drug delivery for cancer, diabetes, and arthritis; and developing rational designs based on whole body and cellular pharmacokinetic-pharmacodynamic profile of drugs. She has two patents on anticancer drugs, over 125 publications in peer-reviewed international journals of repute, and 12 book chapters to her credit. She has been conferred with

various prestigious awards, notably Charles Darwin Gold Medal in 2009; Best Paper Award from a journal in 2016; Best Teacher Award in 2017; Best Researcher Award in 2018; and the **Distinguished Collaborator Award**, 2019, from UCLan, UK. Dr. Verma is a member of many national and international scientific societies and organizations, notably *Pharmaceutical Research* and others. Dr. Verma has been a member of the selection committee for the prestigious Khorana Scholar Program partnered by the Department of Biotechnology (DBT), Government of India; Indo-U.-S. Science and Technology Forum (IUSSTF); and WINStep Forward for 4 years. She has supervised 14 Ph.D. students and is currently supervising 6 doctoral and 2 postdoctoral students.

Indrakant K. Singh received his Ph.D. in Zoology from the Department of Zoology, University of Delhi (DU). Dr. Singh is currently working as Professor (Zoology), Deshbandhu College, and Fellow, Delhi School of Public Health, Institute of Eminence, University of Delhi, New Delhi, where he enjoys teaching immunology, genomics, computation biology, and medical diagnostics. He has also worked as a visiting professor at Division of Medical Oncology, Keck School of Medicine, University of Southern California, USA. He is a recipient of the **Young Scientist Award/Fellowship** (2013–2016) by **Science and Engineering Research Board (SERB)**, **Raman Post-Doctoral Fellowship** (2016–2017), **Max Planck Society Visiting Post-Doctoral Fellowship** (2019), **ICMR-DHR Long-Term International Fellowship for Young Biomedical Scientists** (2019–2020 and 2022–2023), prestigious **Delhi University Excellence Award for Teacher in Service** (2021), and **DHR Long-Term Fellowship for Training in Indian Institute** (2022). He has also been awarded with **Best Researcher**, 2017, and **Best Teacher**, 2018, by Grace India Trust. Dr. Singh has also filed two patents (India and the USA) on *Mycobacterium tuberculosis*. He is a life member/fellow/member of many prestigious academic societies including the RSB, AMCA, IBS, AIMI, ESI, BIDDS and BSI. Dr. Singh has published more than 60 quality research articles in peer-reviewed journals of international repute, like *Scientific Reports* and others. He is an

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Recent Advances on the Discovery of Plants Derived Bioactive Scaffolds/Extracts Against Parasitic Diseases

1

Charu Upadhyay, Sumit Kumar, and Poonam

Abstract

Infectious parasitic diseases are still a serious menace to the health of humankind, specifically across tropical regions, and are becoming a major challenge due to the increase in financial and mortality rates. Approximately, one billion people across the globe are affected by parasite-borne diseases such as leishmaniasis, malaria, and chagas disease. Currently, available antiparasitic drugs are having limitations like high cost, drug resistance, and side effects which promote the discovery of new potent drugs with better activity and target-parasite specificity. For decades, plant-derived natural products are known as the source of drugs and medicines throughout the world due to their low cost and high potency. Secondary metabolites originated from plant extraction like flavonoids, terpenes, chalcones, xanthones, and alkaloids are reported for their medicinal properties and treatment against various parasitic diseases. Investigation and screening of natural products can provide promising routes and better opportunities in the field of developing antiparasitic pharmaceuticals. In this chapter, we have discussed the recent advancement of plant-derived natural products and their derivatives as promising antiparasitic agents mainly focusing on leishmaniasis, malaria, and chagas disease.

Keywords

Leishmaniasis · Malaria · Anti-parasitic drug · Flavonoids · Chalcones · Xanthones

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_1

1.1 Introduction

Natural products are considered as the root of innovative and a promising lead in the field of drug designing and application in pharmaceutical areas (Davison and Brimble 2019). The fascination with natural products, especially plant-derived ones, has been observed since ancient times and is still of interest in the research field (Kayser et al. 2002). Parasitic diseases are a major and considerable menace for mankind, particularly in tropical areas across the globe. Most infectious diseases caused by parasites include leishmaniasis, schistosomiasis, malaria, and trypanosomiasis which seriously affect human health (Simoben et al. 2018). Unfortunately, many antiparasitic drugs have developed resistance, that has created more challenges for the eradication of such tropical parasitic diseases. According to the literature, approximately 60% of people in the entire world is dependent on folk medicinal plants to fulfill their health necessities. The strategy of plant extraction for the isolation of secondary metabolites such as chalcones, flavonoids, alkaloids, xanthones, terpenes, and coumarins is highly favorable in the pharmaceutical field against parasitic diseases like leishmaniasis, malaria, and chagas disease (Fig. 1.1).

Plant-based compounds containing biologically active moieties/phytochemicals are found to be a source of drug development and are highly efficacious against various parasitic diseases (Mathew and Negi 2019). Antimalarial drugs like artemisinin (isolated from *Artemisia annua*) **(1)** and quinine **(2)** (isolated from chinchon bark) (Fig. 1.2) are plant-derived medicines (Builders 2019).

Various compounds showing antiplasmodial activity are isolated from phenolic compounds such as chalcones, xanthones, flavonoids, and coumarins. Some naturally occurring chalcones as antimalarials are Medieagenin **(3)**, Menchiwanin **(4)**, and Licochalcone A **(5)** (Fig. 1.3) (Qin et al. 2020). In recent years, various plants and their modified scaffolds have shown better activity against leishmaniasis (Vechi et al. 2020).

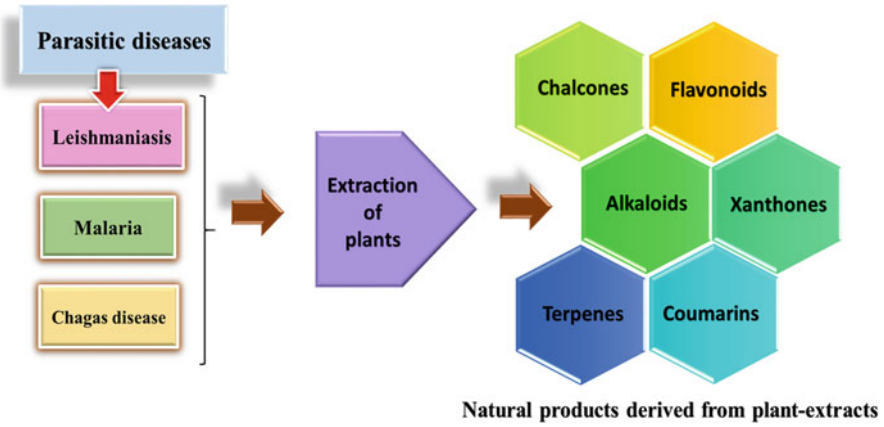


Fig. 1.1 Natural products against parasitic diseases

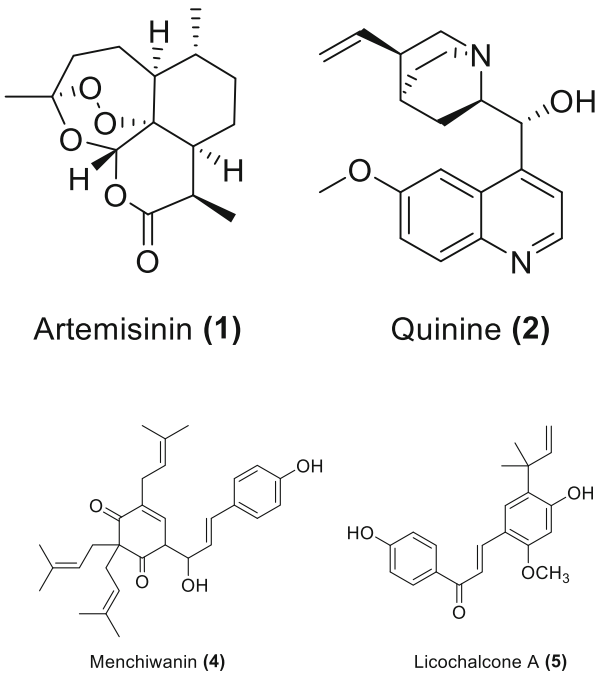


Fig. 1.2 Plant-derived antimalarials

Fig. 1.3 Naturally occurring chalcones as antimalarials

Natural products such as flavonoids, chalcones, saponins, quinolones, and diterpenoids (Bekhit et al. 2018) derived from plants are widely studied against leishmania infection. Other than malaria and leishmaniasis, chagas disease, also known as American Trypanosomiasis, is a parasitic disease which is mainly observed in the South American subcontinent (Álvarez-Bardón et al. 2020). Previous studies on Chagas disease have mentioned many natural products such as flavonoids, catechins, diterpenes, sesquiterpenes, monoterpenes, and xanthones as interesting bioactive scaffolds against it (Izumi et al. 2011). Despite the drugs available in the market, resistance and the absence of vaccines are still problems to be considered. Exploration of natural products and their derivatives can enlighten a path for successful drug discovery. In this chapter, we have discussed the recent advances in the plant-isolated compounds active against parasitic diseases viz.; leishmaniasis, malaria, and chagas diseases which are mentioned in Table 1.1.

Table 1.1 Plant containing natural products effective against parasitic diseases

Plant/plant species containing natural product	Diseases	Natural products present in the plant
P. marginatum	Leishmaniasis	Alkaloids, terpenes, steroids, chalcones, phenylpropanoids, flavones and flavonones
Calophyllum brasiliense	Leishmaniasis	Coumarins, triterpenes, steroids and xanthones
Curcuma caesia	Malaria	Sesquiterpene
Camu-camu	Malaria	Flavanols and flavonoids
Vitex doniana	Malaria	Flavonoids, terpenes, terpenoids
Hypoestes forskaolii	Malaria	Alkaloids
Carica papaya	Malaria	Alkaloids
Curcumin	Malaria	Flavonoids
Feretia canthioides Hiern	Malaria	Terpenoids, alkaloids, steroids and flavonoids
Artocarpus altilis	Malaria	Flavonoids and terpenes
Amaryllidaceae	Chagas	Alkaloids
Asteraceae	Chagas	Sesquiterpene lactones

1.2 Plant-Extracted Natural Products Against Leishmaniasis, Malaria and Chagas Diseases

1.2.1 Leishmaniasis

Leishmaniasis is a neglected infectious protozoan parasite-borne disease of the genus *leishmania* and is spread among human beings by the bite of an infected female insect vector, phlebotomine sandfly (Bongiorno et al. 2019; Cortes et al. 2020). Leishmaniasis is mainly found in three forms: cutaneous leishmaniasis (CL), visceral leishmaniasis (VL), and mucocutaneous leishmaniasis (MCL) (Raj et al. 2020). VL is commonly known as kala-azar (Bhunia and Shit 2020) and is the most fatal form that affects the vital internal organs like the spleen, liver, lymph nodes, and bone marrow (de Souza et al. 2020) while CL is the least acute form (Çizmeci et al. 2019) among all three forms of diseases. Across the globe, undeveloped and developing countries are considered to be endemic for this infectious disease. People infected with HIV (human immunodeficiency virus) are at high risk of getting infected from leishmaniasis due to poor immunity systems (Botana et al. 2019). Moreover, factors such as malnutrition, population mobility, and environmental conditions are also responsible for leishmaniasis infection. Leishmania parasite has two forms of development, these are known as amastigotes (non-flagellated spherical cells) and promastigote forms (thin elongate cells). The length of the diameter of the promastigote form (5–14 μm) is longer than the amastigote form (2–4 μm) (Thakur et al. 2020). According to the WHO report 2018, approximately one million cases of CL and 30,000 new cases of VL are observed annually (World Health Organization 2020). After malaria, leishmaniasis is placed second in terms of mortality rate among all the parasitic diseases (Pasandideh et al. 2020). For the

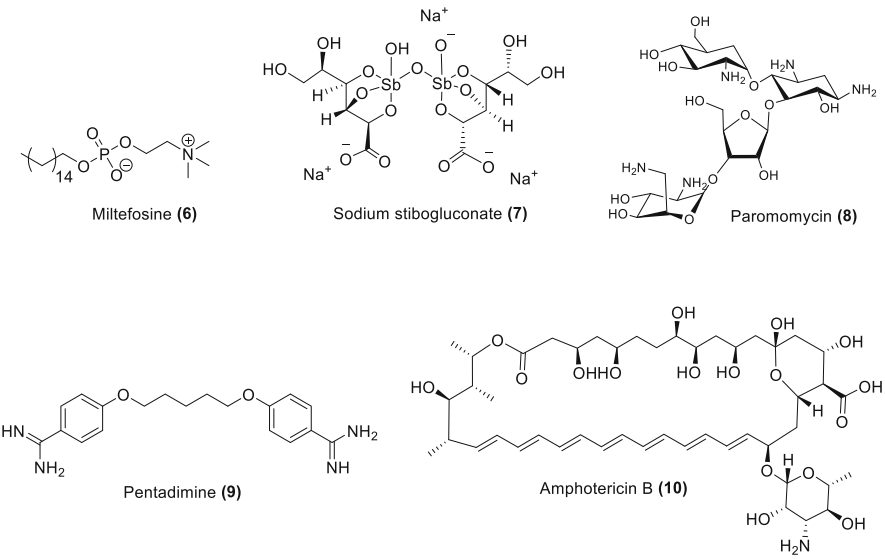


Fig. 1.4 Drugs available against leishmaniasis

treatment of leishmaniasis, currently, five drugs are known which include miltefosine **(6)**, pentavalent antimonials **(7)**, paromomycin **(8)**, pentamidine **(9)**, and amphotericin B **(10)** (Fig. 1.4).

However, eradication of the disease is still challenging due to the various side effects such as high toxicity, drug resistance, and high cost of the drugs (Hendrickx et al. 2019). Also, no effective vaccine is developed for leishmaniasis so far (Tiwari et al. 2018; Mbekeani et al. 2019). These drawbacks have alarmed an urgent need for the discovery of more potent and effective drugs against leishmaniasis (Passero et al. 2018). Keeping in focus, the recent findings related to plant-based bioactive analogs effective against leishmaniasis, the latest discoveries mentioned are as follows:

1.2.1.1 Naturally Derived Phenolic Substances

Recently Garcia et al. (2019) evaluated the inhibition of leishmania infantum arginase in vivo by a set of 14 naturally obtained phenolic substances containing flavonoids, stilbene, coumarins, and phenylpropanoids. Arginase is a metalloenzyme containing manganese that acts as a catalyst in the urea cycle in mammals and converts L-arginine into L-ornithine and urea (Malik et al. 2019). In the case of leishmania species, arginase plays a crucial role in regulating the growth of parasites and their survival inside the host body (Pessenda and da Silva 2020). Being the first enzyme of the polymine pathway, it supports the amazonensis replication of leishmania (da Silva et al. 2012). In this work, Garcia et al. also performed molecular docking to analyse the mechanism of enzyme inhibition and further investigated the effect of these 14 natural compounds on parasite biology (Garcia et al. 2019). Initially, the inhibition % against the *Leishmania infantum* arginase was analysed.

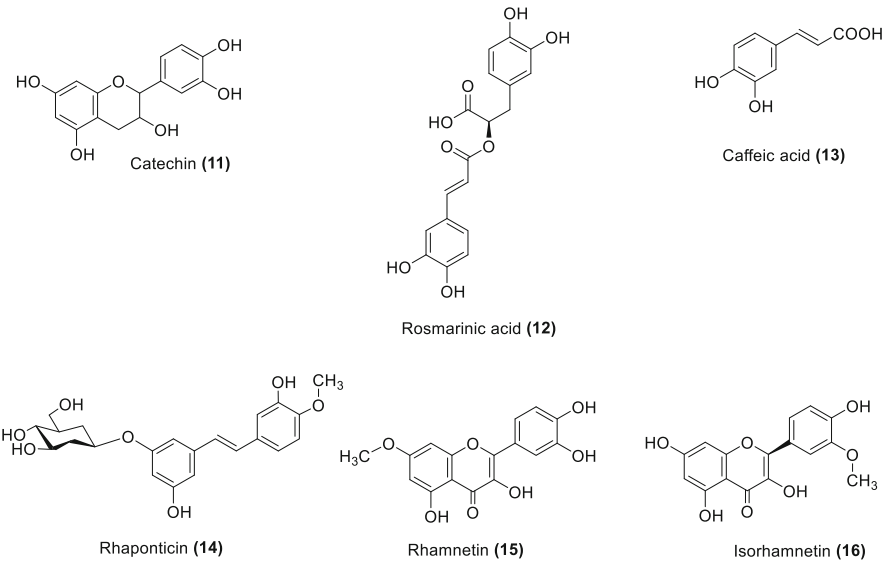


Fig. 1.5 Antileishmanial naturally occurring phenolic compounds

Out of 14 phenolic compounds, compounds that displayed more than 50% of *Leishmania infantum* arginase inhibition activity were further chosen for in vivo experiments (Fig. 1.5). Among all seven (selected) phenolic analogues tested, rosmarinic acid **(12)** [IC_{50} (half maximal inhibitory concentration) = 57.3 ± 2.65 μM] and caffeic acid **(13)** (IC_{50} = 60.8 ± 11 μM) showed the high selectivity with the most potent in vivo antileishmanial activity against promastigote form of *Leishmania infantum* and showed inhibition % of 71.48 ± 0.85% and 56.98 ± 5.51%, respectively at 100 μM concentration. However, rhamnetin **(15)** and isorhamnetin **(16)** displayed a lesser IC_{50} value of 832 ± 6.9 μM and 818 ± 30 μM respectively but were found to be very less toxic for RAW 264.7 macrophages leading to high selectivity. Catechin **(11)** showed an IC_{50} value of 395 ± 50 μM although rhaponticin **(14)** was inactive against *Leishmania infantum*. Compounds such as catechin **(11)**, rosmarinic acid **(12)**, caffeic acid **(13)**, and rhamnetin **(15)** showing good in vivo results shared a common moiety of catechol group suggesting that it is an efficient core for the arginase inhibition in other *Leishmania* species.

All these results signify that a combination of these phenolic natural products can emerge as promising scaffolds with less toxicity and more activity for the treatment of leishmaniasis (Garcia et al. 2019).

1.2.1.2 *P. marginatum* Extracts

A large number of plant species are known of the genus *Piper* from the Piperaceae family which are broadly used as food and also for the treatment purpose for various diseases (Takooree et al. 2019). This class of genus has a wide variety of constituents of natural products such as alkaloids, terpenes, steroids, chalcones,

phenylpropanoids, flavones, and flavanones which are well known for their biological applications (Parmar et al. 1997). *P. marginatum*, is a plant species that belongs to the *Piper* genus, showing a wide range of antimicrobial and bactericidal activity and contains medicinal properties like healing (Durant-Archibold et al. 2018). Essential oil and crude extracts of *P. marginatum* are still not explored extensively, hence can be studied further for the development of antiparasitic diseases. Keeping all these facts, Macedo et al. (2020) investigated the in vitro experiments of essential oil, ethanolic extract, and different fractions of *P. marginatum* against *Leishmania amazonensis.* All the samples were tested at concentrations 1, 10, and 100 μg/mL for 48 h and a cytotoxicity assay was performed against macrophages. When compared to the reference drug, Pentamidine, samples like methanolic, hexane, ethyl acetate fractions, and ethanolic extract showed less toxicity to macrophages. Among all the samples, essential oil and ethanolic extract showed significant inhibition against the growth of promastigotes forms of *L. amazonensis* (Macêdo et al. 2020). This experiment describes that *P. marginatum* can be considered as a promising candidate in the field of leishmaniasis treatment.

1.2.1.3 *Calophyllum brasiliense* Extracts

Calophyllum brasiliense is a plant species of *Calophyllum* genus and *Calophyllaceae* family which has been used as a medicinal plant against leishmaniasis (Domeneghetti et al. 2018). Various plant-derived natural products such as coumarins, triterpenes, steroids, and xanthones can be isolated from this plant (Gupta and Gupta 2020). Previous studies have shown that coumarins isolated from *Calophyllum brasiliense* were found to be active against *Leishmania amazonensis* (Brenzan et al. 2007).

Silva et al. (2021) continued their studies by isolating two geometrical isomers of coumarins from the stem of *Calophyllum brasiliense*, compound **(17)** and **(18)** (Fig. 1.6) by bioactivity-guided fractionation.

Further, a cytotoxicity assay was performed against the NCTC-L929 clone cell line for both compounds and their activity against *L. infantum* was tested. The result showed that both compounds were non-toxic up to the concentration of 200 μM. The EC_{50} values for compounds **(17)** and **(18)** were found to be 29.1 and 37.1 μM in the biological experiment conducted against amastigotes forms of *L. infantum*. In conclusion, compounds **(17)** were more significant as they displayed a better EC_{50} value and selectivity index (Silva et al. 2021). These biological data give a path to the extension of coumarins study in order to design better scaffolds for leishmaniasis disease.

1.2.1.4 Aporphine Natural Products

The aporphines are considered to be a type of alkaloid that possess quinoline core showing many pharmacological features mainly against such as anticancer, anti-inflammatory, and antiparasitic activities but are most commonly studied against parasitic diseases. Pieper et al. (2020) reported the biological evaluation of four

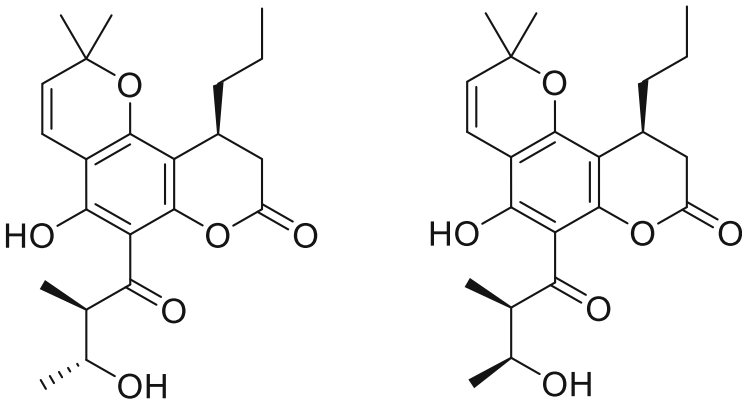


Fig. 1.6 Geometrical isomers of coumarins

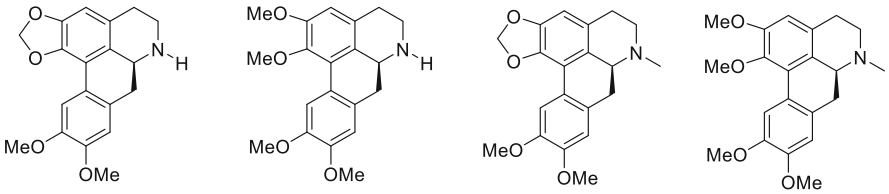


Fig. 1.7 Aporphine natural products

aporphine compounds, Nordicentrine **(19)**, Norglaucine **(20)**, Dicentrine **(21)**, and Glaucine **(22)** against *L. infantum* (Fig. 1.7).

In this work, they tested the cytotoxicity of all these four natural products against NCTC cell lines and further evaluated their EC_{50} values against the intracellular amastigotes forms of *Leishmania*-affected macrophages. Two of the four compounds **(20)** and **(21)** were found to be active *L. infantum* amastigotes. Compound **(20)** was more effective and displayed an EC_{50} value of 21.7 μM against the intracellular amastigote form of *L. infantum* along with a significant CC_{50} value (71 μM) while compound **(21)** showed an EC_{50} value of 10.5 μM against the *L. infantum* amastigote forms (Pieper et al. 2020). Further studies on aporphines can lead a path for the development of these forms of alkaloids as antiparasitic agents.

Apart from the biological evaluation, docking studies of natural products like flavonoids have also given effective results against leishmaniasis. Mercado-Camargo et al. (2020) analysed docking results with glycoprotein 63, a metalloprotease responsible for the pathogenesis and virulence of leishmania.

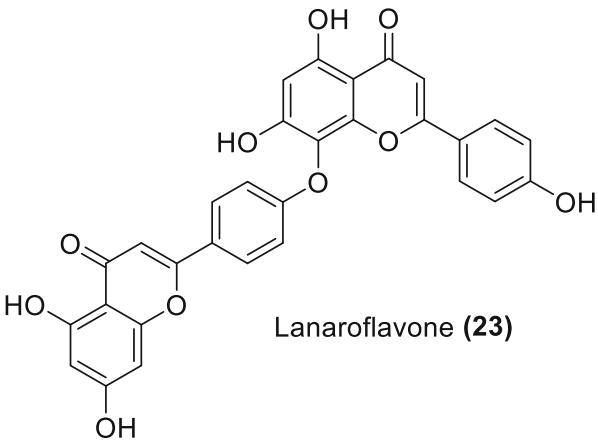


Fig. 1.8 Structure of Lanaroflavone

Based on the binding energy, it was observed that among all the flavonoids studied, Lanaroflavone **(23)** (Fig. 1.8) showed the lowest binding energy and was effective against *L. major* (Mercado-Camargo et al. 2020).

1.2.2 Malaria

Malaria is the most deadly parasitic infectious disease that is caused by protozoan parasites of the Plasmodium genus (Singh et al. 2015; Sharma et al. 2019). Malaria in humans is responsible due to five types of parasite species namely, *Plasmodium falciparum* (*Pf*), *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium knowlesi* and *Plasmodium malariae* with *Pf* as the most lethal form which is responsible for the higher mortality rate (Upadhyay et al. 2020). All of these parasites have a unique life cycle with different stages (sexual and asexual) inside the host body (Poonam et al. 2018; Singh et al. 2018). Approximately, 228 million cases are reported in 2018 globally (World Health Organization 2019). Most of the endemic areas are covered by the region of Africa. Despite drugs available in the market, the emergence of drug resistance demands the development of more effective antimalarial drugs for multiple life stages of malaria infection (Singh et al. 2019). Natural products are a great origin for the development of medicines and drugs (Kumar et al. 2008). Indoloquinoline based natural-product isolated from roots of *Cryptolepis sanguinolenta* plant such as cryptolepine **(24)**, ocryptolepine **(25)**, and isocryptolepine **(26)** have shown antimalarial activities and are potential leads in future development (Fig. 1.9) (Sydnes 2020).

Recently, many researchers have reported antimalarial activity of plants and their extracts like *Salvadora persica* and *Balanites rotundifolia* (Gebrehiwot et al. 2019), *Entandrophragma angolense* (Kamkumo et al. 2020), *Rauvolfia caffra* (Tlhapi et al. 2019), *Senna occidentalis* (Daskum et al. 2019), *Vernonia amygdalina* (Airaodion et al. 2019). Natural products like dihydroisocoumarin derivatives (Ghosh and Lee

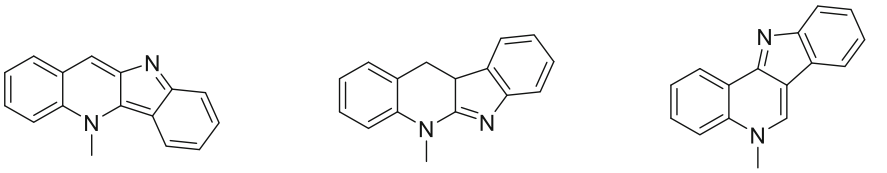
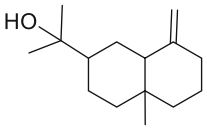


Fig. 1.9 Naturally occurring indoloquinolines

Fig. 1.10 Structure of β-selinenol



2019), Sesquiterpenoid (Wu et al. 2020), alkaloids (Cheenpracha et al. 2019; Uzor 2020) are found in some potent scaffolds against malaria parasites.

1.2.2.1 *Curcuma caesia* Extracts

Curcuma is a perennial herb that has been used for medicinal mainly against antiparasitic diseases (Haddad et al. 2011). Chaturvedi et al. (2020) studied in vitro as well as in silico activity of ethyl acetate and methanol extracts of *Curcuma caesia* against CQ (chloroquine) sensitive (*Pf*3D7) and CQ resistant (K1) strains of malaria infection. The IC_{50} value of the ethyl extract fraction was found to be 3.37 μg/mL (*Pf*3D7) μg/mL and 1.53 μg/mL (K1) against the plasmodium parasite. The methanolic extract of *Curcuma caesia* displayed an IC_{50} value of 8.57 μg/mL (*Pf*3D7) μg/mL and 18.29 μg/mL (K1) respectively. Among all 31 ligands analysed from *Curcuma caesia* extract of methanol and ethyl acetate, five of them showed the highest binding energy. The highest negative value of binding energy corresponded to β-selinenol **(27)** (−6.76 kcal/mol, an oxygenized sesquiterpene) (Fig. 1.10) (Chaturvedi et al. 2020).

Still, there is a need for further studies of the compounds isolated from *Curcuma caesia* and evaluation of their potency as antimalarial agents.

1.2.2.2 Camu-Camu Seeds

Myrciaria dubia is a tree, commonly known as camu-camu, found in the Amazon forest and used to produce food products (Hernández et al. 2011). However, Camu-camu seeds are valuable for their medicinal properties such as antioxidant, anti-inflammatory, and antimicrobial but the area of parasitic diseases is still unexplored. Making an effective attempt, do Carmo et al. (2020) stated the antimalarial activity of the five extracts of camu-camu seeds with different fractions of water and ethyl alcohol. In vivo assay resulted in the IC_{50} values from 24.2 to 240.8 μg/mL against *Pf*W2 and *Pf*3D7 strains of plasmodium parasites (do Carmo et al. 2020).

1.2.2.3 *Coccinia barteri* Leaf Extract

Coccinia barter is a remedial plant from the family of cucurbitaceae, which is used as a drug in the treatment of malaria fever (Orabueze et al. 2017). Orabueze et al. (2020) evaluated the in vivo antimalarial activity of crude extracts and solvent fractions of *Coccinia barteri* leaf against CQ-sensitive strain of malaria, *Pf*NK65. Results of parasitemia level and mean survival time of animal models support the extension of *Coccinia barte* fact-findings as antimalarial templates.

1.2.2.4 *Vitex doniana* Extract

Vitex doniana is a native perennial tree found in South western Nigeria (Dadjo et al. 2012) and is used in curing the diseases such as diarrhea, dysentery, and liver diseases (Rabiat et al. 2013).

Uzoho et al. (2020) analysed the in vivo antimalarial inhibition of methanolic and aqueous fractions of the root, leaves and stem bark of *Vitex doniana* as per the result noted, the parasitaemia level of the infected animals showed a reduction that supports the further research of *Vitex doniana* to give potent antimalarial scaffolds.

1.2.2.5 *Hypoestes forskaolii* Extract

Hypoestes forskaolii (Vahl) is a perennial herb that belongs to the family Acanthaceae and is found in the region of Ethiopia (Gebremedhn and Tesfay 2012). Abdel-Sattar et al. (2020), for the first time, reported the antimalarial activity of the alkaloids isolated from *Hypoestes forskaolii* plant against the CQ resistant strain of malaria parasite. In their experiment, they screened the antimalarial activity of 18 plant extracts belonging to nine families. Based on the results, *Hypoestes forskaolii* was selected for its further biological evaluation since it showed the most interesting result in the first screening. Methanolic extract of *Hypoestes forskaolii* was tested against K1 and FCR3 strains of malaria and demonstrated the highest in vitro activity for the solvent fraction of chloroform and ethyl acetate. Different chromatographic sub-fractions of chloroform and ethyl acetate fraction led to the isolation of an active antimalarial scaffold **(28)**, a phenanthro-quinolizidine alkaloid (Fig. 1.11a).

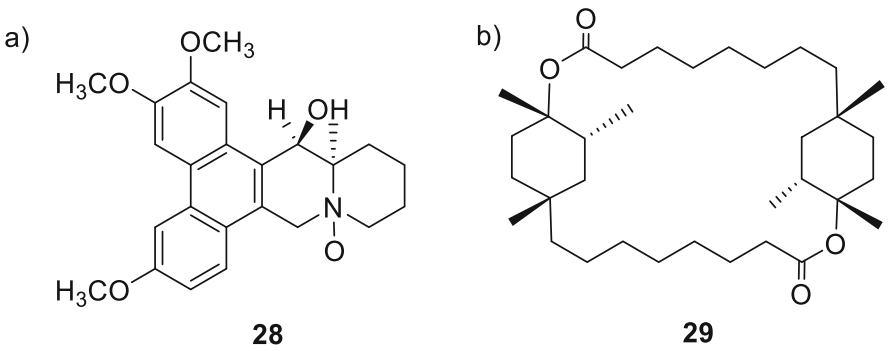


Fig. 1.11 (**a**) Phenanthro-quinolizidine alkaloid. (**b**) Carpaine

This compound was active against K1 strain [IC_{50} = 2.5 ng/mL (6.11 nM)] as well as FCR3 strain [IC_{50} = 2.1 ng/mL (5.13 nM)] of *Pf* (Abdel-Sattar et al. 2020). The detailed study of such new phenanthro-quinolizidine alkaloids can emerge as a hope for finding potent antimalarials.

1.2.2.6 *Carica papaya* Leaf Extracts

Carica papaya is a well-known fruit tree that is found in the tropical and subtropical regions of the world and is used as a food product with medicinal properties like antiviral, antibacterial, and antifungal due to the presence of essential vitamins and enzymes (Vij and Prashar 2015). Teng et al. (2019) in their work, extracted and screened the cytotoxicity against NL20 cell lines and antimalarial activity of samples of *Carica papaya* leaf extracts against *Pf*3D7 and *Pf*Dd2 strains of plasmodium by conducting bioassay-guided fractionation. Among all the extracts, alkaloidal hexane extract was the most active sample, from which the most potent compound (Fig. 1.11b), carpaine **(29)** was isolated. Carpaine **(29)** displayed an effective inhibition against *Pf*3D7 and *Pf*Dd2 strains with an IC_{50} value of 4.21 μM and 4.57 μM respectively (Teng et al. 2019). The non-toxic behavior of carpaine to RBCs, its activity against the strains of *Pf*, and its high SI value (128) promotes it for further development of its structure–activity relationship by synthesizing its derivatives.

1.2.2.7 Curcumin Analogues

Curcumin is a bright yellow powder chemically extracted from the root of *Curcuma longa* (turmeric plant) and is well known for its therapeutic effects against Alzheimer's, Parkinson's disease, diabetes, and arthritis (Sudhanshu and Raj Kumar 2016). Balaji et al. (2019) synthesized curcumin analogues and evaluated schizonticidal activity with an IC_{50} value of 1.48–23.09 μM. These curcumin analogues were classified into three different functionalized derivatives (carboxamide, methanone, and pyrimidine). Among carboxamide derivatives, **(30)** showed the most effective activity ($IC_{50} = 9.87 \pm 2.21$ μM) whereas compound **(31)** ($IC_{50} = 4.21 \pm 0.62$ μM) displayed the best result among the methanone analogues (Fig. 1.12).

However, compound **(32)**, a pyrimidine analogue displayed the maximum activity among all the 19 curcumin derivatives with an IC_{50} value of 1.48 ± 0.10 μM and hence can be explored as an interesting lead for further optimization (Balaji et al. 2019).

1.2.2.8 *Feretia canthioides* Hiern Extracts

Feretia canthioides Hiern is a medicinal herb that belongs to the Rubiaceae family of plants and the stem bark of this plant is often used for the cure of malaria fever in Nigeria (Egbubine et al. 2020). Egbubine et al. (2020) carried out in vitro assay against antimalarial activity by screening extracts of bark of *Feretia canthioides* Hiern with *n*-hexane, dichloromethane, ethyl acetate and methanol solvent. The methanolic extract contained natural products like terpenoids, alkaloids, steroids, flavonoids, glycosides, and saponins. In addition, one compound, betulinic acid **(33)**

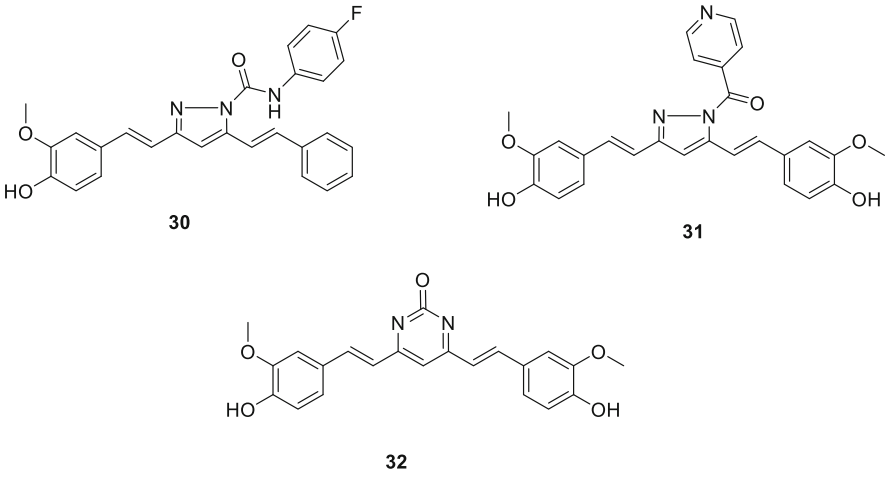


Fig. 1.12 Curcumin analogues

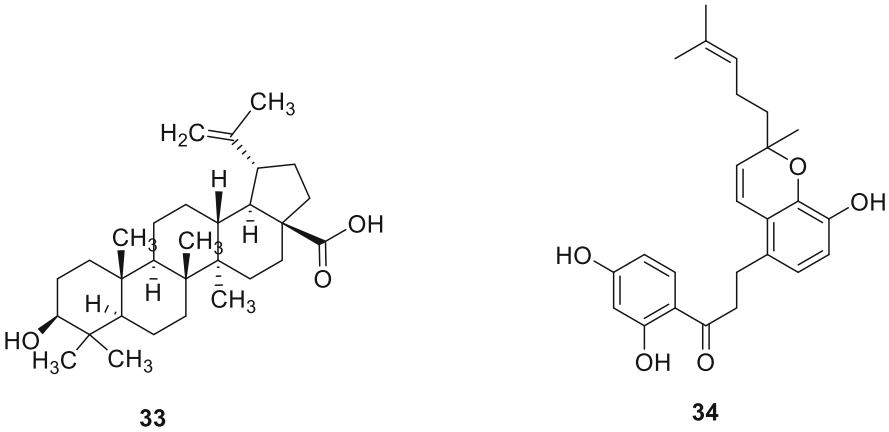


Fig. 1.13 (**a**) Betulinic acid. (**b**) Dihydrochalcone

(Fig. 1.13a) was also isolated. The in vitro results demonstrated that the methanol extract was exhibiting the most significant activity with an IC_{50} value of 7.76 μg/mL among the four solvent fraction. When compared to the chloroquine phosphate (positive control), betulinic acid (compound isolated from the methanol extract), a pentacyclic triterpenoid, displayed fair antimalarial activity results with an IC_{50} value of 12.60 μg/mL (Egbubine et al. 2020). All these phytochemical screening of the plant extract point towards the research of this plant in serving as an efficient agent for malaria therapeutics.

1.2.2.9 *Artocarpus altilis* Extracts

Artocarpus altilis, also known as breadfruit belongs to the family Moraceae and is rich in phenolic compounds like flavonoids and terpenes (Badrie and Broomes 2010). The pharmacological activities of *Artocarpus altilis* such as anti-inflammatory, antifungal, and antioxidant are widely studied (Sikarwar et al. 2014). Agriana Rosmalina et al. (2020) reported the isolation of an active compound using chromatography methods from the ethanol extract of *Artocarpus altilis* leaves and its antiplasmodial activity. Six fractions were obtained by the extraction of this plant and one of the fractions indicated the presence of a flavonoid compound by the TLC method. Further separation of this fraction yielded an active flavonoid compound **(34)** from the class of dihydrochalcone (Fig. 1.13b), confirmed by the NMR and MS spectra data. For further studies, molecular docking and antimalarial assay were performed. In vitro assay result explained the inhibition of growth of *Pf* displaying an IC_{50} value of 1.05 μM. Molecular docking experiments revealed that the compound showed a strong interaction with the 3BPF, a falcipain-2 receptor, an inhibitor of cysteine protease (Agriana Rosmalina et al. 2020). Both these experimental results strongly recommend the in vivo study of this compound and advance research on *Artocarpus altilis* as a new mode of antimalarial scaffolds.

1.2.3 Chagas Disease

Chagas disease is an inflammatory parasitic disease triggered by the protozoan parasite, *Trypanosoma cruzi* (*T. cruzi*), unicellular protozoa of genus Trypanosoma and family Trypanosomatidae. This neglected tropical disease nearly kills 10,000 people every year, however, it can be cured if diagnosed in the early stages of infection. During its life cycle, *T. cruzi* exhibits three forms which are epimastigote, trypomastigote, and amastigote forms of parasite where the amastigote form is difficult to be cured by drugs. Currently, two drugs, Benznidazole **(35)** and nifurtimox **(36)** (Fig. 1.14) are used against chagas disease (Álvarez-Bardón et al. 2020).

Benznidazole **(35)** is used for acute stages while nifurtimox **(36)** is a second-line option for the treatment. But the need for new promising chemotherapy is observed since the issue of drug resistance and side effects of these drugs have emerged.

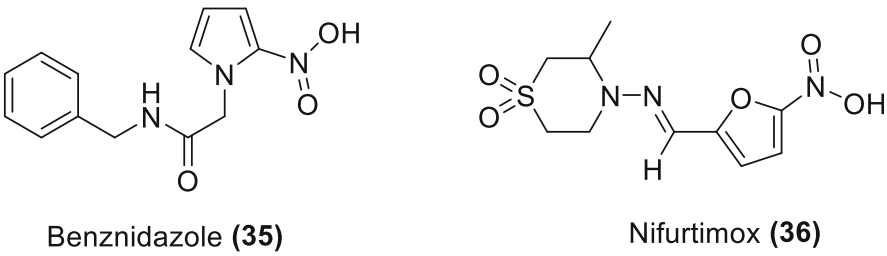


Fig. 1.14 Drugs known for chagas disease

Natural products such as quinolines, triterpenoids, flavonoids, and xanthones are previously reported as against *T. cruzi* (Cockram and Smith 2018; Simoben et al. 2018). Extensive study of natural products and plant extractions can be worthy in the discovery of new drugs that can affect all the morphological forms of *T. cruzi*.

1.2.3.1 Plant-Derived Alkaloids of the Family Amaryllidaceae

Amaryllidaceae is a family of herbs mainly flowering plants which are considered for biological applications due to the presence of alkaloids in them (Cahlíková et al. 2019; Chahal et al. 2020). Plants belonging to this family are a potential source of anti-parasitic, antimicrobial, anti-inflammatory, and anticancer properties (Presley et al. 2016). Martinez-Peinado et al. (2020) analysed the activity of nine alkaloids derived from the plants of this family against *T. cruzi* by performing a growth inhibition phenotypic assay. One compound, hippeastrine **(37)** (Fig. 1.15), was found to be potent against the amastigote stage of *T. cruzi* with an IC_{50} value of 3.31 μM with the high selectivity indexes against Vero (12.7) and HepG2 (35.2) cells.

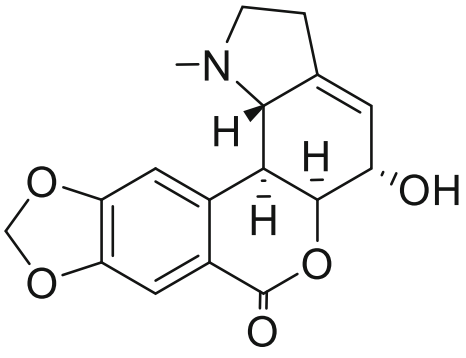
Cytotoxic results confirmed the low toxicity of the compound against Vero cells (TC_{50} = 45.99 μM) and HepG2 cells (TC_{50} = 128.1 μM) respectively (Martinez-Peinado et al. 2020). Extending the library of alkaloids seems promising for anti-*T. cruzi* activities.

1.2.3.2 Estafietin (A Sesquiterpene Lactone) and Its Analogues

Sesquiterpene lactones are naturally occurring compounds that are generally found in the family of asteraceae plants of herbs and flowers (Sepúlveda-Robles et al. 2019). These types of secondary metabolites are well known for their pharmaceutical role. Sülsen et al. (2019) isolated estafietin **(38)** from the plant Stevia alpine of the same family and further synthesized its four derivatives (Fig. 1.16).

The biological experiment of these compounds was carried out against trypomastigotes and amastigotes forms of *T. cruzi*. The IC_{50} value of estafietin

Fig. 1.15 Hippeastrine



Hippeastrine **(37)**

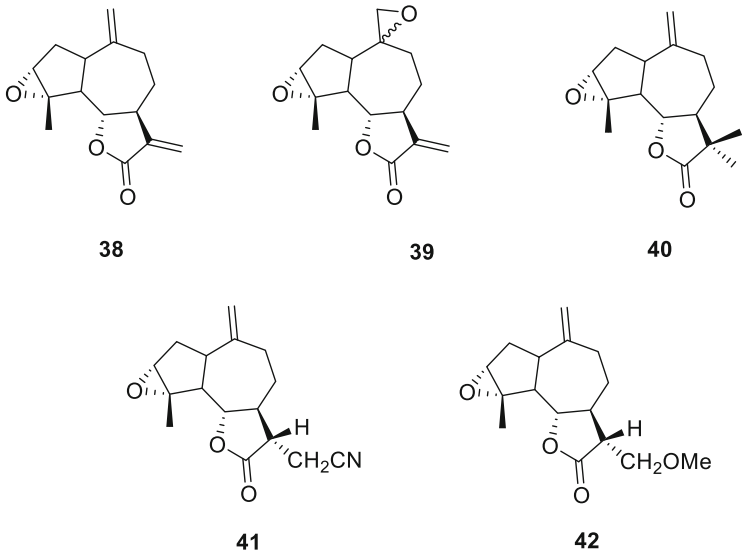


Fig. 1.16 Estafietin and its derivatives

(38) was 25.2 g/mL against the bloodstream trypomastigotes form of *T. cruzi*. After comparing with the reference drug, Benznidazole **(35)** ($IC_{50} = 11.6$ g/mL), the IC_{50} values for compound **(40)** ($IC_{50} = 97.1$ g/mL) and **(41)** ($IC_{50} = 78.1$ g/mL) were less active while compound **(42)** was inactive against *T. cruzi* trypomastigotes. In addition, all the compounds were also tested against the amastigotes form of *T. cruzi*. The IC_{50} values of estafietin **(38)** (28.8 g/mL) and compound **(42)** (30.5 g/mL) were similar, however compound **(40)** and **(41)** were less active with an IC_{50} values of 83.0 g/mL and 99.0 g/mL respectively against *T. cruzi* trypomastigotes. Among all the analogues, the epoxyestafietin compound **(39)** showed the best activity against the trypomastigotes as well as amastigotes forms of *T. cruzi* with an IC_{50} value of 18.7 and 2.0 g/mL, respectively (Sülsen et al. 2019).

1.3 Conclusion

Since ancient times, natural-based products have had a history in medications and therapeutics. Even in today's pharmaceutical world, 80% of the human population depends on medicinal plants and their combination with other drugs for the sake of human health, especially in developing countries that are facing more economical issues. From the above discussion, it can be observed that extraction of medicinal plants, herbs as crudes, solvent fractions, samples, and isolated compounds are effective against parasitic diseases with reduced toxicity. Two common drugs against malaria, quinine, and artemisinin are derived from medicinal plants. Since no effective vaccine has been developed yet, the search for small molecules as drugs

is still under the area of interest. Hundreds of plant extracts have displayed interesting in vitro and in vivo results, further clinical trials in animal models are still needed. The presence of phytochemicals in the form of secondary metabolites is highly effective with fewer side effects and toxicity when tested against parasites like *Leishmania infantum*, *Pf* and *T. cruzi* as discussed in this chapter. Hence, plant-derived natural products could be considered as the safer and more prominent path to identify novel drug leads against various parasitic diseases. Moreover, the combination of natural products or their derivatives with different drugs available in the market can be an additional approach for more surprising results in pharmaceutical industries.

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Applications of Computational Methods in Natural Products Based Drug Discovery

2

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Abstract

Since antiquity, natural resources, mainly plants, have been used for medicinal purposes. The primitive usage was based on a trial-and-error strategy. When time passed, humans started to look deeper into the actual elements responsible for the cure. They started using plant extracts as medicines. In 1806, there opened a new window in the area of natural product-based medicines when Friedrich Sertürner, a German pharmacist, isolated morphine from the poppy plants. This is just the beginning of a new era. Soon, the extraction of phytochemicals i.e., plant-based chemical compounds, became common and chemists started looking for new ways of manipulating them and synthesizing them in laboratories. Charles Frédéric Gerhardt first synthesized acetylsalicylic acid, the wonder drug Aspirin by treating acetyl chloride and sodium salicylate in 1853. This was a revolution in drug discovery which is still running towards more advancements and developments. Later on, computers came into the picture, and chemists and molecular biologists started to visualize and analyze chemical compounds. The emergence of advancements in computers set the foundation stone for a new field of science called bioinformatics. Soon began the applications of computers in medicinal research and a new trend of computer-aided drug discovery started. Which broadly changed the face of the natural product (NP) based drug discovery process. We have huge libraries of NP-based compounds utilized to discover new drugs against several life-threatening diseases, including cancer. This chapter

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_2

talks about applications of computational methods mainly belonging to Bioinformatics and Chemoinformatics that are applied towards NP-based drug discovery.

Keywords

Plants · Natural products · Drug discovery · Bioinformatics · Chemoinformatics · Machine learning

2.1 Background

Since ancient times humans have been exploring natural sources like plants and herbs for medicinal purposes. The primitive usage of these products was limited to the direct application of plant leaves, barks, extracts, fruits, seeds, etc., as medicines. The earliest traces of the medicinal usage of plants are found in the Sumerian civilization's Clay tablet records (Petrovska 2012). Historical records from ancient Egypt, Greek, and the ancient Indus valley civilization have shed light on the pre-historic usage of plants for therapeutic aspects (Bernardini et al. 2018; Dias et al. 2012). However, for a long time and still, to some extent, these techniques have been considered a clear example of medicinal witchcraft despite their success in treating certain ailments of humans and animals. The emergence of molecular biology has made some remarkable discoveries that emphasized the importance of natural resources as medicinal agents.

When we talk about natural products (NPs) in the context of drug discovery, the majority of instances come from the plant kingdom or medicinal plants. Phytochemicals are prominent examples of NPs in drug discovery. Besides, fungi, bacteria, certain insects, and other vertebrates have also been reached for medicinal purposes. There are so many comprehensive reviews that shed light in detail on the types of NPs (Cragg et al. 1997; Harvey et al. 2015; Romano and Tatonetti 2019). In the chapter, our focus will mainly be confined to plant-based NPs or phytochemicals and various techniques in Bioinformatics and Cheminformatics that have been applied so far to develop and design new drugs.

2.2 Historical Overview of Drug Discovery from Natural Products

Although the usage of natural products for the therapy of various ailments has pre-historic traces, from the modern perspective, drug discovery from NPs ways back to the nineteenth century when extraction and chemical analysis came into existence, for a comprehensive review of the usage of NPs for medicinal purposes, readers are suggested to refer to (Petrovska 2012). This journey began with the isolation of alkaloids from plants. Friedrich Sertürner, a German pharmacist, first isolated morphine from the poppy plants in 1806 (Pathan and Williams 2012). This is the first-ever instance of extraction of NP from a plant in modern pharmacology.

After a decade, some more alkaloids were extracted from the plants like ipecacuanha and strychnos in 1817 (Petrovska 2012). The credit from the commercial extraction of alkaloids, mainly morphine, goes to Heinrich Emanuel Merck in 1826 in Germany (Atanasov et al. 2015).

The second phase of advancements in the exploitation of NPs in drug discovery began with synthesizing various phytochemicals and their derived products in the laboratory. The most remarkable example of such experiments is acetylsalicylic acid, aka Aspirin, the wonder drug used as a pain killer. Charles Frédéric Gerhardt first synthesized acetylsalicylic acid by treating acetyl chloride and sodium salicylate in 1853 (ref). Sodium salicylate is a sodium salt of salicylic acid. Salicylic acid has been mainly extracted from the Willow tree and other salicin-rich plants. Willow bark is known for medicinal purposes in the ancient Sumerian and Egyptian civilizations (Norn et al. 2009). Later on, the commercial synthesis of Aspirin began in 1897 by Felix Hoffmann in the Bayer Company.

Moreover, the discovery and synthesis of Aspirin from plant-based extracts have ignited a rapid growth and development of NP-based drug discovery, which is still on the path of progress with modern strategies and techniques. In the next section, we will discuss some strategies for NP-based drug discovery, mainly focusing on the computational and data-driven methods using Bioinformatics and Cheminformatics'.

2.3 Strategies for Drug Discovery Through Natural Products

In this section, we will discuss some modern strategies applied in NP-based drug discovery and design. These strategies make use of established Bioinformatics and Cheminformatics methods. Some leading methods incorporated in these approaches are QSAR analysis, pharmacophore modeling, molecular docking, molecular dynamics simulation, gene expression-based drug discovery, NP library construction, biomarker identification for NP-based drugs, NP-derived database development, big-data-driven drug discovery, machine learning-based methods, combinatorial library construction, fragment-based drug discovery, pharmacokinetic properties prediction and so on (see Fig. 2.1 for a generalized in silico drug discovery process).

2.3.1 Quantitative Structure-Activity Relationship (QSAR)

QSAR is a well-known technique applied in structure-based drug discovery. Although, the formal definition and technical details of QSAR are disseminated in a wide range of literature published previously. But for the brief overview for the reader, QSAR is applied to correlate the activity of a chemical compound with its structure. This uses a set of "predictor variables," which are molecular descriptors as physicochemical properties of the compounds in the context of chemical compounds. Based on these values, the biological activities of new chemical

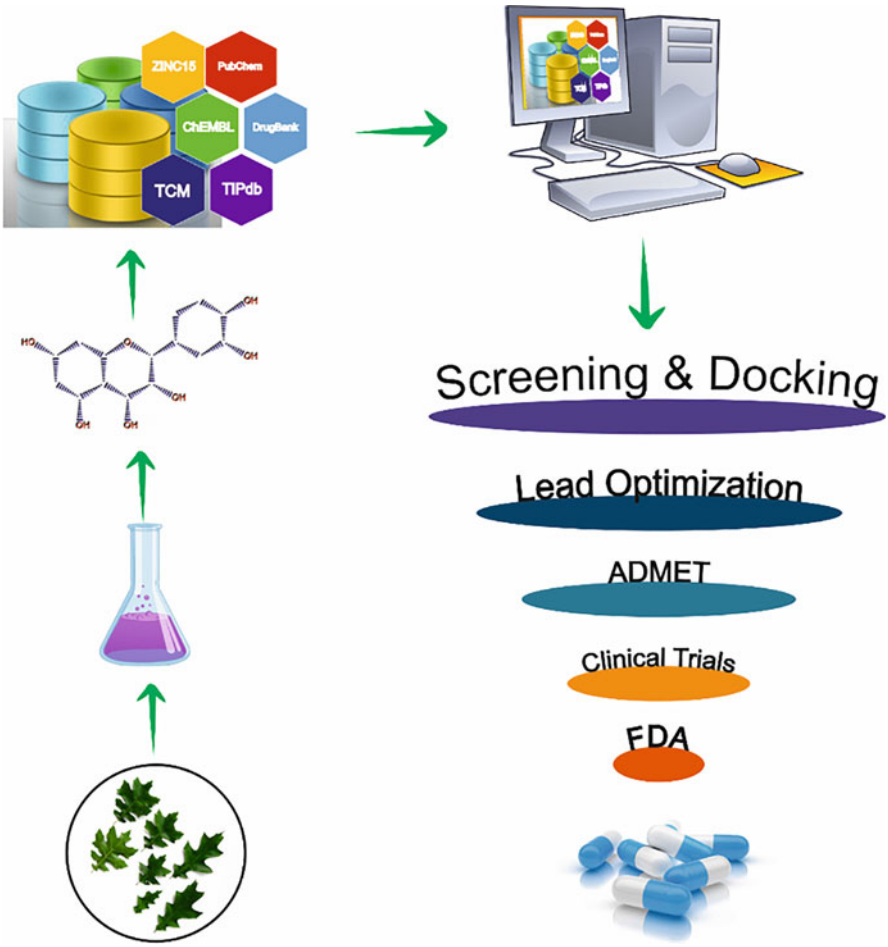

Fig. 2.1 Graphical representation of in silico drug discovery process from NPs

compounds can be predicted (Verma et al. 2010). The applications of QSAR in NP-based drug discovery we established and have come forward with promising results (Ref).

2.3.2 Pharmacophore Modelling

Pharmacophore "*is the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target structure and to trigger (or to block) its biological response (IUPAC).*" Pharmacophore modeling and its application in drug discovery have seen a rise during recent years. Similarly, NP-based drug discovery has also been benefited

from the powers of pharmacophore modeling. The detailed description of pharmacophore approaches is beyond the scope of this chapter. The reader is suggested to refer to (Leach et al. 2010; Yang 2010) for a thorough understanding of the various aspects of pharmacophore modeling and its application in drug discovery.

2.3.3 High Throughput Virtual Screening and Molecular Docking

Screening of 1000 compounds against a target molecule in the laboratory is costing too much of money and time. To overcome the complications of this approach and make the processing time and cost-effective, high throughput virtual screening (HTvS) has been applied to scan a library of bioactive compounds with a biomarker for drug discovery. Subsequently, a selected set of possible candidate compounds are taken for docking analysis which further elucidates the patterns of binding and interaction with the target molecule. Docking helps identify the possible physical interaction between ligand and receptor while suggesting conformational poses that might fit into the binding cavity of the receptor. The combination of HTvS and molecular docking are well-established methods applied in computer-aided drug discovery in general and for NP-based drug development in particular.

2.3.4 Molecular Dynamics Simulations

The first successful application of MD simulation to study protein folding in 1979 opened a window for theoretical chemists to develop further the technique to study more intricate biological processes (i.e., protein–ligand interaction) at the atomic level that are otherwise difficult to study in lab conditions. With this, MD simulations became the most desired tool to study the conformational effects of ligand binding on the target molecule. MD simulations, QSAR, pharmacophore modeling, and molecular docking are widely applied in silico drug discovery studies. The mechanistic insights into the binding of a drug molecule to its target are crucial to understanding the intricate mechanism of how the drug works on its target, thus leading to the development of more specific ligands. NP-based drug discovery and development have largely benefitted from the strengths of MD simulations.

2.3.5 Prediction of Pharmacokinetic Properties and Toxicity

Knowledge of pharmacokinetic properties i.e., Adsorption, Distribution, Metabolism, and Excretion (ADME), and the safety in terms of toxicity of the NP-based compounds, is a challenge in the drug discovery process. There are several statistical and knowledge-based methods for the in silico prediction of these properties, which help eliminate the signs of “unlikely” candidates from the pool of NPs. By convention, ADME properties of a candidate molecule depend on the physicochemical

properties such as molecular weight, lipophilicity, number of hydrogen bond donors, and acceptors of the compounds. In practice, "Lipinski's Rule of Five" is applied as a filter to separate candidates that violate these parameters. Although, the application of the rule of five needs a significant amount of caution and decision making when relatively large NP-based compounds are in the picture. Hence, the rule of five has its limitations.

2.3.6 Computational Combinatorial Chemistry and Library Design

The emergence of combinatorial chemistry in the 1980s (Liu et al. 2017) opened channels for developing chemical libraries of structurally diverse chemical compounds using the structural and chemical properties of known bioactive compounds. Since then, with the advancements of computers, several methods have been developed that use combinatorial chemistry approaches to generate thousands of synthetic compounds and develop ready-to-screen chemical libraries for drug discovery purposes. In the context of NPs, this approach has been applied in recent years to develop chemical libraries of candidate compounds using naturally occurring compounds scaffolds (Grabowski et al. 2008; Mang et al. 2006). As a part of this, *computational mutagenesis* is a recent technique that involves mutating specific structural features of the target NPs to generate libraries of novel candidate compounds (Chen et al. 2002; Romano and Tatonetti 2019).

2.3.7 Machine Learning Approaches for NP-Based Drug Discovery

Machine learning algorithms have found their way through almost every domain of human life where human–machine interaction is possible. The area of computer-assisted drug discovery is also not remained untouched with applications of machine learning methods. In today's time, various aspects of drug discovery take help from the strengths of machine learning algorithms, whether in target identification or validation, de novo inhibitor design, virtual screening, docking, and ADMET property prediction (Vamathevan et al. 2019). Similar trends are also observed in NP-based drug discovery during recent years, where machine learning algorithms are applied to reduce the intricate decision-making process in drug discovery. There are discussions about possible applications of machine learning methods in predicting the functions of NPs using their two-dimensional structures (Liu et al. 2019). Moreover, manifold developments in the strengths of machine learning methods while addressing their limitations in dealing with NP-based drug discovery suggest potential success shortly.

2.3.8 Big Data and Data-Driven Drug Discovery

This section, however, is an extension to the last section, where we have discussed the applications of machine learning in drug discovery. Since these algorithms use an ample amount of predictive data that need both accuracy and integrity to reduce the possibility of false-positive prediction. In the context of drug discovery, big data refers to the huge amount of chemical information piled up in publicly accessible databases such as ZINC, PubChem, ChEMBL, and DrugBank, etc., which store millions of active compounds both naturally occurring and synthesized (Thomford et al. 2018). Big data also encircle a large amount of clinical data stored in Electronic Health Records (EHRs). Besides, disease biomarker databases, disease pathways, protein–protein interaction networks, protein–drug interaction networks, cancer gene expression data, etc., add more to the paradigm of big data. This leads to a novel but challenging aspect of drug discovery, i.e., data-driven drug discovery.

2.4 Tools and Databases for NP-Based Drug Discovery

This section will catalog various tools and databases based on or otherwise strategies above applied in NP-based drug discovery. Herein, the tools and databases discussed are generally used in computer-aided drug discovery and those sources specific to NP-based drug development. Table 2.1 provides a detailed list of available sources along with their brief description. In the following table, we have provided information on the tools and databases widely applied in the drug discovery process in general and particularly for NPs. However, the reader is suggested to refer to (Chen et al. 2017; Lagunin et al. 2014; Ma et al. 2011; Naqvi and Hassan 2017; Naqvi et al. 2018; Nguyen-Vo et al. 2020) for comprehensive details on the sources for in silico drug discovery.

2.5 Recent Advances in Drug Discovery from Natural Products

This section will explore and discuss recent case studies that have focused on natural product-based drug discovery using *state-of-the-art* in silico methods that we have discussed in the previous sections. This will direct the reader to understand and observe the current state of NP-based drug discovery. The studies discussed in this section are both purely computational technique-based and have a hybrid approach of integrating laboratory techniques with the in silico methods.

1. Monoamine oxidase B (MAO B) is associated with the catalysis of aryalkylamines neurotransmitters. Its malfunction is said to have possible involvement in the development of Parkinson's disease. In an attempt to develop potential inhibitors of MAO B, Mladenović et al. (2017) have applied, in a hybrid in vitro in silico approach, 3D-QSAR models for the evaluation of the biological activity of coumarin based compounds. Coumarin is a phytochemical which is

Table 2.1 List of tools and databased for natural product-based drug discovery

S. no.	Tools/ databases	Description	URLs
1.	AutoDock 4.2.6	It is an open-source docking tool with an improved free energy scoring function	http://autodock.scripps.edu/downloads/autodock-registration/autodock-4-2-download-page/
2.	AutoDock Vina	It is an advanced and improved version of AutoDock4	http://vina.scripps.edu/download.html
3.	FlexX	It is a highly benchmarked virtual screening and docking program for flexible docking	https://www.biosolveit.de/FlexX/
4.	Glide	Glide is a high-speed virtual screening and docking platform developed by Schrödinger	https://www.schrodinger.com/glide
5.	GOLD	It is a commercially available docking software for virtual screening, docking, and lead optimization	https://www.ccdc.cam.ac.uk/solutions/csd-discovery/components/gold/
6.	LigandScout	It is suitable for pharmacophore-based screening	http://www.inteligand.com/ligandscout/
7.	SwissADME	It is a web server for the prediction of ADMET parameters. The Swiss Institute of Bioinformatics moderates it	http://www.swissadme.ch/
8.	PreADMET	A web server for ADMET property prediction	https://preadmet.bmdrc.kr/
9.	CarcinoPred-EL	It is an ensemble machine learning-based web application for carcinogenicity prediction	http://112.126.70.33/toxicity/CarcinoPred-EL/
10.	PASS online	It is a web-based biological activity prediction server. It is capable of predicting 4000 kinds of biological activities	http://www.pharmaexpert.ru/passonline/
11.	NPASS	It is a web server cum database specifically for natural product activity prediction	http://bidd.group/NPASS/
12.	Discovery studio	Commercial package for QSAR and pharmacophore modeling along with docking and molecular dynamics simulations	https://www.discngine.com/discovery-studio
13.	GUSAR	QSAR modeling and molecular descriptor prediction tool	https://genexplain.com/gusar/
14.	Molinspiration	Chemoinformatics package for various purposes, including QSAR modeling	https://www.molinspiration.com/
15.	QSARPro	Tool for 2D/3D QSAR modeling	https://www.vlifesciences.com/products/QSARPro/Product_QSARpro.php

(continued)

Table 2.1 (continued)

S. no.	Tools/ databases	Description	URLs
16.	ZINC15	It is a database of commercially available compounds. It also contains a large number of natural products	https://zinc15.docking.org/
17.	PubChem	It is a database of chemical compounds and chemical activities. It contains around 111 million compounds	https://pubchem.ncbi.nlm.nih.gov/
18.	ChEMBL	It is a manually curated database of drug-like compounds. It contains around two million compounds	https://www.ebi.ac.uk/chembl/
19.	DrugBank	It is a comprehensive database of approved drugs	https://go.drugbank.com/
20.	Super Natural II	It is an exclusive database of natural products. It contains 325,508 natural compounds	http://bioinf-applied.charite.de/supernatural_new/index.php
21.	TCM Database @Taiwan	It is a database of Chinese medicines derived from natural products	http://tcm.cmu.edu.tw/
22.	CMAUP	Collective molecular activities of useful plants is a collection of around 47,000 natural compounds	http://bidd.group/CMAUP/
23.	DNP	Dictionary of natural products is a web source for commercially available natural products	http://dnp.chemnetbase.com
24.	IBS	InterBioScreen natural products library	https://www.ibscreen.com/
25.	MMPD	Myanmar medicinal plant database	https://www.tuninst.net/MMPD/MMPD-indx.htm
26.	TCMID	It is an integrated data source on traditional Chinese medicines	http://119.3.41.228:8000/tcmid/
27.	TIPdb	It is a comprehensive source of anti-cancer, anti-platelet, and anti-tuberculosis phytochemicals from indigenous plants in Taiwan	https://cwtung.kmu.edu.tw/tipdb/
28.	TMDB	It is a database of tea metabolites	http://pcsb.ahau.edu.cn:8080/TCDB/f

found in tonka beans in high concentrations. In this approach, they developed a combination of structure-based and ligand-based 3D-QSAR models and eventually deduce six relatively active inhibitors of MAO B, which might act as potential lead candidates for drug development for Parkinson's. Dhiman et al. (2018) have reviewed the application of 3D-QSAR on a diverse set of NP-based compounds such as coumarins, morpholine, piperine, naphthoquinone,

amphetamine moreover flavonoids, caffeine, and curcumin, etc., as potential MAO inhibitors. They conclude the effectiveness of QSAR and molecular docking and COMFA in finding selective and highly active inhibitors of MAO.

2. As discussed in the previous sections, molecular docking in combination with molecular dynamics simulations has proven very effective in computer-aided drug discovery studies. In recent years, many successful experiments have been conducted. These tools have been applied to discover potent and effective inhibitors for several known biomarkers of life-threatening diseases. Khan et al. (2009) studied the inhibitory effects of flavonoid derivatives quercetin, rutin, kaempferol 3-*O*-beta-D-galactoside, and macluraxanthone using molecular docking and enzyme inhibition assays against the activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). In this study, they found that macluraxanthone shows effective binding against both enzymes. Moreover, quercetin also exhibited strong intermolecular interactions with both these enzymes.
   Cozza et al. (2006) identified ellagic acid as an effective inhibitor of casein kinase 2 (CK2) using virtual screening and molecular docking methods. In a recent study, Zhang et al. (2020) screened a library of 2080 NPs to discover their efficacy as potential antiviral compounds. The study aimed to further utilize the compounds as a potent HIV-1 capsid (CA) protein inhibitor. Based on molecular docking, they found compounds rubranol and hirsutanonol showing strong intermolecular binding with HIV-1 CA.

   Ebola virus nucleoprotein (EBOV NP) is significant for its proliferation. To develop effective inhibitors against EBOV NP, Nasution et al. (2018) screened a library of 190,084 NPs from the ZINC database. To evaluate top-scoring compounds' binding affinity and effectiveness, they applied a flexible docking approach and molecular dynamics simulation. Eventually, α-lipomycin and 3-(((*S*)-1-amino-1,2,3,4-tetrahydroisoquinolin-5-yl)methyl)-5-((5-((5*R*,7*S*)-5,7-dihydroxy-3-oxodecyl)-2-hydroxyphenoxy)methyl)pyrrolo[3,4-b]pyrrol-5-ium were found showing strong binding thus posing as potent candidates as anti EBOLA drug.
3. Machine learning algorithms coupled with QSAR or molecular docking have proven very effective in elucidating the inhibitory effects of NPs towards the identification of novel drug candidates (Korotcov et al. 2017; Lavecchia 2015). Classical MD simulation when integrated with machine learning-based methods, enhances the performance of in silico drug discovery processes (Perez et al. 2018). Shi et al. (2020) applied machine learning models to discover New Delhi metal beta-lactamase (NDM-1) inhibitors. NDM-1 producing bacteria are crucial in drug-resistant bacterial infections. They screened a library of NP-based compounds using prediction models and also compared their performance with the virtual screening and docking strategy. As a result, machine learning models exhibited 90.5% accuracy in predicting the potent inhibitors in comparison to 69.14% accuracy by the traditional docking approach.
   Besides inhibitor discovery, machine learning is also applied to ADME property prediction and toxicity profiling of the NPs. In an attempt to assess the efficacy of

machine learning in the toxicity profiling of natural compounds, Onguene et al. (2018) carried out a toxicity assessment of three compound libraries of African flora, which have anti-malarial and anti-HIV activity. When compared to available experimental data for toxicity, machine-learning models were found to agree with the compounds' predicted toxicity.

4. Biological and chemical data during recent years has seen a manifold increase during recent years. Moreover, clinical data for several patients in the for EHRs is also available worldwide. This has opened a new window in the realms of drug discovery, called "data-driven drug discovery." The astronomical amount of data, preferably referred to as "big data," has a tremendous scope towards novel drug discovery (Lusher et al. 2014). During recent years, efforts have been made to study the drug effects and their interactions with pathogenic drug targets using the available information stored as clinical records and EHRs (Tatonetti et al. 2012; Yao et al. 2011). Despite the effectiveness of this approach in revolutionizing the drug discovery era, there are certain limitations, such as hurdles in accessing the clinical records or EHRs or limitations in the understanding of clinical data by informatics researchers. However, projects like Electronic Medical Records and Genomics (eMERGE) network (McCarty et al. 2011) and Observational Health Data Sciences and Informatics (OHDSI) (Hripcsak et al. 2015) are moving towards removing these barriers.

2.6 Challenges and Prospects

In this chapter we have discussed methods and strategies to find out potential drug candidates from NP-based compounds. Most of these approaches seem promising in providing relevant answers to drug discovery problems both in traditional laboratory-based methods and computational techniques. But then the question arises. Do the drug candidates and so-called "potential" inhibitors reach their destination? Destination as in as marketed drugs in the real world treating real diseases. If we see the statistics, the results are somehow satisfying. According to the survey of Newman and Cragg (2016), out of 1562 drugs approved between 1981 and 2014, 646 drugs are either NPs or NP-derived. Another survey suggests that around one-third of the new molecular entities (NMEs) approved by the FDA belong to NPs (Patridge et al. 2016). The statistics for the success of drug candidates discovered through in silico methods are also promising (Zhu et al. 2018).

Despite all the success of in silico drug discovery studies in general or in particular for NP-based compounds, some challenges still need to be addressed for a better future of NP-based drug discovery. These challenges cover the under or overuse of computational methods for discovery, unequal distribution of chemical data over several sources, thus limiting the access in most cases, controlled or no access to clinical data such as in the case of EHRs, etc. However, with the rapid development of computer hardware to mimic more intricate biological and molecular processes, processing large chemical libraries, and providing better solutions to

the challenging problems faced in computer-aided drug discovery, the future of in silico methods for NP-based drug discovery is bright and promising.

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Natural Product-Based Drug Designing for Treatment of Human Parasitic Diseases

3

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Abstract

Medicinal plants are the source of various therapeutic agents, including crude extracts and pharmacologically active compounds. Many novel targets and ligands are being identified daily to treat various diseases from AIDS to Alzheimer's to cancer with the application and techniques of drug designing. Natural products act as pillars for traditional medicine and are involved in the identification of lead compounds which could plausibly act as potential drugs in the area of parasitology. Computational biology has successfully expanded its arms in numerous ways in the process of drug discovery, from the identification of novel targets and biomarkers for rapid screening of large compounds to drug design assistance in clinical trials. The use of in silico suites like Schrodinger's Maestro, Discovery Studio, and software like grid computing and window-based general PBPK/PD modelling for visualization software along with these, the explosion of biological data (genome sequences and information on proteins, etc.) has also led to the enhancement in the designing of effective treatment methodologies. In this chapter, we explicitly focus on the ancient drug discovery

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_3

methods to advanced computational methods, which have led to an inclination of drug discovery towards a data-driven approach and using natural products to identify lead molecules and small molecule drug candidates for parasitic disease. It will also deal with the present informatics knowledge gaps and other barriers that need to be overcome for the complete reliability of computationally generated leads for drug discovery in pathogenesis. Finally, this chapter will provide a summary of commercially available important nature-based drugs and helpful software.

Keywords

Medicinal plants · Natural products · Drug discovery · Data mining · Computational tools · CADD

3.1 Introduction

Have you ever wondered how plants benefit us in a variety of ways? In this twenty-first century, we are all, directly or indirectly, dependent on plants for various functions in our daily lives. Today majority of the world population is dependent on medications derived primarily from plant products. Herbs is a commonly used term nowadays, which is derived from the general term Herbal. *Herbs are the plant part or plant products mainly valued for their Medicinal, Aromatic, and Savory qualities or properties.* These herbs can be used in various ways because of their antioxidant, immunomodulatory, antifungal, antimicrobial, cardiovascular, and sometimes anticancer properties. If a plant possesses any of these properties that can be useful for curing humans in different ways, these plants are known as Medicinal Plants or Herbal Plants, as we all know that plants have been used for medicinal purposes since prehistoric times. It was seen that the earliest record of natural products as described on the clay tablets in cuneiform from Mesopotamia (*around 2600 B.C*) documented oils from *Cupressus sempervirens* (Cypress) and myrrh which are still used to treat cough, inflammation, and cold. Similarly, an Egyptian pharmacological record known as Ebers Papyrus (2900 B.C.) was found to have documented over 700 plant-based medications, which range from pills to ointments. Chinese Materia Medica (~1100 B.C.) Wu Shi Er Bing Fang, with 52 prescriptions, and Tang Herbal (659 A.D. 850 drugs) are also popular documented records for natural products. Natural products have always been a reliable source of potential drug leads. Unique structural diversity is being provided by natural products in competition with combinatorial chemistry, allowing for the development of the best innovative medication, especially with low molecular weight lead compounds. It is known that traditional systems of medicine continue to be widely practised on many accounts. Population rise, inadequate supply of drugs, the prohibitive cost of treatments, Side effects of the various drugs, and development of resistance to currently used drugs for infectious and non-infectious diseases have led to increased emphasis on the use of plant materials as a source of

medicines for a wide variety of human ailments. It is estimated that around 80% of the world's human population depends on herbal medicines in their day-to-day life (*as mentioned by WHO*). *Furthermore, it is believed that over 21,000 plant species* can be employed as Herbal Plants (Sellami et al. 2018).

Day by day, research is being conducted to show how phytochemicals can save us from many fatal diseases, like reducing the mortality rate from 25% by consuming flavonoids in plant food (Pathak et al. 2012; Chetry et al. 2018; Sellami et al. 2018). Since early times, it has been recognized that drug discovery is time-consuming and highly expensive. From normal drug development to a drug reaching the market takes around 15 years with an approximate cost of around ~1 billion U.S. dollars. However, with the development and advancement of new algorithmic and computation tools in cheminformatics and bioinformatics, the time it takes to identify a novel medicine has fallen dramatically, as has the cost. Multiple screenings of the compound have become far too simple in a single instance. The computational technique has almost created a new protocol for discovering a novel medicine from target identification to drug approval. Bioinformatics has a diverse scope and has an essential role in the computational approach for building a novel drug. It is difficult to find and develop new medicines that are effective against parasitic protozoa in humans. The intricacy of the challenge is exacerbated by differences in the clinical manifestations of each illness, pharmacokinetic needs, pre-existing medication resistance, limited ability to grow some organisms, and problems in genetically modifying particular parasites. Widespread drug resistance, severe side effects, long treatment durations, unfavourable toxicity profiles, and complex drug delivery methods are some of the drawbacks of modern chemotherapeutic drugs, which may be a problem in resource-poor areas plagued by these parasitic diseases (Godreuil et al. 2007; Das et al. 2021). Another reason for not having a potential drug for these neglected diseases is the likelihood of low financial returns by big pharmaceutical firms that make an investment in some therapeutic regions unattractive. Different organizations have adopted many new strategies, leading to an increase in the development of drugs for antiparasitic diseases (McKerrow 2005). Natural products are generally more readily absorbed than synthetic drugs. So, to overcome these limitations posed by parasitic diseases, drugs with a plant origin can be used to treat these diseases (Pokorný 2007; Ganesan 2008).

This chapter introduces the computational approach of discovering a novel drug-using plant-based products or simply phytochemical via different means and methods from ab initio to high throughput techniques via different working software. This chapter also explains how plant products or natural products can be used to build a drug-using computational technique for these parasitic diseases.

3.2 Phytocompounds

Phytocompounds, often known as phytochemicals, have a considerable role in the domain of Medical sciences. It is known that for decades people have been using plant-based products as therapeutic remedies. The innate defence mechanism in

plants contributes to the natural healing capacity against various human health problems (Sudipta et al. 2012; Swamy et al. 2017). Phytocompounds are categorized based on their chemical structures and functional groups into terpenoids, polyphenols, and alkaloids. Terpenoids are mainly found in plants in lipids and are the largest phytochemicals with multi-cyclic rings derivative of five-carbon isoprene units. These are primarily used in medicines as natural flavours and nutraceutical agents (Freeman and Beattie 2008). Plants produce terpenes to protect themselves from various enemies like bacteria, fungi, and viruses (Wink 2008; Mazid et al. 2011). Polyphenols are found in the edible parts of plants, including fruits, seeds, nuts, flowers, and sometimes stems. They make up a considerable proportion of phytocompounds. This compound contains a hydroxyl group bound to the aromatic hydrocarbon (Schulze-Kaysers et al. 2015). More than 8000 phenolic compounds have been identified (Lin et al. 2008). Polyphenols are mainly secondary metabolites, thereby having a role in taste, colour, and flavour. Non-communicable illness can be prevented by consuming foods rich in polyphenols, such as tea and coffee, whereas phenolics also possess anti-inflammatory properties (Siracusa and Ruberto 2019).

Tannins, coumarins, stilbenoids, and phenolic acids are considered to be non-flavonoid phenols. Tannins are water-soluble and precipitate the proteins by binding to them. On the other hand, Anthoxanthins and Anthocyanins are considered to be a part of flavonoid phenols in which Anthocyanins are considered to possess anti-neurodegenerative, anti-inflammatory, anticancer, antioxidant, and antimicrobial properties (Sun-Waterhouse 2011; Gupta et al. 2013). Alkaloids are naturally occurring chemical substances made up of heterocyclic nitrogen-containing bases and are primarily found in Angiosperms. They have a significant effect on humans, e.g. Morphine which is isolated from the Opium plant is used as a narcotic in pain relief. Many other effective alkaloids found are caffeine, nicotine, ephedrine, piperine, and quinine (Table 3.1) (Anaya et al. 2006).

3.3 Pharmacognosy and Role of Pharmacognosy in Medical Sciences

Pharmacognosy is a Greek word that is made up of *Pharmakon (*remedy*)* and *gnosis (*Knowledge*) or gignoso* (to acquire knowledge). It means to acquire knowledge or science of drugs (Kinghorn 2002). The term Pharmacognosy was first used by J. A. Schmidt in his work '*Lehrbuch der Materie medica*' in the year 1811. In pharmacognosy, we study natural drugs and crude drugs obtained from natural sources. To get into this topic we first have to understand what a drug is. In simple words, a drug is a natural or synthetic chemical substance, which is used for the prevention, diagnosis, and treatment of a disease. So, what exactly is a crude drug then? It is a naturally occurring substance that is derived from plants, animals, minerals or their derivatives and is further used in its Natural state without any processing for treatment, prevention, and diagnosis. Any plant part plant that contains the active phytocompound can be used for the development of the drug, such as a flower, fruits,

Table 3.1 Table representing various phytocompounds with their source and action

Plant source	Drug	Action
Aesculus hippocastanum (horse chestnut)	Aescin	Anti-inflammatory
Frazinus rhychophylla	Aesculetin	Anti-dysentery
Agrimonia supatoria	Agrimophol	Anti-helminthic
Rauvolfia sepentina	Ajmalicine	Treatment for circulatory disorders
Anabasis sphylla	Anabesine	Skeletal muscle relaxant
Anisodus tanguticus	Anisodamine	Anticholinergic
Anisodus tanguticus	Anisodine	Anticholinergic
Atropa belladonna (deadly nightshade)	Atropine	Anticholinergic
Areca catechu (betel nut palm)	Arecoline	Anthelmintic
Betula alba (common birch)	Betulinic acid	Anticancerous
Ananas comosus (pineapple)	Bromelain	Anti-inflammatory, proteolytic
Camellia sinensis (tea, coffee, cocoa)	Caffeine	CNS stimulant
Camtotheca acuminate	Camptothecin	Anti-cancerous
Papaver somniferum (poppy)	Codeine, morphine	Analgesic
Colchicum autumnale (autumn crocus)	Colchiceine amide, colchicine	Anti-tumor agent
Convallaria majalis (lily of the valley)	Convallatoxin	Cardiotonic
Cassia species	Danthron	Laxative
Rauvolfia canescens	Deserpidine	Anti-hypertensive, tranquiliser
Cephaelis ipecacuanha	Emetine	Amoebicide, emetic
Ephedra sinica (ephedra, mahuang)	Ephedrine	Sympathomimetic, antihistamine
Octea glaziovii	Glasiovine	Anti-depressant
Gossypium species (cotton)	Gossypol	Male contraceptive
Hydrastis canadensis (goldenseal)	Hydrastine	Hemostatic, astringent
Piper methysticum (kava kava)	Kawain	Tranquilizer
Tabebuia species (trumpet tree)	Lapachol	Anticancer, antitumor
Nicotiana tabacum (tobacco)	Nicotine	Insecticide
Larrea divaricate (creosote bush)	Nordihydroguaiaretic acid	Anti-oxidant
Sophora pschycarpa	Pachycarpine	Oxytocic
Coptis japonica (Chinese goldenthread, Huanglia)	Palmatine	Antipyretic, detoxicant
Hydrangea macrophylla (Bigleaf hydrangea, French hydrangea)	Phyllodulcin	Sweetener
Cinchona ledgeriana (quinine tree)	Quinidine, quinine	Anti-arrhythmic, antimalarial
Rauvolfia serpentine	Reserpine	Anti-hypertensive, tranquiliser
Lonchocarpus nicou	Rotenone	Piscicide, insecticide

(continued)

Table 3.1 (continued)

Plant source	Drug	Action
Salix alba (white willow)	Salicin	Analgesic
Cassia species (cinnamon)	Sennosides A, B	Laxative
Theobroma cacao (cocoa)	Theobromine	Diuretic, vasodilator
Thymus vulgaris (thyme)	Thymol	Topical antifungal

leaf, bark, the aerial part of the plant, roots, and rhizomes, and rarely a complete plant, as in the case of Punarnava and Vinca. Alternatively, the fresh part and the secretion of the plant are occasionally used as crude drugs.

Pharmacognostic is the systematic and scientific study of the crude drug in a detailed manner and is hence sometimes known as the pharmacognostic scheme. Since ancient times, humans have utilized a variety of methods to investigate the therapeutic qualities of medicinal plants in various ways including a general Hit and error guesswork for the treatment of a disease, the discovery of therapeutic use of plant parts by nomads while searching for food, or by observing the activities of animals for the cure of a specific illness they encounter (zoo pharmacognosy). Nature may sometimes reveal a plant's therapeutic properties and provide clues as to where and how it may be utilized to heal humans. It is seen when the fruit of a plant is morphologically similar to a human part, it is frequently utilized to treat that organ, which is known as the Doctrine of Signature, for example, the Horsetail plant which is similar in structure to the cartilages of humans is used for the treatment of cartilages and other connective tissue. One by one we will be discussing each one of them. Drugs are even discovered inadvertently and unexpectedly through diverse plant components. Quinine (derived from Cinchona bark), an antimalarial medicine, and Penicillin, an antibiotic, are drugs discovered by accident.

3.4 Advancement in Drug Designing with Modern Equipment

With the help of scientific knowledge in medical treatments and medicinal preparation, there was a huge advancement in the domain of surgery, physiology, anatomy, and medical treatments. From late 1800, herbs were traditionally used for making drugs from botanical sources. The establishment of the pharmaceutical industry began in the 1900s because of the chemical methods used in synthetic drugs. Drugs were researched and produced only for therapeutic purposes but do not completely cure the disease. Since the year 1930, there was a main focus on natural product screening and further, isolation is done for the active chemicals to treat the particular disease (Grabley and Thiericke 1998). These were known as (NCEs) which stands for the New Chemical Entities which go for the tests and iteration to specifically ensure that the drug is effective, safe, and potent. In the late 1970s, the rDNA was implemented for the production of drugs by using various microorganisms and bacteria. During this period the pharmaceutical industries grew up. The advancement in Gene therapy and the prediction of the main cause

of the disease, and with the help of HGP (Human Genome Project) lead rise to the development of the drug.

3.5 Innovation in Extraction Technique and the Role of Multi-Omics in Drug Discovery

Extraction of components from natural products as potential drugs is the first step for the purification of chemical constituents. It is also a great challenge as in certain cases this might render the constituents non-reactive or reduce the reactivity by the interference of possible components (Tebani et al. 2016). Hence, innovative extraction techniques have resulted in higher hit leads and extraction of most components with shortened extraction time. These techniques include:

- Semi-bionics extraction.
- Enzyme-assisted extraction.
- Molecular distillation.
- Membrane separation technology.
- Supercritical fluid extraction.
- Microwave-assisted extraction technique.

Natural products, despite being a significant source of human therapeutics, lost their essence in the 1990s. But with the advent of new technologies, natural product discovery has been geared up. Advancements in the latest technologies such as computational biology techniques, quantum computing, profiling techniques, microfluidic technology, big data, and artificial intelligence; have led to a combinatorial approach to drug discovery. These advancements have led to the development of improvised drugs like quinine and artemisinin which are now used for the treatment of parasitic diseases. Apart from the traditional top-down approach, the new bottom-down discovery approach for natural products has emerged as an important approach to unveil new natural products. These approaches first identify the gene cluster followed by various gene manipulation techniques for potential drug discovery and thereby provide a combinatorial approach to extract the therapeutic properties of plant-based natural products. Systems biology-guided approach provides a different angle in natural products pharma-sciences. A systems biology approach coupled with omics will potentially pave the way for innovative drug design leading to a better drug candidate (Thomford et al. 2018). A multidisciplinary approach to innovative drug discovery will allow for the development of next-generation drugs to combat ever-increasing health challenges.

- **Genomics**

Genomics is an important method for the identification of plant products and biomarkers.

- DNA barcoding: this technique is an important identification technique utilizing genomics that relies on sequence diversity for identification.
- Markers developed by genomic techniques are used in the authentication of plant species. The use of biomarkers in DNA chips provides a high throughput tool for genotyping.
- Bio farming—this is used to show consistency in the species and pharmacological molecules from natural products.

- **Transcriptomics**

Innovative transcriptomic technology is gene expression estimation using microarray analysis that allows fast and effective analysis of many transcripts. Combining transcriptomic analysis with the whole-genome sequence can help in the better exploration of drug targets.

- **Proteomics**

Proteomics is used in the identification of biomarkers and in describing the mechanism of action of many natural products.

- **Metabonomics**

The term metabonomics is a newly introduced term that incorporates a system biology-guided approach to study the function and perturbations of a biological system. The metabonomics profiling is done by techniques like ultra-performance liquid chromatography.

3.5.1 Bigdata

Due to the amiss analysis, a large amount of complex multi-variant data is generated that requires computational tools for handling. The use of multi-variant tools and bioinformatics allows for the application of omics. The application used drug discovery processes such as docking and virtual screening can make use of novel machine learning algorithms such as deep learning (Zhang et al. 2017; Carpenter and Huang 2018; Das et al. 2021; Ramlal et al. 2021). Computational-based screening of candidate compounds makes use of big databases to identify compounds of similar activity.

Mass Spectroscopy This utilizes isotope tags and 2D electrophoresis and gives insight into quantitative protein profiling which generates quantitative data on a scale and sensitivity comparable to which is generated at the genomic level.

3.6 Computational Approaches to Designing Drugs Against Human Parasitic Diseases

Drug designing is the designing of small molecules that are either involved in any signaling or metabolic pathway or are an inhibitory or activator of a protein target. Drug designing by computer modeling techniques is called computer-aided drug design (CADD). The technique CADD is an emerging advanced technique that relies on a computer modeling technique for finding the compound by the topology of the target (Baldi 2010). A computational chemist uses knowledge of molecular interactions, drug activity, geometric considerations, and visualization techniques to search for a potential drug (Kumar et al. 2012). In today's fast-growing world, with the marvelous ongoing growth in the field of pharmaceutical drugs, most of the drugs have been discovered to cure an enormous variety of diseases. To save time and money, Hansch and Fujita developed computational techniques in the 1960s for studying medicines and disease molecular pathways, as well as for predicting the activities of compounds, a process known as computer-aided drug creation (CADD). The emerging tool to rationalize drug discovery, development, and optimization is CADD. It is being utilized to facilitate the target identification, validation, and optimization of the ADMET (absorption, distribution, metabolism, excretion, and toxicity) profile of a drug and safe drugs. A large number of selected compounds which can be either natural products or synthesized compounds were tested through biological assays or screens, which takes a long time. But, CADD can produce and verify the biological activity of a large number of compounds within a framework of time than biological assays. With the help of CADD, it is possible to explain the molecular basis of the therapeutic activity.

3.6.1 Ligands-Based Drug Design

This method also called the indirect method, relies on the designing of drugs based on the knowledge of the 3D structure of biomolecular targets. In this method, a pharmacophore model is built based on the binding interaction of new molecular entities with the target and this model defines the minimum necessary structural characteristics a molecule must possess to bind to it (Merz Jr et al. 2010). A drug can also be designed by modeling a QSAR model (quantitative structure–activity relationship) which is built by finding a correlation between calculated properties of molecules and extensively determined biological activity (Bacilieri and Moro 2006).

Methods including quantitative structure-activity relationship (QSAR), pharmacophore, reverse docking, and target fishing, are used for designing a drug based on ligand binding.

(a) QSAR: quantitative structure-activity relationship (QSAR) model is a regression model that is widely used now for modeling and virtual screening of large data sets of diverse chemical structures. This method builds a mathematical

model that finds a statistical correlation between chemical structure and biological activity. Thereby helping in hit identification and lead optimization.

(b) Pharmacophore: pharmacophore model is used to identify novel ligands that bind to the same receptor and is a combination of the steric features and electronic features of the target that are used to explain the concept of binding of different ligands to a common receptor. It can be built by exploration of the conformational space of ligands or by 3D target information.

(c) Target fishing: target fishing also called polypharmacology prediction or target prediction is used for discovering new targets for small molecule drugs. This may bring about repositioning the medication in another sign or improving our present comprehension of its viability and reactions.

(d) Reverse docking: reverse docking is a method that finds protein targets that can bind to a particular ligand. This is done by first preparing data sets and then searching for the appropriate ligand pose. This method is a powerful technique for drug repositioning.

3.6.2 Structure-Based Drug Design

Drug designing that relies on the 3-dimensional structure of biological targets is called structure-based drug design. The 3D structure is either obtained through methods such as X-ray crystallography or homology modeling (Merz Jr et al. 2010). In this method, the structure that is predicted to bind with high affinity and selectivity to target are candidate drugs.

There is basic 3 category for the identification of new ligand in structure-based drug design

1. Identification of a new ligand for a receptor can be done by searching large databases of 3D structures to find the small molecule that fits the binding site of the receptor. This is known as virtual screening and the available compounds are collected using docking software.
2. De-nova design of new ligands: This could be done by software like BOMB that helps in the construction of analogs by adding substituents to a core that has been placed in a binding site.
3. Optimization of known ligands, is achieved by evaluating proposed analogs within the binding cavity.

3.7 Tools for Target Identification and Validation

(a) **Microarray:** The microarray technique for gene/protein expression profiling is a well-established technique for target identification. The nucleic acid microarray technique, using short oligonucleotides, is a useful technique for identifying targets for drug design. Array-based gene expression analysis has enabled parallel monitoring of cellular transcription at the level of the genome.

A protein microarray is used for examining DNA–protein or protein–protein interactions. Issue and cell microarrays are also used to characterize a large number of gene products to discover prospective therapeutic targets.

(b) **DARTS:** (Drug affinity responsive target stability): this method for target identification relies on the on-resistance of drug-induced protease. It is used for the identification of protein targets for small molecules. This process relies on the stability of protein due to its binding to ligands. In this method, the first step includes the selection of a protein source and then selecting the small molecule for binding of the protein. This process is also helpful for the validation of protein–ligand interaction.

(c) **Affinity chromatography:** affinity chromatography is the most popular approach for drug target identification. In this method, the first step includes the study of the structure-activity relationship (SAR); by which various functional groups of small molecules are removed for determination of the required drug activity. The major disadvantage of affinity chromatography is that the small molecules of interest have to be derivatized.

(d) **Screening:** A large number of analysis assays are involved in the screening of bioactive compounds. These assays could be done by analyzing the whole cell or at molecular levels. The antiparasitic assays are screened by the phenotypic drug discovery method is important because the targets of these parasitic diseases lack validation. The major 3 disease-causing parasitic groups that are kinetoplastid parasites, *Plasmodium*, and helminths, have different screening technologies for drug discovery. These are:

(e) **Compound library:** The most common drug discovery screening technique involves searching for active compounds from the synthetic as well as a natural compound library. The species of helminths are screened due to their characteristic of not being able to grow freely in the laboratory along with the high-content imaging techniques. During the fluorescent-based assays and light field assays, certain specific dues are added that allow the speedy identification of parasites. These dyes could be MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), alarm blue, or acid phosphatase. Imaging assay of the inhibitory action of parasite growth in asexual erythrocytic-stage parasites dyed with MitoTracker or Sybr Green dye is used in the screening of malarial disease (Table 3.2).

3.8 Molecular Docking

One of the best and strongest methods for the drug discovery process, Molecular docking, predicts the actual binding site of the ligand on a required compound molecule. Many drug discovery approaches, such as ligand-based and structure-based drug discovery, are reliant on docking (Bacilieri and Moro 2006; Speck-Planche et al. 2012; Das et al. 2021). The process of molecular docking is cost-efficient and works basically **in silico** medium and further has gained more popularity because it does the effective screening of compounds in less time. The algorithm

Table 3.2 Showing the different drug libraries with corresponding operators and entries of molecules

S. no.	Drug library	Operator	Information about	Entries
1	BindingDB (binding database)	Skaggs of School of Pharmaceutical Sciences at University of California	Small drug-like molecules	41,300
2	ChemSpider	Royal Society of Chemistry (RSC)	Structures of molecules	107 million
3	DrugBank	Dr. David Wishart's lab, University of Alberta	Drug and drug targets	14,544
4	PubChem	National Centre for Biotechnology Information (NCBI)	Chemical molecules and their bioassays	109,920,381
5	TTD (therapeutic target database)	Innovative drug research and bioinformatics group (IDRB) at Zhejiang University and drug design group at the National University of Singapore	Therapeutic proteins and nucleic acid targets	5059 patented drugs
6	T3DB (toxin and toxin target database)	Canadian Institute of Health Research, Canada foundation for innovation and by The Metabolomics Innovation Centre (TMIC)	Toxin data with toxin targets	3678 toxins
7	SPRESI	All-Russian Institute of Scientific and Technical Information of the Russian Academy of Sciences, Moscow (VINITI) and Zentrate Information Verarbeitung Chemie, Berlin (ZIC)	Organic substances	5.8 million molecules
8	SuperDRUG2	Structural Bioinformatics Group, Charite University Medicine Berlin	Drug targets	4605 drug targets
9	Zinc database	Shoichet Laboratory in the Department of Pharmaceutical Chemistry, University of California	Small organic molecules	13 million compounds
10	NPC browser	Therapeutics for Rare and Neglected Diseases (TRND) program	Approved and investigational drugs	2750 approved small molecular entities

of docking involves the receptor-ligand-based complex via intermolecular interactions. Molecular docking allows several other features too, based on binding modes it generally ranks the conformation of the ligand and also displays the corresponding energies (Table 3.3).

It should be kept in mind that molecular docking is a precious step in the process of drug discovery and when the process of molecular recognition, there is

Table 3.3 Showing the list of different molecular docking software

Software	Description	Website
AutoDock	Automated flexible docking	http://autodock.scripps.edu/
FlexiDock	GA-based flexible docking	https://www.ks.uiuc.edu/
Gold	GA-based small ligand to macromolecules docking, gold scoring	https://www.ccdc.cam.ac.uk/
HADDOCK	High ambiguity-driven protein–protein docking	https://www.bonvinlab.org/software/haddock2.2/
DOT	Daughter of Turnip	https://www.sdsc.edu/CCMS/DOT/
Glide	High-throughput Monte Carlo sampling	https://www.schrodinger.com/glide
GRAMM	Global range molecular matching	http://reco3.musc.edu/gramm/
Hex	Uses spherical polar Fourier correlation for docking	http://hex.loria.fr/
Hint	Hydropathic interaction, calculate log *P*	www.edusoft-lc.com › hint

conformational change due to the receptor and the enzymes, sometimes the modification is too small (as shown in Fig. 3.1).

When it comes to molecular docking, two major steps are generally seen i.e.

1. ***Molecule preparation*:** it includes the binding site or an active site prediction, protonation of receptors and rotamers, and finally the aromaticity.
2. ***Identification of protein–ligand complex: an*** important step of the post-docking analysis, which generally predicts protein–ligand complex accurately with its energy of conformation.

Molecular docking is the method in which there is an evaluation of the finest conformation at minimum energy levels by the scoring algorithms.

3.9 Molecular Dynamics Simulation and Its Importance

It should be kept in mind that molecular docking is a precious step in the process of drug discovery and when the process of molecular recognition, there is conformational change due to receptor and the enzymes, sometimes the modification is too small and with low mobility, the ligand perfectly fits into the binding site of the target molecule. Some of the proteins also play a role in the modification of the conformation that contains the secondary and the tertiary components. The ligand usually stabilizes a subset of several feasible receptor conformations, shifting the balance to the minimum energy structures (Salsbury Jr 2010). In these kinds of situations, alternative states of conformity may be produced by the M.D. simulations which correspond to those structures which are induced by the ligand. If the structure has no or very poor binding locations then M.D. simulation can be used by which many useful docking structures are produced. M.D. produces the states of potential

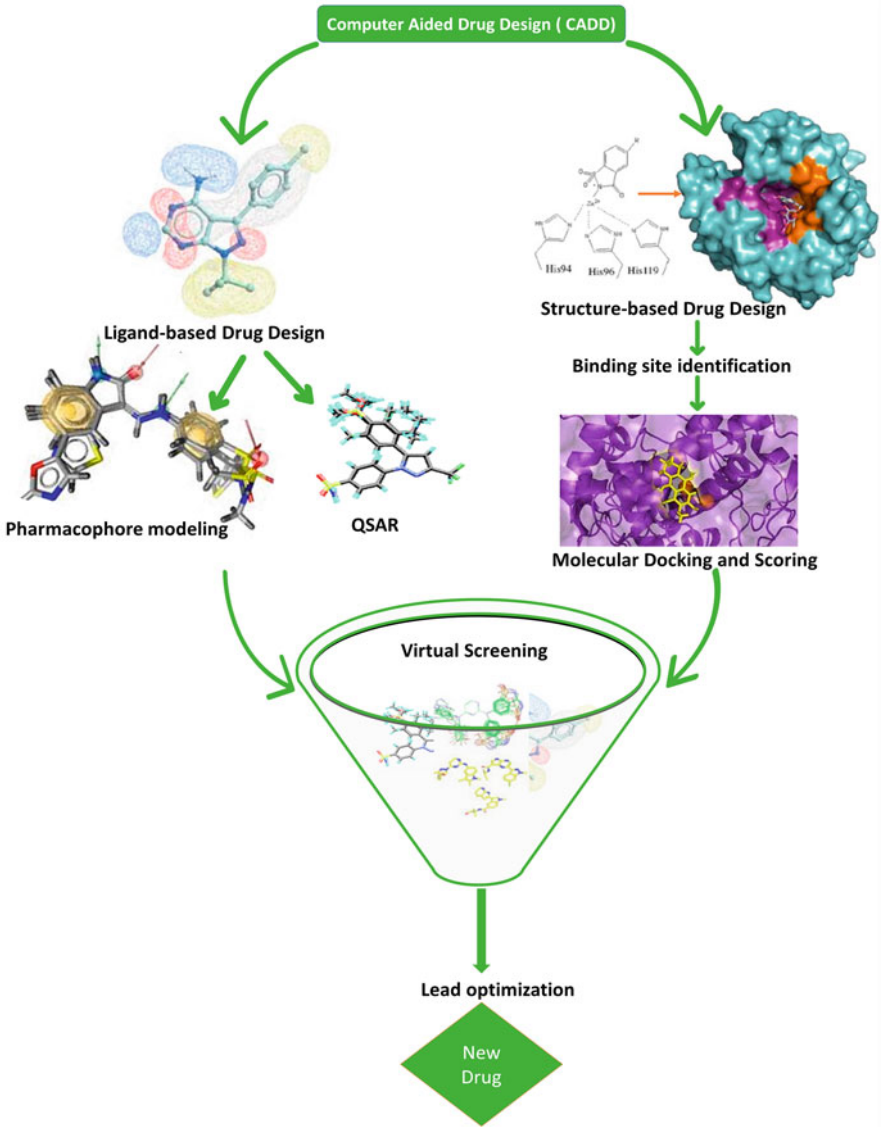

Fig. 3.1 Showing the pipeline for ligand-based and structure-based drug designing

conformational which are based on the crystallographic information available, so those structures with well-defined and affordable binding cavities are further selected for the process of molecular docking. In addition, the stability of the ligand–receptor complex in molecular docking can be assessed using M.D. If there is a deviation into the docking solution by more than an RMSD (Root Mean Square Deviation) of the

ligand conformation generated by M.D. then the complex may be considered as unstable. Since it is important to know that Newton's motion equation for classical mechanics is being used and due to this the position and speed of every atom are being predicted and specified and are further being studied. Hence temporal evolution and the trajectory of the ligand–receptor complex are easily examined. Initially, the atoms are assigned a particular setup to reproduce the actual system's temperature and pressure (Nichols 2011; Lopes et al. 2021). The kinetics (i.e. velocity and position) of each atom can be predicted after the calculation of the forces which are acting on each particle and further, these calculations are continuously conducted till the moment when the interval integrates the molecular trajectories. From the potential present in the molecular interaction, the forces acting on the structure are predicted which is parameterized by the quantum chemical or information from the experiment. Hence the prediction of the overall function of each interaction is done by this set of parameters. CHARMM, GROMOS, and AMBER are the diverse available force fields and are used in molecular dynamics simulation. M.D. has made contributions to the structured based drug design.

Simulation of biological molecules was kind of unknown till as late as the 1950s but within the next 10 years from then, it was one of the hottest topics in the research world. The literal meaning of 'simulation' as we all know, is the imitation of an anticipated event. *Molecular Dynamic Simulation* is the primary tool that uses computer techniques to apprehend the dynamicity of biological molecules by allowing the atoms and molecules to interact for a secure period and analyzing their physical movement and chemical interactions. They provide detailed information on the structure, fluctuations and conformational changes, dynamics, and thermodynamics of biological molecules and their complexes. Understanding these complex biomolecular motions is doubtlessly pertinent to drug discovery. The initial lock and key mechanism of ligand binding proposed by Emil Fischer in 1890 (Mertens 2019), in which a motionless, fixed receptor was assumed to house a small molecule without going through any conformational rearrangements, has now been abandoned to accept new binding models that consider not only the conformational changes but also the random motions of receptors and ligands, thus proving Richard Feynman's statement true. He was a recipient of the Nobel Prize (1965) in Physics and said 'All things are made of atoms, and everything that living things do can be understood in terms of the jiggling and wiggling of atoms. Today Biophysics is a devoted field that aims at comprehending the true essence of this jiggling and wiggling of biological molecules. Docking is the process by which two or more molecular structures orient themselves in such a way that they bind to each other to form a stable complex. M.D. simulation is widely used to study the protein–ligand, protein–protein, DNA–protein, and DNA–ligand interactions. Researchers, in general, are always excited to study the effect of new kinds of molecular interactions. For this purpose, docking is performed followed by M.D. simulation so that one can know the effect of interactions on a temporal scale (Alonso et al. 2006; Roy et al. 2021).

The goal of Molecular Dynamic Simulation is to predict the behavior of atoms in a biological system and how they move as a time-dependent function thereby providing the ultimate details concerning that atom based on algorithms of physics that govern the interatomic interactions. Through this, we hope to understand the properties of molecules concerning their structure and their conduct under different conditions. It serves as an important suffix to the lab experiments thus saving time, cost, and labor of the scientists and bridging the gap between the latest technological advancements in the modern scientific community and the conventional experimental scientists. It aims at lowering the amount of guesswork and fittings traditional scientists make and helps them get an idea about the simulations that are difficult or impossible in the laboratory. We should always keep in mind that it is possible that one might not necessarily have a flawlessly realistic molecular model but the model should be able to portray the essential properties of physics and chemistry and also follow the concerned laws of mathematics along with possessing the correct biological attributes and that should be enough.

Molecular Dynamic Simulation was first introduced by Alder and Wainwright in 1957–1959 to study the interactions of hard spheres. Even though proper simulation was first performed in 1964 by Rahman et al. in using a realistic potential for liquid argon, the numerical methods used for this process were developed much before, preceding the use of computers as well. In 1969, Barker and Watts first performed the Monte Carlo simulation of water, following which McCammon et al. in 1977, performed the first M.D. protein simulation of the bovine pancreatic trypsin inhibitor (BPTI). Duan and Kollman in the 1990s made an amazing revelation by discovering the folding mechanism of villin protein using molecular dynamics simulation and this achievement is considered a landmark event in this field. Now you must be wondering what Monte Carlo Simulation is. For that, we need to understand that there are two main classes of simulation techniques- the Molecular Dynamic (M.D.) Simulation and Monte Carlo (MC) Simulation, in addition to which there are other composite techniques that integrate the features of both these M.D. and MC depending upon the need of the research. For a simulation of low-density systems like gas, where the molecules possibly get trapped in low energy conformations, Monte Carlo simulations are preferable, while M.D. simulation is the technique of choice for the simulation of liquids. It is a difficult effort to use computer simulation to investigate the dynamic behavior of molecules to unravel the mystery of the biological world's intricacy. It demands the use of properly designed models capable of simulating the cellular environment, physical forces that can replicate the laws of physics and thermodynamics and offer dynamicity to the model, as well as intensive calculations that take into account the technique's temporal component. Today, tools for molecular modeling, energy calculations, algorithms to mimic the chemical aspect of actual systems, docking-scoring procedures, and other approaches have been created, making the entire method more robust. The structure is immersed in a "bath" of thousands of water molecules to make the simulation more lifelike. Let's start with a basic understanding of this incredible technology (as shown in Fig. 3.2).

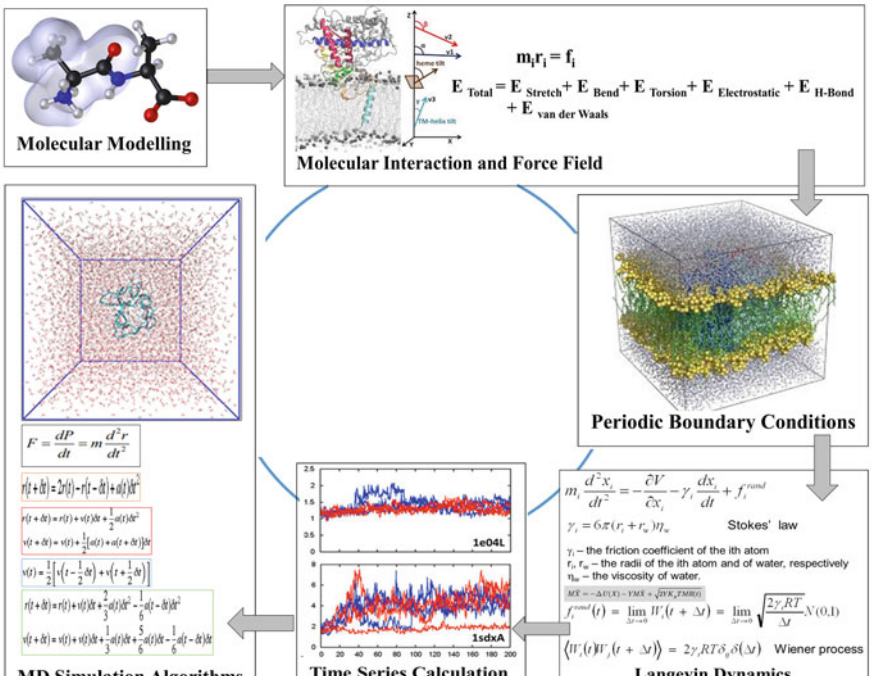

Fig. 3.2 Concept of molecular dynamics simulation and important algorithms

3.9.1 Applications of M.D. Simulation

M.D. simulations have a wide range of applications not only in the field of biological science but in any field one can imagine ranging from physics, chemistry, biology to climatology and meteorology, video games to film industries. Let us focus on the applications of M.D. simulations in biological complexes.

(a) Determination of structures and movements of biomolecules:
As already mentioned, we now know that the most common application of M.D. simulation in biomolecules is to study, analyze and mimic the flexibility, movements, and interactions of and among the different proteins. Structures determined by experimental studies by X-ray crystallography or NMR studies reveal only an average approximation of what the real thing could be, but with the usage of computational simulation techniques, one could make an even more precise approximation of the types of structural fluctuations in the molecules undergo. By just examining a simulation of these structures, one can quantify the movements of various regions of the molecule at equilibrium and the types of structural fluctuations that occur. Such simulations also can show the dynamic behavioral properties of water molecules and salt ions, the effect of

which are often critical for the proper functioning of protein and also for ligand binding (Berneche and Roux 2001; Alonso et al. 2006).

(b) Assessment of accuracy and refinement of modeled structures
This method can also be used to assess the accuracy of already modeled structures or even to refine the structures built using molecular modeling techniques or experimentally in the lab. For example, it is frequently seen that experimentally determined X-ray crystal structures are refined by a computational M.D. simulated annealing protocol and fit the model to the experimental data even more precisely while simultaneously maintaining a physically stable structure (Brunger and Adams 2002; Afonine et al. 2012). One advantage of this approach is that it this approach has been shown to control model errors that are otherwise present. Let us consider another example. It is possible that a membrane protein suffers from artifacts due to the absence of a lipid bilayer or crystal structure suffers from such errors as a result of the crystal lattice packing but owing to the lucidity of the near accuracy of the simulated structures, it is now possible to correct such artifacts by performing a simulation inappropriate solvation environment as per the requirements of the structures one is working with (Linnemann et al. 2015). Though MD simulations are extremely useful in the refinement of existing homology models, quite a several attempts to do this have been unsuccessful (Raval et al. 2012; Mirjalili and Feig 2013). M.D. simulations have also been used to retrieve ensembles of conformations, against a single structure, from NMR data (Lindorff-Larsen et al. 2005). In each of these cases, the molecular mechanic's force field is augmented by terms that have to be taken from experimental data, which results in lower energy for structures (or structural ensembles) that agree better with the.

(a) The flexibility of molecules:
The flexibility of biomolecules directly modulates their association with the neighboring atoms, molecules, and ions, and thus plays an active role in cellular function. We have already studied that molecular dynamic system gives us clear insights into the dynamic evolution of any system, it can also be seen as reflecting its flexibility to an extent. Techniques such as Anisotropic Network Model (ANM), Elastic Network Model (ENM), and Principal Component Analysis (PCA) among others have recently been developed which can extrapolate the prime contributing motions of the system under study (Bakan and Bahar 2009).

(b) Another interestingly important application of M.D. simulation is to ascertain the mechanism in which a biomolecular system will respond to perturbation. Say, for example, someone changes the molecular environment of the protein like the salt concentration or lipid composition, or adds a ligand where there was originally no ligand present or replaces a bound ligand with a different ligand (Fribourg et al. 2011; Tan et al. 2018) or changes the amino acid residues present in a particular protein by mutating them or by changing the protonation state of the amino acid, In all the above-mentioned cases, simulations help one in getting a thorough understanding of the system under study. One thing to be kept in mind while performing such simulations is that one should perform it several

times by using both perturbed and unperturbed systems to get clear insights into the consistent differences in the results and thus ascertain one's results.

(c) Analysis of results of M.D. simulation of different systems helps one to answer such questions about the role of structure, flexibility, and the interactions among different biomolecules that are experimentally very difficult to address. Since simulations can take place at the scale of femtoseconds, we can observe such biological processes which occur in a jiffy, like the order in which the substructures form during protein folding (Snow et al. 2002; Lindorff-Larsen et al. 2011). One can also thoroughly study processes like ligand binding, protein folding, conformational changes, membrane transport, etc. They also help us understand the factors controlling ligand binding and disassociation kinetics, the process of assembly of disordered proteins to form fibrils (Nguyen and Hall 2004; Buch et al. 2011; Lindorff-Larsen et al. 2011; Wacker et al. 2017). Simulations may capture an entire process in one go, or they may capture it in parts which can then be used to reconstruct the entire process (Angeli et al. 2015; Harpole and Delemotte 2018).

3.10 Challenges, Limitations of These Computational Methods

Scientific study on medicinal plants is a rising requirement of an hour at many research institutes, universities, pharmaceutical laboratories, and clinics in the contemporary age in many industrialized countries. Based on the survey and literature prior knowledge, this research primarily focuses on bioactive molecules of plants that have long been known and used for their healing properties. The discovery of new medicinal plants with new bioactive chemicals, new bioactivity, and new medicines from more remote parts of the world has resulted from the second phase of fundamental research. The use of natural products for drug discovery is a challenging task because of the complexity of the chemistry of the plant products and even the slower time taken to deal with them. Ayurvedic, Unani, and Siddha medicines require scientific research before being put forth for testing and confirmation. This is a positive trend that contributes to health development by combining ancient practices with current understanding. The World Health Organization has underlined the need of adopting contemporary procedures to assure the quality control of herbs and herbal formulations. Almost all nations have their herbal pharmacopoeias, updated regularly with new monographs and processes to preserve the high quality of herbal products that serve the general public. Over the years, computational approaches have become essential for drug discovery using natural products. The following are some of the current barriers to natural product use and acceptance of their medicinal efficacy: (1) a lack of standardization methods (2) a lack of pure chemical products or compounds to isolate (3) the absence of biological mechanism elucidation and infrequently conducting so-called controlled and (4) recorded clinical studies following "standards". The advancement in technology promises faster identification of drug targets, but the conceptual validation of these techniques is limited as there are many obstacles and challenges yet to be solved.

Targeting flexibility is one of the most challenging aspects of CADD. The ligand is given much flexibility in most molecular docking technologies, while the protein is maintained more or less fixed, with only limited freedom for the residues within or near the active site. Various attempts have been made to give the protein total molecular flexibility. However, this progressively increases the computation's space and temporal complexity. It is also possible that designing single, rigid structure inhibitors or medication molecules would result in an erroneous outcome. Another important limiting factor is the validity and precision of the methodologies used to predict drug-protein or drug-disease signatures. While docking strategies have improved, they still have a high rate of false positives, raising the question of whether our understanding of ligand–protein binding is complete enough and whether the focus on ligand–protein signatures is sufficient for accurate pharmacodynamics clinical outcomes.

Proteins make up the majority of phytochemicals, and many of them have several domains. Understanding the foundations of most of life requires determining the structure of multi-domain complexes at atomic precision. However, there are still significant problems in multi-domain docking prediction. The global spread of multidrug-resistant and extensively drug-resistant bacteria represents a danger to human health, necessitating the development of novel, effective, and low-cost antibacterial medicines. Structures or substructures are connected to chemical behaviour, and activity is the enigma of chemicals and chemistry. Any change in the chemical structure causes the chemical behaviour to alter. Predicting how the presence of ligands alters the chemical structure and behaviour of other molecules is of significant interest. If we can do so, we will be able to produce more effective medications and more effective but safer chemicals for societal use.

3.11 Conclusion

There are more chances to investigate the therapeutic and other biological characteristics of previously inaccessible natural products now as there is a rising interest in herbal medicine development with minimal adverse effects. It is necessary to concentrate on the visualization and identification of underutilized herbal plants worldwide to determine their utility. In drug screening and design, computational approaches have become more critical. Drug binding sites on target macromolecules can be identified, and drug action processes can be elucidated using multiscale biomolecular simulations. Virtual screening can explore vast chemical databases for lead compounds quickly and efficiently. De novo drug design is a solid alternative for designing pharmacological compounds from the ground up, employing building pieces summarised and abstracted from prior successful drug discoveries. Most computational approaches in drug screening and design are being revolutionized by machine learning, which has the potential to dramatically increase efficiency and precision in the big data age. Different models or efficient methods (e.g., dimensionality reduction) must be appropriately integrated to accomplish a complete study of biological processes at many scales as well as accurate and

effective analysis. The combined computational approaches will speed up drug development and aid in identifying successful treatments with unique action mechanisms that may be used for a range of complicated biological systems in the future.

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Secondary Metabolites of Plant Origin in Parasitic Manifestations

4

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Abstract

Malaria, leishmaniasis, and kala-azar are three major parasitic diseases affecting major population of the world but still remain as neglected tropical disease. Severe side effects from conventional treatment of these diseases and development of drug-resistant strain have opened an outlook for alternative means. The use of natural plant products as therapeutic or prophylactic measures against these diseases has been known and practiced for centuries. It is now known that plant extracts used in traditional medicines are majorly secondary metabolites. Approximately 2,140,000 plant secondary metabolites are reported. Their diverse nature and pharmacological attributes against many aliments including parasitic diseases with little to no side effects have invited numerous scientific investigations. This chapter, therefore, is an attempt to ravel antiparasitic secondary metabolites, their source, status of therapeutic application, and potential of development to novel drug against malaria, leishmaniasis, and kala-azar.

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_4

Keywords

Plant · Secondary metabolites · Malaria · Filariasis · Kala-azar · Parasites

4.1 Introduction

Plants synthesize diverse groups of organic compounds and intermediate compounds of small molecular weight called metabolites. Metabolites vital in performing basic biological functions of cell division, reproduction, growth, respiration, and storage of energy reserves are designated as primary plant metabolites (PPMs). Plant secondary metabolites (PSMs) are the ones not essential for growth or reproduction but produced to give discreet advantage to the plant. PSMs are diverse in nature and are involved in a multitude of actions that are determined by their interactions within their environment. Such advantages include defense agent against microbes, repellent, or attracting compounds for pollination and seed dispersal agents, interfere with molecular targets in cells and tissues, active agent in the transportation of metals, agent of symbiosis, and effector molecules of tissue differentiation (Wink 2018). Moreover, secondary metabolites derived from plants have a rich tradition in medicine as immunosuppressant, antitumor, antiaging, antiviral, antimicrobials, antiprotozoal, antihelminth, etc. (Vaishnav and Demain 2011).

The knowledge of antiparasitic properties in PSMs derived from medicinal plants and its use as therapeutic or prophylactic measures has been known and practiced traditionally for centuries. In recent times, the focus on secondary plant metabolites as an alternative source against parasitic diseases has gained momentum due to ineffectiveness of vaccines and prominent instances of drug resistance against generic drugs in most cases of endoparasitic infections (protozoa, helminths, nematodes, cestodes, trematodes) (Wink 2012). Despite the growing popularity of PSMs as an alternative measure against endoparasitic disease; its potential in therapeutic application has not been explored to its best. Few studies have attempted the isolation, characterization, and in vitro screening of the various constituents present in these extracts, but in vivo studies using isolated compounds are very scarce. Therefore, the present review work highlights various secondary metabolites derived from plants that are identified to be an effective antiparasitic compounds, its status of therapeutic application and potential of developing effective novel drug against three major neglected tropical disease: malaria, lymphatic filariasis, and kala-azar.

Chemistry Approximately 2,140,000 PSMs reported are classified based on the compound's structure, its biosynthesis pathways, and the functions they perform (Thirumurugan et al. 2018). Majorly secondary metabolites are classified into four main classes (Fig. 4.1). They are:

1. Terpenes—monoterpenes, diterpenes, etc.
2. Phenols—coumarin, tannins, flavonoids, etc.

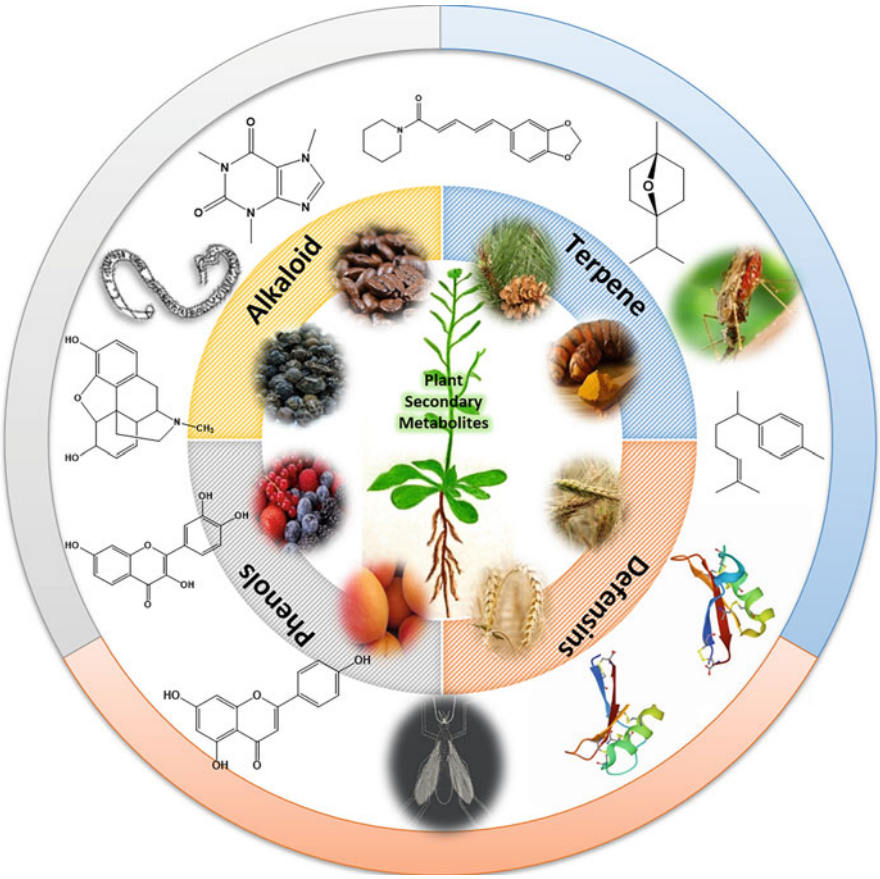

Fig. 4.1 Overview of plant secondary metabolites, chemical classes, and sources

3. Nitrogen-containing compounds—alkaloids, etc.
4. Sulfur-containing compounds—glutathione, defensins, etc.

4.1.1 Terpenes

Terpenes or isoprenoids are one of the most diverse compounds that occur naturally. Isoprenoids are made up of isoprene units with the molecular formula $(C_5H_8)n$. The number of isoprene units and their organization forms the basis of their classification. Terpenes are shown to play medicinal uses apart from their function as components of signaling pathways and heat protectants of plants (Cox-Georgian et al. 2019). Terpenes are classified as (Fig. 4.2):

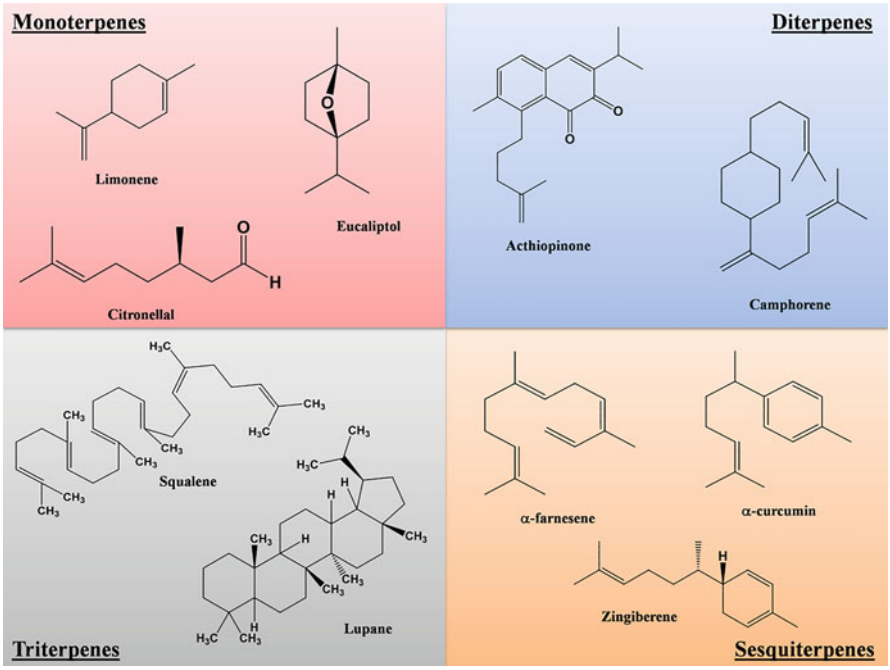

Fig. 4.2 Chemical structure of terpenes

Monoterpenes Monoterpenes are the smallest terpenes. They exist as isoprenoid units ($C_{10}H_{16}$) either in cyclic or acyclic form. Monoterpenes are the major components of essential oils that exist in diverse form and distributed widely in plant kingdom. They possess the most fragrant quality in all of terpenes which imparts the role of attracting pollinators or repelling predators in plants (Loreto et al. 1998). In humans, pharmacological properties of antiviral, antibacterial, antifungal, anti-inflammatory, anticancer, antispasmodic, and local anesthetic effect have been observed in natural and synthetic monoterpenes (Koziol et al. 2015).

Sesquiterpenes Sesquiterpenes are naturally occurring secondary plant metabolites that act as pheromones for attracting mates in insects or a defense system. They are much more stable and larger compounds compared to monoterpenes with the molecular formula $C_{15}H_{18}O_3$. Sesquiterpenes like abscisic acid are not unique to plants but are also found to be present in the animal kingdom. Their roles as pro-inflammatory compounds in immune response as cytokines and stimulatory role of insulin release are well documented (Chadwick et al. 2013). Many flowering plants of the Asteraceae family are not only a very rich source of sesquiterpene lactones but also possess the medicinal properties. The usage of sesquiterpenes for treating cancer, plasmodial diseases, and inflammation-related ailments has been immense in traditional medicine. Artemisinin is an important sesquiterpene found in

the roots and shoots of *Artemisia annua* that is being used extensively in treating malaria (Chadwick et al. 2013).

Diterpenes Diterpenes are compounds made up of isoprene units with the molecular formula $C_{20}H_{32}$. Diterpenes act as growth hormones and are involved in the regulation of flowering in plants, germination of seeds, and alternation of generations in plants (Lee et al. 2015). Diterpenes have been shown to possess various therapeutic properties as antitumor, cytotoxic, anti-inflammatory, and anticancer. The drug "Taxol" also contains diterpenes (Vasas and Hohmann 2014). Diterpene alcohols are also found in the form of oils such as cafestol and kahweol obtained from coffee beans. These diterpenes have a protective role in stroke, diabetes, and liver diseases which are attributed due to their antioxidant and anti-inflammatory properties.

Triterpenes Compounds with 3–6 isoprene units are defined as triterpenes with the molecular formula $C_{30}H_{48}$. They structurally resemble hormones in the human body, thus occupy a vital role in therapeutics. It has been observed that several triterpenes have diuretic action and detoxification properties of saponins (Nazaruk and Borzym-Kluczyk 2015).

4.1.2 Phenols

A large number of chemical compounds present in plants contain phenol groups. Thus, many phenol derivatives are also available, including lignin, tannins, isoflavones, benzoic acid derivatives, anthocyanin, etc. Phenol derivatives are explored largely in therapeutics as antioxidant and anti-inflammatory compounds (Pandey and Rizvi 2009).

Lignin Phenolic compound lignin is a product formed in the phenylalanine/tyrosine metabolic pathway of plant cells. As one of the main components of the plant cell wall it plays an important role in plant heavy metal tolerance, plant drought and salt stress tolerance, and plant temperature stress adaptability. Lignin can be divided into three types according to the different plant species: softwood, hardwood, and grass lignin. Softwood lignin consists exclusively of coniferyl alcohol, hardwood lignin consists mainly of coniferyl alcohol and sinapyl alcohol, grass lignin has three types of monomers (coniferyl, sinapyl, and *p*-coumaryl alcohol) (Liu et al. 2018).

Flavonoids Flavonoids are 15-carbon polyphenolic PSMs that have two aromatic rings linked by 3 carbon-bridge (Fig. 4.3). Roughly about 5000 compounds derived from plants have been characterized as flavonoids. They are responsible for the pigmentation of flowers, e.g., blue color results from the presence of anthocyanin. They give natural protection against UV rays by acting as UV filters with an absorption spectrum ranging from 280 to 315 nm. Flavonoids are well known for their antioxidant activity. Luteolin, quercetin, genistein, daidzein, and

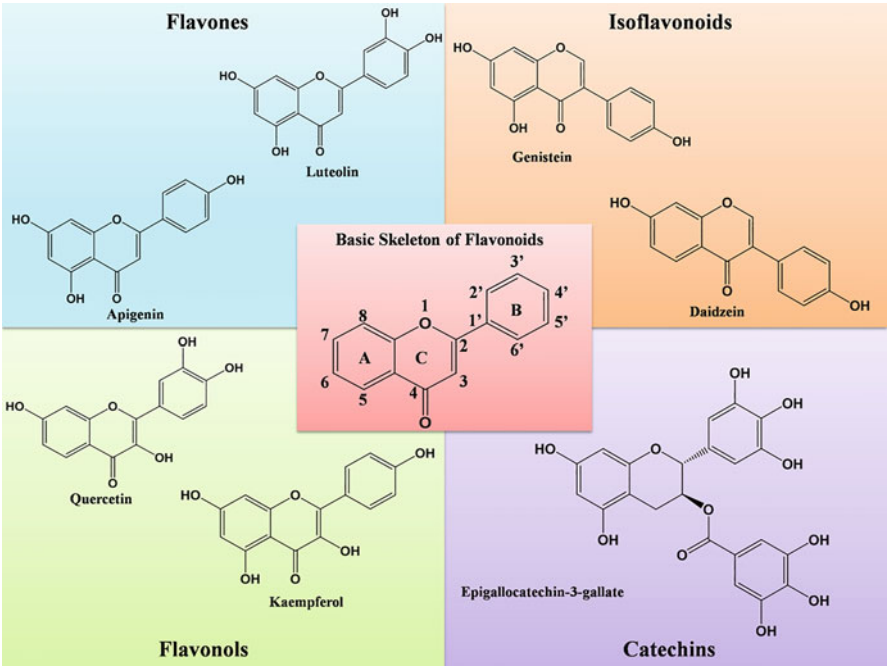

Fig. 4.3 Chemical structure of flavonoids

epigallocatechin-3-gallate are a few examples of flavonoid derivatives that have exhibited anti-inflammation, immune response modulation, anticancer, and free radicals scavenging activities (Janicijevic et al. 2007).

Tannins Tannins are phenolic compounds having a molecular weight ranging from 500 to 3000 Da. Tannins are water-soluble in their free form; however, on binding with protein the tannin–protein complex may or might not be water-soluble. They play important role in plants' defense mechanisms against other species (Hassanpour et al. 2011). They are mainly found in *Sericea lespedeza* and *Lotus* spp. (Hassanpour et al. 2011).

4.1.3 Alkaloids

Alkaloids are the end products of metabolism in plants (Fig. 4.4). They contain the basic nitrogen group, but the difference between alkaloids and other nitrogen-containing natural compounds is not very clear-cut. They are distinct among other PSMs by their large characteristic structural diversity and so, there is no uniform classification of alkaloids. They mainly serve as growth regulators and a defensive compound against predators in plants (Ncube and Van Staden 2015).

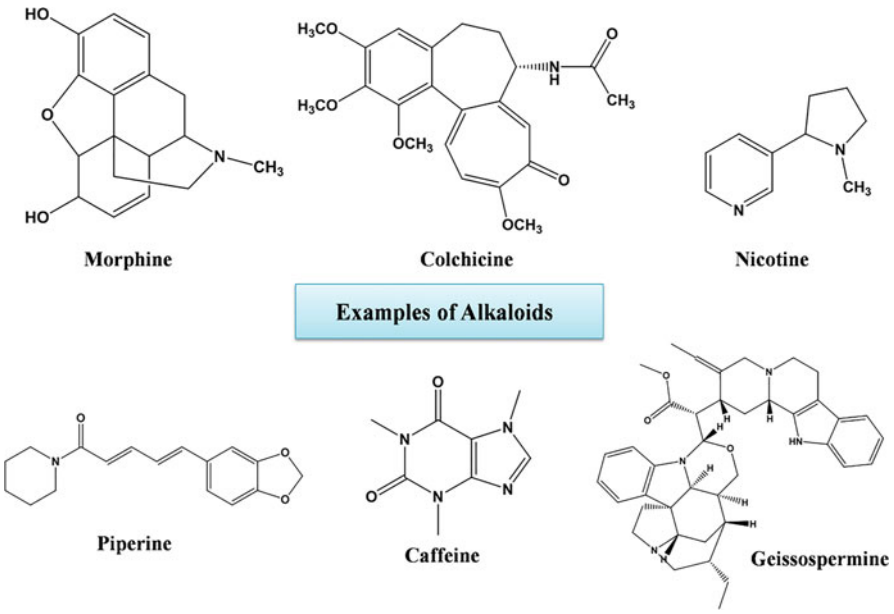

Fig. 4.4 Chemical structure of alkaloids

4.1.4 Defensins

Defensins are cationic cysteine-rich peptides of 45–54 amino acid chain containing 8 cysteine residues with 4 disulfide bonds. Defensins were discovered from wheat and barley plants; however, it is now known that a wide range of tissues from plants express defensin. According to Rosengren et al. (2009), the biological activities of defensins are wide range in nature including their role as a target against plant pests from insects and fungi to bacteria (Rosengren et al. 2009).

4.2 Malaria

According to World Malaria Report (2019), malaria infects more than 200 million and causes the death of 0.4 million per year globally with 93% of the cases from Africa and Southeast Asian countries. Though there is a reduction in the number of incidence per 1000 population from 71 to 57 malaria cases during the period 2010–2018, still it remains the most severe problem of public health especially in developing countries (WHO Global Malaria Programme 2020).

4.2.1 Malaria Parasite

Malaria parasites are protozoans belonging to the genus Plasmodium. There are more than 25 species of malaria parasites, Plasmodium is identified to cause malaria in primates. However, in humans, only four Plasmodium species are responsible for causing malaria disease. They are:

1. *Plasmodium falciparum*: Incidence of malaria from this species is most prevalent in tropical and subtropical countries. They are the most fatal type causing the majority of death globally.
2. *Plasmodium ovale*: *P. ovale* is mostly concentrated in West Africa and islands of Western Pacific regions (Arrow et al. 2020; Antinori et al. 2012).
3. *Plasmodium malariae*: *P. malariae* is distributed worldwide but represents only a small percentage of infection.
4. *Plasmodium vivax*: This species is most prevalent in South America and South-east Asia. They are the second most significant species in the global malaria burden.

Additionally, another Plasmodium species which normally infects animals are observed to cause malaria in humans. Therefore, the fifth species infecting humans is *P. knowlesi* also known as monkey malaria or traveler's malaria (World Health Organization 2023). *P. knowlesi* are prevalent, especially in Malaysia. In the Indian subcontinent, cases have been reported only in Andaman and Nicobar Islands (Subbarao et al. 2019).

4.2.2 The Anopheles Vector

Malaria is transmitted through the saliva of infected female Anopheles mosquito bite. Over 400 species of Anopheles mosquito are known, from which 70 species are reported to be major vectors of malaria (Pimenta et al. 2015). In India, six species of Anopheles are described as primary vectors. They include *A. baimaii, A. culicifacies, A. fluviatilis, A. sundaicu, A. stephensi,* and *A. minimus*. Malaria transmission by secondary vectors such as *A. annularis, A. nivipes, A. philippinensis,* and *A. varuna* is limited to a small local area and occurs in association with one to two primary vectors (Subbarao et al. 2019).

4.2.3 Malaria Pathogenesis

The life cycle of all species of the Plasmodium genus in humans is almost similar, which is characterized by asexual phase in the host and sexual phase in the vector. The asexual phase is completed in the host, i.e., humans, and so termed as an endogenous phase, while the sexual phase occurs outside the host, i.e., in female Anopheles therefore known as an exogenous phase. The asexual life cycle in the host

is completed in two phases which can be divided into liver and intra-erythrocytic (Centers for Disease Control and Prevention 2020).

4.2.4 Global Framework for Malaria Elimination

A comprehensive framework of guidelines for accelerating progress in the effort for the elimination of malaria globally is in force under WHO Global Technical Strategy 2016–2030. The World Health Assembly endorses the strategy in GTS which has set the target of reducing the global incidence of malaria and mortality rate by 90% till 2030. Under this framework, participating countries irrespective of their relevance would have to set their targets at the national or subnational level and accelerate their programs in eliminating transmission and prevention of re-establishment of the disease (World Health Organization 2021a). The required guidelines for strategies, tools, and activities for achieving the aims and objectives of the framework across the globe are provided under the latest WHO-Framework for Malaria Elimination (2017) (World Health Organization 2017a).

4.2.5 Drug Resistance Threat and Need for New Discovery

Currently, medicines used in malaria for prophylactic and curative treatment either as a single or combination of compounds are listed under the WHO Model List of Essential Medicines. The name of 14 medicines used for curative treatment and 4 for prophylactic are listed under the essential medicines list of WHO (World Health Organization 2021b). The available antimalarial drug is broadly classified under three categories based on their mode of action and nature of chemical structure (Saifi 2013):

1. **Class-I:** Medicine that are aryl amino compounds. They include chloroquine, quinine, quinidine, mefloquine, tafenoquine, amodiaquine, piperaquine, lumefantrine, mefloquine.
2. **Class-II:** Medicines that are antifolate compounds also known as antifols. They include trimethoprim, pyrimethamine, chlorproguanil, proguanil.
3. **Class-III:** Medicine that is artemisinin compounds. They include artemisinin, artesunate, dihydroartemisinin, artemether.

The present predicament of drug resistance in antimalarial drugs has contributed to the surge of mortality and morbidity rate. Therefore, the quest for antimalarial agents or the development of novel/new drugs is pertinent at every level. Advances in combating malaria worldwide have gained stride in the recent past. The latest and most promising candidate vaccine against *Plasmodium falciparum*, RTS,S/AS01 has reached stage-III clinical trials (Pan et al. 2018). Yet, the effort of developing a vaccine against malaria commercially is still underway.

One of the most promising avenues is plant-based natural products in the fight against the disease. Several compounds isolated from plants have in vitro anti-plasmodial effect on *Plasmodium* spp. Further, the potential of the antimalarial agents from the plant's repertoire has not been fully explored (Mojab 2012). Therefore, plant secondary metabolites would be a paramount field in the discovery of antimalarial drugs at the pre-clinical level.

4.2.6 Anti-plasmodial Activity of Secondary Metabolites

Thousands of compounds are isolated and studied for their anti-plasmodial activity, but those showing promising results in terms of IC50 values or in vivo activity will be discussed here. Among all the secondary metabolites showing activity against Plasmodium, 38% are alcohols, 35% are terpenoids, and 17% are quinones (Bekono et al. 2020).

The first drug to be used for treating malaria is quinines obtained from *Cinchona officials*, native to South America locally known as Lojabark. The bark of the tree contains the alkaloid quinine, which has been used traditionally in treating malaria. To date, quinine is used exclusively or in combination with other drugs to treat a severe case of *P. falciparum* infection. More importantly, derivatives from quinine have led to the development of other antimalarial drugs. These drugs derived from quinines includes chloroquine, quinidine, mefloquine, tafenoquine, amodiaquine, piperaquine, lumefantrine, mefloquine.

Artemisinin is considered the principal antimalarial drug from plants. It was first isolated from *Artemisia annua* commonly known as sweet woodworm or sweet sagewort. The use of this plant in treating fever and chills has been known from Chinese traditional medicine. The derivatives of artemisinin have shown to be effective against *P. falciparum* especially in drug-resistant cases. Several derivatives of the original compounds such as artesunate, artemisinin, artemether, artelinate, dihydroartemisinin, artemotil are presently in use in a variety of formulations. Artemisinin and its derivatives are reported to be the most effective drug in reducing the number of parasites from the blood streams per asexual cycle (parasitemia). It is observed that artemisinin is activated by heme where the parasite actively metabolizes which then inactivates the protein in the plasmodium body thereby killing them (Creek et al. 2008). Artemisinin also interacts with the non-heme proteins such as glutathione-*S*-transferase (Eckstein-Ludwig et al. 2003), Ca-ATPase of muscle endoplasmic reticulum (Eichhorn et al. 2013), and translational tumor protein (Lisewski et al. 2014). However, the exact mechanism is still to be deciphered.

An important plant secondary metabolite exhibiting anti-plasmodial activity is the Terpenes. Terpenes have the property to bind with the hemin part of the hemoglobin in infected erythrocytes. Iron forms an important component for the development of plasmodium in the erythrocytes. The binding of terpenes with the hemin of the infected erythrocyte kills the parasite (Kayembe et al. 2012). Further, the terpene–hemin complex not only binds with phospholipids of cell membranes (Ginsburg and

Demel 1984) but also disturbs carbohydrate metabolism (Rodriguez and Jungery 1986) which could lead to lysis of the parasite.

One of the most abundant terpenes, Limonene is an important secondary metabolite that exhibits anti-plasmodial properties. The anti-plasmodial activity of limonene is from its affinity to bind with the intermediates of the parasite isoprenoid pathways. The isoprenoid pathway is central to the survival of parasites since they mediate protein translation, cell signaling, and other important biological pathways (Jordão et al. 2011). Terpenes inhibit the synthesis of dolichol and ubiquinone which are products of the isoprenoid pathway. Administration of limonene as a drug does not affect the host since the parasite's isoprenoid pathway is distinct from those found in mammals (Rodrigues Goulart et al. 2004). This makes limonene a highly reliable potential antimalarial drug candidate.

Another class of terpene, pinenes are monoterpenes that are commonly found in pine trees. Two classes of pinene derived from pine extracts that show antimalaria activities are α-pinene and β-pinene. The two pinene classes are observed to be active against chloroquine-resistant *Plasmodium* sp. The antimalarial activity of (+)-α-pinene is 250 times higher than (+)-β-pinene (van Zyl et al. 2006; Salehi et al. 2019). Limonoids isolated from *Vepris uguenensis, Khaya grandifoliola, Entandrophragma angolense,* and *Ekebergia capensis* showed good anti-plasmodial activities against *P. falciparum* FCR-3, chloroquine susceptible strain at IC50 values of 1.25–9.63 μM (Amoa Onguéné et al. 2013). A number of active antimalarial limonoids, neemfruitin A, and anthothecol have been isolated from *Azadirachta indica* and *Khaya anthotheca*, respectively. These isolates showed antimalarial properties against chloroquinone sensitive strain D10 and chloroquinone resistant strain W2 of *P. falciparum* in an in vitro assay within the IC50 value of micro to submicromolar range (Chianese et al. 2010; Lee et al. 2008). Another class of terpenes, caryophyllene which is a sesquiterpene is known to have a prophylactic and remedial property to malaria. It is also an active repellant agent for mosquito and blood-sucking dipterans (Maia and Moore 2011). In a recent development, highly sensitive anti-*P. falciparum* silver nanoparticles synthesized from caryophyllene ensure a possibility of cost-effective malaria therapy (Kamaraj et al. 2017).

One of the secondary metabolites possessing the basic structure of diterpene, Clerodanes isolated from *Nuxia sphaerocephala* showed anti-plasmodial properties in an in vitro assay against *P. falciparum* FcB1 strain. The dose of the isolates was tested at IC50 values ranging from 14.6, 4.3, 8.0, 7.3, 11.4, 21.0, 16.0 μg/mL. Isolates of sesquiterpenes derived from *Vernonia* spp. at 4–11 μg/mL of IC50 showed anti-plasmodial effect against MDR *P. falciparum* K-1 strain (Amoa Onguéné et al. 2013).

Flavonoids have the highest antioxidant activity among plant secondary metabolites as shown by the level of oxygen radical absorbance capacity (ORAC). The Oxygen radical absorbance capacity of *Artemisia annua* leaf extract were found to be highest in comparison to other extract's. *Oregano* spp. herb is reported to have the highest ORAC in herbs which is still lower by 2/3rd of the leaves (1123 μmol Trolox eq/g) and inflorescence (1234 μmol Trolox eq/g) of *Artemisia annua*. This enormous antioxidant property of *A. annua* is attributed to the high content of

phenolic compounds. Flavonoids are described as good metal chelators. Therefore, the use of flavonoids in Fe-chelating therapy for malaria patients is highly recommended. The possibility of using flavonoids as artemisinin synergists is from the fact that the latter could reduce Fe^{3+} to Fe^{2+}, which is necessary for the bioactivity of artemisinin. Thereafter, artemisinin releases short-lived free radicals—an antimalarial mode of action (Ferreira et al. 2010). So, a combinational use of artemisinin and flavonoids might be an effective malaria treatment regime.

Isolates of several flavonoids extracted from the bark of *Milletia usaramensis* stem were effective against *P. falciparum* stains that are chloroquinone resistant, chlorosensitive. The flavonoids isolates that showed anti-plasmodial activities include: prenyl and non-prenyl flavones, isoflavones, flavens, pterocarpenes, and chalones (Yenesew et al. 2003, 2004).

Further, flavonoid extract from leaf, the bark of roots, and stem of angiosperm *Uvaria* spp. of Tanzania showed anti-plasmodial activities in an in vitro assay against multidrug-resistant strain *P. falciparum* K1 at IC50 values from 5 to 500 μg/mL (Nkunya et al. 1991).

Synergistic activity with first line anti-malarial drugs has been reported with Phenolic compounds extracted from the bark of Kigelia africana. At the dose of 0.42–0.71 μM IC50 values, the isolates were effective against multidrug-resistant *P. falciparum* W2mef stain with no toxicity sign to LLC-MK2 kidney cells of monkeys (Zofou et al. 2012). Several alkaloids especially indoles obtained from 3 *Alstonia* spp., *Isolona,* and *Mondora* spp. are highly effective as anti-plasmodial agents in *P. falciparum* CQR strain at a very low dose of 360–144 nM IC50 values (Keawpradub et al. 1999). Similar activities are also reported with naphthylisoquinolines derived from *Triphyophyllum* spp., *Ancistrocladus* spp., *Dioncophyllum* spp. and were found to be effective against *P. falciparum* K-1 and NF54 strain to 0.1–16 μM at IC50 values (Bringmann and Ancistrobertsonines 1999). Other alkaloids like Cryptolepines extracted from *Cryptolepis sanguinolenta* reported in vivo antimalarial properties in mice at a dose less than 50 mg/kg p.o at ED50 and 10 mg/kg i.p at DE50 (Cimanga et al. 1997).

4.3 Leishmaniasis

Leishmaniasis is a common neglected tropical disease prevalent in under-developed and developing countries (Mitra and Mawson 2017). This neglected disease is known by numerous names, viz., Calcutta ulcer, oriental sore, black fever, Jericho button, Aleppo boil, Uta, kala-azar, Dum-dum fever, etc. (ul Bari 2006). Three different forms of leishmaniasis are known: visceral leishmania, cutaneous leishmanial, and mucosal leishmaniasis. According to WHO, out of 200 countries, 98 countries are declared to be endemic for this disease. In 2020, 90% of visceral leishmaniasis cases were reported from ten countries including India and Nepal, Iraq, and the remaining from East African countries. And 87% of cases of cutaneous leishmaniasis for the same year were reported from South America, Central Asia, and East Africa. The third type of leishmaniasis, i.e., mucosal/mucocutaneous cases

was mostly concentrated in South America. Over 90% occur in Brazil, Bolivia, Peru, and Ethiopia from Africa (World Health Organization 2021c). According to the analysis of WHO, every year about 0.9–1.7 million individuals are infected. However, it is estimated that only a small fraction of the infected cases are likely to develop the disease and roughly 30,000 may succumb to the disease (World Health Organization 2021c).

4.3.1 Parasite

The causative agent of Leishmaniasis is a protozoan *Leishmania*, which belongs to Trypanosomatidae. More than 20 species of Leishmania infect mammals including humans. This protozoan parasite is categorized into two species: the old world for those found in Europe, Asia, and Africa and the new world existing in Latin America (Cox 1993). Out of the 20 parasite species, 18 species are zoonotic and 2 are anthroponotic, namely *Leishmania donovani* and *Leishmania tropica*. The zoonotic species include three old world species (*L. aethiopica, L. infantum, L. major*) and the remaining species are included in new world leishmaniasis species. An instance of coexistence between zoonotic and anthroponotic species in certain geographical location exhibiting different epidemiological cycles is reported for *L. infantum* (zoonotic) and anthroponotic species *L. donovani* in Arabian Peninsula (Steverding 2017).

4.3.2 Vector

The transmission of leishmaniasis in humans occurs from the bite of the infected female sandfly. More than 600 species of sandfly that are found are divided into five genera: *Lutzomyia, Warileya, Brumptomyia* from the New World, and *Sergentomyia, Phlebotomus* in the Old world (Ready 2013; Maroli et al. 2013). Infection of human occurs through the bite of infected female sandfly in the old world is caused by Phlebotomus and Lutzomyia in the new world (World Health Organization 2010).

4.3.3 Leishmania Pathogenesis

Leishmania completes its life cycle in two stages vis-a-viz in the vector sandfly and the host human. The parasite Leishmania exists in two forms: Promastigote that grows and develops externally in the vector sandfly; Amastigote form, which multiplies in the host reticulo-endothelial cells. The reservoir host for leishmaniasis includes mammals like the fox, rodents, domestic animals, and humans. However, in anthroponotic leishmaniasis humans are the exclusive reservoir hosts. Visceral Leishmaniasis is endemic to India and its subcontinents where human is the exclusive reservoir host for the disease (Sunter and Gull 2017).

4.3.4 Global Framework for Leishmaniasis Elimination

In line with the resolution of WHA60.13 on control of leishmaniasis, WHO has spearheaded an awareness campaign on leishmaniasis burdened member states toward control of the disease by establishing guidelines and system for data collection, analysis, and surveillance (World Health Organization 2007). The resolution also advocates WHO to extent support to the member states in reinforcing efforts of setting up national programs for efficient detection, affordable diagnosis and treatment measures. Therefore, WHO under Global Leishmaniasis Program has developed standard tools for collecting sets of indicators annually from its member States. In addition, more detailed indicators are prepared especially for the high burden members states and high burden countries as well (World Health Organization 2017b). The collection of indicators is accessible through Global Health Observatory (GHO); however, only six are available to the public (World Health Organization 2021c).

4.3.5 Drug Resistance Threat and Need for Discovery of Novel Drugs

The primary drug for the treatment of Leishmaniasis is sodium stibogluconate (SSG) also known as antimonials, which have been in use since the early 1920s. Though the therapeutic windows of these compounds are very narrow due to their toxic nature along with widespread resistance to SSG. They are still used for treating the disease in America (New World region) and East Africa (Karamian et al. 2015), while their use is outdated in the Indian subcontinent. Sodium stibogluconate, which is administered intramuscularly has been replaced by miltefosine as the first line of treatment due to an increased rate of resistance for SSG (Sundar and Singh 2013). However, the setback has drawn attention to the use of miltefosine from twofold increase of failure rate in a clinical cohort test (Sundar et al. 2012) and an increase in relapse rate by 20% (Singh et al. 2016), a decade after its introduction. In addition, a long period of medication (28 days) combined with the inherent long half-life of miltefosine might favor the development of resistant strains. The best possible approach for the continual use of miltefosine clinically to save lives would be a combinational therapy.

In a recent development, liposomal amphotericin B under the trade name (AmBisome®) has been adopted as the treatment of choice for visceral leishmaniasis (Ponte-Sucre et al. 2017). The current AmBisome® treatment regime is a one-dose intravenous injection to evade the risk of drug resistance emergence and the results have been reported to be excellent (Sundar and Singh 2013). The greatest concern primarily in remote areas endemic to leishmaniasis is the lack of effective vaccines, resistance to treatment drugs, high cost of treatment, possible toxicity of some drugs, and parenteral drug administration (Oryan 2015). Therefore, the challenges of finding novel agents with leishmanicidal and/or anti-leishmaniasis is a worldwide concern (Mahmoudvand et al. 2014; Ogeto et al. 2013). Workers across the globe are

in the race to find alternative sources of drugs that are natural products. One of the most important alternative sources of the drug for anti-leishmaniasis could be plant-based natural products since they are considered to be bioactive agents for antiprotozoal and anti-inflammatory properties (Oryan 2015; Gamboa-Leon et al. 2014).

4.3.6 Pharmacological Activity of Secondary Metabolites Against Visceral Leishmaniasis (Kala-Azar)

Numerous compounds from natural products that exhibit anti-leishmanial properties are plant secondary metabolites (PSM). These PSM include flavonoids, lignans, monoterpenes, chalones, toxoids, iridoids, curcumin, coumarins, polyketides, and quinoline alkaloids. To evaluate compounds' anti-leishmanial properties certain criteria such as IC50, MIC, and selectivity index (>10) are considered (Mahmoudvand et al. 2014).

Monoterpenes linalool isolated from leaves of *Ocimum basilicum* and *Croton cajucara* essential oils exhibited anti-leishmanial activities against *Leishmania amazonensis* and *L. donovani* promastigotes in an in vitro assay (Rosa et al. 2003). Citral, an essential oil extracted from lemon grass *Cymbopogon citrates* showed anti-leishmanial activity against *L. infantum* amastigotes and promastigotes. The extracted oil was found to be *cis*-1-neral and *trans*-1-general, which showed effective anti-leishmanial activity at 8–25 μg/mL (IC50). Other compounds such as *R*-(+)-limonene, *S*-(–)-limonene, α-phellandrene, *S*-(–)-carvone *p*-cymene, γ-terpinene, citral, thymol, carvacrol, sabinene, alpha, and beta pinene showed anti-promastigotes and amastigotes form of *Leishmania donovani, L. amazonensis, L. chagas, L. major* at IC50 values ranging from 8 to 335 μg/mL (Schmidt et al. 2012). Leishmanicidal activities against promastigotes of *L. donovani* by labdane diterpenes obtained from methanolic extraction of *Aframomum sceptrum* rhizomes were reported to be effective at the IC50 value ranging from 5 to 25 μM (Cheikh-Ali et al. 2011).

In vitro anti-leishmaniasis activity against promastigotes was reported for a new diterpene compound extracted from *Aeonium lindleyi* leaves. This compound was identified as 7-Oxo-Labd-8-en-15-ol skeleton diterpene elucidated based on 2D NMR and spectroscopic data. The new diterpenes, labdan-8α-15 diol, and labden-8α-en 3β-15 diol showed the anti-promastigotes form of *Leishmania tropic* and *Leishmania braziliensis* at IC50 values 68 and 77 μM, respectively (Cheikh-Ali et al. 2011; Sob et al. 2007). Triterpenes with anti-leishmaniasis properties were reported for compound LLD-3(1) isolated from a plant extract of *Lophanthera lactescens*. The isolated compound LLD-3(1) when tested in vivo against *L. amazonensis* intra-macrophage amastigote with 0.41 μg/mL at IC50 showed no cytotoxicity to mouse cells, further it did not interfere with immunoglobulin synthesis and multiplication of B and T cell population (Danelli et al. 2009). Interestingly, Danelli et al. (2009) further observed that application of compound LLD-3(1) enhanced the

leishmanicidal effect or efficacy of glucantime, leishmaniasis first choice of drug (Danelli et al. 2009).

Ogungbeand et al. (2014) studied 352 phenolic phytochemicals and identified those compounds that demonstrated leishmanicidal properties. The phytochemicals with anti-leishmaniasis properties include 6 flavonoids (cannflavin A, diplacone, quercetin trimethyl ether, 3-*O*-methyldiplacol, 3-*O*-methyl diplacone, 4-*O*-methyl diplacone), 5 coumarins (umckalin, scoparone, mammea A/AA, B/BB, B/BBA cyclo-F), 3 lignans (TM-epoxy lignan, DD-epoxy lignin, and aristolignin), 2 aurones (DPH-benzofuranone and bacterin triacetate), 1 stilbenoid (machaeriol), 1 isoflavonoid (sophoronol E), and 1 chalcone (crotaorixin) (Ogungbe et al. 2014).

Another secondary plant metabolite, flavonoids such as quercetin, luteolin, flavones (7,8-hydrox), and fisetin are inhibitors of arginase enzyme in *L. amazonensis* (Oryan 2015). One of the adaptations to evade the host immune system by the parasites are ROS and polyamine synthesis which is dependent on arginase. Therefore, the inhibitory properties of flavonoids on arginase make it an important candidate for the development of leishmanial drugs.

The scope of finding potential compounds against leishmaniasis is very bright. Several studies have reported leishmanicidal properties from plant extract but have yet to identify and characterize the bioactive agents. For instance, Chandrasekaran et al. (2017) work on *L. donovani* treated with alcoholic leaves extract from *Withania somnifera* in an infected peritoneal macrophage and immunodeficient mice (Chandrasekaran et al. 2017). In their observation, alcohol extract fractions F5 and F6 showed a wide range of anti-leishmanial activities such as significant reduction in amastigote count, IL-10 mRNA expression, and inducing reactive oxygen species in *L. donovani* infected macrophages. A similar, anti-leishmanial effect was also observed with purified withaferin A. The F5 and F6 extracts were administered orally and withaferin-A through dietary supplements in vivo studies in mice period of 10 days. The in vivo study of the *Withania* extract reported significant immune-modulatory and anti-leishmanial activities such as reduction in cytokines IL-4, IL-10, expression of TGF-βmRNA increased ratio of IFN:IL-10, and increased production of IgG2a.

Similar studies have shown inhibitory activities against the parasites. Promastigotes of *L. major* when treated with an alcoholic extract from *Satureja khuzestanica* leaves showed significant inhibition of the parasite at IC50 values ranging from 0.3 to 0.6 mg/mL (Sadeghi-Nejad et al. 2011). Amin et al. (2017) tested aerial parts of 15 plants family; Euphorbiaceae, Brassicaceae, Solanaceae, Acanthaceae, Chenopodiaceae, Zygophyllaceae, Asteraceae, and Gamineae against *Leishmania donovani* (Amin et al. 2017). The alcoholic extracts fractions of the plant parts of these families demonstrated growth inhibition (0–100% inhibition) comparable to amphotericin B. Further exploration of the phytochemicals of the extracted has identified four compounds (Simiarerol, β-sitosterol, β-sitosterol-3-*O*-glucoside, hexacosanol) that can potentially be used for leishmaniasis treatment as a lead drug.

4.4 Lymphatic Filariasis

Lymphatic filariasis is a disease from a parasitic infection of nematodes (Filarioidea). It is commonly known as elephantiasis. The disease arises from an impaired lymphatic system that causes engagement of body parts along with pains and severe disability often associated with the social stigma. As of January 2020, 859 million population from 50 countries are at risk from lymphatic filariasis. An estimate of 25 million with hydrocele, 15 million lymphoedemas, and 36 million people are with disease manifestation (World Health Organization 2020). This disease is endemic to the tropic and subtropical regions of Africa, the Indian Subcontinent, South America, Southeast Asia, and Pacific islands (Ngwira et al. 2002).

4.4.1 Parasite

Three nematode species belonging to the family filarioidea responsible for filariasis are *Wuchereria bancrofti, Brugia malayi,* and *Brugia timori*. The major nematode that causes the disease in humans is *Wuchereria bancrofti*. It is reported that 90% of filariasis is caused by *W. bancrofti* and humans are the exclusive host. Other species like *Brugia malayi* are responsible for the remaining 10% case. Apart from humans, domestic animals and wild animals are known *B. malayi* host. *B. timori* causes a disease known as "Timorian filariasis" which is limited to Sunda Island in Indonesia (Ramaiah and Ottesen 2014).

4.4.2 Vector

The parasitic nematodes of lymphatic filariasis are transmitted from the bite of infected mosquitoes. The species of vectors responsible for transmission may vary according to geographical locations. In general, five species of mosquitoes are known to be the main vectors: Coquillettidia, Aedes, Culex, and Mansonia. Humans are the definitive host for the parasite especially for *W. bancrofti,* while domestic animals and wild animals also serve as hosts for *B. mayali*. However, replication of adults does not occur in humans.

4.4.3 Lymphatic Filariasis Pathogenesis

The lymphatic filariasis larvae deposited on the skin during the mosquito bites gain entry to lymphatic vessels through wounds inflicted in the bite. These larvae travel to the lymphatic vesicle and live in the lymph nodes for 6–12 months to mature into adult female and male worms. A mature female in her 7th-year cycle can release on an average up to 10,000 microfilaria per day. The released microfilaria is then carried to the bloodstream by the lymph through the lymphatic circulation. This microfilaria tends to remain in large blood vessels during the wake period of the host. However,

during sleep, the microfilariae migrate toward surface vessels or capillaries, thereby creating the opportunity to be injected during the bite. Once ingested by the vector, microfilaria undergoes several molting and development process within 10–12 days to be equipped for human transmission (Lourens and Ferrell 2019).

Most often transmission of visceral filariasis occurs during the childhood period and the prevalence of the disease increase with age (Malhotra et al. 2003). Predominantly, this disease is seldom detected among travelers nor do they develop chronic conditions from high worm burden because of insufficient exposure. Therefore, the extent of this disease is subjected to the level of exposure to the parasite, degree of infestation, and level of antigens from adult worms (Dietrich et al. 2019).

4.4.4 Global Framework for Lymphatic Filariasis Elimination

The effort to eliminate the health burden of the disease, the Global Programme to Eliminate Filariasis was initiated in 2000 under WHO. The program was aimed to eradicate filariasis by interrupting transmission through MDA along with the provision of basic care facilities to the infected person (World Health Organization 2000). Since then, advancement in achieving the goal of lymphatic filariasis elimination has been validated by 11 countries till 2017. Further, ten countries have achieved a full mass drug administration process in all their endemic areas. Nonetheless, the target to eliminate lymphatic filariasis by the year 2020 is still a distant dream. Therefore, WHO has proposed 2030 as the year of the target for lymphatic filariasis elimination as public health burden across the globe (Modelling Consortium Lymphatic Filariasis Group-NTD 2019).

4.4.5 Drug Resistance Threat and Need for New Drug Discovery

The standard operation protocol in the 1980s was through administration of diethylcarbamazine or DEC for 12–14 days after diagnosis. The diagnosis mainly depended on the microscopic identification of the microfilariae in the blood specimen. Further, the nature of treatment was solely for infected individuals (World Health Organization 1984). This treatment regime was able to reduce the microfilariae level significantly but not a complete cure (Ottesen 1985). Three lymphatic filariasis drugs including ivermectin, albendazole, and diethylcarbamazine, are still active and administration of any two combinations of these drugs was found to be significantly more effective in clearance of microfilariae than single use or monotherapy. This evidence of the drug's effectiveness in clearing microfilariae paved the way for achieving lymphatic filariasis eradication (Gyapong et al. 2005).

Therefore, the focus on the fight against lymphatic filariasis has shifted to the prevention of infection in communities. It was anticipated that 4–6 rounds of mass drug administration would be adequate in reducing the level of microfilariae for active transmission. This would, however, be influenced by the efficacy of the available drug and the life span of the worm (Ottesen et al. 2008). At present,

countries endemic to lymphatic filariasis follow a single dose of two drug regimens to attain elimination of the disease. Multiple drug therapy not only enhances the efficacy of the drugs but also minimizes the chance for drug resistance development among the infected population (Gyapong et al. 2018).

Conversely, the effectiveness of the three drugs against the adult worm is subtle be it combinational or single-dose treatment (Behera and Bhatnagar 2018). Report of albendazole singly or in combination with other drugs being ineffective against microfilariae occurrence for over 2 weeks to 12 months after treatment has been recorded (Macfarlane et al. 2019). In contrast to the previous observation, albendazole was found to be effective when used as monotherapy (Behera and Bhatnagar 2018). Regrettably, the available synthetic drugs for lymphatic filariasis treatment are accompanied by adverse side effects. Therefore, the quest for therapeutic drugs that are effective on microfilariae and adult worms at the same time without adverse side effects is the need of the hour. The potential avenues for finding such attributes of therapy for lymphatic filariasis could be elucidated from insights of traditional medicines, which are primarily plant based.

4.4.6 Pharmacological Activity of Secondary Metabolites Against Lymphatic Filariasis

Majority of the plant spp. with phenolic compounds have shown antifilarial activity. Increased lipid peroxidation and protein carbonylation due to the presence of these polyphenolic compounds present in various sp. result in the mortality of worms. The methanolic leaves extract of *Aegle marmelos* Corr. is known to possess coumarins and polyphenolic compounds that can induce 100% motility loss within 48 h against microfilariae of *Brugia malayi* (at 100 ng/mL) with IC50 = 70 ng/mL (Sahare et al. 2008a, b).

Among various compounds isolated from *A. nepalensis*, diarylheptanoid has demonstrated in vitro filaricidal properties against *B. mayali* adult worm. Interestingly, in vivo anti-filariasis activity of the plant extract showed differential efficacy with different alcoholic fractions. Chloroform extract fraction of *A. nepalensis* showed more than 50% macrofilaricidal activity, while *n*-butanol and methanolic fraction macrofilaricidal activity ranged from 38 to 40% in addition to the display of sterilizing effects on female worm. In vitro study against *B. malayi* with the compounds Withaferin A isolated from *Withania somnifera* with monoterpene, 2-isopropyl-5-methyl phenol isolated from *Trachyspermum ammi* in an MTT assay exhibited inhibition of formazan formation and adulticidal property. Further in vivo studies with these compounds showed significant macrofilaricidal and female sterility against *Brugia malayi* at 7.8 μg/mL (Withaferin A). This compound further exhibited microfilariae number reduction to 63% along with inducing defective embryos up to 62% (Mathew et al. 2008; Kushwaha et al. 2012).

Pentacyclic triterpenoids, oleanonic acid, and oleanolic acid extracted from *Lantana camara* stem exhibited sterilizing effect on female adult and considerable filaricidal effect on adult *B. malayi*. Further in vivo studies also showed similar

antifilarial effects on adult worms and sterilization of the surviving female worms (Misra et al. 2006). Crude extract from *Plumbago indica* roots exhibited immobilization effect on adult cattle filarial worm *S. digitata* up to 83% within 6 h at low concentration of 0.01 mg/mL. The bioactive compound was isolated from the extract through column chromatography in petroleum ether:chloroform solvent. The isolated compound when subjected to MTT assay, plumbagin demonstrated antifilarial activity of worm immobilization and significant inhibition on formazan formation even at very low concentration of 0.0006 mg/mL (Mathew et al. 2002).

Gedunin and its derivatives are pentacyclic triterpenoid found in *Azadirachta indica* and *Cedrela odorata* and *X. granatum*. In vitro studies of gedunin and its derivative photogedunin isolated from *X. granatum* fruit exhibited antifilarial activity at IC50 values ranging from 0.213 to 0.240 μg/mL. Further, in vivo studies of gedunin and its derivative against *B. malayi* transplanted in jirds peritoneal cavity resulted in 80% worm mortality when 10 mg/kg body weight dose was given for 5 days subcutaneously (Misra et al. 2011). The treatment regime resulted in 70–80% mortality of adult *Brugia malayi* worm in jirds. Therefore, these compounds open the opportunity for use as filarial adulticidal drugs. Studies with essential oils from alcoholic extracts (100 mg/kg) of rhizomes of *Z. officinale* exhibited reduced microfilarial concentration in blood (Datta and Sukul 1987). The bioactive components of the essential oil included borneol, linalool, limonene, zingiberol, and β-elemene.

Lakshmi et al. (2010) studied the effect of six flavonoids on lymphatic filarial nematode *B. malayi*. The six flavonoids were Naringenin, Naringin, Chrysin, Hesperetin, Flavone, and Rutin. Two flavonoids, naringenin, and hesperetin showed a filaricidal effect on adult worms, and in vitro formazan formation was inhibited by 60% with IC50 value 7.8 and 31.2 g/mL respectively. More than 80% inhibition of formazan formation was recorded for female adult worms at IC50 = 31.2 g/mL of flavones. Naringenin (IC50—2.5 g/mL), hesperetin, rutin, and chrysin showed microfilariae (mf) cidal activity at 250–500, 62.5, and 250 g/mL respectively. Adulticidal effect on *B. malayi* was observed in naringin in vitro assay at 125 g/mL. In vivo studies showed antifilarial activities by flavones and naringenin only. They exhibited a filaricidal effect on adult worms and sterilizing effect on adult females at a dose of 50 mg/kg (Lakshmi et al. 2010).

In an efficacy-safety study, a double-blind clinical trial with flavonoid daflon isolated from *Rutaceae aurantia* and diethylcarbamazine (DEC) was conducted by Das et al. (2003). The purified flavonoid fraction, daflon at 500 mg in combination with DEC at 25 mg and DEC exclusively at 25 mg was administered to 26 lymphoedema patients from *W. bancrofti* infection for 90 days twice per day. Patients receiving daflon with DEC showed edema reduction by 64% in volume in 360 days. In docking analysis on alkaloidal compounds derived from *Rauvolfia tetraphylla* fractions F1 = Curan-17-oin acid, F2 = 18,19-secoyohimban, F3 = Reserpiline exhibited dock score of −5.14 for F1, −7.19 for F2, and −7.2 for F3 compound on glutathione-*S*-transferase enzyme. In vitro study of the three compounds on glutathione-*S*-transferase enzyme activity reported inhibition of the enzyme activity by 35.78%, 78.22%, and 64.21%, respectively (Behera and Bhatnagar 2019).

4.5 Conclusion

The review, as mentioned above demonstrates the use and importance of secondary metabolites of plant origin in three parasitic infections of paramount importance. The life cycles of three parasites, namely malaria, kala-azar, and human lymphatic filaria in the host and their vectors, have been discoursed in this chapter. Further, the plethora of drug resistance to the existing treatment regimen, necessitates the need to explore secondary plant metabolites were also presented. The unavailability of an effective vaccine against most of the neglected tropical diseases calls for the search of alternative means to cater the unmet need of health care. Despite our rich biodiversity, the majority of the plant products are either unexplored or are not scientifically validated. Bio-activity-guided fractionation, isolation and characterization of secondary metabolites is the only key to these questions. Thus, our report reiterates the importance of the in-depth study of plant products in parasitic diseases through in vitro, in vivo studies followed by clinical trials.

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5 Exploring Marine Biodiversity as Alternative Resources for Treatment of Human Parasitic Diseases

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Abstract

Protozoan-dependent ailments such as leishmaniasis, malaria and trypanosomiasis continue to be considered as the most neglected diseases, especially among economically deprived populations in developing sub-tropical and tropical belts of Asia, America and Africa. It is also known as the "disease of poverty" as most of the victims belong to the poorest communities. Among the prevalent protozoan parasitic diseases, leishmaniasis ranks second after malaria. Owing to toxicities and adverse effects as well as drug resistance to conventional treatment, new modalities for the treatment of parasitic infections are being continuously explored especially from naturally available bioactive compounds. These have impelled researchers to discover innovative medicines from natural biodiversity as alternative resources from marine environs that are economical, effective, and safe.

Marine environment is recognized with a variety of substances from the classes predominately from invertebrates including tunicates, sponges, bryozoans, molluscs marine cyanobacteria and bacteria, which targets many diseases including the three protozoan diseases mentioned above. As the diseases progress and develop resistance to current treatments, the marine world provides pioneering alternatives against parasitic diseases. The chapter explains the life cycle and geographical distribution of Leishmania, Malaria, and Trypanasoma and the novel marine metabolites that are being exploited for their anti-parasitic activity. Enhanced global awareness regarding the novel anti-infective remedies that are being explored from the incredible diversity of natural marine products

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_5

will assimilate the augmented efforts to utilize marine products in clinical applications.

Keywords

Marine biodiversity · Natural products · Trypanosomiasis · Leishmaniasis · Malaria

5.1 Introduction

Ocean covers more than 70% of the earth's surface and represents the greatest diversity of life by possessing 34 out of 36 phyla of life. It is the habitat of greater than 300,000 explored species of animals and plants (Costello and Chaudhary 2017). Among them, some macroscopic animals and plants have covered entire oceanic regions including tropical, temperate, and polar areas. The species diversity comprises coral reefs variety of approximately 1000 species per m^2 at large extents in some areas and the greatest tropical biodiversity in marine areas of the world comes under the Indo-Pacific Ocean (Donia and Hamann 2003). Overall represents over 95% of the whole biosphere and become a cherished useful products awaiting to explore for several infectious disease treatments (Obura et al. 2019). Due to the limitations on the terrestrial derivatives, ecological pressures involving space competition, surface fouling, predation, and the reproductive rate at the peak, all these values have directly led to the unique secondary metabolites evolution possessing numerous biological activities (Petersen et al. 2020). These secondary metabolites play an important role in controlling parasitic and infectious organisms, but it was ignored for several years (Petersen et al. 2020).

Protozoan diseases induce high rates of death and morbidity all over the world. As nature has harboured the richest biodiversity in marine (Fletcher et al. 2012), in this chapter we worked with the marine-derived natural products facilitating in vivo efficiency or significant in vitro activity against parasitic protozoan infections caused by *Plasmodium* sp., *Leishmania* sp., and *Trypanosoma* sp. etc. Apart from these sources, various bioactive compounds are summarized in the Table 5.1 showing anti-parasitical activity.

5.2 Leishmaniasis

It is a vector-transmitted infection communicated by an obligatory intracellular protozoan parasite *Leishmania* (class Kinetoplastea), transmitted by sand flies. Human infection is caused by more than 20 species. It is very difficult to distinguish *Leishmania* species morphologically.

But, on the basis of isoenzyme analysis, monoclonal antibodies and molecular methods, differentiation can be possible (Cecílio et al. 2022). *Leishmania* variates causes a variety of diseases including cutaneous leishmaniasis (self-healing) to

Table 5.1 Various bioactive compounds isolated from marine environment exhibited anti-parasiticidal activity

Marine sources	Functional group	Class	Bioactive compounds	IC50	References
Pandoras acanthifolium	Steroid	Sponge	Pandaroside	0.051 μM	Regalado et al. (2010)
Agelas mauritiana	Alkaloid	Sponge	Ageloxime D	29.28 μg/mL	Yang et al. (2012)
			Ageloxime B	28.55 μg/mL	
Agelas conifera	Alkaloid	Sponge	Hymenidin	29.87 μg/mL	França et al. (2017)
Axinella verrucosa	Alkaloid	Sponge	Bromoaldisin	>90 μg/mL	Scala et al. (2010)
Ircinia spiculosa	Alkaloid	Sponge	Tryptophol	9.6 μg/mL	Davies-Bolorunduro et al. (2021)
Plakortis simplex	Polyketide	Sponge	Simplexolide B	13.82 μg/mL	Davies-Bolorunduro et al. (2021)
Spongia sp. and *Ircinia* sp.	Terpene	Sponge	Furospinulosin-1	14.2 μg/mL	Davies-Bolorunduro et al. (2021)
			Furospongin-1	4.8 μg/mL	
			Heptaprenyl-*p*-quinol	18.9 μg/mL	
Lyngbya majuscula	Peptide	Cyanobacterial	Dragonamide E	5.1 μM	Balunas et al. (2010)
			Dragonamide A	4.25 μg/mL	
			Almiramides	2.4 μM	
Schizothrix	Peptide	Cyanobacterial	Gallinamide A	9.3 μM	Veerabadhran et al. (2014)
Oscillatoria sp.	Peptide	Cyanobacterial	Viridamides A, B	1.5 μM	Simmons et al. (2008)
Lyngbya majuscula	Peptide	Cyanobacterial	Dragonamide E	5.1 μM	Sanchez et al. (2010)
			Dragonamide A	4.25 μg/mL	
			Almiramides A–C	2.4 μM	

disseminating visceral leishmaniasis (many times deadly) due to lack of proper treatment (approx. 95% of cases) (Torres-Guerrero et al. 2017). In spite of all the diseases, mucocutaneous leishmaniasis is very rare and transferred from cutaneous parasites only. This variety is defined as all-out or incomplete obliteration of the throat, mouth and nose mucous layers. Another type including adult visceral leishmaniasis (also known as AIDS-related opportunistic disease) occurs because of immunosuppression (Lindoso et al. 2016).

Out of all the types, mucocutaneous leishmaniasis is an uncommon and carried by the cutaneous form of the parasite. It is simplified by the incomplete or all-out destruction of the mucous lining of the nose, mouth, and throat (Garrido-Jareño et al. 2020). Another variety, called adult visceral leishmaniasis is predicted as an AIDS-associated opportunistic disease, mostly due to the recurrence of latent infections caused by immunosuppression (de Figueiredo et al. 2022).

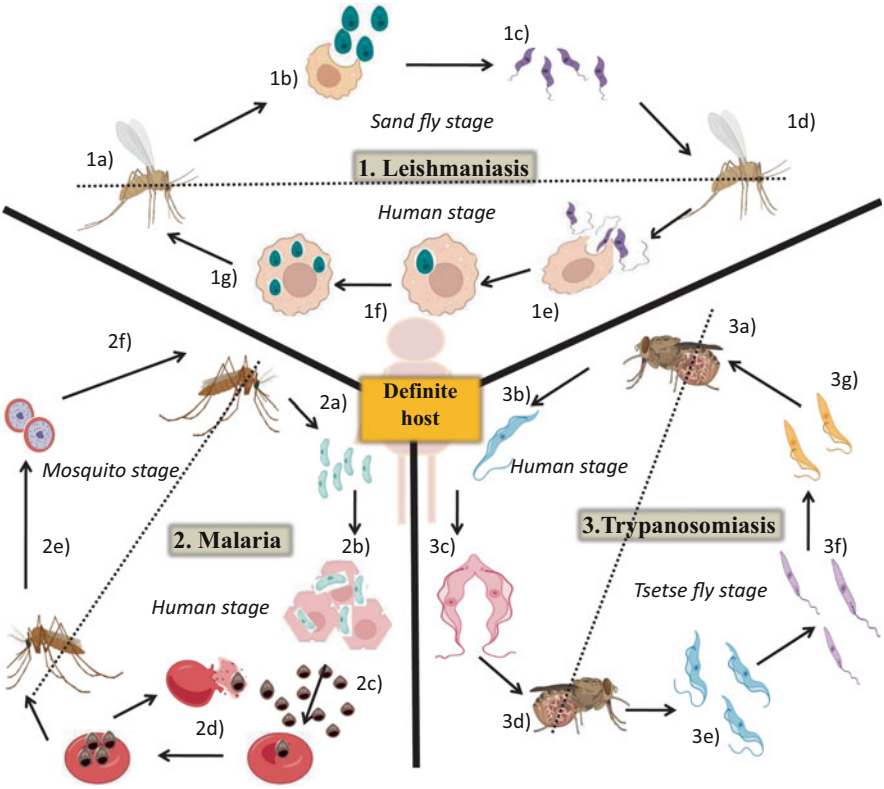
There are various factors which have a connection to this disease involves socio-economic conditions likewise environmental changes, population displacement, immune systems, hygienic conditions, poverty, malnutrition, poor housing and domestic hygienic conditions, urbanization and climate change (Costello and Chaudhary 2017).

5.2.1 Geographical Distribution of *Leishmania*

The infection randomly occurs in subtropical and also in tropical regions worldwide, especially in Southern Europe, South America, North America, Asia and Africa, where major fatalities are reported, a range of 700,000–1 million which shows a death range of 20–30,000 (Aversi-Ferreira et al. 2014). Twenty different kinds of *Leishmania* species are found only in Mediterranean countries where 90% of case report comes from India, Ethiopia, South Sudan, Brazil and Bangladesh. In report obtained from India shows 90% of VL cases directly from Bihar (a northern region), and other countries contribution like Bangladesh and Nepal, provide rest of the burden for the Indian subcontinent (Deb et al. 2018; Singh and Singh 2019; Aversi-Ferreira et al. 2014). Similarly, western states like Italy, Spain, France, Europe and Spain reported endemic cases of human and canine leishmaniasis (Aversi-Ferreira et al. 2014).

5.2.2 Life Cycle of *Leishmania*

Leishmaniasis is spread in the human population through the bite of infected female phlebotomine sand flies. The first stage of the life cycle is to inject the infective stage called as, promastigotes through the proboscis during blood meals by sand flies (Fig. 5.1). Then the promastigotes moved to the puncture wound and are destroyed by macrophages and other types of mononuclear immune cells. These Promastigotes now change in the form of amastigotes in immune cells also called as diagnostic stage (Teixeira et al. 2013). This stage can multiply by asexually and proceed to



1.Life cycle of **Leishmaniasis** parasite [1a-1g]:
1a) Sandfly takes a blood meal ingesting macrophages infected with amastigotes;1b) Release of amastigotes; 1c) Amastigotes transform into metacyclic promastigotes in midgut; 1d) Sandfly infect host by infecting metacyclic promastigotes when taking a blood meal; 1e)Promastigotes are phagocytized by macrophages; 1f) Promastigotes transform to amastigotes in macrophages; 1g) Amastigotes multiply in macrophages of various tissues.
2. Life cycle of **Malaria** parasite [2a-2f]:
2a) Transmission to sporozoites human; 2b) Sporozoites enter liver and infect hepatocytes; 2c) Liver cell rupture and merozoites released; 2d) Intraerythrocytes cycles; 2e) sexual cycle; 2f)transmission to mosquito
3. Life cycle of **Trypanosomiasis** parasite [3a-3g]:
3a) Tsetse fly takes the blood meal (inject metacyclic trypomastigotes); 3b) infect metacyclic trypomastigotes transform into blood stream trypomastigotes which are carried to other site; 3c) Trypomastigotes multiply by binary fission in various body fluid; 3d) Circulating trypomastigotes in blood during acute phase usually undetectable in latent phase; 3e) Tsetse fly takes the blood meal (blood stream trypomastigotes are injested); 3f) Blood stream trypomastigotes transform to procyclic trypomastigotes, further transform to epimastigotes; 3g) epimastigotes multiply in salivary gland, transform to metacyclic trypomastigotes.

Fig. 5.1 Life cycle of protozoan parasite. (**1**) Life cycle of **Leishmaniasis** parasite: (**1a**) Sandfly takes a blood meal ingesting macrophages infected with amastigotes; (**1b**) Release of amastigotes; (**1c**) Amastigotes transform into metacyclic promastigotes in midgut; (**1d**) Sandfly infect the host by infecting metacyclic promastigotes when taking a blood meal; (**1e**) Promastigotes are phagocytized by macrophages; (**1f**) Promastigotes transform to amastigotes in macrophages; (**1g**) Amastigotes multiply in macrophages of various tissues. (**2**) Life cycle of **Malaria** parasite [**2a**−**2f**]: (**2a**) Transmission to sporozoites human; (**2b**) Sporozoites enter liver and infect hepatocytes; (**2c**) Liver cell rupture and merozoites released; (**2d**) Intraerythrocytes cycles; (**2e**) sexual cycle; (**2f**) transmission to mosquito. (**3**) Life cycle of **Trypanosomiasis** parasite [**3a**–**3g**]: (**3a**) Tsetse fly takes the blood meal (inject metacyclic trypomastigotes); (**3b**) infect metacyclic trypomastigotes transform into bloodstream trypomastigotes which are carried to other sites; (**3c**) Trypomastigotes multiply by binary fission in various body fluid; (**3d**) Circulating trypomastigotes in blood during acute phase usually undetectable in latent phase; (**3e**) Tsetse fly takes the blood meal (bloodstream trypomastigotes are ingested); (**3f**) Bloodstream trypomastigotes transform to procyclic trypomastigotes, further transform to epimastigotes; (**3g**) epimastigotes multiply in the salivary gland, transform to the metacyclic trypomastigote

infect other mononuclear phagocytic cells. The resulted symptomatic conditions and type of leishmaniasis is depended on Parasite, host, and other factors. Sand flies again get infection from receiving blood meals from infected individuals. In this stage, the amastigotes develop into promastigotes and grow in the gut, and also species affect the region at which part of gut is to be developed as hindgut for leishmanial organisms in the *Viannia* subgenus; in the midgut for organisms in the *Leishmania* subgenus, and finally the promastigotes drift to the proboscis from gut (Teixeira et al. 2013; Machado de Assis et al. 2012). Therefore, the parasite switch between two life stages, promastigote which develops in the alimentary gut of the vector and differentiates into amastigote (an infective form) which represents the clinically applicable parasite stage which develops in the mammalian host. These promastigotes are destroyed by neutrophils, dendritic cells and macrophages (Machado de Assis et al. 2012).

5.2.3 Treatment and Control of Leishmaniasis

The management and control of disease are centre at the periphery of chemotherapy, and the treatment depends on economic factors because infected persons mostly belong to lower economic categories so, we need to explore cheap elements (antimony) used in medications where vaccination is very poor or not done at all. The five valance elements such as meglumine antimonite and sodium stibogluconate possess cardiotoxic adverse effects at the prescribed doses and therefore, describes the quick requirement for alternative treatment. The treatment methods we used till date cause serious issues, involving safety concerns due to harmful toxic effects, high drug cost even for the developing realm and the long-term administration of pentavalent drugs induces resistance against the disease (Davies et al. 2003).

The commonly used antimonial also have side effects like arthralgia, myalgia, hepatotoxicity, pancreatitis and nephrotoxicity etc. apart from developing resistance. This has developed the concept of alternative therapy utilizing the mode of oral administration (Ponte-Sucre et al. 2017). Paromomycin is an aminoglycoside antibiotic produced by *streptomycetes* when used in combination with miltefosine and is effective against visceral leishmaniasis. The hurting intramuscular injections, ototoxicity and nephrotoxicity are one of the usual side effects reported for this drug as well as the teratogenic effects of miltefosine make it unsuitable during pregnancy. A highly effective drug, Amphotericin B (AmB) has also similar adverse effects. Common adverse effects are intestinal cramps, vomiting, diarrhea and anorexia (Laniado-Laborín and Cabrales-Vargas 2009). The problems remain the same of developing resistance and the frequency of treatment failure is a major concern for these drugs too. As every drug has its own side effects, there is a need to search for alternative treatment methods (Ponte-Sucre et al. 2017). Above the development of resistance, protozoans have also advanced their adaptive metabolic enzymes, optimal targets of specific drug design.

Natural resources have been a solution for many diseases since ancient times, now the exploration of marine derivatives is another big scope as marine possesses

the highest diversity of life. This is a reason for concern as its urgent to discover natural sources relevant to anti-Leishmanial chemotherapeutics posing lower cytotoxicity and reduced or no side effects (Sundar and Singh 2018).

5.2.4 Marine Metabolites for Leishmaniasis Treatment

Marine environment provided us with a variety of natural products which possess anti-leishmanial activity. The organisms that lived in marine habitats such as cyanobacteria, sponges, fungi, bacteria and seaweeds, are gifted with the mechanism for a variety of compounds having specific chemical structures showing significant anti-leishmanial activity (Sundar and Singh 2018).

5.2.4.1 Microbial Derivatives

The presence of a variety of micro-organisms forms an inexhaustive collection of novel science, providing us with an important invention field for creative biotechnology. A lot of secondary metabolites had been extracted from marine microbes as important antimicrobials.

During past years, around 1980 the microbial space has declined attempts in this field contributing to the evolving awareness that this resource has been briefly studied. Whereas dealing with challenges facing toward human pathogens, scientists again started doing new experiments for novel drugs isolated from marine bacteria as well as fungi which became very important sources for novel antibiotic discovery due to their huge biodiversity and capacity to grow very fast giving high yield using bioreactors (Sundar and Singh 2018).

One of the major groups of microbes is actinomycetes listed for the eventual composition of 45% of total metabolites extracted from bioactive microbial fungal population. Microbes contributed greater than 23,000 secondary metabolites having bioactive components showing anti-leishmanial activity (Valli et al. 2012; Selim et al. 2021). Staurosporine, a compound obtained from 11 (GU214750) strain from *Streptomyces* sp. extracted from an unidentified Mediterranean sponge had potent activity of low EC50 as 5.30 μM for promastigotes of *L. major*. Several other biosynthetic compounds with potent bioactivities against bacteria isolated from *Streptomycetes* are the main source for primary antibiotic production possessing highly valuable in the pharmaceutical industry (Davies-Bolorunduro et al. 2021). Around 7600 compounds were obtained from this single species. Secondary metabolites obtained from the species categorized into various groups on the basis of their functional groups such as peptides, terpenoids, terpenes, alkaloids, lactams, trioxacarcins, polycyclic xanthones, macrolides, polyketides and many more but only a few are listed.

The products obtained from *Streptomyces* don't stop by the above metabolites. *Streptomyces* sp. of strain ISID311, which lived in symbiosis with a fungus-growing ant. These compounds are highly active against *Leishmania donovani amstigotes* with IC50 values of 2.32, 0.091 and 0.73 μM respectively and possesses high selectivity indexes (Ortega et al. 2019).

5.2.4.2 Sponges

There are reports for the production of substances like cyclic peroxides from *Plakortis* genus of sponges. *Plakortis* aff *angulospiculatus* Palauan sponge produces two peroxides and shows anti-*Leishmania* activity for *L. mexicana* (Donia and Hamann 2003). The cyclic peroxide is a very active compound at 1.0 g/mL concentration possess 0.29 g/mL LD50 value causing cell membrane lysis after 24 h and a gradual reduce in motility after 30 min second product Ketoconazole is much more potent than the initial compound showing LD50 0.06 g/mL and is also known for the same production of cyclic peroxides which is active against *Leishmania mexicana*. *Pandoras acanthifolium, Agelas mauritiana, Agelas conifera Spongia* sp., and *Ircinia* sp. *Dysidea avara* are those sponges other than *Plakortis*, which also produce different classes of bioactive compounds like steroid, alkaloid, quinone etc. (Donia and Hamann 2003).

5.3 Malaria

Malaria is another neglected protozoan parasitic disease caused by *Plasmodium* species (*P. vivax, P. ovale, P. falciparum,* and *P. malariae*), with most severe infection and most reported mortality reported under *P. falciparum* (Pf) species (Fattorusso and Taglialatela-Scafati 2009). According to the estimated cases of the year 2019, the WHO world malaria report has an approximation of 228 million people suffering from malaria and 405,000 deaths reported in the whole world in 2018 (Monroe et al. 2022). Another serious problem with this disease is that the victim of the disease in children below 5 years, reporting mortality for every 30 s.

There are certain drugs which have been used to treat the disease, but the troublesome increase in the number of fatalities in recent years may be due to the spread of multi-drug resistant strains of *Plasmodium.* Because of the cosmopolitan nature of malarial infection and around 50% of the world's population is prone to get malaria infection, eradication of malaria is one of the major concerns for WHO for whom various programmes are organized each year. After so many years invested towards malarial infection and awareness programmes, still there is a such specific vaccine for *Plasmodium* species and also various mutative strains originating with time cause difficulties in treatment strategies (Tougan et al. 2020).

5.3.1 Geographical Distribution of Malaria

It often causes serious problems, particularly in the region of sub-Saharan Africa, and also a major concern for public-health issues in a few areas of South America and South-East Asia. Unfortunately, this disease is still a common cause of death in the above regions, the tropical countries of America, Asia and Africa, affecting 300–500 million people every year with a mortality of 1–3 million per year (Snow et al. 2005).

5.3.2 Life Cycle of *Plasmodium* sp.

The parasite is transmitted via vector to humans, the female *Anopheles* mosquitoes feed on blood meals then it circulates in erythrocytes and punctures them causing anaemia; the lysis of erythrocytes release them into the circulatory stream causing a spike in fever patterns. In severe cases, protozoans produce a specific protein which integrated into the infected erythrocyte cell membrane due to which erythrocyte sticks with the vessels leading to vessels obstruction (Fig. 5.1). When this condition is generated into the central nervous system causes unconsciousness as the first appearing symptom but if treatment of brain affecting malaria has not been given at times, then it might turn into mortality (Venugopal et al. 2020).

Since complete elimination of the vector *Anopheles* mosquito is not possible, there is an urgent need for new antimalarial drugs with novel mechanisms of action to reduce resistance to currently using drugs such as sulfadoxine–pyrimethamine, quinine, chloroquine, and mefloquine (Belete 2020).

5.3.3 Treatment and Control of Malaria

Malaria treatment is basically carried out by natural sources, in 1820, quinine was an anti-malarial agent extracted from the bark of *Cinchona* tree, and till the twentieth century, various novel drugs were developed via quinine structural components. Quinine and its analogues like, chloroquine have been the widely used drug for malaria treatment, whereas WHO has suggested these drugs use as first-line treatment as malarial drugs and various monotherapies have reduced because of resistance development from the 1980s onwards. Artemisinin, another novel drug for malarial treatment, has no such major adverse effects but might be causing reproductive and neurological toxic effects at suggested non-clinical doses (Belete 2020).

As marine invertebrates harbour a list of microbes including fungi, cyanobacteria and bacteria and sometimes, related microbes in their tissues become 40% of biomass (Hentschel 2002). The bacteria constitute more than two or three times the environmental residing seawater composition. The higher potential of every single marine organism (including soft corals, tunicates, sponges and most invertebrates) to secrete a variety of secondary metabolites can be shown by targeting secondary metabolites usual features present in nearly every living organism also including important features of the marine environment (Sehnal et al. 2021). So, various antimalarial novel products have been obtained from marine environment microbes including manzamine A, 10,11-lepadins D-F,12 6-bromoaplysinopsin,13 and venturamides A and B.14. (García and Monzote 2014). These products are largely separated into some classes as listed below: Isonitrile-containing derivatives; Alkaloids; Endoperoxides and some miscellaneous groups.

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5.3.3.1 Isonitrile-Containing Derivatives and Their Analogues

Secondary metabolite nature consisting Isonitrile compounds obtained from various marine sources. The invertebrate of sponges group including Halicondridae and Axinellidae constitute major secondary metabolite.

Axisonitrile-1 In 1973, *Axinella cannabina* sponge produces which shows a resemblance with axisothiocyanate-1. These also relate with other compounds obtained from the same source including isothiocyanate-, isonitrile- and formamide-containing sesquiterpenoids products like axamide-3, axisonitrile-3, axisothiocyanate-3, axisonitrile-2, axamide-1, axamide-2 and axisothiocyanate-2- (Fattorusso and Taglialatela-Scafati 2009). From the chemical constituent of *Cymbastela hooperi* (Axinellidae) sponge, a variety of diterepenrs were obtained on the concept of neoamphilectane, isocycloamphilectane and amphilectane skeletons having isothiocyanate, isonitrile and sometimes isocyanate functionalities. These compounds possessed selective and significant in vivo activity against malaria and their various co-occurred analogues suggested few relations with structure-activity. *Cymbastela hooperi* (axinellidae, halichondrida) also produces Di-isocyanoadociane and showed anti-malarial capacity showing IC50 at 0.005 g/mL, and selectivity that proved the in vitro data get from chloroquine and artemisinin, antimalarial drug uses in clinical trials (König et al. 1996).

Kalihinane Diterpenoids Many isonitrile-analogues showing antimalarial activity isolated from the Japanese sponge *Acanthella* sp. shows Kalihinane diterpenoids class showing antifouling, antifungal and anthelmintic compounds (Malve 2016). These compounds are in the category of marine secondary metabolite possessing isonitrile group having isocycloamphilectane skeleton of diterpenes (e.g. kalihinol A) shows significant anti-plasmodial activity at a very low dose of IC50 = 0.4 ng/mL (Kapoor and Ghorai 2021).

5.3.3.2 Alkaloids

The second category of drugs is alkaloid base compounds. The commonly used derivatives are mostly sponges and a few of them are also derived from other microbes.

Manzamines The most common alkaloids used in malaria treatment after isonitriles. These are unexceptionally the most potent and useful alkaloids extracted from marine sources, showing antimalarial activity (Fattorusso and Taglialatela-scafati 2012). In 1986, Higa and coworkers isolated multifaceted polycyclic (seven to eight rings or more) alkaloid from *Haliclona* genus (Okinawan sponge) showing the characteristic of an internal five-cyclic heterocyclic system which connects using β-carboline moiety. Various different manzamine derivatives are obtained from different genera of sponges fitted with different taxonomically unrelated sponges including *Amphimedon, Xestospongia* and *Ircinia* and various other order belonging organisms. One of the bacterial species producing this compound is *Micronosphora* sp. which provides evidence for manzamines are posing symbiotic origin instead of a

true sponge origination scale (Alamgir 2018). The manzamine A and its related analogues shows significant anti-malarial activity which provides a promising compound against infections leads extracted from the marines and understanding its component structures provide a lead towards designing of novel safer manzamine-related anti-malarial drug. In vitro activity of Manzamine A towards D6 clone of *Plasmodium falciparum* shows 0.0045/mL of MIC (minimum inhibition concentrations) when compared using artemisinin and chloroquine MICs values of 0.010 and 0.0155 g/mL respectively. This compound also works against *Plasmodium berghei*, a rodent malaria parasite by reducing the growth when worked in in vivo models. Manzamine A oil suspension when given via oral administration, also inhibits parasitaemia. Similarly, hydroxyl derivatives of manzamine A and dimer neo-kauluamine, enhance the life span of mice showing infection with *P. berghei* when compared with two crucial human antimalarial drugs as 100 mol/kg of one IP (intraperitoneal) dose (Donia and Hamann 2003).

Chloroquine and Manzamine A show a similar therapeutic index where 50 mol/kg dose becomes toxic as compared to 500 mol/kg dose required to diminish the parasite concentration when given with 2 days intervals of three times administration. Manzamine A and its derivatives are much more potent due to their remarkable bioavailability and continued antiparasitic activity involving less or negligible toxic effects (Donia and Hamann 2003; Ang et al. 2000). Manzamines pharmaceutical properties not only limited for antimalarial but also as effective as antituberculosis, antibacterial, antifungal, and anti-inflammatory agents as well as also active against *Toxoplasma gondii* (AIDS opportunistic pathogens) (Donia and Hamann 2003). Current research provided evidences for inhibitory activity against GSK-3 (glycogen synthase kinase-3) where the enzyme is included in pathological hyperphosphorylation of the protein tau, which provides a new pathway for designing novel drugs related to Alzheimer's disease too (Donia and Hamann 2003).

Lepadins These compounds derived from a new marine tunicate species, reported to have some antimalarial property and these are linear eight-carbon chain decahydroquinoline derivatives extracted from *Didemnum* sp. and *Clavelina lepadiformis,* Australian originated invertebrate species with antimalarial activity having 400 ng/mL of its IC50 value (Fattorusso and Taglialatela-Scafati 2009). Whereas, its analogues lepadin D and B are nearly inactive compounds. The activity of the first compounds comes due to the presence of 2*E*-octenoic acid ester at the position of secondary alcohol. The Lepadin B causes blocking of neuronal nicotinic acetylcholine receptors (Fattorusso and Taglialatela-Scafati 2009).

Salinosporamide A One of the marine actinomycete *Salinispora tropica* derivative, which produces this compound. Salinosporamide also called as Marizomib which inhibited the *Plasmodium falciparum* human malrail erythrocytic phase via interaction with 20S proteasome. Another discovery involves two and four skeletons containing alkaloids with indolactam and carbolines properties showing potent antimalarial activity, which was obtained from SCSIO 00652 strain of

Marinactinospora thermotolerans found on remote ocean South China Sea soil sediment (Prudhomme et al. 2008).

Bromo Tyrosine Derivatives Sponges are also responsible for compounds like bromotyrosine derivatives from *Hyattella* sp. by mass-directed fractionation on the large-scale. This resulted into the production of Psammaplysin, a derived bromotyrosine product with past time extracted product psammaplysin F possesses potent antimalarial activity when tested using Dd2 strain of *Plasmodium falciparum,* a chloroquine-resistant type (Carroll et al. 2022). Previous studies were conducted using HEK293 cell line for human cell analysis showed that at 40 μM there was inhibition up to 98% as well as no cytotoxicity was reported. The same experiment was repeated with 3D7 strain of *P. falciparum* which is a chloroquine-sensitive strain (Xu et al. 2011). Another product obtained in 1985 is a simple indole derivate, 6-Bromoaplysinopsin, a sponge-derived compound and in later days obtained from *Smenospongia aurea* sponge shows antimalarial property against *P. falciparum* D6 clone strain at 340 ng/mL of IC50 (Rahman 2002).

Phloeodictynes Another compound extracted from *Oceanapia* sponge genre possesses branching of 1,2,3,4-tetrahydropyrrolo-[1,2-*a*]-pyrimidinium at C6 carbon with alcohol function group and CH_3 chain of variable number as well as $N-1$ having guanindine group with four/five methylene chains. This compound had potent activity (IC50 = 300 ng/mL) against FGB1 chloroquine-resistant strain of *Plasmodium falciparum*. These compounds are an alkaloid belonging to marine microbes which shows chemical resemblance with lepadins with both possessing skeleton of nitrogen compounds with a long alkyl chain in common (Mancini et al. 2004).

Halichondramide The next alkaloid drug is antifungal-based macrolide halichondramide, a type of sponge derivative that shows significant novel activity against D6 clone of *P. falciparum* possessed 0.002 g/mL of IC50 value which is approximately similar to mefloquine, a clinically approved drug (IC50 0.0003 g/mL) (Donia and Hamann 2003). But, the compound has a very less selectivity index in comparison with current drugs in this field still, shows a new window for designing of novel compounds in the field of alternate chemotherapy for malaria treatment which required futuristic work in this field (Donia and Hamann 2003).

Heptyl prodigiosin A type of pigment isolated from α-proteobacteria culture which was again isolated from marine tunicates showed significant potential activity towards 3D7 chloroquine-sensitive strain of *P. falciparum* malaria (Fattorusso and Taglialatela-Scafati 2009). In vitro experiment results were 20 times more significant as compared to in vitro cytotoxic activity of mouse lymphocytes and also shows similarity with quinine (Fattorusso and Taglialatela-Scafati 2009).

5.3.3.3 Endoperoxides

The considerable development in malarial chemotherapy is the invention of artemisinin obtained from leaves of *Artemisia annua* (Compositae) which is a type of endoperoxide cadinane sesquiterpene lactone having 1,2,4-trioxane domain, possess antimalarial activity at nanomolar scale against chloroquine-resistant strains of *Plasmodium* sp. The compound has a specific juxtaposition of peracetal and acetal known as sweet wormwood, sweet annie, sweet sagewort, annual mugwort or annual wormwood, is a common type of wormwood native to temperate Asia and lactone functional groups which fascinate organic chemists for their novel drug designing (Rudrapal and Chetia 2016). Because of complete synthetic pathway for artemisinin synthesis development, still their complex nature suggested that its not a clever option to replace natural drug resources with the synthetic ones. Another research for natural resources of drug development is endoperoxide derivatives consisting of an important other resource to artemisinin is on the way to develop at the periphery of the world (Rudrapal and Chetia 2016). On the basis of origination, this compound structural formula of endoperoxide categories into two polyketides and terpenoids.

Polyketides Plakinidae family marine sponges consist of a list of simple endoperoxide derivatives which have been marked as polyketide metabolites having five or six-membered 1,2-dioxygenated rings (1,2-dioxolane or 1,2-dioxane, respectively). Plakortin extracted from *Plakortis halichondroides* shows potent antimalarial properties and is currently again isolated in huge amounts from *Plakortis simplex,* a Caribbean sponge (Aguiar et al. 2021). Various other similar components such as plakortide Q, 3-epiplakortin and dihydroplakortin extracted from the same sponge and possess significant anti-plasmodial activity for a chloroquine-sensitive strain (D10) and a chloroquine-resistant strain (W2) with high potent activity for second W2 strain of IC50 ~180 ng/mL as well as zero or no toxicity (Aguiar et al. 2021).

Terpenoids Sigmosceptrellin A functionally a form of norsesterterpene shows potency towards *P. falciparum* at IC50 value of 450 ng/mL. Sigmosceptrellin B, an epimer, has been experimentally approved to possess very low capacity comparing to the previous one (Fattorusso and Taglialatela-Scafati 2009). The stereochemistry of the compound also helps in the determination of the antimalarial potential in 1,2-dioxane derivatives series. Another sponge derivative methyl-3 epinuapapuanoate, extracted from the New Caledonian sponge *Diacarnus levii* is a norditerpene showed an average in vitro activity against chloroquine resistant strains of *Plasmodium falciparum* (IC50 = 1.2 μg/mL) (Fattorusso and Taglialatela-Scafati 2009).

Unluckily, only few terpenoids having endoperoxide structure had been extracted from ocean sources and only few of them tested for their antimalarial activity.

5.3.3.4 Other Miscellaneous Compounds

These are marine secondary metabolites without an isonitrile or endoperoxide group or an alkaloid structure that have antimalarial action. These compounds can be

further grouped into two functional categories, such as peptides-quinones and phenols (Fattorusso and Taglialatela-Scafati 2009).

Quinones and Phenols The marine sponge *Dactylospongia elegans* from Australia contains a marine quinone called ilimaquinone. Since then, many quinone derivatives known as alisiaquinones—which share structural similarities with xestoquinone—have been discovered from an uncategorized New Caledonian sponge (Chen et al. 2022). These substances displayed micromolar antiplasmodial activity in addition to effectiveness against Pfnek-1. Next compound, (*S*)-Curcuphenol, is a sesquiterpene phenol that was extracted from various sea sponges of the genus *Didiscu*s. This compound showed significant in vitro antimalarial property with MIC of 1.8 μg/mL towards the W2 clone and 3.6 μg/mL against the D6 clone of *P. falciparum*.

15-Oxopuuphenol is a phenol-containing antimalarial marine metabolite derived from genus Hyritos sponges, representative of a sponge metabolite distinct family that comprises quinol-quinone pair of avarol and avarone. This compound exhibited in vitro MIC activity of 1.3 μg/mL against the W2 clone and MIC activity of 2.0 μg/mL against the D6 clone (Kapoor and Ghorai 2021).

Peptides Two modified cyclic hexapeptides are the metabolites that have been isolated from the marine cyanobacterium and are reported to possess antimalarial properties, one is isolated from the marine cyanobacterium *Oscillatoria* sp. and *the* other is dragomabin a linear alkynoic lipopeptide isolated from a Panamanian strain of the marine cyanobacterium *Lyngbya majuscule* (Kang et al. 2015). Characterization of the above compound showed the presence of unusual 4-(*S*)-amino-2-(*E*)-pentenoic acid subunit and an *N,N*-dimethyl isoleucine terminus, which demonstrated a moderate in vitro antimalarial activity (IC50 = 8.4 μM) (Kang et al. 2015).

Along with the sponges, a bacterial secondary metabolite called a polyether that was isolated from the marine strain H668 of *Streptomyces* sp. showed considerable in vitro antimalarial activity (IC50 150 ng/mL) and high selectivity (Kang et al. 2015).

5.4 Trypanosomiasis

The flagellated protozoa *Trypanosoma brucei* and *Trypanosoma cruzi*, which cause human South American trypanosomiasis (Chagas disease) and African trypanosomiasis (sleeping sickness) respectively, are the causes of trypanosomiasis. Major health and economic issues are being brought on by African trypanosomiasis in rural Sub-Saharan Africa. *Trypanosoma brucei* has subspecies called *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense,* both causes human sleeping sickness. The tsetse fly spreads the parasites across vertebrate hosts (*Glossina* sp.) (Büscher et al. 2017).

5.4.1 Geographical Distribution of Trypanosomiasis

In the 1960s, a combination of strategies involving patient treatment, active case discovery, and vector control helped to control human African trypanosomiasis (HAT). However, the disease has dramatically re-emerged since the 1970s (Franco et al. 2014). It became endemic in over 30 African nations, posing a threat of around 60 million people, and reached epidemic ration in a few states including southern Sudan, Uganda, Angola, and the Democratic Republic of the Congo. Nearly 45,000 reports of HAT were documented in 1999, but the WHO believes there were actually between 300,000 and 500,000 cases, as only 3–4 million at-risk individuals are regularly examined or have access to health facilities (Franco et al. 2014). Although originally it was reported to be endemic in Latin America, *Trypanosoma cruzi* has spread around the globe as a result of population movements, creating an urgent global public health concern. According to the World Health Organization, *Trypanosoma cruzi* infects 8 million individuals and causes more than 7000 fatalities per year (Rassi and de Rezende 2012).

5.4.2 Life Cycle of *Trypanosoma*

HAT is brought on by infection with *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense,* whereas Chagas disease is brought on *by Trypanosoma cruzi*. Which required *Glossina* sp. (Tsetse flies) as the vectors for HAT, while certain bug species of *Triatoma* genre transmit *T. cruzi.* African trypanosomes go through life cycles that alternate between the tsetse fly and a vertebrate host body fluids including blood and other fluids of gut and salivary glands also (Fig. 5.1). To live in varied conditions involving the mitochondrial system and the surface membrane, the parasite goes through distinct metabolic and morphological modifications (Tyler and Engman 2001).

The vector ingests bloodstream trypomastigotes while feeding on the blood of an infected mammal. In a matter of 3–4 weeks, the parasites replicate in the fly and go through a number of growth phases. In the fly's midgut, the parasites transform into procyclic trypomastigotes, multiply by binary fission, leave the midgut, and transform into epimastigotes. The epimastigotes reach the fly's salivary glands and continue multiplication by binary fission. When the fly feeding on blood bites another mammal, it transmits metacyclic trypanosomes into the skin where they develop into bloodstream trypomastigotes and then spread through the lymphatic system into the circulation. The parasites spread in waves and constantly alter their antigenic coat of varied surface glycoproteins to evade the host's immune system (Tyler and Engman 2001). One to three weeks after inoculation, the disease's first haemolymphatic stage is marked by vague symptoms including erratic fever bursts, headaches, joint discomfort, and itching that are frequently mistaken for the flu or malaria. Trypanosomes enter the central nervous system by breaching the blood–brain barrier after many days or even sometimes required months, which depends on the variety of the parasite involved. This causes severe meningo-encephalitis that in

due cross progresses to encephalopathy. Headaches, neurological symptoms, personality and behaviour changes, poor coordination, variation in sleep patterns (which give the condition its name), and body wasting are common symptoms of this later stage of HAT. If untreated, these conditions can progress to a final sleepy state and ultimately succumbed. Thus, early diagnosis and therapy are crucial for improved control of the disease's second stage. Rarely, *Trypanosoma brucei gambiense* may be acquired congenitally if the mother is infected during pregnancy (Tyler and Engman 2001).

Contrary to HAT, acute Chagas disease frequently has no symptoms, making it difficult to identify. One-third of those who are infected continue to get the severe type of the illness, which can go without being symptomatic for 10–30 years. That severe condition may lead to cardio-digestive disorders (megaeosphagus, megacolon) or cardiac disorders, or a mix of the two. The mortality caused by Chagas is related to heart disease in endemic areas. The disease's acute phase is marked by peaks of parasites in the bloodstream that cause vague symptoms that are difficult to diagnose. Without a specialized course of treatment, the majority of cases progress to cardiomyopathy, gastrointestinal (megacolon, megaesophagus), and neurological diseases (Fenn and Matthews 2007).

5.4.3 Treatment and Control of Trypanosomiasis

The medications required to solve these conditions, such as suramin and pentamidine, need to be administered by the parenteral route and are only effective against the early haemolymphatic stage of the illness. Melarsoprol, an arsenical medication for treating later conditions of CNS infections, is available but still, induces reactive encephalopathy which is challenging to utilize. New drugs with different structures and mechanisms of action from those currently in use are required because the heterocyclic drug benznidazole, used for the treatment of initial stages of Chagas disease, is ineffective against the chronic situation of the disease and is badly tolerated (Legros et al. 2002).

The problem in the treatment of *Trypanosoma* is the limitation of current chemotherapeutic options and toxic side effects. There are only four approved drugs for the disease, suramin, pentamidine, melarsoprol and eflornithine, among which three were created more than half a century ago. The effectiveness of medications and duration of treatment also dependent on the duration of the infection, the longer the *Trypanosoma cruzi* infection persists, the less effective are the two available medications, nifurtimox and benznidazole (BZN). These medications have extensive dosing regimens that call for bi- or tri-daily treatment for 60–90 days. Patients often reported experiencing hepatic intolerance, nausea, seizures, vomiting, and skin disease symptoms as a result of the medication's side effects (Calvet et al. 2020).

The *Trypanosoma cruzi* strain affects the response of the treatment. Drugs used in treating animal illnesses like homidium, isometamidium, and diminazene aceturate have varying sensitivities for distinct developmental stages. The only medications that can be utilized in second-treatment stage are melarsoprol and eflornithine, with

eflornithine being only fruitful in *Trypanosoma brucei gambiense* infection. With the exception of eflornithine, which blocks the polyamine production pathway, the mechanisms of action of these medications are still poorly known. Reactive encephalopathy is caused by the arsenic-base medication melarsoprol, which is used to treat late-stage central nervous system infections. One of the main drawbacks of these medications, aside from their potentially fatal side effects, is their difficulty in administering via long-term injection, which necessitates the use of medical facilities and specialist personnel, both of which are frequently lacking in rural regions. Additionally, there are more and more cases of therapy failures, particularly with melarsoprol. The availability of these medications further hinders treatment because the production unit frequently stops making them. In order to combat sleeping sickness, it is urgently necessary to develop new molecules that are secure, efficient, affordable, and simple to administer, as well as new leads with novel mechanisms of action (Calvet et al. 2020).

5.4.3.1 Natural Marine-Products with Anti-Trypanosoma Activity

As evidenced by the abundance of plants, bacteria, and marine organisms in nature, these organisms have the capacity to contain an infinite number of molecules with a wide range of pharmacological activity. Marine creatures like sponges, colonial tunicates, and brown algae have shown their chemical potential for novel antitrypanosomal compounds, which is one of several study areas that may result in the identification of new substances with anti *T. cruzi* activity. A compound is only classified as a "hit" in drug development screening works for HAT and Chagas disease if its IC50 is less than 10 μM. Antitrypansomal structures are divided into five groups according to their functional groups into five categories: terpenes, quinones, alkaloids, phenolic derivatives, and other metabolites (Calvet et al. 2020).

Alkaloids Many purines and indole-based (bromopyrrole) alkaloids have demonstrated a variety of potency towards *Trypanosoma* activities. A compound indole alkaloid tryptophol, extracted from *Ircinia spinulosa,* Turkish Aegean Sea sponge, shown broad-spectrum inhibitory efficacy for variety of anti-parasitic microorganisms, including *T. b. rhodesiense* with IC50 of approx. 36.6 M and for L6 cells show negligible toxicity (SI >11). Some additional indole alkaloids derived from *Bacillus pumilus*, a marine bacterium and extracted using black coral *Anthiphates* sp., (a Panamanian collection) are 3-formylindole, *N*-acetyl—oxotryptamine and 3-hydroxyacetylindole, showed negligible activity against *T. cruzi* with IC50 values of 26.9, 19.4 and 20.6 μM respectively, still the compounds selectivity was very low (SI <4).

Three alkylguanidine structural analogues—carboline alkaloids, designated opacalines A, B and C, were discovered from *Pseudodistoma opacum*, an ascidia found in New Zealand. Opacaline A and its *N*-hydroxy counterpart, opacaline B, only slightly inhibited *T. b. rhodesiense* with IC50 values of 30 and 27 μM, but possess low selectivity (<5) (Jones et al. 2013). de-Bromo analogue is a synthetic analogue known as Compound 32, of **30** had enhanced *T. b. rhodesiense* activity with an IC_{50} value of 12 μM and a bit increased in SI of 7 versus mammalian L6

cells. Other related substances include bromopyrroles 35–38, which were discovered through research on Turkish sponges from the genera *Agelas* and *Axinella*. These compounds were tested using an assay in which trypomastigotes from the *T. cruzi* parasite were causes killing of infected host cells before the compound was added. When compared to mammalian L6 cells, the oroidin dimer dibromopalauamine from *Axinella verrucosa* showed sub-micromolar selective action against *T. b. rhodesiense* with an IC50 value of 0.8 M and a SI of 10. Other alkaloids having significant anti-trypansomal activity are decahydroquinoline alkaloids lepadins D-F extracted from ascidian *Didemnum* sp. *Lepadins D*33, E 34 and F35 possess an unusual decahydroquinoline skeleton and show significant and selective anti-trypanosomal activity in vitro (Jones et al. 2013). The pentacyclic bis-indole alkaloid fascaplysin, another product of the sponge Hyrtios, was isolated from a Fijian collection and showed a wide range of biological activity, including strong, selective action against *T. b. rhodesiense* with an IC50 value of 0.46 M and SI of 15 vs. L6 cells. *Aspergillus fumigatus*, a marine-derived fungus, was the source of a number of compounds including, fused penta- and hexacyclic, spiro-pentacyclic, dimethyl-thio, and diketopiperazine compounds. These compounds shown variable activity against *T. brucei* with IC50 values of 12.9, 6.4, 5.7, 8.5 and 19.5 M, respectively (Jones et al. 2013).

Peptides Next category is peptides. The marine cyanobacterium *Oscillatoria* sp. from Panama produced two compounds, venturamides A and B are of cyclic hexapeptides nature. These compounds had average cytotoxicity toward mammalian kidney epithelial cells (Vero monkey) and average activity against *T. cruzi*, with IC50 values of 86 and 56 M and 14.6 and 15.8 M, respectively (Gogineni and Hamann 2018). Aerucyclamides B and C, two related cyclic peptides, were similarly found to have anti-trypanosomal action when they were extracted from the *Microrcystis aeruginosa,* a cyanobacterium species with IC50 values for *T. b. rhodesiense* were 15.9 and 9.2 M, respectively (Kang et al. 2015). Two linear peptides, almiramides C and B, were isolated from marine cyanobacterium *Lyngbya majuscule* taken from Panamanian collection possess low micromolar inhibition (IC50 = 6 and 3 M) against *T. b. brucei.* In comparison to Vero cells, Almiramide C showed a SI of 11, while Almiramide B's SI was a little lower at 9. With the development of molecular tools, it was demonstrated that the drugs disrupt the parasite's glycosome activity by a number of fluorescence site localization imaging and target-based affinity probes experiments. In trypanosomatids, glycolysis is a crucial metabolic pathway, and glycosomal enzymes have been recognized as a significant novel drug for trypanosomes (Kang et al. 2015).

5.4.3.2 Other Metabolites

As a result of bio-guided fractionation, klaivanolide (5-acetoxy-7-benzoyloxymethyl-7*H*-oxepin-2-one), a seven-membered lactone with moderate activity on *T. b. brucei* bloodstream forms (IC100 = 33.2 M), was isolated from a methylene chloride extract of the stems of *Uvaria klaineana* (Annonaceae). As a Ras farnesyltransferase inhibitor, the antibiotic manumycin A, which is made by

Streptomyces bacteria, was strongly active in vitro against the growth of both the bloodstream and procyclic forms of *T. b. brucei* (IC50 = 1.5 and 0.4 M, respectively) (Kang et al. 2015). A chemically understudied marine cnidarian called *Macrorhynchia philippina* has shown in vitro activity against trypomastigote and intracellular amastigotes of *T. cruzi* with IC50 values of 32 and 40 M, respectively. There has been no human cytotoxicity reported so far (>200 M). The active ingredient, Bisaprasin, is a biphenylic dimer of psammaplin A that was isolated and characterized from the marine sponge *Aplysinella rhax* (de Laubenfels 1954) that was procured from the Fiji Islands. It had a modest activity with an IC50 against *T. cruzi* (Lima et al. 2019).

Thirteen terpenoids obtained from sponges, including the furospinulosin-1, furospinulosin-2, furospongin-1, furospongin-4, and demethylfurospongin-4 linear furanoterpenes; 4-hydroxy-3-octaprenylbenzoic acid, 4-hydroxy-3-tetraprenylphenylacetic acid, and 2-(hexaprenylmethyl)-2-methylchromenol are four linear meroterpenes,a linear triterpene, squalene; two spongian-type diterpenes dorisenone D and 11β-acetoxyspongi-12-en-16-one ; a scalarane-type sesterterpene; 12-*epi*-deoxoscalarin, as well as an indole alkaloid, tryptophol were screened for their in vitro activity against four parasitic protozoa. All compounds were active against *T. brucei rhodesiense*, with compound (Orhan et al. 2010).

Ascosalipyrrolidinone A is a structurally unique tetramic acid metabolite, produced by a endophytic and obligate marine fungus *Ascochyta salicorniae*, isolated from the green alga *Ulva* sp., have a Minimum Inhibitory Concentration (MIC) of 1.1 g/mL, whereas benznidazole (control) has a Minimum Inhibitory Concentration (MIC) of 30.0 g/mL. The reported cytotoxic properties of 3–7 g/mL of this compound against the rat skeletal myoblast cells serves as the principal obstacle to future development. Sigmosceptrellin-B, isolated from the Red Sea sponge *Diacarnus erythraeanus*, exhibited strongly in vitro activity against *T. gondii* at a concentration of 0039 g/mL, without causing serious adverse effects (Osterhage et al. 2000). Sigmosceptrellin-B exhibited 84–99% inhibition against the parasite in human diploid fibroblast cells. A cyclic peroxylactone, Plakortide derived from *Plakinastrella onkodes*, a Jamaican sponge, exhibited a significant in vitro activity with IC50 value of 0.023 g/mL against *T gondii.* Up to a concentration of 0.12 g/mL, host cells are not adversely affected. The marine bacteria *Bacillus pumilus*, which was isolated from the black coral Antipathes sp., was fractionated, and the result was the discovery of chemicals that can suppress the growth of *T. cruzi* (Martínez-Luis et al. 2012). Aytarabine (Ara-C) and vidarabine (Ara-A) are the first FDA-approved marine-derived drugs, that are synthetic pyrimidine and purine nucleosides, respectively, but have been developed from the naturally occurring nucleosides initially isolated from the Caribbean sponge *Tethya crypta*. Although many extracts have shown anti-trypansomic activity, very few have been given FDA approval, so it is vitally important to discover new molecules that are safe, effective, cheap and easy-to-administer against sleeping sickness. The marine environment is a reservoir of enormous biodiversity have the potential of harbouring innumerable molecules of importance with a wide range of structural and pharmacological properties, it provides us with many animals that may be sources of new molecules that may

have unique modes of activity. Around two-thirds of the world's population still relies on plants based traditional medicinal therapies, as pharmaceutical medications are not always accessible and affordable. Manzamines, salinosporamide, some polyketide endoperoxides, and several isonitrile containing diterpenes could be suggested as viable options for future innovations. The possibility of manufacturing these compounds by bacterial fermentation and genetic engineering is crucial in this regard to raise the likelihood of their thorough pharmacological assessment and potential application in therapy (Fattorusso and Taglialatela-Scafati 2009). High morbidity and mortality rates are caused by diseases brought on by protozoan parasites around the world. Marine-derived natural products show promising efficacy against the protozoal infections caused by *Trypanosoma* sp., *Leishmania* sp. and *Plasmodium* sp. Since leishmaniasis is a disease caused by an obligate intracellular parasite, it is critically essential to develop novel medications that can successfully eliminate relapses caused by tissue cysts, especially for patients who are intolerant to folate inhibitors (pyrimethamine, trimethoprim), which commonly have unfavourable side effects (Fattorusso and Taglialatela-Scafati 2009).

5.5 Conclusion

Increased global awareness about the need for novel anti-infective medicines and the tremendous diversity of natural marine products will together bring increased efforts to utilize marine products in clinical applications. It has been estimated that presently only 5% or less of the marine bacterial samples observed microscopically maybe cultivated under optimal conditions. Molecular techniques provide encouraging alternatives by using the biosynthetic gene clusters in a suitable vector for large-scale fermentation that may further help to get over the challenges associated with cultivating the symbiotic bacteria. Undoubtedly, oceans present worldwide may have an important role in the near future in controlling worldwide burden of infectious-disease. Even though considerable advancement has been reported in identifying innovative leads in forming drugs from marine resources, prodigious efforts are urgently required to progress to clinical applications.

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Marine Bioprospecting for the Treatment of Human Parasitic Diseases

6

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Abstract

This chapter provides information on sulfated polysaccharides (SPS), which are found in marine hydrobionts including algae and invertebrates that may be used to treat and prevent protozoa and helminthiasis. The pathogenetic targets of the protozoa in the cells of the host and their antiparasitic activity through polysaccharides from different algae from marine ecosystems are included in this chapter. Additionally, a summary of information has been provided regarding the mechanisms of action of these special chemicals in disorders brought on by protozoa. High antiparasitic action, good solubility, and nearly no toxicity are what set SPS apart. Long term, makes it possible to view these substances as desirable and efficient building blocks for pharmaceuticals, biologically active food additives, and functional food items with antiparasitic properties.

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_6

Keywords

Sulfated polysaccharides · Marine hydrobionts · Parasitic disease · Antiparasitic activity

6.1 Introduction

The term "parasite" refers to a broad category of creatures that have evolved different ways to infect hosts and feed off them. They can parasitize a variety of tissues along with organs, including the liver, lungs, intestines, blood, and the central nervous system (Combes 2020). Parasites are unicellular protozoa causing protozoal infection and are known for common human diseases. In addition to domestic and wild animals, protozoa also cause serious and frequently fatal infections in humans (McDougald et al. 2020). There are about 50 types of protozoa that can harm humans. These pathogenic germs can infect people through the gastrointestinal system, arthropod vectors, and sexual contact. Protozoa, helminths, and ectoparasites are the three primary classes of human pathogenic parasites (Sharon and Regev-Rudzki 2021).

The most important protozoal illnesses in terms of medicine and society are intestinal protozoa, Trypanosomiasis, Leishmaniasis, and Malaria. There are many drugs that are used for the treatment of parasitic disease, but due to their toxicity effect and loss of emergence, they are not used very efficiently (Eberle and Voehringer 2016).

Natural substances, synthetic compounds, or already-approved medications with a wide range of uses can all serve as potential sources for novel therapies for the treatment of protozoal diseases. To effectively treat protozoal infections, new drugs are required that have few to no adverse effects, or none at all, and to which protozoa do not quickly acquire resistance. Numerous seaweed extracts have been tested on a variety of human parasites, including protozoa. These extracts exhibit strong antiparasitic activities (Rizwan et al. 2021). Algae extracts can prevent parasites from attaching to target cells or directly poison protozoa. In addition, 44% of the seaweed species under study showed significant or moderate antitrichomonal activity (Torres et al. 2014).

Sulfated homo- and heteropolysaccharides, which act as agonists of innate and adaptive immune cell receptors, are of particular relevance. They stand out for their low toxicity and lack of negative effects. Antiviral, antibacterial, anti-inflammatory, and immunomodulatory activities are only a few of the biological effects of these biopolymers. Sulfated polysaccharides (SPS) are a broad category of anionic polymers found in a variety of marine creatures, such as macroalgae and mammals, but not in terrestrial plants (Besednova et al. 2021).

6.2 The Nature and Scope of Marine Bioprospecting

Exploring biological material in the marine environment in search of genetic and biochemical traits that have commercial value is a field of study and industry that is fast growing. While bioprospecting in the terrestrial environment has a long history, the collection and screening of commercially valuable samples from the marine environment dates only as far back as the 1950s, thanks to the advent of technological innovations like SCUBA diving. Due to its high levels of biodiversity and endemism, which carry the promise of biologically active chemical entities for the production of novel natural goods, the maritime environment offers significant economic potential (Hunt and Vincent 2006). Extremophiles, or marine animals that thrive in harsh conditions including hydrothermal vents, seamounts, cold seeps, and underwater trenches, are of particular interest because they force organisms to adapt to novel biochemical pathways that produce intriguing substances. Many marine creatures manufacture natural products as a chemical deterrent against predators or in reaction to interspecies competition for scarce resources. Marine macroalgae were a primary focus of marine natural product research in the 1970s and 1980s, but microorganisms and marine invertebrates have subsequently been the focus of bioprospecting efforts (Bhatia and Chugh 2015).

The potential of marine microalgae is also receiving more attention, particularly in the food and cosmetics industries. More and more evidence suggests that symbiotic bacteria, which biosynthesize the natural chemicals associated with their hosts (particularly those found in sponges), are the source of these bigger species' secondary metabolites (Paul et al. 2021). As a result, marine bacteria are now the subject of much more investigation. The bulk of microorganisms are still largely unknown, and prokaryotes contribute 50% of the marine genes. As a result, there is significant potential for the creation of valuable goods (Morgado and Vieira 2021).

6.3 Antiparasitic Activity

Antiparasitic medications are used to get rid of parasites from host organisms. Nematode parasitism, which affects the commercial livestock business and significantly worsens human hunger and disease, is a major problem. Nematodes are unsegmented worms that belong to the phylum helminth. A powerful antiparasitic and antifungal agent is Jasplakinolide (Iordache et al. 2015).

Trichostrongylus colubriformis and *Haemonchus contortus* both have fatal doses of 50%. This tetrahydrofuran prevents the growth of eggs for these two species' third, infectious free-living stage. Levamisole and closantel, two commercially available nematocides, both exhibit similar levels of nematocidal activity (Pena-Espinoza et al. 2018).

The sponge *Amphimedon* sp. was obtained in the Great Australian Bight, and the amphilactams were extracted from it. They contain different and unique skeleton of carbon and lactam moiety. The parasitic nematode *H. contortus* exhibits nematocidal activity against the free-living stages. Although it has little to no effect on nematode eggs, this chemical slows the development of larva at the L1 stage. This level of

in vitro action is comparable to that of already available commercial antiparasitics, like levamisole and closantel, and it is believed to warrant in vivo testing (Tian et al. 2008).

6.4 Diseases and Their Treatment

6.4.1 Malaria

The bites of female *Anopheles* mosquitoes carrying the disease, where people are exposed to the potentially fatal parasite disease known as Malaria. Children under the age of 5 are the group most susceptible to this condition. All *Plasmodium* species have a highly plastic genetic makeup that enables parasites to adapt to shifting environmental conditions and develop resistance to antimalarial drugs quickly. Malaria is caused when the Plasmodia of the Malaria at the stage of sporozite enters the bloodstream through the saliva of the insect when the female mosquito bites a person. Before settling in the liver cells, they move freely for 25 min in the bloodstream (Tuteja 2007). Once the infected liver cells have been killed, the tissues that are active divide after 14 days and produce numerous tissue merozoites, which enter the circulation after 4 weeks. The second cycle of erythrocytic schizogony starts when the merozoites enter the erythrocytes (Le Roch et al. 2003).

Several merozoites develop into gametocytes as seen in Fig. 6.1. Gametocytogeny is the name of this process. In the deep arteries of interior organs,

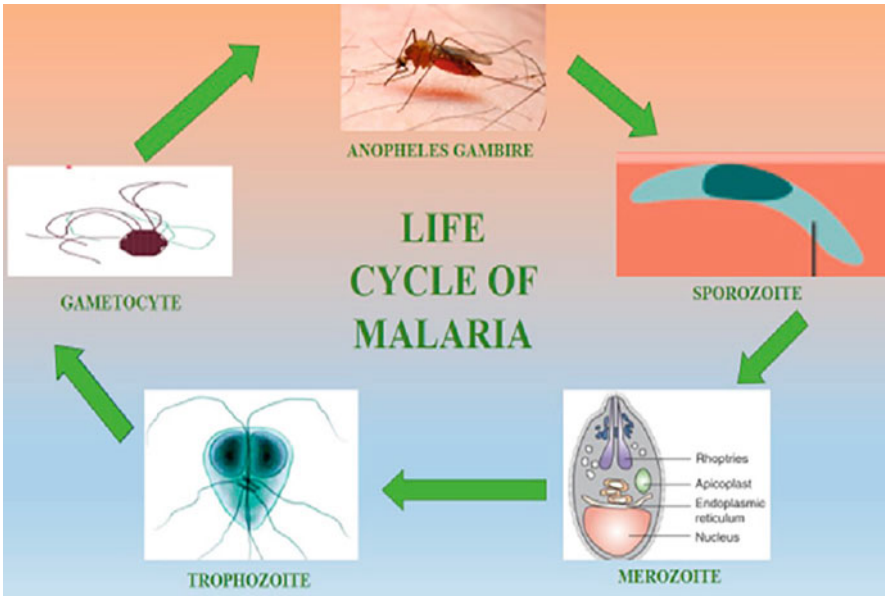

Fig. 6.1 Life cycle of malaria

P. falciparum gametocytes grow. They develop throughout the course of 13 days before emerging in the peripheral blood, where they can survive for a few days up to 6 weeks. The species of *Plasmodium* gametocytes grow in peripheral vessels over the course of 2–3 days before maturing and dying a few hours later (Eckhoff 2011).

When the parasite infects erythrocytes, it binds to endothelial cells in capillaries so that it can multiply and avoid being eliminated in the spleen. Erythrocytes with infections can attach to those without, generating rosettes that can clot by attaching to the other red blood cells through platelets, which can obstruct the circulatory bed. The parasite's capacity to develop resistance to antimalarial medications like chloroquine and sulfadoxine–pyrimethamine is a significant issue. A component of the mission to eradicate Malaria, artemisinin, also experiences resistance. The discovery of new antimalarial drugs with novel mechanisms of action is necessary in light of this worrying situation (Nureye and Assefa 2020).

Several methods can be employed in the development of antimalarial medications, from altering already-existing components to create novel components that work for different targets. The majority of currently available licenced antimalarial medications target the parasite's intra-erythrocytic stage. However, numerous authors' investigations have demonstrated that the invasion of merozoites is an important stage in the development of parasites—has a significant chance of influencing the surface proteins of plasmodia. As a result, it is conceivable to combine medications designed to kill intraerythrocytic parasites with treatments that prevent the invasion of merozoites (Howick et al. 2019).

SPS and heparin-like compounds can prevent the invasion of merozoites, which includes complex ligand–receptor interactions and various parasite invasion routes in host cells. The parasite's surface antigens are crucial to the invasion process of *Plasmodium*. Studies have demonstrated the antimalarial effects of SPS, particularly heparin. Heparin blocks cyto adhesion, which lowers parasitemia. In this instance, the parasite's apical surface is the only factor limiting localization, and the polysaccharide's impact on erythrocytes is minimal. Heparin equally inhibits *P. falciparum* merozoites' invasion of sialic acid-dependent and sialic acid-independent sites. Heparin also prevents the erythrocyte membrane from deforming when it is present. Heparin, however, was stopped because it caused patients to bleed to death (Marealle et al. 2018).

The method by which additional SPS and related compounds exhibit antimalarial effects has been clarified through studies using the heparin model and *P. falciparum* merozoites.

SPS from marine invertebrates and plants, which has heparin-like molecular structures, also demonstrated an anticoagulant action. These substances, which have structures resembling pRBC-binding GAGs, are an interesting and potential replacement for heparin in the treatment of Malaria. It has been demonstrated that fucoidan (a brown alga's SPS) inhibits plasmodia's ability to produce erythrocytes (Gunasekaran et al. 2014).

6.4.2 Leishmaniasis

Leishmaniasis occurs in the intestine of the carrier in both humans and other animals (dogs, rats, and monkeys) in the immobile stage. Leishmaniasis enters the intestines of mosquitoes when they bite diseased mammals, which serve as a reservoir for around 30 species of animals, including humans. In an insect's digestive system, amastigotes are changed into promastigotes, which the female mosquito spits up at the site of the bite on a human body, and 100–100,000 amastigotes penetrate the wound. They grow in this location in the cytoplasm of the reticulo endothelial system cells (Efstathiou and Smirlis 2021). Neutrophils that arise at the entrance gate absorb parasites that do not develop in these cells. When neutrophils undergo apoptosis, macrophages take them in and within 4 days, Leishmaniasis transforms into the amastigote, an internal morphological form devoid of a flagellum. Amastigotes have a 24-h breeding cycle. Due to its resistance to the macrophages' NO and ROS-based microbicidal processes, the parasite replicates and lives in their phagolysosomes. The process starts in the skin at the parasite's place of penetration, where a granuloma forms (leishmanioma) (Motazedian et al. 2006).

There is a limited number of antileishmanial medications now on the market that target different metabolic pathways used by the parasite, and their increasing resistance is worrisome. Miltefosine, pentavalent antimony compounds, amphotericin B, lipid versions of amphotericin B, and azole preparations are the principal medications used to treat Leishmaniasis. Antimony does, however, have a long list of undesirable side effects. These conditions have made it necessary to look for safer ways to treat and prevent Leishmaniasis.

Compounds found in marine macroalgae and crustaceans can inhibit some diseases. There is proof that Leishmania's surface contains heparin-binding proteins (HBP), which may be important to the parasite's life cycle and affect how successfully it binds to the host tissue. In order to separate two heparin-binding proteins that were present on the promastigote surface, fractionated *L. braziliensis* promastigotes (Gharirvand Eskandari et al. 2020). These proteins are necessary for the adherence of these parasites to heparin-coated surfaces or to Lulo cells, an insect cell line that can be used as a model for researching how insects and Leishmania interact. Leishmania's growth was inhibited and the existence of abnormally round parasites occurred at an SPS concentration of 20 g/mL. Leishmania cells were spherical, aggregated, and contained individuals without flagella in cultures with a polysaccharide content of 80 g/mL. It was unable to locate complete protozoa in cultures with an SPS concentration of 160 g/mL; only apoptotic bodies were identified. It is noteworthy that SPS from *S. muticum* and *U. pinnatifida* did not affect *Trypanosoma cruzi* at the same concentrations, indicating their particular effect against Leishmania (Table 6.1) (Davies-Bolorunduro et al. 2021).

Table 6.1 Marine-based antileishmanial medications

Source	Class	Bioactive compound	References
Marine seaweed			
Canistrocarpus cervicornis (Brown seaweed)	Terpene	(4*R*,9*S*,14*S*)-4α-Acetoxy-9β,14α-dihydroxydolast-1(15),7-diene	Camara et al. (2011)
Laurencia dendroidea	Terpene	Obtusol	De Oliveira et al. (2015)
Marine algae			
Dictyota spirali	Terpene	Spiralyde A	Davies-Bolorunduro et al. (2021)
Stypopodium zonale	Terpene	Atomaric acid and its methyl ester derivative	Dorta et al. (2002)
Marine cynobacterial			
Lyngbya majuscule	Peptide	Dragonamide A	Chai et al. (2016)
Oscillatoria sp.	Polyketide	Coibacin A–D	Balunas et al. (2012)
Marine sponges			
Axinella verrucosa	Alkaloid	Bromoaldisin	Scala et al. (2010)
Tedania braziliensis	Alkaloid	Pseudoceratidine	Parra et al. (2018)
Marine bacteria			
Paenibacillus polymyxa	Alkaloid	Paenidigyamycin A	Osei et al. (2018)
Streptomyces sp. EIIB strain	Coumarins	Crude extract	Davies-Bolorunduro et al. (2021)

6.4.3 Trypanosomiasis

The genus Trypanosoma causes a group of flagellate protozoal diseases known as Trypanosomiasis. *Trypanosoma cruzi*, which causes American Trypanosomiasis, is native to the continent of Latin America and is transmitted through the bite of a triatomine bug. However, numerous European nations, including the United States, Canada, and Canada, have reported cases of the disease recently. Approximately, 65 million individuals worldwide are at risk of contracting Trypanosomiasis, and there are now six to seven million cases of the disease. These parasites develop and diversify in the insect's digestive system before migrating to the hindgut (Nussbaum et al. 2010).

SPS derived from the brown algae *Lessonia* spp. was used as the agent for treating the problem. Brown algae also include sulfated polysaccharides that mostly consist of L-fucopyranose residues coupled by 13 and 14 glycosidic linkages, in addition to alginic acid. The sulfated polysaccharide was employed as the raw material for the creation of an anti-trypanosome nanopreparation in an effort to

broaden the scope of practical applications for SPS. The scientists created a mixture comprising fucoidan from the brown algae *Spatoglossum schroederi* and silver nanoparticles to boost SPS activity (de Borba Gurpilhares et al. 2016).

6.4.4 Schistosomiasis

Schistosomes, often known as blood flukes, are the parasitic organisms that cause Schistosomiasis. In 2019, it is estimated that nearly 236.6 million people needs therapy, but only one hundred five million of them are able to receive it. Helminthiasis is a tropical and subtropical disease that mostly affects underdeveloped countries. A dominant CD4+ Th2 immune response is regulated by InterLeukin-4 and InterLeukin-13 which occurs at the same time that leads to the formation of granulomas and fibrosis. M2 phenotype macrophages are a crucial cell group in the development of liver fibrosis. These cells are important for immune response because they contribute to the TH2 response while also suppressing the Th1 response (McCarthy et al. 2004).

Fucoidans are known to have anti-inflammatory properties, reducing LPS-induced aggravation in macrophages and blocking the nuclear factor NF-kB, TLR4 signalling pathway. A commercially available fucoidan extract from the brown alga Sargassum hemiphyllum reduced the levels of cytokines and therefore the severity of lung fibrosis in rats. Fucoidan, a natural anti-inflammatory and hepatoprotective compound derived from the brown alga *F. vesiculosus*, was used in experimental Schistosomiasis (500 mg/kg twice a day for 40 days). Fucoidan was used for treating mice infected with Schistosomes during the infectious phase, and this significantly decrease the size of the liver granulomas and the level of fibrosis. The levels of mRNA and anti-inflammatory cytokines (IL-4 and IL-13), as well as the infiltration of Treg cells into the liver and spleen tissue, all increased (Bai et al. 2020). The number of Treg cells rose in splenocytes activated in vitro by fucoidan, and responsible for the expression of chemokine receptors CCR4 and CXCR5 on Treg cells. In addition to this, macrophages showed a rise in the levels of IL-4 and IL-13 mRNA. As a result, using fucoidan to treat Schistosomiasis lowers degenerative alterations in the liver and delays the progression of the disease caused by *S. japonica*, which could be a new treatment option for people suffering from this helminthiasis in the future (Sullivan et al. 2014).

6.4.5 Cryptosporidiosis

Toxic protozoa condition known as cryptosporidiosis, which affects mammals including humans, is brought on by *Cryptosporidium parvum*. Cryptosporidia have the ability to replicate both inside and outside of cells. Infected food and water are the main sources of cryptosporidiosis transmission. The agent causes acute watery diarrhoea in species, and it is quite dangerous for those who have impaired immune systems. Because it is the most prevalent enteric pathogens in farm

animals and poultry and causes large financial losses. It is a major problem in animal agriculture. Each year, Europe reports 4.7 million cases of sickness, with 500 fatalities on average. Nitazoxanide, which has a low effectiveness, is the only generic medication approved by the Food and Drug Administration for the treatment of immune-compromised individuals with cryptosporidiosis. For both the treatment and prevention of cryptosporidiosis, like with other parasite disorders, effective medications are being sought after. The host animal becomes infected with cryptosporidium when it consumes the pathogen's oocysts, which then release sporozoite as they pass through the gastrointestinal system. Sporozoite bind to the intestinal epithelium, encase the host cellular membranes, and mature into trophozoites (Willis et al. 2013).

Sporozoites, on the other hand, are unable to interact directly with intestinal epithelium. Instead, they have a glycocalyx, a branched carbohydrate filamentous layer. This serves as a barrier of protection and contains a lot of proteoglycan-type transmembrane mucin glycoproteins. A membrane or secreted protein is covalently attached to one or more glycosamine chains in proteoglycans, which are macromolecules found on the cell surface or in the extra-cellular matrix. These structures are used by different pathogens to enter host cells. *C. parvum* may interact with GAG on host cells, some polysaccharides may prevent the parasite from adhering to cells. The most effective treatment in this case was of heparin at a dose of 1 g/mL. Fucoidan, one of the SPS under study, demonstrated a dose-dependent inhibitory action and, at a dosage of 100 g/mL, reduced parasite penetration by around 50%. Additionally, some research results suggested that polysaccharides, specifically those as seen in the fucoidan model, contend with some component(s) on the parasite's surface and are involved in the invasion of HCT-8 cells. Heparan sulphate on the surface of cells has also been shown to support *C. parvum* growth in vitro. The antiparasitic effect of fucoidan was not specifically studied. However, it was demonstrated how SPS interacts with parasites and cells and the need for further study in this area to develop new, effective medications to treat cryptosporidiosis (Hares et al. 2021).

The effects of native and desulphated fucoidans on the adherence of cryptosporidium to the primary culture of the human intestinal mucosa and the infectious process in newborn mice. The sporophylls of *U. pinnatifida* were used to produce native fucoidans. Fucoidan was applied to intestinal cells at a modest dose (1%, 50 g/mL), which significantly decreased *C. parvum* adhesion to the cells. The number of *C. parvum* oocysts was almost five times lower in mice who received fucoidan as compared to control (animals) that did not get the polysaccharide. Thus, the scientists have successfully demonstrated that fucoidan effectively reduces *C. parvum* development in mice and also inhibits parasite attachment to human intestinal epithelial cells. Fucoidan, which directly binds to functional mediators generated from *C. parvum* in the epithelial cells of intestine of newborn mice, can suppress cryptosporidium at the same time. Desulphated fucoidan, however, had no impact on parasite development. Chitosan has potential as a treatment for cryptosporidiosis. To determine the effect of different chitosans on oocyst formation in new-born CD-1 mice, *C. parvum* oocysts were orally injected and treated with

chitosan, then compared to those of untreated animals. The control was paromomycin, a common drug in veterinary medicine. Treatment with both chitosan's and paromomycin significantly decreased the discharge of parasites in afflicted animals that received chitosan (56%, 34.5%, and 58%, respectively). In the in vitro tests, a considerable drop in the vitality of the cryptosporidium oocysts (>95%) was seen after 24 h of incubation at 37 °C. Additionally, the growth of *C. parvum* in the NCT-8 and Caco-2 cell lines was suppressed in a dose-dependent manner by paromomycin, chitosan NAG, and chitosan mix (Hu et al. 2013).

6.5 Marine Sponge Derived Compounds

Marine sponge derived compounds which contains halenaquinone and xestoquinone shows various enzymes inhibitory properties in addition to their inhibitory activity against phosphatidylinositol 3-kinase and topoisomerase I and II. Compound xestoquinone inhibited skeletal muscle myosin on the Ca^{2+} and K^{+}-ATPase levels. SAR studies revealed a significant reduction in Ca^{2+} ATPase activity for halenaquinone and three synthesized analogs with a quinone structure. On the other hand, four xestoquinone analogs in which the quinine structure was altered to quinol dimethyl ether did not decrease the Ca^{2+} ATPase activity. With IC50 values of less than 10 mm, halenaquinone, halenaquinol, and 14-methoxyhalenaquinone demonstrated the most impressive protein tyrosine kinase (PTK) inhibitory activities. The other analogs were either ineffectual or less potent, and a justification for this SAR pattern was also provided. Additionally, xestoquinone showed significant protein kinase inhibitory action towards Pfnek-1, a serine/threonine malarial kinase, with an IC50 value of roughly 1 mm, and moderate activity towards PfPK5, a member of the cyclin-dependent kinase (CDK) family (Mehbub et al. 2014).

A crucial structural component for this increased activity appeared to be the dihydro-benzothiazine dioxide found in the molecules adociaquinone A, adociaquinone B, and adociaquinone A. The most efficient inhibitors of the Cdc25 B phosphatase inhibitory activity were adociaquinone B and 3-ketoadociaquinone B. Histone deacetylase generated by K562 human leukaemia cells was inhibited by four cyclostellettamines: cyclostellettamine A, cyclostellettamine G, dehydrocyclostellettamine D, and dehydrocyclostellettamine E. The IC50 values for these compounds ranged from 17 to 80 mm. Xestospongic acid ethyl ester (207) was found to inhibit the Na^{+}/K^{+} ATPase (Zhang et al. 2017).

6.6 Conclusion

SPS from marine hydrobiota are promising antiparasitic agents. The therapeutic efficacy of current therapies is lost as a result of parasites acquiring drug resistance. The toxic and adverse side effects of a number of medications used to treat parasitic infections have also inspired interest in alternative therapies. Actively screening

physiologically active natural chemicals, including the metabolites of marine aquatic organisms from which effective pharmaceuticals can be created, has led to the development of new methods for the treatment and prevention of parasitic disorders (Strobel and Daisy 2003).

SPS produced by algae and marine invertebrates are generally innocuous and only very infrequently mildly dangerous to humans. One polysaccharide that has been widely used by the populace of many countries and is produced as a food additive is fucoidan, for instance. However, little is known about the molecular and cellular mechanisms that underlie the antiparasitic activity of these unique compounds. It can be stated that there is presently a collection of information, but efforts are made to understand various processes, considering that every type of parasitic interacts with each SPS differently and requires a distinct strategy. It is of great interest to examine how bacteria interact with polysaccharides using contemporary methods in order to understand the nature of the direct effects of SPS on protozoa. Injuring the cellular membranes or permitting nutrients to exit from the bacterium, these chemicals adhere to the surface of the bacteria. This is confirmed by the discovery of nucleic and proteins generated after bacteria were treated with SPS. The same inquiry can employ both monocellular and multicellular parasites. Furthermore, it's probable that SPS binds and holds onto ambient nutrients, lowering their bioavailability for parasites thus diminishing or even eradicating protozoa's viability (Mutanda et al. 2011).

Despite the combination of new techniques, such as genetic manipulation of pathogens, protein interaction, biomedical imaging, and the use of physical and chemical methods for research, which will lead to the standardisation of anticipated platforms in drug development, will undoubtedly make it possible to apply these strategies for the development of antiparasitic drugs based on biologically active components from marine hydrobionts (Reed 2005).

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Microorganisms-Derived Biochemicals: Potential Drugs for Human Parasitic Diseases

7

Kuntal, Seema Kalra, and Madhu Yashpal

Abstract

Infectious parasites cause a large number of human diseases which is one of the chief global problem. Parasitic infections result in many mortalities and disabilities worldwide. Parasites depend on host organisms for their survival. Two types of significant parasites which cause diseases in humans are protozoa and helminths. There are numerous promising biomolecules in microorganisms with structural and functional diversity. These biomolecules show antifungal, antibacterial and antiparasitic bioactivity also and act as a remarkable class of therapeutics in medicine and scientific research. This chapter summarizes the significant biochemical and antiparasitic compounds isolated from microorganisms as potential drugs against human parasitic diseases.

Keywords

Microorganisms · Anti-protozoal · Drugs · Parasitic · Antihelminth · Anti-malarial

7.1 Introduction

A large variety of human diseases caused by parasites is one of the main public health problem worldwide. As the world is getting smaller with faster modes of transportation, many newer types of parasitic diseases have appeared or been

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_7

introduced at places unknown places. Parasites live in or on a host organism and are dependent on it for their growth, multiplication and survival. Many parasites can spread diseases without killing their host; however, some may be fatal. Parasites known to be infectious in humans are categorized into protozoa, helminths and ectoparasites, significant types of human parasites.

Protozoa are acellular organisms which can invade and live in different tissues and cells of an organism and spread from one person to another through contaminated water and food, insect bites and person-to-person contact. Some common examples of infectious protozoa are *Plasmodium malariae*, *Escherichia histolytica*, *Trichomonas vaginalis, Giardia lamblia, Balantidium coli* etc.

Helminths are multicellular organisms generally known as worms. These infect the digestive tract of humans and eventually pass through a person's stool. These include hookworm and roundworm parasites e.g. *Ascaris lumbricoides, Ancylostoma duodenale, Trichuris trichiura, Wuchereria bancrofti etc.*

Ectoparasites as the name suggests live outside of the body, for example, like fleas, ticks and lice.

Parasitic diseases are very prevalent and associated with debilitating health consequences causing a threat to the global healthcare system. Treatment relies primarily on the use of antiparasitic drugs and chemotherapeutic approaches. While chemotherapy is associated with several toxicities and cases of resistance to many of the antiparasitic drugs being used are already being reported. Also, the lack of vaccines warrants the need for developing new medications or alternate options for treatment. As the noteworthy parasitic diseases are caused by protozoa and helminths, we will emphasise these two in this chapter. Table 7.1 lists the major disease caused by protozoa and helminths.

Microbial products got recognition after the discovery of penicillin from *Penicillium notatum* by Alexander Fleming in 1928. Since then the utility of microorganism-derived compounds has been explored in agriculture, pharmaceutical and food industries. Compounds isolated from microorganisms have divergent chemical structures and biological properties activities like anti-cancerous, antiparasitic, anti-inflammatory, and immunosuppressive. Many of these compounds have demonstrated anti-parasitic activities and have been successfully used for the treatment of parasitic diseases. These drugs inhibit the parasite's growth and paralyze it so that they cannot attach themselves to the host.

Antiparasitic drugs are classified into the following categories based on the type of parasite they treat

- Antiprotozoal agents, which treat protozoan infection in humans;
- Antihelminthic agents treat infections caused by parasitic worms
- Ectoparasiticides kill scabies, lice and other ectoparasites.

In this chapter, we shall review the compounds derived from microorganisms which have been or can be used as potential drugs against human parasitic diseases.

Table 7.1 Major parasitic diseases, commonly used drugs and their disadvantages

Disease	Causative parasite	Widely used drugs or drug combination	Mode of transmission	Disadvantages
Malaria	*Plasmodium* spp.	Chloroquine, sulphadoxine/ pyrimethamine, mefloquin, artemisinins, artemether/ lumefantrine. Chlorproguanil	Vector borne–Mosquito bite	Reporting of resistance to drug due to prolonged usage and adverse effects by some patients. High cost and difficult to isolate
Leishmaniasis	*Leishmania* spp.	Pentamidine, pentavalent antimony, liposomal amphotericin B, mitelosine	Vector borne–Phlebotomus or Lutzomyia genu flies	Efficacy loss/drug resistance, high cost for liposomal amphotericin B. Adverse effects, Miltefosine cannot be used in young women
African trypanosomiasis	*Trypanosoma brucelgambiense*	Suramin, pentamidine, melarsoprol, eflomithine	Tsetse fly bites	Severe adverse effects. Suramin and pentamidine have no effect in an advanced stage of disease, eflomithine expensive and only effective against *T. gambiense*
Chagas' disease	*Trypanosoma cruzi*	Nifurtimox, benznidazole	Vector borne–Triatominae insect bite, food borne, blood transfusion	Elongated treatment and adverse effects, do not work in late-stage disease
Schistosomiasis	*Schistosoma mansoni*, *S. haematobium*, *S. japonica*	Oxamniquine, praziquantel	Exposure of skin to contaminated fresh water carrying certain types of snails	Oxamniquine effective only against *S. mansoni. Praziquantel* ineffective against immature worms, reports of drug resistance
Lymphatic filariases	*Brugia malayi*, *Wuchereria bancrofti*	Diethylcarbamazine (DEC), ivermectin, albendazole/DEC, albendazole/ivermectin	Mosquito bite	Diethylcarbamazine is not recommended in *O. volvulus*-endemic areas. Combination therapy is successful in the case of albendazole. Ivermectin is not effective for adult worms
Onchocerciasis	*Onchocerca volcvulus*	Ivermectin	Bite by black flies of genus Simulium	Does not eliminate adult worms

7.2 Anti-protozoal

Protozoans are unicellular eukaryotic organisms and have one motile stage in the life cycle. They are ecologically diverse and widely dispersed. Protozoan parasites belong to four different groups: amebae, ciliates, flagellates, and sporozoan. Pathogenic protozoans cause human diseases like Amoebiasis, Giardiasis, Trypanosomiasis, Leishmaniasis, Malaria, Balantidial Dysentery and Toxoplasmosis and result in a significant number of deaths each year. The treatment for protozoan infection is a challenging task due to rapidly developing parasite resistance to the existing drugs and slow development of vaccines. Current treatment includes the use of anti-protozoal agents synthesized chemically or derived from microbes or other plants. Here, we will discuss the drugs of microbial origin.

7.2.1 Anti-malarial

Plasmodium is the most prevalent protozoan parasite that infects man. There are six species of Plasmodium which cause malaria in humans (Sutherland et al. 2010). *Plasmodium vivax* and *Plasmodium falciparum are the two most notable species*. According to WHO, with 241 million malaria cases reported in 2020, it poses a global threat and in 2020. The treatment of malaria depends on chemotherapy like other protozoan infections of man. However, poor efficacy has been achieved in producing a malarial vaccine (Agnandji et al. 2011; Abdulla et al. 2013). This means that drugs persist as the best option for malaria treatment.

The action of antimalarial drugs on the hepatic stage is not well characterized. Most of the currently available antimalarial drugs target the symptomatic phase (erythrocytic stage) of infection. Therefore, to treat malaria it is necessary to treat acute blood stage infection caused by all the malarial species. If *Plasmodium vivax* or *Plasmodium ovale causes infection then* combination therapy (terminal prophylaxis with drug) is which should be active against the dormant stage (hypnozoites) for months in the liver occasionally.

Recently, a concern was raised that due to prolonged usage, the microorganism is developing resistance to many of the currently available drugs, therefore, the treatment is ineffective. Stress is on monitoring the continent-wide capacity for antimalarial resistance since resistance spreads among parasites. It may develop independently in a particular region or may spread to broad geographical areas with the travelling and migration of malaria-infected persons. Chloroquine (quinine), a widely used drug for its treatment is not being discussed here as it is a plant product, therefore is not within the scope of this topic.

7.2.1.1 Tetracycline

Tetracyclines are derived from actinomycetes *Streptomyces* spp. These and their semi-synthetic derivatives have been used against many bacteria, chlamydiae and mycoplasmas. These are also used in treating malaria along with quinine as an

effective regimen for prophylaxis of *P. falciparum,* a multi-drug resistant species (World Health Organization 1995).

According to Chopra and Roberts (2001), tetracyclines have been also used to treat other protozoan infections like amebiasis, leishmaniasis with Giardiasis and Toxoplasmosis. Smith and Rajan (2000) studied humans who were infected with filarial nematodes, numbers of adult worms and microfilariae were decreased after taking a tetracycline dose.

Plasmodium, Eimeria, Toxoplasma, and Cryptosporidium constitute apicoplast. Apicoplast is an organelle which is necessary for the formation of infective stages of these parasites. Walsh (2003) observed that tetracyclines inhibit protein synthesis by blocking the binding sites on the ribosomes. Lin et al. (2002) and Dahl et al. (2006) reported that tetracyclines have inhibitory action against the growth of Plasmodium.

Gaillard et al. reported doxycycline, other derivatives of tetracycline as an alternative for malaria prophylaxis and treatment. Doxycycline is used much in the areas to prevent malaria where chloroquine and multidrug-resistant *P. falciparum* parasites are prevalent. It has been reported that doxycycline has been proven effective in combination with quinine and resistance to doxycycline is not well described.

7.2.1.2 Apicidin

Fungus *Fusarium pallidoroseum* produces apicidin and it has been used to develop anti-protozoan drugs. Darkin-Rattray et al. (1996) observed wide antiprotozoal activity against Apicomplexan parasites in vitro and active against *P. berghei* malaria in mice when introduced orally and parenterally. It inhibits the action of enzymes in treated parasites.

***Fusarium pallidoroseum* also produces other peptide antibiotics which showed anti-malarial activities such as**

- Takaokamycin (Otoguro et al. 2003)
- Enniatins (Nilanonta et al. 2003)
- Leucinostatin, efrapeptins and peptaibols (Nagaraj et al. 2001)

Streptomyces azureus produces thiostrepton that is found to be effective against infection with *Plasmodium berghei* in mice. It inhibits protein synthesis by interacting with the GTPase binding domain of the apicoplast large subunit rRNA and Sullivan et al. (2000).

Nocardia sp. (actinomycetes) produces thiolactomycin which limits the growth of *Toxoplasma gondii* and *P. falciparum* (Roberts et al. 2003). It acted against *Trypanosoma brucei* also.

7.2.1.3 Fosmidomycin

Fosmidomycin is a product of *Streptomyces lavendulae* that inhibits DOXP reductoisomerase in *Plasmodium* (Jomaa et al. 1999). Fosmidomycin was effective in the treatment of acute uncomplicated *P. falciparum* infection in clinical trials. It is a safe antimalarial drug, although the occurrence of recrudescent infections resists its

use as a single agent. However, Lell et al. (2003) and Fernandes et al. (2015) reported that the role of combination therapy should be invested more.

Bormann et al. (2004) demonstrated that Fosmidomycin has fast action and sufficient tolerability. But after a short-term interval reappearance of a disease has been seen by its use alone. However, its introduction with clindamycin was more effective due to synergistic activity against *Plasmodium falciparum.* Fosmidomycin–clindamycin has emerged as a potent novel treatment option for malaria.

Building blocks for isoprenoids are synthesized by DXR enzyme of MEP pathway. Wang et al. (2016) designed and created a series of analogues of fosmidomycin which inhibit DXR in *P. falciparum.* More current antimalarial compounds can be designed as crystal structures of fosmidomycin-bound complexes PfDXR has been discovered by Umeda et al. (2011).

7.2.1.4 Scyphostatin

Fungus *Trichopeziza mollissima produces* scyphostatin (Hanada et al. 2002). It is a mammalian-neutral sphingomyelinase inhibitor. It also inhibits the intra-erythrocytic proliferation of *P. falciparum*.

7.2.1.5 Borrelidin

Streptomyces spp. produces borrelidin (Berger et al. 1949). It demonstrates antiviral, insecticidal, antimicrobial, herbicidal, and antitumor activities. Otoguro et al. (2003) reported that borrelidin showed outstanding antimalarial activity against both resistant *P. falciparum* and chloroquine-sensitive and in vitro. The effective dose was also found to be lower than that of chloroquine, artemether and artesunate.

7.2.1.6 Secondary Metabolites Derived from Marine Microorganisms Against Malaria

Secondary metabolites from marine microorganisms are emerging as an enriched resource with the potential for new drugs. They have also been explored for their anti-malaria activity (Prudhomme et al. 2008).

Ahmad et al. (2017) reported a compound, Gancidin-W isolated from *Streptomyces* with anti-malaria properties. Recently, Waluyo et al. (2021) utilized Indonesian bioresources and developed an Indonesian microbial depository. They isolated fungi and characterized actinomycetes strains from five locations in different Indonesian geographical areas. The anti-malarial activities of secondary metabolites from these are being explored using enzyme-based screening against two enzymes from *Plasmodium falciparum* and an assay was developed.

7.2.2 Anti-amoebiasis

Entamoeba histolytica is a protozoan parasite which causes amoebiasis worldwide. The infection leads to amoebic colitis or extra-intestinal abscess resulting in up to

100,000 deaths annually (Choudhuri and Rangan 2012). There are two types of amoebiasis; tissue and luminal which determine the dose of antibiotics.

Metronidazole was found to be the first treatment of amoebiasis. However, due to increased side effects and pathogen resistance to this drug; alternative therapeutic agents or drugs with new modes of action are being explored. Medications such as metronidazole or nitazoxanide are effective in combination (Gonzales et al. 2019). Other drugs include ornidazole, tinidazole, chlororquine and aromomycin. Nitroimidazole therapy is most effective in most of the patients with amebic colitis but it does not work for intraluminal parasites.

Espinosa et al. (2012) isolated Echinomycin A and tirandamycin A from marine derived actinomycetes and screened for ant amoebic and antibiotic properties. These were found to inhibit in vitro growth of an *E. histolytica* strain and a clinical isolate.

Two enzymes serine acetyltransferase and cysteine synthase catalyse reactions of L-cysteine biosynthetic pathway. Defense of *E. histolytica* is dependent upon these two reactions. It is suggested to be a normal drug target counter to amebiasis because this pathway does not occur in humans. Tsuge et al. (2018) discovered new compounds by screening already-known compounds and extracts of microbial culture. These compounds act on cysteine biosynthesis (de novo) of *E. histolytica*. Five novel compounds deoxyfrenolicin, chaetoglobosin A, aspochalasin B, cerulenin and prochaetoglobosin III found to distress the development of the trophozoites differentially in the medium (cysteine-absent) compared to the cysteine present medium were isolated.

7.2.3 Anti-Leishmaniasis

Leishmania is a protozoan disease prevalent in tropic and sub-tropic regions and southern Europe. It is characterized by weight loss, enlarged liver and spleen, bleeding and fever. It is of two types: visceral and cutaneous leishmaniasis. Amphotericin B produced by *Streptomyces nodosus* has antifungal properties (Sternberg et al. 1956). Ergosterol-related sterols occur in the cell membrane of *Leishmania* parasites which are similar to those of fungi. Therefore, amphotericin B can kill the parasites by binding to the cell membrane of Leishmania. Aamphotericin B or liposomal amphotericin B have been used for kala-azar (visceral leishmaniasis) and mucocutaneous leishmaniasis which is indifferent to antimony compounds (Adler-Moore and Proffitt 2002).

More recently, microorganisms (fungal and bacterial) named endophytes living on plant tissues (Molinar et al. 2012; Baptiste et al. 2021) have been found to have anti-trypanosomal and anti-leishmanial effects and are being explored as potential drug candidates. For example, *Penicillium janthinellum* (Endophytic fungus) was extracted from fruits of *Melia azedarach* and produced polyketide citrinin. These compounds showed good activity against promastigotes of *L. mexicana* (do Rosário Marinho et al. 2005).

Chan-Bacab et al. (2021) reviewed the current state of the bacterial and fungal metabolites derived from heterotrophic bacteria, cyanobacteria and filamentous

Table 7.2 Microorganisms and their antiprotozoal metabolites

Microbial species	Metabolite(s)	Target parasite
Bacteria		
Acinetobacteria		
Micromonospora sp.	Manzamine A	*L. donovani*[a]
Micromonospora sp.	Lobosamide A	*T. b. brucei*[b]
Streptomyces sp.	Valinomycin	*L. major*[a]
T. b. brucei[b]		
Streptomyces axinellae	Tetromycin 1	*T. b. brucei*[b]
Streptomyces sp.		
ICBG292	Nigericin	*L. donovani*[a,c]
Streptomyces sp. ICBG233	Dinactin	*L. donovani*[a,c]
Actinokineospora sp. EG49	Actinosporin A	*T. b. brucei*[b]
Cyanobateria		
Symploca sp.	Symplocamide A	*L. donovani*
Oscillatoria sp.	Venturamide A	*T. cruzi*[c]
Lyngbya majuscula	Dragonamide A	L. donovanid
	Dragonamide EH	
	erbamideBAlmir	
	amideBAlmiramide C	
	L. donovani[d]	
Schizothrix sp.	Gallinamide A	*L. donovani*[d]
Okenia sp.	Janadolide	*T. brucei*[b]
Fungi (Ascomycetes)		
Nigrospora sphaerica	Aphidicolin	*L. donovani, L. infantum, L. enriettii*
Penicillium janthinellum	Citrinin	*L. mexicana*
Alternaria sp.	Altenusin	*T. cruzi*
Cochliobolussp.	Cochlioquinone AIs, ocochlioquinone A	*L. amazonensis*[d]
Penicillium sp.	Mycophenolic acid	*T. brucei, T. cruzi*

[Adapted from Chan-Bacab et al. (2021)]
[a]promastigotes; [b]trypomastigotes; [c]intracellular amastigotes; [d]axenic amastigotes

fungi with demonstrated antileishmanial and antitrypanosomatid activity (Table 7.2).

Ma et al. (2004) isolated Hypocrellin A and hypocrellin B perylene quinonoid pigments from fungus *Hypocrella bambusae*. These two metabolites showed antileishmanial activity against *L. donovani*. The antileishanial activity of both was powerful as compared to amphotericin B and pentamidine. *Cochliobolus* sp. (endophytic fungus) produces cochlioquinone A and isocochlioquinone A. Both quinonoid compounds had antileishmanial activity against

L. amazonensis, with insensitivity in human cell lines which may point that these could be developed into potential drug targets (Campos et al. 2008).

7.2.4 Anti-trypanosomal

Sasaki et al. (1973) isolated Ascofuranone from the culture broth of the fungus originally *Ascochytaviciae*. Nihei et al. (2002) further worked on Ascofuranone which inhibited a terminal oxidase (trypanosome alternative oxidase) of the respiratory chain of *Trypanosoma brucei*. It showed satisfactory therapeutic effects for African trypanosomiasis in mice.

Three indole alkaloids (Table 7.2; Chan-Bacab et al. 2021) were produced from *Bacillus pumilus* and isolated from *Antiphates* sp. These alkaloids showed particular activity against *T. cruzi* amastigotes expressing β-galactosidase in Vero cells. A similar response against trypanosoma was shown by three compounds *N*-acetyl-β-oxotryptamine, 3-hydroxyacetylindole, and 3-formylindole. Martínez-Luis et al. (2012) reported that due to similar structure it showed antiprotozoal activity.

Janadolide also showed excellent antitrypanosomal activity against the *T. brucei* which was isolated from a *Okenia* sp. (Ogawa et al. 2016). Athawale et al. (2018) observed which group in structure is important for parasitic activity. Other scientists manufactured janadolide, in vitro which had antitrypanosomal activity against *T. cruzi* and *T. b. rhodesiense* (Chung et al. 2020).

7.3 Anti-helminths

Helminths include roundworms (nematodes), flukes (trematodes), and tapeworms (cestodes). Helminths cause many infections in humans as well as animals. For example, nematodes that cause many zoonotic diseases in humans are *Ancylostoma duodenale* (hookworm), *Ascaris lumbricoides* (roundworm), *Gnathostoma spinigerum*, *Trichinella spiralis* (Trichina worm) and *Halicephalobus gingivalis*.

7.3.1 Avermectins and Milbemycins

Avermectins and milbemycins are a group of spiro-ketal 16-membered ring macrolides (Fig. 7.1) which show nematocidal and insecticidal. Two differ in the presence of hydroxyl group at position 13. A sugar moiety at C-13 is present in avermectins, but milbemycins don't have sugar moiety due to the absence of hydroxyl groups.

Streptomyces produces both avermectins and milbemycins. However, many strains of *Streptomyces* spp. produce milbemycins, those producing avermectins are rare (Shoop and Soll 2002). Mutants of *S. avermectinius* produced about 40 avermectin homologs and more than a hundred milbemycin homologs.

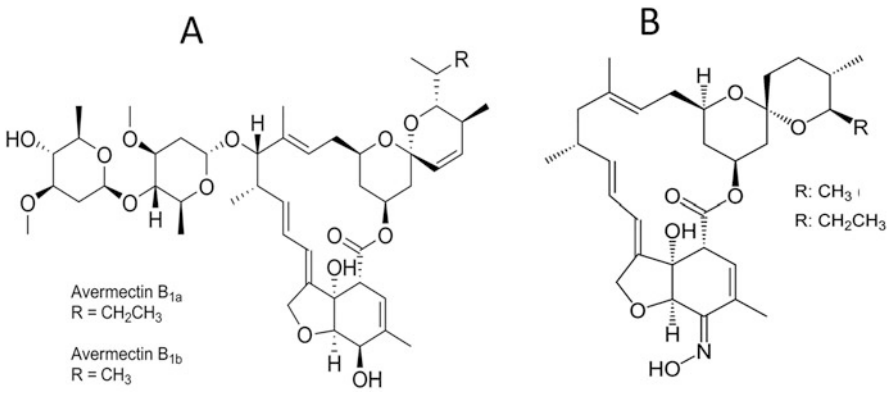

Fig. 7.1 General structures of avermectins (**a**) and milbemycins (**b**)

Ivermectin is effectively used for the treatment of nematode infections in humans. Human onchocerciasis (river blindness) is an microfilariae infection which is endemic to certain parts of Africa and America. *Onchocerca volvulus* causes river blindness and is seen by chronic eye and skin lesions. Richards et al. (2001) described that these endemics have been effectively treated with ivermectin. *Brugia malayi and Wuchereria bancrofti* causes Elephantiasis (lymphatic filariasis) and it can also be treated by ivermectin. Paromomycin is used to treat human and animal cestode infections (Salem and El-Allaf 1969).

7.3.2 Potential Anti-helminths Candidates Derived from Marine Microorganisms

Nematodes that cause many zoonotic diseases in humans are *Ancylostoma duodenale* (hookworm), *Ascaris lumbricoides* (roundworm), *Gnathostoma spinigerum*, *Trichinella spiralis* (Trichina worm) and *Halicephalobus gingivalis*. Bérdy (2005) explored microbial compounds on land for therapeutics and drug development. Xiong et al. (2013) reported more beneficial bioactive metabolites; some of which have strong nematotoxicity through different modes of action (Table 7.3, Salikin et al. 2020).

Newly discovered antinematode compounds from marine epiphytic bacteria represent a promising repository. These bacteria are plentiful on marine surfaces and bacterivorous predators graze continuously upon them e.g., nematodes and protozoans (Ballestriero et al. 2014; Esposito et al. 2018). These bacteria have established protection approaches to combat grazers by making toxic bioactive compounds (Penesyan et al. 2010; Rocha-Martin et al. 2014). These marine microorganisms derived bioactive compounds are called blue gold from the ocean. Therefore, these bioactive substances can be used as a new source of antinematode drugs (Table 7.3; Salikin et al. 2020).

Table 7.3 Microorganisms and their antinematode metabolites

Microbial source	Compound	Mechanism of action	Target nematode
Bacillus thuringiensis	Crystal toxin Cry5B, Cry21A	Intestinal cell membrane integrity is compromised due to binding of toxin to nematode glycoconjugate receptor resulting in smaller brood size and increased death rate	*Ancylostoma ceylanicum, Ascaris suum, C. elegans*
Streptomyces avermectinius	Avermectin and ivermectin	Pharyngeal paralysis leading to death	*Haemonchus contortus, Brugia malayi, C. elegans*
Pseudomonas aeruginosa	Phenazine toxin	Neurodegeneration, disruption of protein homeostasis	*C. elegans*
Pseudomonas aeruginosa	Exotoxin A and other undetermined effectors	Buildup of bacteria in the gut, eventually resulting in paralysis of nematode and death	*C. elegans*
Pseudomonas aeruginosa	Chitinase enzyme	Disrupts of nematode cuticle, intestine and egg shell leading to the animal death	*C. elegans*
Microbulbifer sp. *D250*	Violacein	Damaged cell and cell death due to colonization of bacteria	Algae *Delisea pulchra*
Pseudoalteromonas tunicata D2	Tambjamine	Nematode death due to bacterial accumulation	Algae *Ulva australis*

(Adapted from Salikin et al. 2020)

It is surprising that in spite of the fact that antiparasitic diseases are a global burden and so many natural and microbial resources have been explored with compounds which are potential drug candidates, not many new drugs have come to market. The development of drug resistance to those drugs is increasing the problem already in the market. Despite need for the urgent innovation, only three new anthelminthic compounds reached the market after 2000:

- emodepside (Zahner et al. 2001)
- monepantel (Kaminsky et al. 2008)
- derquantel (Woods et al. 2012)

These products were introduced for animal diseases and have not been tested for humans yet.

Drug discovery is a lengthy and expensive process and costs about $50–100 million for animal health products (Yarbrough 2016) and over U.S.$ 2.5 billion for human drugs (DiMasi et al. 2016). Value of the goods, availability of investment partners and return on investment are the major driving factors for drug development. After world war II, a large proportion of the human population suffered from tropical diseases, which drove the industry, government and research communities

to look for drug candidates to meet the challenge and antimalarial and anti-schistosomal drugs for humans were discovered (Beaumier et al. 2013).

Many a time, standard industrial processes may pose a challenge for converting a potential lead molecule to a drug. Even though a good drug target may be identified, the evaluation methods may not be standardized. For example, it is the *Plasmodium* spp. that infects humans with malaria, but in laboratory conditions, standard animal models for malaria infection are *P. berghei, P. chabaudi, P. yoelii.* Similarly, the *Onchocerca gutturosa* worms for onchocercal infection used as in vitro models but they are parasites of cattle rather than humans.

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Plant-Based Vaccines Against Human Parasitic Diseases

8

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Abstract

Historical evidences substantiate the vulnerability and susceptibility of humans to major pandemics. Worldwide, outbreaks of infectious diseases caused increased mortality and morbidity of millions. Despite improved hygiene, medical care, advanced medicines, and sanitary conditions in "modern" era, developing countries witness mortality due to a variety of infectious diseases. Globally, it is extremely difficult to combat the enhanced recurrence of infectious diseases that include avian influenza, Ebola, and Zika. Vaccination is considered one of the best alternatives for control of parasites in the future. To overcome the burden of infectious diseases, individuals are subjected to mass immunization drive with the aim to develop immunity in the community. Occurrence of innumerable variants of the infectious agent necessitates intensive research on vaccines. Success in vaccine development against parasites is severely limited by innumerable unknown, unidentified antigens and complete lack of understanding of the kind of immune response essential for protection. Regardless of these barriers, several vaccines are under different phases of development against several parasitic diseases. Other limitations include high cost of vaccine production, maintenance of vaccine depots, costs involving distribution, and degeneracy. Further, proper management to maintain biosafety and biosecurity is detrimental to the success of vaccines. The past decade has witnessed resurgence in

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_8

developing plant biotechnology that offers innovative approaches for vaccine development based on gene transfer strategies for engineering newer recombinant vaccine in plants with added advantages in the form of improved production, better isolation, purification, and enhanced efficacy with least immunological side effects. The production of plant-based vaccines provides a promising alternative to create affordable biological products. Such recombinant vaccines can irrefutably offer us novel standards and authorized regulations for better approval, licensing, distribution, and marketing of plant-based vaccines. The chapter elaborates on various strategies based on recombinant DNA technology and plant biotechnology for exploiting plant-based vaccine research for therapeutic management of infectious diseases.

Keywords

Plant vaccines · Infectious diseases · Immunization · Vaccine recombinant technologies

8.1 Introduction

Vaccines and vaccination were the initial attempts to prevent infectious diseases in human host. In late eighteenth century, smallpox became a weapon of war. On May 14, 1796, Edward Jenner demonstrated that infection with cowpox could give protection against smallpox infection. In 1803, the term *vaccination* was coined based on the Latin for cow-"*vacca*." This paved the way for vaccine development against dreadful diseases like polio, influenza, cholera, measles, rabies, hepatitis, and many more all over the world and saving innumerable lives globally. The major disadvantages of these vaccines are their high cost of production and purification and maintenance of vaccines in cold storage that requires highly skilled labor. Gradually, plants are being used as vaccine bio-factories for expressing foreign antigens and corresponding antibodies using genetic engineering technologies. There is an inherent advantage in using plants for the production of vaccines as they are cost-effective, with inexpensive upscaling as greenhouses or bioreactors. Plants can express complex antigens that can carry human pathogens or endotoxins inherent to the bacterial, insect, or mammalian cell systems. Plants act as bioreactors wherein larger recombinant proteins could be produced with no contamination from humans or animal pathogens. Their potential low cost, ease to administer, high scalability, and ready acceptance by patients in the form of carrier plants put them at an advantage over the conventional vaccines. Apart from these cited advantages, they can be easily frozen and stored (Egelkrout et al. 2012). Plant genetic engineering technology has given fresh insight on vaccine research via gene transfer technology that can incorporate the desired gene in plants. In 1986, Barta and colleagues reported chimeric gene expression of human growth hormone and nopaline synthase in tobacco plants and sunflower with Ti plasmid (Barta et al. 1986). Different plants such as peanut, tomato, tobacco, maize, lettuce, carrot, rice, and soybean are often

referred to as hosts for addition of preferred gene of interest. Criteria that should be taken into consideration while developing plant-derived vaccines for commercial purposes include identification of the genes to be transfected, high expression of recombinant genes, enhanced stability, and a high shelf life of antigens with zero contamination with live pathogens. Finally, ease of manufacturing and newer targetable antigens against variant pathogen subtypes are desirable.

8.1.1 Production of Plant-Based Vaccines by Recombinant Technologies

Plant-derived vaccine research and technologies necessitate the incorporation of the preferred gene of interest encoding the antigen protein into the plant genome.

The transgene of interest is introduced into the vector for further expression in plants either by stable transformation or via transitory transformation mechanism within plant cells. A steady and ephemeral gene expression can be attained through gene delivery methods. Broadly, it can be categorized as direct gene delivery and indirect gene delivery method (Fig. 8.1).

(a) Direct method for delivery of gene, where the nucleic acids (DNA/RNA) are straight incorporated into the plant cells with biolistic method, where two different kinds of antigen expression within transgenic plants can occur such as nuclear transformation and chloroplast transformation.
(b) Indirect method for delivery of gene provides evidence for more considerable vaccine production as it engages exploitation of plant bacteria, chiefly the *Agrobacterium* species and other plant viruses, which unsurprisingly contaminate the plant cells and are employed on work to amalgamate the desired gene of interest into plant genome.

8.2 Human Parasitic Diseases

The past two decades have witnessed a meteoric rise in infectious diseases that often go undiagnosed and are fatal if left untreated (Fig. 8.2). Parasitic diseases are a great burden in tropical regions where the morbidity index and mortality rate indicate ~1.1 million deaths occurring annually. Major causes of reported deaths are malaria and schistosomiasis. Drugs administered are ineffective, probably due to developed parasitic drug resistance. Hence, there is an urgent need to look for alternative approaches like development of vaccines for the parasitic diseases. Clinical trials for vaccines against malaria and leishmaniasis are also under trials, and vaccines for treatment of schistosomiasis are in their phase trials (I/II) (Shahid and Daniell 2016). "Classic" vaccines are based on attenuated infective stages of protozoan, and helminths like *Coccidiosis*, *Toxoplasmosis*, and *Dictyocaulus* are very unstable and expensive. Recombinant technology has enabled quick processing of protective antigens in bulk amounts. Cultivation, purification, and processing of the

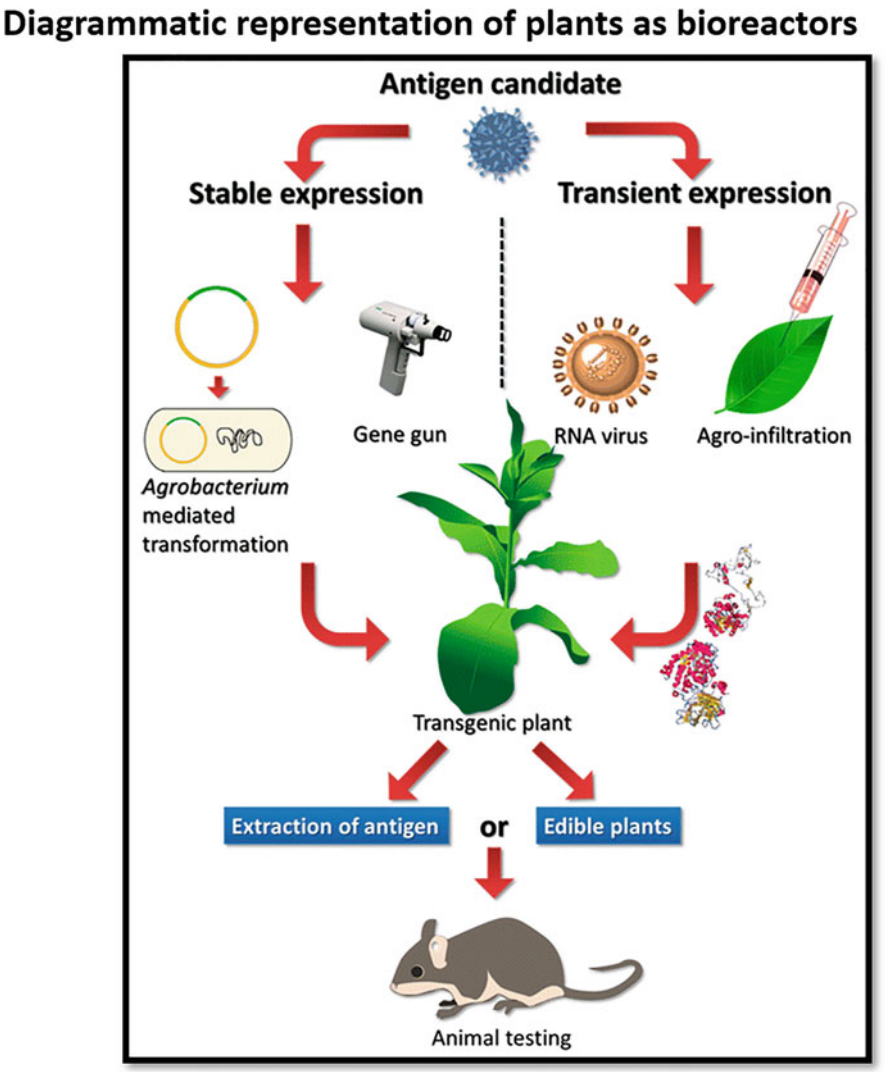

Fig. 8.1 Diagrammatic illustration of plant-based bioreactors

recombinant protein are less expensive than the maintenance of host animals and isolation of protective antigens from harvested parasites. But till date, there are no vaccines that can be used against the life-threatening advanced stages of malaria, leishmaniasis, schistosomiasis, trypanosomiasis, toxoplasmosis, cryptosporidiosis, and many other diseases. At present, TickGARD is the only anti-parasite recombinant protein vaccine that is commercially available (Kumar and Ghosh 2016). Edible vaccines, based on transgenic plants that express the protective parasitic antigens, present alternative approaches in the research for anti-parasitic vaccines and may

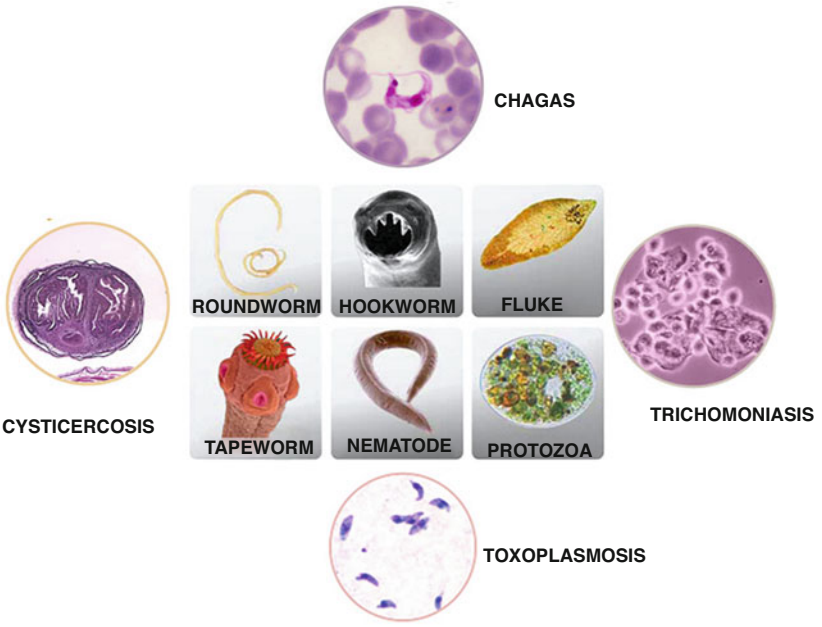

Fig. 8.2 Parasites and parasitic infections

also be important against gastrointestinal parasites (Zahmanova et al. 2022). Such vaccine prototypes have also been assessed at preclinical levels. Predominantly, these plant-based vaccines target various parasitic infections with foremost potential in the form of immunogenicity and protection. Vast number of human parasites that cause infection can be grouped under the following categories:

8.2.1 Protozoa

Protozoan disease occurrence is centered around tropical and subtropical areas of the globe. Protozoans have characteristic life cycles where it is able to switch between active infectious and inactive (cyst) form. Table 8.1 represents key pathogenic protozoa responsible for the occurrence of various diseases. Flagellates, Leishmania, and Trypanosoma are proficient enough in invading the blood and tissue of human host, producing severity. Occurrence of *Trichomonas vaginalis* and *Giardia lamblia* in the reproductive and gastrointestinal tracts does not cause mortality but is surely responsible for moderate morbidity. In contrast, sporozoan produces two most lethal diseases such as malaria and toxoplasmosis (del Yácono et al. 2012). Further, appearance of HIV has provided a new dimension to be looked upon, i.e., "opportunistic" parasitosis. Treatment and prophylaxis of protozoan-inflicted diseases have been reliant upon the type of drugs, many of which have become less effective, thereby imposing urgency in exploration for alternative systems.

Table 8.1 Important pathogenic protozoa and diseases caused by them

Type and location	Species	Disease
Urogenital tract	*Trichomonas vaginalis*	Trichomoniasis
Blood and tissue	*Plasmodium* species *Toxoplasma gondii* *Trypanosoma* species *Leishmania* species *Naegleria* species *Acanthamoeba* species	Malaria Toxoplasmosis Trypanosomiasis Leishmaniasis Amoebic meningoencephalitis Amoebic meningoencephalitis
Intestinal tract	*Entamoeba histolytica* *Giardia lamblia* *Cryptosporidium parvum* *Balantidium coli Isospora belli* *Cyclospora cayetanensis*	Amoebiasis Giardiasis Cryptosporidiosis Balantidiasis Cyclosporiasis

8.2.2 Pathogenic Free-Living Amoebae

Various free-living amoebae live in different habitats such as soil and water habitats. Certain amoebic species including Naegleria, Acanthamoeba, and Balamuthia are also known as facultative parasites for human host. Human infections that are caused due to amoebae are attained with time on exposure to contaminated water; secondly, it can also be inhaled in the form of cysts that are present in dust. *Naegleria fowleri* causes acute primary amoebic meningoencephalitis and brain abscesses in immune-incompetent individuals. Treatment of such free-living amoebic related infections is largely unsuccessful.

8.2.3 Pathogenic Flagellates

8.2.3.1 Trichomonas

The trophozoite of *Trichomonas vaginalis* is mainly present in the urethra and vagina of the female individual, as well as in the urethra and prostate gland of individual men. Their proliferation causes inflammation, and the presence of trophozoites is also found in part of tissues and secretions from the glands. Early signs of vaginal or vulvar pruritus are in the form of sudden discharge during or after menstruation; this also increases vaginal pH. Vaginal secretions are greenish to pale yellowish color, or at times it may be frothy in nature, and with a foul smell. Infections that occur in male are more latent, with negligible symptoms.

8.2.3.2 Leishmania

L. donovani, causative agent of kala-azar ("black sickness") commonly called as dumdum fever, is persistently taking place in diverse regions of Africa and Southeast Asia. Annually, ~12 million suffering individuals are reported to be infected with

three different clinical forms such as visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (MCL). All three of them have separate immunopathogenesis, mortality, and morbidity rate. As parasites invade the host body, it proliferates and infects several cells of different organs such as liver and spleen, resulting in organ enlargement and leading to subsequent weight loss. Its persistence further leads to post-kala-azar dermal leishmaniasis. Untreated visceral leishmaniasis on the other side may be fatal because of the occurrence of secondary infection. Drugs such as pentavalent antimonials (Pentostam™; GSK) and meglumine antimoniate (Glucantime, Aventis), amphotericin B and its lipid formulations, pentamidine, and ketoconazole are considered effective treatments (Sundar and Chakravarty 2013). Diamidine-pentamidine is extremely toxic. The antimonials have erratic efficacy against leishmaniasis. Futile therapeutic regime with antimonial drugs is also known (Lira et al. 1999), while varied results have been observed with ketoconazole treatment (Herwaldt et al. 1992; Ozgoztasi and Baydar 1997). Amphotericin B is effective against *Leishmania donovani* VL. Although there has been an upsurge of innovations in the development of antileishmanial drugs, their progression is restricted because of the differential chemosensitivities of *Leishmania* species (Croft and Coombs 2003).

8.2.3.3 Trypanosoma

Trypanosoma is the causative agent of African sleeping sickness. Around 60 million sub-Saharan Africans are threatened by this infection caused by *Trypanosoma gambiense* alone, and it is responsible for 95% of the cases (Brun et al. 2011; Simarro et al. 2012) (Checchi et al. 2008). *T. b. rhodesiense* affects the central nervous system, and the parasites are able to cross the blood–brain barrier (Grab and Kennedy 2008). Till date, there is no vaccine available for trypanosomiasis. The remedial approach relies on the infecting species and the occurring stage of the disease. Administration of pentamidine or suramin to patients at an early stage of *T. b. gambiense* and *T. b. rhodesiense* infections is more effective (Steverding 2010). With the advancement in disease, the treatment relies on melarsoprol or eflornithine, where the former can cause severe adverse effects including reactive heart failure, encephalopathy, and even death (Balasegaram et al. 2009; Burri and Brun 2003). Eflornithine is less toxic as compared to melarsoprol, but it is not cost-effective and is difficult to administer (de la Cruz et al. 2019). In addition, resistance to a particular drug can also develop in patients. With the serious limitations in current therapies, further research for the advancements of therapeutic management of sleeping sickness is essential and indispensable.

An estimate of ~7 million people worldwide, predominantly in Latin America, are infected with *Trypanosoma cruzi*, a causative agent for Chagas disease. The main transmission route for *T. cruzi* to humans is vector-borne through the insect triatomine bug. Currently, there are no certified DNA vaccines for the management of *T. cruzi* infection in humans due to the inadequate immune responses in the infected host. Nonetheless, recombinant protein vaccines, Tc24 and its variants, are being administered to test their potential in mice where they have shown their

efficacy to elicit a heightened immune response to combat and control the elevated infection (de la Cruz et al. 2019).

8.3 Medically Important Ciliates

8.3.1 Sporozoa

The genus *Plasmodium* is the prototype of this class and causes malaria. It is found in poverty-stricken tropical and subtropical areas worldwide and affects ~3.3 billion individuals causing approximately 1.1 million deaths annually. Six species of *Plasmodium* are accountable for occurrence of malaria in human hosts (Garrido-Cardenas et al. 2019; Sutherland et al. 2010). Amongst them, *P. falciparum, P. vivax*, and *P. falciparum* are the chief sporozoans that invades both young and older erythrocytes. Globally, treating drug-resistant infections caused due to *P. falciparum* presents a unique challenge. While *P. vivax*, *P. ovale*, and *P. malariae* infect only the mature erythrocytes. quinine is the recommended treatment for severe malaria, and artemisinin derivatives are also used (Paddon et al. 2013). Primaquine is the only drug against *P. vivax* infection and is known for its adverse effects in inhabitants with glucose-6-phosphate dehydrogenase insufficiency (Von Seidlein et al. 2013; Krishna and Kremsner 2013). The present drugs for malaria treatment have been allied with acquired drug-resistant parasites (Krishna and Kremsner 2013; Ariey et al. 2014). Thus, there is an urgent requirement for the development of novel antimalarial drugs that are effective against multidrug-resistant (MDR) parasites.

Vaccination has been the key to reduce the adverse effects of many human infectious diseases and has even led to the eradication of few. The WHO has recommended for RTS,S/AS01 malaria vaccine. "Mosquirix" has been developed as a vaccine and is given to children aged between 6 weeks and 17 months to protect against malaria caused by the parasite *P. falciparum*. This vaccine has shown 30% efficacy in severe disease cases. Recent studies from Africa indicated a significant reduction of ~70% in malaria, if the vaccine was given in combination with an antimalarial medication (Cotton 2020).

Advances in vaccine technology can impact transmission and occurrence and enable targeted management of sexual and oocyte stages. Preerythrocytic and erythrocytic vaccines that can aid in reducing transmission rate have been designed. The accelerated costs for a successful immunization plan and long-scale vaccine production method, allocation, and deliverance are the key obstacles in the expansion of subunit malaria vaccines. However, plant-based expression systems offer significantly reduced costs, enhanced efficacy, and amplified scalability. Till now, expression stratagem has been standardized in plants for antigens against *Plasmodium* to elicit a substantial immune response in mice.

Consequently, plant bioreactors present excellent opportunities for developing commercial vaccines. They help to attain a high expression level of recombinant genes, will be able to rapidly design and easily produce new antigens in response to unique pathogen subtypes, and, lastly, identify the genes to be transfected and

warrant the safety of the produced proteins for use in both animals and humans. Additionally, proficient malaria antigen expression in the chloroplast of lettuce and nuclear transformation in tobacco and seeds of *Arabidopsis thaliana* for oral immunization are landmark improvements that permit the oral administration of subunit vaccines in combination with an adjuvant (Laguía-Becher et al. 2010; Lau et al. 2010). These conclusions provide a rationale for the advancement of a plant-derived oral vaccine against infectious malaria.

8.4 Other Coccidian Parasites

Toxoplasma gondii is another protozoan causative agent for toxoplasmosis. Over 25–30% of the global population is affected with it (Baril et al. 1999). It has both definite and intermediate hosts, where the former is cat and the latter host is human. Its vertical transmission can infect the fetus through mother (Lima and Lodoen 2019). Toxoplasmosis can have complications in the immunocompromised individuals, e.g., AIDS, with fatal consequences (Luft and Remington 1992). The global burden indicates a shocking increase in congenital toxoplasmosis (1.2 million DALYs) (Torgerson and Mastroiacovo 2013). The current treatment approach for toxoplasmosis is quite inadequate. Therapy for severe diseased forms may consist of a combination of pyrimethamine and sulfonamide. For prevention of transmission to fetus in a pregnant female, spiramycin and leucovorin are often prescribed. Severe side effects to pyrimethamine combinations accentuate the requirement for developing alternative therapeutic approaches (Farthing et al. 1992).

8.5 Helminthes

Helminthes are common parasitic worms. They have the high global morbidity and mortality as quite often they are the root source of anemia and malnutrition-related ailments in individuals, with few that are life-threatening. Helminthes can penetrate the host body via the skin, mouth, and respiratory tract through inhalation of airborne eggs. The helminthic parasites are categorized into three main classes—Trematodes (flukes), Cestodes (tapeworm), and Nematodes (roundworms).

8.5.1 Flukes

Flukes reside in the alimentary canal, liver, bile duct, ureter, and bladder of craniate animals. Depending on the sites they enter, flukes are categorized into four groups: intestinal flukes, blood flukes, lung flukes, and liver flukes. Blood flukes like *Schistosoma* cause schistosomiasis bilharziasis, liver flukes are commonly known as *Fasciola hepatica*, and also sheep liver fluke is a familiar and globally distributed parasite. Various studies reported that immune response elicited by orally administered plant-based vaccine that expresses the recombinant cysteine protease

against *F. hepatica* metacercaria infection in rats shows significant effects (Kesik-Brodacka et al. 2017).

8.5.2 Nematodes

This class includes the filarial worms—the guinea worm (*Dracunculus medinensis*) and *Trichinella spiralis*. Filariasis is an infectious disease commonly seen in tropical climates. The filarial worms reside in the lymphoid immune system and the subcutaneous tissues of human and can lead to lymphedema (fluid retention) or hydrocele (swelling in the scrotum). The microfilariae form the early stage in the life cycle of nematodes (facultative parasite), and the adults live in the tissue or the circulatory system of vertebrates (definitive hosts) where these develop into filariform larvae forming the infective stages. Humans get infected by *Wuchereria bancrofti*, another filarial worm that resides in the host lymph nodes and lymphatic vessels and leads to lymphatic filariasis. Around 1.5% of the global population is reported to be infected (Katiyar and Singh 2011). According to the WHO, over 880 million people are presently at the threat of acquiring lymphatic filariasis (LF) in over 52 countries worldwide. Though medication can kill the worms, prevent them from spreading the infection to someone else, and reduce the symptoms of filariasis (Chavda et al. 2021), current approaches to control LF are short of the anticipated goal. The complex behavior of this parasite is evident as yet there is no single vaccine for filariasis.

8.6 Cestodes (Tapeworms)

Worldwide prevalence of taeniasis is caused by tapeworms (*Taenia saginata*, *T. solium*, and *T. asiatica*). *T. solium* is frequently reported in underdeveloped communities with deprived sanitation facility, mostly where people have adapted to eating raw food, especially undercooked pork. A disease called cysticercosis occurs due to the ingestion of *T. solium* eggs. The adult tapeworms are generally delivered to host via portal blood supply to the various organs such as lungs, liver, and brain, which causes extraintestinal diseases. In rare cases, it can also lead to intestinal blockage and thinning and shortening of the intestinal ducts like bile duct or pancreatic duct that can be fatal (Gonzales et al. 2016). The frequent management of tapeworm infection involves oral medications including praziquantel (Biltricide), albendazole (Albenza), nitazoxanide (Alinia) that target the adult tapeworm (Lloyd et al. 2014).

Development of effective delivery systems for vaccines is a priority in vaccinology. Transgenic papaya callus lines expressing the components of the S3Pvac vaccine constitute a stable platform to produce an oral vaccine against cysticercosis caused by *T. solium* or *T. crassiceps*. The parasitic disease adversely affects human health and acquires a lethal form when the cysticerci are blocked in the central nervous system of the infected host, causing neurocysticercosis. Here,

pigs are known as the obligate intermediate hosts to complete the parasitic life cycle. Vaccination of pigs significantly amplifies the antibodies and triggers mononuclear cell proliferation that can help in curbing human transmission by reducing cysticercosis in pigs. S3Pvac-papaya vaccine is under evaluation for understanding the cost-benefit of developing a delivery system and also a dose range (Fragoso et al. 2017).

Diseases caused by parasitic protozoans are responsible for ill-health and put an immense social and economic burden particularly in tropical areas of the world. To add to the woes, these parasitic pathogens acquire drug resistance, and they recur with superior virulence. The unavailability of an accredited vaccine for several human parasitic diseases, collectively with a paucity of inexpensive, secure, and operative drugs for some diseases, or challenges posed by parasite drug resistance necessitate steering an exploration for new anti-parasitic agents. Vaccination to control helminthes has been an important part of an integrated veterinary and public health policy. Attempts are being made to recognize more efficient, economical, and effortlessly deliverable mucosal vaccines. One such research area that is presently under development focuses on genetic plant modifications for large-scale production of immune-protective proteins that has progressed dramatically over the last quarter of the century. Edible vaccines offer pertinent solutions for treating known diseases where its therapeutic management is restricted by the intrinsic constraint of traditional vaccines, like cost of production, storage problem, and expensive logistics. Sixteen foods by now are known for producing antigens to counter human and animal diseases. However, using plants for generating drugs against human parasitic diseases will be extremely important. In summary, to minimize the upsurge of infectious and parasitic diseases globally, edible vaccines have the potential to mitigate and avert parasitic diseases in countries where conventional vaccination approach is still inadequate.

8.7 Future Potential

Plant-based vaccines are emerging as a novel alternative to traditional vaccines with greater therapeutic potential to treat infectious and parasitic diseases. A transient and stable gene expression has been achieved with the aid of genetic engineering. Chloroplast transformation via particle bombardment gene delivery method or through biolistic has been deliberated upon as a promising alternative for improving the production of plant-based vaccines. Nevertheless, efficient and optimum vaccine production requires continued development and improvement of suitable gene delivery methods. The bioethical issues arising from the production of plant-based vaccines include the risk of transferring the allergens from transgenic plants to humans and animals. As virus and bacteria are used as the vectors to produce plant-based vaccines, the pathogens might be reverted to its pathogenic form and infect other organisms. The benefits of plant-based vaccines will overwhelm the challenges faced by this interesting biological product. Thus, it is anticipated that regulatory approvals will be granted ultimately to help in the global disease control. There is an urgent need to generate bio-pharmed vaccines that can respond to the

sudden outbreaks of emerging parasitic diseases. Increasingly, the generation of plant-based "bio-betters" opens novel pathways to facilitate biopharming that is rapid, safe, and easily scaled up to manufacture high-value biopharmaceuticals. In view of the enhanced development of plant-derived vaccines, regulatory agencies must advance their knowledge about the latest advanced emerging technology and adapt accordingly.

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Nanotechnology: Its Usages in Drug Delivery for the Treatment of Human Parasitic Diseases

9

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Abstract

The occurrence of infectious parasitic disease is the leading driver of mortality worldwide. Treatment of such parasitic disease is challenging due to the minimal target bioavailability of antiparasitic drugs, poor cellular uptake, nonspecific distribution at the target site and rapid elimination from the body. Further antiparasitic drug toxicity and prolonged therapeutic regimens also concerns us. Leading trends in nanotechnology can overpower these shortcomings in the form of an ideal nanocarrier system that can be designed and fabricated accordingly, where new formulations and the existing antiparasitic drugs in nano-sized delivery vehicle can be more promising in terms of minimized non-specific drug accumulation, desired antiparasitic drug availability at the site of action, reduced therapeutic dose and duration that is to be delivered etc. Through this chapter, we have highlighted the major challenges of conventional treatment approaches and presented nanotechnology as an imminent alternative treatment approach for the infectious parasitic disease. However, the unification of these two-research areas as "nano-antiparasitic medicines" can progress as a therapeutic strategic plan, minimising the burden of individuals suffering from this worldwide.

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_9

Keywords

Nano carrier · Antiparasitic drugs · Nano-antiparasitic medicines · Therapeutic regimens

Abbreviations

AmB	Amphotericin B
Aphi	Aphidicolin
Ber	Berberine
CR	Curcumin
Lip	Liposomes
NE	Nano emulsion
NLCs	Solid lipid nanoparticles
NPs	Nanoparticles
PDT	Photodynamic therapy
PEG	Polyethylene glycol
PLGA	Poly(lactic-*co*-glycolic acid)
PZQ	Praziquantel

9.1 Introduction

Infectious parasitic diseases are of major concern to public health worldwide. Their occurrence and pathological prevalence are closely related to geographic and socio-economic factors (Cable et al. 2017). However, in India, their pervasiveness is determined by climate change, as such changes turn on favorable condition for the spread of vectorborne infectious parasitic diseases, it accounts for 17% of all infectious diseases. The most common vector-borne infectious parasitic diseases are visceral leishmaniasis, malaria, dengue, chikungunya, Japanese encephalitis, lymphatic filariasis etc. (Leal Filho et al. 2022). Further, the causative agent (pathogen) for infectious parasitic diseases is usually known as parasites. These are broadly been classified as eukaryotic organisms ranging from single cellular protozoans, to large multicellular helminths responsible for severe disease onset in both animals and the human population. The various class of parasites that cause disease in the human population are protozoans, helminths, ectoparasites etc. Amongst protozoans there is subdivision based on their mode of movement this includes Sarcodina (*Entamoeba*); Mastigophora (*Giardia* and *Leishmania*); Ciliophora (*Balantidium*); Sporozoa (*Plasmodium, Cryptosporidium*). Similarly, sub division inhelminths is based on their shape this includes platyhelminths (Trematodes (flukes) and Cestodes (tapeworms)); acanthocephalins (worms reside in the gastrointestinal tract); nematodes (worms reside in the gastrointestinal tract, blood, lymphatic system or subcutaneous tissues). Under protozoan parasites severe health illness in human is

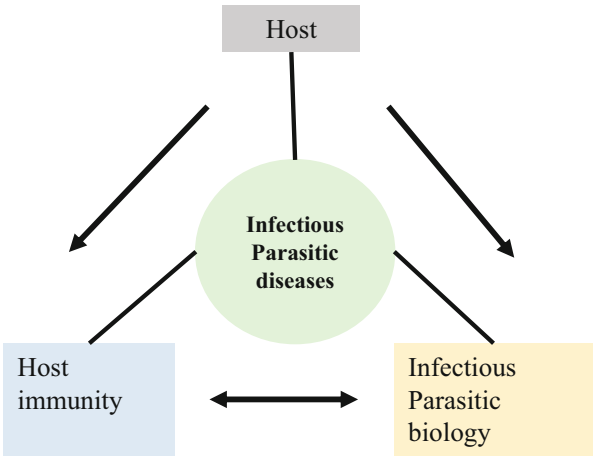

Fig. 9.1 Schematic representation of host parasite interaction

majorly caused by genus *plasmodium, entamoeba*, *acanthamoeba, leishmania, trypanosoma* and *toxoplasma* (David Sibley 2011). While for helminths the human health complications are mainly related to genus of *ascaris, schistosoma* and *tenia* (Jiménez et al. 2016). For host-parasite interaction (Fig. 9.1).

The conventional treatment approach for controlling infectious parasitic diseases is dependent on the currently available antiparasitic drugs. Major issues with the conventional treatment approach are due to its insoluble nature, shorter half-life, and minimized bioavailability of antiparasitic drugs to the target site. However, for enhanced effective therapeutic response frequent long-term booster dosage is required based on parasitic life cycles. Such treatment repetitions might introduce deleterious consequences such as stress, drug resistance, etc. (Vercruysse et al. 2007).

To overcome such limitations there is a prerequisite need for novel therapeutic approaches in the form of nanotechnology, integration of nanotechnology with parasitic disease management can design and fabricate nanomedicines with nanoparticles ranging from 1 to 1000 nm. It can have a substantial impact on parasitic diseases and its presence can aim for enhancing the efficacy of antiparasitic drugs at the target site. There are various types of nanomaterials such as organic nanocarriers that are made from desired synthetic or natural polymers, cholesterol, phospholipids, solid lipids etc. They can be designed in the form of nanospheres, nanoparticles, micelles etc. Other than this inorganic nanocarriers like metallic and non-metallic nanoparticles are also used (Sun et al. 2019). The loading of antiparasitic drugs into the nanocarrier system is a physical or chemical reactive event which occurs through adsorption, encapsulation and conjugation process. Further, its release at the target site can be a sequential event that might occur through desorption, dissolution or degradation of antiparasitic drug from nanocarrier system. These nanocarriers can easily infiltrate into the biological system where it can shield the antiparasitic drug from enzymatic degradation causing sustainable, controlled release and accumulation of antiparasitic drugs at target site etc. (Negi

et al. 2013; Das and Chaudhury 2011; Chen et al. 2015). However, effectiveness of therapeutic approach dose not completely depend on type of nanocarrier system and the properties of drug it also depends on the route of administration etc. (Chen et al. 2015, 2017). At present, nanoparticles that have been explored so far exhibit the forthcoming potential for development of "nano-antiparasitic medicines" further it also highlights its other broad developmental aspects of antiparasitic drug delivery application.

9.2 Nanoparticles Physiochemical Characteristics and Its Effect on Activity of Antiparasitic Drugs

The physico-chemical characteristics of nanoparticles has an effect on the activity of antiparasitic drugs. Enhanced efficacy of antiparasitic drugs is also dependent on the size of nanoparticle as it plays crucial role in transportation of antiparasitic drugs and distribution in an in vivo system. Thus, can be assumed to have diverse inhibitory effect on parasites. Work led by Liu and colleagues have determined that variation in the size of radiolabelled liposomes will alter the distribution in an in vivo mouse model, where in the bloodpost 4 h treatment 60% of liposomes in the size range of 100–200 nm were identified and small size nanoparticles were easily eliminated through excretion. Antileishmanial effects of gold nanoparticles of smaller size was also reported in an in vitro system (Liu et al. 1992; Want et al. 2021). Hence, selection of an appropriate size can be promising for longer retention and sustained targeted distribution of antiparasitic drugs. Therefore, optimization of the nanoparticles based on the shape and size can enhance the cellular entrance ability thus, can be promising for treating intracellular parasitic infections. Similarly, surface charges can also have an effect on the activity of antiparasitic drugs. Surface hydrophilicity or hydrophobicity can influence the kinetics in an in vivo system, its impact on protein binding extents will enable easier attaining of the estimated distribution and kinetics, modification with a desired polymer like polyethylene glycol (PEG) will increase nanoparticles surface hydrophilicity. It can significantly prolong the residence time, half-life and bioavailability of antiparasitic-loaded nanoparticles (Kumar et al. 2017; Pensel et al. 2015; Fülöp et al. 2018). Therefore, such modification of nanoparticles can be an important parameter for satisfactory sustained-release antiparasitic drugs (Fig. 9.2).

9.3 Nano-Assisted Therapeutic Regime for Parasitic Disease

Current advancements and innovations in the nanotechnology field have been extensively studied in parasitic diseases, to overcome the several frailties associated with conventional diagnosis and therapeutics. As such, anti-parasitic drug delivery systems gained attention to ameliorate their bioavailability, controlled release and intracellular penetration activity. Therefore, drug-loaded nanoparticles hold significant promise to enhance efficacy and reduce the dose and side effects of drugs. This

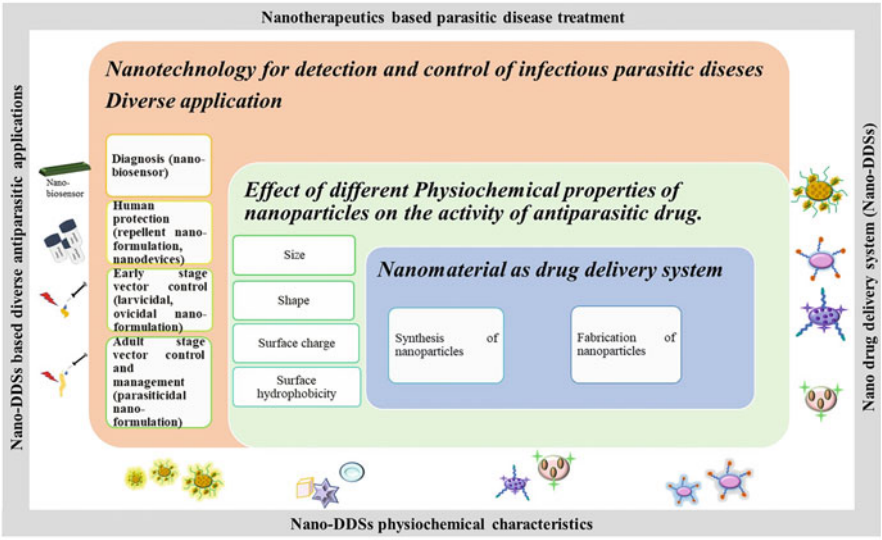

Fig. 9.2 Schematic representation of the effect of nanoparticles physiochemical properties on the activity of anti-parasitic drugs

section focuses on a variety of nanoparticles enlisted in Table 9.1 used in drug delivery systems and therapeutics diagnostics include liposomes, polymeric nanoparticles, SLNs, nanosuspensions, metallic nanoparticles and others (Fig. 9.3).

9.3.1 Liposomes

Liposomes are small artificial vesicle consisting of two or more lipid bilayer, which immobilized the drugs in bilayer or inner core when employed as a nano-carrier system, facilitates the targeted, controlled release of drug with decreased cytotoxicity. Currently, Calvo et al. constructed berberine (ber) loaded liposomes against *Leishmania infantum* infected Balb/c mice. This study demonstrated that Ber-loaded liposomes exhibited higher biocompatibility by increasing its selective index more than sevenfold in macrophages as confirmed by in vitro cytotoxicity assay and reduced parasitic burden in liver and spleen when compared to free drugs (Calvo et al. 2020; Frezza et al. 2013). The evaluation of praziquantel loaded liposomes (Lip-PZQ) on *Schistosomiasis mansoni* (BH strain) showed the following dose for 45 days to mice model decreased the parasitic egg counts and worm in intestine and liver as compared to free drugs. Therefore, this formulation enhanced the bioavailability in host organisms via targeted delivered to liver site where it is absorbed by the tegument of *S. mansoni* (Frezza et al. 2013). Additionally, Voak et al. studied the biodistribution and pharmacodynamics of Amphotericin B encapsulated liposome nanoparticles (AmBisome) at different stages of *Visceral leishmaniasis* infected Balb/c mice. It has been reported that a higher dose (10 mg/kg) of AmBisome

Table 9.1 Tabular representation of different nanoparasitic formulations studied in different parasitic diseases

Nanoparticles	Modification	Drugs	Parasite	Experimental model	Inference	References
Niosomes	–	Praziquantel (PZQ)	*Schistosoma mansoni*	*Biomphalaria alexandrina* snail host and mice infected with *S. mansoni*	PZQ-encapsulated noisome are capable of successfully overcoming the tolerance of *S. mansoni* to PZQ in mice infected with cercaria with decreased sensitivity to PZQ	Amer et al. (2022)
Liposomes	*p*-Aminophenyl-α-D-Mannopyranoside	Andrographolide	*Leishmania donovani*	Hamster Balb/c peritoneal macrophages	Targeted drug delivery to phagocytic macrophage, were found to be most effective in lowering the parasites load in the spleen as well as in lowering the hepatic and renal toxicity	Sinha et al. (2000)
Nano-emulsion	–	Curcumin	*Toxoplasma gondii*	Acute and chronic toxoplasmosis infected Balb/c mice	CR-NE possessed the enhanced anti-toxoplasma activity in both acute and chronic phase, by eliminating the latent bradyzoites in the brain	Azami et al. (2018)
Solid lipid nanoparticles	Heparin	Chloroquine	*Plasmodium falciparum*	Chloroquine-sensitive (CQS) D6 and chloroquine-resistant (CQR) W2 strains	Collaborative effect of CQ-loaded heparinized solid lipid nanoparticles (Hep-SLN), meaning that combining heparin and CQ in solid lipid nanoparticles has useful	Muga et al. (2018)

					effects, including potential for specific targeting of parasitized red blood cells as afforded by heparin by killing 50% of population	
Gold nanoparticles	Glucose	Ciprofloxacin	*Plasmodium falciparum*	Asynchronous intracellular *Plasmodium falciparum* and asexual stages of *Plasmodium falciparum* in blood	Ciprofloxacin-loaded nanoparticles rendered the moderate antimalarial drug more water-soluble, enhanced its effectiveness against *Plasmodium falciparum* through a combined effect of selective targeting, leading to accumulation of drug on the parasite, combined with better hydrophilicity to prevent the formation of drug masses	Varela-Aramburu et al. (2020)
Chitosan–gold nanoparticles	Protein-A	–	Cystic Echinococcosis	Immuno-dot-blot assay (biosensor)	This biosensor signal intensity was proportional to the amount of active anti-*Echinococcus granulosus* antibodies present on the surface of nanoparticles, antibodies titre in the sera samples, and amount of Ag B	Safarpour et al. (2021)

(continued)

Table 9.1 (continued)

Nanoparticles	Modification	Drugs	Parasite	Experimental model	Inference	References
					coated on the nitrocellulose membrane	
Gelatin	Mannose	Amphotericin B	*Visceral leishmania*	J774A.1 macrophage cells	AmB loaded f-GNPs exhibited remarkable anti-leishmanial activity and promising carrier for specific delivery of AmB to macrophages for effective treatment of VL	Nahar et al. (2010)
Copper oxide		Albendazole	*Setaria cervi*	Filarial parasite *Setaria cervi*	CuO NPs as a effective adjuvant with ABZ against filariasis along with increased antifilarial activity of nanocomposite under the UV light by increased ROS production and decrease of parasitic-GST and GSH levels were detected and as well DNA fragmentation	Zafar et al. (2016)
Poly-DL-lactic-*co*-glycolic acid (PLGA)	Chitosan	Albendazole sulfoxide	*Echinococcus granulosus*	Cystic echinococcosis infected mice	ABZ-SO-loaded CS-PGLA NPs therapeutic effect of ABZ-SO-loaded CS-PGLA NPs in the weight and volume of cysts were statistically significant when	Darvishi et al. (2020)

					compared with that in the control group ($p < 0.05$)	
Polymer nanoparticles (polycaprolactone)		Amphotericin B	*Leishmania tropica* KWH23 and *Leishmania donovani*	Leishmania infected macrophages	Anti-leishmanial activity of Amp B was significantly enhanced by macrophage targeting through drug-loaded formulations for the inhibition of intracellular parasites. Maximum parasite inhibition was provided by prepared drug-loaded formulation for anti-leishmanial activity against infected macrophages	Saqib et al. (2020)

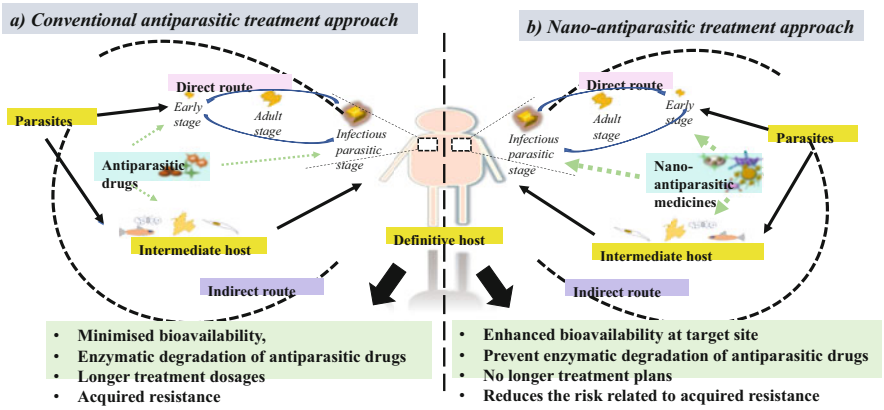

Fig. 9.3 Schematic representation of (**a**) conventional antiparasitic treatment approach, (**b**) nano-antiparasitic treatment approach [line in green color show effectiveness of anti-parasitic drugs while the line in green color with increased width shows enhance effectiveness due to the existence of nanocarriers as a drug transfer system for antiparasitic drugs distribution to target site]

kills parasite within the 48 h of 14 days of infection while drug potency was decreased when administered after 35 days of advanced infection. Drug accumulation distribution was found to be lower in spleen and liver on 35 days when compared to 14 days. This finding suggested that organ enlargement and other physiological factors are responsible for the distribution and potency of single dose of AmBisome (Voak et al. 2017). Moreover, Khodabandeh et al. demonstrated combinatorial therapy against resistant visceral leishmaniasis on 2-year-old boy. The patient was given treatment with liposomal amphotericin B, allopurinol and interferon gamma for 3 weeks. So, therefore together this treatment accelerated the complete cure. This study further suggested to identify resistant species of leishmania along with their response to different treatment (Khodabandeh et al. 2019).

Apart from other, liposomes play a lead role in providing specific and controlled distribution, toxicity reduction, prolonged circulation and minimize side effects of anti-parasitic drugs. Their potency is further increased through modification of the surface by coating with suitable moieties.

9.3.2 Solid Lipid Nanoparticles (SLNs)

Solid lipid nanoparticles (SLNs) are solid core lipid nonvehicle rapidly developed in current years. Mainly it is based on synthetic or natural lipids as a platform to endow drugs adsorption and encapsulation. The SLNs consolidate the advantages of conventional colloidal nano-carriers such as liposomes, oil-in-water emulsions and polymer nanoparticles, i.e., facile mass productions, highly compatible and degradable at physiological conditions. Currently, Khosravi et al. synthesized mannosylated functionalized solid-lipid nanoparticles loaded with paromomycin

(PM-SLN-M) to combat acute toxoplasmosis. Although PM has high killing rate of intracellular *Toxoplasma* in low amount in comparison to PM-SLN-M but it possessed low cytotoxicity on Vero cells. This study suggested that PM-SLN-M exhibited remarkable activity against Toxoplasma without harming the cells of the host (Khosravi et al. 2020). 5-Hydroxy-3methyl-5-phenyl-pyrazoline-1-(*S*-benzyl dithiocarbazate) (H2bdtc) loaded solid lipid nanoparticles (H2bdtcSLNs) to target *Trypanosoma cruzi*. Comparative study of benznidazole and H2bdtc-SLNs from in vitro and in vivo outcomes revealed that H2bdtc-SLNs mediate the parasitaemia reduction in mice compared to benznidazole. This study concluded that H2bdtc-SLNs formulation prevent inflammation and lesion in the liver and heart, which enhanced overall survivability in *T. cruzi* infected mice (Carneiro et al. 2014). Moreover, Radwan et al. prepared formulation of praziquantel solid lipid nanoparticles (SLN-PZQ) against murine *S. mansoni* infection. The SLN-PZQ demonstrated superior anti-schistosomal activity along with significant bioavailability and sustained release of drug in advanced *Schistosoma mansoni*-infected groups were showed remarkable inhibition and reduction of worm population in both hepatic and intestinal tissue in comparison to free PZQ (Radwan et al. 2019).

In brief, the anti-parasitic activity has not been predominately studied. Therefore, SLNs offers promising substitute besides some other nanoparticles by facilitated the sustained drug release and specific targeting.

9.3.3 Nanosuspensions

Nanosuspensions are defined by very fine submicron-dispersed colloid of solid drug nanoparticles stabilized by surfactants in aqueous medium. The potential advantages of nanosuspension offer efficient solubility, absorption, dissolution percentage and rate of drugs as well as mediates the prolonged release and reduces the systematic toxicity of drugs. It is an ideal operation to use nanosuspension apart from conventional dosage due to its affordable cost, high loading capacity, facile production and negligible adverse effects. In a study, Kayser prepared aphidicolin-loaded nanosuspensions (Aphi-loaded NSs) against leishmania-infected macrophages. This finding suggested that Aphi-loaded NSs easily phagocytosis by murine macrophage via passive target and enhanced the anti-leishmania activity approx. 140 times. This indicated that nanosuspension increased the payload of drugs and augment the sustained release of drugs to the infected site (Kayser 2000). Recently, curcumin-nanoemulsion (CR-NE) was synthesized to treat acute and chronic toxoplasmosis in mice. This study revealed that CR-NE possessed the significant anti-toxoplasmosis by inhibiting the growth of tachyzoites in Peritoneum were observed in both acute and chronic phase compared to curcumin (CR). In addition, decreased in cyst count were determined by the downregulation of BAG1 were maximum in CR-NE treated mice than CR (Azami et al. 2018). Zarenezbad et al. study the leishmanicidal activity using nanoemulsion-based nanogel *Citrus limon* essential oil against *Leishmania tropica* and *Leishmania major*. This finding suggested that the toxic effect of *Citrus limon* was found to be more significant compared to *Mentha*

piperita, *Anethum graveolens*. Moreover, an 80 μg/mL concentration of CLN gel responsible for the complete inhibition of both species of leishmania in in vivo mice model (Zarenezhad et al. 2021).

9.3.4 Polymeric Nanoparticles

Polymeric NPs are solid colloidal suspensions consisting of natural and synthetic polymers used for nanosized drug delivery system. NPs allows therapeutic agents could be encapsulated, entrapped, dissolved and conjugated to their surface. For the drug delivery, polymeric NPs can be employed in variety of forms such as in NPs, nanospheres, or nano-capsules based on preparation methods and their physiochemical properties. In the golden age of pharmaceutical nanocarriers, polymeric NPs have been considered for sustained drug release, and targeted delivery to specific organs and tissues and due to such versatile nature, it offers multiple cargo (proteins, peptides and genes) delivery.

Currently, Elmi et al. synthesized biogenic Chitosan nanoparticles from *Penicillium* fungi to target human protozoal parasites—*Giardia lamblia*, *Plasmodium falciparum* and *Trichomonas vaginalis*. This study demonstrated that nano-chitosan exhibited excellent anti-parasitic activity by inhibiting the growth rate of cultivated *P. falciparum, T. vaginalis* and *G. lamblia* by 59.5%, 99.4%, and 31.3%, respectively with negligible toxic effects. This finding concluded that green synthesized Chitosan nanoparticles combat parasitic infection based on dose-dependent (Elmi et al. 2021). Although, polymer-based nanoparticles are extensively used to carry the drugs for intracellular parasites, for example, amphotericin B for *Leishmania* as reported in Asthana et al. (2015), and chloroquine and artemisinin for intracellular targeting of Plasmodium (Tripathy et al. 2013). In addition, layered assembly of gelatin nanoparticle encapsulated phthalocianato [bis(dimethylaminoethanoxy)] silicon (NzPC) and modified with polyelectrolytes (polystyrene sulfonate/polyallylamine hydrochloride) [PGN-NzPc] for PDT (photodynamic therapy) implementation in combating promastigote form of *Leishmania amazonensis*. The PGN-NzPc facilitated the lower toxicity in the dark while in the presence of PDT trigged the 80% killing of *Leishmania* promastigotes by altering the morphology (de Souza et al. 2021). Further Lima et al. prepared lignan (−)-6,6′-dinitrohinokinin (DNHK) loaded into poly(lactic-*co*-glycolic acid) nanoparticles (DNHK-loaded PLGA) against *Schistosoma mansoni*. This study concluded that DNHK-loaded PLGA NPs augmented the sustained release of DNHK to the infection site and showed remarkable potency in killing 100% population of adult worms. This DNHK-PLGA NPs have enhanced anti-schistosomicidal activity (Lima et al. 2017).

9.4 Future Perspective and Conclusion

Despite the quick expansion of parasitic diseases worldwide, exciting therapeutic approaches are essential to manage these diseases. But the existing conventional drugs for treating parasitic diseases are outdated, weak, ineffective with deadly toxicities, adverse effects and inducing elevated resistance to disease-causing pathogens. Various reports of re-purposing of conventional drugs via nano-formulations have shown significant responses by reducing their toxicity and enhancing the therapeutic efficacy coupled with low cost. Since, reports suggest that nanoformulations will increase efficacy by higher targeting efficiency, site-specific delivery, and enhanced bio-availability of the drug at the disease site. Therefore, more emphasis is to be given to systematic investigation and research related to the selection designing and fabrication of nanocarriers. Here, the physiochemical characteristics of nanoparticles can be a conclusive factor playing a decisive role in determining the effectiveness of nanocarriers in delivering antiparasitic drugs at a desired locus with a hope for improved pharmacokinetic and pharmacodynamic properties. Moreover, the research method with powerful technologies together can advance the treatment approach with more precision in in vivo research and clinical studies. Currently, no nano-formulations of anti-parasitic drug is available in the marketplace, lots of them are still in the process of better clinical trials, formulation designs, preclinical studies, and commercialization under the name of "antiparasitic nanomedicines". With constant efforts, efficient therapy will progress as an inevitable trend in pharmaceutical industries with antiparasitic nanomedicine shaving an infinite future against parasitic diseases controlling their spread.

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Therapeutic Potential of Benzopyrones Against Antiparasitic Diseases

10

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Abstract

Parasitic diseases remain a significant concern for public health in almost every part of the world. Epidemiological studies have shown that several factors i.e., environmental conditions, parasite pathogenicity, social condition, and host health are responsible for the infection of human beings and the expansion of the related diseases. In the past, many research activities were carried out on benzopyrone derivatives and proved their potential against various diseases. Benzopyrone analogs have been demonstrated effective against various parasitic

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_10

diseases. Here, in this chapter, we provided an overview of benzopyrones and discussed their promise as a treatment option for the parasitic diseases.

Keywords

Benzopyrones · Parasitic diseases · Therapeutic potential · Pathogenicity

10.1 Benzopyrone: Introduction and Types

Benzopyrones belong to a diverse class of naturally occurring and synthetic polyphenolic compounds expected to be chemically and biologically distinct. They comprised the fusion of the benzene ring **(1)** and pyrone ring **(2)** (El-Sawy et al. 2021). Benzopyrone is a core moiety on which multiple substitution sites are present, which creates diverse product classes for a broader exploration of biologically relevant chemical scaffolds (Fig. 10.1). The benzopyrone derivatives are mainly found in two forms: benzo-**α**-pyrone or coumarins (1-benzopyran-2-one), and benzo-**γ**-pyrone or chromones (1-benzopyran-4-one). In addition, flavonoids an important class of secondary metabolites also have benzopyrone skeletal within their structural network (Edwards and Howell 2000).

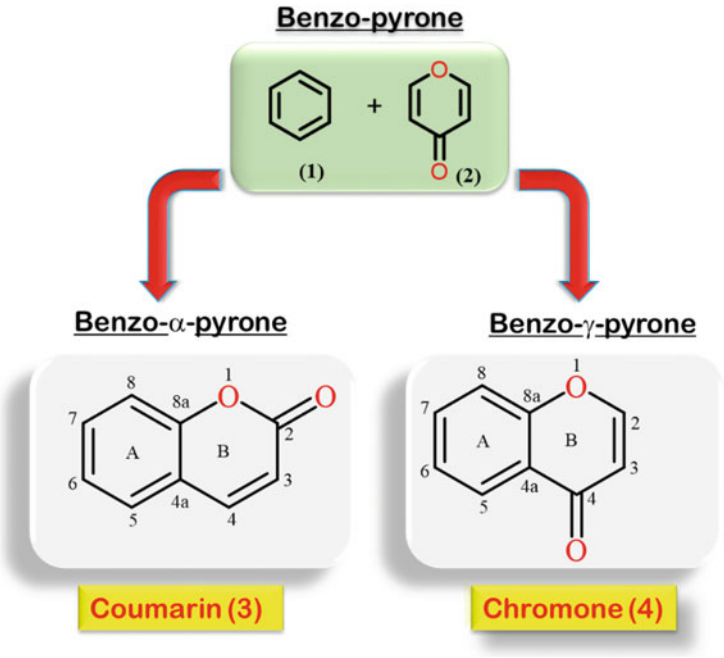


Fig. 10.1 Structural representation of benzopyrones

10.1.1 Benzo-α-Pyrone (*Coumarin)*

Coumarin **(3)** belongs to benzo-α-pyrone and is classified as 1,2-benzopyrone and systemically nomenclature chromen-2-one by IUPAC. The history of coumarins is more than 200 years old. These are a divergent class of oxygen-substituted heterocycles; coumarin and its derivatives are widely occurring secondary metabolites found as natural products in various plant families and essential oils (Murray et al. 1982). A total of 800 derivatives of coumarin are obtained from 600 genera of 100 families (Murray et al. 1982). The variety of coumarin derivatives extracted from many plants' seeds, roots, and leaves belong to the dicotyledons class of Rutaceae and Apiaceae families (Manolov and Danchev 2003). Tonka bean is a rich source of coumarin and roots of *Dipteryx odorata (*Lončarić et al. 2020*)*.. In plants, the benzene ring with pyrone ring condensation, accomplished by the Shikimic acid pathway **(**Fig. 10.2**)**, resulted in the coumarin compounds.

Based on the substitutions, coumarins are further subdivided into different groups such as simple coumarin (Hoult and Payá 1996), pyrano-coumarins (Thant et al. 2021), furanocoumarins (Rodrigues and Rodrigues 2021), phenylcoumarins (Chatterjee et al. 1976), biscoumarins (Sarmah et al. 2022), and triscoumarins (Kielesiński et al. 2019; Rubab et al. 2022). (Fig. 10.3).

Naturally occurring coumarins also show variation in the functional groups i.e., alkoxy/hydroxy coumarins (Ngoc Toan and Dinh Thanh 2020), thiocoumarins (Maresca et al. 2010), formyl/carboxy coumarins (Borges et al. 2005), arylcoumarins (Roussaki et al. 2010), halocoumarins (Soussi et al. 2011), nitrocoumarins (Sau and Mal 2021), alkylcoumarins (Mitra et al. 1980). Coumarin analogs possesses hydroxyl groups that probably facilitates their solubility in organic solvents in comparsion of water. Several methods, including maceration, reflux, ultrasonic-

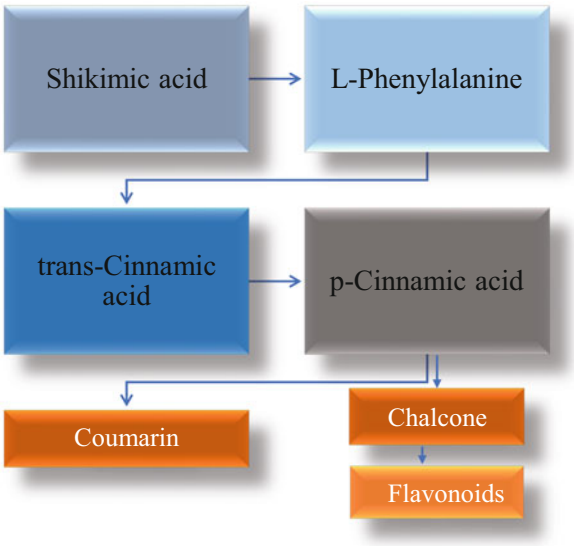


Fig. 10.2 Shikimic acid pathway for the coumarin synthesis

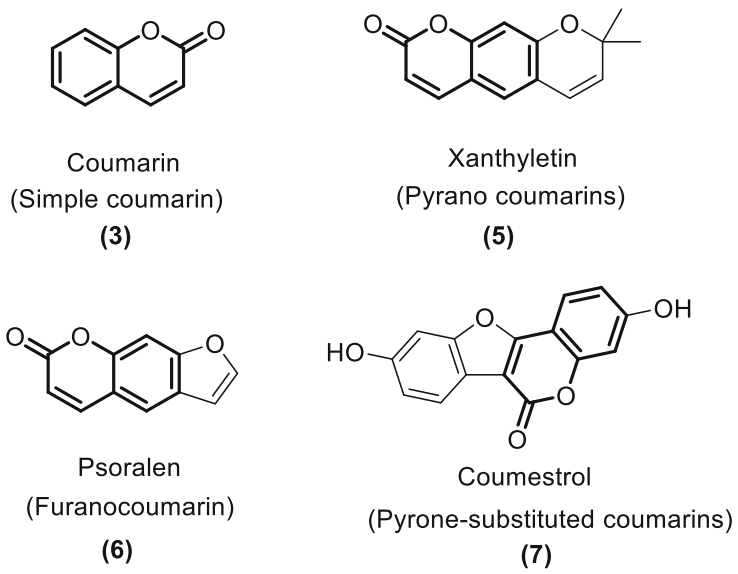


Fig. 10.3 Examples of various coumarin compounds based on structural differences

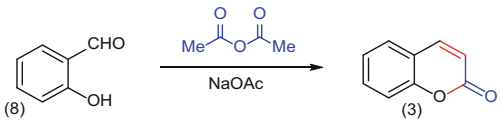


Fig. 10.4 Synthesis of coumarin molecules by Perkin reaction

assisted, and microwave extraction have been utilized to separate and purify the compounds (Lozhkin and Sakanyan 2006; Dong-wei et al. 2021; Martino et al. 2006).

In addition to a natural occurrence, the synthesis of coumarin can be achieved with well-known name reactions i.e., Perkin, *von*-Pechmann, Knoevenagel, Reformatsky, and Wittig reactions (Lončarić et al. 2020; Molnar et al. 2020).

10.1.1.1 Perkin Condensation Reaction

The most popular way to make coumarins includes the Perkin reaction i.e., reaction of aromatic aldehyde (*o*-hydroxybenzaldehyde) and acid anhydride. Several reports in the literature suggest the synthesis of coumarins by this method (Fig. 10.4) (Augustine et al. 2012; Calcio Gaudino et al. 2016; Francisco et al. 2019).

The classical method for synthesizing coumarin from salicylaldehyde and acetic anhydride in the presence of a base is ubiquitous. The yields can be improved by using anhydrous sodium fluoride (Banothu et al. 2014) or dibenzo-18-crown-6 (Zacharis et al. 2008) as the catalyst.

10.1.1.2 Pechmann Reaction

Pechmann reaction is a valuable method for coumarins preparation which involves the condensation of phenols with different β-ketoesters (Heravi et al. 2014; Potdar et al. 2001). When the starting material is replaced with acetoacetic esters and their derivatives, the reaction is termed as Pechmann-Duisberg reaction (Fig. 10.5) (Bulut and Erk 1996).

This method is mainly used to synthesize various naturally occurring coumarins for biological and industrial purposes (Khan et al. 2016).

10.1.1.3 Knoevenagel Reaction

Knoevenagel reaction is the condensation of aldehydes with active methylene compounds in the presence of ammonia or different primary and secondary amines. A suitable combination of carboxylic or Lewis acids and amines is necessary for the presence of a catalytic amount of weak base (Fig. 10.6) (Vekariya and Patel 2014; Bogdał 1998).

Coumarins are mainly used for the fragrance in food and cosmetic products (Wisneski 2001). Coumarin compounds have a vast array of biological activities such as antiparasitic (Di Pisa et al. 2017), antileishmanial (Brenzan et al. 2007; Muzitano et al. 2009), antifilarial (Tripathi et al. 2000), anticoagulant (Abdelhafez et al. 2010), anti-inflammatory (Selim and Ouf 2012; Kontogiorgis and Hadjipavlou-Litina 2005; Silván et al. 1996), antibacterial (de Souza et al. 2005; Lee et al. 2003; Qin et al. 2020), antioxidant (Borges Bubols et al. 2013; Yu et al. 2005), anti-tumour (Küpeli Akkol et al. 2020; Liu et al. 2014; Cao et al. 2016; Majnooni et al. 2019; Wu et al. 2020), antiviral (Hassan et al. 2016; Mishra et al. 2020) and enzyme inhibition (Monczor 2010). Coumarins are also being used in the field of drug delivery by employing a pro-drug strategy. In this approach, the modification in the unwanted physico-chemical parameters of the drug molecule resulted in the drug's approach to its target site of action.

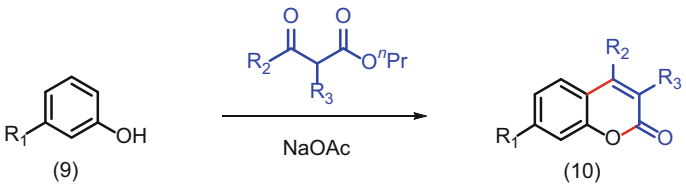


Fig. 10.5 Coumarins synthesis by Pechmann reaction

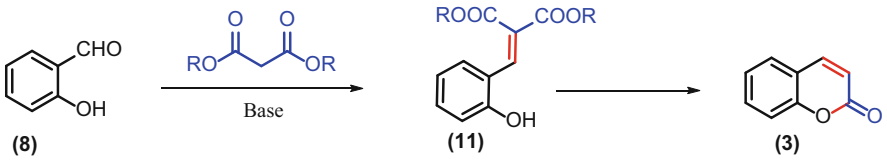


Fig. 10.6 Synthesis of coumarins by using Knoevenagel reaction

10.1.2 Benzo-γ-Pyrone (Chromone)

Chromones **(4)** and coumarins are isomers with a substituted keto group on the pyran ring of chromones. The term chromone originates from the Greek word chroma, which denotes "color" and shows that most chromone and its derivatives have a wide range of colors. Chromones are oxygen-containing heterocyclic chemical scaffolds that consist of a parent component, i.e. benzoannelated-γ-pyrone ring. These are naturally occurring chemicals which are abundant in plants, and integrative features of various medicinal agents. Chromones are secondary metabolites occurring widely in a broad range of plant families (Gaspar et al. 2014). Structure-wise, chromones are divided into three groups *i.e.*, chromanones, chromones, and combined chromones (pyrano and furano chromones). Claisen condensation, Baker Venkatamaran, Kostanecki Robinson reaction, and the Vilsmeier Haack reaction are the most popular traditional approaches for the synthesis of simple chromones from ortho-hydroxyaryl alkyl ketone (Gaspar et al. 2014). Many chromone derivatives and chromone glycosides obtained from natural or synthetic origins are discovered. Plant species like *aloe (*Grindlay and Reynolds 1986*)*, *aquilaria*, *cassia* (Agarwood), *Hypericum* (Klamath weed), and *polygonum* (knotgrass) are credible sources of some natural chromones. Moreover, many fungi genera also produce bioactive chromones, such as *aspergillus*, *orbiocrella, penicillium and Mycoleptodiscus.* Chromones are also a part of a healthy human diet (Semwal et al. 2020) and display pharmacological importance in multiple fields like antioxidant (Jovanovic et al. 1994), anti-fungal (Malefo et al. 2020), anti-cancer (Al-Amiery et al. 2012), anti-HIV, anti-ulcers (Meydani et al. 2019), wound healing, anti-inflammatory (Silva et al. 2016), immune-stimulatory, and antiparasitic (Reis et al. 2017; Coa et al. 2017a). Intriguing reports have been published over the years, which demonstrate that chromones have been used as antidiabetics and cardiovascular agents (Nazreen et al. 2014; Lynch et al. 2006). They are also crucial as carbonic anhydrase, NADH: ubiquinone reductase, aldose reductase, calcium antagonists, and ligands for orphan nuclear receptors like retinoid receptors (Fonseca et al. 2017). Various chromone molecules can treat neurological and mental disorders, reduce oxidative damage, and allergies, inhibiting cancer, infection, and inflammation (Semwal et al. 2020).

10.1.3 Flavonoids

Flavonoids **(12)** are secondary metabolites primarily composed of a benzopyrone ring with phenolic or polyphenolic groups at various locations (Panche et al. 2016). Flavonoids are mainly found in vegetables, fruits, roots, stems, bark, and beverages. They play an essential role in the cosmetic products, pharmaceutical industries, and medicinal fields (Batiha et al. 2020; Martens and Mithöfer 2005). Quercetin (Lakhanpal and Rai 2007) **(21)** moiety is an essential polyphenolic flavonoid which has shown activity against antiallergic, anti-cancer, antiviral, metabolic processes and inflammatory disorders, vision-related and cardiovascular diseases, also exhibits beneficial effects against Alzheimer's disease (AD) and arthritis (Anand

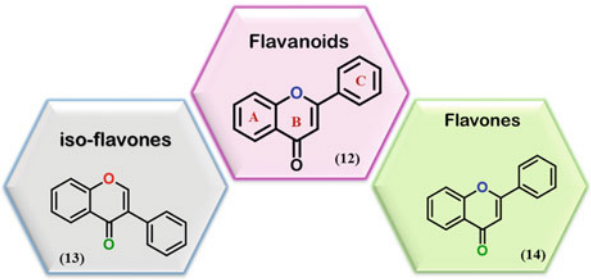


Fig. 10.7 The general structure of flavonoids and their sub-groups

David et al. 2016). This broad range of medical importance of quercetin has shown its inhibitory effect against acetylcholinesterase (Batiha et al. 2020). Quercetin displays biological efficacy against a variety of microorganisms and parasites, including some pathogenic viruses, bacteria, and *Plasmodium* parasites (Azeem et al. 2022). Some reports revealed that metal-complexed flavonoids show improved biological activity compared to the parent flavonoids. Complexed flavonoids with different metal-ions exhibit anti-cancer and antidiabetic therapeutic applications, i.e., vanadium (Selvaraj and Krishnan 2021). However, the increased toxicity of metal ions and their derivatives creates limitations for their therapeutic use. Based on the attached functional groups to the aromatic rings, flavonoids are categorized into sub-groups, viz. flavones, and isoflavones (Fig. 10.7).

10.1.3.1 Flavones

Flavones **(14)** are the most critical and significant sub-class of flavonoids. Flavones are generally found in citrus fruits, herbs, spices, and cereals. Chrysin **(15)**, Apigenin **(16),** and Luteolin **(17)** are some examples of flavones. They are mainly known for the antioxidant activity against diseases that originate from oxidative stress. Flavopiridol **(22)** had shown potency as an anti-cancer agent (Verma and Pratap 2010). Apigenin **(16)** shows the anti-parasitic activity (Mead and McNair 2006).

10.1.3.2 Iso-Flavones

Iso-flavonoids or iso-flavones **(13)** are a particular class of flavonoids as these are less abundant in plants and found in some microbes. Daidzein **(18),** Genistein **(19),** and biochanin A **(20)** are examples of isoflavonoids (Fig. 10.8). Iso-flavones act as chemotherapeutic agents against various diseases such as anti-malarial, anti-leishmanial (Sartorelli et al. 2009), anti-cancer, anti-diabetic (Hussain and Green 2017).

Benzopyrones have been widely explored in the medicinal chemistry against different diseases. These scaffolds have shown potent biological efficacy as antiparasitic, anti-tumor (Jain and Joshi 2012; Rawat and Reddy 2022), and anti-inflammatory agents (Küpeli Akkol et al. 2020; Yan et al. 2022). Here, our primary focus is on parasitic diseases like antiparasitic, leishmaniasis, schistosomiasis, and filariasis, accountable for billions of human infections every year. Currently, the medicines available in the market to treat these parasitic diseases were introduced decades ago. Also, the region for these parasitic infections is not limited to tropical

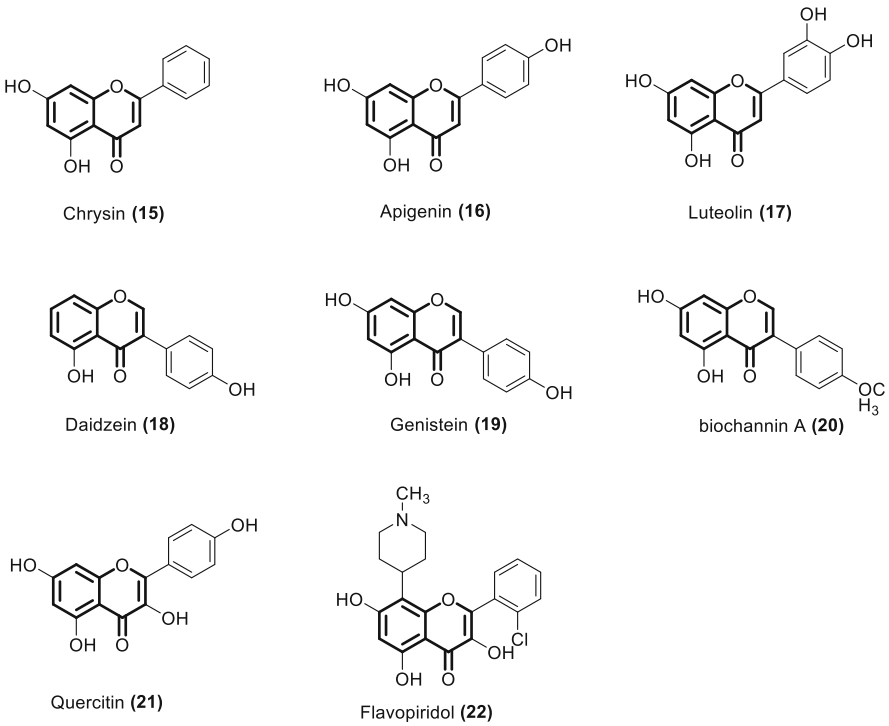


Fig. 10.8 Some examples of naturally occurring flavonoids

areas only, as they have a severe effect in subtropical and temperate regions, where they tend to infect immigrants and travellers (Short et al. 2017). Therefore, in light of the urgent discovery of new therapeutics for these parasitic diseases, the benzopyrone class of molecules offers some advantages because of their high medicinal chemistry value. In this chapter, we have discussed the antiparasitic activity of benzopyrones such as coumarins, chromones, flavonoids, and flavanones etc.

10.2 Overview of Parasitic Disease (Malaria, Leishmaniasis, Chagas)

Parasitic diseases are considered as one of the significant threats to the population of poor countries from Asia, Africa, and Latin America. The parasite burden in the tropical areas of these countries often causes several devastating diseases. These diseases are termed as Neglected Tropical Diseases (NTDs) and are not given much importance for treatment. According to WHO, almost half the world's population is at risk of these infectious diseases. Parasitic diseases are mainly transmitted from other animals to humans. The complex life cycle of the parasites and emerging drug

resistance to the currently available drugs led to complications in drug discovery. The high cost and low availability of the drugs make them out of reach to poor people. It creates a great demand for inexpensive drugs or naturally occurring molecules to reduce the burden of Tropical parasitic diseases. Some major NTDs are malaria, leishmaniasis, schistosomiasis, and Chagas disease, in which different naturally occurring molecules are helpful for their treatment.

10.2.1 Malaria

Nowadays, malaria is becoming a severe disease caused by protozoan parasites and a matter of concern for public health all-round the globe (Singh et al. 2018; Upadhyay et al. 2020). The protozoan parasite responsible for the disease belongs to the genus plasmodium, and transmission of the disease in humans takes place by the bite of an infected female *Anopheles* mosquito (Sharma et al. 2021). This neglected disease requires proper medical attention; otherwise, it can be fatal. The number of cases was estimated to be 228 million in the year 2018 as compared to 251 million cases in the year 2010 and 231 million cases reported globally in 2017 (World Health Organisation 2019). There has been a reduction in both cases and the number of deaths, which are reduced to 4,05,000 in 2018 in comparison to 5,36,000 in 2011 (Bansal et al. 2021). However, the feasible cure options were declined due to the compromised effectiveness of drugs. Therefore, there is an immense need to develop new treatment therapies.

10.2.2 Leishmaniasis

Leishmaniasis, caused by Leishmania parasites and transmitted through phlebotomine sand fly bites (Torres-Guerrero et al. 2017; Maroli et al. 2013), constitutes a wide array of diseases ranging from the one which is usually self-healing but potentially disfiguring cutaneous leishmaniasis (CL) (Bilgic-Temel et al. 2019) over the highly disfiguring mucocutaneous (MCL) (Gillespie et al. 2016), to the visceral leishmaniasis (VL) (Alves et al. 2018), which is invariably fatal if not treated appropriately. An estimated 600 million people are at risk of VL, according to the World Health Organisation (WHO). The report suggested that 50,000–90,000 new cases are recorded annually, giving rise to 26,000 to 65,000 deaths annually (WHO 2020; Le Rutte et al. 2018). Currently, available treatments have severe limitations ranging from adverse toxicity over complex administration to emerging resistance (Halder et al. 2020). Current drugs recommended for treating leishmaniasis include Sb (V)-based compounds (Ali et al. 2013), amphotericin B (Mosimann et al. 2018), paromomycin (Matos et al. 2020), and miltefosine (Iranpour et al. 2019). However, there are disadvantages and side effects of these drugs, i.e., considering high toxicity and low affordability, pentavalent antimonial drugs are

compromised by clinical resistance that increased during the past 10 to 20 years, as observed, for example, in the Bihar state of India (Ponte-Sucre et al. 2017). Sb-based molecule shows toxicity, and they are expensive too. Amphotericin B can cause acute toxicity, and its chronic adverse effect is nephrotoxicity and renal injuries by different mechanisms (Laniado-Laborín and Cabrales-Vargas 2009). Other drugs, paromomycin, and miltefosine, also reveal some side effects, including nausea, diarrhea, and abdominal cramps (Wiwanitkit 2012). All these situations warrant the discovery of new, effective and safe therapeutics to treat antiparasitic diseases.

10.2.3 Chagas

Chagas disease also belongs to the neglected tropical disease. It is also referred to as American trypanosomiasis (Echeverria and Morillo 2019) and is endemic in south and central America. The disease is caused by the protozoan parasite *Trypanosoma cruzi (*Abras et al. 2022*)* and transmission occurs through an insect vector called triatomine or reduviid bug (Kieran et al. 2021). Symptoms of the disease include pain in the abdomen or chest, a high fever that last for a long time, headache, muscle pain, swelling of the liver, spleen, and lymph nodes, trouble breathing, swelling, and subcutaneous edema. At the moment, there is no reliable treatment for the fatal Chagas disease (Añez et al. 2020). Nowadays, it spreads all around the globe through the migration of infected humans (Harrison et al. 2020). Approximately 6 to seven million people infected globally are reported, 70 million are at risk, and 12,000 deaths are recorded annually (World Health Organization 2022). Complex life cycle of the parasite and the already available drugs show many drawbacks, so there is an immense need for new drug molecules (Kourbeli et al. 2021). The symptoms of the disease have two stages, i.e., acute stage (Paiva et al. 2018) and chronic stage (Álvarez-Hernández et al. 2021), based on the severity of the disease. In most individuals, if the parasite load is small, the infection is termed acute Chagas disease infection, usually found asymptomatic (Michel-Todó et al. 2019). The two drugs that were developed four decades ago, i.e. benznidazole (2-nitroimidazole) and nifurtimox (5-nitrofuran), are still used as current frontline treatment (MacLean et al. 2018). So, to avoid the disease's severe conditions, discovering some new effective therapeutics is required.

10.3 Benzopyrone-Based Compounds Activity Against Parasitic Diseases

Benzopyrone-based compounds have shown activity against numerous diseases, as mentioned in the above sections. Here in this section, we have summarised their activity against parasitic diseases based on their structure and occurrence.

10.3.1 Benzo-α-Pyrone (Coumarin)-Based Analogs Against Parasitic Diseases

Coumarins and their derivatives have shown activities against various parasitic diseases, we have compiled them based on their synthesis or isolation from natural sources.

10.3.1.1 Synthetic Coumarins Against Parasitic Diseases

Recently, coumarin-based derivatives have been explored against malaria parasites to check their biological efficacy (Ren et al. 2018; Taha et al. 2018; Mustafa and Abdulaziz 2020). Some of the featured reports include the potency of synthetic coumarin-triazole analogs by Yadav et al. (Yadav et al. 2018) and coumarin-annulated ferrocenyl 1,3-oxazine derivatives by Mbaba et al. (Mbaba et al. 2021) Yadav et al. have explored the coumarin against malaria parasite with its fusion with triazole and synthesized a total of twenty-two compounds through the click chemistry method on 7-(prop-2yn-1-aryloxy)-2*H*-chromen-2-one derivative. Biological evaluation of synthesized compounds takes place for their potency against the chloroquine-sensitive strain of *plasmodium falciparum* (3D7). Best result among all the synthesised compounds was displayed by (7-[1-(2, 4-dimethoxy-phenyl)-1*H*-(El-Sawy et al. 2021; Edwards and Howell 2000; Murray et al. 1982) triazol-4-ylmethoxy]-4-methyl-chromen-2-one; (**23**) with 50% inhibitory concentration (IC_{50}) of 0.763 ± 0.0124 μg/mL (Yadav et al. 2018). Mbaba et al. have explored coumarin-based analogs in combination with ferrocenyl 1,3-oxazine. The authors have also synthesized a series of novel compounds and reported the biological efficacy of fourteen compounds against the *Pf*3D7 strain of malaria parasites. Among all these compounds, the best result was displayed by compound (**24**), with IC_{50} value of 1.73 μM. However, the authors did not carry out any animal model experiment, and no study was conducted on the synergistic effect as combination partners to the artemisinins.

Several reports have been published on the biological efficacy of coumarin-based scaffolds as leishmaniasis agents, and some of the highlighted reports are covered in this chapter. Mandlik et al. (Mandlik et al. 2016) have explored coumarin-based compounds for *in-silico* studies and further evaluated the compounds for cell-based assay for their anti-leishmanial properties, and the top candidate was also considered for the animal model in BALB/c mice. The authors have selected compounds that are either commercially available or otherwise the synthesis was procured externally. Sigma Aldrich, USA, synthesized the top hit the compound of the study (**25**). The compound **(25)** exhibited leishmanicidal activity of 524 μM, with the percentage viability of the macrophages around 65%. No significant macrophage toxicity was observed for the compound **(25)** at IC_{50} concentrations. Nano-liposomal formulations of compounds have also been developed. Treatment of cutaneous lesions with the compound **(25)** showed a significantly decreased lesion size as compared to untreated mice ($p < 0.05$), this study suggests that the compound **(25)** may have anti-leishmanial drug properties (Mandlik et al. 2016).

Khatoon et al. (Khatoon et al. 2021) designed and developed novel coumarin-isatin hybrids and performed molecular docking studies of these compounds, which revealed some lead compounds. Further, the authors have evaluated all ten synthesized scaffolds for their efficacy as anti-leishmanial agents. Their *in-silico* studies indicated that three highly active compounds show potency after biological evaluation. One compound **(26)** showed higher conformational stability within the protein's active site during an MD simulation of 50 ns. The remarkably higher activities shown by these three hit compounds at the micromolar range concentration against both stages, i.e. promastigotes and amastigotes (Khatoon et al. 2021). Overall, the results from the report indicated that three compounds were highly efficacious, non-toxic in nature, and biocompatible as antileishmanial agents. However, these compounds need to be evaluated in an animal model to validate in vitro results. Brancaglion et al. (Brancaglion et al. 2018) discovered a novel molecule i.e., 8-methoxy-3-(4-nitrobenzol)-6-propyl-2*H*-chromen-2-one **(27),** and evaluated it in vitro and in vivo biological efficacy as the trypanocidal agent. The compound was screened for safety profile on the H9C2 cell line and found to have CC_{50} value of >200 μM. Next, the in-vitro studies were performed to test the compound's efficacy against *T. cruzi amastigote*, and the compound displayed IC_{50} value of 13 μM. Further, the authors evaluated the mice model and the infected mice with trypomastigote forms orally treated with the compound. The results show that the compound reduced parasitemia load as compared to the group of untreated mice. The report's findings suggest that synthetic novel chromones derivatives possess potential therapeutic applications. The coumarins-based top hit compounds having potential as antiparasitic agents are represented in Fig. 10.9.

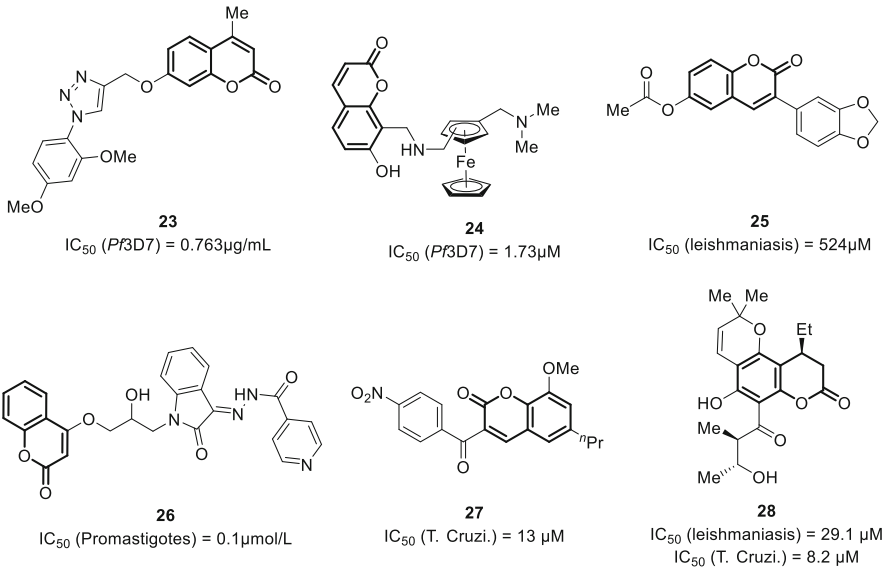


Fig. 10.9 Coumarin-based biological effective antiparasitic inhibitors

10.3.1.2 Naturally Occurring Coumarins

Silva et al. (Silva et al. 2021) explored the two derivatives of coumarins extracted from the plant *Calophyllum Brasiliense* Cambess. The authors have explored these coumarins for cell-based assay against specific *amastigote* stages of *Trypanosoma cruzi* and *Leishmania infantum* (*L. infantum*). Both compounds were isomers, with one being trans/anti and the other being syn, and the biological profile of both was evaluated, which displayed that the trans/anti isomer **(28)** was more active than the syn isomer. Syn isomer showed EC_{50} values of 12.1 μM and 37.1 μM against the *T. cruzi* and *L. Infantum* species, respectively whereas trans-isomer displayed improved EC_{50} values of 8.2 μM and 29.1 μM. Authors evaluated the therapeutic index of both the isomers and found that trans isomer is better with specific values of >24.4 against *T. cruzi* and >6.9 against *L. infantum (*Silva et al. 2021*)*.

In total, all the findings from the report have suggested that these naturally occurring and synthetic coumarin derivatives can serve as primary scaffolds for further designing new potent inhibitors for leishmaniasis and Chagas disease.

10.3.2 Benzo-γ-Pyrone (Chromones) Based Analogs

10.3.2.1 Chromones

Several reports and findings have been published on the medicinal chemistry importance of chromones-based scaffolds, particularly as antiparasitic agents. Here, we have highlighted the latest literature reports on chromones as antiparasitic agents such as leishmaniasis, malaria, and trypanocidal.

Coa et al. (Coa et al. 2017b) have explored chromones-based scaffolds in fusion with quinoline. The authors have synthesized five quinoline-chromones hybrids and explored them against *amastigote* forms of *T. cruzi* and *Leishmania*. The most active compound among five newly synthesized analogs was 7-[4-(quinolin-8-yloxy) butoxy]-4*H*-chromen-4-one **(29)**, which displayed IC_{50} values of 11.32 and 16.91 μM against *T. cruzi* and *Leishmania* respectively. The compound's biological efficacy was better than benznidazole, an anti-trypanosomal drug. However, there was some sign of toxicity for the hit compound for mammalian U-937 cells. Further, optimizing hit compounds can lead to a potential antileishmanial or trypanocidal drug candidate. The chromone-based top hit compounds having potential as antiparasitic agents are represented in Fig. 10.10.

Lerdsirisuk et al. (Lerdsirisuk et al. 2014) have explored chromones-based compounds against the malaria parasite. The authors have synthesized a novel series of chromone-based molecules possessing inhibitory activity against HIV-1 protease and evaluated them against *P. falciparum* to determine the potency of compounds as antimalarial. The most active scaffold among the series was compound **(30)**, with IC_{50} values of 0.95 μM against *P. falciparum*, while the positive controls, primaquine, and tafenoquine, showed IC_{50} values of 2.41 μM and 1.95 μM, respectively. The authors have also performed the molecular docking for these analogs against plasmepsin II, indicating that compound **(30)** exhibited a higher binding affinity value i.e., −13.24 kcal/mol (Binding Free energy). Another, Indian research group has worked on nitrone-based chromone derivatives. They synthesised novel

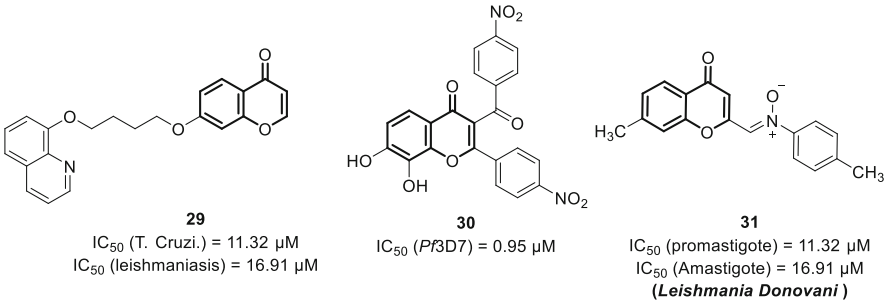


Fig. 10.10 Chromones-based biological effective top antiparasitic inhibitors

nine chromone analogs and evaluated them for their in-vitro antileishmanial activity. Among the series of nine compounds one compound **(31)** is showing potential to inhibit both promastigote ($IC_{50} = 17.1$ μM) and amastigote ($IC_{50} = 8.53$ μM) forms of *leishmania donovani*. The molecule demonstrated the capacity to inhibit the intracellular amastigotes and shows in-vitro inhibition against pentavalent antimonial responsive-strain and pentavalent antimonial resistant-strain of promastigotes. Overall, the compound is showing good activity against the leishmania parasite (Mallick et al. 2011). The chromone derivatives possessing HIV-I protease inhibitory activity maybe optimized to find a lead drug candidate against the malaria parasite.

10.3.2.2 Flavonoid-Based Analogs

Sulsen et al. (Sülsen et al. 2007) reported flavanoids like hispulidin and Santin extracted from *Ambrosia tenuifolia and Eupatorium buniifolium. During cell-based* assays, they emerged as a potential trypanocidal and leishmanicidal candidate. The in-vitro parasiticidal effect of naturally extracted products has been quantified using a sensitive technique. This technique takes advantage of ((Murray et al. 1982) H) thymidine uptake by dividing trypanosomatids. The authors have reported that hispidulin and santin showed good biological efficacy against *T. Cruzi* epimastigotes with IC_{50} values of 46.7 μM and 47.4 μM, respectively, and against trypomastigotes, IC_{50} values for hispidulin and santin were 62.3 μM and 42.1 μM, respectively. These compounds were also tested for *Leishmania mexicana* promastigotes, hispidulin ($IC_{50} = 6.0$ μM) was found to be more active than santin ($IC_{50} = 32.5$ μM). Further, these compounds were also evaluated for their toxic effect on lymphoid cells, which indicated that hispidulin and santin showed no cytotoxic effect. Overall, the authors showed that these flavonoids have trypanocidal and leishmanicidal properties. All the observed results indicated the possibility of these lead compounds for developing novel natural medications (Sülsen et al. 2007).

Marin et al. (Marin et al. 2009) reported a novel class of flavonoid derivatives extracted from the species *Consolida oliveriana* (i.e., quercetin, trifolin, acetyl hyperoside, and their O-acetyl derivatives) having potential as anti-leishmaniasis agents against the two different forms of leishmania species i.e., promastigotes as well as amastigote forms. The authors reported that compounds Penta-O-acetyl

quercetin (IC_{50} = 11.18 μm) and Hepta-O-acetyltrifolin (IC_{50} = 10.53 μm) were highly potent against Leishmania *perviana.* These compounds also show good activity against Leishmania *brazilienins* i.e., IC_{50} values are 46.78 μm and 8.72 μm for Penta-O-acetyl quercetin and Octa-O-acetyl hyperoside, respectively. These synthesized compounds of the flavonoid series were found to be non-toxic to the cell line and efficacious at doses comparable to the available reference medications (pentosan and glucantim) (Marin et al. 2009).

Tasdenuir et al. (Tasdemir et al. 2006) reported a variety of flavonoid series, which consists of aglycones and glycosides, as well as a group of additional phenolic and phenyl propanoid-related compounds. Also, they assayed them for in vitro activity against *Trypanosoma brucei rhodesiense, Trypanosoma cruzi,* and *Leishmania donovani*. Approximately, >100 substances with flavone, biflavone, flavanone, flavan-3-ol, isoflavones, and coumarin moieties were evaluated for their cytotoxicity profile in mammalian L6 cells. Further, antiparasitic activity was compared. The best result was displayed by 7,8-dihydroxyflavone **(32)** for cell-based assay against the trypanocidal activity for the parasite *T. brucei rhodesiense* (IC_{50} = 68 ng/mL), after this some other molecules i.e., 3-hydroxy flavone **(33)**, rhamnetin, and 7,8,3,4-tetrahydroxyflavone **(34)** (IC_{50}s = 0.5 μg/mL), and catechol (IC_{50}s = 0.8 μg/mL). Only chrysin dimethyl ether and 3-hydroxydaidzein derivative had IC_{50}s smaller than 5.0 μg/mL, indicating moderate efficacy against *T. cruzi*. The bulk of metabolites tested has leishmanicidal solid properties. The most potent compounds were fisetin, 3-hydroxy flavone **(33)**, luteolin **(17)**, and quercetin **(21)**, with IC_{50}s values of 0.6 μg/mL, 0.7 μg/mL, 0.8 μg/mL, and 1.0 μg/mL, respectively. In animal models, molecules 7,8-dihydroxyflavone **(32)** and quercetin **(21)** emerged to reduce parasite infections. However, compounds were found to have toxic behavior when evaluated for their cytotoxicity (Tasdemir et al. 2006). Therefore, the compound shown in the figure below (Fig. 10.11.) needs to be optimized for toxicity and biological efficacy to find a lead compound for antiparasitic applications among the flavonoids.

Rudrapal et al. (Rudrapal and Chetia 2017) have compiled an exciting review article covering the importance of bioactive flavonoids in medicinal chemistry, which are isolated from plants. The authors have laid down the focus on the antimalarial flavonoid molecules. The authors have discussed these types of antimalarial flavonoids in their report that were naturally present in plants/plant medicines. These compounds comprise numerous polyphenolic compounds with a comprehensive structural diversity and pharmacological potential in diverse therapeutic areas. The top hits indicated in their review report are highlighted in Fig. 10.12. below.

Overall, the authors have indicated that identifying a biological target and the specificity of that target for antimalarial action is crucial as this approach could be implemented for establishing plant-based flavonoids as future antimalarial hits.

Kuhn et al. (Kuhn et al. 2010) have explored the flavonoids-based compound as potential candidates against the NAD^+ catabolizing enzyme of *Schistosoma mansoni* (SmNACE). The authors have followed a high-throughput screening approach to identify hit compounds among a diverse chemical library of approximately 14,300 molecules. Further, a secondary assay identified two naturally extracted product inhibitors, i.e., cyanidin, and delphinidin. Both the inhibitors possessed IC_{50} values

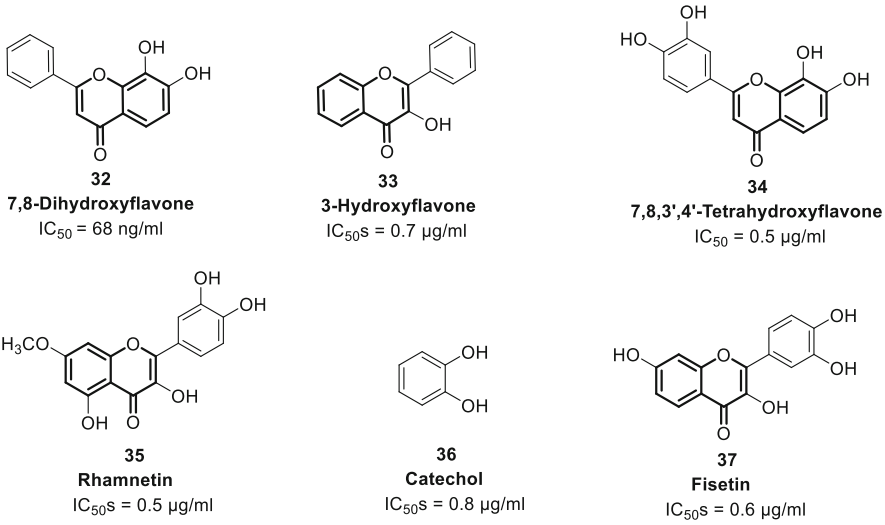


Fig. 10.11 Examples of flavonoid aglycones and glycosides with antiparasitic activity

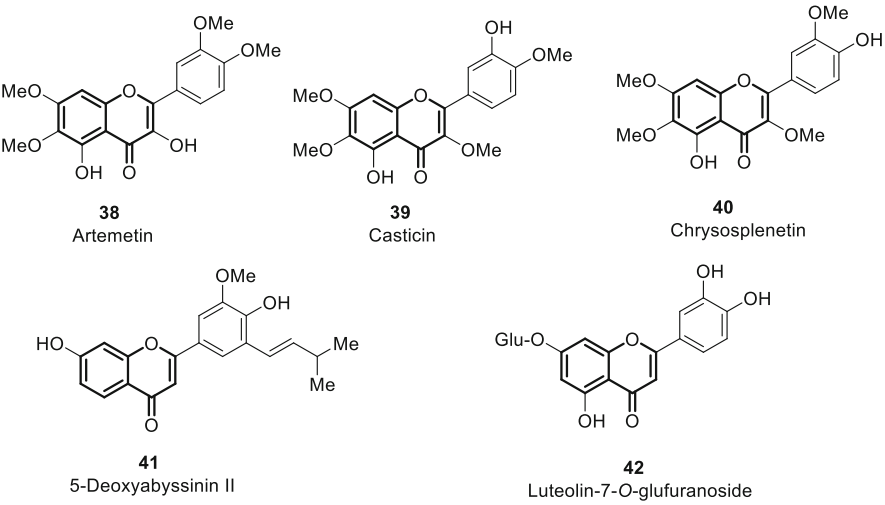
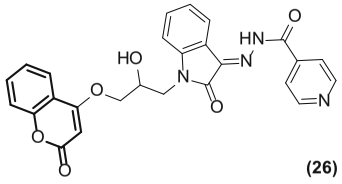
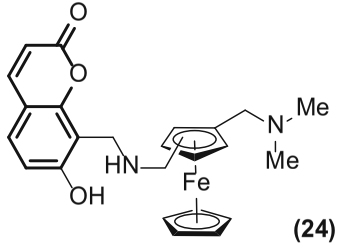
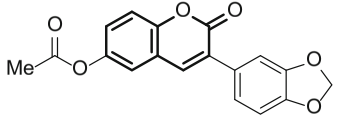


Fig. 10.12 Top hit flavonoids-based compound extracted or isolated from plants with potential antimalarial activity

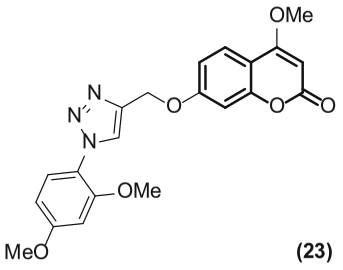
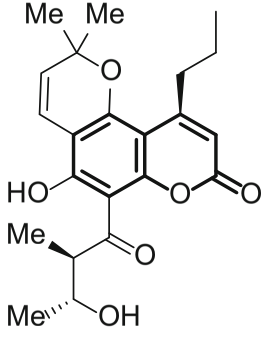
in the low range of micromolar values against SmNACE. Further, the authors have performed the structure-activity relationship and tested more related flavonoids, which led to the identification of 15 more inhibitors. Next, the authors performed the molecular docking studies for these inhibitors, revealing the pharmacophores feature required for SmNACE active site recognition. Overall, the authors followed an excellent approach to shortlisting an extensive compound library (Table 10.1).

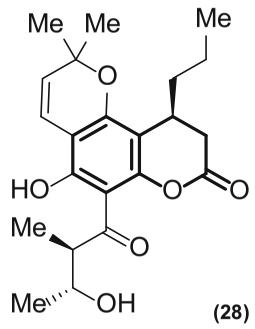
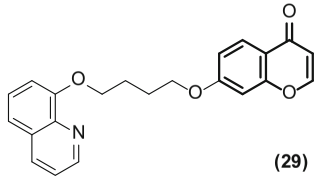
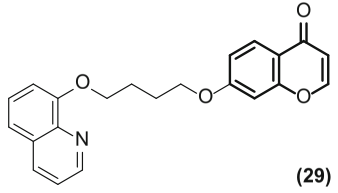
Table 10.1 Examples of Benzopyrone derivatives and their biological properties against antiparasitic diseases

S. no	Type of benzopyrone	Structure of benzopyrone derivative	Name of diseases	IC_{50}/EC_{50} value	Reference
1.	Coumarin	 (26)	Leishmaniasis (promastigote)	0.1 μmol/L	Khatoon et al. (2021)
2.	Coumarin	 (24)	Malaria	1.73 μM	Mbaba et al. (2021)
3.	Coumarin	 (25)	Leishmaniasis	524 μM	Mandlik et al. (2016)

(continued)

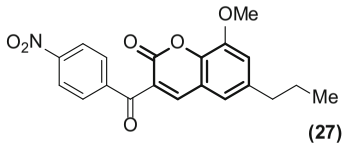
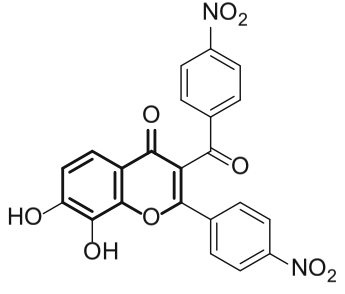
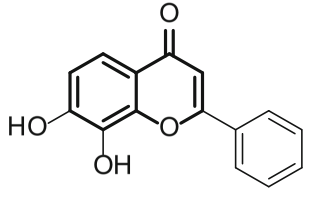
Table 10.1 (continued)

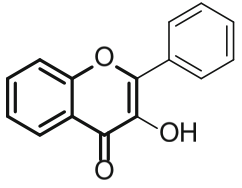
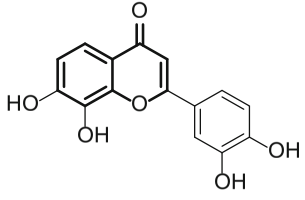
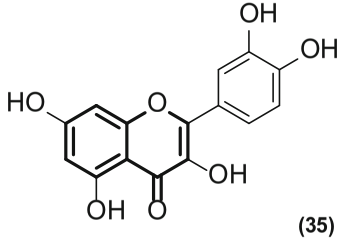
S. no	Type of benzopyrone	Structure of benzopyrone derivative	Name of diseases	IC_{50}/EC_{50} value	Reference
4.	Coumarin	 (23)	Promastigotes	0.763 μg/mL	Mbaba et al. (2021)
5.	Coumarin	 (28)	Leishmaniasis	29.1 μM	Silva et al. (2021)

6.	Coumarin	 (28)	Trypanosoma cruzi	8.2 μM	Silva et al. (2021)
7.	Chromones	 (29)	Trypanosoma cruzi	11.32 μM	Coa et al. (2017b)
8.	Chromones	 (29)	Leishmaniasis	16.91 μM	Coa et al. (2017b)

(continued)

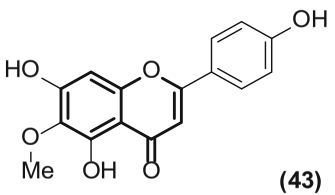
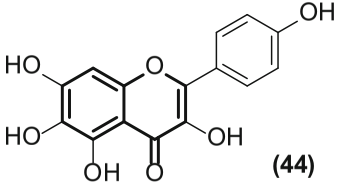
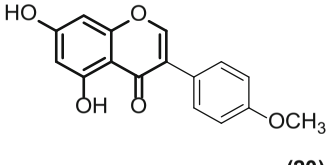
Table 10.1 (continued)

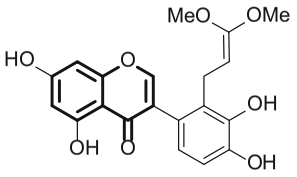
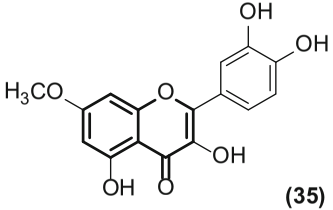
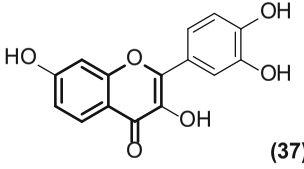
S. no	Type of benzopyrone	Structure of benzopyrone derivative	Name of diseases	IC_{50}/EC_{50} value	Reference
9.	Chromones	 (27)	Trypanosoma cruzi	13 μM	Brancaglion et al. (2018)
10.	Chromones	 (30)	Malaria	0.95 μM	Lerdsirisuk et al. (2014)
11.	Flavonoid	 (32)	Trypanosoma brucei rhodesiense	68 ng/mL	Tasdemir et al. (2006)

12.	Flavonoid	 (33)	Trypanosoma brucei rhodesiense	0.7 μg/mL	Tasdemir et al. (2006)
13.	Flavonoid	 (34)	Trypanosoma brucei rhodesiense	0.5 μg/mL	Tasdemir et al. (2006)
14.	Flavonoid	 (35)	Leishmaniasis	1.0 μg/mL	Tasdemir et al. (2006)

(continued)

Table 10.1 (continued)

S. no	Type of benzopyrone	Structure of benzopyrone derivative	Name of diseases	IC_{50}/EC_{50} value	Reference
15.	Flavonoid	 (43)	Leishmaniasis	6.0 μM	Sülsen et al. (2007)
16.	Flavonoid	 (44)	Leishmaniasis	32.5 μM	Sülsen et al. (2007))
17.	Iso-flavonoid	 (20)	Leishmaniasis	18.96 μg/mL	(Sartorelli et al. 2009)

18.	Iso-flavonoid	 (45)	Leishmaniasis	13.0 μM	(Salem and Werbovetz 2006)
19.	Flavonoid	 (35)	Leishmaniasis	0.5 μg/mL	(Tasdemir et al. 2006)
20.	Flavonoid	 (37)	Leishmaniasis	0.6 μg/mL	(Tasdemir et al. 2006)

10.4 Conclusion

Recent studies have focused on searching for an antiparasitic agent to treat various parasitic-based diseases. In this regard, numerous benzopyrone-based compounds have shown good behaviour in inhibiting parasitic diseases during in vitro (cell-based assay) and in vivo (animal model) studies. These molecules affect diseases at various stages of their complex life cycle. Another factor that can contribute to the speedy discovery of benzopyrone-based inhibitors as antiparasitic agent is the use of computer-aided drug discovery. Researchers have adopted approaches such as molecular docking and molecular dynamics simulation studies for an extensive library of compounds to shortlist some of the top hits. Further, these hits can be evaluated for their biological efficacy, such as in vitro and in vivo, to validate the computational result as an explorative drug.

Benzopyrone-based compounds have been explored as potential HIV agents. HIV inhibitors are known for their high medicinal values. Recently, during the pandemic time, some HIV drugs were repurposed against SARS-CoV-2 and indicated the potential efficacy of the compounds. Therefore, a similar approach can be implemented for these HIV-based inhibitors against various parasitic diseases, and with proper optimization of their SAR, we can find a lead compound against targeted parasitic diseases.

Acknowledgement We acknowledge BRICS Research Project (RFBR 20-53-80002 & DST/INT/BRICS/COVID-19/2020-Part-1) and MB is thankful to ICMR, New Delhi for Senior Research Fellowship.

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Antimicrobial Peptides (AMPs): Current State and Future Prospects for the Treatment of Human Parasitic Diseases

11

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Abstract

Antimicrobial peptides (AMPs) are natural compounds that are primarily used in the development of new therapeutic components for a variety of illnesses, such as infectious diseases, parasitic illnesses, cancer, etc. Due to the rising occurrence of drug resistance, AMPs are used the same as antibiotics. Since the discovery of the first AMP, numerous studies have been conducted to determine the importance of amino acid residues showing antimicrobial activity and to develop and characterize potential AMPs. Since wet-lab experimental identification requires a lot of effort and cost, in silico studies are widely used to identify novel AMP candidates. In silico studies aid in the identification and modeling of AMPs. However, wet-lab studies using assays are assisting in characterizing the newly identified AMPs and their therapeutic potential. Thus, a number of researches helped to uncover the diversity of AMPs as well as the different classes within them. In this book chapter, we cover a wide range of AMP classifications, the

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_11

mechanism of action of AMPs, various AMP-based drugs identified so far for treating parasitic diseases. Also, we illustrate how the approach of AMPs has helped in finding out remedies for Malaria. Additionally, we explain computational methods and resources for better research in the field of AMPs studies.

Keywords

Antimicrobial peptides (AMPs) · AMP classification · peptide modelling · defensin · malaria · parasites

11.1 Classification of Antimicrobial Peptides (AMPs)

Antimicrobial peptides (AMPs) are a class of components with significant innate immune properties. These are small natural compounds found in many microbiological organisms, including bacteria, fungus, and viruses, as the name implies. Insects, being a highly biodiverse group are abundant and most inventive sources of AMPs due to their biodiversity. AMPs have so many features in common such as; having a length of around 10–50 amino acids, a high proportion of hydrophobic amino acids, cationic nature and their immune modulatory abilities. However, a lot of dissimilarities exist between them which enable the classification of AMPs. One method of classifying AMPs is depending on whether synthesized by ribosomes or not. Accordingly, there are two classes—ribosomally synthesized and non-ribosomally synthesized AMPs. Non-ribosomal AMPs, such as vancomycin, are not synthesized using the protein translation machinery, instead, they are made using non-ribosomal peptidyl synthetases. A number of important classes of AMPs is discussed below.

11.1.1 Based on the Source

AMPs can be broadly classified into bacterial, plant and animal AMPs based on their biological source. Bacteriophages are found to serve as a source of AMPs, leading to two classes- phage-encoded lytic factors and phage tail complexes. Bacteria produce both ribosomal and non-ribosomal AMPs. Bacteriocins, the ribosomal AMPs generated from gram-positive bacteria, can be classified into lantibiotics, non-lantibiotics, large-sized bacteriocins and uniquely structured bacteriocins. The AMPs found in gram-negative bacteria can also be classified further into colicins, colicin-like, microcins and phage tail-like bacteriocins. Plants host a range of AMPs that are rich in cysteine and disulphide bridges, which provide protection against proteolytic degradation.

The animal AMPs can still be classified into insect, amphibian and mammalian AMPs. Insect-derived AMPs help the insects to adapt to their survival. E.g. Cecropin A. Among amphibians, the main sources of AMPs are frogs, the most important AMP being magainin. Mammalian AMPs, of which a large proportion is formed by

human host defence peptides, are distributed not only in humans but also in other vertebrates. Two classes of AMPs termed Cathelicidins and Defensins are prominent among mammalian AMPs. Cathelicidins are found in secretory granules of neutrophils, T-cells, B-cells, mast cells and epithelial cells. E.g. LL-37. Defensin was the first reported AMP in animals and is found in neutrophils, monocytes, macrophages, keratinocytes and epithelial cells (Hirsch 1956). E.g. Human Beta-defensin-1,2,3 is present in the mucosal membranes which ensures protection against microbial attack (Ghosh et al. 2019). Another human AMP is histatin which is a short peptide with anti-microbial and anti-fungal activity found in saliva.

11.1.2 Based on the Structure

AMPs belong to any of the four structural categories and correspondingly there are four classes- α-helix (e.g. LL-37), β-sheet (e.g. Gomesin), linear extension structure (e.g. Indolicidin) and αβ consisting of both α-helix and β-sheet (α1-purothionin). α-helix is the most abundant in nature and hence the most studied structural class of AMP. A fifth class with unique structural features, cyclic and unusual or complex AMPs has also been proposed (Koehbach and Craik 2019).

11.1.3 Based on Biological Functions

AMPs can be classified based on their biological activity against bacteria, virus, fungi or parasites. The relative proportion of the various AMPs classified depending on their biological function is represented in Fig.11.1. A large proportion of AMPs is contributed by antibacterial AMPs, which are the most thoroughly studied AMPs. A majority of them are cationic and hence can kill the bacteria by disrupting the negatively charged lipid bilayer (Hiraki et al. 2000) . Some antibacterial peptides also act by targeting intracellular pathways and some others use both these mechanisms. These are generally amphipathic and exhibit broad inhibitory activity

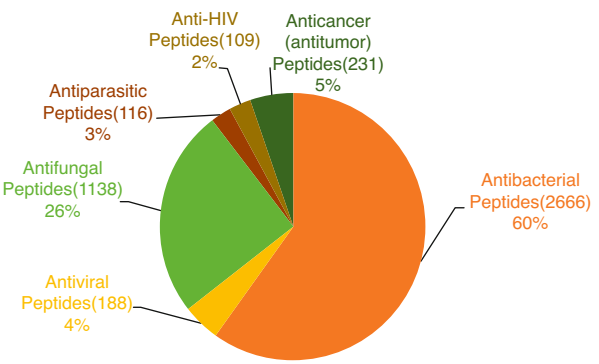

Fig. 11.1 The relative proportion of the various classes of AMPs categorized on their biological functions (Huan et al. 2020)

against pathogenic bacteria. One example is nisin, produced by *Lactococcus lactis* which is used to prevent food spoilage in food industry.

Anti-viral peptides (AVP) that can be therapeutically used against viral diseases act by different mechanisms such as inhibition of attachment and fusion with the membrane, degradation of the viral envelope or inhibition of the viral replication. Anti-lipopolysaccharide AMPs, a class of antiviral AMPs are reported to inhibit the replication of influenza virus (Hoffmann et al. 2014). Another subclass of AVP is anti-HIV drugs, a commercial example being Fuzeon™ (enfuvirtide). AMPs that act against fungal infections exhibit increased drug resistance and are used in clinical practice against common fungal infections such as *Candida albicans*. Three possible mechanisms have been reported for the action of antifungal peptides, namely, barrel-slave, carpet-like and torroidal pore (Galanth et al. 2009; Gray et al. 2012; Li et al. 2019). In carpet-like model, there is a parallel arrangement of AMPs in a parallel fashion which covers the surface of the cell membrane like a carpet. In torroidal pore model, the vertically embedded AMPs in the cell membrane come together to form a ring hole whereas in the barrel model, AMPs aggregate to form channels.

AMPs with antiparasitic activity have been successfully explored for their therapeutic potential against Malaria and Leishmaniasis. For example, the development of *Plasmodium berghei* is inhibited by the host defense peptide defensin. Several defensins are also known to kill Leishmania, possibly by inducing apoptosis. We will be discussing the AMPs with antiparasitic activity in detail in ensuing sections.

11.1.4 Based on Properties of the Peptide

Based on aminoacid composition, there are proline-rich AMPs which enter the bacterial cell through membrane transporter SbmA and then interfere with the translational machinery (Mattiuzzo et al. 2007). These are mainly involved in the killing of Gram-positive bacteria and some of these are also found to be immunostimulatory. Tryptophan and Arginine rich AMPs such as lactoferricin are another class and synthetic peptides with potent antibacterial activity have been designed using short Trp and Arg rich sequences (Bacalum et al. 2017). Histidine rich peptides exhibit good membrane penetration and this property has been made of use in increasing the cell penetration capacity of AMPs (Lointier et al. 2020). Another class is Glycine rich peptides which have a markedly characteristic tertiary structure.

11.1.5 Based on the Pattern of Covalent Bonding

In earlier times, before the 3D structure and biological activity of AMPs are established, the nature of covalent bonding was utilized to classify AMPs. Accordingly, the universal system of classification identifies four different types of AMPs. (i) Class I (UCLL) - Such AMPs have one linear chain which may be chemically modified or even two linear peptides not connected via a covalent bond.

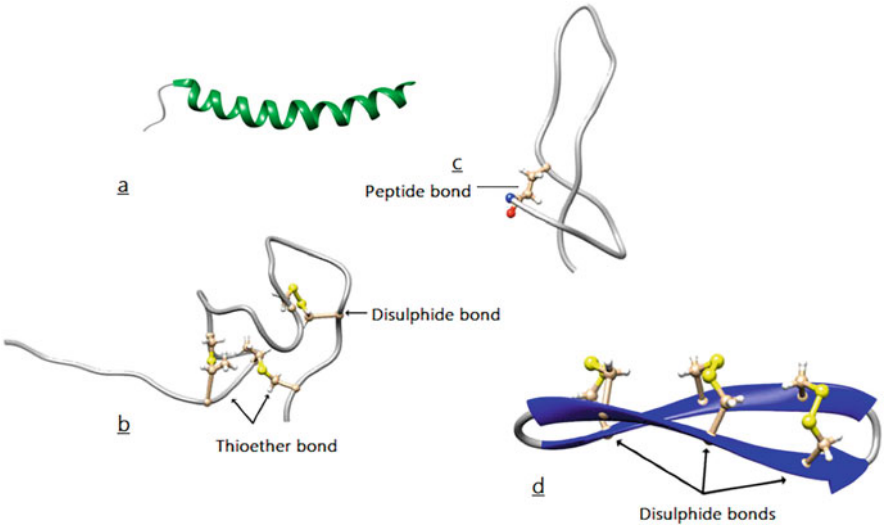

Fig. 11.2 (**a**) Class I (ULCL) (**b**) Class II (UCSS) (**c**) Class III (UCSB) (**d**) ClassIV (UCBB) (Bin Hafeez et al. 2021)

e.g. magainin. (ii) Class II (UCSS) - Consists of AMPs that have linkages between side chains either within a chain or between two chains. e.g. defensin. (iii) Class III (UCSB) - these AMPs have a linkage between the side chain and backbone of the same peptide chain e.g. fusaricidins. (iv) Class IV (UCBB) - circular AMPs with a peptide bond between the amino- and carboxy-termini of the peptide and may also contain additional modifications such as disulphide bond e.g. enterocin. The basic structural characteristics of the four classes of AMPs are illustrated in Fig.11.2.

11.1.6 Based on Molecular Targets

AMPs can be classified into two groups based on the molecular target on which they act: membrane targeting and non-membrane targeting. The non-membrane targeting peptides are further classified into those that execute inhibition of protein synthesis, biosynthesis of DNA and RNA, protease activity and cell division.

11.2 Mechanism of Action of AMPs

AMPs execute their action by mechanisms involving direct killing and by immune modulation. Direct killing is achieved by targeting the membrane and by processes that do not target the membrane (Kumar et al. 2018).

11.2.1 Membrane Targeting Mechanisms

AMPs interact with the target cells by processes that are mediated by receptors and also by mechanisms that do not involve receptors (Corrêa et al. 2019). AMPs formed by bacteria adapt receptor-mediated pathways. Binding of these AMPs to receptors is facilitated by the presence of specific domains in their structures and thus make them more specific. Antimicrobial property of these peptides is retained even when these domains are lost, though at higher concentration and acting via non-receptor mediated mechanism (Holdbrook et al. 2018).

11.2.1.1 Receptor-Mediated Mechanism

Receptor-mediated AMPs include Nisin Z, mesentericin Y and polymyxin B. Lipid II, which is a cell wall precursor anchored to the membrane, acts as a receptor for nisin Z, a product of gram-positive Lactococcus lactis and approved by FDA for use as preservatives in dairy products. Nisin forms pores in bacterial membrane, upsets membrane integrity, impedes bacterial growth and leads to lysis of cell Polymyxin B interacts with membranes of microbes with the help of LPS, phosphatidyl glycerol and cardiolipin-specific receptors to interact with membranes of microbes. Mesentericin binds to a receptor that is solely found in Listeria sp. (Corrêa et al. 2019; Kumar et al. 2018).

11.2.1.2 Non-receptor Mediated Mechanism

Non-receptor-mediated AMPs are comparatively more abundant, and they target cell membranes without any specific receptors and by interacting with membrane components. Cationic AMPs can interact with teichoic acid and lipopolysaccharides present on the exterior of gram-positive as well as gram-negative bacteria and impart negative charge to them. Magainin and alamethicin, products of Xenopus laevis and Trichoderma viride respectively belong to this category of AMPs (Kumar et al. 2018). Other than membrane charge, membrane curvature also plays a significant role in the initial interaction of AMPs with bacterial membranes. For example, membrane leakage is induced by AMP magainins in liposomes composed of phosphatidyl glycerol (PG) and that by Polybia-MP1 in unilamellar vesicles containing phosphatidyl serine (PS) (Alvares et al. 2017; Matsuzaki et al. 1998) This is because PS and PG displays different membrane-curvature properties (Jouhet 2013). AMPs can differentiate between invading microbial cells and self-cells of the organism. Membrane composition, hydrophobicity and charge are involved in this process and influence the interaction of AMPs and the target membrane (Jouhet 2013). The AMPs establish weak hydrophobic interactions with animal cell membranes and strong electrostatic/hydrophobic interactions with bacterial cell membranes. This is because the external membrane of bacteria is comprised of lipids and negatively charged molecules like PG and cardiolipin (Zhang et al. 2001), whereas animal cell membranes consist of zwitter ion phospholipids like phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), shingomyelin and neutral molecules such as cholesterol (Guilhelmelli et al. 2013).

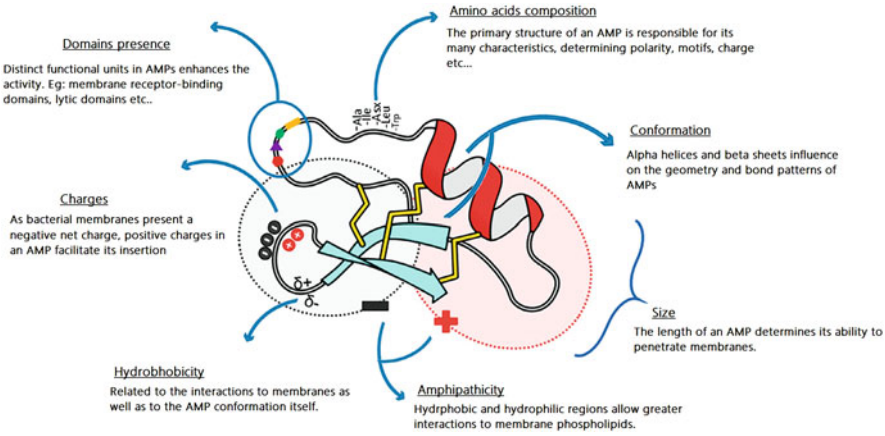

Fig. 11.3 Factors that affect the antimicrobial property of AMPs (Kessedjian et al. 2010)

Various structural factors such as amino acid composition, conformation, size, amphipathicity, hydrophobicity, presence of domains and charge determine the antimicrobial property of AMPs, Fig. 11.3.

The conformation of AMPs is associated with the three-dimensional topology of the sequence of amino acids of the peptides. There are α-helix, β-pleats and peptides rich in certain specific amino acids that exhibit specific folds involved in targeting. Cathelicidin PR-39 from pigs is rich in lysine and arginine which exemplifies the last group of AMPs. The amphipathic and cationic α-helix has higher activity and thus has greater industrial application (Dullius et al. 2018). Both α- and β- structured peptides are involved in leading to pore formation in target membranes (Lee et al. 2019). Porcine cathelicidin protegrin-1 and bovine milk lactoferricin B belong to β-structured AMPs.

As for the charge, most of the AMPs are positively charged due to the presence of cationic domains. Antimicrobial property of the AMPs increases with cationicity up to a certain limit, +5 charge being optimal. Positive charge facilitates the initial electrostatic interaction of peptides with target membranes (Dathe et al. 2001) The percentage of hydrophobic amino acid residues determine hydrophobicity of AMPs, which reflects upon their ability to cleave the lipid bilayer of target membranes. (Andreev et al. 2018). Hydrophobicity is related to amphipathicity which is theoretically suggested in all AMP-membrane interactions. Both α and β structures acquire amphipathic structures while interacting with microbial membranes (Hollmann et al. 2018).

11.2.1.3 Models Describing Action of AMPs

After electrostatic and hydrophobic interaction, AMPs self-organize onbacterial cell membranes on achieving a particular concentration. Several models proposed to explain the action of AMPs have been broadly categorized into two. One is the

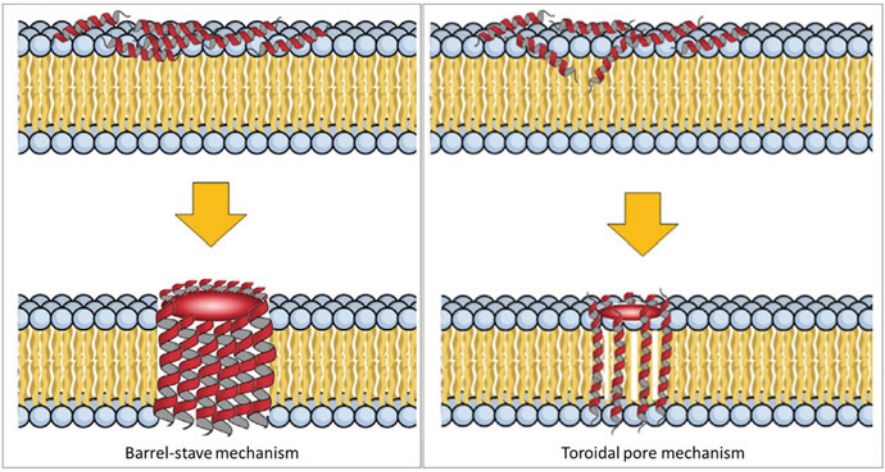

Fig. 11.4 Shows Barrel-stave and Toroidal pore mechanism. In the barrel-stave mechanism, AMPs collect on the membrane surface and then get inserted followed by formation of an aqueous pore in the target membrane. In the toroidal mechanism, AMPs interact with lipids of the membrane forming toroidal pore and induce a local curvature of the lipid bilayer (Corrêa et al. 2019). In the Carpet model (Fig. 11.5) which is a non-pore model, there is no channel formation or insertion of the peptides into target membranes. At a threshold concentration, AMPs form a cover on the membranes like a carpet and destabilize the bilayer by changing its fluidity and then disintegrate into several micelles. The final detergent-like effect caused by AMPs results in the collapse of the membrane and so the final stage is called detergent-like model (Fig. 11.5). Here also peptide-peptide interaction is lacking (Hollmann et al. 2018; Huan et al. 2020; Kumar et al. 2018; Wimley 2010; Zhang et al. 2021). Examples of AMPs that follow the carpet model for antimicrobial property include cecropin, indolicidin, aurein 1.2 and cathelicidin LL-37 (Kumar et al. 2018)

transmembrane pore model and the other non-pore model. The transmembrane pore model is again subcategorized into barrel-stave pore model and toroidal pore models (Kumar et al. 2018; Wimley 2010). In the barrel-stave model (Fig. 11.4) the AMPs are first positioned on membrane surface in a parallel manner and then inserted perpendicular to the lipid bilayer, which helps lateral peptide-peptide interaction. The amphipathic structure of AMP, be it α-helix (minimal length ~ 22 residues) or β-pleats (minimal length ~ 8 residues), is essential in pore formation. The nonpolar region of AMP interacts with the core of the lipid bilayer whereas the polar region is oriented outwards forming the lumen of the channel (Corrêa et al. 2019; Kumar et al. 2018). At times, such AMPs can cause a collapse of cell membranes and result in cell death (Huan et al. 2020). Alamethicin, pardaxin and protegrins adapt barrel stave mechanism, where protegin-1 is a hairpin AMP that forms β- barrels and half barrels (Huan et al. 2020).

Toroidal pore model (Fig. 11.4) which is also called wormhole model, initially orients the peptides like barrel stave model, but the peptide-peptide interaction is lacking here. Peptides interact with lipids of the membrane forming a supramolecular structure called toroidal pore and stimulates a local curvature of the lipid-bilayer.

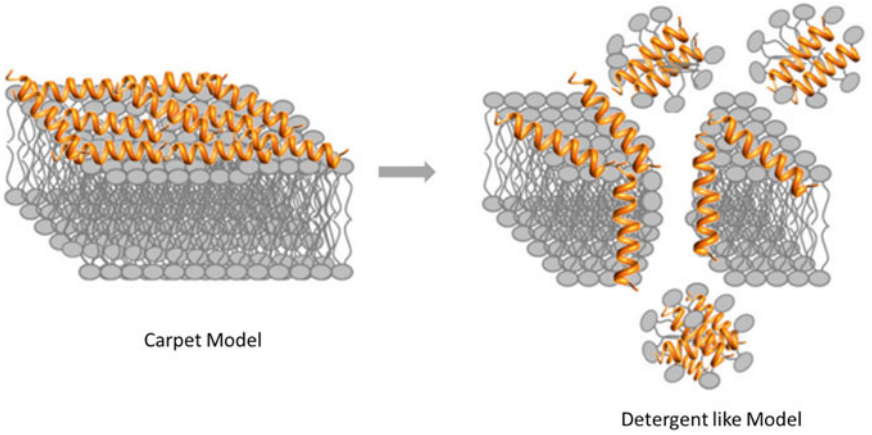

Fig. 11.5 Shows the carpet model. In the carpet model, AMPs form a cover on the membranes like a carpet, and destabilize the bilayer by changing its fluidity and then disintegrates into several micelles. This is followed by detergent model due to the detergent-like effect caused by AMPs results in the collapse of the target membrane (Kumar et al. 2018). There is yet another membrane targeting model called the Aggregate model, in which AMPs bind to the membrane forming peptide-lipid complex and channels so formed results in leakage of the intracellular content and thus cell death (Zhang et al. 2021)

Unlike the Barrel-stave model, the polar-nonpolar assembly of the bilayer is disturbed in toroidal pore model. The destabilization of target membrane leads to cytoplasmic extravasation and lysis (Huan et al. 2020; Kumar et al. 2018 ; Zhang et al. 2021) . Magainin 2, aurein 2.2, lacticin Q, melittin and arenicin are examples of AMP that adapt toroidal pore model (Kumar et al. 2018; Zhang et al. 2021).

11.2.2 Non-membrane Targeting Mechanisms

These AMPs are classified into two: peptides that target the bacterial cell wall and ones having intracellular targets. AMPs inhibit cell wall synthesis by targeting and interacting with precursor molecules necessary for the process. For example, human α-defensin-1 and β-defensin 3 confer bactericidal properties by binding to lipid II (Kumar et al. 2018; Münch and Sahl 2015). Other examples of AMPs inhibiting cell wall synthesis include Cycloserine, HNP1, Teixobactin (Zhang et al. 2021).

AMPs have several intracellular targets affecting numerous processes that lead to target cell death. Ribosomes are targets of several AMPs that bind to them and inhibit protein synthesis. Proline and arginine rich PR-39, oncocin-type peptide, apidaecin-type peptide, Bac5, Bac7 (1–35), Turl 1 A, etc. belong to this category of AMPs. These peptides bind to various parts of ribosomes there by tampering with translation (Zhang et al. 2021). Tryptophan rich AMPs like indolicidin, Tissue-factor-pathway inhibitor-1TC24 (an AMP from tongue), buforin II (AMP having

sequences which are similar to part of histone 2A), peptide P2, isolated from Xenopus laevis skin etc. target nucleic acid biosynthesis (Huan et al. 2020; Zhang et al. 2021). There are AMPs that inhibit activity of intracellular enzymes of bacteria thereby causing cell death. For example, pyrrhocoricin inhibits ATPase, microcin J25 inhibits RNA polymerase, LL-37 inhibits palmitoyl transferase PagP, NP-6 inhibits β-galactosidase (Zhang et al. 2021). AMPs affect metabolic activity by inhibiting activity of proteases. Histatin 5, eNAP-2, indolicidin, Cathelicidin-BF etc. belong to this category (Huan et al. 2020).

AMPs can also impede cell division by affecting DNA replication, repair, cell cycle and chromosomal separation (Lutkenhaus 1990). This group of peptides include APP (Li et al. 2016) and Mother Cell Inhibitor of FtsZ (MciZ) (Cruz et al. 2020). AMPs like Histintin 5 interacts with mitochondria leading to formationof reactive oxygen species thus inducing cell death (Huan et al. 2020). AMPs like Pyrrhocoricin and drosocin employ antibacterial property by interacting with heat shock protein DnaK of bacteria. Another antibacterial mechanism of action includes co-aggregation of amyloidogenic peptides and amyloids (Zhang et al. 2021).

11.3 Immune Modulation

AMPs are involved in modulating both the innate and adaptive immune system. Peptides produced by macrophages and neutrophils confront attacking microbes and they can produce immune responses such as activation and differentiation of white blood cells, inducing angiogenesis and downregulating expression of proinflammatory chemokines. Human AMPs like β-defensin and LL-37 act as chemoattractants of immune cells. Synthetic AMPs like innate defense regulators are found to reduce inflammatory responses. The latter along with anti-malarial agents could decrease fatal neural inflammation associated with malaria. Studies also demonstrate that AMPs can also act as vaccine adjuvants (Kumar et al. 2018; Liang and Diana 2020).

11.4 Microbial Resistance to AMPs

Microorganisms adopt various mechanisms to develop resistance towards antimicrobial agents that are a threat to their existence. One of the important approaches includes modification of membranes affecting the insertion and permeabilization of the peptides (Maria-Neto et al. 2015). The resistance strategy can be either be constitutive or induced. The former, also called passive resistance is dependent on the inherent properties of the microorganisms irrespective of presence of the peptides whereas the latter is initiated by the presence of AMPs. Constitutive resistance involves mechanisms like energy change in microbial membrane and electrostatic shield formation there by disrupting binding of AMP (Zawack et al. 2018). In induced resistance, there is mobilization of proteases, activation of efflux pumps and modification of target by mutation (Corrêa et al. 2019) Studies are extensively

carried out in bacteria in connection with resistance to AMPs and several mechanisms are described. Proteases that degrade AMPs are secreted by gram-positive as well as gram-negative bacteria thereby providing resistance to these peptides. These degradative proteins include aureolysin, SepA, V8 protease, cysteine protease SpeB, aspartate protease belonging to omptin family etc. (Sieprawska-Lupa et al. 2004). Protease mediated inactivation of AMPs is extremely dependent on the target peptide structure.

Bacteria that are embedded in matrices composed of extracellular proteins, DNA and exopolysaccharides, which are collectively known as bacterial biofilms, decrease the penetration of AMPs thereby exhibiting several fold resistance to AMPs (Otto 2012). Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, Klebsiella pneumoniae, or Pseudomonas aeruginosa etc. impede the action of AMPs by this mechanism.

Bacteria resist AMPs also by applying various strategies to modify their cell surface. Alanylation of teichoic acid in cell walls of Gram-positive (Staphylococcus, Streptococcus and Bacillus) and Gram-negative bacteria (Bordetella pertussis), offer resistance towards AMPs by reducing the surface permeability of the microbes. Gram negative bacteria like Pseudomonas aeruginosa and Salmonella typhimurium modify lipopolysaccharide content (LPS) lipid A of their cell surface by attaching aminoarabinose. Certain other gram-negative bacteria like Francisella novicida, Bordetella species and Acinetobacter baumannii add galactosamine/glucosamine to lipid A. S. typhimurium, Neisseria gonorrhoeae and A. baumannii attach phosphoethanolamine (PEA) to phosphates of LPS whereas Vibrio cholerae adds glycine. All these modifications increase positive charge of cell surface there by diminishing attraction of AMP (Whitfield and Trent 2014). Modification of cell membranes also upset AMP attraction to bacterial cells. Amino-acylation, especially lysylation, of phosphatidyl glycerol (PG) head group produce cationic surface barrier to AMPs. Most of the bacteria like P. aeruginosa, Caulobacter crescentus, and Rhizobium tropici adapt this mechanism for resisting AMPs. PG palmitoylation is another mechanism of AMP resistance (Band and Weiss 2015).

AMP efflux pumps on the surface also have a significant role in peptide-resistance of bacteria (both gram positive and gram-negative) bacteria. Bacteria resist AMPs like cathelicidin, defensin,nisin,vamcomycin, indolicidin etc. via this mechanism (Delmar et al. 2014).

To minimize the instability in anionic homeostasis and energy-requiring processes, some of the bacterial AMP resistance mechanisms are closely regulated by sensors. Gram positive pathogens like Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Listeria monocytogenes, Clostridium difficile and Bacillus anthracis, are found to possess antimicrobial peptide sensor (Aps) homologous system that regulates expression of genes associated with mechanisms of AMP resistance (Li et al. 2007). PhoPQ,a histidine kinase sensor present in gram-negative bacteria like Shigella flexneri, Salmonella typhimurium, and Yersinia pestis (Derzelle et al. 2004).

11.5 Antimicrobial Peptides as Drug Candidates against Parasitic Diseases

Parasitic diseases cause significantly high morbidity and mortality in Man, especially in tropical and subtropical regions. These disease-causing parasites belong to two categories viz., protozoan parasites and helminth parasites. While *Plasmodium*, *Trypanosoma*, *Leishmania*, *Entamoeba* and *Trichomonas* are the major protozoan genera, *Schistosoma*, *Ascaris*, *Trichuris*, *Wuchereria*, *Brugia*, *Taenia*, *Onchocerca*, *Ancylostoma*, *Necator* and *Fasciola* are the most important helminth genera. Among all the parasitic diseases the leading agents in terms of morbidity and mortality are malaria, Soil Transmitted Helminth infections (*Ascaris lumbricoides*, *Trichuris trichiura* and hookworm) and schistosomiasis. According to an estimation done in 2004 by World Health Organization the global disease burden due to these three diseases were 46 million, 2.9 million and 1.7 million disability- adjusted life years (DALYs) respectively. This estimation was based on both morbidity and mortality (Pullan and Brooker 2008). Since Malaria is the most dreaded parasitic disease in terms of both morbidity and mortality, the discussion on Anti-microbial peptides in this chapter is restricted to this parasitic disease alone. Malaria continues to be the leading parasitic disease in the world, more so in the sub-Saharan Africa. According to World Malaria Report 2021, there were 241 million cases and 627,000 deaths due to malaria in 2020 (World malaria report 2021, WHO, 2021). Numerous other AMPs have been proven to be helpful against various parasites (Table 11.1).

11.5.1 Malaria Parasites

Human malaria is caused by five species of *Plasmodium* viz., *P.vivax*, *P. falciparum*, *P. malariae*, *P. ovale* and *P. knowlesi*. They have a highly complex life cycle with two hosts (Man and *Anopheles* mosquito) and multiple stages (Tissue schizont and merozoites in human liver, erythrocytic schizont, trophozoites and gametocytes in blood, gametes, ookinete, oocyst and sporozoite in mosquitoes) (Fig. 11.6). This complexity enables the parasites to evade anti-malarial drugs by mutation. Similarly, it also opens up possibility for multiple drug targets (Vale et al. 2018).

Currently, several anti-malaria drugs are available for clinical use. However, many of them have issues related to either toxicity or resistance (Table 11.2) Hence, the search for new anti-malarial drugs is absolutely necessary to ensure the availability of effective drugs. One of the emerging candidates is AMPs.

11.5.2 Antimicrobial Peptides (AMPs) Having Anti-Malarial Properties

Based on the target stages of Plasmodium, AMPs with anti-malarial properties reported so far can be classified into two groups- those against mosquito stages (sporogonic stages) and human stages (erythrocytic stages).

Table 11.1 Antimicrobial peptides (AMPs) against various parasitic diseases

Disease	Name of the parasite	Type of parasite	Route of transmission/ infection	Antimicrobial peptide (AMP)	Reference
Malaria	*Plasmodium falciparum* *Plasmodium vivax* *P. ovale* *P. malariae* *P. knowlesi*	Obligate unicellular protozoan parasite	Vector borne-female anopheles mosquito	Ankyrin NK-2 Phylloseptin-1 (PS-1) CA (1–13) M(1–13) Cecropin B Defensin A DermaseptinDS$_3$ DermaseptinDS$_4$ D-HALO-rev Drosomycin Gambicin IDR-1018 Dermaseptin K4K20-S4 Dermaseptin K4-S4(1–13)a Dermaseptin NC7-P Magainin 2 NK-2 SB-37 Scorpine Shiva-1 Vida 1 Vida 2 Vida 3 Mtk-1 Mtk-2	Arrighi et al. (2002), Vale et al. (2014), Tonk et al. (2019)
Cryptosporidiosis	*Cryptosporidium parvum*	Apicomplexan protozoan parasite	Fecal-oral route	Lactoferricin B, cathelicidin LL37, indolicidin, β-defensin 1, β-defensin 2, lysozyme, phospholipase A2, phosphatidylinositol-specific phospholipase	Giacometti et al. (2003), Carryn et al. (2012)

(continued)

Table 11.1 (continued)

Disease	Name of the parasite	Type of parasite	Route of transmission/ infection	Antimicrobial peptide (AMP)	Reference
Trypanosoma	*Trpanosoma brucei* *Trypanosoma cruzi* *Trypanosoma gambiense*	Unicellular flagellated protozoan	Vector borne-tsetse fly	Attacin Defensin Diptericin Cecropin Stomoxyn Beta-defensin-1 Beta-defensin-2 Cryptin-4(alpha-defensin) LL-37 SMAP-29 Novispirin Protegrin-1 OaBAC-5-mini Indolicidin BAC-CN BMAP-27 BMAP-18 TP10 Pleurocidin CP-26 V681 Nonamer peptide A (D-aminoacids) Leucinostatin A Alamethicin I Tsushimycin Vasoactive intestinal peptide Adrenomedullin	Harrington (2011)

Leishmaniasis	*Leishmania donovani* complex	Obligate intracellular protozoa	Vector borne-sandflies	Melittin Cecropin-A Cecropin-D LL-37 RI-BMAP-28 D-BMAP-28 MBD1 MBD2 MBD3 Vu-Def MG-H1 MG-H2 F5W-magainin 2 Pexiganan Temporin A Temporin B Temporin- she SHd DS 01 Dermaseptin S1 Eumenitin Eumenitin-F Eumenitin R Hst5 D-Hst Dhvar4	Lynn et al. (2011), Lewies et al. (2015)
Trichomoniasis	*Trichomonas vaginalis*	Unicellular protozoa	Sexually transmitted infection (STI)	Tritrpticin Epinecidin-1 D-Hectate Prophenin 2	Mutwiri et al. (2000), Infante et al. (2011), Huang et al. (2019), Liu et al. (2019)
Toxoplasma	*Toxoplasma gondii*	Protozoan	Cat litter-fecal contamination	HPRP-A1/A2	Liu et al. (2019)

(continued)

Table 11.1 (continued)

Disease	Name of the parasite	Type of parasite	Route of transmission/ infection	Antimicrobial peptide (AMP)	Reference
Schistosomiasis	Blood fluke/ *Schistisoma*	Helminthic-flatworms	Snails-intermediate host	Dermaseptin 01	de Moraes et al. (2011)
Cysticercosis	*Taenia solium*	Helminthic-tapeworms	Fecal-oral route	Temporin A Iseganan IB-367	Landa et al. (2009)

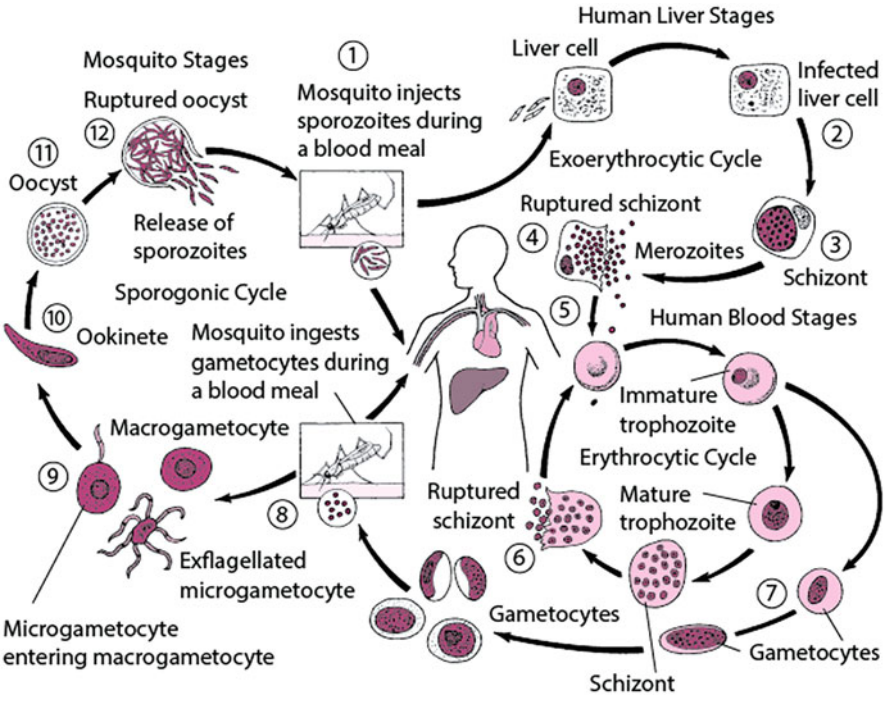

Fig. 11.6 Life cycle of *Plasmodium*

11.5.2.1 Antimicrobial Peptides Against Sporogonic Stages

1. Cecropin B: Cecropins are naturally occurring peptides in the humoral immune system of insects. Cecropin B is a synthetic Cecropin. Gwadz et al. (1989) tested its efficacy against the sporozoite development of *P. falciparum* in *Anopheles gambiae* and *An. freeborni*. Though there was no direct effect on sporozoites, the majority of oocysts failed mature. This demonstrates its efficacy as a potential transmission-blocking drug (Gwadz et al. 1989).
2. Defensin A: Defensins are AMPs in the innate immune system. Kokoza et al. (2010) demonstrated overexpression of Defensin A along with Cecropin A in transgenic *Aedes aegypti* which blocked the development of *Plasmodium gallinaceum* in the midgut. Subsequenty, they demonstrated complete blocking of transmission of *P. gallinaceum* by these transgenic mosquitoes. However, studies on human malaria parasites have not been done so far (Kokoza et al. 2010).
3. Drosomycin: It is an antifungal defensin and a part of *Drosophila* innate immune system. Tian et al. (2008) demonstrated the anti-ookinete activity of Drosomycin and Drosomycin 2 in *Plasmodium berghei*, a rodent *Plasmodium* species (Tian et al. 2008).
4. Gambicin: It is an immune responsive AMP isolated from the notorious African malaria vector mosquito *Anopheles gambiae*.

Table 11.2 Profiles of important anti-malarial drugs

Drug	Target stage of *Plasmodium*	Severe adverse reactions	Resistance status
Chloroquine	Erythrocytic schizonts	Death from overdose	Resistance in *P. falciparum* everywhere except Central America and in some areas of south West Asia
Sulfadoxine-pyrimethamine	Erythrocytic schizonts	Stevens-Johnson syndrome	Resistance in reported *P. falciparum* reported from Thailand and high rates of treatment failure in some parts of Africa, Southeast Asia and South Asia
Quinine	Erythrocytic schizonts	Hemolytic anemia, coma, respiratory arrest, renal failure	Resistance and treatment failure reported sporadically from various parts of the world
Mefloquine	Erythrocytic schizonts	Psychosis	Resistance in. P. *Falciparum* reported from some areas of Thailand and Africa
Atovaquone-chlorogunanide	Erythrocytic schizonts	None	No evidence of resistance
Artemether-lumefantrine	Erythrocytic schizonts, gametocytes	Impaired hearing	Partial resistance in *P. falciparum* reported from some areas of Africa
Artesunate-mefloquine	Erythrocytic schizonts, gametocytes	None	Partial resistance in *P. falciparum* reported from some areas of Africa
Halofantrine	Erythrocytic schizonts	Cardiac arrest	No evidence of resistance
Primaquine	Tissue stage schizonts (Hypnozoites), gametocytes	Hemolytic anemia	High rates of treatment failure

5. Magainin 2- Magainins are amphibian peptides originally isolated from *Xenopus laevis.* Gwadz et al. (1989) used the synthetic carboxy amide of Magainin 2 against *P. falciparum* during his studies on Cecropin B discussed earlier. The result was the same as obtained for Cecropin B (Gwadz et al. 1989).
6. Scorpine: Scorpine is a peptide present in scorpion venom originally isolated from *Pandinus imperator.* Conde et al. (2000) showed growth inhibition of ookinetes and gametes of *P. berghie* (Conde et al. 2000).
7. Vida1 Vida 2 Vida 3: Vida 1 to 3 are synthetic hybrid peptides designed by Arrighi et al. (2002). These were tested against the sporogonic stages of *P. berghie* and *P. yoelii nigeriensis*. (Doi: 10.1128/AAC.46.7.2104–2110.2002). Vida 3 showed the highest level of activity both in vitro and also in vivo in *Anopheles gambiae* (Arrighi et al. 2002)

11.5.2.2 Antimicrobial Peptides Against (AMPs) Erythrocytic Stages

1. Cecropin A (1–13)Melittin (1–13): It is a hybrid peptide of the naturally occurring antibiotic peptides Cecropin A and Melittin. As mentioned earlier, the former is an important component of the humoral immune system of insects and the latter is a venom toxin. The anti-malarial property of the hybrid peptide was demonstrated by Boman et al., in 1989. They had assayed the reinvasion of the human erythrocytes by the blood stages of *P. falciparum* and recorded a reduction of 50%. Later Wade et al. (1990) synthesized the 'all D enantiomer' of Cecropin A (1–13) Melittin (1–13) and demonstrated a 20% increase in efficacy than its naturally occurring L-isomer counterpart (Wade et al. 1990).
2. Dermaseptins: Dermaseptins are naturally occurring peptides isolated from the skin of *Phyllomedusa,* a genus of tree frogs. Ghosh et al. (1997) demonstrated the efficacy of two Dermaseptins DS3 and DS4 against *P. falciparum* infected human RBCs. They found that both DS3 and DS4 were effective against the intraerythrocytic parasites. However, DS4 was also found toxic against uninfected RBCs. Hence, DS3 is a better anti-malarial peptide (Ghosh et al. 1997). Another couple of Dermaseptins showing anti-malarial properties are the DS4 derivatives Dermaseptin K4K20-S4 and Dermaseptin K4-S4(1–13)a. As demonstrated by Krugliak et al. (2000) the substituted S4 analogue K4K20-S4 was more potent than Dermaseptin K4-S4(1–13)a. Both peptides inhibited the growth of trophozoite stage than the ring stage of *P. falciparum*. They lysed only the infected RBCs (Krugliak et al. 2000). The fifth Dermaseptin to exhibit anti plasmodia activity is Dermaseptin NC7-P. Experiments done by Efron et al. (2002) showed that this derivative peptide was more potent than previous two S4 derivatives. Besides, the hemolytic activity was also less, making it safer (Efron et al. 2002).
3. D -HALO-rev: It is a designed histidine-rich amphipathic cationic peptide. Mason et al. (2009) tested the efficacy of various histidine-rich amphipathic cationic peptides and found D -HALO-rev to be the most potent in killing the erythrocytic stages of *P. falciparum* (Mason et al. 2009).
4. NK-2: The peptide NK-2 is a component of the mammalian lymphocytic effector protein NK-lysin. An experiment conducted by Gelhaus et al. (2008), showed that RBCs infected with *P. falciparum* were lysed by this peptide. However, non-infected RBCs were spared (Gelhaus et al. 2008).
5. SB-37 and Shiva-1: These are synthetic lytic peptide analogs. Shiva- 1 was twice as effective as SB-37 (Fan et al. 2018).

11.6 Disadvantages of Antimicrobial Peptides (AMPs)

None of the peptides discussed above have entered clinical trials so far. The major reasons for the hesitation of pharma companies to go for large-scale clinical trials are believed to be the higher molecular weight of peptides and the high cost involved in their production. Due to their high molecular weight, peptides are not easily

bioavailable. Besides, they are also easily prone to enzymatic degradation and binding to plasma proteins (Vale et al. 2018).

11.7 Computational Methods for AMPs and Drug Discovery Research

11.7.1 AMP Databases

Drug-resistant pathogens are a threat to the entire globe, and AMPs have been suggested as a potential drug component in the fight against diseases (Mahlapuu et al. 2016; Zainal Baharin et al. 2021). The design and development of new AMPs will greatly benefit from studying the existing peptides and their human action mechanisms.(Chen and Lu 2020). In order to facilitate such research, it is necessary to store and manage the increasing number of AMPs that have been identified. As a result, AMP databases play a significant role in drug discovery. A list of important AMP databases is shown in Table 11.3.

11.7.2 3D Modelling

A step toward understanding the structure of a peptide is its design, which aids in understanding how to improve its therapeutic properties (Chen et al. 2022). There are numerous approaches to studying structurally unknown AMPs. Ab initio 3D

Table 11.3 List of important AMP databases

S. no	Name of the database	Webserver Link	Reference
1.	The antimicrobial peptide database (APD)	https://aps.unmc.edu/	Wang et al. (2016)
2.	CAMP (collection of antimicrobial peptides) database	https://www.camp.bicnirrh.res.in/	Waghu and Idicula-Thomas (2020)
3.	AVPpred	https://crdd.osdd.net/servers/avppred	Thakur et al. (2012)
4.	Database of antimicrobial activity and structure of peptides (DBAASP)	https://dbaasp.Org/	Pirtskhalava et al. (2021)
5.	Antifp: A prediction server for antifungal peptide	https://webs.iiitd.edu.in/raghava/antifp	Agrawal et al. (2018)
6.	AntiBP2 server, antibacterial peptide prediction	http://crdd.Osdd.Net/raghava/antibp/	Lata et al. (2010)
7.	iAMPpred	http://cabgrid.res.in:8080/amppred/	Meher et al. (2017)
8.	DRAMP 2.0	http://dramp.Cpu-bioinfor.Org/	Kang et al. (2019)
9..	LAMP	http://biotechlab.Fudan.Edu.Cn/database/lamp	Zhao et al. (2013)

modelling is one method, which analyzes the predicted folds of modelled sequences to similar ones (Wu et al. 2007).

11.7.3 Docking Studies

The development of new drugs that utilize AMPs to treat diseases has been accelerated by computational and structural biology techniques (Ahmed and Hammami 2019). Structural biology studies are helpful in retrieving and analyzing data from data repositories for further analysis in drug discovery research. Thus, using AMPs data for docking and dynamics studies are important part of the drug discovery process.

11.7.4 Molecular Dynamics (MD) Simulations

Molecular dynamics simulations (MD) is a method that allows peptides or proteins to analyze their changes in conformation over time (Hollingsworth and Dror 2018). Atoms are simulated to determine the stability of newly modelled folds and their fold matches in the case of proteins or peptides using an atomic-level representation (Zhang et al. 2003).

11.7.5 Machine Learning Techniques

A deeper knowledge of diseases and the biological events that underlie them is provided by machine learning techniques, which also support preclinical wet-lab studies and clinical trials (Réda et al. 2020). Machine learning is helpful to identify new and novel AMPs from the genomic sequences of organisms in order to develop the development of new AMPs (Lee et al. 2017). Specialized databases will be used to collect and manage the details. These programs will make quick searches and data processing possible. Additionally, there are numerous programs that aid in evaluating the effectiveness of screening AMPs.

11.8 Conclusion

Several microorganisms synthesize antimicrobial peptides (AMPs) as their first line of defense. Few amino acids that constitute AMPs have various mechanisms. These AMPs have innate immune properties. The existence of databases is helpful. AMP databases aid in the management and utilization of AMP data for studies on drug discovery. We have discussed the classification of AMPs, the nature of various AMPs, the pharmacological components used to treat parasitic infections, and the disadvantages of AMPs. We could see that, AMP research will have much importance and relevance in medical research especially in parasitic diseases. Finally, we

had a brief discussion on some of the significant databases used in AMPs research. In order to properly understand how AMPs can be applied in various clinical settings, more research is required.

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Pathway to Register Natural Product-Derived Therapeutics

12

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Abstract

Natural products (NPs) are biochemical compounds or molecules produced by living organisms. They are obtained from diverse sources ranging from small microbes to large plants or animals. NPs have diverse structural and biochemical properties and possess great utility for human beings. Among them, pharmacological properties make them highly demanding as medicinal and therapeutic applications to cure, treat and protect from various diseases, infections, and illnesses by interacting with specific targets present in the cell of the diseased person or pathogen. The NPs identified and known to us can be grouped into three categories as pure natural products, semi-synthetic NPs and natural product-derived compounds. Technological advancement has made possible faster and efficient identification, isolation and characterization of NPs that can be used as novel drug candidates or knowledge about NPs structure or biochemistry help to develop modified analogues or derivatives. The finding of such novel NPs molecules leads to innovations. In present knowledge based socio-economic conditions, innovations have economic potentials and are protected under intellectual property rights (IPRs) laws in every jurisdiction and well supported by international legal frameworks. These rights are intangible assets as created by human intellect and creativity and have great socio-economic value. These rights are related with copyrights, Patents, Trademark, Trade secrets, GI, PVPFR, Design. IP rights refers to the exclusive rights granted by the state to their citizen or innovator/creators over their creativity and innovations. There is great potential between the NPs and the IP rights associated with them as these NPs are novel and obtained by application of human intellect and creativity. Patent is one of the

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_12

key IP Right sought for protection of the innovation related with NPs which gives a bundle of rights, an exclusive legally enforceable power to IP owner over their invention for a limited period of time (20 years) in exchange of full disclosure of his invention to prevent others from using own intellectual creation. The Indian Patent Act, 1970 and subsequent amendments along with Patent Rules 2003 and judicial interpretations and directions provide the path for registration of NPs and regulation over it at national level. These laws are made in tune with international IP rights regime laid down by WTO-TRIPS rules and PCT. Any violation of IP rights of inventors granted over NPs are dealt with under these legal regimes mixed with civil and criminal remedies associated with the laws. These laws are acting as catalysts for further innovations and development of therapeutic products based on NPs.

Keywords

Intellectual property rights · Innovation · Natural products · Patent · Therapeutics

12.1 Introduction

Natural products (NPs) are biochemical compounds or molecules produced by living organisms ranging from small microbes to large plants or animals. These products are structurally and biochemically diverse in nature and possess diverse utility for human beings due to their pharmacological, food, coloring agent properties. The pharmacological properties of NPs make them a source of therapeutic agents. NPs from animals, microbes, plants, and minerals have been sources of human disease treatments (Shinde et al. 2019). Advancement in technology has led to the development of modified analogues or derivatives of NPs. Such modified analogues show better pharmacological potential and are chosen as novel drug candidate (Chopra and Dhingra 2021). The finding of such novel NPs molecules leads to the emergence of intellectual property rights (IPRs) of innovators as the invention is made. If such inventions are brought to the IP office for claiming the rights, after testing the application on certain criteria, the IP office issues the IP rights to innovators for their invention. These rights are classified as copyrights, Patents, Trademarks, Trade secrets, GI, PVPFR, and Design. There is a strong interrelationship between the NPs and the IP rights associated with them as these NPs are obtained and tested by the application of human intellect and creativity that leads to the generation of the IPRs, especially in the fields associated such as the pharmaceutical and biotechnology industry (Ajeet 2012). IP rights refer to the exclusive rights granted by the state to their citizen or innovator/creators over their creativity and innovations. These IP rights can be given for industrially useful products or services such as industrial designs, patents, trademarks, trade secrets, new plant varieties and geographical indications, (GI), copyrights and related rights. The NPs therapeutic value makes them very much an integral part of the IP system. As these NPs become the source for obtaining IP rights. The sustenance of the pharma, biotech industry, and allied

companies economic and business is pivoted on IP based model as it provides resource investment for further research and development, innovative capacity, and competitiveness. Companies earn revenues further by relying upon licensing in or licensing out of IP rights.

12.2 Natural Products and Their Types

NPs have been used as therapeutic agents since time immemorial because of their chemical diversity. These chemicals are initially used as crude but as technology evolved, their purified versions become available as they work with more efficacy and potency (Firn and Jones 2003). The chemical diversity is generated in living organisms for their better survival against biotic and abiotic pressure and requirement as per their environmental setup for a better competitive edge (Lee 2010). These NPs have great therapeutic potential as they interact with specific targets present in the cells of diseased persons or pathogens. These NPs can be categorized as NPs, Semi-synthetic NPs and NP-derived compounds (Fig. 12.1).

NPs are those chemicals that are derived from natural sources (animals, microorganisms and plants) and have the ability to show biological effects. The chemical compounds derived from an NP template using a semi-synthetic process are called semi-synthetic NPs whereas synthetically derived or sometimes inspired from semi-synthetic NPs are called NP derived compounds (Mishra and Tiwari 2011). CRISPR-Cas9 is a genome-editing tool that helps in obtaining and optimizing NPs have the potential as the therapeutic value against cancer and other medical conditions (Dey and Nandy 2021). Alzheimer's Disease (AD), a neurodegenerative disease, causes progressive neural degermation leading to damage of memory and cognitive function. The AD has no successful therapy till date. It is known that medicinal herbs rich in diverse compounds can be advantageous because of multitargeted nature in comparison to single targeted synthetic drugs and may be a promising source of disease cure (Koynova and Tenchov 2018). Many medicinal plants and isolated compounds from them are used for treating AD (Bui and Nguyen 2017). Herbal medicine and NPs derived from them are seen as beneficial roles in

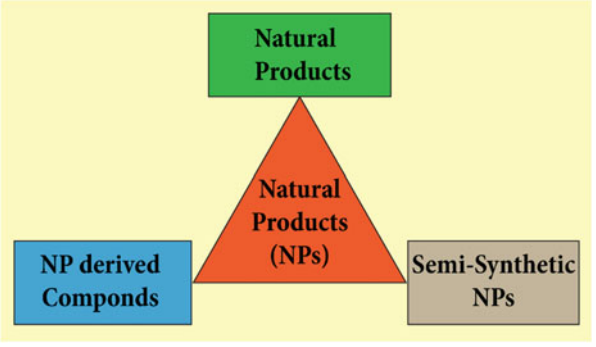

Fig. 12.1 Natural products and their types

CNS Disorders and Neurodegeneration (Ahmadi 2021). NPs (alkaloids, flavonoids, lignin, polyphenols, sterols, triterpenes and tannins) based therapeutics offers diverse options because of multitargeted nature, to reduce the progress and symptoms of various diseases because of their anti-amyloidogenic, anticholinesterase, anti-inflammatory and antioxidant.

As the generic medicine concept is applicable in classical drug system, biosimilar concept is a fast emerging threat in IP system. The reason behind that the product such as interferons, growth hormones (GH) and epoietins are product of recombinant DNA technology (RDT) and their Patent rights period ended which opened up the introduction of similar products by the competitors. Without proper clinical trial and immunogenicity test, their application may lead to greater threat as seen in upsurge of pure red cell aplasia (PRCA), an anaemia of severe nature associated with the use of epoietin-alpha. Therefore, there is a great need to take care of tough legal regime of IP rights (Schellekens 2004). These are not exact copies of the original, but, like generic drugs, biosimilar drugs have to demonstrate equivalence to the reference drugs in quality, safety and efficacy. Nevertheless, despite their importance and contribution to the sustainability of the health system, doctors are sometimes unaware of differences between them, and their impact in terms of clinical and economic effects (Villamañán et al. 2016). Receptor Tyrosine Kinases (RTKs) are involved in diverse cellular processes such as growth development, angiogenesis, and cancer. Among RTKs, Mesenchymal-Epithelial Transition Factor (c-Met) kinase is one of the types showing potential targets for antitumor drugs having great prospects in tumor prevention, chemotherapy, biotherapy, and especially in tumor resistance. It has been found that all c-Met inhibitors published in patents database since 2015, two kinds of c-Met inhibitors, one is from NPs and the other one is of synthetic origin. (Xu and Yao 2020).

Marine organisms are excellent producers of natural chemicals with diverse structures and pharmacological activities. Diverse NPs obtained from marine organisms have potential for therapeutics from marine-based drugs for anticancer, antimicrobial, antiviral and anti-inflammatory drugs and treatments as a great number of marine NPs are entering into clinical trials (Shinde et al. 2019). The US Supreme Court's decision in *Association for Molecular Pathology v. Myriad Genetics, Inc.* held that isolated human genes cannot be patented on the ground that "*products of nature*" were not eligible to be patented unless they were "*isolated and purified*" from their surrounding environment. Myriad Genetics, is a company who obtained patents on genes (BRCA1 and BRCA2) useful in testing early onset of breast and ovarian cancer, which led to great social and political criticism (Sherkow and Greely 2015). Antibiotic resistance and tolerance are major global problems. As bacteria employ diverse mechanisms to break the antibiotic used during treatment. It requires innovative therapeutic strategies to address the challenges associated with pathogenic bacteria. NPs have been used since time immemorial and have played a great role in discovery of new antibiotics (Abouelhassan et al. 2019). The bacteria (*E. Coli*) acted as host for the heterologous biosynthesis of NPs having therapeutic value. The problem is associated with its access challenges posed by native production hosts (Ahmadi and Pfeifer 2016). Despite immense potential of NPs, very few

bacterial genera have been investigated, therefore more research is required to overcome the urgent threat of antibiotic resistance by increasing the organism range including anaerobes, pathogens, and symbionts of humans, insects, and nematodes to opens the way for a new golden age of microbial therapeutics (Challinor and Bode 2015).

Cardiovascular disease (CVD), is one of the causes that has a significant impact on human morbidity and mortality. NPs derived medicines have been greatly used to cure this disease for centuries and accounts for over 50% of newly developed cures for CVD. There is great future prospect for developing NPs-based safer and effective CVD drugs (Zhao et al. 2020).

Malaria, caused by *Plasmodium* parasites, is another devastating global health issue especially in developing countries because of the absence of broad and effective drug and drug resistance. NPs derived antimalarial drugs such as artemisinin combination therapies (ACTs) have historically been an excellent source to treat malaria. Two compounds, alstonine and himbeline have displayed better activity against *P. falciparum* versus two human cell lines and *P. falciparum* multidrug-resistant lines showed no cross-resistance (Arnold et al. 2021). Historically, medicinal plants have been a valuable source as NPs with therapeutic potential, and research continues to identify novel drugs. Pharma industries have developed a great number of synthetic or analogue compounds to be used as drugs because of their easy production, cost efficiency and compatibility with established high throughput screening (HTS) platforms. But because of issues associated with synthetic drugs, once again the trend is up for drug discovery from NPs and biotechnological approaches are used as alternatives and total organic synthesis. Current research trends clearly indicate that NPs will be among the most important sources of new drugs in future also (Atanasov et al. 2015). Actinomycetes are one of the important groups that are known to produce over two-thirds of all known secondary metabolites. The metabolic engineering of *Streptomycetes* has led to the synthesis of natural products and increased production of nystatin and teicoplanin (Bilyk and Luzhetskyy 2016).

Recently, the novel coronavirus infection (COVID-19), which started in Wuhan, China in late 2019 created havoc around the globe in a very short span of time. Several NPs were reported from plants (*Allium sativum, Camellia sinensis, Echinacea spp., Glycyrrhiza glabra, Hypericum perforatum, Nigella sativa, and Scutellaria baicalensis, Zingiber officinale*) having antiviral activities in different studies These NPs reported to improve the immune response and fight against virus by preventing viral replication. Alkaloids such as homoharringtonine, lycorine, and emetine have also been reported to have strong anti-coronavirus effects. The NPs targets are S protein and replication enzymes suggesting their use as preventive and therapeutic agents (Boozari and Hosseinzadeh 2021). The use of NPs in traditional medicine have served as starting points for new therapeutics and can help further by providing information for future drug discovery. Recent technological advancements provide the framework to leverage ethnopharmacologic data for further boosting of drug discovery. Loss of traditional medicinal knowledge and extinction of organisms are two key threats that are affecting the discovery process (Buenz et al. 2018).

As the US legal system has clearly demarcated what is a natural product and laid down criteria for registration, a similar situation is not very clear in the Indian legal system. The Indian Patent Act, Section 2(1)(j) lays down the definition of '*Invention*' that says "*any new product or new process without differentiating between 'product of nature' or 'man made products*'." Along with this, Section 3 lays down what are not patentable inventions. Reading these two sections together makes sense that NPs can be patented if necessary human intervention is made while obtaining, creating or producing the product (natural) or its process. A patent is the most sought IP right for NPs where exclusive rights are granted for an innovation that passes the filter test prescribed under the Act or judicial decisions. The exclusive rights legally protect the invention from being copied or reproduced by others. But in return to grant a patent, the patentee has to disclose the invention to the public in their application presented to obtain patent rights in such a manner which is sufficiently clear and complete and enables it to be replicated by a person with an ordinary level of skill in the relevant field.

12.3 Natural Product, Therapeutic Value and IPR

In today's knowledge-driven industrial economies, IPRs play an integral and pivotal role for encouraging innovation, creation. IPRs are making more lucrative domains for public as well as private investment for more research and development (R&D). IP Rights are a bundle of rights that gives an exclusive legally enforceable power to IP owners over their invention for a limited period of time in exchange of full disclosure of his invention to prevent others from using their own intellectual creations. Various IP rights are available depending upon the domain sought by the applicant for their own inventions or creativity. In the absence of a strong IP regime, investment from investors can't be done for further research and inventions because in the absence of protection, anyone can exploit the invention.

Constitution of India also accept and provides such IP rights and has included a provision as entry no 49 under union list of the seventh schedule. It gives power to the union government to enact any laws related to Patents, inventions and designs; copyright; trademarks and merchandise marks (Constitution of India 1949). The IP domain has been enlarged over the period and as India became a party, member of multilateral treaties, institutions or agreements at the global level. World Intellectual Property Organization (WIPO) World Trade Organization (WTO), Trade Related Intellectual Property Rights (TRIPS), Patent Cooperation Treaty (PCT) etc. are the key instrumental bodies responsible for laying down laws, rules and regulations related to IP rights. India has adopted them and amended them accordingly in their domestic laws. (For detailed information about WIPO, WIPO—World Intellectual Property Organization; WTO/TRIPS,www.wto.org; PCT, Patent Cooperation Treaty (PCT) (wipo.int)).

Here in this chapter, the discussion will be restricted to the procedure related to patent protection for NPs associated with therapeutic potentials. Patent protection is available for novel processes and products associated with NPs.

12.4 Natural Products and Registration Pathways

12.4.1 Registration Requirements

As with other products or processes, patents on NPs are treated alike. To obtain a patent, any invention based on NPs having therapeutic value will become eligible for grant of patent only when it satisfies the patentability requirements as prescribed in Indian Patent Act 1970 [1] and is consistent with judicial decisions. Indian legal system keeps following four requirements or touchstone:

1. Novelty.
2. The inventive step.
3. Capable of Industrial application.
4. Must be patentable subject matter.

12.4.2 Novelty

As per Indian Patent Act, an "*invention*" means a new product or process involving an inventive step and capable of industrial application and becomes eligible for patent only if it is new in the light of prior art, or is not anticipated by prior art (Section-2(1)(j). The assessment of novelty in inventions based on NPs shall be done as done for other inventions. To pass this stage, the prior art is to be construed as per Section 13 (read with Sections 29 to 34) of the Indian Patent Act. All information and knowledge relating to the invention, available to the public in publication before the date of priority of patent application constitutes prior art. Thus, the Indian Patents Act, 1970 does not have any explicit provisions with respect to novelty of NPs inventions.

12.4.3 Non-Obviousness/Inventive Step

NPs having therapeutic value should have or show inventive steps to secure a patent. Not many case laws are available related to NPs which make the picture a little bit obscure on inventive steps. Indian patent office has released a guideline as the Manual of Patent Office Practice & Procedure (2013) (hereinafter the manual) that helps in the assessment of inventive steps of inventions.

The Patents Act says that an invention will have inventive steps if the invention involves (a) technically advanced as compared to existing knowledge or (b) having economic significance or (c) both, and that makes the invention not-obvious to a person skilled in the art (Section −2). The Manual says that isolated gene sequences

[1] All Sections and Rules quoted are taken from Indian Patent Act, 1970 and Indian Patent Rule 2003 except otherwise mentioned.

and protein sequences will be considered to have an inventive step in the light of their naturally existing counterparts. The economic significance requirement is relatively easy to prove for inventions based on NPs due to their medical sector applications. Apart from that reasonable expectation of success, predictability of the field like principles are applied to assess the inventive steps on NP based inventions as applied on other inventions.

12.5 Industrial Application

The criteria "capable of industrial application" in relation to invention based on NPs means the invention is capable of being made or used in an industry (Section-2(1)(ac)). The invention granted is liable to be revoked if the invention is not useful. Apart from that, patent application containing the specifications should disclose the usefulness and applicability in a distinct and credible manner (Section—64(1)(g)).

12.6 Patentable Subject Matter

Eligible subjects matter to get patents are very broadly worded in the Indian Patent Act and consider any product or process irrespective of the technology.

There is a list under S-3 of the patent Act which can't be protected under the patent regime. In other words, these items are excluded from the patentable subject matter and equally applies to NPs also. Patent examiner while examination of patent applications is rejected even though the inventions pass trio tests viz., novelty, inventive step, and capability of industrial applications. The list includes the following inventions under exclusions that are:

1. Not contrary to morality or which cause serious prejudice to human, animal or plant life or health or environment (Section 3(b));
2. Discovery of any living thing or non-living substance occurring in nature (Section 3(c));
3. Mere discovery of new form of a known substance which does not result in enhancement of known efficacy or mere discovery of any new property or new use for a known substance (Section 3(d));
4. Mere admixture resulting only in the aggregation of the properties (Section 3 (e));
5. Method of agriculture and horticulture (Section 3(h));
6. Method of treatment and diagnosis (Section 3(i));
7. Plants and animals in whole or any part thereof other than micro-organisms, but including seeds, varieties and species, and essentially biological processes (Section 3(j)):
8. Inventions which are in effect traditional knowledge (Section 3(p));
9. Apart from that patent must disclose sufficiently with the best method of performing the invention (Section 10(4));

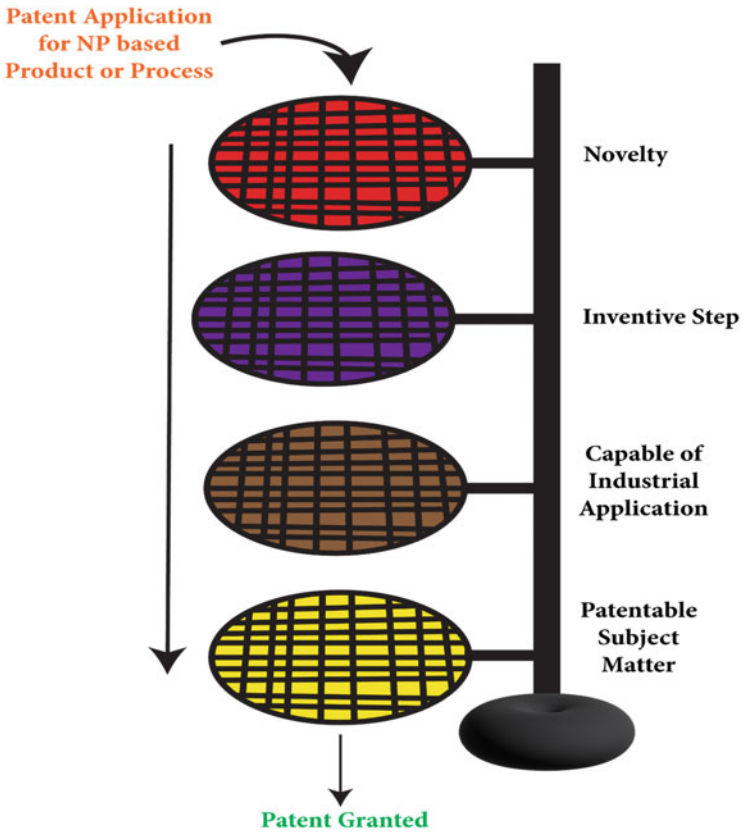

Fig. 12.2 Assessment criteria for obtaining process or product patents

10. Along with unity of invention and clarity, succinctness and support of the claims (Section 10(5)).

Discovery of any living thing occurring in nature is not patentable matter in India. However, microorganisms and microbiological processes are patentable subject matter. Genetically modified multi-cellular organisms including plants, animals, human beings and their parts are excluded from patentability in India. Gene sequences and DNA sequences having disclosed functions are considered patentable in India. However, human beings and embryonic stem cells are not patentable (Fig. 12.2).

To summarize, an invention based on NPs must satisfy substantial, credible and specific utility to satisfy the utility requirement. General uses of the invention will not be accepted for the purpose of utility and specific uses must be shown. Key information regarding product and process patent are depicted as an example in Table 12.1.

Table 12.1 An example of process and product patent on natural products

S. no.	Details	Process patent	Product patent
1.	Patent description	The invention related process for obtaining hexane bioactive fraction from the roots of an aromatic plant (*Vetiveria zizanioides*), having potential for inhibiting the growth of drug resistant bacterial infections in humans and animals	The invention related with an antioxidant extract (product) from sesame seed/cake containing sesamol and other compounds, prepared by employing selective extraction techniques and purification methods and having industrial applications as a substitute for synthetic antioxidants for the protection of vegetable oils, foods, cosmetic/ pharmaceutical preparations etc.
2.	Patent assignee	Council of Scientific & Industrial Research (CSIR)	Council of Scientific & Industrial Research (CSIR)
3.	Patent no./ application no.	IN231854 [India/2565/DELNP/ 2004	IN227580 [India]/1002/DEL/ 2003
4.	Inventors	Suman Preet Singh Khanuja; Suchi Srivastava; Tiruppadiripuliyur Ranganathan Santha Kumar; Madan Mohan Gupta; Arvind Kumar Tripathi; Monika Singh; Janak Raj Bahl; Raj Kishori Lal; Madendra Pandurang Darokar; Ajit Kumar Shasany; Sushil Kumar	Ananthasankaran Jayalekshmy; Chami Arumughan; Kizhiyedathu Polachira Suja
5.	Issue date	27-03-2009	30-01-2009
6.	Filing date	01-09-2004	14-08-2003

Source: Patents, India Science, Technology & Innovation (ISTI); https://www.indiascienceandtechnology.gov.in/innovations/patents

12.7 Enablement and Written Description

Indian Courts and Patent Offices have laid down standards of written description and enablement requirements for inventions including NPs-based inventions and insist on detailed invention descriptions, research data and examples. Enablement is commonly not assessed through supplementation of prior art unless specific reference is made in the written description. The written description and enablement requirements may be satisfied by the deposit of biological materials or submission of sequence listings in case of genetic material inventions related to NPs. The manual lays down that invention to be described completely in the specification to enable a person skilled in the art to be able to carry out the invention by reading the specification.

12.8 Drafting of a Patent Specification

An applicant willing to get patent rights for his invention related to NPs must disclose his or her invention to the patent office and subsequently to the public. Such disclosure is done by presenting or producing a document called specification along with a patent application. The application provides the details of an invention and on the basis of that claims are made. The application claims are assessed by the patent office and accordingly, patent rights are given or rejected.

12.8.1 Patent Specification

It is a description or techno-legal document that discloses inventions made by inventors to the patent office as well as the public. The patent office receiving applications must contain specifications. The specification should relate to a single invention. There are two types of patent specifications namely,

1. Provisional specification.
2. Complete specification

12.8.2 Provisional Specification

It is a type of techno-legal document that does not disclose an inventive concept completely. The aim of filing provisional specification (PV) is mainly to secure a "*priority date.*" The legal provisions related with specifications are Section 9, 10, 57 and 59 of the Indian Patent Act 1970 and corresponding rules are available as Rule 13, 14, 24A of the Patent Rules 2003. Extra time to carry out more work as well as introduction of further development on inventions becomes available after filing provisional applications that can be finally added in complete specification. Drawings related to invention can also be submitted, and forms part of provisional applicants can also be submitted.

12.8.3 Complete Specification

It is a description or a techno-legal document. It discloses all details of an invention in such a way that makes it clear and complete so that a person skilled in the art can practice the invention. Twelve months are time-space available to file a complete specification after filing of the provisional application. Every complete specification must conclude with a claim or set of claims. It is important to note that each claim in specification defines an invention and thus a respective priority data associated with it.

There are two ways of filing the complete specifications:

1. Direct filing.
2. Subsequent filing.

Direct filing requires no corresponding provisional specifications and is filed straightaway as complete specification to the patent office. In case, the provisional specification is filed first and the complete specification is filled subsequently and claiming priority from the corresponding provisional specification is called as subsequent filing.

12.8.4 Specification Filing Format

Form-2 is prescribed under the Patent Act and Rules to be used for filing of the provisional or complete specifications. It is to be submitted along with an application Form-1 and other documents with the requisite fee prescribed in the First Schedule. Drawings are also prepared on A4 size paper (29.7X 21 cm, margin 4 cm on top and left-hand part, 3 cm on the bottom and right-hand part). The drawing material is submitted as a different set of papers and does not appear in specification itself.

12.8.5 Components of Complete Specification

Form −2 is used for submission of drafted complete specifications to the patent office and contains following parts:

1. Title.
2. Applicant(s).
3. Preamble.
4. Description.
   (a) Technical field.
   (b) Background of the invention.
   (c) Objects of the invention.
   (d) Statement of the invention.
   (e) Brief description of the drawings.
   (f) Detailed description of the invention.
5. Date and signature.
6. Claims.
7. Abstract

12.8.5.1 Title

The specification should begin with the title indicating the subject matter related to invention and should not be more than fifteen (15) words.

12.8.5.2 Applicant(S)

This section is followed by the title and contains the name of the applicant(s), their nationality and address.

12.8.5.3 Preamble to the Description

The preamble to the description contains provision for both provisional as well as complete specification. The provisional one states that "*the following specification describes the invention*" while the complete one states that "*The following specification particularly describes the invention and the manner in which is to be performed*" The title, applicants and preamble should appear on the first page of From 2.

12.8.5.4 Description

Description is also known as a technical field. It will mention the field of invention as to what field the invention belongs to and states as to the subject matter of the invention. For example, "*The invention generally relates to dispensing machines and more particularly to a machine which dispenses coffee.*" The description shall start in the next page or second page and describe the invention background to distinguish what is novel and what is already being practiced or known currently. The description also contains the invention's objective, advantage or solution provided by the invention. Description contains the objectives of the invention that tells the necessity of the invention, brief description of the drawing including figures and finally detailed description of invention. Detailed description is followed by claims.

12.8.5.5 Claims

Claims are part of the specification and crucial part of the patent specification and become the base of rights claimed. As each claim and associated right is independent therefore not held invalid for other claim(s) being held invalid. If claims are not made in the specification description, it will be considered disclaimed or donated to the public. A claim is a sentence, hence should start with a capital letter and end with a full stop. The claims should start with the preamble "I claim," "We Claim" on a separate page after the "Detailed description of the invention" section. The claims section follows with the claim listings. There will usually be several claims, some of which may be alternative. Often, the first claim will be developed and expanded upon by subsequent claims which may enlarge the first claim, particularize it or give specific embodiment of it.

12.8.5.6 Abstract

It is a summary of the matter contained in the specification of an invention and starts with the invention title. One hundred fifty (150) words is the maximum limit provided under the abstract. Reference numbers are required to be mentioned in the abstract for the drawing feature and submitted separately along with complete specification.

12.9 Patent Procedure in India

Patent procedure refers to rules related to the filing process and prosecuting patent applications in a particular jurisdiction. The patent procedure in India can be divided into the following stages (Fig. 12.3):

1. Patent filing at Patent Office.
2. Request for Examination (RFE).
3. Publication.

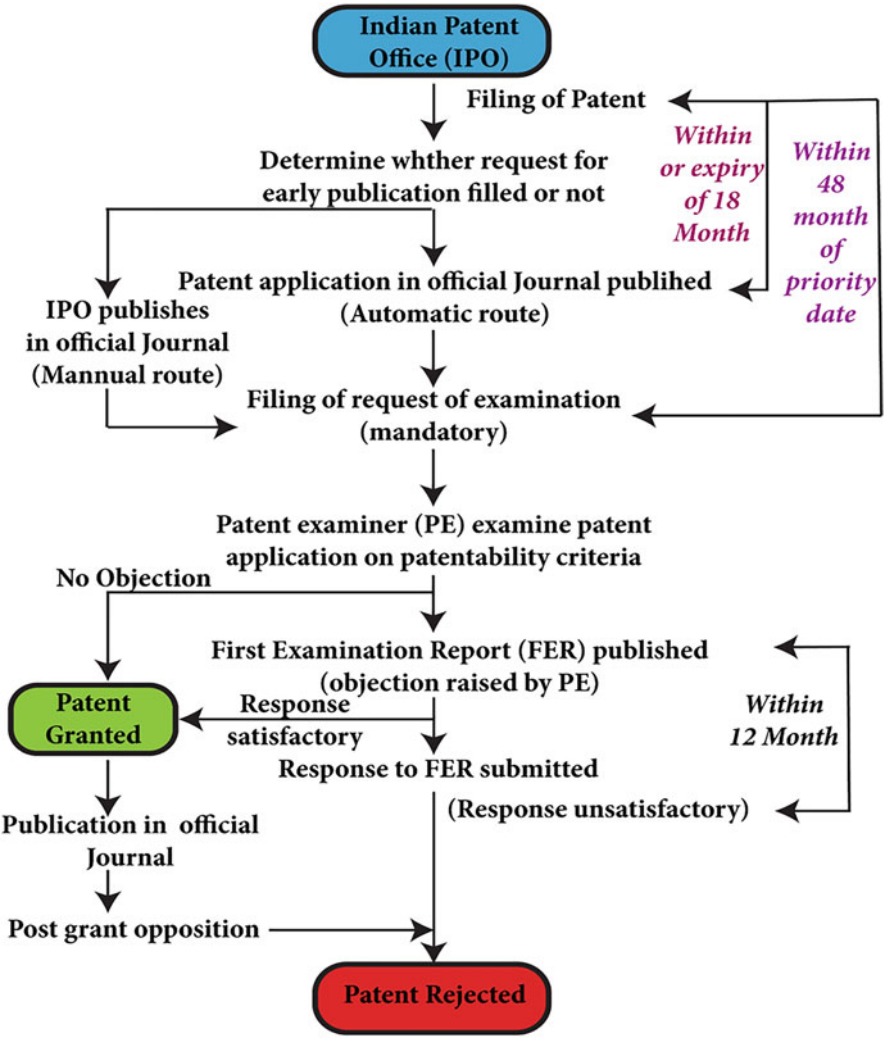

Fig. 12.3 Schematic representation of patenting procedure of India

4. Examination.
5. Opposition.
   (a) Pre-grant Opposition.
   (b) Post-grant Opposition.
6. Registration and Renewal fee payment.

12.9.1 Filing

Patent application filing is a first step to get a patent for an invention. It can be done by:

1. A person who claims to be "*the true and first inventor*" of the invention.
2. The "*assignee*" of the true and first inventor.
3. The "*legal representative"* of any deceased person who was entitled to file such an application immediately before the death of the true and first inventor.
4. The patent can be filed *alone or jointly* with another person.

The application is filled at the patent office which has four regional offices viz. Delhi, Mumbai, Kolkata and Chennai. Patent applications originating out of a particular geographical area can be filed only in their respective branch office. For example, a patent application emerging from Kerala can be filed only in the Chennai branch office.

India recognizes two types of patent filings, such as:

1. **Provisional Application:** It is less formal, intended as a relatively inexpensive and expeditious way of embarking on patent protection where inclusion of claims are not necessary but then a complete application must be filed within 12 month otherwise it will be deemed abandoned.
2. **Complete Application:** It is an application that is complete in all respects i.e., a detailed description enabling a person to practice the invention containing claims, all embodiments, best mode etc. and need to be filed within 12 months from the filing date of the provisional application.

12.9.2 Request for Examination

Request for Examination (RFE) is a mandatory requirement at the Patent office. It is filed at the option of the applicant (within a non-extendable period of 48 months from the priority date of the application) whenever the applicant wants his application to be examined. The patent office does not take up the application for examination *suo motu* and if not filed with this time limit, the application is considered as deemed withdrawn.

12.9.3 Publication

It is a crucial step in patent procedure where an application filled at patent office gets published upon expiry of 18 months from the priority date except in following three situations where the application:

1. Secrecy directions are imposed by authority under Section 35 of Patent Act.
2. Abandoned as per Section 9(1) of Patent Act,
3. Withdrawn 03 months prior to the period specified under Section 11 A(1) of patent Act.

Publication of application takes place after cessation of the secrecy directions or after 18 months whichever is later. After publication and till the date of patent grant, applicant gets the equivalent rights and privileges as available to with granted except institution of infringement proceeding which becomes available from the date of actual granting of patent by patent office (Section 11A(7) and proviso). If it is a divisional application, it is done after 18 months from priority of parent application or immediately after filing divisional application whichever is later. As the application is filed, the determination is made by the examiner that the application contains one or more than one invention and accordingly an application will be created for additional inventions.

An international treaty known as PCT allows filing of single patent applications which can be base for relining upon for later filing in member countries of the treaty. National phase of PCT application makes it publishable immediately after filing. In case the application is not published at the end of 18 months it is considered deemed to publish and will have the same effect as publication.

The two major reasons that make the publication date a really vital one is:

1. The Patent applicant rights commence from the publication date i.e., once the patent is granted and the patent owner sues someone for infringement then he will receive damages/account of profits from the date of the publication.
2. Opposition opportunity at the pre-grant level becomes available for third parties to oppose the patent grant from the publication date.

In case an applicant does not wish to wait for 18 months for the publication to occur, there is an option filing a request for early publication that ensures publication of application within 1 month from the date of request made. Given the significance of the publication data, it becomes really important for an applicant to decide whether to *opt for* early publication or not. At one end early publication will ensure that the rights of the applicant will commence early while on the other hand the application will be open for opposition sooner than the normal publication procedure.

12.9.4 Examination

As a RFE can be filed at any time after the patent application is filed after that examination will be done by the controller with coordination of the patent examiner. The Controller will refer the application, to an examiner generally within one (1) month from the date of publication or request for examination is made, whichever is earlier. The examiner will review the application and make a report in respect of the following:

1. Requirement laid down in the Patent Act and Rules. Whether the application form and specification are in accordance with the requirements of the Patent Act and the Rules,
2. Invention satisfies the patentability requirement such as subject matter, novelty, inventive step, industrial application and specification.
3. Other requirements such as unity of invention and such other prescribed in Acts and Rules.

The First Examination Report (FER) is made available to the patent applicant or authorized person/agent. In case, the RFE is filed by any other interested person (third party), only intimation of such examination will be sent to such person and FER will be sent to applicant or his authorized agent.

12.9.5 Putting Application in Order for Grant

Once the FER is issued by the patent office, the application has to comply with all the requirements mentioned in the FER and subsequent examination report within 12 months from the issue date of the FER. This is known as putting the application in order for grant. If all the requirements are not met within these 12 months, the application shall be deemed to have been abandoned.

12.9.6 Opposition

Indian Patent Act makes provisions for pre and post-grant opposition. The aim of such an opportunity is to prevent an unlawful grant of patents. Pre-grant opposition can be filed in writing at any time up till the grant of the patent and after that post-grant opposition can be filled but within 1 year of the patent grant. This two-stage opposition was streamlined by making an "integrated system" as per TRIPS agreement by making amendments in Section 25 of the Act. As per Section 25 of the Act, pre-grant opposition can be made on the following 11 grounds such as:

1. Wrongfully obtaining the invention.
2. Anticipation by prior publication.
3. Anticipation by prior date, prior claiming in India,

4. Prior public knowledge or public use in India,
5. Obviousness and lack of inventive steps,
6. Non-patentable subject matter,
7. Insufficiency of the description of the invention,
8. Non-disclosure of information as per the requirement or providing materially false information by an applicant,
9. Patent application not filed within 12 months of filing the first application in a convention country,
10. Nondisclosure/wrong mention of the source or geographical origin of biological material used for the invention,
11. Invention anticipated with regard to traditional knowledge (TK) of any community, anywhere in the world.

A post-grant opposition may be filed by any interested person by giving opposition notice to the controller after the publication of the patent grant but within one (1) year from the date of such publication. The grounds for filing post-grant opposition are the same as the grounds of pre-grant representation. 'Person interested' includes 'a person engaged in, or in promoting research in the field of the invention'. Such a person must have genuine interest in the invention and the onus of providing lies on him. The procedure for opposition involves constitution of the Opposition Board by the Controller. The Opposition Board consists of three members including the Chairman. The Board conducts the examination and submits the recommendation report to the Controller. The Controller thereafter fixes a date for the hearing and gives parties at least ten days' notice. After hearing the parties and decisions are made for grant, amend or revoke the patent.

12.9.7 Registration and Renewal Fee Payment

Once the application is published in an official journal and objections raised in the examination report (if any), are met, and no pre-grant representation is pending then the application proceed towards registration. In case there is a pre-grant representation, the patent is granted after the opposition is dismissed or satisfied. After grant of the patent, a prescribed renewal fee has to be paid for maintaining the patent for 20 years. A detailed flow diagram of the patent procedure in India has been provided in the Draft Manual of Patent Practice and Procedure issued by the Indian Patent office which is adopted here and given below.

12.10 Conclusions

NPs are diverse, obtained from nature and widely used for medicinal purposes since time immemorial. These NPs are the source of inspiration and innovation of new drug discovery or synthetic drug development. But during the recent past rapid surge in search, isolation and characterizations of many NPs having therapeutic value has

taken place. These NPs constitute almost one-half of all the drugs currently used across the globe. To get these valuable products from nature having great therapeutic potential with an economic return, a great investment is made in research and development, production, advertisement and marketing by pharma and associated R & D companies and institutions. To secure these investments and further profits on innovation-related aspects with NPs, IP rights are secured at local and global levels. The patent is one of the key IP rights which are most sought over NPs along with trademarks, trade secrets etc. These IP rights are further paving the way for generating knowledge and investment for increased innovations related to NPs as therapeutic agents. Legal protection for innovations related to NPs is given relevant Indian IP rights laws, rules and shaped by judicial interpretations and orders. As India is a party to international legal institutions such as the UN, WTO, framework and legal instruments (TRIPS), domestic laws are made coherent with international legal regimes by amendments made from time to time. As the future is based upon the knowledge economy and facing great impacts of climate change, demand, development and legal protection on the innovations based on NPs are inevitable.

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Recent Advances in the Treatment of Parasitic Diseases: Current Status and Future

13

Archna Kumar, Deepika, Shivani Sharda, and Anupama Avasthi

Abstract

As per World Health Organization (WHO), parasitic diseases significantly threaten global health. Diminishing boundaries have contributed to its spread beyond the geographical limits of the tropical and subtropical regions. Additionally, the overuse of conventional antiparasitic drugs has led to a reduction in their effectiveness in the treatment of these diseases. Advances in metabolomics and system biology approaches have led to a better understanding of parasite biology for identifying appropriate drug targets. DNA/RNA-based molecular techniques, Proteomic approaches, Bioinformatics tools, advanced Nano-technological fabrications and Epigenetics based host–pathogen interactions tactics offer new dimensions towards treating parasitic diseases. Drug repurposing is also emerging as an effective alternative in discovering/developing drug molecules with new pharmacological/therapeutic indications. This chapter explores various strategies for assessing and developing new drug discovery patterns. In this chapter, we have emphasized recent technological advances in the treatment of parasitic diseases through the identification of new drug targets, structural genomics, Nanotechnology based therapeutic options (dendrimers, micelles, lipid NPs, capsules etc. as potential delivery systems), Metabolic pathways (Meglumine antimonite, Paromomycin for treating leishmania) and molecular dynamics (MD) with simulations.

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_13

Keywords

Parasitic diseases · Epigenetic landscape · ncRNA · Metabolomics · Network neology · Interactome networks · Herbomics · Drug repurposing · Nanotechnology · Natural products · Antimicrobial peptides

13.1 Introduction: Challenges and New Perspectives

The host response analysis and immunopathology were already well accepted for disease etiopathologies by researchers and medical associations. Genetic and non—genetic features for establishing their discreet role in creating the landmarks of disease etiopathologies have been appended recently to allow thorough diagnostic and therapeutic consequences. The clinico- molecular interventions designate the role of studying the host/parasite-specific parameters as well as to include the cross-talk of host-pathogen interactome analysis (Mocumbi et al. 2019). The sole dependency of either of these classes of factors has led to a lacunae of the checkpoints of disease progression. Such investigations thus call for addressing the missing link to furnish the minimalist details for handling disease theranostics to debunk the old theories towards novelty in precision medicine. Further, the research regarding the disease prevention and health monitoring requires a thorough structuring of the pharmaceutical interventions with both modern as well as traditional therapies. (Kumar et al. 2020). The recent indictment of the pandemic had precipitated the bioprospecting and re-purposing of drugs to avail a spectrum of therapeutic strategies towards the existing or unwarranted disease burden (Kollmer et al. 2020). The chapter enlightens the aspects of the last decade of research and clinical applications of parasitic disorders to apprise the reader towards the most relevant outcomes.

13.2 Advanced Molecular Methods for Precision Therapeutics in Parasitic Diseases

Existing chemotherapeutics regimes for the treatment of parasitic diseases have serious consequences as well as limitations and hence there is a dire need to translate the existing biomedical information into impactful therapeutics (Singh 2020). Molecular and precision medicine approaches relying on information inscribed in genome and signalling pathways need further investigations to provide solutions. Various Genomics and bioinformatics strategies are increasingly being employed to offer new potent therapies to combat parasitic infections in humans (Argüello-García et al. 2020). These include epigenetics-based, ncRNA based and metabolic pathways-based approaches that have opened the new potential for theranostics of parasitic diseases (Fig. 13.1).

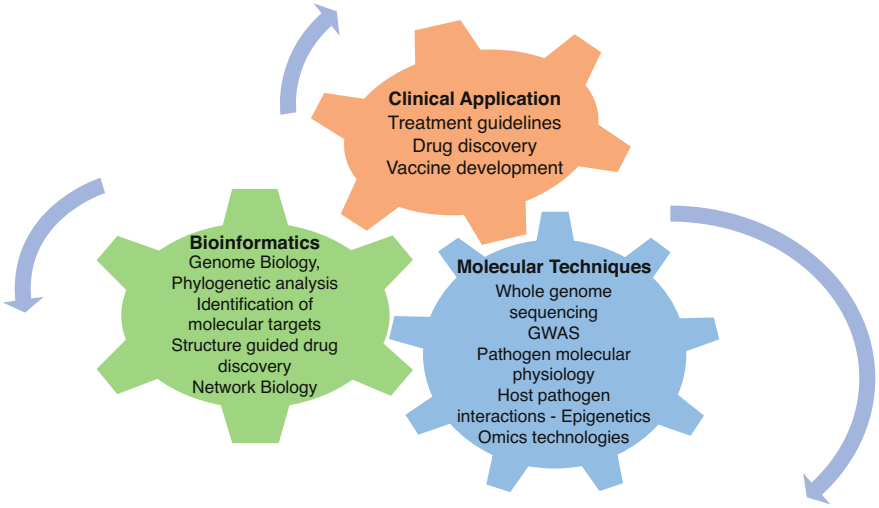

Fig. 13.1 Advanced molecular methods for precision theranostics: advanced molecular and bioinformatics-based approaches have helped in drug discovery, vaccine development and formulation of treatment guidelines

13.2.1 Parasite Epigenomics Landscape

Epigenetics consists of multiple categories of transferable alterations changes in gene expression, which may arise due to various factors including structural changes in chromatin amongst others (Pagiatakis et al. 2021). Other factors that operate in sync include multiple types of non-coding (ncRNA) including microRNAs, circular RNAs (circRNAs), and other remodeling proteins which structurally alter the DNA methylome and chromatin. These factors work in utmost coordination to create transient as well as stable (with memory) changes in the genome of the individual to create individual as well as transgenerational signatures during evolution.

13.2.2 Paradigms of Anti—Parasitic Therapeutics

Parasites are eukaryotic organisms and, as such, share common histone marks and epigenetic mechanisms with humans (Fleck et al. 2021). Epigenome dynamics in human parasites consist of interactions between transcriptional regulation and epigenetic mechanisms and play a significant role in regulating life cycle progression, host-parasite interactions and parasite adaptation to the host environment. Modulations of epigenetic responses by intracellular parasites can not only manipulate host cells altering the host genome expression and signaling pathways but can also exhibit altered genome functions of the parasites too during these interactions. To bypass the host immune response, apicomplexan parasites synthesize epigenetic modification enzymes, secrete epigenators into the host cells, inhibit host signaling

proteins, and also deploy non-coding RNAs to alter gene and protein expression (Villares et al. 2020). Rational epigenetic drug discovery using validated targets is now a reality and studies on the epigenetics of parasites and host interactions can therefore provide critical leads and targets for therapeutic interventions (Dahlin et al. 2015).

13.2.3 Epigenetic Manipulations as Therapeutic Option

Information from the Parasite- Epigenomics landscape has offered potential opportunities towards drug discovery and development by targeting the host epigenome rather than the parasite (Argüello-García et al. 2020). Post-translational modifications of histone including acetylation, methylation, phosphorylation, SUMOylation, ubiquitinylation, which, together with other "histone marks" constitute a complex "histone code" has a central role in regulating chromatin architecture, the binding of protein cofactors, the regulation of gene expression and its dysregulation in pathology (Sadakierska-Chudy and Filip 2015; Veluchamy et al. 2015). The enzymes that effect these modifications, "writers" and "erasers" of histone marks, and, increasingly, "reader" proteins that interact with them, are privileged targets for chemotherapeutic interventions. In particular, the writers, readers and erasers associated with histone acetylation and methylation have been under active investigation as prospective drug targets (Allis and Jenuwein 2016; Biswas and Rao 2018). The key to advances in treatment would involve identifying molecules capable of inhibiting DNA methylation ultimately leading to the killing of the parasites (Jones et al. 2019).

Plasmodium falciparum depends on epigenetic mechanisms by encoding multiple histone-modifying enzymes that comprise histone deacetylases (HDACs), histone acetyltransferases (HATs), histone methyltransferases (HMTs, including lysine HKMT), protein arginine methyltransferases (PRMTs), and histone demethylases (HDMs) (Coetzee et al. 2020). These have roles in the control of gene expression and cell cycle progression and regulation of variable surface gene expression involved in immune evasion by the parasite. Hence, inhibitors of histone-modifying enzymes have been investigated as novel molecules towards antimalarial drug discovery (Ghosh et al. 2021). The discovery process involved screening through the chemical library of such inhibitors and has been worked out even for various *P. falciparum* strains including artemisinin -resistant *P. falciparum* parasites at both in silico and in vitro levels using more than 70 methylation-inhibiting molecules which were found to be as effective as chloroquine (Saxena et al. 2019). Given the treatment failure in endemic areas, in particular, this could pave the way for new drugs that, combined with artemisinin, could eliminate resistant parasites (Nsanzabana 2019). The future steps could involve optimizing the selectivity and efficacy of the most promising molecules and to identify molecules that may act on other development stages of the parasites responsible for transmission. Similarly, HDACs including include hydroxymate-based inhibitors like SAHA (sub-eroylanilide hydroxamic acid, Vorinostat and its derivates) and TSA (Trichostatin A), cyclic tetrapep-tides

like apicidin amongst others can inhibit asexual *P. falciparum* and *P. vivax* stages and gametocytes as evidenced through recent clinical trials through transcriptional activation (via H3K4me marks) and repression (via H3K9me marks), and can be a promising drug targets. Drug BIX01294 has been proposed as potential HKMTi demonstrating inhibition of asexual *P. falciparum* proliferation as well as its viability (Almela et al. 2015). As a part of drug repurposing, the library of anticancer compounds (Cayman Epigenetics Screening Library, Cayman's Chemicals, USA) has been also investigated for its antiparasitic role. The library mainly consisting of 39% HDACi and 15% HKMTi apart from other epigenetic regulators, and Hydroxamate-based HDACi and 4-quinazolinamine-based HKMTi were identified as the promising scaffolds with low cytotoxicity. Many of these are already clinically approved for other diseases like cancer and some were derivatized and repurposed (Kirtonia et al. 2021). In another recent clinical trial in Mali, it has been shown that reprogramming of monocytes is also regulated through epigenetic modificications caused by immune dysregulations. This has been evidenced through RNA-seq analysis of monocytes isolated from PBMCs where expression of genes for TNF and IL6 was upregulated indicating the 'reprogramming' of bone marrow progenitor cells due to previous parasite exposure.

On a similar note, even *Leishmania donovani* can utilize epigenetic machinery to bypass the host immune response. A summary of these responses are presented in Table 13.1. Specific histone lysine methyltransferases/demethylases regulate LPS, IFN and IL-10 expressions. Smyd2 can induce dimethylation of H3K36, while Ezh2 can induce trimethylation of H3K27 (Salles et al. 2021). *L. donovani* infection can also regulate H3K4 and H3K27 trimethylation via Kdm5b and Kdm6b. The mechanism involves signalling cascade involving multiple genes, transcription factors and proteins like IL-10, IL12, TNF-a, and arginase-1 promoters, Kdm5b and Kdm6b, transcription factor MeCP2 amongst others. (Parmar et al. 2020). Similarly, DNA methylation of macrophage genome at loci LAR2, HDAC4, CDC42EP3, IRAK2 and ADPRHL1 (Sharma et al. 2016). These switches help in supporting macrophage survival. Additionally, these switches inhibit the activation of the inflammasome so that the host response is further de- escalated (Olson et al. 2017). Hence, targeting the inhibition of inflammasome activation can be a way for the development of therapeutics.

Schistosomiasis is a neglected tropical disease (NTD) caused by infection with macro-parasitic blood fluke schistosomes. Epigenetic inhibitors targeting histone readers, writers and erasers obtained from the Structural Genomics Consortium have been explored for their anthelmintic activities (Whatley et al. 2019). Development of high throughput Roboworm platform has led to identification of inhibitors of several readers, writers, and erasers of epigenetic signatures including reader -NVS-CECR2–1, writer- LLY-507 and BAY-598 and eraser GSK-J4. The inhibitors include molecules like LLY-507/BAY-598 (a class of SMYD2 histone methyltransferase inhibitors) and GSK-J4 (a JMJD3 histone demethylase inhibitor). These are reported to be critical for schistosome development. Oviposition and packaging of vitelline cells into in vitro laid eggs was also found to be significantly affected by GSK-J4 (Amaral et al. 2020). These results provide support for the

Table 13.1 The details of such epigenetic landscape factors are summarized in the following table

Epigenetic landscape contributing factors	Nomenclature	Organism	Effective cells including innate as well as adaptive immunity cells	Role/function/mechanism	References
miRNA	miR – 9/9-5p, miR-21/21a, 23a/a-p, 23b-5p, 24-2, 26a2, 27a/a-5p, 29a, 29b1, 34a, 92a, 124, 125a/a-3p/a-5p,125b, 127, 130b,132, 142-3p, 146 a/b, 147-3p, 155, 155-3p/5p, 181, 195-5p, 200-c, 222, 223, 320a, 324-5p, 451,511-3p, 551-5p/3p, 574-5p, 720, 1931, 3473e/f, 3783p, 2128, 5128, 6994-5p, 7093-3p, 7235, let-7a, let-7c/7c-1-3p, 7d-5p, 7e	*L. donovani*	Macrophages	Modulation of macrophage plasticity/phenotypic switching between M1/M2	Neeraj et al. (2017)
miRNA	miR-9, miR-22, miR-124, miR 130a, miR 708	*L. donovani*	Dendritic cells	Th1/2 phenotype switching	Neeraj et al. (2017)
miRNA	miR-16, 17/17-5p, 19a/b, 20a/b, 21, 22, 29a/c, 34a, 99b, 125a, 146 a/b, 148a, 155, 221, 342, 422b, 424/424-b, let-7e	*L. donovani*	Inflammatory monocytes		Neeraj et al. (2017)
DNA Methylome	HDAC, H3K4, H3K9, H3K27, H3K36 and H3K79	*L. donovani*	DNA methylation		Parmar et al. (2020)
lncRNA	NONSHAT022487	*T. gondii*	UNC93B1 immune related genes	Negative modulation of IL-12, TNF-a, IL-1b and IFNg secretions through UNC93B1	Liu et al. (2018)

lncRNA	mir17hg	*T. gondii RH*	mir17 microRNA gene cluster in host	Induction of apoptosis	Menard et al. (2021)
lncRNA	NONMMUT014792.2, NONMMUT061096.2, NONMMUT057813.2, NONMMUT057813	*S. japonicum*	Modulation of TGFb-1, JAK3, STAT1 chemokine C motif receptor 1, VCAM1	Involved through liver pathogenesis	Xia et al. (2020)
lncRNA	NONMMUT021591	*E. granulossus*	cis-regulation of retin-oblastoma gene, Rb1	Dysregulated differentiation of MDSCs	Yu et al. (2018)
lncRNA	XLOC_030813, XLOC_510697, XLOC_237221	*Toxocara canis*	Regulation of ubqln1, inhibition of sox4 Expression IL-21 gene localization	Immune/inflammation	Zheng et al. (2021)
lncRNA	Csf1-lnc and Socs2-lnc	*T. gondii*	Kinase ROP16	Up-regulation of Csf1-lnc and Socs2-lnc	Menard et al. (2018)
lncRNA	NR_045064	*C. parvum*	Csf2, Nos2, and Cxcl2	Promotion of antimicrobial defense	Li et al. (2018)
lncRNA	Sense, antisense, intergenic, divergent and intronic	*C. parvum*	Hedgehog, Wnt signaling pathways, tight junction	Maintenance of intestinal epithelium integrity for infection prevention	Liu et al. (2018)
lncRNA	NeST, MEG3, MIR17HG, lnc-SGK	IId subtype *T. gondi* tachyzoite	Th1 and Th17	Immunomodulation	Rochet et al. (2019)
lncRNA	lncRNAs, cirRNA	*C. baileyi*	?	Increased IgA production cytokine-cytokine interactions	Ren et al. (2018)
lncRNA	NONGGAT004163.2, TCONS_00018115, NONGGAT001393.2	*E. necatrix*	Ring finger protein 152 type I interferon rec- eptor subunit 1	Apoptosis induction	Fan et al. (2020)

(continued)

Table 13.1 (continued)

Epigenetic landscape contributing factors	Nomenclature	Organism	Effective cells including innate as well as adaptive immunity cells	Role/function/mechanism	References
lncRNA	Long antisense ncRNA	*P. falciparum* trophozoite schizont merozoite	Var genes PFF0845c PFD1005c	Gene expression regulation	Epp et al. (2009)
lncRNA	lncRNA-TARE	*P. falciparum*	DNA replication in parasites	Parasite development in blood	Broadbent et al. (2015)
lncRNA	Var antisense lncRNA	*P. falciparum*	Parasite var. genes	Transcriptional upregulation of var. and increased promoter activity	Jing et al. (2018)
lncRNA	IncRNAs	*P. falciparum*	?	Host- parasite interaction, proteolysis, cell adhesion, locomotion, pathogenesis, metabolism	Liao et al. (2014)
lncRNA	MIAT	*T. cruzi*	?	Chagas disease leading to chronic cardiomyopathy	Frade et al. (2016)
miRNA	miR-146a ↓ (source placental samples)	*P. falciparum*	IRAK1 and TRAF6 (target genes)	TLR-receptor signaling pathway modulation	Van Loon et al. (2019), Taganov et al. (2006)
miRNA	miR-361-3p ↑ (source skin biopsies sample)	*L. braziliens*	TNF, GZMB, and FLG2 (target genes)	Tissue necrosis	Lago et al. (2018)
miRNA	miR-526b-5p ↑ (source epithelial colon cells	*E. hystolytica*	XIAP, BAK1, BNIP3L (target cells)	Inhibition of cell cycle, induction of tumor suppression, and apoptosis	López-Rosas et al. (2019)

miRNA	Let-7 g-5p ↑ (source whole blood samples)	*E. granulosus*	IL-13, IL-10, and IL-6 (target genes)	Macrophage activation and proliferation inflammatory response, induction of apoptosis and net oxidative damage	Mariconti et al. (2019)
miRNA	miR-150-5p (source liver cells)	*S. japonicum*	KANK4, DRD1, and MT1H (target gene)	Actin reorganization and modulation of cell contractility	Cabantous et al. (2017)
HDAC	PfHDAC1, PFI1260c	*P. falciparum*		Expressed in asexual and gametocyte stages; nuclear localization (asexual stage); inhibited by apicidin, TSA and SAHA	Andrews et al. (2012)
HDAC	TgHDAC3 TGME49_027290	*T. gondii*		460% amino-acid identity to hHDAC1; associated with bradyzoite-specific promoters; absent from tachyzoite-specific promoters; nuclear localized (not nucleolus); forms complex with actin, TgHSP70a and TgHDP70b; inhibited by FR235222 in tachyzoites; amino-acid T99A and T99I mutations affect basal activity22,50,96	Andrews et al. (2012)

development of next-generation drugs targeting schistosome epigenetic pathway components.

Toxoplasma, Theileria, and Cryptosporidium are also able to control the host epigenome through methylation signatures. H3K4 methylation and transcriptional activation by SET and SMYD3 in Theileria, methyl transferases expression and secretion by parasites (Toxoplasma-secreted TEEGR (Toxoplasma E2F4-associated EZH2-inducing gene regulator), and subsequent enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) expression and polycomb-mediated H3K27me3 repression) in *Cryptosporidia* are just some of the displayed epigenetic signatures.

Apart from transcriptional modulations through epigenetic modifications, the Post-translational modification of proteins (through acylation and phosphorylation) also has a significant role in modulating metabolism and signal transduction, thereby affecting protein conformation, stability, and function (Heinemann and Sauer 2010). The transcriptional and post-translational modifications can work in sync to up or down-regulate the expression of important genes involved in parasite-host interactions (Kouzarides 2007; Croken et al. 2012). Lactylation signatures during *Trypanosoma brucei* have recently been identified and so has been the histone modifications altering the chromatin structure in *M. incognita*, similar to that in model organism *Caenorhabditis elegans*. The chromatin structure modulation can occur at both the developmental stages: eggs, and pre-parasitic juveniles (Gallegos et al. 2018).

13.2.4 ncRNA Based Theranostics

Non-coding RNAs are essential transcriptome components that have gained prominence in the recent studies of biomolecules as they regulate the host-parasite interface, host responses, parasite differentiation as part of disease progression. There have been ample scientific evidence depicting pathogen-induced or transferred non-coding RNA molecules to the host cells thereby modulating their physiological responses and functions (Olajide et al. 2021). Knowingly, such a phenomenon is achieved through entities like extracellular vesicles, small membrane vesicles secreted by the microorganism. The microenvironment of the host-infected with parasite promulgates a two-way exchange mechanism i.e. from parasite to host or vice-versa, leading to either beneficial or detrimental outcomes to the parasite (Bayer-Santos et al. 2017). The non-coding RNAs are segregated as subtypes known as short non-coding RNAs (sncRNAs) and long non-coding (lncRNAs), a classification based on the sequence length of the nucleotides and their adapted structures and functions (Laurent et al. 2015). Broadly as per literature, sncRNAs range from 20–300 bases, further sub-divided into mainly small interfering RNA (siRNA) and microRNA (miRNA). The studies pertaining to protozoan parasites vividly emulate RNA interference (RNAi) pathways and complex miRNA repertoires, with minimal homology to prevalent miRNAs of plants and animals. Such miRNA subsytems have been designated to modulate the expression of host miRNAs associated with

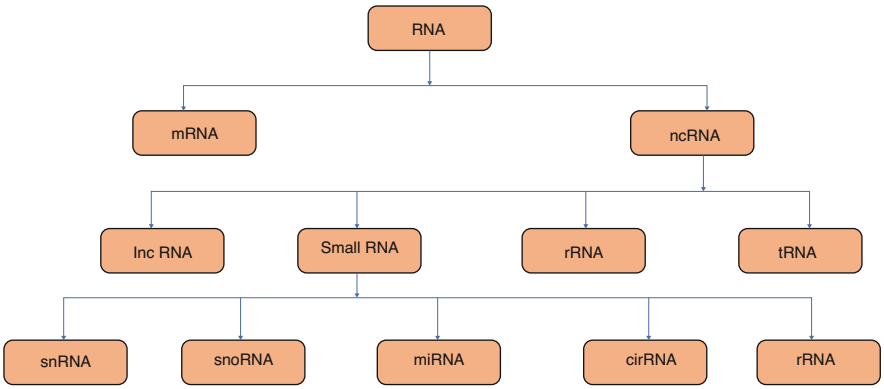

Fig. 13.2 Classification of noncoding RNAs (ncRNAs): Messenger RNA (mRNA is translated to proteins and structure and regulatory functions are performed by ncRNA which includes ribosomal RNA (rRNA), transfer RNA (tRNA), small RNAs including majorly micro RNAs (miRNAs) and long noncoding RNAs (lncRNAs). The intracellular parasite can transfer non-coding RNA molecules to the host cells to modulate their functions via extracellular vesicles. The exchange of information may take place in both directions, from parasite to host or vice-versa

different biological processes as part of their survival mechanisms in the host intracellular environment. This is in contrast to host miRNAs, which strive to inhibit microbial proliferation by targeting the virulence and essential genes of the parasite (Asgari 2011) (Fig. 13.2).

There is an interplay between parasite, vector and mammalian cells during the pathogenesis and apicomplexans including *Plasmodium spp*., *Trypanosoma gondii*, and *Cryptosporidium parvum* have been reported to alter miRNA expression profile. The transfer and transition of 100 s of human miRNAs to the infecting *P. falciparum* have been marked to occur through the intraerythrocytic cycle. The erythrocyte miRNAs play a significant role in communication between host cells, e.g. let-7i and miR-451 miRNAs are found to be transferred from sickle cell erythrocytes to the infecting parasite *P. falciparum* negatively regulating parasite growth. Infected erythrocytes are also known to release functional miRNAs that are subsequently internalized by endothelial cells. Experimental studies designed to block miR-155 function utilizing gene knockout or pre-treatment with miR-155 antagomir had provided evidence supporting enhanced endothelial quiescence, and maintenance of the integrity of blood-brain-barrier leading to host survival. At another level, altered miRNAs expression of four miRNA in the midgut of *A. gambiae* infected with *Plasmodium* was also observed that effected a two-fold increase in the number of oocysts in the mosquito illustrates an interdependent regulation between the key players of the pathogenesis (Bayer-Santos et al. 2017).

Similarly, *T. gondii*-infected fibroblasts have altered miRNA expression by as much as 14% that included miR-146a and miR-155, which play an imperative role in modulating host responses post *T. gondii* infection.

The infectivity of mammalian hosts by trypanosomatids *T. cruzi* and *Leishmania* spp. is conducted through insect vectors. Some of these parasites have functional siRNA pathway e.g. *L. braziliensis*. However, others like *T. cruzi, L. major,* and *L. amazonensis*, not possessing this machinery can still release EVs containing different types of small RNAs like stRNAs and TcPIWI-tryp. Even during *T. cruzi* Chagas disease cardiomyophaty (CCC) EVs play a significant role during inflammation with long ncRNA MIAT (myocardial infarction-associated transcript) overexpression. Detailed studies by (Linhares-Lacerda et al. 2015) have provided further validation in this regard.

In leishmaniasis, ncRNAs packaged in EVs can induce negative modulation of miR-122 in host cells effectuated using Zn-metalloprotease surface glycoprotein GP63 that directs pre-miRNA processor Dicer1 aimed at prevention of miRNP formation while stimulating enhanced parasite burden. Molecular mechanisms marking maturation block on endosomes toward late endosomes and MVBs via endosomal protein hepatocyte growth factor regulated tyrosine kinase substrate (HRS) are also reported to be involved. Downregulation of HRS inhibits uncoupling of mRNA-AGO2 interaction, thereby thwarting degradation of translationally repressed messages and recycling of miRNPs. This in the cascade, in turn, fails to repress IL-6 mRNA leading to its enhanced translation in the host thereby suppressing host macrophage activation. Another study has reported amplified MIR30A-3p expression during *L. donovani* infection, thereby negatively regulating autophagy promoting protein BECN1/Beclin1. These points toward therapeutic target as BECN1 impacting the treatment regime of visceral leishmaniasis. The above studies have fuelled the research of host miRNA dysregulation inherently coordinating impaired immune response followed by increased host colonization by the pathogen. Conversely, such reports are also indicating the employment of host miRNAs as a defense mechanism against the parasite. A similar mechanism in the induction of cellular miRNAs is deciphered for *Toxoplasma* and *Crytosporidiae*, as these intracellular parasites steer clever strategies to hijack host gene expression. Semin Immunopathol:1–12. (Villares et al. 2020). The findings of parasite interface at the miRNA scales are fascinating to provide for the diagnostic and prognostic tools, as novel miRNAs and other ncRNAs get accommodated as potential targets for chemo and immunotherapies for parasitic diseases.

Apart from miRNAs, long non-coding (lnc)RNAs have also emerged as critical regulators of gene expression and play a crucial role in Vector–Host–Pathogen Triad (Fischer 2020). lncRNAs diversity and abundance ctares to their unequivocal molecular transitioning at the lncRNA:RNA, lncRNA:protein and lncRNA:chromatin interface. The archetypical molecular designs followed by lncRNAs include: prototype archetype-lncRNAs molecular signals, mostly transcribed only during specific cell processes; decoy archetype-lncRNAs, regulating binding and titrating proteins and regulatory RNAs; guide archetype-lncRNAs, binding and directing localization of ribonucleoprotein complexes; and scaffold archetype-lncRNAs, generating a functional platform allowing assembly of molecular components (Lucero et al. 2021).

Understanding the molecular and cellular interplay of lncRNAs at the vector–host interface is crucial during pathogenesis (Ahmad et al. 2021). These are routed using extracellular vesicles, that are engulfed by neighboring cells (in autocrine and paracrine communication) or by remote recipient cells (in endocrine communication). These long noncoding entities may be transported between organisms, potentiating their contribution to genetic and epigenetic memory and exchange between hosts. Exosome secretion in arthropod saliva, releasing molecular contents including exogenous lncRNAs is a direct transmission between vertebrate hosts. The dynamic interactions between vector and host have shown that depletion of certain lncRNAs resulted in the increased replication of the parasite. Long non-coding RNA discovery across the genus anopheles reveals conserved secondary structures within and beyond the Gambiae complex (Jenkins et al. 2015). Similarly, in *T. gondii*, Tg-ncRNA-1 is reported to help in recruiting histone modification complex to regulate gene expression bradozoite formation. In *Cryptosporidium parvam*, host lncRNAs like Cdg7_FLc-0990 are reported to be used by the parasite to interact through H3K9 methylation protein complex. Another lncRNA Cdg7_FLc-1000 is known to suppress genes involved in cell adhesion and migration resulting in increased migration of host epithelial cells. In apicomplexes, many lncRNAs have been reported but their role still remains unclear. Natural antisense transcripts (NATs) are significant in *Plasmodium* and many sense-antisense pairs are reported to be upregulated during intraerythrocytic development. Circular RNAs (CircRNAs) have also been reported in *Plasmodium*.

Current knowledge about lncRNAs is limited and needs further intensive study. Techniques such as ribosome profiling to understand ribosompathies in parasite invasion, chromatin isolation by RNA purification (ChIRP-Seq), crosslinking immunoprecipitation (CLIP), RNA structure mapping, CRISPR toolkit for targeted genome engineering and phylogenetic lineage tracing may provide deeper insights and allow us to unravel the multi-facated interaction nodes of lncRNAs and other RNA types or proteins at the vector, pathogen, and host levels (Ahmad et al. 2021).

13.2.5 Metabolic Pathways as Therapeutic Target

Advances in high throughput whole genome sequencing and complete metabolomic profiling have given valuable insights towards new and more effective drug targets. (Mukherjee et al. 2016). The genetic, genomic, and biochemical approaches to identify other pathways and mechanisms used for adaptation and growth by the parasites provide valuable insights. Although, the carbon metabolism essentially remains the same in both: the host and the parasite, however, there are also some signature metabolic adaptations unique to each. Understanding these unique metabolic signatures can facilitate new drug development processes (J Timson 2016). This strategy can specifically target the parasite without causing much damage to the host cells. Pyrimidine nucleotide metabolic pathway has recently been investigated for the same (Fig. 13.3).

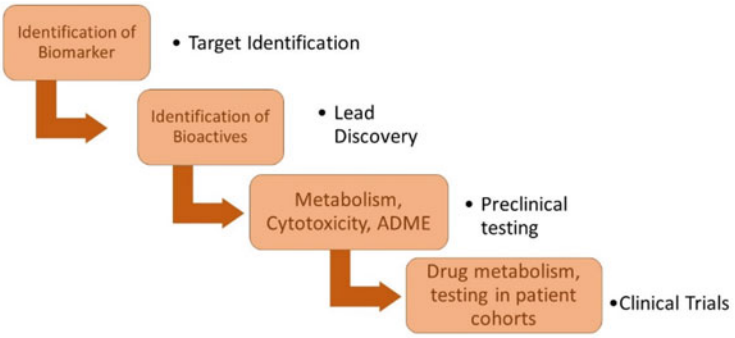

Fig. 13.3 Summary of metabolomics-based pathway in four stages of drug discovery and development. The processes include target identification, identification of bioactive compounds biomarker identification and drug metabolism during both preclinical and clinical testing

In this context, the mitochondrial metabolism and apicoplast metabolism are closely related. The PDK/PDH axis and its inhibitor dichloroacetate (DCA) have been established as therapeutic targets in cancer/agent in cancer. The same is also being explored for various parasitic infections (Oberstaller et al. 2021). DCA has been used to treat lactic acidosis during *Plasmodium* infection apart from being specifically cytotoxic to to *T. gondii* without causing any collateral effect to the host cells. In the case of Trypanosomas, the fatty acid metabolic pathways are substantially different from higher eukaryotes, and therefore may be valuable for the identification of potential new drug targets (Raj et al. 2020).

Nucleotide metabolism for all organisms comprise de novo as well as salvage Pathways with the salvage pathway being more active (Krungkrai and Krungkrai 2016). However, in *Plasmodium* the salvage pathway enzymes are absent. Hence, the difference in key enzymes of the de novo pathways is being investigated as drug targets. One of the enzymes of this pathway dihydroorotate dehydrogenase (DHOD), has been identified as a potential target for anti-malarial drug development. The advantage of using this enzyme as target is that it the binding site for antiproliferating leflunomide inhibitor is entirely different in the host and the parasite (Löffler et al. 2015). Another enzyme of the same pathway orotidine 5′-monophosphate decarboxylase (OMPDC) has also been studied as a target in a recent study to design inhibitors using structure-based drug-design approach of the parasite enzyme. The de novo pyrimidine and de novo folate biosynthetic pathways are linked in *P. falciparum*, (Bhattacharya et al. 2021) and are being investigated as a potential drug target for antifolate drugs. Triazolopyrimidine inhibitors, namely DSM265, targeting DHOD of the de novo pyrimidine pathway are already in phase I clinical trials against artemesin reistant *Plasmodium*. Structure-based design of antimalarial drug development is in the developmental stage for OMPDC, and there is a strong potential for developing lesser cytotoxic drugs with high therapeutic potential against malaria.

Similarly, polyamine biosynthesis pathway is emerging as an important target for the development of antiparasitic drugs in *Trypanosoma brucei* and *Leishmania* and inhibitors of polyamine pathway enzymes represent a rational paradigm for the treatment of parasitic diseases. D,L-α-difluoromethylornithine (DFMO), a suicide inhibitor of ornithine decarboxylase (ODC), has shown remarkable therapeutic efficacy agianst*Trypanosoma brucei gambiense* and other protozoan parasites in mouse and hamster models. Arginase (ARG) is also under investigation being validated for its antiparasitic role. An additional feature is the discrete cellular segregation of ARG in glycosome in *Leishmania* with respect to other enzymes being present in cytosol. Mislocalization of ARG to the cytosol in both *L. mexicana* and *L. amazonensis* has been reported to significantly reduce the parasite burdens. (Boitz et al. 2017).

Not much information is available on comparative differences between human and helminthic parasitic enzymes. Investigations regarding subtle differences between them can reveal more effective and more specific enzyme inhibitors. Hexokinase (EC 2.7.1.1) has been recently validated as a target in *T. brucei* through RNAi studies. The inhibition of upto 60% for this enzyme could be achieved through RNA silencing in *T. brucei.* Comparative sequencing for this enzyme in *Haemonchus contortus* and humans has identified insertions on the enzyme surface and these can be explored for designing and targeting specific inhibitors against the protozoan parasite. However, this strategy has proved harmful against *S. mansoni* hexokinase inhibitor, 5- thio-D-glucose, as this compound inhibits mammalian hexokinase too (Zuurbier et al. 2020).

As the new research is still in the developmental stage only, additional metabolic targets are also expected from other organelles and other sub-cellular compartments of parasites including mitochondrion, kinetoplast, apicoplast, glycosome, hydrogenosome and acidocalcisome (Mukherjee et al. 2016).

13.2.6 Nontargeted Metabolomics

Structurally and metabolically, parasites are quite diverse from humans and metabolic differences can be exploited for better understanding of their interactions. Complete and extensive metabolic profiling can identify unique parasitic markers or signature metabolites through various MS techniques and can provide insights into the mechanistic aspects of drug- protein interactions. This robust platform can be further utilized to study signalling synthetic pathways of systems and molecules under different metabolic conditions (e.g., diseases) providing valuable unique biomarkers (Townsend 2021). This way crucial check points in metabolism to both parasite and the host are identified. As an example, compartmentalized and constraint-based models have been developed for *Brugia malayi* and essential genes involved in switching metabolism from aerobic to anaerobic mode and novel pathways converting glutamate to aspartate were identified (Curran et al. 2020). Metabolic flux analysis (Fluxomics) can be a significant tool to measure and trace the analytes through the pathways and this strategy has identified biomarkers like

N-acetyltyramine-O-β-glucuronide (NATOG) for helminthic diseases. Recent studies utilizing the power of LC-MS and support of EM and optical microscopy have revealed the tegumental damage and altered metabolite profile of both sexes of *S. mansoni* during adult schistosomes and PZQ metabolites interactions which could pave the way for antischistosomal drug development.

Significant changes in metabolic profiles related to galactose, sphingolipid, valine, leucine and isoleucine biosynthesis pathways have been observed in *Leishmania* parasites also. Upon infection, the decriminating metabolites included acetate, β- alanine, citrate, creatine, creatine phosphate and many others. Westrop et al. 2015 identified 64 metabolites which showed up to a three-fold difference upon infection with *L. donovani* (Atan et al. 2018). In another study, 876 metabolites were detected during miltefosine administration after infection and differential levels of thiols and polyamines could help distinguish between antimonial sensitive and antimonial resistant *L. donovani* isolates.

Metabolic engineering studies based on kinetic modelling of pathways to study the regulatory components through pathway flux (flux control coefficient) and metabolic intermediate concentrations (concentration control coefficient) have also been used in recent times to provide further insights (Pineda et al. 2015). These studies identify two or three enzymes of the metabolic pathway that have highest control of flux and hence their inhibition will have higher repressive effects on the pathway function. This strategy has been applied to study trypanothione metabolism in *T. cruzi* with subsequent validation in vivo experiments too. The results have identified three out of total of 10 enzyme reactions which were controlling the trypanothione metabolism. This is a further step in target prioritization for drug development against trypanosomatids and other parasites. A similar approached may be used to target other parasitic diseases also. (Saavedra et al. 2019).

13.3 High Throughput Screening and Systems Approaches in Parasitic Diseases

Advanced technologies involving the utilization of database repositories and toolkit generated through Bioinformatics allow Genomics, Proteomics and allied high throughput analysis in deciphering complex infectious diseases caused by parasites. High throughput technologies and screens involve the much-studied genome, trancriptome and proteome footprint of pathogens along with untargeted metabolomics and predictive analytics are also adding to our information pool. Preparation of metabolic networks and identification of key check points, target-based drug discovery and other computational tools have contributed to research progress in inpretation of stratified mechanisms of parasite-triggered complex diseases. The biological sample analyses that involve a multi-factorial detailing at the gene, transcript, protein, metabolite and their interaction network level have revealed novel targets (Paananen and Fortino 2020). These can be effectuated utilizing complete genome and transcriptome profiling constituting the genome-wide association studies (GWASs), for configuring or validate contemporary drug

targets, invented through systematic analysis evaluating their therapeutic efficacy and related side effects. The core bioinformatic analyses undertakes Genome scale metabolic reconstruction of the involved parasites appended by constraint-based modeling, differential expression analysis that provide a knowledge base for identifying critical metabolic enzymes and pathways in infectious diseases.

13.3.1 Disease Network Biology in Parasitic Infections- Multi Omics Approaches

The infectious exemplars in the contemporary "omics" era is directed towards crafting the genetic landscapes of pathogenic parasites following technologies to discretely identify polymorphisms and structural and copy number variations, factors that contribute vitally towards parasite evolution. The majority of such detailing is processed through sequencing consortia such as the Malaria GEN, with deep sequencing platforms targeting *Plasmodium* genome related polymorphism frequency rates depicting higher recombination events through parental generations and their progenies (Miles et al. 2016), that could be quantified as potential biomarkers. Similar strategy with additional input from microarray data was useful in identifying regions with molecular changes like deletions that encode for immune evasion associated multigene families (Bopp et al. 2013). Detailed studies performed on intra-host diversity related to clonal variations, multiplicity of number of clone ratios, and within-host diversity have added to available data on parasite exemplars. (Duffy et al. 2017).

Parasitic diseases genomics has also been employed to reveal various genetic loci pertaining to novel malaria resistance loci in humans, that could give insights for prevention from severe malaria amounting to 33% protection, while generating information on the loci associated with severe disease form as documented in recent research i.e. chromosome position 1q32 within the ATPase Plasma Membrane Ca^{2+} Transporting 4 (ATP2B4) gene as also the 16q22.2 linked to a tight junction protein known as MARVELD3 (Network MG 2019). The genome wide association studies on malaria serotypes through longitudinal surveillance led to detection of K-13 signatures, identifying Kelch variant, implied to be potential modulator of artemisinin resistance (Cerqueira et al. 2017).

Bioinformatics based tools have also provided us valuable information towards construction of putative resistant mechanisms affecting prevalent drug mechanism utilized in malaria therapy e.g. chloroquine and evolving resistance to artemisinin-based combination therapies (ACT). NGS platform was deployed to study multiclonality patterns, parasite population genetics and drug-resistant genotypes to stratify mutations prevalent in the K-13, Kelch propeller domain contributing towards artemisinin (ACT) resistance in Cambodia (Ariey et al. 2014; Straimer et al. 2015). Further, illumina sequencing platform was deployed for sequencing of various resistance genes likewise of multidrug resistance (pfmdr1), chloroquine resistance transporter (pfcrt), dihydropteroate synthetase (dhps), dihydrofolate reductase (dhfr), and *P. falciparum* Kelch protein 13 (pfk13)] genes to exhibit the

ecological segregation of artemisinin resistant K-13 variants being largely absent in Africa (Malaria GE 2016; Nag et al. 2017).

Paraiste immune-pathophysiology profiling through transcriptomic and proteomic analysis append to the bioinformatics and statistical models, portraying the the genome-wide translational dynamics of *P. falciparum*. Such studies have corroborated the tightly regulated molecular coupling of parasite transcription and translation, endorsing high resolution expression profiles of parasite genes involved in host infection (Caro et al. 2014). Other platforms of transcriptome profiles generated through ChIP-Seq and RNA sequencing involve exploring the polysome profiles linked to the regulation of gene expression of the parasite Plasmodium species infecting humans. Bunnik et al. (2013) detected delay in peak polysomal transcript abundance while performing relative analysis of polysomal genes as compared to the mRNA fraction. These were registered as alternative polysomal mRNA splicing events linked to non-coding transcripts.

Microarrays are increasingly being used to configure parasite transcriptomes, postulating key patterns of differentially expressed genes during cerebral and asymptomatic malaria. RNA seq furthermore had augmented deciphering molecular patterns in the infectious exemplars charting the alternative splice events, newer gene transcripts, and predicted untranslated regions of putative parasite infection-related genes projecting novel findings on parasite biology (Otto et al. 2010). Another detailing of the clinical samples for parasite-host transcriptome spatiotemporally connected through simultaneous analysis, has uncovered unique details about several genes of human host as well as parasite e.g. Toll-like receptor 2 and TIR domain-containing adapter molecule 2 (TICAM2) (Yamagishi et al. 2017). Transcriptome profiling of *P. vivax* through RNAseq uncovered vir gene hotspots on chromosome 2, species-specific virulent genes as well as novel gene transcripts (Zhu et al. 2016). Relative comparison study design of Chloroquine sensitive and resistant parasites transcriptomes identified regulatory patterns comprising resistance linked to 89 upregulated and 227 downregulated genes (Antony et al. 2016). The parasite biology and host-parasite network profiling display insightful impacts of this interplay that would label putative drug and vaccine candidates towards a modernistic diagnostic and therapeutic regime (Lee et al. 2018).

13.3.2 Multi-Host Directed Therapeutics—Host-Pathogen Interactome Networks and Integromic Strategies in Targeted Therapeutic Designs

Disease networks have been inundated to include the plausible factors that drive the instigation, progression, development, regression or recurrence of any disease condition. The iterative learning of the host immune systems in parasitic infections has grossed the need of fine tuning the transversal information that can become the training set for the disease models (Vijayan et al. 2021). The parasitic infections work on a dual path of addressing the niche interactions in both host and parasite separately as well as in co-existing mechanisms. This derives that the disease model

in parasitic triggered diseases has much branching in the involved organisms and requires a more systematic workflow in its analyses.

The caricature of the interactions that include the genetic, epigenetic and protein molecular players, in the realm of parasitic interactions would include the generation of networks of each such contributor to the overall drifts that encompass the network of networks. The multi-pronged analysis thus, includes the host—parasite, host—microbiome, host-parasite-microbiome, interactomes at the levels of gene regulations, protein—protein interactions, transcriptome networks, gene interaction networks to recreate the genotype to phenotype linkage.

13.3.3 Host—Parasite Interactome Networks

Biological interactions in any organism are systems level drivers of evolution, physiological stability and allows effective predictions as per altering environmental conditions. The complexity of any system or ecosystem, in conjunction with its interacting features is established as per quantitative levels and their direct versus indirect links amongst interacting entities. Network analysis in parasitology and infectious human diseases has been in limelight from the past decade that is allowing to synthesize the nodal targets in direction of disentangling host—parasite processes. Network analysis caters towards a versatile tool accounting for all interactors (host or parasites) within a system or community—an entity (taxa) or organism that represent framework of edges or links and nodes. The complex host-parasite interaction study using networks was supported as the over-simplified experimental or mathematical models of disease could not suffice the interplay of the expanse of interactions that happen during infection, innate and adaptive immune responses in reaction. The qualitative and quantitative assessment with spatiotemporal details of the bipartite/multi-partite networks is allowing in the last decade to understand the dynamics of parasite survival and disease progression. This would finally strategize and synthesize the deterministic role in therapeutic regimes.

Parasites playing an integral and peripheral role in shaping the host immunity as a trade-off between alternative hosts (interaction edges with different hosts), lead to variant host-specific survival tendencies during such evolutionary changes. The functional changes that decipher the host-specific interactions drive toward network structures with high partitioning or modularity and low nestedness. Such interaction patterns are mostly observed for ectoparasite in comparison to endoparasite communities. The host-parasite networks could define the trade-offs in the transmission of parasites, as co-evolution would mark the nestedness (high or low) transmission rates (Graham et al. 2009). Mostly, when the parasite is segregated into different life stages, network analysis includes parasites being considered as differential interactors, following the varied life stages that interact with different subsets of host taxa. These network connections are suggestive of the immense role played by such interactions that shape its parasitism, evolutionary outcomes and genetic diversity of multiple host species. The selective pressures of such factors account for the modulations of also host characteristics, thereby impacting the topology of

host-parasite networks. The network organization also reflects the diversity of host characteristics, co-evolutionary processes and simultaneously the modulation of the host behaviour by parasite. Similarly, the host phylogeny and phenotypic characteristics also structure the topology of the host-parasite networks that further dictate parasite transmission. This was exemplified by a mammal-flea network (Dallas et al. 2017), where close phylogenetically linked hosts could be part of the interactors, with different parasites or species of the parasite. Parasites, also in contrast affect the host behaviour generating heterogeneity due to their exposures to parasites and molecular modulations at the interaction interface. Parasite transmission and survival rely on direct or indirect contact between hosts to transmit, thereby making network analysis of host/host (eco-social networks) and host/parasite (molecular networks) creating the epidemiological and immunological context.

Network based approaches to establish the pattern of host-parasite interactions, that are direct, indirect or non-obvious has been henceforth implicated in generating the high throughput knowledge base representation. The data communicated through various omics' studies have been used to structure the differential levels of relationships between these bio-entities (ref). Networks, containing the most vital chords of nodes and edges can help in simplifying the heterogeneity and complexity of such multi-layered biological systems, with a myriad of knowledge creating computational and corroborative experimental analysis. The networks represented here are the Gene Regulatory Networks (GRNs), Protein-protein Interactions (PPIs), Metabolic networks. GRNs are directive of regulatory events between transcriptional regulators like transcription factors (TFs), non-coding RNAs (ncRNAs) and protein coding genes. The correlation of differential expression data with the GRNs, can be used to layer the condition-specific networks at various stages of parasite infections, pointing towards specific disease markers or potential pharmaceutical targets. Similarly, the non-directed protein-protein interactions and directed metabolic reactions can also be employed to sketch the connection of disease development, heterogeneity and epidemiological information. The integration of such multitude of networks or a network of networks can facilitate the complex biological information to decipher the check-points of host responses, where nodes and edges are grouped as per the heterogeneity of the network nodes and their inter-relations as discussed previously. The similar network elements in such network of networks are aligned together and connected by edges linking the different types of elements. Such approaches have been utilized in creating the niche of therapeutic targets for host-directed anti-leishmanial therapies, where the infection dynamics was followed in human macrophages, in conjunction with multi-layered network approach to map metabolic or signalling pathways and drug-target identification. The identified candidates can thus be effectively targeted using drug repurposing with enhanced anti-parasitic therapy regimes (Martinez-Hernandez et al. 2021).

Such network approaches have also been followed to trace the tropism and interconnection between parasites affecting the human population in an extensive study that relayed the crosstalk of parasitism, and pathological outcomes (Cuesta-Astroz et al. 2019).

Similar host-parasite interactome networks have been curated for *Plasmodium falciparum*, with functional interactions provided through the careful assemblage of data-driven approaches providing holistic details of disease pathogenesis, host genetic susceptibility/resistance, drug resistance and novel drugs or drug re-purposing pathways (Agamah et al. 2021).

13.3.4 Herbomics and Structure-Guided Drug Discovery

Identification of new macromolecular targets and small-molecule modulators and has been facilitated through omics-based rational strategies. GenBank3 (Benson et al. 2004), WormBase5, EuPathDB (4formerly ApiDB), Virus Pathogen Database and Analysis Resource (ViPR) etc. database consists of information about complete genome sequences of the pathogens and host provide a led to marvelous advancements in the exploration for new vaccine targets and drugs. High throughput in silico screening using the QSAR (Quantitative Structure-Activity Relationship) framework for the identification of drug and vaccine targets can act as a prologue to expensive and time-consuming laboratory screening and can reduce the time and cost of drug development against parasites. Topological indices (TI) can be to explain macroscopic and macromolecular systems epitomized by composite networks of interaction mechanism i.e., links between the different parts of a system i.e., nodes e.g. protein-protein, drug-target, metabolic, brain cortex, host-parasite, parasite disease spreading, Internet, social networks etc. The structure-based focusing of orthologous pathogen proteins quickens the finding of new antiparasitic drugs.

Comparative genomics and phylogenetic tree analysis have helped in identification of CPKs (calcium-dependent protein kinase) and phosphoisomerase and carboxylase as potential drug targets for parasite inhibition (Amenga-Etego and Awandare 2020). Comparative transcriptome analysis too can provide useful information as has been evidenced in case of Plasmodium species during blood-stage infection, where 800 genes had similar expression pattern across six plasmodium species and out of these 240 were identified as potential through screening by drug target prioritization databases (Hoo et al. 2016). Similar strategy has also been utilized in case of *Filariasis* to provide information on ideal and novel molecular signatures which can support the thernostics of Filaria infection (Armstrong et al. 2016). Information derived from genome-wide RNA-interference data of *C. elegans* (model organism) has identified 3059 genes in *B. malayi* of which 589 can be possible drug targets through validated arrangement algorithms.

Recent studies using Brookhaven protein data bank structure to study seven Fe-SOD enzyme compounds those are reflected as inhibitors of parasitic efficiency of anti- *L. donovani, T. cruzi, and L. infantum* activity and have been projected as goals for antiparasitic drugs. (Yunta and Dietrich 2019). Another approach, fragment-based drug finding may be beneficial in recognizing small-size molecules as ligands. Fragment-based libraries either using natural product-like compounds or natural products can be made and can be used in high throughput screening. This strategy has been effectively utilized to identify benzhydryl ethers along with anti-

protozoal activity and low toxicity against TbrPDEB1, a necessary cyclic nucleotide phosphodiesterase (PDE) from *Trypanosoma brucei*. (Blaazer et al. 2015).

Energy metabolism enzymes in *Leishmania* are deliberated as potential targets and in the study conducted in 2015, various in silico methods have identified 94 genes and 93 energy metabolism sites through TriTrypDB database. The predicted peptide sequences could be further utilized in other relevant databases like DrugBank, Therapeutic Target Database (TTD), and PubChem. This strategy led to the screening of 44 targets for inhibition, which were further shortlisted to 11 for use in humans (including Lonidamine (Doridamina) (LND, (1-[(2,4-dichloro-phenyl)methyl]-1H-indazole-3-carboxylic acid), Nadide, Saframycin A, Sulfacetamide, Morantel tartrate and others) (Silva et al. 2015).

Target fishing/screening is another strategy that utilizes the current information on the biological activity of small molecules and the chemical similarity principle, and this approach has been used for docking molecules similar to strychnobifavone (a drug with high druggability and fitness score) to be used against *L. infantum* where NADPH oxidase, Aldo-keto reductase and Aldose reductase were identified as a putative target which could cause inhibition of the methylglyoxal degradation super pathway. (Chávez-Fumagalli et al. 2018).

Omics techniques and bioinformatics tools can be further utilized to identify virulence and pathogenesis genes and proteins. Efficient epitope prediction tools are available which can aid in effective drug discovery and synthesis (Bah et al. 2018) for optimal vaccine designing process.

To combat the problem of antibiotic resistance, the identification of genes responsible for drug resistance can be determined and utilized for designing and synthesizing new derivatives based on the available structural information. This strategy has been utilized for the work for the design of novel 3,3′ -disubstituted pyrrolidines targeted against *S. mansoni, S. japonicum and S. haematobium*. (Simoes-pires et al. 2014).

Utilization of various bioinformatics tools as crosstalk between other omics-based technologies has the potential to mitigate issues related to detection, design and therapies for parasitic diseases.

13.3.5 Drug Repurposing/Repositioning

In modern day drug development, repurposing/repositioning of drugs can be utilized so that the drugs already tested and validated for other diseases can be utilized for treatment of parasitic diseases also. Comparative genomics data and target homology information (chemo-genomics approaches) from various databases like Therapeutic Targets Database and DrugBank provide the necessary inputs to explore already available drugs. This strategy is fast and economical and hence most pharma industries are already exploring alternative applications of their developed products (Silva et al. 2015).

This strategy extremely helps in minimizing the cost of the process of new drug discovery. These drugs to be utilized in repurposing are those that are already

approved by regulatory agencies such as FDA, EMA, MHRA etc. As the first step in the commercialization process of new drugs is their entry into the market and here it requires, to following strict regulations. To resolve the glitches related to cost, and development time and to accomplish the persistently increasing demand for several medicines drug repurposing is an extremely economical solution (Laura Sbaraglini et al. 2016; Zheng et al. 2018; Shirley et al. 2021). For the treatment of parasitic diseases, drug repurposing is a widely applied approach nowadays which utilizes existing drugs. For example, Sterol alpha-14 demethylase (LdSDM) inhibitors like Zafirlukast and Avodart have been approved by FDA against *Leishmania donovani.* In fact, Avodart has demonstrated high reduction of intra-macrophagic amastigotes and has also demonstrated increased reactive oxygen species (ROS) generation in the parasites. The sequence of LdSDM (https://www.ncbi.nlm.nih.gov/protein/XP_003859085.1) exploited to form the model of the three-dimensional structure by the Modeller 9.24 18 using sterol 14α- demethylase (PDB ID: 3L4D) of *L. infantum* which has 100% sequence similarity with it. Avodart caused the induction of apoptosis like cell death in the parasites by inducing ROS as evidenced through V/PI staining (Rudrapal et al. 2020; Tabrez et al. 2021).

To resolve the issue of drug resistance, drug repurposing could be an effective and highly valuable mode. In a recent study, screening of 400 compounds from pathogen Box (PBox) were screened against *L. braziliensis* with 24 compounds showing promising results even against intracellular amastigotes and antimony resistant *L. braziliensis*. Many of these were found (Silva et al. 2021) to be active against kinetoplasticds. Additionally, MMV676477 and MMV688703 (common to tuberculosis and toxoplasmosis disease) were also found to be effective. ADMET prediction tool results have been validated through in vivo studies. Additionally, CYP450 (LbrM.30.3580), CRK3 (LbrM.35.0660) and PKA (LbrM.18.1180) have been identified as potential targets through molecular docking analysis in *L. braziliensis.*

Combination of oral disulfiram and zinc has been described to be extremely active in contrast to human amebic colitis. Zinc-ditiocarb complex (ZnDTC) was found to be effective in inhibiting *E. histolytica* parasites even at nM concentrations, and it performed even better than metronidazole, the latest drug of choice to treat amebiasis (Shirley et al. 2021). Sterol C-24 methyltransferase (LdSMT) of *L. donovani* is a key target for inhibiting *Leishmania*. Screening of the Food and Drug Administration (FDA)-approved drug library against LdSMT revealed that simeprevir (an antiviral drug) has a significant binding affinity with LdSMT as reflected by high binding score and also repressed *L. donovani* growth of promastigotes. The proposed mechanism has been validated as evidenced from increased ROS generation in upto 44.7% of parasites at 125-μM concentration. Simeprevir has emerged as promising anti Leishmanial drug (Tabrez et al. 2021).

Through the "drug repurposing" approach, histone deacetylase inhibitors (HDACi), which are currently clinically accepted for cancer use, are now examined for several parasite infections (Hailu et al. 2017). Lysine Deacetylase Inhibitors in Parasites: Past, Present, and Future Perspectives, Gebremedhin S. Hailu, Dina Robaa, Mariantonietta Forgione, Wolfgang Sippl, Dante Rotili, and Antonello

Mai, Journal of Medicinal Chemistry 2017 60 (12), 4780–4804, DOI: 10.1021/acs.jmedchem.6b01595). A similar strategy was also utilized in a recent study, where six compounds efficient in anti-tumoral research were used to be tested as anti-parasitic agents in vitro models of *T. brucei, L. infantum P. falciparum* and *T. cruzi*, dichloroacetic acid (DCA), 3-bromopyruvic acid (3BP), 2-deoxy-D-glucose (2DG), lonidamine (LND), metformin (MET), and sirolimus (SIR). (Simoes-pires et al. 2014).

Even though drug repurposing is not a new approach, its deployment to search for antiparasitic drugs in recent years has provided new impetus in drug discovery.

13.3.6 Nanotechnology Based Therapeutic Options

Pharmacological treatment of parasitic diseases is usually hindered by drug toxicity. This problem has been of prime interest to researchers in past decades. In this regard, combinatorial approach combining therapeutic drugs and Nano-drug delivery system may provide slow and spatio-temporal release of cytotoxic drugs to prevent unwanted side effects. One such useful carrier consists of a central polymeric molecule with functional group as branches (dendrimers). Advantages of dendrimers include higher water solubility and biocompatibility along with enhanced flexibility in terms of the load to be delivered. Recently polyamidoamines (PAMAM), polyamines, polyamides (polypeptides), poly(aryl ethers), polyesters, carbohydrates and DNA have been formulated as dendrimers with wide available variability in terms of synthesis and surface hydrophobicity (Folliero et al. 2021).

Amphiphilic dendritic derivatives as nanocarriers for the targeted delivery of antimalarial drugs chloroquine (CQ) and primaquine (PQ). These dendritic derivatives are specifically targeted towards Plasmodium-infected red blood cells (pRBCs), with reduced in vitro ICs of CQ and PQ by 3- and four-fold down to 4.0 nM and 1.1 jiM. respectively (Movellan et al. 2014).

Recently, PEGylated dendritic polyglycerol-based conjugate (PG-PEG) that colonizing intracellular parasites have been investigated for enhanced interactions once mannosylated and it was observed that enhanced specificity was indeed observed upon mannosylation studied for the effect of the surface decoration with mannose units on the conjugates (Vossen et al. 2020).

The Antiparasitic Activity of copper and silverOxide Nanoparticles against *Entamoeba histolytica* and *Cryptosporidium Parvum* Cysts. The average sizes of synthesized Ag NPS and CuO NPs were 9 & 29 nm respectively and a reduction for cysts viability ($p > 0.05$) was observed for CuOPs against E histolytica cysts and Ag NPs against C parvum oocysts (Saad et al. 2015). The effectiveness of benznidazole nanoparticles (BNZ nps), on trypomastigote form and on intracellular infection in primary cardiac myocyte cells and mammalian cells. The use of benznidazole nanoparticles is useful and attractive approach to treating Chagas disease in infected mice (Scalise et al. 2016). *Trypanosoma brucei*, causative agent of African sleeping sickness, is a serious pathogen and Fluorescence quenching and FRET studies have showed interactions of parasite arginine kinase with Ag and Au nanoparticle via

cysteine residues that regulates the electrophilic and nucleophilic characters of the substrate arginine-guanidinium group critical for enzymatic phosphoryl transfer between ADP and ATP (Adeyemi and Whiteley 2014).

13.3.7 Meta Barcoding

The molecular detailing of the parasite landscape is required while ascertaining the role of phenotypic characteristics observed at the varied parasite level. The taxonomic and phylogenetic profiles of the geographic and population-specific genera/species of the parasites have become pertinent in the present scenario that includes the genomic imprints to be ascertained to deconvolute the plethora of similar parasitic species. Meta-data analyses through barcoding are coming in handy to provide such details that have also benefitted many marker genes/proteins associated with the species-specific profiling.

13.4 Bio Prospecting

13.4.1 Marine Natural Products

Searching for new marine products as treasured therapeutic options have increased significantly in the past decade (Lindequist 2016). Benthic marine algae and seaweeds have arisen as promising sources of novel biochemically active compounds, especially with antiprotozoal activity (Torres et al. 2014). MTT assessment of compound present in *n*-Hex extract of *Cystoseira baccata* i.e. meroditerpenoids, (3R)- and (35) tetrapreny Italuquinol (a/b) and (3R) and (35)-tetraprenyltoluquinone (2a/26) against amastigotesand promastigote stage of *Leishmania infant,* signposts its inhibitory potential. (R) and (38)-tetraprenyltoluquinolta/lb) decreased the intracellular infection index (0 = 25.04.1 μM), while (3R) and (35)-tetraprenyltoluquinone (Ca/2b) abolphed 50% of the intracellular amastigotes at a concentration > 88.0 M. Both compounds inhibited the growth of Cystoseira baccata the *L. infantum* promastigotes and were found liable for cytoplasmic vacuolization and disruption of the mitochondrial membrane potential (de Sousa et al. 2017).

Similarly, sponges derived manzamine derivatives including Zamamidines A-C have demonstrated moderate anti- leishmanial activities against *L. donovani* promastigotes. Amphimedon sp. sponges have also been reported as source of anti-*Trypanosoma brucei.*

manzamine alkaloids. Bromotyrosine psammaplin P and two other analogs, psammaplin O and 3-bromo-2-hydroxy-5-(methoxycarbonylbenzoic acid alkaloids isolated from the marine sponge Aplysinella showed moderate activity against *Trypanosoma* and *P. falciparum* (Ashok et al. 2015). In another study, the efficiency of marine-derived natural products (MNPs) like alkaloids, terpenes and terpenoids, amino acids, peptides and polyketide, quinones, macrolide, lacton and sterol have

been reported to be active against malaria, *Leishmania*sis, and trypanosomiasis (Blunt et al. 2018).

The Ghanaian *Paenibacillus* sp. isolated from the mangrove rhizosphere soils is a source of various potential antiparasitic alkaloids active against *Leishmania* and *Trypanosoma* (Tetevi et al. 2019). Even other uncommon sources like benthic marine algae including red algae (Phylum Rhodophyta), brown algae (Phylum Heterokontophyta) and green (Phylum Chlorophyta) algae are also reported to be valuable source of secondary metabolites having antiprotozoal activities. Some of these include terpenes, halogenated triterpenes, sulfated polysaccharides, acetogegin and polyphenols. Amongst these, ETC uncoupler diterpene (4R.95.14S)-4a-acetoxy-9, 14-dihydroxydolast-1(15),7 diene, from *Canistregarpus cervicornis* has demonstrated high potential (Álvarez-Bardón et al. 2020), Sulfated polysaccharides from various marine hydrobionts (algae and invertebrates) are also useful as anti-protozoal and anti-helminthic agents (Besednova et al. 2021). Rampant antimicrobial resistance against anti-helminthic agents have necessitated the need for bioprospecting and novel drug development and their complementary strategies of using conventional treatments with novel drugs from natural sources may provide more effective treatments. Tunicates and coral, from Australian sea waters have also been a good source of natural compounds with nematocidal properties (Taki et al. 2021), Molluscan shellfish of classes Bivalvia and Gastropoda are of particular interest, and secondary metabolites from *Haliotis tuberculata* extracts have proven to be useful against *Caenorhabditi elegans*. Marine actinomycetes are an extremely important group of bacteria for antibiotic production and marine-derived natural products from actinomycetes have anti *Leishmania*l bioactivity potentials (Davies-Bolorunduro et al. 2021).

Apart from various marine natural products, several studies have well defined the role of numerous plant extracts as a moderate source of antiparasitic activities. Along with these few more sources like antimicrobial peptides (Wardana et al. 2018) flavonoid obtained from various herbs (Bolaños et al. 2015) and specific use of glycolytic enzyme was also evolved (Vique-Sánchez et al. 2021).

13.4.2 Antimicrobial Peptides as Therapeutic Target

Antimicrobial peptides (AMP) are increasingly being considered a novel drug candidate. They are found in every organism and show high structural and functional diversity. Apart from the direct antimicrobial activity, AMPS also have properties like immunomodulation (Fjell et al. 2012), which make them extremely interesting compounds for the development of novel therapeutics and many of these are already introduced into the market, and many AMPs are currently being tested in clinical trials (Fox et al. 2013). The mechanisms of AMPs are related to the disruption of the normal mycobacterial cell membranes, interaction with the intracellular targets, modulation of innate immunity, and adaptive immunity's promotion. In recent years synthetic amino acid peptides utilization in treating human parasitic diseases have been arose as a new strategy to control the parasitic disease. (Robles-Loaiza

et al. 2021). Currently, these are being used in the development of inventive therapies for assorted health conditions, like Leishmaniasis. Berrocal-Lobo et al. (2009) tested thionins defensins, plant antibiotic peptides (PAPs) against *L. donovani* and reported thionins and defensins were active against this human pathogen even at a low micromolar range of concentrations. In a similar study, the activity of the synthetic amino acid peptides lysine. Glutamic acid (KDEL) and aspartic acid, aga *Leishmania* trentolae (promastigote and amastigote stages) were tested to reveal that Larentolae was significantly susceptible to KL peptides in a dose-dependent manner, and KDEL peptides were able to put the surface membrane integrity and cause cell apoptosis. The author verified the therapeutic potential a new anti- Leishmanial drug in Pseudomonas aeruginosa-derived AMP KDEL. In a study conducted by (Kumar and Chugh 2021) peptide Tachyplesin was used against *L. donovani* to validate the dual use of Tachyplesin as an anti- Leishmanial peptide as well as a cargo delivery vehicle making the marine peptide Tachyplesin an attractive therapeutic target against visceral Leishmaniasis. In a recent study in 2021 on transcription factors of sandy by Kykalová et al. gut-specific defensin gene was found to be upregulated during L. major infection, and in combination with gut bacteria, maybe a capable target for parasite disease control approaches. Current therapeutic strategies cannot avert or reverse the heart damage triggered by the parasite of Chagas disease. Aspirin-triggered resolvin D1 (AT-RvD1) is a pro-resolving mediator of inflammation that acts through N-formyl peptide receptor 2 (FPR2). AT-RvD1 contributes in the alteration of cytokine assembly, inhibition of leukocyte selection and efferocytosis, macrophage switching to a nonphlogistic phenotype, and the promotion of healing, thus restoring organ function. In a study led by (Carrillo et al. 2021), AT-RvD1 was projected as a probable therapy aid to control the pro-inflammatory state during the chronic phase of Chagas disease. Victoriano Corpas proposed drug Targets in *Trypanosoma brucei* by Thermal Proteome Profiling. They used eflornithine that is a known ornithine decarboxylase (ODC) suicide inhibitor and expected this as a promising tool for improving drug development pipelines for *T. brucei* and related kinetoplastid diseases (Vincent et al. 2010).

It seems that peptides may represent the basis for modeling new and safe anti-parasitic drugs. Further studies are needed to assess the activity of several peptides against the various stages of *Leishmania, Trypanosoma* and other such parasites and their therapeutic activity in animal models of Leishmaniasis.

13.5 Public Health Perspective in Disease Management—PPP Models

The open-source model appears working well for NTDs, as most of the stakeholders involved are nonprofit or philanthropic organizations collaborating with pharmaceutical or biotech companies (Weng et al. 2018). The Pathogen Box comprising of about 400 compounds against these diseases from Medicines for Malaria Venture (MMV) has been proven to be a successful open-access for NTDs drug research.

Researchers around the world can freely request a Pathogen Box without charge, which will promote the establishment of an open and collaborative forum of DDD for NTDs. Recently, the three kinetoplastid chemical boxes offered by GlaxoSmithKline has become an open resource for future lead discovery programs. They were assembled with approximately 200 compounds each and are freely available to academic researchers. Free flow of information is one of the goals of this model: it would not only facilitate innovation but also encourage competition. The latter is instrumental to the production of less expensive and more accessible medicines: two major targets in combating NTDs (Dans et al. 2020).

EC-funded project, SEtTReND project has particularly focussed on systematic study of selective epigenetic inhibitors (HDACi) as potential drugs wherein library of potential lead compounds (active against *Plasmodium falciparum* and other strains and *Leishmania*) were rapidly tested against other important human parasites. These enzymes are increasingly targeted in the therapy of a variety of pathologies including cancer, which allows us to benefit from the expertise and inhibitors already developed as starting points for developing anti-parasitic drugs. The project employed two complementary strategies: phenotypic screening using focused HME inhibitor libraries and a structure-based approach involving validated target enzymes and high-throughput or in silico screening. Bioguided optimization of the hits identified fed into lead development, basic ADMET and in vivo testing to generate lead compounds. Knockdown by RNA interference was used to identify three HMEs, HDAC8, the lysine demethylase KDM1 and the arginine methyltransferase PRMT3 as valid targets in *Schistosoma mansoni*, the flatworm parasite causing intestinal schistosomiasis.Trichostatin A (TSA) and the approved cancer drug Vorinostat are effective against *P. falciparum* and *S. mansoni*, but less so against Leishmania sp.. However, these are pan-HDAC inhibitors that are active against all the isoforms of HDACs and inhibit human HDACs very effectively. The key to using HME inhibitors as a basis for new drugs against parasites is to develop compounds that are selective for parasites: i.e. that selectively inhibit a particular HME isotype and which also show species selectivity.

These strategies will go a long way in boosting the drug discovery process across the globe through collaborative endeavours between large pharmaceutical companies and other key actors in the healthcare ecosystem, i.e., academic institutions, small and medium enterprises, patients, and regulatory authorities.

13.6 Conclusion

A highly diverse requirement pertinent to a thorough biological understanding requires a fresh look into the perspectives of co-evolution, co-existence as well as survival. The distinctive characteristics for such phenomenon is important to create an environmental niche of life sustenance and health. The chapter broods on detailing the host-parasite analysis, interactions, and deliberations of the molecular and cellular events that define the survival, immunological tolerance and etiopathologies of pathogen-induced disbalance and disease. With the recent

detailing of the epigenome contributions and the host-pathogen interactome in defining the plasticity and immune behavior, we have addressed the last decade of the research in disease prognosis, distinctive immunopathologies, treatment regimes followed for human—parasite infections predicting disease models. The clinico-molecular analyses further induce the façade of therapies, drugs/vaccines that needs to be re-purposed for alleviating the parasite burden on the hosts entailing a major socio-economic and medical impact in the field.

The structured details of the strategies and the upcoming technologies and regimes to tackle the parasite-driven multitude of factors encompassing epigenetics, non-coding RNAs, metabolomics, network of networks, nanotechnology formulating the basis of an integromics approach using the multifarious precision medicine tagging along the traditional medicine practice showcases the outreach of our presented chapter. Furthermore, analyzing the natural products not just from medicinal plants but also from marine sources and adding up the conclusive evidence from the trends alternatively anti-microbial peptides and proteasomes, have been appended to nest the overall effects of the paradigms of parasite treatments. We conclude these latest details of effective management using the much-required public–private partnership model for the discussed parasitic diseases.

Acknowledgments The authors acknowledge Dr. A.K. Chauhan, Founder President, Amity University, for his continuous motivation and encouragement and Dr. Balvinder Shukla, Vice Chancellor Amity University and Officiating Director, Amity Institute of Biotechnology, Amity University for providing the supporting infrastructure for preparation of this review chapter.

Glossary

Antimicrobial peptides Class of small peptides that widely exist in nature.
Apicomplexa Are a large phylum of parasitic alveolates.
Artemisinin A terpene-based antimalarial substance used in Chinese medicine.
Bioprospecting Exploration of natural sources for small molecules that change into commercially valuable products.
Cytotoxicity Toxicity caused due to action of chemotherapeutic agents on living cells.
Dendrimers A synthetic polymer with a structure of repeatedly branching chains.
Drug Repurposing Drug utilized for treatment for a different diseases from the one it was initially discovered or designed.
Epigenetics Study of heritable phenotype changes.
Epigenetic landscape Is concept representing embryonic development.
Etiopathologies Consideration of the cause of an abnormal state or finding.
Interactome networks Network that contains ideally all possible PPIs in a specific organism.
Macrolide Class of natural products that consist of a large macrocyclic lactone ring to which one or more deoxy sugars.

Metabolomics The scientific study of the set of metabolites presents within an organism, cell, or tissue.

Macrophage A large phagocytic cell found in stationary form in the tissues.

Microarrays A set of DNA sequences representing the entire set of genes of an organism.

Miltefosine Treat leishmaniasis and free-living amoeba infections.

nc RNA Non-coding RNA.

Nanotechnology Branch of technology that deals with dimensions and tolerances of less than 100 nanometres.

Natural products Small molecules produced naturally by any organism.

Parasitic diseases Any illness that is caused by a parasite, an organism that lives in or on another organism.

Schistosomes A parasitic flatworm which needs two hosts to complete its life cycle.

Simeprevir Medication used in combination with other medications for the treatment of hepatitis C.

Terpenoids Any of a large class of organic compounds including terpenes, diterpenes, and sesquiterpenes.

Theranostics The combination of using one radioactive drug to diagnose, and a second radioactive drug.

Transcriptome The sum total of all the messenger RNA molecules expressed from the genes of an organism.

Trypanosomatids Protozoan parasites.

Vitelline cells A membrane enclosing an egg that comprises the zona pellucida in mammals.

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Strategies and Challenges for Developing Plant-Based Therapeutics Against Protozoans

14

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Abstract

Several protozoan parasites are known to cause severe human and zoonotic infections as well as life-threatening diseases including trypanosomiasis, leishmaniasis and malaria. These diseases cause high mortality and morbidity in developing countries across the world and yet remain a neglected public health issue. Currently, available chemotherapeutic drugs against these parasitic protozoans are either ineffective or exhibit severe side effects and also result in the development of resistance. To overcome these problems, phytochemicals have shown an ingenious way to provide innumerable molecules exhibiting great potential for the management of protozoan infection and diseases along with safety for humans and the environment. Several studies across the globe have provided evidence for the presence of bioactive components through in vitro, in vivo and clinical screening of phytochemicals. These bioactive compounds present in the crude extracts and essential oils of medicinal plants are quintessential components for strategies to develop plant-based therapeutics against Protozoans. This chapter highlights the potential of plant-based compounds as powerful anti-protozoan drugs and future challenges to fight against parasitic infection.

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_14

Keywords

Protozoa · Phytochemicals · Chagas disease · Leishmaniasis · Trypanosomiasis

14.1 Introduction

Protozoan parasites cause severe infections and deadly diseases in humans which continue as an unsolved public health problem, predominantly in tropical and sub-tropical countries and remain neglected for several decades. The protozoan diseases continue to be a major cause of substantial morbidity and mortality, particularly in underdeveloped or developing countries across the world. Disease such as malaria, Chagas disease, African trypanosomiasis and leishmaniasis are considered among Neglected tropical diseases (NTDs). The significant mortality caused by these protozoans is associated with poor sanitation and a lack of appropriate prophylactic measures (Pink et al. 2005). These diseases mostly influence deprived populations in underprivileged countries and are therefore unattractive to private industries for investment under the research and development sector.

Trypanosomiasis constitutes the two main human systemic trypanosomiases (Kennedy 2013; Bern 2015). American trypanosomiasis is caused by *Trypanosoma cruzi*, which is usually transferred through contact with the excrement of hematophagous *Triatomine* insects, infecting 6 to seven million people worldwide, more frequently in Latin America (WHO, 2016, 2021). These disease became endemic for 21 continental Latin America countries which due course of increase migration with time frame extends across Japan, Canada, U.S. and Europe (WHO 2021).

African trypanosomiasis is caused by *Trypanosoma brucei gambiense* and *T. brucei rhodesiense*in most of Africa region (Giordani et al. 2016) which is transmitted by tsetse fly of thegenus *Glossina* (Sudarshi and Brown 2015). Leishmaniasis is caused by *Leishmania* is commonly transfer using sandflies as vector, either *Lutzomyia* (New World) or *Phlebotomus* (Old world). This protozoan is listed as the much divergent of human pathogens, in criteria including geographical dispersal and a variety of clinical manifestations. The disease nativity extends to around 88 countries, the higher percent relates to the developing world while other lie down in the criteria of least developed countries. The approximated annual report of visceral leishmaniasis (VL) is around 5x 10^5 in 61 countries with only 5 countries access 90% of the confirmed cases namely India especially Bihar and its surroundings, North Eastern Brazil, Sudan, Nepal, and Bangladesh. When relations form for the cutaneous form, 1.0–1.5 million cases are reported annually which comprises 90% of the cases reported around 8 countries, 6 being in the Old World (Syria, Algeria, Iraq, Iran, Saudi Arabia,and Afghanistan) and 2 in the New World (WHO 2007; Jhingran et al. 2008).

The chemotherapeutic drugs currently available for treatment of these neglected diseases were introduced decades ago and most of them causes serious disadvantages, have complicated side effects, may be ineffective and in cases where drug treatment is available, caused development of drug resistance in

protozoan parasites (Coura and De Castro 2002; Fairlamb 2003; Burrows and Waterson 2011). As the main prevalence of parasitic diseases occurs in the poorest areas of the world, the market has not enough strength to drive the development of new drugs. In 2000, only about 0.1% of investment in health research was transferred for tuberculosis, leishmaniasis, trypanosomiasis and malaria, whereas 5% of the share was done in the global disease burden which additively contribution of all the above listed diseases (Pink et al. 2005).

So, its an urgent need to explore new drugs with cost-effective, safe and alternative methods to control protozoan diseases. The experiments conducted on host-parasite interaction at the molecular level have been explored by the involvement of reporter genes, which produces fluorescent in the sequence of β-gal to luciferase follows fluorescent proteins. The complete assemblance and sequenced genomes availability of *Leishmania, Plasmodium*, and *Trypanosoma*species suggest multi-disciplinary pathways lie on function-structural studies of proteins and drug libraries screening involving various high techniques. In addition, newly discovered targets for drug development also emphasise on anti-oxidant nature of defense mechanism against Trypanosomatidae because of the mechanism of trypanothione-dependent detoxification of peroxides, a unique pathway, not found in vertebrates (Santi 2022). As the drug availability is very low for this disease treatment including resistance development of parasites and side effects of the drug, the research field of plant products-based sciences which mainly deal with bioactive compounds and their properties such as phenolics, terpenoids, sterols and alkaloids etc. with a capacity to control protozaons numbers has been explored for the designing of new drugs with maximum efficiency to provide more accessibility to the patients (Alviano and Alviano 2009). This section of the book deal with some plant-based therapeutics against protozoans.

14.2 Recent Treatments Approaches and Their Limitations

14.2.1 Drugs and Their Side Effects

African trypanosomiasis infections lead to CNS infections, meningoencephalitis related to various other neurological disorders and the most dangerous of sleeping sickness having high mortality rate (Kennedy 2013; WHO 2016). Still in the current scenario, no such availability of vaccine neither any future agenda for the development of vaccines (Goupil and McKerrow 2014). Treatment offered to patients involves single or combined chemotherapy drug treatment involving merlasoprol, nifurtimox, pentamidine, and eflornithine. The process of these drugs to administer in patients is complicated and highly toxic with no such guaranteed treatment, chances increase with the severity of infections (Kennedy 2013; Giordani et al. 2016; WHO 2016). Suramin and pentamidine are currently used as stage 1 drugs whereas eflornithine and melarsoprol are stage 2 drugs (Nanavaty et al. 2016; Steverding 2010). Suramin first introduced in 1922 is a polysulfonated drug with side effects of vomiting, nausea, shock and fatigue, which on later stages of

prolonged exposure also shows neurological complications and renal toxicity (Brun et al. 2010; Barrett and Croft 2012). Melarsoprol, organo-arsenical medicine which is a single drug found effective in *T.b.gambiense* and *T.b. rhodesiense* both but the side effects includesfatal encephalopathy in 5% of cases (Barrett et al. 2007; Robays et al. 2008). 2-(difluoromethyl) ornithine with market name of Eflornithine (trypanocide) side effects involves neutropenia, fever, infections, diarrhea, fever and seizures. The most relaxing concepts with these drugs of reversible side effects after completion of treatment (Brun et al. 2010). Combination therapy of Nifurtimox_eflornithine (NECT) was used by WHO for possbility in *T.b.gambiense* late stages control programs (Alviano et al. 2012). In India, Miltefosine has been licensed as oral formulation relates to Visceral Leishmaniasis treatment. This drug has longer treatment time, teratogenicity and of long-term residence in the body which decreases drug efficacy as well as the median half life of 150 h help the parasite to develop resistance against the disease (Perez-Victoria et al. 2006).

In South Africa country, Chagas disease causing non-ischaemic cardiomyopathy which has taken way of many lives because of dilated cardiomyopathy, thromboembolic and dysrhythmias as well as congestive heart failure events reported in 30% of patients infected with those protozoans (Bern 2015). Acute, early chronic as well as reactivated infections of up to 60% are curable with drugs benznidazole and nifurtimox but as same of above, no guarantee of complete cure for severe cases as of 10–20% (Cançado 2002) only, and various side effects attached automatically with the treatment, no specific vaccines designed against those protozoans and also, low tolerance power with multiple side effects which reduces the efficacy of the drugs (Jackson et al. 2010).

14.2.2 Intracellular Localization of Protozoans

In various cases of pathogen infections, the protozoans invade intracellular locations which became problematic for the drugs to internalize in the host cells via host mechanism eg includes, anti-leishmanial drugs. Although, various other factors also contribute to increasing the issues for the drug to get internalized involves, resistance for intracellular degradation, causing host cytotoxicity, uptake kinetics, and intracellular trafficking. Leismaniaparsite involves more than 20 species of a broad spectrum for sign and symptoms involving L. donovani complex causing life-threatening visceral Leishmaniasis (VL), chronic of *L. braziliensis* causes mucocutanous infections to acute, self-healing infection skin ulcers by L. major and other dermatropic species. Therefore, drug designing for these protozoans involves, extracellular promastigotes for easier pathways, providing us a promising compound with high therapeutic index for intracellular amastigotes involves complicated processes. During treatment, the given drugs include first-line drugs of sodium stibogluconate and meglumine antimoniate whereas the second line drugs involve pentamidine and amphotericin B. These antimonial drugs used in the treatment are toxic as well as shows resistance pattern over a longer duration causing enhanced treatment failures (Delgado et al. 1999; Rijal et al. 2003) even toxicity was reported with use of

pentamidine. Current anti-Leshimanials advancement of drugs involves an alkylphosphocholine, miltefosine(an anticancer drug) has active components against visceral-Leishmaniasis and CL. But the drug shows teratological potential, so restricted for the treatment of pregnant women and even 28 days treatment leads to resistance of drug (Ouellette et al. 2004; Sundar and Murray 2005).

14.3 Plant-Based Therapeutics for Protozoans Management

As from above explained paragraph, it has been cleared that not much effective treatment is available for some severe protozoan diseases. The gap in treatment involved unknown mechanism of their action and unfavorable toxic profiles. These drugs are very costly and the production rate also less so sometimes, became out of reach for poorly resourced communities. The most common method evolved to cure this disease radiation involves removal of their vector which help in transmission via using insecticide which is again harmful for the environment or also causes resistance development against insecticide (Cheuka et al. 2016).

Plants derived bioactive compounds are unique and mysterious compounds of so many chemical compositions in a single plant product as in the form of secondary metabolites and many more other forms also. These compounds possess high therapeutic potential and medicinal properties for the exploration of variety of new drug forms for the treatment of protozoal infections. Plant-derived natural products (NPs) as eg. alkaloids quinine from *Cinchona* sp. Emetine for amebiasis treatment from *Cephaelis ipecacuanha* (Cheuka et al. 2016), and a sesquiterpene lactone artemisinin from *Artemisia annua*. These NPs functions as probes for semisynthetic and synthetic drugs with enhanced safety, efficacy and pharmacokinetic results (Table 14.1).

14.3.1 African Trypanosomiasis

T.b. brucei species including two subspecies *T.b.gambiense* (West African sleeping sickness) and *T.b. rhodesiense*(East African sleeping sickness) are the reason for Human African Trypanosomiasis causing 3% and 97% of reported cases. The transmission mode includes tsetse flies (*Glossina* species) bites which became vector either from animal harboring parasite or human itself. During the initial days of infection, the parasites multiply inside inner layer of skim around the bite area showing a lesion, known as chancre or trypanoma (Brun et al. 2010) followed by invading into circulatory system showing glycoprotein coating on the surface which helps in escape from the immune machinery of animals (including humans) (Sternberg 2004). Continuation of exposure to these parasites makes immune cells dysregulation as well as an imbalance in cytokine production causing immunosuppression (Barry and Corrington 2004). The infected areas at the initial stages include, lungs, brain, heart and lymphoid system which on longer exposure leads to meningoencephalitic stage due to blood-brain barrier crossing of the parasites followed by

Table 14.1 Showing plant products with their bioactive component nature, IC_{50} values and activity against protozoans

S. no	Plant name	Plant part used in extraction/extract solvent/nature of extract	Compounds with their IC_{50} values	Activity	References
1.	*Vernonia lasiopus O. Hoffm*	Dichloromethane	11,13-dihydrovernodalin, vernodalol (0.255μM), vernodalin, vernomenin, vernolepin (0.185μM), 8-desacylvernolide (2.53μM)	Anti-african trypanosomiasis	Schmidt et al. (2002)
2.	Arnica species	Sesquiterepene	Helenalin (0.051μM)	Anti-african trypanosomiasis	Schmidt et al. (2002)
3.	*Azadiracta indica*	Aqueous	1000 mg/kg	Anti-african trypanosomiasis	Ngure et al. (2009)
4.	*Artemisia sieversiana*	Whole plant	Sesamin (2.4–10.0μM)	Anti-african trypanosomiasis	Banzragchgarav et al. (2016), Buyankhishig et al. 2020; Nurbek et al. 2020
5.	*Solanum incanum*	Fruit and leaves extract	(27.3μg/mL)	*T.brucei*	Anwar (2018)
6.	*Peperomia obtusiflora*	Leaf extracts	Peperobtusin A (3.1μM)	*T. cruzi*	da silva (2009)
7.	*Aristeguietia glutinosa*	Hydroethanolic extract	(1)-13,14,15,16-tetranorlabd-7-en-l7,12-olide (62.9μM) and (1)-15-hydroxy-7-labden-17-al (9.8μM)	*T. cruzi*	Varela et al. (2014)
8.	*Corydalis govaniana*	Alkaloids extracted	Tetrahydro-protoberberine(0.18μg/mL)	Anti-Leishmaniasis	Callejon et al. 2014
9.	*Sterculia villosa Roxb*		130–0 μg/mL	*L. donovani* promastigotes	Das et al. (2017)
10.	*Piper rusbyi*	Flavanoid	Chalcone flavokavain B (11.2μM)	*L. donovani* promastigotes	Flores et al. (2007)
11.	*Artemisia roxburghiana*		0.42μg/mL	Anti-malaria	Dua et al. (2011)

12.	*Diospyros melanoxylon*	Aqueous and ethanol extracts		Anti-plasmodial activity.	Saxena et al. (2011)
13.	*Crotalaria anixensis, C. medicagenia, C. ramosissima*		Phenylated chalcones(50, 10 and 2μg/mL)	*Plasmodium falciparum*	Narender et al. (2005)
14.	*Salaginella bryopteris*	Biflavonoid	Amentoflavone and hinokiflavone, methylamentoflavone(0.26μM)	Anti-protozoan, K1 strain of *P. falciparum*	Kunert et al. (2008)
15.	*Artemisia roxburghiana*	Chloroform extract	0.42μg/mL	*P. Falciparum*	Dua et al. (2011)

various CNS-related neurological changes including, mood alterations, poor coordination, sensory disturbances, behavioral changes, mental confusion, and so on. The acute to chronic stage transformation involves months to years or sometimes causing death of the patients.

14.3.1.1 Plant-Derived Anti-Trypanosomias Products

Vernonia lasiopus O. Hoffm plant dichloromethane extract shows 6 elemanolide-type sesquiterpene lactone showing in vitro activity against *T.b. rhodesiense*. These compounds are 11,13-dihydrovernodalin, vernodalol, vernodalin, vernomenin, vernolepin, 8-desacylvernolide. The experimental results showed that Vernolepin with high potential IC_{50} values of 0.185μM and SI of 14.5, the second potent extract was vernodalol (IC_{50}, 0.255μM and SI, 14.4) which further followed with 8-desacylvernolide (IC_{50}, 2.53μM and SI, 13.7). The blood stream forms of *T.b. rhodesiense treated* with Arnica species sesquiterpene lactone, Helenalin was found with 0.051μM IC_{50} values. The mechanism of action of these compounds involves α,ß-unsaturated carbonyl group which causes alkylation of nucleophiles in biological system of infected blood stream (Schmidt et al. 2002). The same experiment performed with another set of secondary metabolites, flavonoids and their analogs the order of compounds are 7,8-dihydroxyflavone (0.27μM IC_{50}), second one of rhamnetin (1.7μMIC50), third one of 7,8,30,40-tetrahydroxyflavone (1.7μMIC50), and the last one is of 3-hydroxyflavone (2.0 μMIC50) was obtained. A lot of alkaloids, naphthylisoquinoline obtained from genus *Ancistrocladus*includes ancistrocladidine, ancistrotectorine ancistrolikokine D, 6,40-O-didemethyl ancistrocladinium A**,** 50-Odimethyl-ent-dioncophylleine, ancistrocladinium A, 40-O-demethyl ancistrocladinium (Bringmann et al. 2003, 2004, 2005, 2011). Another naphthoquinone includes 2-phenoxy-1,4-naphthoquinone (0.08μM of IC_{50}) (Pieretti et al. 2013), *Juglans regia* L. products of SM showed for STIB900 strain of *T.b. rhodesiense* are1,4-napthoquinone (0.58μM of IC_{50}), juglone (1.62μM of IC_{50}) and hydrojuglone glucoside (6.12μM of IC_{50}) but also showing antileishmanicidal properties (Ellendorff et al. 2015). Ngure et al. (2009) studied using *Azadiracta indica* bark aqueous extracts of 1000 mg/kg for anti-trypanosomal effects showed promising results with mice infected with *T.b. rhodesiense* through enhanced life span of mice, control weight loss, control parasitemia level as in comparison with a trypanocidal drug, suramin. *T. b. brucei* infected rats was treated using leaf extracts of *Saba florida* and *Cissus multistriata* which showed that later plant extract was more effective than former one. The *Psidium guajava* ethanolic leaf extract used for treatment of rats infected with *T.b. brucei*shows zero parasitemia as well as extend the life span up to 32 days as compared to untreated group with 8 days of survival only (Stephen et al. 2009). *Annona senegalensis* Pers stem bark, root, whole root and leaves extract was capable of complete cured of mice infected with *T.b. brucei*as well as cerebrospinal fluid and subinoculation of blood was also transfuse to infected mice from the cured one and no infection was reported up to 60 days from post-inoculation with the parasite (Ogbadoyi et al. 2007). In Nupeland, Nigeria, conventional treatment methods involve six plants in which *Heterotis rotundifolia* (whole plant), *Bombax*

buonopozense (stem bark), and *A. nilotica* (stem bark), which found to be effective and completely removes parasite circulating in blood fluids for up to 30 days (Nwodo et al. 2015).

One of the known capable anti-*Trypanosomal* are lignans and their analogs. These are isolated from *Brachanthemum gobicum* (Asteraceae) plant parts that showed characteristic acrylated lignan. These dimers are of phenylpropanoid nature and another product from *Artemisia sieversiana* of the same plant family, the Sesamin as well as other flavonoidal products showed trypanocidal activity of IC_{50} at 2.4–10.0μM (Banzragchgarav et al. 2016; Buyankhishig et al. 2020; Nurbek et al. 2020). Eleven 2,5-diphenyloxazoles compounds isolated from *Oxytropis lanata*, of which six products are isoflavanoids nature. The one of lethal disease caused by *Trypanosoma congolense*, can be prevented using Oxazole-type alkaloids which are very rare in nature and shows high trypanocidal activity (Murata and Batkhuu 2021).

14.3.2 American Trypanosomiasis or Chagas Disease

A life-threating potential disease, Chagas disease or American trypanosimiasis occured due to protozoan parasite *T. cruzi.* Involves triatomine bug, a blood feeding organism of triatominaeand Reduviidae family which requires blood for their complete life cycle. This CD involves two phases, of acute and chronic subsequently. The first phase of acute which persist for around 2 months after infection which involves, parasites high number circulating in blood fluid which followed by chronic by subsequent days involving hidden nature of protozoans in digestive and heart muscles which finally leads to death of patients from heart failure, arrhythmias and vascular cerebral accident, sometimes in initial stages life (Rassi Jr et al. 2010).

14.3.2.1 Plant-Derived Antichagasic Products

Three stages involved in *T. cruzi* are Epimastigote, replicating culture in semi-defined and defined media and same as life cycle of vector. Trypomastigote involves non-replicating population of 24 to 48 hrs in medium. Finally, Amastigote phase replicating enormously which grown in fibroblast, muscle cells and macrophages and leads into trypomastigotes infection. The time period of 4 to 5 days is there when amastigotes replicate via epimastigote stage reverse into trypomastigotes escaping stage from the cells. This time is used for checking drug activity. But the complications arise in data interpretation due to trypomastigotes floating in blood, low infection rate, and the dividing host cell population (Croft 1986).

Albaha region plant of *Solanum incanum* fruit and leaves extract was shown in vitro antiprotozoal and cytotoxic activity when used in MRC-5 cell-lines and *T. cruzi* and *T. brucei* species treatments respectively (Anwar 2018). The data analysis of the above showed that both extracts have the same range of IC_{50} of 27.3μg/mL against *T.brucei* but in case of *T. cruzi* leaves has IC_{50}: 6.0μg/mL and fruits with IC_{50}: 9.3μg/mL reported whereas this plant leaves (IC_{50}: 293.2μg/mL) showed lesser antioxidant activity but for fruits extracts (IC_{50}: 98.7μg/mL) the range is high.

Peperomia obtusiflora leaf extracts has active compounds including a chromane compound, peperobtusin A is more potent against trypanosomes with report of 3.1μM IC_{50} values for *T. cruzi*epimastigote forms (da silva et al. 2009). Root bark extracts of Maytenusilicifolia has two compounds, pristimerin and maytenin (quinonemethide triterpene) shows anti-protozoan activity for trypanosomes and Leishmania both and 0.25 and 0.30 nM IC_{50} values against *T. cruzi* epimastigotes respectively.

In vitro experiments conducted using *Curarea toxicofera* and *Ambelania duckei* extracts shows concentration dependent inhibition of 50+/−5 and 221+/−29 IC_{50} values respectively whereas *Aspidosperma excelsum* and *Abuta grandifolia* of >500μg/mL IC_{50} values. These all extracts possess no cytotoxicity against MRC5 and HepG2 cell lines (Arias et al. 2021). The aerial parts of plant *Aristeguietia glutinosa* hydroethanolic extract were tested for in vivo anti- *T. cruzi* activity (Varela et al. 2014). The extracted bioactive compounds are (1)-13,14,15,16-tetranorlabd-7-en-l7,12-olide (IC_{50}, 62.9μM against epimastigotes) and (1)-15-hydroxy-7-labden-17-al (IC_{50}, 9.8μM against epimastigotes) were tested in CD acute mouse models for higher dose of 30 mg/kg and lower dose of 10 mg/kg. The mechanism of action involves reduced dehydrogenases activity in mitochondria as well as sterol synthesis inhibition.

14.3.3 Leishmaniasis

This disease is occurred by protozoan parasites from greater than 20 Leishmania species (Banuls et al. 2007). These protozoans are communicated when an infected female sandfly bites to healthy human. The parasite completed its development in two stages i.e., the amastigote stage lived in the reticulo-endothelial cells of mammals whereas; the flagellated promastigote stage grows in the vector gut (sand fly). Leishmaniasis exhibit three forms: cutaneous, mucocutaneous and visceral leishmaniasis (Alviano et al. 2012). *Leishmania tropica* effects known as cutaneous leishmaniasis which results in the form of ulcers on arms, face and legs. The mucocutaneous leishmaniasis diversified from localized cutaneous leishmaniasis to anergic diffuse cutaneous leishmaniasis and is caused by *Leishmania braziliensis*. It leads to the damage of mucous membranes of mouth, throat cavities and nose. Visceral leishmaniasis or kala-azar is caused by *L. donovani* and considered as most dangerous disease than other forms which may cause death if remains untreated (Alviano et al. 2012). Generally, it infects children however, can also be seen in adults. It is reported endemic to Indian subcontinent and East Africa. The major symptoms consist of fever, weight loss, splenomegaly, pallor, hepatomegaly, cough, asthenia, vomiting and anorexia. If it remains untreated, the mortality can reach up to 100% in developing countries.

14.3.3.1 Phytochemicals as Antileishmanial Agents

The plant secondary metabolites such as flavonoids, glycoflavones (quercitrin and isoquercitrin), aglycone and quercetin reported to exhibit leishmanicidal activity

(da Silva et al. 2012). The flavonoids reported to inhibit the activity of arginase enzyme in *L. amazonensis.* The IC_{50} values for quercetin, isoquercitrin, and quercitrin were estimated to be 3.8, 4.3, and 10μM, respectively (da Silva et al. 2012). The quercetin functioned as mixed inhibitor, however, isoquercitrin and quercitrin reported as uncompetitive inhibitors of arginase enzyme in *L. amazonensis*. The leishmanicidal activity of various alkaloids extracted from plant *Corydalis govaniana* was evaluated. The tetrahydro-protoberberine type alkaloid exhibited highest activity with IC_{50} of 0.18μg/mL which may be due to the presence of methylenedioxy moiety in the alkaloid (Callejon et al. 2014). Screening of several plants belonging to Asteraceae family leads to isolation of 8-epixanthatin-1β,5-β-epoxide which significantly reduced the leishmania parasite, *L. donovani* with IC_{50} value of 0.6μM (Nour et al. 2009). *Sterculia villosa Roxb* (SVE) was found with reversal manner of dose dependent activity against *Leishmania* when experiments performed with 130 μg/mL concentration used for *L. donovani* promastigotes. The IC70 and IC_{50} values are 10 μg/mL and 17.5 μg/mL respectively. The IC_{50} values showed that increases ROS levels, DNA fragmentation, lipid peroxidation and superoxide and zero cytotoxicity against promastigotes (Das et al. 2017).

Two plant derived quinonemethide triterpenes were found to cause higher mortality in *L. amazonensis* and *L. chagasi* even in nanomolar concentration (Dos Santos et al. 2013). Moreover, phytocompound Maystenin also found to exhibit stronger activity against promastigotes and epimastigotes forms of *L. amazonensis* with IC_{50} of 0.09 and 0.47 nM, respectively and *L. chagasi* with IC_{50} of 0.46 and 0.25 nM, respectively. The plant compound aloe-emodin reported to effectively minimize population growth of *L. major* amastigotes in in-vitro condition. The in-vitro study of essential oil extracted from *Artemisia absinthium* found to significantly inhibit the growth of promastigotes and amastigotes form of *L. amazonensis* (Monzote et al. 2014). The chalcone flavokavain B as an active ingredient derived from leaves of *Piper rusbyi* exhibited promising Leishmanicidal compound (IC_{50} of 11.2μM) against promastigotes form of *L. donovani* (Flores et al. 2007).

Plant nectar composition also involves anti-Leishmania components which decrease growth of protozoan, and used in human infection treatments. When infected sand flies feed on these nectar plants, the infection of protozoans decreases automatically. By analysis existing data of nectar chemistry and *Leishmania* sensitivity, it has been concluded that, the nectar components reduced the growth of insect-stage *Leishmania* (Palmer-Young et al. 2022).

14.3.4 Malaria

Plasmodium parasite infection and associated diseases combine to form the complex disease known as malaria. A significant portion of mortality in children under the age of five and pregnant women are caused by organ-specific syndromes, which are driven by immune responses to infection and parasite-induced red blood cell perturbations. Acute renal failure, hypoglycemia, severe malaria anemia, cerebral

malaria and acute respiratory distress syndrome/acute lung injury, are a few of the notable illness manifestations that are a result of the multi-factorial pathogenesis.

Human malaria infections are caused by five primary Plasmodium strains from the Phylum Apicomplexa (Sporozoa), primarily P. falciparum, P. ovale, P. malariae, P. vivax, and a zoonotic parasite called P. knowlesi, with varying clinical consequences. Therefore, malaria clinical presentations of different intensities are determined by parasite sub-species, species, host genetics, and demography of the host at the location of infection intensities (Vandermosten et al. 2018).

As a result, the pathophysiology of malaria, or malaria illness (Mavondo et al. 2016) exhibits immunological peculiarities, inflammatory aberrations, and hemolysis that may result in severe malaria anaemia (SMA) (Buffet et al. 2011), acute kidney injury (AKI) (Khan et al. 2013), malaria cachexia leading to cardiac failure (Onwuamaegbu et al. 2004), hypoglycaemia (White et al. 1983) acute respiratory distress syndrome (ARDS) (Van den Steen et al. 2013), acute lung injury (ALI) (Mohan et al. 2008), cerebral malaria (Lin et al. 2017), hyperlactaemia with non-respiratory acidosis.

14.3.4.1 Plant-Derived Antimalarial Products

Phytochemicals like asiatic acid (AA), artemisinin, masilinic acid (MA) and oleanolic has been reported to possess both anti-malarial and anti-inflammatory activities that ultimately established their anti-disease action in malaria. A secondary plant metabolite called asiatic acid (AA) has inherent antioxidant and oxidative capabilities that may prevent the proliferation of parasites and host inflammasomes. AA is tightly bound to albumin, it may have a longer bioavailability, extending the duration of the parasite-drug interaction. Neurite elongation, impaired nerve regeneration, and selective apoptotic events on activated Th1 and macrophages Improvement of AA leads us to believe that the phytochemical has an impact on malaria.

It is well acknowledged that artemisinin exerts its antimalarial effects via (i) haem or iron in free state dissolving the peroxide bridge, which causing molecular structure of artemisinin to degrade and produce the nucleophilic radical metabolite with the C4 centre. (ii) afterwards, the free radical will attack macromolecules containing electrophilic groups or centres while serving as an alkylating agent, which will finally result in the parasite's death (Vennerstrom et al. 2004; Robert et al. 2005). AA demonstrates capacity as an Reno protective, anti-parasitic, anti-inflammatory, anti-disease, immunomodulatory, antioxidant and malarial disease elixir due to its selective enzymatic inhibition propensities, apoptotic influences, and improvement in malaria-induced systemic metabolic derangements.

In in-vitro study, phytochemical phenylated chalcones isolated from plant *Crotalaria anixensis* exhibited strong antimalarial activity against *Plasmodium falciparum*. Similar effect was observed with the plant *C. medicagenia* and *C. ramosissima* at three concentrations of 50, 10 and 2 μg/mL (Narender et al. 2005). Eleven biflavonoids including amentoflavone and hinokiflavone derivatives extracted from *Salaginella bryopteris* (Kunert et al. 2008) were found to show antiprotozoan activity in in-vitro condition. Antiprotozoan activity of methylamentoflavone against K1 strain of *P. falciparum* was found significantly

higher with an IC_{50} of 0.26μM. Moreover, chloroform extract of *Artemisia roxburghiana* also found effective against *P. falciparum* (Dua et al. 2011) with IC_{50} of 0.42μg/mL. In addition, aqueous and ethanol extracts derived from *Diospyros melanoxylon*, exhibited antiplasmodial activity (Saxena et al. 2011).

14.4 Mechanisms of Action of Plant Derived Compounds

Phytochemicals derived from different plants are known to exhibit potent antiprotozoan activity. These plant secondary metabolites include several compounds such as phenolics, Tannins, Flavonoids, Saponins, Alkaloids, Chalcones, Lignans, Terpenes and miscellaneous sources of plant secondary metabolites.

An oxygenated chalcone, Licochalcone A, extracted from the plant *liquorice* root was found to adversely affect the mitochondrial structure of protozoan parasite (Zhai et al. 1995) and inhibit dehydrogenases enzymes (Zhai et al. 1999) more specifically, fumarate reductase involved in parasite respiration (Chen et al. 2001). In addition, macrophages activation has also been proposed as an alternative pathway (Zhai et al. 1999). Flavonoid such as Luteolin and Quercetin reported to inhibit DNA synthesis of protozoan parasite by inhibiting the topoisomerase II mediated linearization of kDNA minicircles, collecting in arresting of growth of cell cycle (Mittra et al. 2000). Additionally, quercitin also chelate iron, leads to reduced concentration of the iron-dependent ribonucleotide reductase, a rate limiting enzyme for DNA synthesis (Sen and Majumder 2008). High concentration of flavonoid found in leaf extract of plant *Kalanchoe pinnata* possess antileishmanial activity, by enhancing reactive nitrogen generation intermediates (Gomes et al. 2010). An ethanolic extract of *Piper betle* caused mitochondria-mediated apoptosis in *Leishmania* (Sarkar et al. 2008).

Plant secondary metabolites such as Plumbagin isolated from *Pera benensis* caused free radicals generation in parasites as well as it also lead to topoisomerase II mediated DNA cleavage in mammalian cells imply its cytotoxicity towards host cells (Fujii et al. 1992). Alkaloid Berberine chloride extracted from *Berberis aristata* induced caspase-independent apoptosis-like cell death in promastigotes (Saha et al. 2009). Berberine chloride causes oxidative burst which is a part of apoptois in infected neutrophils and leads to reduction in parasite load but in case of infected macrophages, changes occur in regulatory enzymes, mitogen-activated protein kinases (MAPKs) and inflammation which is followed by p38 MAPK phosphorylation increment and ERK1/2 signal reduction and finally MAPKs signaling pathway targeted for Leishmaniasis (Saha et al. 2010).

Artemisinin and its related sesquiterpene lactones have been identified as endoperoxide bridge as the structural components which enhances free radical generation in the parasite bodies (Krishna et al. 2004); iron causes induced apoptosis in parasites as well as Dihydrobetulinic acid, a terpene compound leads to DNA topoisomerases targeting and protects cleavage of DNA, finally induces apoptosis in *L. donovani* (Alakurtti et al. 2010). oleanolic acid and ursolic acid extracted from

Pourouma guinensis also functions via macrohapges-induced phagocytic acitivity to inhibit parasite growth (Torres-Santos et al. 2004).

Momordica charantia derived aqueous extract (Momordicatin) reserved iron-containing parasite superoxide dismutase (SOD), causes no disturbance to host SOD (Gupta et al. 2010). SOD is a crucial enzyme for reducing oxidative stress, therefore its suppression would result in an increased production of free radicals, which would be harmful to the parasite, especially given that it is known to have a weak antioxidant system (Jaeger and Flohé 2006). By boosting the generation of ROS and NO, an EtOH extract and butanol fraction extracted from Tinospora sinensis caused macrophages to undergo an oxidative burst, which killed the parasite (Singh et al. 2008). Ascaridol (22%), the essential oil of Chenopodium ambrosiodes contains an endoperoxide that assisted in the generation of oxygen-centered radical intermediates that are attributed to its anti-parasitic activity. (Monzote et al. 2009). By inhibiting DNA topoisomerase I activity, the peganine hydrochloride dihydrate derived from Peganum harmala caused the death of parasitic cells in an apoptotic manner (Khaliq et al. 2009).

14.5 Conclusion

Protozoans are trepidation for the communities living in developing countries with improper sanitary conditions and adversely influence notable population of the world. The chemotherapeutic drugs used to manage protozoan diseases exhibit high toxicity related with adverse side effects upon longer use and also lead to drug resistance. Another way to control these protozoans could be done by eliminating their vectors using synthetic insecticides. However, this causes deleterious effects on the environment and also leads to drug resistance in protozoans. Therefore, the search for affordable, safe and efficacious drugs to fight NTDs is of supreme importance.

bioactive compounds obtained from plant parts play an immense diversified role that provides specific and myriad scope for discovering new drugs. Conventional and also, ethnomedicinal usage cumulatively including laboratory findings at earlier stages have illustrated the important role of plants in the treatment and prevention of protozoal diseases. Later work relates with explore of a variety of plant species for the isolation of more effective drugs, their targeting pathways, and in vivo studies for compounds and extracts with low IC_{50} and eco-friendly safer to humans is urgently required.

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Recent Approaches to Combat *Toxoplasma Gondii* with Plant-Derived Alternatives

15

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Abstract

Toxoplasmosis is considered as one of the widely distributed and predominant parasitic ailments. This Zoonotic infection caused by *Toxoplasma gondii* as an opportunistic protozoan pathogen affects both humans and animals to sustain their life cycle. The *T. gondii* is foodborne virulent strain that severely affects the immunocompromised individuals and pregnant women that may result in serious complications—abortion, stillbirth and multiple disorders that can be lethal if left untreated. Globally, one-third of populations are recurrently affected by this virulent infection. It is also a major health concern with pet dogs and cats. Therefore, pyrimethamine and sulfadiazine are used as gold-standard drug therapy to overcome toxoplasmosis. But there are still some adverse side effects and not effective in completely eradicating T. *gondii* bradyzoites and achyzoites. Therefore, there is an emergent need to employ medicinal plants to address this outcome. Traditional herbal plants have a versatile property to cure multiple parasitic disorders with negligible side effects. This chapter provides a glimpse of the biology of *Toxoplasma gondii* and their recent advancement in clinical effects of various alternative plant-derived products to treat Toxoplasmosis.

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_15

Keywords

Toxoplasmosis · *Toxoplasma gondii* · Zoonotic infection · Medicinal plants · Sulfadiazine · Pyrimethamine

Abbreviations

AgNPs	Silver nanoparticles
AIDs	Acquired immunodeficiency syndrome
BSO	Black seed oil
GE	Ginger extract
HFF	Human foreskin fibroblast
NPs	Nanoparticles
T. gondii	*Toxoplasma gondii*

15.1 Introduction

Toxoplasma gondii (T. gondii) is one of the world's most frequently reported intracellular obligatory protozoan parasite that affects major groups of homeotherms mammals such as birds, and humans and causes Toxoplasmosis. This broad distribution is dependent upon its lytic life cycle, which involves several opportunities for their transmission to animal hosts. It includes two main host such as definitive host (sexual) and all hosts (asexual) to complete its life span by two modes of reproduction (Al-Malki 2021). *T. gondii* consists of three major infectious forms—tachyzoites (in clusters or clones), bradyzoites (within tissue cysts) undergo asexual reproduction and the sporozoites (in oocysts) are sexually reproduced for their development (Mévélec et al. 2020). The Toxoplasmosis infection caused in humans, is usually spread orally by absorption of oocysts within water and soil, eating undercooked and infected meat or shell fish, intake of contaminated water with sporulated oocyst and via transplacental infection from mother-to-child transmission cause congenital toxoplasmosis which is an important concern to be considered from clinical point of view, because of the severe consequences may occurred in new born baby or foetus (Mévélec et al. 2020) (Sarman %J Tropical parasitology Singh 2016). But, accidental exposure or ingestion of *Toxoplasma* via blood products or in laboratories that works on this parasite is quite rare (E, 2021). In fact, according to the Centre for Disease Control (CDC) involves *T. gondii* as a virulent pathogen, along with *Listeria* and *Salmonella*, as accountable for 70% cases of foodborne mortality in U. S. (Bintsis 2017).

However, *T. gondii* considered as the most opportunistic parasite that infected more than 60 million of the human population and most of them are in asymptomatic phase (Al-Malki 2021). The prevalence of infection rates is reported to be higher in continental Europe and central and South America (60–80%) (Galeh et al. 2020).

T. gondii includes two clinical stages in intermediate host i.e., acute phase, the tachyzoite multiplies rapidly and spread all over the body and while in chronic phase, the bradyzoites forms a tissue cyst, predominately in the neural tissue and skeletal muscle, which persist dormant within the host lifespan (E, 2021). In Latin America, it has been studied that virulent strain of *T. gondii* caused toxoplasmosis, most commonly occurred in immunocompetent individuals and resulted in disseminated aliment, acute pneumonia and even death (Montoya and Contopoulos-Ioannidis 2021). During acute phase, the toxoplasmosis infection is generally mild in immunocompromised patients and directs asymptomatic disease in tissues includes skeletal and encysts in vascular muscles, parenchyma, retina and neural tissues that eventually caused life-threatening brain damage and loss of visual insight, if adequate treatment is not administrated on time (Ildiko Rita Dunay et al. 2018). In order to measure the accurate physical or commercial burden of the toxoplasmosis infection is still not be possible due to the inadequate information existing on the overall prevalence and their peculiar signs.

Currently, the recommended gold-standard drugs against toxoplasmosis cause serious inflammation side effects and are usually expensive for least developed areas. Such drugs are teratogenic in nature and thus could not be prescribed during early pregnancy. Together this factor responsible for the emergence of drug-resistant strain of *T. gondii* will initiate the urgent need to be addressed the demand for novel anti-parasitic therapies (Wei et al. 2015). Consequently, there is no current therapy approved for the treatment of encysted bradyzoite form (Cerutti et al. 2020). Therefore, intensive research should be focused to developed innovative drugs to cure both acute and chronic stages of *T. gondii* parasite.

Plant-derived compound have been extensively investigated for mankind to cure wide variety of diseases due to its pharmacological properties to effectively combat the *T. gondii* infection. The various medicinal herbs, such as *Artemisia annua, Zingiber officinale, Sophora flavescens Aiton* and etc., have been exploited and reported to be applicable against RH stain of *T. gondii* (Al Nasr et al. 2016*).* This chapter, deliberately highlights the recent status and occurrence of toxoplasmosis and also demonstrated the current therapeutics application of herbicidal plants and their underlying mechanisms to combat Toxoplasmosis.

15.2 Stages of *T. Gondii* Life Cycle

The lifespan of *T. gondii* based upon two mode of reproduction includes asexual and sexual. There are three altered infective stages such as aggressive tachyzoite, bradyzoite and the sporozoite as discussed above in introduction section (Al-Malki 2021). The sporozoite form of life is safeguard in the oocyst and also known as environmental stage that takes 1–5 days to sporulate in nature and become ineffective. It characterized as almost 2 m wide and 5 m length, crescent-shaped cells with pointed anterior end and rounded at posterior end. The cytoskeleton structures govern the mobility and structural integrity of sporozoite (Al-Malki 2021). Intermediate host in ecosystem includes birds and rodents that become infected due to

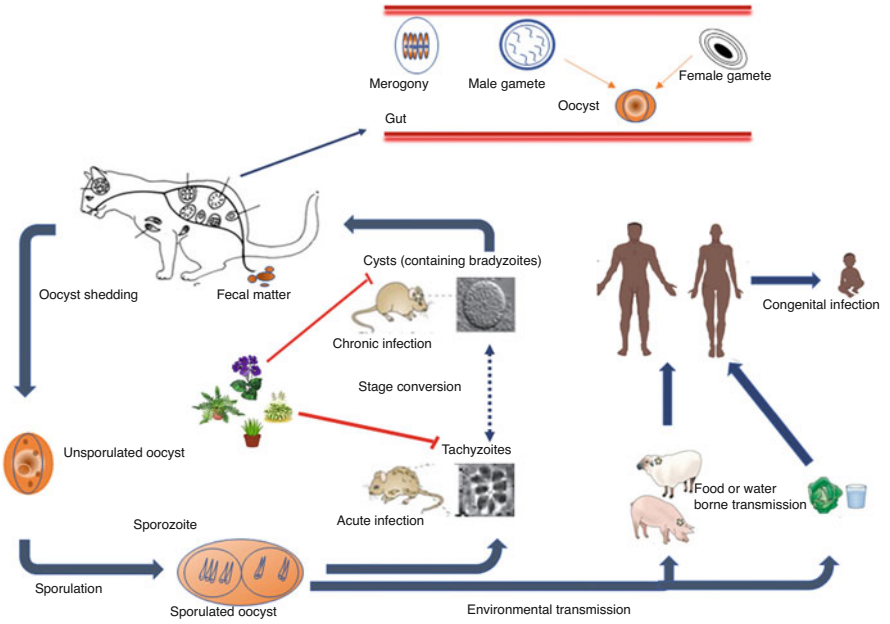

Fig. 15.1 Diagrammatic illustration of infective stages of *T. gondii* life cycle in their respective hosts

ingestion of oocysts contaminated soil, water or plant materials. These oocysts convert into tachyzoites just after absorption (E, 2021). Tachyzoites is highly energetic phase of parasitic life, and can efficiently invade and infect in various orders of vertebrate. These tachyzoites confine to neural and muscle region and mature to tissue cyst bradyzoites. Bradyzoites cysts mature into less spheroid in neural cells and elongated in muscular cells. It is well adapted to sustain for long time in intracellular region. The animal (non-primates) become infected by ingesting the tissue cyst of intermediate hosts or by directly consume sporulated oocysts in the environment (Attias et al. 2020). While in human host, the *T. gondii* form tissue cysts and localized to brain, eyes, skeletal muscle. *T. gondii* has commonly found in pork, undercooked meat, lamb and cat faecal matter. The mode of transmission patterns for the existence of *T. gondii*, may vary corresponding to the physical character and structure of both population of intermediate and definitive host (Fig. 15.1) (Cerutti et al. 2020).

15.3 Clinical Manifestation of Toxoplasmosis

In the majority of healthy persons, the toxoplasmosis is usually symptomless because of the strong immune system. But the symptoms may be prone as a serious and lethal in immunosuppressed patients, include as those suffered from AIDs, organ

Table 15.1 Diseases and clinical symptoms linked with Toxoplasmosis

S. no.	Disease/clinical signs	Ref.
1.	Congenital toxoplasmosis (chorioretinitis, encephalitis, neonatal mortality))	Lindsay and Dubey (2011)
2.	Granulomatous liver disease, liver cirrhosis, impede liver function	Alvarado-Esquivel et al. (2011)
3.	Hashimoto's thyroiditis	Kaňková et al. (2014)
4.	Graves' disease; thyroid adenoma	Cosme Alvarado-Esquivel et al. (2019)
5.	Hearing loss	Salviz et al. (2013)
6.	Crohn's disease	Wu et al. (2021)
7.	Ocular toxoplasmosis (uveitis, retinochorioiditis, blurred vision, floaters, retinitis pigmentosa, retinal necrosis, blindness, macular scars, contract visual acuity, strabismus, neuroretinitis, nystagmus, retinal detachment, congenital cataract, vitritis, vasculitis, atrophic optic papilla, papillitis, scleritis)	Park and Nam (2013)
8.	Schönlein-Henoch purpura	Wang et al. (2020)
9.	Diabetes mellitus type 1 and 2	Prandota 2013, Anand et al. (2015)
10.	Celiac disease	Rostami Nejad et al. (2011)

transplants and cancer. Infected individuals may suspect fever or cervical lymphadenopathy, even so often related to myalgia, asthenia, or other non-specific signs (Daher et al. 2021). The clinical symptoms may continue for some months, mimicking mononucleosis infection that can be spotted on blood smears (Weiss and Dubey 2009). In addition, both immunocompromised and component individuals can also lead to retinochoroiditis (ocular toxoplasmosis). In immunocompetent patients, the acute disease may cause visual loss and blindness. For example, in Australia, the clinical effects of toxoplasmosis reported that 50% of human population suffered from *T. gondii,* which result in approximately 0.67% or 1 per 149 persons (Ferreira et al. 2022). Primary infection of *T. gondii* in pregnancy has been associated with, spontaneous abortion, premature birth. A new born foetus exposed to *T. gondii* infection during pregnancy via transplacental transmission leads to high risk of congenital abnormalities (Goldstein et al. 2008).

Emerging evidence suggested that there are various symptomatic condition and the development of clinical symptoms is linked with *T. gondii* disease as represented in Table 15.1.

Some of the clinical signs of toxoplasmosis is caused by the intense communication of virulent strain with almost 3000 host genes and proteins probably due to the antigen homology of host/pathogen that results in disrupting/generating and activating host-specific signalling pathways that are eventually responsible for the evolution of endophenotypes of various ailments (Carter 2013).

Most intracellular parasites, concomitant viral and different bacterial infections enter to host cells and avoid them to be killed by reactive species by scavenge the

essential elements for their survival and in return offer the other composites to the host that result in unwanted outcomes. Moreover, chronic *T. gondii* infection produced autoantibodies show an impressive effect in the host infection caused by various parasite (Carter 2013).

15.4 Treatment for Toxoplasmosis

A standard drug for the prophylaxis of toxoplasmosis infection should exhibit the parasiticidal activity against both acute and chronic phases of the parasitic life span by effectively penetrating into the cyst and assembling to placental barrier to deliver it to the targeted site of foetal infection to prevent foetal lethality and teratogenicity. Currently, existing therapies do not offer these adequate criteria (Gallant 2015). Drugs such as Pyrimethamine (dihydrofolate reductase inhibitors) and Sulfadiazine used as ideal anti-parasitical therapy against toxoplasmosis but it is specifically favorable for actively dividing parasitise during acute phase of infection (Rajapakse et al. 2013). Therefore, almost drugs effective in killing tachyzoite stage but not for cyst forming bradyzoites (Tedford and McConkey 2017). While in chronic stage, various drugs are used such as atovaquone, diaminodiphinylsulfone, clindamycine, and spiramicyne, however, these drugs are limited due to low efficacy, develop resistance variants strains and their serious consequences on the foetus during pregnancy (Montazeri et al. 2018). In addition, sulfadiazine shows low anti-parasitic properties if it is employed without pyrimethamine. The combination of Sulfadiazine and pyrimethamine were also increase the chances of kidney failure, allergic reactions, and other forms of liver/kidney complications. Pyrimethamine is a folic acid agonist and is associated with adverse events (AEs) that are majorly responsible for the repression of bone marrow functioning and eventually results into neutropenia. Therefore, this current treatment cannot be applied for managing the congenital toxoplasmosis and early fetal development due to depletion of folate results into serious consequences (Alday and Doggett 2017). Hence, some antibiotics drugs include, fluoroquinolone, epiroprim (DHFR inhibitor) and atovaquone are successful in combating *T. gondii* infection as confirmed by in vitro and in vivo studies, but its not be recommended during pregnancy because of the safety point of view (I. R. Dunay et al. 2018). Therefore, an urgent need to develop effective treatment based on the drug safety, drug potency and duration of therapy to prevent Toxoplasmosis.

15.5 Anti-Parasitic Medicinal Plants: History and Success

Poor anti-parasitic control is a major global concern. From ancient times there is the trend for ancient traditional medicinal system because of easy availability of herbal plants. India and bordering nations such as China, Japan, Pakistan, Sri-Lanka and Thailand all follow this medicinal system. China alone has a consumption of approximately 40% of total herbal medicinal, such herbal medicines are provided

as nutritional products in the form of extracts from herbal plants, vitamins, phytochemicals and minerals for prophylactic and therapeutic management against chronic toxoplasmosis. As this approach can be employed as anti-cyst nutritional products to affect with the tachyzoite stage. Traditional medicine plant *Cinchona officinalis* belong to family of Rubiaceae and was first drug to be used for treatment of malaria in Central and South America. Cinchona officinalis and Artemisia annua L. belong to family Asteracea along with other plant atovaquone of family Bignoniaceae are well known against parasitic infection (Efferth et al. 2011). Further Curcuma and other similar species of Zingiberaceae are known for their targeted action of mechanism against Trypanosoma (Haddad et al. 2011; Hoet et al. 2004). Baccharis retusa and Kalanchoe pinnata belong to Asteraceae and Crassulaceae were screened to exhibit anti- leishmanial activity (Hoet et al. 2004). Plant species Streblus asper from Moraceae is considered effective for anti-filarial activity validated through in vitro and in vivo (Raoof and Mohamed 2020). Ascaridole is herbal extract obtained from Chenopodium ambrosioides of family Amaranthaceae was potentially known for its enhance therapeutic effect against hookworm-related infection (Monzote et al. 2018). Antiparasitic potential of Ginger root extract was also considered effective from in-vitro and in-vivo studies performed against T. gondii where inactive expression of apoptotic protein and inhibition of inflammatory cytokine reduces the infection rate (Choi et al. 2013). The anti-toxoplasmic action of spiramycin and Myrrh was determined using RH strain of T. gondii (Al-Zanbagi 2007). Nutmeg (Myristica fragrans Houttuyn), seed kernels with essential oils obtained from it show anti- T. gondii activity and reduced cytotoxic to nearby normal cells. Such activity of nutmeg can be an attribute of presence of different active compounds present in it such as myristicin, limonene, eugenol and terpinen-4-ol etc.(Pillai et al. 2012). *Nigella sativa* common name is black seed or black cumin, also has anti-inflammatory, immuno-potentiating effects along with anti-helminthic and anti-protozoal activities (Hanan and Heba 2011). Azadirachta indica commonly known as neem, Melia azedarach popular as cinnamon, are also effective herbal medicine, their leaves stem bark etc. exhibit immunomodulatory, anti-inflammatory, nematocidal, antiparasitic and antioxidant properties due to presence of over 50 different bioactive compounds such as terpenoids and many others that interfere with intracellular development of T. gondii (Tauheed et al. 2022).

Phytochemicals alone are known for its broad-spectrum secondary metabolites with activity similar to anti-parasitic action (Anchal Singh et al. 2020; Abdullahi et al. 2020). Phytochemical belonging to different groups such as flavonoids, alkaloids, phenols, glycosides etc. derived from plant can perform the antiparasitic action too. Berberine along with its derivatives are known for their anti-parasitic action as they inhibit growth and maturation of various parasitic strains such as *Trypanosoma* and *Plasmodium*. Thus, it can be inferred to have an inhibitory effect against *T. gondii cysts (*Krivogorsky et al. 2012*)*. Mangiferin is glucosylxanthone, another bioactive compound is also of research interest because of its anti-parasitic potential (Yadav et al. 2022). Resveratrol is polyphenol with antioxidant and anti-parasitic properties. Resveratrol is known for downmodulating *Toxoplasma gondii* infection by targeting HMGB1/TLR4/NF-κB signaling pathway (Lu et al. 2021).

15.6 Medicinal Plants and Toxoplasma

The use of drugs against Toxoplasma parasite in long run leads to development of resistance in parasite and it has been developed resistant to a varied variety of inhibitors (Pfefferkorn and Borotz 1994). Besides, the less access to chemical drugs owing to their high cost, the usage of native medicinal herbs for the treatment of toxoplasmosis is increasing particularly in poor countries (Sharif et al. 2016). These native herbs are the foundation of innovative medicines that may advance therapeutic excellence, enhance the standard antimicrobial results, reduce side-effects of drugs and mainly lessen the prices. For the moment, the pharmacological manufacturing units are eyeing for substitute drugs for remedial of parasitic infections or to augment the natural medicinal plants-based drugs against parasitic infections. Recently, several medicinal plants have been explored to identify their parasiticidal activities (Benoit-Vical et al. 2000). The persistent attempts of utilizing existing medicinal herbs, exploring new medicinal product herbs and therefore manufacture and assessment of the anti-toxoplasma outcomes of these natural drugs could be a comprehensive substitute for the traditional chemical drugs (Fig. 15.2).

15.6.1 *Ginkgo biloba*

In the conventional Chinese medicine *Ginkgo biloba sarcotesta* has been exploited for many years ago. Ginkcolic acid (GA) extracted from it possess antitumor and antimicrobial properties (Sheng-Xia Chen et al. 2008). In a study, mice infected with *T. gondii* showed decreased renal impairment after treatment with fresh onion juice

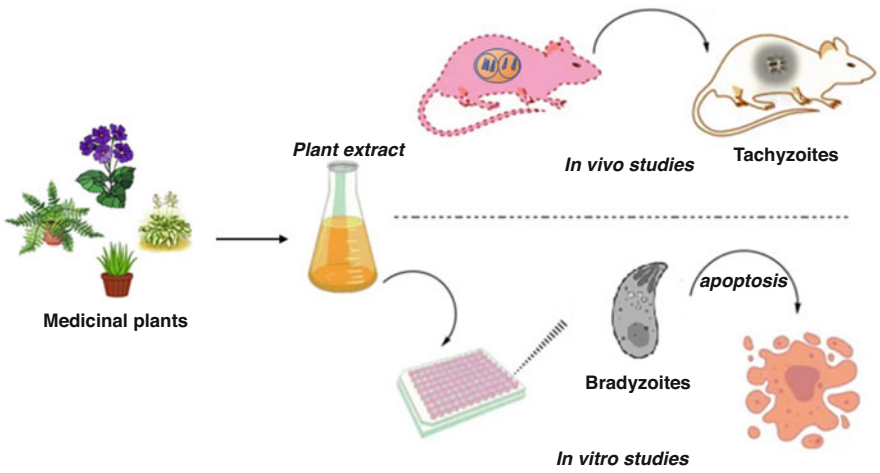

Fig. 15.2 Diagrammatic illustration of various herbicidal plants and their possible specific inhibitory act against *Toxoplasma gondii*

(strong antioxidant) (Gharadaghi et al. 2012). Chen et al. showed the anti-toxoplasmosis properties GA in in-vitro studies. Human foreskin fibroblast (HFF) was affected with toxoplasma and then treated to GA for 48 hr., no trophozoite were viewed in HFF. Moreover, the electron microscopy results showed no change in cell morphology or damage of HFF cells due to multiplication of parasite (S. X. Chen et al. 2008).

15.6.2 *Zingiber officinale Roscoe*

Zingiber officinale Roscoe commonly known as ginger is used in Asian countries for the purpose of cooking and for the cure of digestive problems, headaches, common cold, diarrhea and cough. The anti-bacterial, anti-inflammatory and antioxidant activities of *Zingiber officinale* have already been established (Choi et al. 2013). Choi et al. showed the significance of ginger derivative fraction GE/F1 and ginger extract (GE) against *T. gondii* both in-vitro and in-vivo. In C6 cells, GE/F1 greatly hampered the multiplication of T. gondii and also decreased the inflammatory cytokines in-vivo (Choi et al. 2013).

15.6.3 *Panax ginseng*

Ginseng isolated from *Panax ginseng* has been utilized as a conventional drug for a long time now. This is supposed that ginsenosides (Ginseng saponins) are one of the lively fragments of the root. Ginsenosides are reported to enhance antibodies response against bacterial and viral antigens. Qu et al. demonstrated that recombinant ROP-18 proteins together with ginsenoside augmented the generation of inflammatory cytokine (IFN-γ) and IgG antibodies (IgG1 and IgG2a) in mice. The results showed that the ginsenoside with recombinant ROP 18 protein triggered a vigorous humoral as well as cellular immune response, while the mortality of this group of mice was lesser compared to other groups (Daofeng Qu et al. 2013). Qu showed that the immunization of mice with ginsenoside Rg1 along with recombinant surface antigens of *T. gondii* (rSAG1) with 100 μg/ml and 50 μg/ml concentration leads to an extended survival rate. Moreover, rSAG1/Rg1 treated groups with 100 μg/ml concentration displayed greater cytokine level of IFN- γ (473.0 ± 11) compared to other groups that owing to the capability of ginsenoside Rg1 as a favorable vaccine for toxoplasmosis (Dao-Feng Qu et al. 2011).

15.6.4 *Sorghum bicolor*

Sorghum bicolor is the fifth utmost significant cereal globally and habitually cultivates in tropical areas like South America, Africa and Asia. A study revealed that the prolific isolates of 3-deoxyonthocyanidins from red leaves of *S. bicolor* has an inhibitory effect on the proliferation of T. gondii in-vitro (Abugri et al. 2016). In

another investigation, it is exhibited that crude isolates (oil-like) and portions have effectiveness against the tachyzoite stage of *T. gondii* and the results also revealed great anti-parasitic activities in the isolates with little cytotoxic outcomes (Abugri et al. 2019).

15.6.5 *Cinnamomum zeylanicum*

The leaves of *Cinnamomum zeylanicum* are known to possess anti-microbial, anti-viral, antioxidant, anti-inflammatory, anti-carcinogenic, anti-parasitic and anti-hyperglycemia effects (Melo et al. 2011). A previous study exhibited that treatment of *T. gondii*-infected cells with aqueous isolates of cinnamon and neuem for 24 hr. resulted in significant decrease in number of intracellular tachyzoites and percentage of infected cells. In parasite infected cells, treatment with aqueous isolates of cinnamon (1 mg/ml) and neem (5 mg/ml) lessened the infection by 70% and 85% respectively without no toxicity to the cells (Melo et al. 2011).

15.6.6 *Myrtus communis* and *Artemisia aucheri Boiss*

Myrtus communis is a plant from Myrtaceae family and is utilized for the treatment of hemorrhoids, vaginal discharge, urinary tract infections, dry cough, bronchial congestion, gum infections, ulcers, digestive problems, sinusitis and acne. *Artemisia aucheri Boiss* is a species of the Asteraceae family used as an anti-parasitic, anti-microbial, antiseptic and soothing agent in conventional medicine (Javadi et al. 2017). The study revealed the parasite excretion effects of extract from both the plants, however, EC50 and the qualities of these extracts were inferior compared to pyrimethamine. Therefore, they could not be an effective substitute for anti-toxoplasmosis drugs (Javadi et al. 2017).

15.6.7 *Nigella sativa*

Nigella sativa also known as black seed belongs to Ranunculaceae family possess anti-microbial anti-parasitic, disinfectant and anti-inflammatory properties that are appropriately recognized. Along with this, the anti-coccidial properties of *N. sativa* are also stated. It activates the immune system, boosts the cellular immune reaction and upsurges the interleukin secretion (Hanan and Heba 2011). Rayan et al. studied the role of black seed oil (BSO) against infection of Swiss albino mice with Me49 strain of the *T. gondii*. The results revealed a decreased amount of brain cysts and improved survival time of BSO treated infected mice than control mice. However, a slight encephalitis and meningitis might be realized in BSO treated mice (Hanan and Heba 2011).

15.6.8 *Securinega suffruticosa*

Securinega suffruticosa is combined with various other herbs in conventional Chinese medicine and utilized for the management of several ailments such as inflammation and rheumatism resulted from infective illnesses. Holmes et al. revealed the effects of securinine alkoid isolated from *S. suffruticosa* against *T. gondii*. This study showed the greater anti-toxoplasmosis effects of Securinine compared to pyrimetamine, and also inhibits transformation of tachyzoites into bradyzoites form (Holmes et al. 2011).

15.6.9 *Sophora flavescens*

This plant is used as antitumor, anti-viral and anti-bacterial drug in conventional Chinese medicine. The pharmacokinetics of *S. flavescens* is associated with its alkoids like matrime and oxymatrin, both alkoids possess anti-diabetic, anti-tumor and antioxidant activites (Zhang et al. 2016). Studies on toxoplasma infected mice shows that treatment with alkoids causes reduction of tachzyite, as well as recovers enzymes like MDA, GSH, AST and ALT leading to toxoplasmosis control (Zhang et al. 2016).

15.6.10 *Eurycoma longifolia*

Eurycoma longifolia belongs to Simaroubaceae family and called as Pasakbumi or Tongkat Ali in native areas. This plant is obtained in Asian nations like Philippines, India, Malaysia, China, Thailand and is regularly exploited for the management of fever, swollen lymph and nausea in conventional medicines. The cytotoxic activity of *E. longifolia* has already been confirmed against malaria (Kavitha et al. 2012). Kavitha et al. extracted the crude isolates and portions (TAF 191, TAF 273, TAF 401 and TAF 355) from *E. longifolia* and assessed their role against *T. gondii*. Their results showed that treatment of toxoplasma infected mice with portions TAF 401 (EC_{50} of 0.882 μg/ml) and TAF 355 (EC_{50} of 0.369 μg/ml) resulted in reduction of intracellular tachzyoites form and their multiplication thus, damaging the infected cells (Kavitha et al. 2012).

15.6.11 *Piper betle*

Piperaceae family have 1000 species of plants present in tropical areas of Africa, Southeast Asia and India. Round 40 species of this family have also been found in Thailand. *P. betle* is used as active compounds in conventional Thai medicine for overall health stability, uptake of nutrient, digestion and reduction of stress. The pharmacological functions of these plants include anticancer, lipid protection, anti-bacterial and antioxidant. Moreover, studies have revealed the anti-malarial anti-

leishmaniasis properties of this plant (Leesombun et al. 2016). Another study demonstrated that *P. betle* isolate has great efficacy against *T. gondii* multiplication in HFF cells. In-vivo studies on mice infected with PLK strain (1000 tachyzoites) of *T. gondii* showed 100% survival of mice after treatment with *P. betle* isolates (400 mg/kg) for 7 days (Leesombun et al. 2016).

15.6.12 *Licopodium clavatum*

Lycopodium clavatum is a dry moss that belongs to Lycopodiaceae family and also called as Wolf Paw Clubmoss. Geologically, this plant can propagate in non-fertile soils and is extensively dispersed in the northern hemisphere. In remedy, isolates of *L. clavatum* are utilized in homeopathy and for the management of acute ailments (children's autism) and chronic ailments (Alzheimer's disease) (Pereira et al. 2020). In a study, mice infected with ME49 strain of *T. gondii* were used to evaluate the different outcomes of *L. clavatum*. The G72 group mice were injected with 200dH of *L. clavatum* for 3 successive days before administration of parasitic strain. Another G48 group of mice were infected with parasite first and 200dH of *L. clavatum* was administered on 2, 4 and 6 days after infection. The sores grow graver in genital region and tachyzoites to bradyzoites conversion, brain cyst formation was greater in G72 group as compared to G48 group, also (Pereira et al. 2020).

15.6.13 *Sambucus nigra*

In conventional medicine *Sambucus nigra* is utilized for the management of common cold, rheumatism, burns¸ hemorrhoids and wound injuries. Additionally, the anti-protozoal, anti-diabetic, anti-viral and antioxidant therapeutic properties of this plant have been well noted (Daryani et al. 2015). A study demonstrated the methanol isolates from the leaves and fruits of *S. nigra* at 5 mg/ml and 10 mg/ml concentrations after 60 min and 120 min cause 100% excretion of tachyzoites of *T. gondii*. Moreover, 25 mg/ml and 50 mg/ml concentrations of fruit isolate from *S. nigra* caused 100% killing of tachyzoites. Therefore, fruit isolate from *S. nigra* exhibited a superior parasitic excretion outcome as compared to leaf isolate (Daryani et al. 2015).

15.6.14 *Centaurea lydia* and *Phlomis nissolii*

Centaurea lydia is a member of Asteraceae family generally located in Western and Mediterranean Asia and possesses anti-inflammatory, anti-microbial and antioxidant effects. The compound sesquiterpene derived from *C. lydia* specie has antitumor and cytotoxic effects. *Phlomis nissolii* is a Labiatae family member supplemented with flavonoid and has analgesic, anti-inflammatory and anti-diabetic properties in in-vivo studies, while anticancer, anti-fungal and anti-bacterial properties in

in-vitro studies (ZÖ et al. 2017). The potential of both *C. lydia* and *P. nissolii* against *T. gondii*. *C. lydia* treated mice group caused 85% reduction in tachyzoites and also enhanced the survival period to 7 days than the control group and *P. nissolii* treated mice group. Therefore, the *C. lydia* isolate is a suitable medication for the management of toxoplasmosis (ZÖ et al. 2017).

15.6.15 *Thymus broussonetii Boiss*

Thymus broussonetii Boiss is a type of herb utilized for cooking as well as medicines. This plant is known to have antioxidant, anti-spasmodic, anti-inflammatory and antimicrobial effects. *T. broussontii* one of the species of Thymus genus is exploited for the remedial of fever and diarrhea (Dahbi et al. 2010). Earlier facts have shown that treatment of mice with essential oil extracted from *T. broussonetii Boiss* leads to inhibition of the formation of cystic forms of *T. gondii*. The results revealed that mice firstly infected with cystic form of *T. gondii* does not show any cystic form in brain days after administration of *T. hymus broussonetii* essential oil, that can be due to preventive potential of essential oil in the killing of rigid cysts and discharge of bradyzoites in mice intestine (Dahbi et al. 2010).

15.6.16 *Artemisia annua*

Artemisin is native herb extracted from *Artemisia annua* and used in conventional Chinese medicine. This plant is frequently utilized for the treatment of Plasmodium infection without any cytotoxicity. Studies has already verified the anti-parasitic effects of artesunate against *Trypanosoma cruzi* and Schistosoma (Mishina et al. 2007; Xiao et al. 2000). Another in-vitro and in-vivo study evaluated the excretory potential of artesunate against ME49 and RH strains of *T. gondii*. The survival rate for tachyzoites of RH strain at 0.1 μg/ml of artesunate concentration after 24 and 48 hr. was 41.67% and 16.67% respectively with a substantial variance. The apoptotic killing of tachyzoites was seen with changes in membrane, vacuolization of cytoplasm and wrinkling. The in-vivo study on Swiss albino mice infected with ME49 strains shows decreased quantity of cyst in brain after artesunate treatment (Mahmoud et al. 2016).

15.6.17 *Myristica fragrans Houtt*

Myristica fragrans Houtt also known as Nutmeg, seeds of this plant have been utilized for the remedial treatment of asthma, anti-inflammation, atherosclerosis, rheumatism, abdominal bloating and diarrhea (Pillai et al. 2012). Pillai et al. isolated the essential oils from nutmeg and assessed the anti-toxoplasmosis activities in-vitro, where the EC_{50} of 24.45 μg/ml showed anti-parasitic results that may be due to presence of composites of limonene and myristicin in the essential oil (Table 15.2) (Pillai et al. 2012).

Table 15.2 List of various types of medicinal plants used to combat toxoplasmosis

Medicinal plant	Bioactive component	Animal model	T. gondii stage	Treatment regimes	Observation after treatment	Toxicology	Ref
Curcuma longa (turmeric)	Curcumin (nanoemulsion and pure compund) Curcumin (native compound and nanocomposite)	BALB/c Swiss Albino rats	Tehran cysts ME49 cysts	100 mg/kg BW/day for 30 days, after 4 h post-infection 10 days, after 60 days post-infection	Reduced number and size of brain cysts: Nancemulsion is more effective than native compound Downregulation of BAG1 expression Reduction in brain cysts by 60%: UiO-66-NH2 nanocomposite is the most effective	Oral toxicity studies found no clinical symptoms in infected mice administered with nanoemulsion N.A.	Azami et al. (2018) Anand et al. (2015)
Nigella sativa (black cumin)	Black seed oil	Swiss albino mice	ME49 cysts	Polyphylatic ("P"): 5 mg/kg BW/day for 14 days followed by infection. Therapeutic ("T"): 400 mg/kg BW/day of each extract for 14 days, after 4 days post-infection	Higher survival rate: 100% for "P"; 86.7% for T Reduced brain cysts load Lessened meningitis, encephalitis "P" was more effective than "T" regimen	No mortality or clinically significant toxicity in the form of decreased activity, piloerection, lethargy or weight loss were observed in uninfected mice administered with black seed oil.	Hanan and Heba (2011)
Rosmarinus officinallis (rosemary)	Alcoholic and oil extract from leaves	Swiss albino mice	ME49 cysts	Prophylactic ("P"): 400 mg/kg BW/day of each Extract for 7 days followed by infection Therapeutic ("T"):	Reduced brain cyst burden, BAG1 expression, and cyst viability: Cysts isolated from "P" and "T" groups displayed mutilation in the	No significant toxicity observed	Hamed et al. (2021)

				400 mg/kg BW/day of each Extract for 14 days, on the second week post-infection	surface membrane and were less infective – Lessened histopathological insults in the brain – "T" was more effective than "P"		
Thymus vulgaris	Ethanolic extract from leaves Ethanolic extract from aerial parts	Swiss albino mice Swiss albino mice	ME49 cysts ME49 cysts	Prophylactic ("P"): 500 mg/kg BW/day for 5 days followed by infection Therapeutic ("T"): 500 mg/kg BW/day for 10 days, after 42 days post-infection 400 mg/kg BW/day for 14 days, after 4 days post-infection	Smaller brain cysts with irregular cyst wall, and reduction in cyst count in "P" (24%) and "T" (46%) groups – Amelioration of neuroinflammation and neuronal necrosis Reduction in brain cysts by _47.5% and lessened inflammatory brain lesions	N.A. N.A.	FARAG et al. (2019), Ferreira et al. (2022), Eraky et al. (2016)
Fruits, vegetables, and grains	Quercetin	In vitro human fibroblast cells	ME49 tachyzoites followed by in vitro differentiation		Reduced bradyzoite antigen-positive *T. gondii* vacuoles.	N.A.	Weiss et al. (1998)
Thymus broussonetii Boiss	Oil extract from whole plant	OF1 mice	PRU cysts	20 µg at the point of infection, or 30 min, 5, or 6 days post-infection	Absence of intracerebral cysts.	20 µg of essential oil did not show toxicity in mice	Weiss et al. (1998)

15.7 Conclusion

One of the promising relevance of various medicinal herbs present around the world with anti-*Toxoplasma* properties includes inhibition of re-activation of toxoplasma in immune-compromised individuals and combating hereditary toxoplasmosis. The plant isolates not only decreased the multiplication of parasites but also enhanced the laboratory life span of animals. Presently, chemicals drug used for their anti-toxoplasma effect have various side effects that limit their application. Medicinal herbs against *T. gondii* act as a good source for novel herbal drug production that will possibly reduce the cost and enhance the therapeutic quality with fever off-effects. Constant attempts are required to utilize the existing medicinal herbs with anti-toxoplasma properties and hunt for fresh medicinal herbs. All the medicinal herbs discussed above can lessen or even eradicate the clinical symptoms, whereas in some cases they are not able to eradicate the parasite from the infected host. Though, more investigations are required to detect the mechanism of efficient medicinal plants and their isolates. This involves clinical trials to examine the safety and worth of these herbs for the management of toxoplasma infection in immune-compromised people and widespread exploration of various plant composites that are competent for the elimination of parasites and have the slightest difficulties and cytotoxicity for humans. Further, novel outcomes not only aid the anti-parasitic potential of NPs but additionally reinforce the projections of exploring NPs as an encouraging alternative as anti-parasitic mediators.

15.8 Future Perspectives

Insufficient knowledge related to dosage information of plant isolates limits their use against parasitic infection. A promising alternative strategy that parasitologists have exploited to overcome the prevalence of toxoplasmosis is by using nanoparticles (NPs) to circumvent the adverse effects of anti-parasitic drugs. Anand et al. (Anand et al. 2015) fabricated the encapsulated bovine lactoferrin protein nano-capsules used to kill the *T. gondii* or arrest the growth by elevated ROS level. Biosynthesis of metallic NPs, including biogenic silver NPs.

(AgNp-Bio), have been reported based on their therapeutic activity against parasitic infections (Fanti et al. 2018; OS Adeyemi et al. 2017), that include toxoplasmosis (Machado et al. 2020). Recent reports of inhibition of the proliferation of *T. gondii* in infected human cells of trophoblastic origin have been suggested (Machado et al. 2020). A survey of the literature indicated that silver nanoparticles when used alone or in combination with chitosan have exhibited promising anti-toxoplasma efficacy. The experimental animals that were treated with these NPs showed a significant reduction in the mean number of the parasitic count in the spleen and liver, in comparison to the controls (Jameii et al. 2015). Further, intracellular growth of tachyzoites of *T. gondii* have been reported to be suppresses in in-vitro by AgNPs (Oluyomi Stephen Adeyemi et al. 2018). Furthermore, earlier reports demonstrated that AgNp-Bio decreased e infection, adhesion and

proliferation of *T. gondii* in HeLa cells (Machado et al. 2020). AgNp-Bio have proved to be promising anti-parasitic agents against *T. gondii*, as they can activate the macrophages to combat the pathogens (Park and Hunter 2020). Nevertheless, the impact of AgNp-Bio usage on the innate immune cells infected with *T. gondii* are not known.

Presently, there are no approved vaccines for humans or for most of the livestocks against the parasite. Nanovaccines offers state-of-the-art approaches to overcome the unresolved challenges that are still of major concern due to the homogenous antigen variability. *T. gondii* has adaptive escape mechanisms that evade the host immune surveillance. With the occurrence of neo-antigens, novel adjuvants and therapeutics, vaccines against toxoplasmosis have evolved considerably (Warner et al. 2021). The literature survey reports the evaluation of the immune surveillance triggered by DNA vaccines that encode either a single or multicomponent antigen against toxoplasmosis in mice model. DNA vaccines, which may be just a kind of subunit vaccine as it uses small segments of the pathogen's DNA to elicit an immune response and produce immunity, has revealed variable degrees of efficacy in experiments against infection caused by *T. gondii*. Nanovaccines can lead to advent in medical and pharmaceutical industry designing and fabricating superior nanodrug delivery system for management and control of infectious diseases. Still, there is a need of comprehensive research that focuses on the NPs action for advances in therapeutic approaches.

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Target-Based Rational Improvement Strategies and Pitfalls in *Leishmania* Drug Discovery

16

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Abstract

Worldwide parasite infection, especially *Leishmania*, is the most severe high morbidity and mortality issue. It is one of the leading protozoans parasitic agents responsible for causing the tropical disease leishmaniasis. In the seventh century BC, leishmaniasis comes into account by isolating leishmanial mitochondria DNA from Egyptian mummies circa 2000 BC. Multiple species are listed in this chapter, and their geographical distribution manifests typical diseases. Over 12 million people are currently affected, and around 350 million people are still likely to get infected by leishmaniasis. As leishmaniasis attacks mainly in rural area or the outskirts of cities, where healthcare facilities is a challenging job to hold on, it leads us to discover a therapeutic approach with cost-effective and easy access to the need with significant diagnosis and treatment of the disease. Unfortunately, there is no potential theranostics against *Leishmania* due to the clinical and acquired resistance. Thus, we must find new a weapon or strategies,

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_16

such as drug repurposing by using drugs already available for other diseases. Several antileishmanial drugs are available for treating leishmaniasis, including repurposed drugs. Recently, several alternative therapies have provided a novel approach to drug discovery through rational improvement, including target identification and modeling, compound screening and ligand structure validation by virtual screening against the novel target.

Keywords

Drug discovery · Therapeutic landscape · Target-based · Rational improvement · Hit to lead

16.1 Introduction

16.1.1 The Parasite

Leishmania is a protozoan parasite and the organism responsible for the neglected tropical disease leishmaniasis, which leads to significant morbidity, particularly among the poor. It is one of the leading parasitic agents that causes disease worldwide. Approximately half of the global population are affected by the following five protozoan parasites: *Plasmodium*, *Toxoplasma, Cryptosporidium*, *Leishmania*, and *Trypanosoma* (World Health Organization 2021). Leishmaniasis is a vector-borne multispectrum disease, existing in at least four main forms: visceral leishmaniasis (VL, or kala-azar) and its complication post-kala-azar dermal leishmaniasis (PKDL), cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (MCL, or espundia) (Prevention C-C for DC 2021). Cutaneous leishmaniasis is the most common, but in total, these diseases account annually for 981,000 disability-adjusted life years (Okwor and Uzonna 2016; Hay et al. 2017).

Primarily spread by the bite of the female phlebotomine sandfly, *Leishmania* has been reported in at least 87 countries with nearly 100 species of the genera *Phlebotomus* and *Lutzomyia* identified as vectors for human disease (Steverding 2017; Pratlong et al. 2013). Although widespread, 77% of CL cases and 96% of VL cases in 2017 occurred in "high-burden" countries (Pratlong et al. 2013). Importantly, the geographical distribution varies for each of the 20+ species of *Leishmania*. "Old World" species include *L. donovani*, the organism primarily responsible for visceral leishmaniasis or kala-azar, a term derived in the nineteenth century to mean "black disease" from the Hindi/Urdu name for black (Kala) and the Persian term for disease (Azar) (Steverding 2017). First well described in 1827 by military surgeon William Twinning in an article about patients from Bengal, India, kala-azar is a disease that causes a gray discoloration of the skin, fever, weight loss, and hepatosplenomegaly (Prevention C-C for DC 2021; Steverding 2017). Left untreated, it is fatal in 95% of cases. The natural course of the illness is often unpredictable, and it may be weeks or even decades that the disease manifests after exposure. Visceral leishmaniasis more commonly occurs in the Eastern

Hemisphere, though it is not exclusive. India, Sudan, South Sudan, Ethiopia, Somalia, Kenya, China, and Brazil are considered high-burden countries (Ruiz-Postigo et al. 2020).

Leishmaniasis clinical presentations. Cutaneous leishmaniasis is described in ancient history dating back to the seventh century BC, with accounts of the characteristic ulcerative lesion found in the library of the Assyrian King Ashurbanipal. *Leishmania* mitochondrial DNA has even been isolated from the Egyptian mummies circa 2000 BC (Steverding 2017). Cutaneous leishmaniasis spread across the globe long before the discovery of the "New World," and the species that cause this disease can be divided geographically between the two hemispheres with little crossover. To this day, it remains a debilitating and disfiguring illness, with most cases in 2017 occurring in high-burden countries such as Brazil, Peru, Colombia, Morocco, Tunisia, Algeria, and the Middle East (Steverding 2017). The course of illness also varies from visceral leishmaniasis, with lesions typically developing within weeks of exposure. Over time, papules at the location of the sandfly bite turn to nodular plaques and, ultimately, ulcerative lesions. These may be painful. While some will only have localized lesions, others may have sporotrichosis-like spread along lymphatic channels. Cutaneous lesions may also be a precursor to mucocutaneous disease. Felt to be a metastatic sequela, mucocutaneous leishmaniasis is the most devastating form of cutaneous illness, sometimes causing utter destruction of the nasal septum, lips, or palate (Steverding 2017; CDC n.d.). Certain Viannia subgenus species are prone to causing MCL, and it may occur either following untreated CL or concomitantly (CDC n.d.). See Table 16.1 for a list of *Leishmania* species, their geographical distribution, and typical disease manifestations.

16.1.2 The Disease Epidemiology, Life Cycle, and Treatment

Leishmaniasis currently affects more than 12 million people, and an additional 350 million people are at risk of infection. Annually, it is estimated there are 20,000–40,000 deaths (Dorlo et al. 2012). Like other neglected tropical diseases, the majority of afflicted persons with leishmaniasis reside in low- and middle-income countries (LMIC) where many challenges stand in the way of preventing or treating infectious diseases. Even more, leishmaniasis is primarily a disease of the rural poor or affects those on the outskirts of cities where people have little or no access to healthcare facilities (Dorlo et al. 2012). Ideally, a global vaccine effort to eradicate the disease would be of global interest; however, no such vaccine exists. As it stands, individuals are at the mercy of local healthcare systems for diagnosis and treatment of the disease, both cost and access are significant burdens. One study in Nepal revealed that the cost of treatment for VL was more than the median annual household income, with 75% of this cost being incurred before ever receiving treatment (Okwor and Uzonna 2016).

Beyond costs, treatment of the disease is limited by toxicity, the ineffectiveness of therapy, and often a need for a long treatment course. In the case of early or localized CL, it may be managed with direct therapy such as cryotherapy with liquid nitrogen,

Table 16.1 Geographical distribution, transmission, and disease manifestations of select *Leishmania* species (Okwor and Uzonna 2016; Hay et al. 2017; Pratlong et al. 2013; Prevention C-C for DC 2019; Coulibaly et al. 2016)

Subgenus	Species		Distribution	Disease	Transmission
Old World					
Leishmania	*L. major* complex	*L. major*	Central Asia, Middle East, Northern Africa, East Africa	CL	Zoonotic
	L. tropica complex	*L. tropica*	Central Asia, Middle East, parts of Northern Africa, SE Asia	CL	Anthroponotic[a]
		L. killicki	Northern Africa	CL	Zoonotic
		L. aethiopica	Ethiopia, Kenya	CL	Zoonotic
	L. donovani complex	*L. donovani*	Africa, central Asia, SE Asia	VL, CL	Anthroponotic[a]
		L. archibaldi	East Africa, Middle East	VL, CL	Zoonotic
		L. infantum	Europe, Northern Africa	VL, CL	Zoonotic
New World					
Leishmania	*L. donovani* complex	*L. chagasi*	Central America, South America	VL, CL	Zoonotic
	L. mexicana complex	*L. pifanoi*	South America	CL	Zoonotic
		L. amazonensis	South America	CL	Zoonotic
		L. venezuelensis	Northern South America	CL	Zoonotic
		L. waltoni	South America	CL	Zoonotic
		L. garnhami	South America	CL	Zoonotic
Viannia	*L. [V.] braziliensis* complex	*L. [V.] braziliensis*	*South America, parts of Central America, Mexico*	CL, MCL	Zoonotic
		L. [V.] peruviana	Peru	CL	Zoonotic

(continued)

Table 16.1 (continued)

Subgenus	Species		Distribution	Disease	Transmission
	L. [V.] guyanensis complex	*L. [V.] guyanensis*	South America	CL	Zoonotic
		L. [V.] panamensis	Northern South America, Southern Central America	CL, MCL	Zoonotic
		L. [V.] shawi	Peru, Brazil	CL	Zoonotic
	L. [V.] lainsoni		South America	CL	Zoonotic
	L. [V.] naiffi		South America	CL, MCL	Zoonotic
	L. [V.] lindenbergi		South America	CL	Zoonotic

[V] Viannia, *CL* cutaneous leishmaniasis, *MCL* mucocutaneous leishmaniasis, *VL* visceral leishmaniasis

[a] Human–sandfly–human spread is termed anthroponotic and primarily occurs in South Asia (the Indian subcontinent) (Prevention C-C for DC 2021)

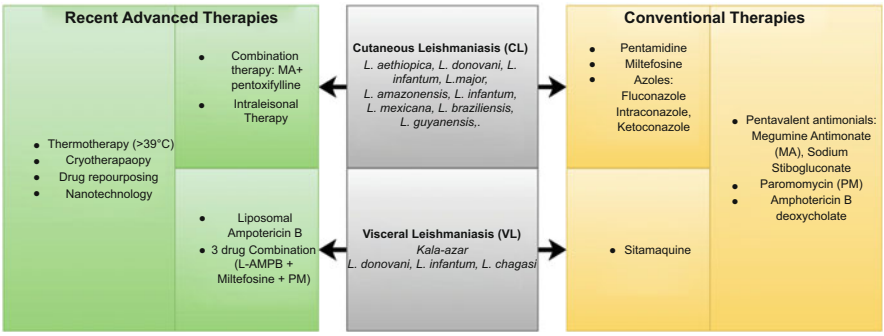

Fig. 16.1 Clinical presentation vs. therapeutic strategies and therapeutic regimens

thermotherapy with radiotherapy, paromomycin, or intralesional pentavalent antimonial (SbV) therapy (Prevention C-C for DC 2019). However, diffuse CL, MCL, and VL will require systemic therapy. While the mainstay of therapy is amphotericin B, the drug of choice ultimately depends upon the resources of the endemic area in which a person is treated. While travelers returning to the United States would receive liposomal amphotericin, natives of endemic areas may only have access to the older, more toxic form of amphoteric B deoxycholate (Prevention C-C for DC 2019) (Fig. 16.1). Additionally, prolonged use of an injectable medication by intravenous route may not be possible, resulting in inadequate therapy and the development of resistance. Other agents with high, irreversible toxicity,

including pentamidine isethionate, are also not used in the USA but are common agents elsewhere. Miltefosine, originally studied as a chemotherapeutic agent for cancer in the 1980s, was eventually approved in 2002 and remains the only oral agent with approval for the treatment of all three primary forms of leishmaniasis (Dorlo et al. 2012), though its efficacy is limited to a handful of New World species of the *Viannia* subgenus (CDC n.d.). Other agents, like the azole antifungals, have mixed data on their effectiveness and are not considered first-line (Prevention C-C for DC 2021).

Vector control strategies have been difficult to sustain. Persons traveling may be told to avoid sandfly bites, use insect repellant, minimize nocturnal outdoor activities, wear protective clothing, and utilize bed nets, but these are not typically feasible tasks for those most at risk, mainly the poor citizens of endemic areas. Given the variabilities among species, complex life cycle, and variable reservoir hosts (maybe rodents, dogs, or even infected humans), preventing transmission and developing new treatment strategies is difficult (Prevention C-C for DC 2021).

Vaccination remains the most economical method for prevention, and some clinical trials have reported promising results. A perfect vaccine would have high immunogenicity to induce a Th1 and cytotoxic T cell response and utilize an adjuvant for stimulating antigen-presenting cell activity. Several antigens and adjuvants (including immunogenic proteins from the saliva of the sandfly) have been proposed (Ghorbani and Farhoudi 2018; Jain and Jain 2015), yet the perfect vaccine is elusive.

The *Leishmania* protozoan parasite has many mechanisms of evasion. The parasite is injected into the host when an infected female phlebotomine sandfly takes a blood meal. This causes promastigote stage parasites to enter under the skin from the proboscis (Prevention C-C for DC 2021). Macrophages take up the infectious stage promastigote into the phagosome, where normally killing would occur after fusion with the lysosome. However, surface molecule LPG inhibits the fusion of the phagosome and lysosome (Jain and Jain 2015). Additional surface protein GP63 inhibits oxidative bursts, allowing the promastigotes time to transform into the tissue stage of the parasite, the amastigote (Prevention C-C for DC 2021). Amastigotes can further avoid detection by preventing macrophage apoptosis and degrading MHCII, inhibiting the interaction with antigen-presenting cells. They also secrete TGF-β, and PGE2, further inhibiting macrophage function. The innate immune response is also thwarted by blocking proteolysis activity of C3b and by the production of protein kinases that inhibit components of both the classical and alternate complement pathways. It is in part for these reasons the vaccine development has been thus far ineffective. In the meantime, new drug development will be key to reducing the global burden of leishmaniasis. This chapter will further describe the current approaches to novel drug development.

16.2 Current Therapeutic Landscape

16.2.1 Drug Targets, Indications, and Adverse Effects

Multiple therapeutic options exist to treat *Leishmania* infection, including several repurposed drugs (Pushpakom et al. 2019; Dhir et al. 2020). Overall, currently employed medications have proven effective, but carry adverse effects and face growing parasitic resistance (Fig. 16.2).

16.2.1.1 Pentavalent Antimony Sodium Stibogluconate

Drug Target and Indications. First-line antileishmanial treatment is pentavalent antimony sodium stibogluconate, a repurposed organometallic prodrug. Two antimonial preparations are currently available: complexes of Sb(V) with N-methyl-D-glucamine (meglumine antimoniate, Glucantime, Specia Rhone Poulenc) and sodium stibogluconate (Pentostam, GlaxoSmithKline) (Frézard et al. 2008). Meglumine antimoniate is available via intramuscular administration and sodium stibogluconate via intravenous and intramuscular administration. The use of mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy has elucidated the structures of these complexes and supports two main models of antileishmanial activity (Frézard et al. 2008; Headley et al. 1995; Roberts et al. 1998; Berman and Grogl 1988; Hansen et al. 2008). According to the active Sb(V) model, Sb(V) has intrinsic antileishmanial activity, while in the "prodrug model," Sb(V) is reduced to Sb(III) to exert antileishmanial activity (Zhou et al. 2004). More recently, the mechanism of action of pentavalent antimony sodium stibogluconate has been demonstrated to be through inhibition of trypanothione reductase (Nagle et al. 2014). Although antimonials are effective, their limited administration preparations

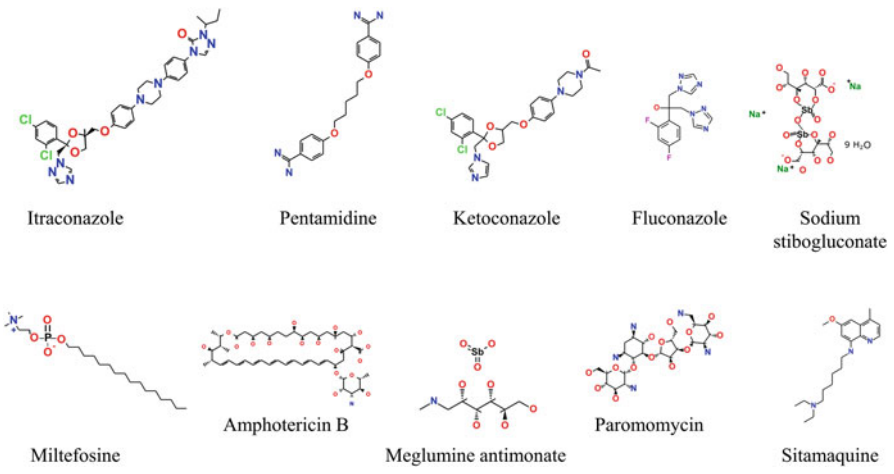

Fig. 16.2 Current antileishmanial drugs

and associated toxicity make them difficult to use (Herwaldt and Berman 1992; Esfandiarpour and Alavi 2002).

Allopurinol, an analog of hypoxanthine, is effective against multiple *Leishmania* spp., but is generally not effective without pentavalent antimony therapy. A combination of allopurinol and sodium stibogluconate was more effective in treating *L. panamensis* infection than monotherapy (D'Oliveira Jr et al. 1997). The leishmanial target for allopurinol is proposed to be adenylosuccinate synthetase or adenine phosphoribosyltransferase (Marr and Berens 1977).

Adverse Effects. Adverse effects of antimonials include fatigue, myalgias, headache, nausea, and rash. More serious adverse effects include hepatitis, pancreatitis, bone marrow suppression, and electrocardiogram changes (QT prolongation). Complications usually resolve upon discontinuation of therapy, so regular monitoring is recommended during its usage. The optimal dosage has varied throughout the decades to balance effectiveness and prevention of adverse effects (Wortmann et al. 2002).

16.2.1.2 Pentamidine

Drug Target and Indications. Second-line treatments for *Leishmania* include pentamidine (PTM) (Fig. 16.2) and amphotericin B. PTM is an aromatic diamidine that is toxic to multiple protozoa, including *Leishmania*. It exerts its antileishmanial effects by inhibiting DNA synthesis pathways (Raj et al. 2020; Sakyi et al. 2021a). In *L. infantum*, PTM was found to inhibit amastigote nucleoside triphosphate diphosphohydrolase 1 (NTPDase 1) (Maia et al. 2019). In addition, PTM is the first-line treatment for cutaneous leishmaniasis used in French Guyana (Nacher et al. 2001; Pradinaud 1994). A study by Soto et al. on *L. panamensis, L. braziliensis*, and *L. mexicana* demonstrated a cure rate of 96% when using a short course of low-dose pentamidine isethionate. This regimen had a similar cure rate and side effects as meglumine antimonate (Soto et al. 2018).

Adverse Effects. PTM is no longer used due to associated toxic side effects in humans and existing drug-resistant parasites. Adverse effects include acute hepatitis, nephrotoxicity, arrhythmias, nausea, vomiting and diarrhea, leucopenia, anemia, thrombocytopenia, cough, bronchospasm, and neurological effects such as confusion, neuralgias, and hallucinations. Low-dose regimens used for CL are better tolerated than higher dosages utilized for VL. At higher doses, PTM may cause diabetes mellitus, and reversible hyperglycemia has been described after just one dose of PTM (Amato et al. 1998; Soto-Mancipe et al. 1993). Therefore, blood sugar levels must be monitored before injecting PTM.

16.2.1.3 Amphotericin B

Drug Target and Indications. Amphotericin B-desoxycholate (Fig. 16.2) is an antifungal agent that has been successfully applied to treat leishmaniasis. The mechanism of action includes inhibition of sterol biosynthetic pathways (Raj et al. 2020; Sakyi et al. 2021a). Amphotericin B also targets *L. donovani* promastigote membrane sterols, resulting in the formation of aqueous pores that are permeable to small cations and anions that facilitate killing unicellular *Leishmania* (Ramos et al.

1996; Saha et al. 1986). In addition, amphotericin B inhibits the entry of *L. donovani* into primary macrophages, possibly by its interaction with leishmanial ergosterol and host macrophages' cholesterol (Paila et al. 2010). Amphotericin B is delivered intravenously to treat VL and is also effective against CL (Kariyawasam et al. 2019).

Adverse Effects. Amphotericin B is associated with a high incidence of adverse effects, including malaise, hypotension, hyperpyrexia, anemia, thrombophlebitis, hepatitis, renal tubular damage, azotemia, and hypokalemia. Lipid formulations of amphotericin B have lower toxicities than the free drug and are effective in treating VL (Frézard et al. 2008). However, amphotericin B treatment is expensive and requires hospitalization due to its lipid formulation (Carvalheiro et al. 2021; Jafari et al. 2021).

16.2.1.4 Miltefosine (MF)

Drug Target and Indications. Miltefosine (MF, hexadecylphosphocholine) (Fig. 16.2) is a phosphocholine analog developed to combat resistance to antimonial drugs. It is the only drug available in oral preparation for VL. MF exhibits antileishmanial effects through various mechanisms, including inhibition of phosphatidylcholine synthesis, inhibition of cytochrome *c* oxidase in the mitochondrion, and activation of plasma membrane calcium channels in *L. donovani*. Altering calcium homeostasis appears to cause rapid alkalinization of *Leishmania* acidocalcisomes and resulting cellular death (Pinto-Martinez et al. 2018). MF also induces apoptosis-like death in *L. donovani* (Verma and Dey 2004). García Bustos et al. demonstrated MF to be safe and effective for treating *L. amazonensis* infection in mice as well as more efficacious than meglumine antimoniate (García Bustos et al. 2014). Recently, MF in vivo activity was enhanced by nanoformulation (Peña-Guerrero et al. 2021).

In addition to VL, MF has been used to treat CL. Its oral administration option coupled with increased efficacy against multiple *Leishmania* spp. and manageable adverse effects make MF a feasible treatment option for CL (Ware et al. 2021). However, the cost and limited local availability of MF may limit its use. Soto et al. successfully treated cutaneous *L. braziliensis* infection with a combination of MF and PTM (Soto et al. 2018). The cost and adverse effects of the drugs were addictive, but the combination is reasonable to consider for rescue therapy as it treats locally and protects against systemic parasite dissemination.

Perifosine, structurally identical to MF, has been repurposed as an antileishmanial candidate. In *L. donovani* and *L. amazonensis*, perifosine decreases cellular ATP levels, reduces mitochondrial membrane potential, and increases phosphatidylserine externalization (López-Arencibia et al. 2017). Perifosine also inhibits phosphorylation of Akt in parasites. This drug may be an alternative to MF.

Adverse Effects. The most common adverse effects of MF are gastrointestinal symptoms, elevated liver enzymes, motion sickness, and headache (Soto et al. 2001). Importantly, MF is teratogenic and should be avoided in pregnant people. Female patients of reproductive age are advised to use contraception during and 5 months after the MF therapy.

16.2.1.5 Paromomycin (PAR)

Drug Target and Indications. Paromomycin (PAR, aminosidine) (Fig. 16.2) is an aminoglycoside antibiotic that was approved in 2006 for the treatment of VL caused by *L. donovani*. It is also efficacious against CL with relatively short treatment duration and is available at low cost (Sundar and Chakravarty 2008). PAR inhibits *Leishmania* protein synthesis by binding to the 30S ribosomal subunit, which locks the 30S–50S ribosomal complex and causes misreading of mRNA template. Proteomic studies have found PAR to affect translation and vesicle-mediated trafficking in *L. donovani* (Jhingran et al. 2009; Chawla et al. 2011). An electron cryomicroscopy study demonstrated the PAR-binding site within the *Leishmania* ribosome, showing that PAR interferes with cytosolic translation and indicating that it targets cytosolic rather than mitochondrial ribosome (Shalev-Benami et al. 2017). Lastly, in combination with benzethonium chloride, PAR inhibits PTEN-induced putative kinase 1, which decreases protozoan mitochondria membrane potential (Hendrickx et al. 2017).

In addition to treating *L. donovani* infection, PAR may be effective against other *Leishmania* spp. A murine model study by Coser et al. demonstrated that PAR has the potential to effectively treat CL caused by *L. amazonensis* in Brazil (Coser et al. 2020). In *L. mexicana*, PAR decreases translation accuracy, protein synthesis, and proliferation rate in promastigotes (Fernández et al. 2011). Furthermore, surface plasmon resonance analysis demonstrated a strong binding between PAR and the leishmanial ribosomal decoding site.

Adverse Effects. Common adverse effects of PAR include rash, heartburn, drowsiness, headache, and gastrointestinal symptoms, such as nausea and vomiting, diarrhea, loss of appetite, and abdominal cramps. In addition, patients may experience injection site pain, ototoxicity, and elevated liver enzymes (Sundar and Chakravarty 2008).

16.2.1.6 Imidazoles

Drug Target and Indications. Imidazoles are antifungal medications that have been used to treat CL. These drugs, which include fluconazole, itraconazole, ketoconazole, and voriconazole (Fig. 16.2), exhibit antileishmanial activity by inhibiting sterol biosynthesis in infected host cells (Hart et al. 1989). The major advantages of using imidazoles are oral administration and a safe side effect profile (Frézard et al. 2008). However, these drugs do not cover all *Leishmania* spp., as outlined below.

Fluconazole is safe and effective for treating CL caused by *L. major* (Alrajhi et al. 2002). Studies in murine models have demonstrated promising results for topical fluconazole in treating *L. major* and *L. amazonensis* (Mussi et al. 2007). There are, however, conflicting data using fluconazole to treat CL caused by *L. braziliensis*; overall, the efficiency of the drug is promising for this species of *Leishmania* (Prates et al. 2016; Bezerra-Souza et al. 2016; Veraldi et al. 2021). Lastly, fluconazole has demonstrated efficacy against *L. tropica* CL (Laffitte et al. 2005). Interestingly, fluconazole has been used in combination with miltefosine and an

immunomodulator, Picroliv, to successfully treat VL and upregulate cell-mediated immunity (Shakya et al. 2011).

Itraconazole and ketoconazole have also been used to combat CL. Itraconazole has proven to be effective against CL and comes with safe alternatives to other antileishmanial drugs (Dogra and Saxena 1996). The drug has successfully been used to treat CL caused by *L. amazonensis*, leading to mitochondrial swelling and membrane rupture, accumulation of lipid bodies, and kinetoplast alterations (de Macedo-Silva et al. 2013). Of note, posaconazole was found to exert similar effects on *L. amazonensis*. In vitro studies on *L. tropica* and *L. major* promastigotes indicate that itraconazole may be an effective option against these species (Khazaeli et al. 2014; Zakai et al. 2003). However, not all leishmanial spp. respond well to this medication, specifically not effective as monotherapy against CL caused by *L. major*, *L. donovani,* or *L. mexicana* (Zakai et al. 2003; Momeni et al. 1996). Thus, itraconazole has been used in combination with other drugs. In contrast to the findings of de Macedo-Silva et al., Anversa et al. found that itraconazole was not effective against *L. amazonensis* alone, but improved the efficacy of antimonial therapy (Anversa et al. 2017). Recently, a ternary treatment composed of itraconazole, miltefosine, and ezetimibe was demonstrated to be a promising therapeutic option for VL (Andrade-Neto et al. 2021).

Ketoconazole is effective for CL caused by several leishmanial spp., including *L. braziliensis, L. panamensis, L. mexicana*, and *L. amazonensis* (Pirson et al. 1990; Saenz et al. 1990). It has also demonstrated improved in vitro antileishmanial efficacy against *L. amazonensis* amastigotes in combination with antimonials (Nunes et al. 2017). Of note, ketoconazole should be avoided in patients with *L. mexicana* infection being treated with amphotericin B (Ramos et al. 1996). Another imidazole, voriconazole, is effective against several *Leishmania* spp. (Kulkarni et al. 2013). A combination of voriconazole and amiodarone was found to be promising for CL caused by *L. major* (Bahrami et al. 2021).

Additional drugs that interfere with ergosterol synthesis have demonstrated antileishmanial activity against *L. amazonensis*, *L. braziliensis,* and *L. infantum*. These include fenticonazole, tioconazole, and nystatin. Yamamoto et al. have found that fenticonazole, tioconazole, and nystatin eliminated promastigote and intracellular amastigotes. The drugs interfered with parasitic mitochondrion, and fenticonazole specifically alkalized infected host macrophages (Yamamoto et al. 2018).

Adverse Effects. Imidazoles are generally well tolerated but have been associated with adverse events. These include rash, headache, dizziness, hair loss, gastrointestinal symptoms, and rarely liver disease. Of note, ketoconazole has the broadest spectrum of activity, but this is accompanied by an increased risk of adverse effects. Therapy is most likely to be limited by hepatotoxicity or gastrointestinal symptoms. In rare cases, ketoconazole has caused anaphylactic shock. Although itraconazole is better tolerated than other imidazoles, it has higher rates of treatment failure than other compounds.

16.2.2 Drug Resistance

Resistance against currently used antileishmanial drugs is highly concerning (Raj et al. 2020; Sakyi et al. 2021a). The pentavalent antimonials, liposomal amphotericin B, and miltefosine remain the primary drugs for the treatment of leishmaniasis. However, *Leishmania* spp. continue to develop resistance against first-line medications (Chakravarty and Sundar 2010; Ponte-Sucre et al. 2017). Moreover, experimental studies have demonstrated the ability of *L. donovani* to develop resistance against drug combinations and observed cross-resistance from one drug combination to another (García-Hernández et al. 2012). Designing effective drugs is made difficult by a large number of evolving leishmanial molecular targets (Akhoundi et al. 2017). Here, we will review drug resistance patterns and mechanisms to the drugs that are outlined above.

Drug	Background	Resistance
Pentavalent Antimonials:	First used in 1945, currently available as sodium stibogluconate and meglumine antimoniate	Resistance was developed in India The first reported resistance came early in the 1980s
Pentamidine:	Pentamidine first reported use for VL was in 1949 in India, and in 1950 in Spain, it was used as the second-line therapy for the treatment of antimony-refractory cases of VL in India	Resistance developed in India
Amphotericin B:	Amphotericin B was traditionally a second-line treatment for VL in India. Then in the 1990s, it has, due to decreased antimonial and pentamidine efficacy, led to the use of amphotericin as a first-line	Resistance was reported in Bihar, India, and different parts of the world
Paromomycin:	Efficacious for the treatment of CL in 1966 and VL in 1990 in Kenya. Then it was introduced for VL in 2006. Paromomycin can be effectively used for patients with other drug resistance to treat VL	The strain of *L. tropica* and *L. donovani* has developed resistance
Miltefosine:	In 2002, it was approved in India as the first oral treatment of VL	Strains of *L. braziliensis*, *L. guyanensis*, and *L. mexicana* developed resistance
Ketoconazole:	As *Leishmania* parasites rely on ergosterol for their sterol needs and share this biosynthetic pathway with fungi, azoles have been explored for their therapeutic potential against *Leishmania* infections	
Nitroimidazole class Oxaborole class Aminopyrazole	DNDI-0690 showed excellent activity against *Leishmania* in vitro, and *L. major* in mice, Oxaborole, DNDI-6148, for preclinical development in	

(continued)

Drug	Background	Resistance
	January 2016, this candidate achieves high levels of parasite burden reduction in mouse and hamster models of VL after 5–10 days of treatment Aminopyrazole, DNDi5561, was nominated for preclinical development in October 2017	

Pentavalent antimony sodium stibogluconate. Pentavalent antimony sodium stibogluconate faces resistance by *Leishmania* spp. through several proposed mechanisms. These include gene amplification, cellular sequestration of a metal–thiol conjugate, decreased activity of the antimony prodrug, and decreased drug uptake. Genomic analysis has revealed gene amplification of *gsh1* and *MRPA* in resistant *Leishmania* (El Fadili et al. 2005; Torres et al. 2010; Douanne et al. 2020). These results were confirmed by parasite proteome analysis studies (Torres et al. 2010; Brotherton et al. 2013; do Monte-Neto et al. 2011). For example, the *gsh1* gene codes for the Ɣ-GCS heavy subunit, the rate-limiting enzyme in the glutathione (GSH) biosynthesis pathway, which plays a role in drug detoxification (Grondin et al. 1997). Similarly, *MRPA* codes for an ABC transporter that helps remove drug molecules from the cytoplasm (El Fadili et al. 2005). Sodium stibogluconate resistance is associated with manipulation of both host and parasite glutathione levels by *L. donovani*, which promotes an oxidative intramacrophage environment (Carter et al. 2006). It should be noted, however, that similar studies completed with *L. donovani* clinical isolates found no appreciable changes in *gsh1* or *MRPA* expression (Vergnes et al. 2007). Thus, mutations among *Leishmania* are hypothesized to be species-specific (Decuypere et al. 2012).

Resistant strains of *Leishmania* spp. have overexpression of ornithine decarboxylase (ODC), which is essential for trypanothione synthesis and subsequent metal–thiol complex formation and drug efflux (Mukherjee et al. 2007). Indeed, increased thiol levels have been observed in resistant strains (Mukhopadhyay et al. 1996). Manzano et al. have found a transporter, ABC14, in *L. major* that is responsible for antimony efflux as a drug–thiol conjugate (Manzano et al. 2013). Cellular sequestration and drug efflux from the leishmanial cytoplasm in the metal–thiol forms are shown to be important for parasitic resistance.

Decreased antimony activation is thought to occur in resistant *Leishmania* strains. The intracellular enzyme responsible for the conversion of Sb(V) to Sb(III), TDR1, is thought to be underexpressed in resistant cells (Denton et al. 2004). In addition, decreased expression of *AQP1* in resistant cells leads to decreased uptake of Sb(III) (Gourbal et al. 2004). Resistance has been demonstrated in *L. donovani* as well as *L. major* and *L. infantum* (Mukherjee et al. 2013b; Mukherjee et al. 2013a).

Pentamidine. Pentamidine (PTM) is an alternative treatment for antimony-resistant VL. Its mechanism of action includes reduction of the mitochondrial membrane potential and inhibition of S-adenosyl-L-methionine decarboxylase activity (Basselin et al. 1997). The main drug target is parasitic mitochondria (Coelho

et al. 2008). PTM resistance is been well documented (Bray et al. 2003; Papadopoulou et al. 1998) and proposed to arise from several mechanisms, including decreased mitochondrial uptake, drug efflux pumps, and alterations in the genome sequence.

Drug uptake studies in resistant leishmanial cells have demonstrated inhibited mitochondrial uptake of PTM, which allows the drug to be rapidly removed from the cytosol (Basselin et al. 2002). Within parasitic cells, PTM noncompetitively inhibits transport of spermidine and putrescine and competitively inhibits transport of arginine. A study of PTM-resistant cells in *L. amazonensis* showed altered spermidine and putrescine uptake (Basselin et al. 1997). In addition, PTM-resistant *L. donovani* and *L. amazonensis* cells demonstrated increased intracellular ornithine and arginine levels and decreased putrescine levels (Basselin et al. 1997). The modified levels of arginine and putrescine are a mechanism to prevent mitochondrial PTM uptake. Furthermore, resistant cells have a lower mitochondrial membrane potential, which aids in drug efflux. Interestingly, a calcium channel blocker, verapamil, has reversed PTM resistance in *L. mexicana*, but has no such effect in *L. donovani* (Mukherjee et al. 2006).

Similar to other drug resistance profiles, ABC transporters play an important role in PTM resistance. The *PRP1* gene in *L. major*, *L. infantum*, and *L. amazonensis* codes an ABC transporter that confers resistance (Coelho and Cotrim 2018). Similar transporters are still being studied (Coelho et al. 2008). Finally, modifications to the kDNA genome sequence confer PTM resistance (Basselin et al. 1997). Molecular modeling studies have demonstrated the interaction of PTM within the kDNA regions (i.e., the minor groove of AT-rich DNA regions). Further molecular studies of PTM-sensitive vs PTM-resistant *L. donovani* and *L. amazonensis* demonstrated major variations in kDNA.

Amphotericin B. Amphotericin B has a high affinity to membrane-bound ergosterol, leading to a porous membrane and subsequent leakage of ions and cellular death (Pourshafie et al. 2004). *Leishmania* spp. resistance to amphotericin is relatively not as common, including *L. donovani* and *L. tropica* (Lachaud et al. 2009; Özbilgin et al. 2020). However, resistant strains of *L. donovani* are emerging and patients with HIV/VL co-infection are at particularly high risk (Jha 2006; Cruz et al. 2006; Távora et al. 2015). Proposed mechanisms of amphotericin resistance include the change in membrane fluidity, drug efflux, and gene amplification (Purkait et al. 2012).

Early studies on amphotericin resistance used flow cytometric analysis to understand the membrane potential of leishmanial cells (Di Giorgio et al. 1999). Resistant *L. donovani* promastigotes are rich in cholesta-5,7,24-trien-3B-ol, which causes increased membrane fluidity. Mutations in lathosterol oxidase can confer amphotericin B resistance in *L. major* via disruption of membrane stability (Ning et al. 2020). Of note, drug-resistant parasites have been found to have decreased infectivity (Al-Mohammed et al. 2005). Change in membrane fluidity may result in nonfunctional membrane receptors in resistant parasites with subsequently increased infectivity (Mbongo et al. 1998). Stigmasterol may serve as a potential biomarker of

resistance in *L. donovani*, but further research is required to validate a diagnostic method (Bansal et al. 2020).

Amphotericin B-resistant *Leishmania* also has increased drug efflux. The MDR1 ABC transporter is expressed four times higher in resistant cells (Purkait et al. 2012). Intracellular levels of thiol and reactive oxygen species (ROS) were low in resistant cells. In addition, RT-PCR studies have demonstrated the upregulation of genes needed for the tryparedoxin cascade and trypanothione biosynthesis. Thus, the tryparedoxin cascade may prevent oxidative damage by ROS. Finally, gene amplification studies were completed in *L. tarentolae* and found extrachromosomal DNA expression. This increased expression was directly correlated with parasite resistance (Singh et al. 2001).

Miltefosine (MF). Miltefosine exerts antileishmanial effects through inhibition of phosphatidylcholine synthesis, inhibition of "cytochrome *c*" oxidase in the mitochondrion, and activation of plasma membrane calcium channels in *Leishmania.* Resistance is caused by several mechanisms, including decreased protein synthesis and degradation, modified energy use through elevated lipid degradation, drug efflux via ABC transporters, altered DNA replication and repair, and resistance to oxidative stress (Kulshrestha et al. 2014).

Several studies have demonstrated the ability of MF-resistant *L. donovani* strains to resist oxidative damage (Das et al. 2013; Yadav et al. 2020). This is largely possible through increased trypanothione metabolism. A metabolomic study utilizing gas chromatography, liquid chromatography, and capillary electrophoresis was used to show impairment of polyamine metabolism from arginine to trypanothione in MF-susceptible *L. donovani*, pointing to the role of ROS damage caused by MF (Canuto et al. 2014). A laboratory study by Mishra et al. showed MF-resistant *L. donovani* are protected against reduced mitochondrial membrane potential and release of ATP cytochrome *c* release into the cytosol (Mishra and Singh 2013). In addition, resistant cells express higher levels of iron superoxide dismutase (*FeSODA*), which helps parasites resist oxidative stress (Veronica et al. 2019). A recent study of the T-complex protein-1 (TCP1) chaperonin protein by Yadav et al. describes the molecular mechanism of TCP1 gamma subunit (LdTCP1γ) overexpression involved in ROS neutralization (Yadav et al. 2020).

MF resistance also arises from decreased cellular levels via ABC transporters and reduced drug uptake (Pérez-Victoria et al. 2006). Decreased cellular uptake is caused by underexpression of the MF transporter, LdMT, and its beta subunit, LdRos3. Interestingly, MF resistance in *L. donovani* has no impact to date on the sandfly vector (Hendrickx et al. 2020b).

In addition to *L. donovani*, other *Leishmania* spp. have developed resistance to MF. A phase II dose-escalation study of MF was conducted in Brazil to test the susceptibility of *L. infantum*, the causal agent of Brazilian VL. The parasite demonstrated resistance, although MF had not been used in Brazil before the trial, suggesting existence of natural resistance (Carnielli et al. 2019). *L. infantum* resistance to MF has been corroborated by other studies (Bhattacharya and Ouellette 2018; Eberhardt et al. 2019). Of note, MF may enhance the fitness of drug-resistant *L. infantum* due to drug dependency. Thus, resistance profiling is critical before

administering the MF (Eberhardt et al. 2019). Carnielli et al. identified a Miltefosine Sensitivity Locus in *L. infantum*, which may serve as a genetic marker to stratify patients before administering treatment (Carnielli et al. 2018). Lastly, experimental MF resistance in *L. major* shows elevated metacyclogenesis. However, these experimental parasites have reduced virulence and normal survival rates in the sandfly vector, indicating no increase in fitness in *L. major* from MF resistance (Turner et al. 2015).

Paromomycin (PAR). PAR exerts antileishmanial effects by inhibiting *Leishmania* protein synthesis and vesicle-mediated trafficking. *Leishmania* spp. has gained resistance to PAR via several mechanisms. PAR-resistant *L. donovani* demonstrates increased membrane fluidity and decreased intracellular drug buildup (Maarouf et al. 1998). In addition, PAR-resistant parasites have improved drug efflux as indicated by increased expression of ABC transporter genes, including *MDR1* and *MRPA*, and protein phosphatase 2A (Bhandari et al. 2014). Drug efflux is accompanied by reduced drug binding on the cell surface (Jhingran et al. 2009).

PAR-resistant parasites also have an increased ability to withstand reactive nitrogen species and host defense mechanisms. Transcriptome profiling in experimental *L. donovani* has identified potential adaptations to confer resistance to PAR (Verma et al. 2017). Recent studies have demonstrated increased aneuploidy in PAR-resistant *L. donovani*, which may lead to a rapid selection of advantageous traits (Shaw et al. 2019). All of these adaptations lead to improved survival capacity for PAR-resistant *L. donovani*.

L. infantum has also demonstrated resistance to PAR, indicating a potential to gain resistance in nature (Hendrickx et al. 2020a). A potential solution is combination therapy with PAR and MF, which delayed experimental drug resistance in *L. infantum* (Hendrickx et al. 2017). Of note, PAR-resistant parasites have demonstrated increased sensitivity to PAR if exposed to the calcium channel blockers, verapamil, and amlodipine, which also modulate ABC transporters (Verma et al. 2017).

Imidazoles: Fluconazole, Itraconazole, Ketoconazole, and Voriconazole. Resistance to imidazoles is less well studied in *Leishmania* spp. The drug class exerts antileishmanial effects by inhibiting sterol biosynthesis in infected host cells. Camizotti et al. isolated nine loci within two genes that confer resistance to itraconazole in wild-type *L. major* parasites (Camizotti et al. 2009). Interestingly, these genes are not related to ergosterol biosynthesis, indicating novel gene systems that may cause imidazoles resistance. Resistance to ketoconazole in *L. amazonensis* was proportional to the upregulation of C14-demethylase (CYP51). A phase II trial of fluconazole in treating cutaneous *L. guyanensis* in Brazil found the drug to be inefficacious (Francesconi et al. 2018). Further studies are needed to understand the resistance mechanisms of *Leishmania* spp. against imidazoles.

16.2.3 Current Drugs Development Pipelines

To combat the oncoming threats of drug resistance, toxicity, and high costs from current leishmaniasis treatment, recent drug development strategies by phenotypic screenings and target-based drug design have been implemented. Although often limited by challenges in the maintenance of *Leishmania* cultures in the appropriate life stage, two common pipelines are used in current leishmaniasis drug discovery. Phenotypic screenings aim at targeting multiple aspects of the parasite with no predetermined target, whereas target-based screenings have a specific molecular target for which the drug will be designed to interact (Singh et al. 2019).

Phenotypic screening. Drug development via a phenotypic screening has the advantage of immediately showing drug effects of the parasitic target as well as effects on the host cell. This allows for the testing of a high quantity of compounds in a short time. Moreover, one can easily assess the compound's toxicity, permeability, and specificity efficiently. However, this method lacks the identification of a specific drug target and, therefore, risks a potential compound developing resistance within a short time frame. If a compound shows antileishmanial phenotypic activity but the drug target(s) is part of a non-conserved system, the compounds will risk decreased activity later on. To combat this risk, the mechanism of action deconvolutions has been often paired with phenotypic screenings (Pena et al. 2015; Ortiz et al. 2017).

Target-based drug discovery. This method of drug discovery requires an evaluation of several molecular targets within the target organism regarding their homology to humans, level of conservation in evolutionary history, and physiological role in parasite survival. Consequently, this method of discovery requires a higher level of curation on the *Leishmania* proteome, which often is not available (Shaw et al. 2019). With ongoing advancements in sequencing techniques and bioinformatics efforts, protein targets of *Leishmania* have been made available and their crystal structures or sequencing data is available to modeling and consequential virtual screenings (Sakyi et al. 2021a; Sakyi et al. 2021b; Broni et al. 2021). The pitfall in this method comes when the compound is tested in the scope of the entire system; although the compound is designed and fitted to one molecular target, it could have a nonspecific binding that is not controlled for. This could lead to higher toxicity levels, adverse effects, or unexplained resistance.

16.2.4 Recent Alternative Therapeutics

16.2.4.1 Multidrug Therapy

Combination drug therapy has proved effective in the past as it has the potential to increase the effectiveness of the drugs due to synergistic effects; however, there comes a risk of increased toxicity. In the case of VL, a combination of Paromomycin (PAR) and sodium stibogluconate (SSG) has been tested and made the first line of treatment in East Africa due to its higher effectivity in combination than using PAR alone, along with no significant differences in toxicity profiles (Mondal et al. 2016). Other studies have shown higher efficacy in the combined use of amphotericin B and

SSG compared to amphotericin B with miltefosine (MF) or MF alone (Bahrami et al. 2021; Mesquita et al. 2014b). Other combination therapies have been tested in the past with known leishmaniasis therapeutics, but they come as a short-term solution for rapid treatment; although often effective, the shortcomings of this treatment option are the same as traditional therapies.

16.2.4.2 Cryotherapy and Immunotherapy

Immunotherapy could provide useful adjuvant to chemotherapy in the treatment of CL. In contrast to chemotherapy that kills the parasite, immunotherapy stimulates the host's immune response to clear the parasite. One such example is imiquimod, which is an imidazoquinoline immunomodulator inducing cytokines (Arevalo et al. 2001). Combination therapy of imiquimod (topical ointment) along with meglumine antimonate showed a positive response with a 90% cure rate for CL in a clinical trial (Arevalo et al. 2001). Other immunomodulators, such as Pentoxifylline, continue to be studied for the treatment of leishmaniasis in combination with other conventional therapeutics. Additionally, cryotherapy by application of liquid nitrogen to the lesion caused by acute CL has shown positive outcomes at a low cost and low risk (Asilian et al. 2004).

16.2.5 Drug Repurposing

Drug repurposing or repositioning means developing new uses of already existing drugs. Some examples of repositioned drugs developed for leishmaniasis include Pentamidine, Miltefosine, Paromomycin, and Amphotericin B (Andrade Neto et al. 2018; Machado et al. 2015; Sundar et al. 2012). Pentamidine was discovered while developing anti-hypoglycemic drugs but is now used to treat antimonial resistance leishmaniasis. Miltefosine, the only drug given orally today for leishmaniasis, was initially developed as an anticancer drug. Paromomycin was developed as an antibiotic but is now one of the most widely and safely used drugs for leishmaniasis, with low cost, better efficacy, and lesser side effects. Amphotericin B is an antifungal medication that is now used for leishmaniasis, especially the liposomal formulation, which is less toxic and can be used for visceral leishmaniasis (Andrade Neto et al. 2018).

16.2.5.1 Strategies for Drug Repurposing

Drug discovery is a highly positive cost-to-benefit strategy exploiting an already worked out therapeutic agent for the treatment of another disease. The strategies for drug repurposing do not vary much from the methods used for initial drug discovery. The goal here is to test compounds, already approved and in use as other treatments, against the *Leishmania* parasite. Table 16.2 shows some antifungal drugs used for specific *Leishmania* species. Clotrimazole combined with ruthenium showed increased antileishmanial activity, especially in species causing cutaneous leishmaniasis. Nystatin inhibits the entry of promastigote into macrophages by sequestering macrophage cholesterol. The use of most of these antifungals for *Leishmania* was

Table 16.2 Antifungal drugs used for specific *Leishmania* species

Drug	Species	Activity	Ref.
Fluconazole	*L. major*		Andrade Neto et al. (2018)
Ketoconazole	*L. mexicana*		Andrade Neto et al. (2018)
Nystatin	*L. major*, *L. donovani*, *L. amazonensis*	Promastigote	Tewary et al. (2006), Ali et al. (1997), Ghosh and Chatterjee (1962)
Itraconazole	*L. amazonensis*	Promastigotes and amastigotes	de Macedo-Silva et al. (2013)
Posaconazole	*L. amazonensis*	Promastigotes and amastigotes	de Macedo-Silva et al. (2013)
Clotrimazole	*L. infantum*	Promastigotes and animal model	Martínez et al. (2012), Iniguez et al. (2016)
Bifonazole	*L. infantum*	Promastigotes	Andrade Neto et al. (2018)
Econazole	*L. infantum*	Promastigotes and intracellular amastigotes	Mesquita et al. (2014a)
Voriconazole	*L. major*, *L. donovani*, *L. amazonensis*		Kulkarni et al. (2013)
Miconazole	*L. amazonensis*		Andrade Neto et al. (2018)
Butenafine	*L. amazonensis*, *L. braziliensis*		Bezerra-Souza et al. (2016)
Terbinafine	*L. amazonensis*, *L. major*		Zakai et al. (2003), Vannier-Santos et al. (1995), Sampaio et al. (2009), Zakai et al. (2003), Simoes-Mattos et al. (2002), Zakai and Zimmo (2000)
Caspofungin	*L. tropica*	Promastigotes	Limoncu et al. (2013)
Posaconazole	*L. donovani*	Amastigotes	Gupta and Kempaiah (2022)

discovered due to similar cholesterol targets of action, like ketoconazole and miconazole that reduce ergosterol synthesis (Andrade-Neto et al. 2016a).

Table 16.3 shows some other antiparasitic drugs used for the treatment of leishmaniasis. Artemisinin decreases the synthesis of nitric oxide synthase mRNA. Metronidazole causes decreased lesion size and number of parasites on histological examination. The use of nitazoxanide showed decreased liver and spleen size in *Leishmania* patients. Imidocarb was also found to reduce skin lesions and parasite load (Andrade Neto et al. 2018).

Table 16.4 shows anticancer drugs being used for specific *Leishmania* species. Hydroxyurea is shown to alter the cell cycle in promastigotes by arresting the G2/M phase of the cell cycle. Cisplatin alters the cell cycle in promastigotes and amastigotes by arresting S2 and M phase and causing loss of mitochondrial membrane potential. It also increases in thiols and reactive oxygen species (ROS) in promastigotes, which leads to enhanced destruction of cells. Carmustine and mitomycin-C act by inhibiting trypanothione reductase. Mitomycin-C has also

Table 16.3 Antiparasitic drugs used for the treatment of leishmaniasis

Drug	Species	Activity	Ref.
Artemisinin	*L. donovani*	In vitro as well as tried with patients	Want et al. (2015), Ghaffarifar et al. (2015)
Chloroquine	*L. amazonensis*	Promastigotes	Khan et al. (2007)
Mefloquine	Cutaneous leishmaniasis	Promastigotes	Correia et al. (1999), Galvão et al. (2000)
Oxamniquine	*L. braziliensis*	In hamsters	Brazil and Gilbert (1976)
Ivermectin	*Leishmania* species, *L. infantum*	Promastigote and amastigote, in vitro and in vivo	Noël et al. (2011), Rasheid and Morsy (1998)
Metronidazole	Cutaneous leishmaniasis		Al-Waiz et al. (2004), Belhadjali et al. (2009), Griffiths and Sodeify (1976)
Nitazoxanide	*L. infantum, L. donovani*	Promastigote and amastigote	Mesquita et al. (2014b), Mesquita et al. (2013), Zhang et al. (2010)
Imidocarb	*L. amazonensis*		Rodrigues et al. (2006)

been entrapped in nanospheres to reduce drug toxicity in host cells. Paclitaxel affects the tubulins and causes the death of promastigotes and intracellular amastigotes, along with arresting the G2/M phase of the cell cycle. Imatinib inhibits Abl/Arg kinase enzymes involved in phagocytosis. Tamoxifen causes apoptosis of infected macrophages and enhances the action of drugs against amastigotes by modifying the alkaline intravacuolar pH. Raloxifene can cause the formation of autophagosomes and mitochondrial damage (Andrade Neto et al. 2018).

Table 16.5 shows antidepressant drugs used for specific *Leishmania* species. Imipramine induces apoptosis in affected cells, alters sterol profile and proton motive force of such cells, and inhibits trypanothione reductase. Clomipramine works by inhibiting L-proline transport, destructing plasma membranes in promastigotes, and inhibiting trypanothione reductase. Amitriptyline also acts by decreasing proline transport and depleting ATP levels in cells. Cyclobenzaprine helps by increasing ROS levels in promastigotes. Sertraline reduces ATP levels and oxygen utilization of promastigotes. Mianserin inhibits the enzyme 3-hydroxy-3-methyl-glutaryl-CoA reductase and destroys amastigotes and promastigotes. Diazepam, on the other hand, interferes with the cell cycle (Andrade Neto et al. 2018).

Table 16.6 shows the antihypertensive drugs used for specific *Leishmania* species. Nimodipine was shown to cause ultrastructural damage to infected cells. Nifedipine and verapamil are calcium channel blockers that are shown to reduce the number of parasites attached to macrophages and the number of infected macrophages themselves. Verapamil was also found to be useful in treating primary drug-resistant leishmaniasis by inhibiting drug efflux pump proteins in resistant parasites. Amlodipine and Lacidipine have been shown to significantly decrease the spleen and liver size, along with parasite burden in infected patients. Antihypercholesterolemic drugs like Ezetimibe interfere with the ergosterol synthesis pathway of parasites. Propranolol decreases parasite burden and increases CD4+

Table 16.4 Anticancer drugs used to treat leishmaniasis

Drug	Species	Activity	Ref.
Hydroxyurea	*L. mexicana*		Martinez-Rojano et al. (2008)
Cisplatin	All	In vitro *and* in vivo, promastigotes and amastigotes	Kaur et al. (2010), Sharma and Kaur (2013), Tavares et al. (2007)
Carboplatin	Visceral leishmaniasis		Kaur et al. (2013)
Carmustine	*L. donovani*	In vitro	Shukla et al. (2012), Shukla et al. (2011), van den Bogaart et al. (2014)
Mitomycin-C	*L. donovani*	In vitro	Shukla et al. (2012), Shukla et al. (2011), van den Bogaart et al. (2014)
Doxorubicin	Visceral leishmaniasis		Sett et al. (1992), Kansal et al. (2014)
Topotecan	*L. infantum*	Promastigotes and intracellular amastigotes	Prada et al. (2013)
Gimetecan			Prada et al. (2013)
Paclitaxel	Visceral leishmaniasis	Promastigotes and intracellular amastigotes	Moulay et al. (1996)
Sinefungin	*L. donovani*	Promastigotes	Moulay et al. (1996)
Sunitinib	Visceral leishmaniasis	In vitro	Sanderson et al. (2014)
Sorafenib	Visceral leishmaniasis	In vitro	Sanderson et al. (2014)
Lapatinib	Visceral leishmaniasis	In vitro	Sanderson et al. (2014)
Imatinib	*L. amazonensis*	In vivo	Wetzel et al. (2012)
Dactolisib	*L. major*, *L. donovani*	In vitro	Diaz-Gonzalez et al. (2011)
Tamoxifen	*L. major*, *L. infantum chagasi*, *L. amazonensis*, *L. braziliensis*	In vitro and in vivo	Eissa et al. (2011), Miguel et al. (2008), Miguel et al. (2009), Trinconi et al. (2014), Trinconi et al. (2016)
Raloxifene	*L. infantum chagasi*, *L. amazonensis*	In vivo	Reimão et al. (2014)

and CD8+ splenic T lymphocytes producing IFN-y, which helps eliminate the parasites, while atenolol shows ultrastructural changes in infected cells. Sodium nitroprusside increases nitric oxide, TNF-alpha, and 3-nitrotyrosine levels, thus decreasing the number of amastigotes. Ketanserin disrupts ergosterol synthesis in parasites by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A reductase enzyme (Andrade Neto et al. 2018).

Table 16.5 Antidepressant drugs used for specific *Leishmania* species

Drug	Species	Activity	Ref.
Imipramine	*L. amazonensis, L. donovani*	In vitro *and* in vivo	Andrade-Neto et al. (2016a), Mukherjee et al. (2012), Mukherjee et al. (2014)
Clomipramine	*L. donovani, L. major*		Benson et al. (1992), Zilberstein et al. (1990)
Amitriptyline	*L. donovani*		Zilberstein and Dwyer (1984)
Cyclobenzaprine	*L. infantum*	In vitro *and* in vivo	Cunha-Junior et al. (2017)
Sertraline	*L. donovani*	Promastigotes and amastigotes	Lima et al. (2018), Palit and Ali (2008a)
Mianserin	*L. donovani*		Dinesh et al. (2014)
Phenelzine	Visceral and cutaneous leishmaniasis		Evans et al. (1989)
Nialamide	Visceral and cutaneous leishmaniasis		Evans et al. (1989)
Paroxetine	*L. infantum*	Promastigotes	Alberca et al. (2016)
Diazepam	*L. mexicana*	Promastigotes	Dagger et al. (1996)

Table 16.7 shows the antibiotics used for specific *Leishmania* species. Most of these antibiotics have impact against intracellular amastigotes. Macrolides have been shown to affect amastigotes and promastigotes and decrease the parasite load (Andrade Neto et al. 2018).

Table 16.8 shows the antiviral drugs used for specific *Leishmania* species. Tucaresol 36 is an immunomodulator being developed for the human immunodeficiency virus, which was found to reduce *Leishmania* parasite burden and suppress liver amastigotes in some studies. Imiquimod 37 is primarily used for human papilloma virus and is found to be effective against *Leishmania* by releasing nitric oxide in infected macrophages (Smith et al. 2000; Buates and Matlashewski 1999).

Table 16.9 shows the miscellaneous drugs used for specific *Leishmania* species. Several other miscellaneous drugs that work against intracellular amastigotes and promastigotes have been used to treat leishmaniasis, by the process of drug repurposing. These drugs have been shown to decrease the parasite load in several studies. Simeprevir has been effective against *Leishmania* by inhibiting the growth of promastigotes and inducing ROS regeneration. Chlorpromazine inhibits trypanothione reductase and hampers the redox potential for the parasites. Mianserin and mevastatin 47 inhibit the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), required for sterol synthesis of the parasite. Amiodarone 48 and dronedarone 49 inhibit oxidosqualene cyclase (OSC) required for ergosterol synthesis of the parasite (Andrade Neto et al. 2018; Sundar et al. 2012).

Advantages of drug repurposing (Charlton et al. 2018): Drug repurposing is a novel technique that helps in developing new treatment strategies for diseases by

Table 16.6 Antihypertensive drugs used for specific *Leishmania* species

Drug	Species	Activity	Ref.
Azelnidipine, cilnidipine, lercanidipine, nicardipine, nitrendipine	*L. amazonensis, L. braziliensis, L. infantum chagasi*, and *L. major*		Tamargo and Ruilope (2016), Reimão and Tempone (2011), Reimão et al. (2011), Reimão et al. (2016)
Bepridil	*L. chagasi*		
Nimodipine	*L. infantum chagasi, L. major, L. amazonensis*	Promastigote and intracellular amastigote	Tempone et al. (2009)
Nifedipine	*L. donovani*		Ganouly et al. (1991)
Verapamil	*L. donovani, L. tropica*	In vitro, promastigotes and amastigotes	Shokri et al. (2012), Valiathan et al. (2006)
Amlodipine	*L. donovani*	Promastigotes and amastigotes.	Palit and Ali (2008b)
Lacidipine	*L. donovani*	Promastigotes and amastigotes.	Palit and Ali (2008b)
Ezetimibe	*L. amazonensis*	In vitro	Andrade-Neto et al. (2021), Andrade-Neto et al. (2016b)
Propranolol	*L. mexicana*		Garcia-Miss et al. (2015)
Atenolol	*L. major*		Karam et al. (2016)
Sodium nitroprusside	*L. amazonensis*		Genestra et al. (2008), Kawakami et al. (2016)
Ketanserin	*L. donovani*	Promastigotes and intracellular amastigotes	Singh et al. (2014)

making use of already existing drugs being used for different indications. Several explored and unexplored properties of these pre-existing drugs can be used to treat the target disease as explained above. This technique can serve as a timesaving, wholesome, and cost-effective method to treat rare tropical diseases, especially, in resource-deficient environments around the world, where underprivileged population groups are affected. Many of these strategies are not only faster but also with lower side effect profiles compared to the primary drugs.

16.2.6 New Drugs

New drugs under trial are listed in Table 16.10 along with the mechanism of action.

Table 16.7 Antibiotics used for specific *Leishmania* species and stages

Drug	Species	Activity	Ref.
Fluoroquinolones (Enoxacin, ciprofloxacin, ofloxacin, lomefloxacin, and norfloxacin)	*L. panamensis*	In vitro	Limoncu et al. (2013), Cortázar et al. (2007), Romero et al. (2005), Farca et al. (2012), Rougier et al. (2008), Vouldoukis et al. (2006)
Aminoglycosides (streptomycin and tobramycin)	*L. donovani*	Intracellular amastigotes	Navin and Pearson (1987)
Azithromycin	*L. amazonensis, L. braziliensis, L. tropica, L. major*, and *L. infantum chagasi*		Amer et al. (2016), Balcioğlu et al. (2012), Krolewiecki et al. (2002), de Oliveira-Silva et al. (2008, Sinagra et al. (2007)
Clarithromycin	*L. tropica* and *L. donovani*		Balcioğlu et al. (2012), Roy et al. (2013)
Clofazimine	*L. infantum* and *L. tropical*		Barteselli et al. (2015)
Tetracycline	*L. major*		Katiyar and Edlind (1991)
Rifampicin	*L. aethiopica, L. donovani, L. tropica*, and *L. major*	In vitro in intracellular amastigotes	El-On et al. (1983), El-On et al. (1984), Kochar et al. (2006), Livshin et al. (1987), Peters et al. (1981)
Delamanid	*L. donovani* and *L. infantum*	In vitro *and* in vivo	Patterson et al. (2016)

Table 16.8 Antiviral drugs used for specific *Leishmania* species

Drug	Species	Activity	Ref.
Tucaresol	*L. donovani*	In vivo	Smith et al. (2000)
Imiquimod	*L. major*	In vitro	Buates and Matlashewski (1999)

Ongoing clinical trials (Rao et al. 2018). The following drugs are under consideration for the treatment of various forms of leishmaniasis (Fig. 16.3):

Pentoxifylline: This drug is a xanthine derivative primarily used for peripheral vascular disease and works by inhibiting TNF-alpha, thereby reducing inflammation. Pentoxifylline is being studied in combination with meglumine antimoniate and glucantime, respectively, for cutaneous and mucocutaneous leishmaniasis, caused by *L. braziliensis.*

Fexinidazole: This drug is 5-nitroimidazole and is used for trypanosomiasis. It is currently being studied at the level of phase II clinical trial, in vitro and in vivo, to be used for visceral leishmaniasis caused by *L. donovani*, because of its activities against the parasite.

Table 16.9 Miscellaneous drugs used for specific *Leishmania* species

Drug	Species	Activity	Ref.
Disulfiram	Visceral leishmaniasis	Promastigotes and intracellular amastigotes	Peniche et al. (2015)
Cyclosporin	*L. donovani, L. major*	Promastigotes	Meißner et al. (2003), Yau et al. (2010)
Hydroxyzine	*L. infantum*	Promastigotes	Peniche et al. (2020), Pinto et al. (2014)
Omeprazole	*L. donovani*	Intracellular amastigotes	Jiang et al. (2002)
Simeprevir	*L. donovani*		Tabrez et al. (2021)
Cinnarizine	*L. infantum*	Intracellular amastigotes	Reimão et al. (2010)
Chlorpromazine	*L. donovani, L. mexicana, L. aethiopica,* and *L. major*	Promastigotes	Pearson et al. (1984), El-On et al. (1986)
Mianserin	*L. donovani*	Promastigotes and intra macrophage amastigotes	Dinesh et al. (2014)
Naloxonazine		Intracellular amastigote	De Muylder et al. (2011)
Loperamide		Intracellular stage	De Muylder et al. (2011)
Mevastatin	*L. donovani*	Promastigotes and intracellular amastigotes	Dinesh et al. (2015)
Amiodarone	*L. mexicana*	Promastigotes and intra macrophage amastigotes	Serrano-Martín et al. (2009a), Serrano-Martín et al. (2009b)
Dronedarone	*L. mexicana*	Promastigotes and intra macrophage amastigotes	Benaim et al. (2014)
Triclosan	*L. amazonensis*, cutaneous leishmaniasis	In vitro and in vivo, intracellular amastigotes	Mesquita et al. (2020)
Disulfiram	Visceral leishmaniasis	Promastigotes and intracellular amastigotes	Peniche et al. (2015)
Cyclosporin	*L. donovani, L. major*	Promastigotes	Meißner et al. (2003), Yau et al. (2010)
Hydroxyzine	*L. infantum*	Promastigotes	Pinto et al. (2014)
Lansoprazole	*L. donovani*	Intracellular amastigotes	Gupta and Kempaiah (2022)

Table 16.10 New drugs for leishmaniasis

Drug	Species	Chemical class	Mechanism of action	Ref.
DNDI-6148	All *Leishmania* species in vitro and against *L. major* in mice. Currently being developed by DNDI for possible treatment for CL	Benzoxaborole	Inhibitor of leucyl-tRNA synthetase	Mowbray et al. (2021), Van Bocxlaer et al. (2019)
DNDI-0690	*L. major* in an animal model		Reduce the 5-nitro group producing active metabolites	Van Bocxlaer et al. (2019), Wijnant et al. (2019)
DNDI-1047	All *Leishmania* species in vitro and against *L. major* in mice. Currently being developed by DNDI for possible treatment for CL	Nitroimidazoles		Van Bocxlaer et al. (2019)
CPG-D35			Immunomodulator by disrupting parasite DNA, RNA, and protein synthesis	Sangenito et al. (2019)
Chitosan	*L. donovani*	Aminopyrazoles	Antimicrobial by altering the permeability of cell membrane	Varshosaz et al. (2018)

16.3 Drug Discovery Through Rational Improvement

There are more than 20 closely related *Leishmania* species causing leishmaniasis and vary in infectivity, virulence, clinical presentation, and zoonotic reservoir range. The *Leishmania* parasites have a complex life cycle (digenetic) existing as a flagellated promastigote (motile) in the digestive tract of the female phlebotomine sandfly vector and as nonmotile, intracellular amastigotes within the parasitophorous vacuole of the macrophage of the mammalian host within which the parasite survives, replicates, and persists.

The discovery of drugs for neglected tropical diseases is often complicated by various factors such as drug cost, stability in remote places lacking specialization, and the requirement to dose children and pregnant women. The standard drug development process mostly begins with molecular biology and biochemistry to identify and validate molecular drug targets. Frearson et al. in 2007 proposed a Traffic-light definition for target assessment (Table 16.11) (Frearson et al. 2007).

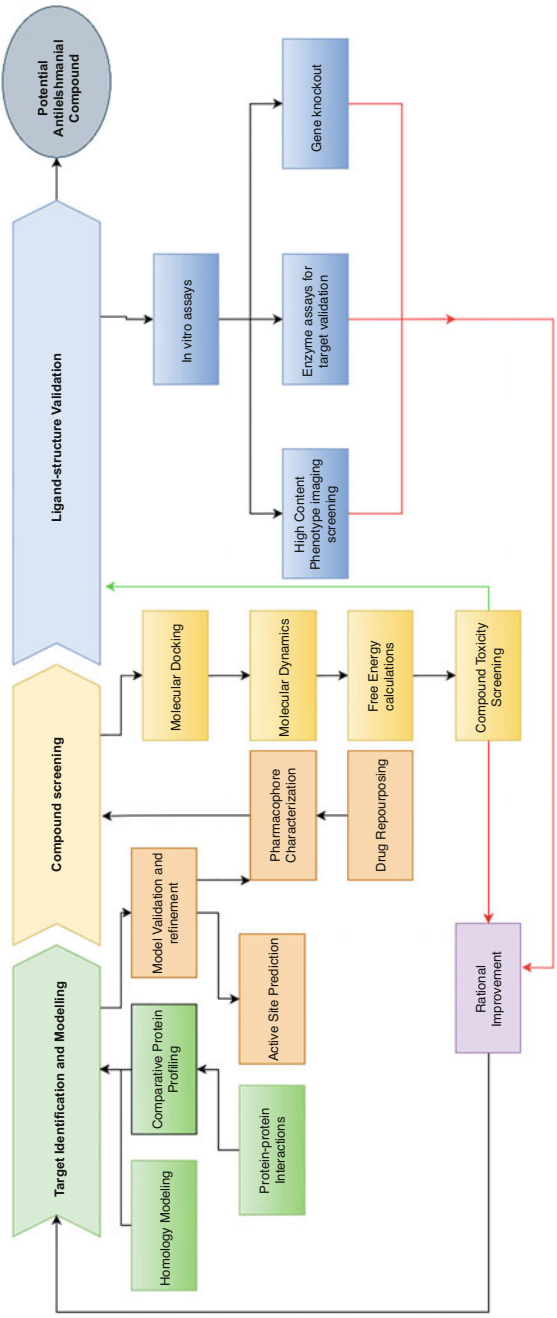

Fig. 16.3 A summary of a common target-based drug discovery pipeline with different approaches for leishmaniasis

Table 16.11 Drug target assessment decision chart (Frearson et al. 2007)

Criterion	Red	Amber	Green
Target validation, i.e., evidence of the target essentiality for growth or survival of the pathogen	No or weak	Either genetic or chemical	Genetic and chemical
Druggability (known inhibitors)	None	Potential (close homolog)	Validated (same genus)
Assay feasibility	No in vitro assay developed or not feasible altogether	Specialized/indirect assay	HTS compatible assay
Toxicity	Human homologs present with the similar active site	Assay with selectivity	No/nonessential human homolog
Resistance potential of target	Multiple genes Copies or isoforms or alternative pathways. Nonessential drug activation/ modification enzymes	Highly similar isoforms are expected to block all, essential drug activation enzyme	Direct inhibition of single gene copy/form target
Structural information	No solved structure of target or closely related homolog	Structure available without ligand/poor resolution (>2.3 Å)/ homology model with C-score of more than 2.1 (I TASSER) (Yang et al. 2015)	High-resolution (<2.3 Å) ligand-bound structure of target/close homolog

These criteria fail in the case of neglected diseases such as leishmaniasis. While treated similarly, the 20 species of pathogenic parasites from genus *Leishmania* have a lot of peculiarities in their genetic compositions, pathobiology, and clinical presentation. Also, very few high-resolution protein structures are known or available, and thus most targets fall in the "Amber" zone (Table 16.11). With new AI-powered protein structure prediction strategies such as "alpha fold," it is believed that the structures as good as crystallographically solved can be constructed (AlQuraishi 2019). However, so far the nearest pathogen covered by alphafold is *Plasmodium falciparum* (Varadi et al. 2021). Also, the AI cannot predict structural changes in the active site during ligand binding, thus limiting virtual screening efficiency. Pink et al. in 2005 defined terminology for compounds at different stages of drug development (Pink et al. 2005). Target assay or antiparasitic active compounds are hits, the animal model tested compounds are leads, and human trial-ready compounds are called drug candidates. Drug development candidate (human trial-ready) is a compound that has emerged from a rigorous lead optimization process and most likely to fulfill the following essential criteria: -

- The in vivo activity comparable or better of standard drugs inanimal models.
- Effective against all species and strains resistant to current drugs.
- Tolerable toxicity/mutagenicity profile.
- Acceptable metabolic profile (toxic metabolite or quick degradation/elimination) in vivo.
- Pass pharmacokinetic checkpoints.
- A cost-effective scale-up is feasible.
- Unraveled mechanism of action synergistic/compatible with current drugs.

The biggest source of drugs in current pipelines of development is high-throughput screening (HTS) (Hu et al. 2008; Reichwald et al. 2008). It is more feasible to screen millions of compounds against host stages (axenic amastigotes) in an HTS format and unravel the mechanisms later. This causes a bottleneck for understanding possible unforeseen side effects in human hosts, including developmental, neuro, or endocrine toxicities that are not frequently assayed before trials. While this is strong advocacy for target-based drug development but in neglected diseases, the lack of advanced proteomic characterizations and structural elucidations makes the classical approach less feasible. The drug target discovery is usually posted discovery of a potent hit against the *Leishmania* or related parasite. This is followed by target-based rational improvement if the chemistry of the hit is amiable for chemical modifications and derivatizations. In case of a complex hit, the pharmacophore is elucidated usually using structural prediction and docking due to lack of crystal structure solved proteins.

The proteomic characterizations and molecular analysis of the *Leishmania* genus have been quite steady. Thus far five leishmanial species, namely, *L. major, L. infantum, L. donovani, L. mexicana,* and *L. braziliensis*, have whole genomes sequenced and annotated. Genomes of *L. major, L. infantum*, and *L. donovani* (subgenus *Leishmania* {*Leishmania*} sp.) consist of 36 chromosomes each. *L. braziliensis*, which has been assigned to a different subgenus *Leishmania* (Viannia) sp., has chromosomes 20 and 34 fused and hence contains only 35 chromosomes. While in *L. mexicana* there are two pairs of fusions (chromosome 8 and 29; chromosome 20 and 36), resulting in 34 chromosomes in total. Even with chromosome number variabilities, all these genomes have on average ~ 8300 functional open reading frames. Based on homology and protein characterization, around 40–45% of these gene products have known assigned functions (Peacock et al. 2007; Smith et al. 2007; Warrenfeltz et al. 2018). Leishmanial genomes consist of several novel metabolic pathways whose enzymes could serve as potential drug targets (Waugh et al. 2014). There are some databases and resources that can be used to shortlist relevant drug targets like the TDR database (Magariños et al. 2012; Urán Landaburu et al. 2020), *Leishmania* small molecule inhibitor database (LeishInDB) (Vijayakumar et al. 2019), Biocyc; LeishCyc database (Doyle et al. 2009), and essential gene database (Luo et al. 2014).

HTS of 1.8 million compounds against *L. donovani* by GSK identified ~67,400 primary hits (4%) with ~32,200 compounds having confirmed activity above the threshold of at least one replicate (Pena et al. 2015). Further ~5500 active

compounds were found highly active against host pathogenesis identical stage (amastigotes) of *L. donovani*, out of which 351 were noncytotoxic. Another major screening was performed by Novartis with 1.5 million initial compounds libraries against three different parasites: *L. donovani, T. cruzi,* and *T. brucei*. This screening resulted in novel antileishmanial and antitrypanosomal agents such as azabenzoxazole and GNF5343 (Singh et al. 2019). Many other such efforts of in vitro HTS with *Leishmania* or closely related parasites have resulted in hundreds of "hit" compounds (Ortiz et al. 2017; Gamo et al. 2010; Duffy and Avery 2012; Sykes et al. 2012). Ex vivo HTS has also been very successful and has generated more than 200 hits (Osorio et al. 2011). A major bottleneck in the hit-to-lead-to-drug candidate pipeline for these hundreds of molecules is a lack of understanding of their mechanism of action (Ortiz et al. 2017). Target(s) identification for the hit compounds is the steppingstone for rational medicinal chemistry or pharmacophore-based virtual screening workflows for ligand improvement in terms of target selectivity, and desirable pharmacokinetics and toxicity profiles matching the clinical presentation, specific bioavailability in the case of *Leishmania*. Also, a known mechanism of action enables optimal clinical utilization and molecular monitoring for efficacy among field strains and tackling resistance.

There are different approaches to unravel targets of hits. Untargeted metabolomics relative quantifications of hundreds of small molecules ($<$ 1500 Da) in vitro under subinhibitory drug concentrations is an upcoming method (Creek and Barrett 2014). This method revealed the target of antimonials (Antimony (III)) in *L. infantum,* which is amino acid depletion due to oxidative stress (Canuto et al. 2014). Another most popular method is through developing a drug-resistant mutant. Whole-genome sequencing is performed with both drug-susceptible and -resistant lines, which only differ on resistance-conferring mutation. This helps immensely to understand mechanistic interactions of the drug (Catta-Preta and Mottram 2018).

There are numerous successful SAR and QSAR studies in developing potent antileishmanial compounds. But to focus on the theme of target-based drug discovery, i.e., target–ligand interaction improvement, we are not discussing these methodologies as it is comprehensively covered elsewhere (Ferreira and Andricopulo 2018; Kwofie et al. 2020; Rivas and Gil 2017; Mowbray 2017).

16.3.1 Virtual Screening against Novel Targets

Virtual screening (VS) is an in silico computational workflow used to fish out matching inhibitors by mimicking chemistry interactions and energy principles. This method has now much better performance with ever-increasing computational power. Advance molecular strain calculations such as molecular mechanics with generalized Born and surface area solvation MMGBSA, MD simulations, and free energy perturbations have greatly enhanced the performance of virtual screenings. For the proteins or their near homologs with a structural characterization, virtual screening is the most cost-effective and rapid method for populating a target-specific hit population. One good example of this approach is γ-glutamylcysteine synthetase

(Gcs, EC 6.3.2.2) inhibitors populated by virtual screening and validated in vitro (Agnihotri et al. 2017). In another study of phosphoglycerate mutase, the available crystal structure was used and compounds were shortlisted by virtual screening against 0.1 million compounds. Many potent antileishmanial agents were discovered with validated enzyme inhibition although their host toxicity parameters were not reported (Fuad et al. 2016). Spermidine synthase (SS) is an enzyme involved in polyamine synthesis, which is an important component of DNA and protein synthesis. Grover et al. found highly selective potent molecules by virtual screening (Grover et al. 2012). Trypanothione reductase (TR) targeting is an example of target repurposing. The same enzyme is responsible for thiol-redox balance and is one of the proposed targets of antimonial compounds. While many in silico prospects have been proposed, there is one compound [6-bromo-3-(4-methoxybenzoyl)-2H-chromene-2-thione] inhibiting amastigotes in the micromolar range (Rai et al. 2022).

Carbohydrate metabolism is very important for an intracellular parasite like *Leishmania* for energy generation. While most of the enzymes from these pathways are highly conserved throughout all kingdoms of life, there are some differences in the binding site architecture from the human host for selective drug targeting. For example, sodium fluoroacetate (NaFAc) was previously reported to selectively inhibit the TCA cycle enzyme aconitase in *Leishmania* (Saunders et al. 2011). There are multiple successful rationally designed compounds targeting the three primary enzymes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Aronov et al. 1999; Aronov et al. 1998), pyruvate kinase (PyK), (Nowicki et al. 2008), and hexokinase (da Matta et al. 2015).

Another important target is amino acid metabolism. Again, due to the intracellular life cycle, *Leishmania* has to scavenge for simpler lipids and amino acids and synthesize the required complex ones. Several antileishmanial compounds have been populated by virtual screening against proteases. But due to lack of specific assays, the target validation is pending and none have progressed to animal testing due to either inadequate potency or toxicities. Epigenetic modulators that inhibit histone deacetylase (HDAC) enzyme responsible for chromosomal coiling and banding topology.

There is a new upcoming trend in drug discovery to have multi-target compounds. One of the prominent examples is spiro-acridine compounds interacting with both essential enzymes trypanothione reductase (TryR), which keeps the intracellular redox balance of the parasite, and DNA topoisomerases (LdTopoI), participating in the regulation of DNA supercoiling (Almeida et al. 2021). LdTopoI is also inhibited by a novel molecule GF1061outperforming AmpB in animal studies (Tavares et al. 2019).

The in silico pipelines that include target–ligand interaction-based strategies are widely used in industry and academia complementary to experimental techniques (Broni et al. 2021; Kwofie et al. 2020; Ekins et al. 2011). Target fishing has been implemented in numerous instances to discover the mechanism of the biological effect of compounds arising from HTS (Erić et al. 2012). The virtual screening starting point is a reliable and high-resolution protein crystal structure that is a

recognized therapeutic target essential for parasite survival and pathogenesis. Several synthetic pathways unique to the parasite metabolome have been the focus of interest, e.g., ergosterol synthesis, hypusine/putrescine (polyamine) synthesis, and folate pathway (nucleic acid synthesis). Several pathways are highly similar to humans but are having structural differences in the components making them specifically targetable, e.g., topoisomerase, histone deacetylase, mitogen-activated protein kinase, and glycosylphosphatidylinositol (GPI) synthesis. Some have parasite-specific functions required for intracellular survival, e.g., transporters of ions/nucleosides or amino acids, proteases, enzymes of thiol metabolism, and methylglyoxal (mutagenic metabolite) reduction pathway.

Table 16.12 represents major virtual screening efforts that have resulted in potent hits/leads against leishmaniasis.

16.3.2 Discovery of New Drug Targets

Unraveling targets for antiparasitic compounds discovered through various screening methods is paramount to further develop those hits into leads through target-based rational improvement. While the hit might have significant concentration dependent inhibition for it to be considered a hit, variables critical for a "lead" such as a potency level, host toxicity, solubility/bioavailability, and sustainable scale-up synthesis dictate a need for target-based rational improvement. There are a variety of methods used for *Leishmania* target identification applied by different groups (Tables 16.13, 16.14, 16.15, and 16.16).

Thus, while the methods are highly specific to the drug–target relationship, often an integrated approach has a satisfactory result.

16.3.3 Rational Drug Improvement

A few reports have performed target-based rational improvement of compounds (Table 16.17). This lack of studies is mostly due to the absence of experimentally solved structures for in silico interaction studies (Fig. 16.4).

16.4 Conclusion

Leishmaniasis remains a leading cause of parasitic disease worldwide with an increased burden of morbidity and mortality primarily placed on low- and middle-income countries. Advances in therapeutic modalities and vaccines have been slow due to problems intrinsic to the causative organism as well as numerous external factors. Several compounds are in the development pipeline for leishmaniasis drug discovery. Many of these compounds have a strong candidature to become the next treatment option alone or in combination with other existing or newer and unique target proteins encoded by parasite genomes. Many researchers have successfully

Table 16.12 A few successful virtual screening pipelines with hits potent in vitro below a sub-micromolar (nanomolar-range) activity markup

Compound(s)	Molecular target	*Leishmania* spp.	Methods	Biological validation	Ref.
Benzoxaboroles.	Carbonic anhydrase	*Leishmania donovani*	Homology protein modeling virtual screening	Enzyme assay	Nocentini and Supuran (2018)
Quinoline-carbaldehyde derivatives	Methionine aminopeptidase 1	*Leishmania donovani*	Homology protein modeling virtual screening	Antiparasitic testing and enzyme assay	Bhat et al. (2020)
BTB13319	Type 2 NADH dehydrogenase (LiNDH2)	*Leishmania amazonensis*	Protein crystallization virtual screening	Antiparasitic testing	Mishra et al. (2017)
	Nucleoside diphosphatase kinase (NDK)				

Table 16.13 Novel compounds with unraveled in silico validated targets, being tested in vitro as antileishmaniasis

Compound(s)	Molecular target	*Leishmania* spp.	Methods	Software used	Ref.
Suramin	Arabinono-1,4-lactone oxidase	*L. donovani*	3D modeling Model refinement	Robetta 3D refine and ModLoop	Adinehbeigi et al. (2020)
Undefined ligand 18	Glyceraldehyde-3-phosphate dehydrogenase	*L. mexicana*	3D pharmacophore Molecular docking Molecular dynamics	ZINCPharmer DOCK & AutoDockVina AMBER	Alves et al. (2020)
Benzimidazole and benzoxazole derivatives	Dihydrofolate reductase-thymidy-late synthase, pteridine reductase 1, and Myo-inositol-1-phosphate synthase	*L. donovani*	Homology modeling Pharmacophore modeling Docking Molecular dynamics QSAR	Modeller Discovery studio CHARMm, CDOCKER, PyRx, FlexX & iGEMDOCK Desmond OMEGA	Kapil et al. (2019), Sinha et al. (2020), Cabrera et al. (2021)
Staurosporine	DEAD box RNA helicase, TRYR, and PEPCK.	*L. braziliensis* and *L. infantum*	Protein–protein and protein–ligand networks	Fpocket tool- to find active site Cytoscape- determine the essentiality of protein	Rezende and dos Santos Vasconcelos (2021)
Lansoprazole, Posaconazole, Iloprost, mupirocin and other FDA approved drugs	Calcium transporting ATPase, lanosterol 14-alpha-demethylase, mitochondrial primase	*L. donovani*	Drug screening and docking Target prediction and sequence analysis Molecular dynamics	LigPrerp- Schrödinger COACH, I-TASSER Stitch, Swiss target prediction, MolTarPrep, super prediction, target hunter Desmond- Schrödinger	Rai et al. (2021)

5' Iodotubercidin, PP2, 42, NSC 699479	*Leishmania* casein kinase 1.2	*L. donovani*	Target-based compound screening Toxicity assay Target deconvolution	Swiss-model PyMOL	Durieu et al. (2016)
Riluzole	Pteridine reductase (PTR1)	*L. major*	Virtual screening Target analysis Target docking	Insight II GRID GOLD and GLIDE	Ferrari et al. (2011)

Table 16.14 Compound validation through gene expression. Expression downregulated (−), upregulated (+)

Compound	Target	Genes	Exp.	*Leishmania* spp.	Ref.
CPE2	Lmj_04_BRCT domain	Cyclin, α-tubulin, Yip 1, ABC transporter H1	–	*L. major*	Peña et al. (2021)
AN2690	Leucyl-tRNA synthetase	LdLRS	–	*L. donovani*	Manhas et al. (2018)

Table 16.15 MoA deconvolution through gene expression, drug network, and pathway annotations

Bioactive compound	Proposed target(s)	*Leishmania* spp.	Method	Brief MoA	Ref.
Riluzole	Pteridine-reductase 1 (PTR1)	*L. mexicana* and *L. major*	Protein pathway assays	Reduction in the oxidation of NADPH which is catalyzed by PTR1	Guerrieri et al. (2013)
N/A	FCGR4, CCL4, CXCL9, Arg1, and IL-1β	*L. major*	• Pathway annotation using expressed genes in mice models • Gene interaction network	Triggering receptor expressed on myeloid cells 1 pathway upregulated in infected mice	Ulusan et al. (2020)

repurposed approved drugs, especially the ones working against other parasites. There is an urgent need to accelerate structural protein research to support drug discovery programs.

Future Perspectives: Advancement in cheminformatics tools, *in silico* validation, and synthetic methodologies allows for rapid improvement of existing and suboptimal drug candidates against the known target. The traditional drug discovery strategies do not always produce highly active compounds due to factors like bioavailability, lack of knowledge on specific targets, or the presence of alternate mechanisms bypassing the blocked target. As such, computational techniques, including visualization, transition state modeling, *ab initio* (quantum mechanics), and molecular dynamics (MD), are being currently used by chemists and biologists for predicting the chemical–structural properties of biomolecules. For example, molecular docking is a computational procedure that attempts to predict the non-covalent binding of "drug-like" molecules (ligands) to larger macromolecules (receptors). An underlying principle of molecular docking is that ligands with similar properties can be rationalized and used to design new similar bioactive compounds. Therefore, the identification of compounds expected to be active against a given target can be justified. These tools can be implemented as a frontline in the design

Table 16.16 Target identification through genetic manipulations or protein profiling

Gene target	Method of discovery	Accession number (s)	*Leishmania* spp.	Ref.	Gene target
1. EF-1β and 2. Tryparedoxin peroxidase	Comparative protein profiling	1. AY763288-GenBank 2. AY753537-GanBank	*L. guyanensis*	Walker et al. (2006)	1. EF-1β and 2. Tryparedoxin peroxidase
LdGS	1.Cloning of protein nucleotide sequence 2. Gene knockout	KT907048.1-GenBank	*L. donovani*	Kumar et al. (2017), Kumar et al. (2021)	LdGS
LdLRS	Gene replacement	XP003859311.1-GenBank	*L. donovani*	Manhas et al. (2018)	LdLRS

Table 16.17 Novel compounds with unraveled in silico validated targets, being tested in vitro as antileishmaniasis

Compound(s)	Molecular target	*Leishmania* spp.	Methods	Activity	Ref.
Chlorhexidine	β-N-Acetylhexosaminidases	*L. donovani*	3D modeling MD simulations and structural refinement	4.0 μM	Dong et al. (2019)
Benzimidazole derivatives	Cysteine-protease type 2 (CPB2.8 ΔCTE)	*L. mexicana*	Ligand improvement		De Luca et al. (2018)
Various	Topoisomerase 1 from *L. donovani* (LdTop1)	*L. donovani*	Scaffold hopping and bioisosteric manipulations	3.51 μM	Mamidala et al. (2016)
Quinolones	Tryparedoxin peroxidase	*Leishmania major*	Docking and rational modifications in ligands	39 μM	Brindisi et al. (2015)

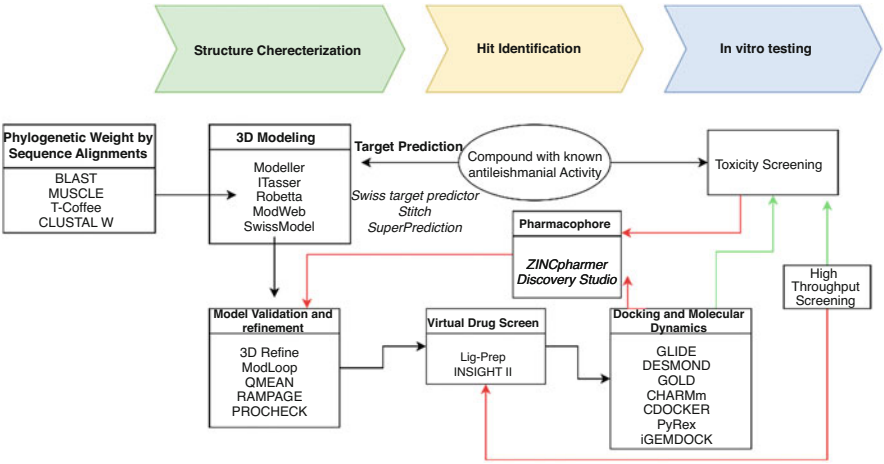

Fig. 16.4 Summary of in silico drug screening pipeline with common software and servers used

and synthesis of new lead molecules based on the interaction of scaffolds with active sites of the target proteins in the parasite. It is also critical to remember that the rationally improved compounds should undergo in vitro evaluations as well as be validated by selection through mutant development to confirm the mechanism of action. It is also important that a target-based rational improvement strategy for drug discovery involves good interactions, to begin with, and thus evades non-synthesizability, cross activities, and possible toxicities of parent compounds. Besides, based on in silico evaluations, it is critical to implement chemical modifications (high-valued bioactive scaffolds) on the identified hits to secure more potent compounds with improved activity, specificity, and efficacy. Usually, ligand improvement studies are undertaken to modify a known drug to work against resistant strains and variants of the target protein. In this chapter, we have illustrated advanced strategies to conduct ligand improvement to achieve maximum efficacy and host tolerance, in the beginning, process to generate highly efficient drug molecules to replace or use in combination with current antileishmanial drugs that are failed to achieve complete cure.

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Versatile Structurally Diverse Natural Products and Their Semisynthetic Analogs as Potential Antileishmanial Drugs

17

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Abstract

Mortalities associated with leishmaniasis are skyrocketing at an alarming rate. Lack of vaccine for leishmaniasis treatment is making the fight against the disease an arduous one. Interestingly, apart from the expensive nature of leishmaniasis treatments, the mono- and combinational chemotherapeutic agents suffer

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_17

drawbacks such as resistance, synergism resistance, systemic toxicity, and ineffectiveness. Due to this, the new paradigm proposed for combating this canker is an efficient therapeutic antileishmanial agent with multitarget inhibitory properties. Though natural products and their derivatives have long been known for their medicinal properties for treating various ailments, little is known for their multimodality antileishmanial effects. A plethora of structurally diverse natural products, their IC_{50}, and their biological targets of inhibition are, therefore, discussed in this book chapter. Structural modifications of these natural products based on pharmacophoric analysis leading to the semi-synthesis of their derivatives are also presented. Additionally, a view on metallodrugs using these versatile natural products and their derivatives coordinated to transition metals geared toward leishmaniasis treatment is proposed.

Keywords

Leishmania parasite · Pharmacophoric analysis · Chemotherapeutic agents · Synergism resistance · Toxicity

17.1 Introduction

Perpetuated by numerous and evolutionary diverse infectious agents, neglected tropical diseases (NTDs) are now one of the major medical and psychosocial problems with a huge socioeconomic burden on low- and middle-income countries around the globe (https://www.paho.org/en/topics/leishmaniasis 2021). Commonly referred to as the diseases of the poor mainly due to the low economic conditions of the people affected, leishmaniasis is one of the devastating NTDs whose current frontiers constitute a major challenge for modern medicine (Okwor and Uzonna 2016; Alvar et al. 2006; Pascual Martínez et al. 2012). Latest estimates indicate that approximately 350 million people are said to be at risk (https://www.who.int/news-room/fact-sheets/detail/leishmaniasis 2021). Additionally, current mortality rate is between 26,000 and 65,000 annually with most deaths recorded in the Asian diaspora, followed by Africa and the Mediterranean basin (https://www.who.int/news-room/fact-sheets/detail/leishmaniasis 2021). Aside the parasite type, gender and age of the victim, poor housing conditions and malnutrition are risk factors that contribute to the development of leishmaniasis (Okwor and Uzonna 2016). However, the three main clinical spectrum, cutaneous (CT), mucocutaneous (MCT), and visceral (VL) as well the post kala-azar (darkening of conspicuous physical lesions after healing from VL) can be date back as far as the pre-Incan civilization period (WHO 2010). Economically, cost of illness studies purports that leishmaniasis puts strain on the already meagre salaries of households in endemic regions (Bern et al. 2008). For instance, in 2016, the direct medical and nonmedical costs incurred by a household affected by VL in Sudan was estimated to be $760 (Sunyoto et al. 2019). This expenditure is expected to rise, signaling a prospected severity and the need for

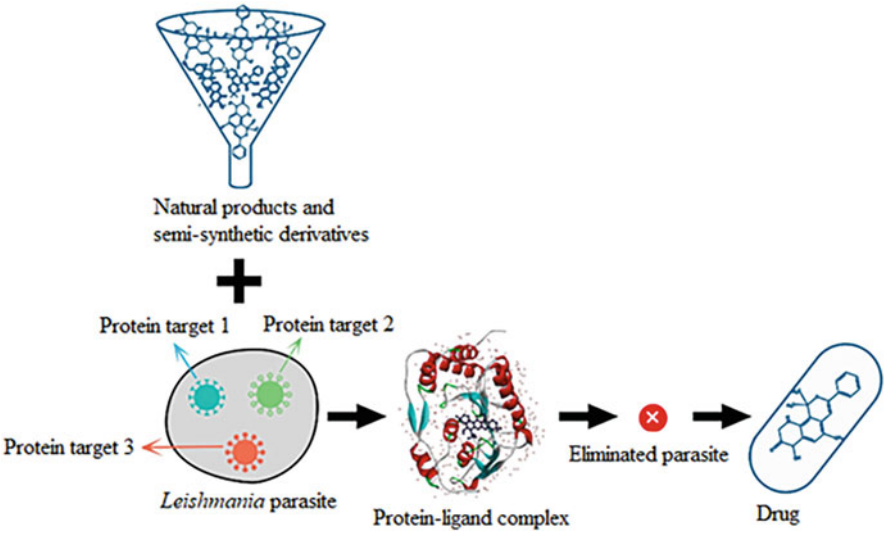


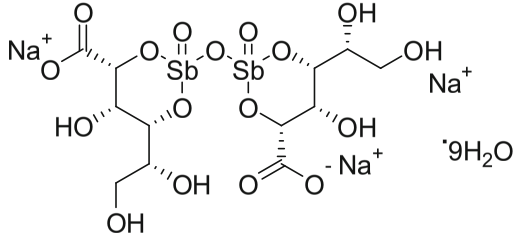
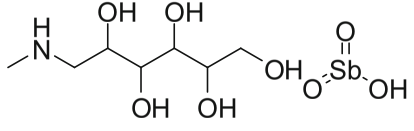
Fig. 17.1 Mode of action of structurally diverse natural products and their semisynthetic derivatives as multitarget antileishmanial agents

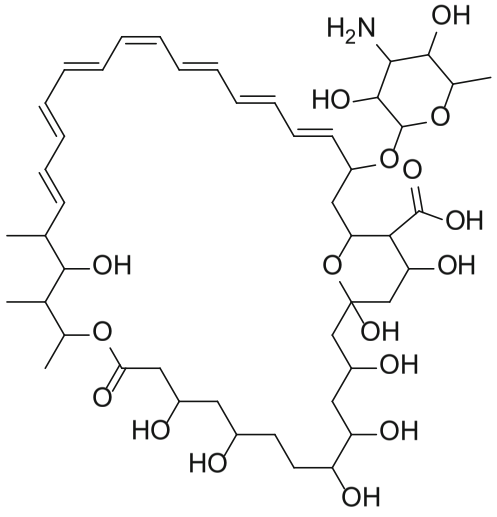
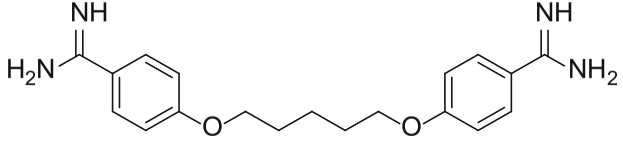
a solution to prevent global spread and increased number of disability-adjusted life years (Bern et al. 2008; Sunyoto et al. 2019) (Fig. 17.1).

Concerted efforts from the scientific community in combating leishmaniasis (cutaneous, mucosal, and visceral) has hovered around four general approaches, namely, diagnostics, vector control agents, vaccines, and drugs, with the latter been the most promising and invested venture (Davies et al. 2003). For more than a decade, the use of chemotherapy against leishmaniasis has been based on pentavalent antimonials (sodium stibogluconate, **1** and meglumine antimoniate, **2**) (Table 17.1), which are inhibitors of trypanothione and glutathione reductase (Kumar et al. 2018).

However, several setbacks with the use of this first-line regiment resulted in the introduction of alternatives like amphotericin B, **3**, pentamidine, **4**, paromomycin, **5**, and the first oral antileishmanial miltefosine, **6** (Table 17.1). These second- and third-line drugs remain ineffective even though efficacy is improved when administered in combination with other drugs compared to their use as monotherapeutic agents. This notwithstanding, compliance difficulties, including the long-standing treatment time, synergism resistance, and toxicities/side effects, have been recorded, making them unfavorable for combating the disease. In view of this gloomy picture associated with the current drugs available for leishmaniasis treatment and in the absence of an effective vaccine, there is the urgent need for new chemotherapeutics with multitarget modulation potentials. This is so because only a well-concerted multipronged approach is more likely to achieve the desired therapeutic effect in infected patients.

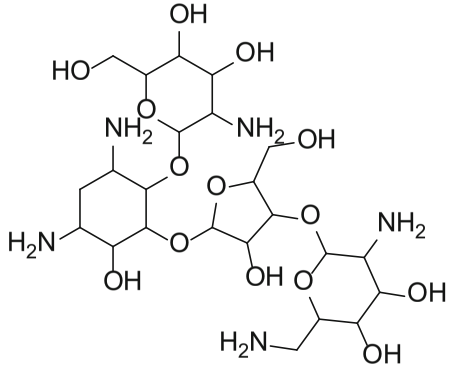
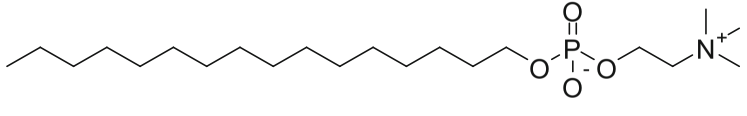
Table 17.1 Various classes of organic and organometallic compounds used as drugs for leishmaniasis treatment and their side effects

Chemotherapeutic agent	Class of organic compound	Mode of action	Side effects	References
 Sodium stibogluconate, **1**	Dicarboxylic acid	Inhibition of trypanothione reductase	Pancreatitis, cardiac arrhythmias	Kumar et al. (2018), Singh et al. (2003), Wishart et al. (2018)
 Meglumine antimoniate, **2**	Hexoses	Inhibition of trypanothione reductase	Pancreatitis, cardiac arrhythmias	Kumar et al. (2018), Singh et al. (2003), Wishart et al. (2018)

 Amphotericin B, **3**	Aminoglycosides	Suppresses ergosterol biosynthesis by complexing with the 24-substituted sterols	Fever, renal complications	Wishart et al. (2018), Sundar et al. (2007)
 Pentamidine, **4**	Phenol ether	Bind to kinetoplast DNA	Pancreatitis, cardiac arrhythmias, gastrointestinal side effects	Wishart et al. (2018)

(continued)

Table 17.1 (continued)

Chemotherapeutic agent	Class of organic compound	Mode of action	Side effects	References
 Paromomycin, **5**	4,5-disubstituted 2-deoxystreptamines	Affect mitochondrion structural integrity	Reversible ototoxicity	Wishart et al. (2018), Jhingran et al. (2009)
 Miltefosine, **6**	Phosphocholines	Inhibition of phosphatidylcholine biosynthesis	Teratogenicity in pregnant women	Wishart et al. (2018), Soto and Soto (2006)

17.2 Natural Products: An Easy Source of Versatile Compounds for Leishmaniasis Treatment

Aside from the complete comprehension of the disease biology in the process of drug development, the source of molecules out of which a drug is discovered is also of great concern. Comparatively, a structurally diverse chemical space increases the chances of discovering new drugs as well as return on investment. The term "drug," used to designate a medicinal preparation for disease treatment, began with the use of herbs (Wadud et al. 2007). Historical records of this, including the use of De Materia Medica, the first encyclopedia related to herbs compiled by the Greek physician Pedanius Diocorides, were as far recorded in the first century after AD (Yarnell and Touwaide 2019). For centuries, traditional medicine has recorded milestone achievements in the fight of debilitating human diseases (Patridge et al. 2016; Ogungbe et al. 2012). Yet in the last two decades, drug discovery approaches have drifted from the exploration from natural sources to the small synthetic compounds (Li and Vederas 2009). Advances in synthetic approaches such as combinatorial chemistry, and computational de novo drug discovery strategies, as well as high-throughput screening (HTS) techniques have become the competitive mainstay for discovering novel drug leads. However, despite these advances, there is still lack of better approaches to effectively harness the full potentials of pharmacological agents from mother nature. It is evident from established literature on approved drugs that natural products still remain the main source for structurally diverse chemotypes suitable for usage as drugs and drug candidates for the treatment of debilitating human diseases (Patridge et al. 2016; Ogungbe et al. 2012; Mishra and Tiwari 2011). In fact, contemporarily, over 80% of the population in developed countries still rely on traditional medicinal practices to meet their primary health-care needs (Karunamoorthi et al. 2021). Additionally, over 69% of new small molecules currently used in chemotherapy have their origin from natural products (Patridge et al. 2016; Ogungbe et al. 2012). Undeniably, the chemical space occupied by natural products is vast and versatile compared to synthetic opponents and even those from diversity-oriented synthesis (Gu et al. 2013). Regardless of the late start of antileishmanial natural products research which became of topical interest during the mid-1980s, natural products still hold the most, percentage wise, in terms of drugs at the forefront of leishmaniasis treatment (Moore and Lockwood 2010; Nagle et al. 2014). With the emergence of high attrition in drug discovery and the skyrocketing rate of the current panorama (leishmaniasis cases) in the clinics, new drugs with multi-inhibitory potentials are needed in great demand, and natural product, an easy source of prototypes, cannot be afforded to be left out.

17.3 Versatile Natural Products and Their Semisynthetic Derivatives as Potential Antileishmania Drugs

Till date, an effective immune protection against leishmaniasis has still not been established. Emerging resistance against the hand few repurposed drugs available for leishmaniasis treatment poses a great threat (Sundar and Singh 2018). Arguably, the developing resistance by *Leishmania* parasites has the potential of destroying the efficacy of current antileishmanial drugs in few years to come. Over the years, new alternatives capable of increasing the efficiency of new drugs has been one of the aims of medicinal chemists. The reductionist drug discovery paradigm "one drug one target-based" has been successful in producing potent drugs (Duval 2018). However, its effectiveness for diseases with convoluted pathogenic mechanisms observed for diseases like leishmaniasis signals the urgent need for new drugs, especially ones that have multitargeting potentials. Despite the challenge posed in the past, off-target effects of single-target drugs has helped in the identification of crucial novel targets within the same and parallel disease pathways. Designing compounds that can modulate multiple targets of relevance to the survival of the disease-causing organism has recently gained prominence in the drug development hub. Apart from the effectiveness associated with this approach of drug design, there is that added advantage of the disease-causing organism finding it difficult to develop resistance. Given this new paradigm, repositioning natural products through the demonstration of their multitargeting efficacy will be a great turning point in molding the rising resistance against current treatment strategies of leishmaniasis. In view of the aforementioned scenarios, this chapter details out the current developments regarding structurally diverse biologically potent natural products and their semisynthetic analogs as potential antileishmanial drugs against all types of *Leishmania* protozoans.

17.3.1 Phenolics

Aside from the consumption of a large number of phenolics in our everyday diet from vegetables and fruits, ethnobiological use of phyto-phenolics in the prevention and treatments of both infectious and noninfectious maladies has been long reported (Pandey and Rizvi 2009). Convincing evidence from pharmacological studies indicates that phenolics remain one of the most versatile natural product pipelines for the development of new drugs (Tungmunnithum et al. 2018). Among the various subtypes, chalcones, flavanoids, biflavones, and synthetic aurones are the most active. However, one prominent flavanoid with promising efficacy in the termination of proliferation for both flagella and intracellular forms of *Leishmania* is quercetin, **7** and its analogs quercetrin, **8** and isoquercitrin, **9** (Fig. 17.2) (Da Silva and Maquiaveli 2012). Studies by various research groups have reported numerous leishmanicidal mechanisms of action for quercetin and its derivatives (Mohajeri et al. 2018). From arginase and trypanothione reductase inhibition, Fe and Mn^{2+} sequestration, topoisomerase I dysfunction, ribonuclease reductase to mitochondria

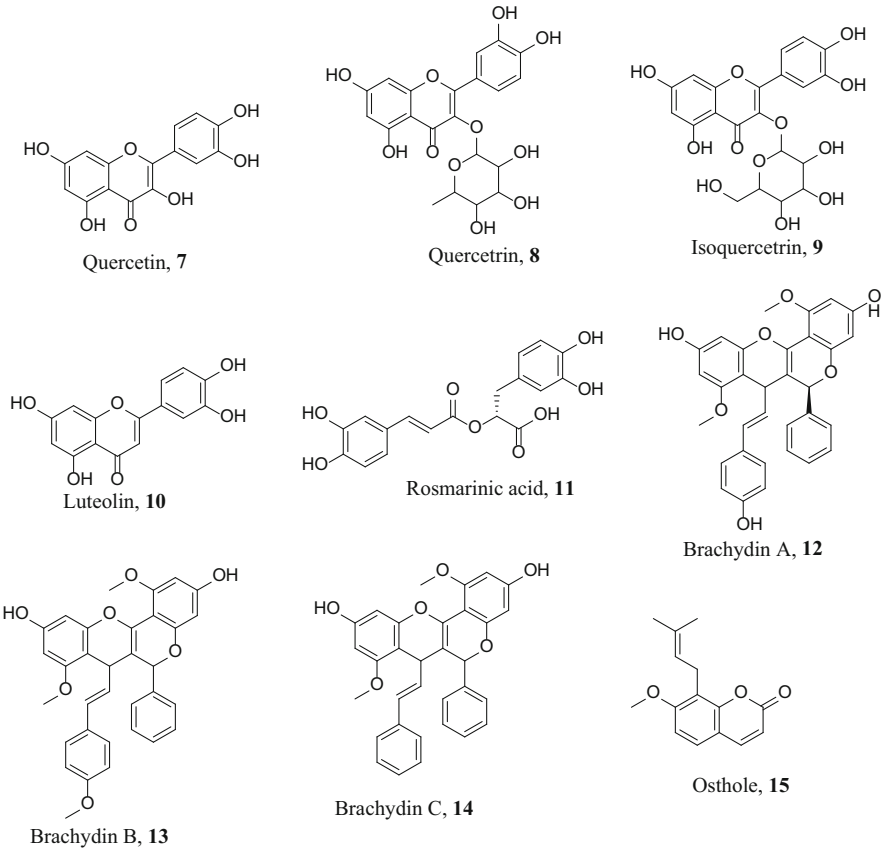


Fig. 17.2 Structurally diverse natural products phenolic compounds and their analogs with antileishmanial potential

ultrastructure destruction as a result of respiratory chain block (Da Silva and Maquiaveli 2012; Mohajeri et al. 2018). But overall, the different multitargeted nature of quercetin derivatives induces parasite death via DNA machinery collapse and mitochondrion potential depolarization (Fonseca-Silva et al. 2011).

Interestingly, another antiparasitic compound worthy of further clinical investigations is the flavanoid luteolin, a dehydroxyl derivative of quercetin. Luteolin, **10** also displays high affinity toward arginase, trypanothione reductase, and its synthetase amidase just like quercetin. At an IC_{50} of 12.5 μM, luteolin suppresses growth of *L. donovani* promastigotes and amastigotes in vitro via inhibition of topoisomerase II (Mittra et al. 2000). Aside from the metal chelation property of rosmarinic acid, **11** impairs growth, an eventual cause of death of *Leishmania* parasite (Antwi et al. 2019). It has also been reported to induce apoptosis with changes in mitochondria integrity and DNA structure at 19.21 μM against *L. donovani* amastigotes (Antwi et al. 2019).

Phytochemical analysis of *Arrabidaea brachypoda,* a native plant used for different therapeutic purposes in Brazil, displayed significant antiprotozoal potency, which signaled natural products chemist to develop an efficient solvent system for the isolation and characterization of the responsible chemotypes (Rocha et al. 2019). In vitro assessment of isolated compounds disclosed only Brachydin B, **13** and C, **14** to be active against *L. amazonensis* amastigotes out of the Brachydin series (A-C) (Fig. 17.2) at 2.20 μM and 6.25 μM, respectively (Rocha et al. 2019). As a known component of most traditional Chinese medicine, Osthole's **15** anti-infective potential against bacteria, fungi, and helminthics prospects it as a good antiparasitic agent (Sun et al. 2021). An effort to improve the efficacy of current antileishmanial regimens led to the evaluation of the antileishmanial potential of the prenylated coumarin, **15**. Its leishmanicidal activity against intracellular amastigote forms of *L. major* was about 61 μM (Kermani et al. 2016). In addition, in vivo studies indicated that, despite the low recovery of treated mice, **15** significantly reduced lesion progression in BALB/c mice compared to the untreated mice (Mandlik et al. 2016). Chalcones, open chain flavonoids characterized with two aromatic rings and an αβ enone, has been reported in numerous antiprotozoals studies (Rammohan et al. 2020). Commonly known as Chinese licorice, Licochalcone A, **16** from *Glycyrrhiza* spp. and its derivatives (**17–19**) (Fig. 17.3) have also displayed strong activity against *L. donovani* amastigotes within sub-micro molar range (Zhai et al. 1999). Their corresponding IC_{50} values were 0.9 μM, 0.4 μM, 0.8 μM, and 0.7 μM, respectively. Results from in vivo studies with **18** and **19** in mice and hamsters infected with *L. donovani* showed significant reduction of the parasite load in the liver (97 and 84%, respectively) and the spleen (88 and 70%) at 5 mg/kg after 6 days of intraperitoneal administration (Zhai et al. 1999). Reported efficacy of naphthoquinones to induce topoisomerase II-mediated DNA cleavage inspired the need to assess the efficacy of three isolated naphthoquinones from the Bolivian plant *Pera benensis* (Santd et al. 1992). After several in vivo trials, 8,8- biplumbagin, **22** demonstrated an inhibition of parasite proliferation at 50 mg/kg/day, which was similar to the drug Glucantime, **2** at 400 mg/kg/day (Santd et al. 1992).

Study was conducted to discover the promising antitrypanosomal potency of dehydrodieugenol B, **23** and its methyloxy analog, **24** isolated from *Nectandra leucantha*. It was found that dehydrodieugenol B, **23** was inactive, but the introduction of the methoxy group at C4 increased the antileishmanial potential against amastigote forms of *L. infantum* (Grecco et al. 2018). Similarly, structural modification using synthetic functionalization approach involving the hydrogenation of the terminal alkene and the substitution of the C5 methoxy group of **23** with a benzyloxy moiety afforded **25.** Out of the four sets of the novel semisynthetic derivatives, only **25** was potent against the amastigotes form of *L. infantum* at 6.1 μM (Amaral et al. 2019). In short, autophagosome formation and the impairment of the cell division cycle visualized from ultrastructure investigation can be ascribed to the destruction of mitochondria and dysfunction of DNA machinery (Amaral et al. 2019).

In an in silico study, 19 multitargeting polyphenolics consisting of 6 flavonoids, 1 isoflavonoid, 3 lignans, 5 coumarins, 1 chalcone, 2 aurones, and 1 stilbenoid after a screening of 352 phyto-phenolics against 24 *Leishmania* enzymes were reported

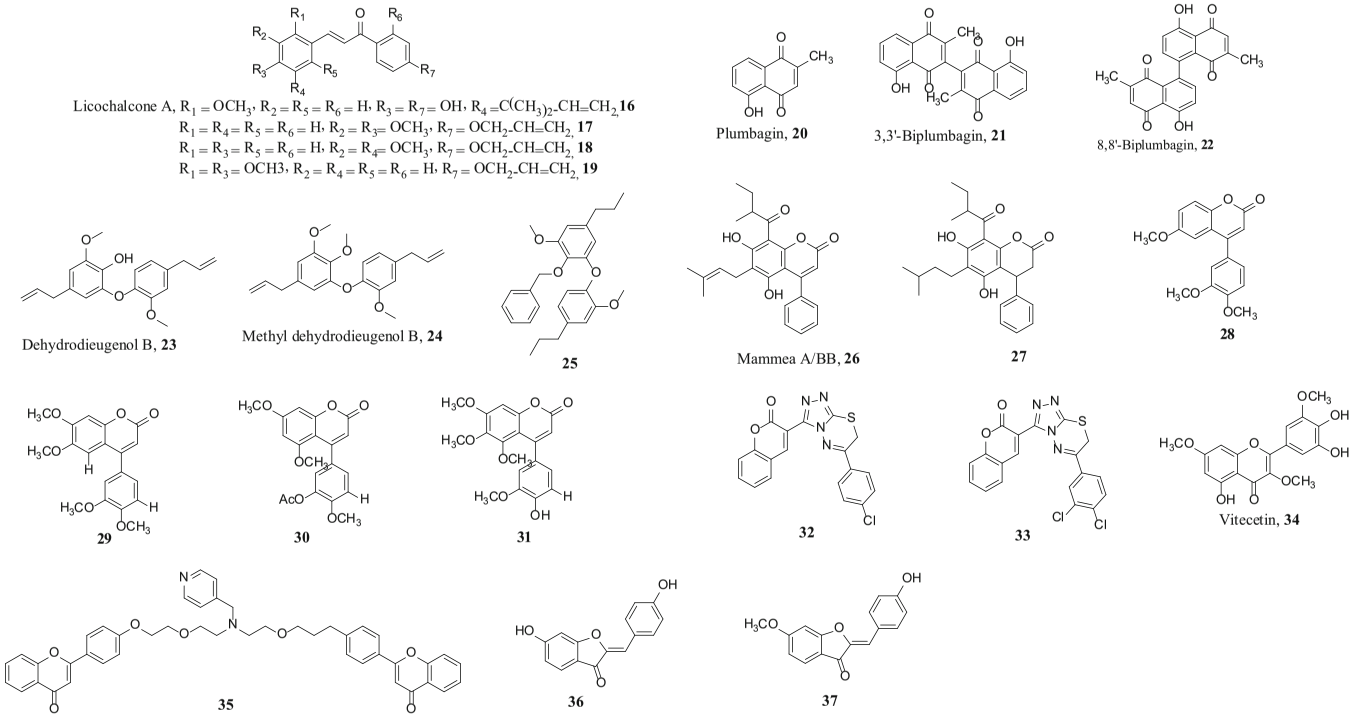


Fig. 17.3 Chalcones, naphthoquinones, coumarins, aglycone, and other polyphenolic compounds evaluated for their multitarget antileishmanial properties

(Ogungbe et al. 2014). Among the 19, one of the most promising was mammea A/BB, **26**, a coumarin isolated from *Calophyllum brasiliense* dichloromethane (DCM) crude extracts. Suppression efficacy of **26** against promastigote and amastigote forms were 7.38 and 2.16 μM, respectively (Brenzan et al. 2007). Due to the promising candidacy of **26**, structure modifications via methoxylation and hydrogenation reactions afforded 4 derivatives. The most potent derivative, **27**, suppressed half of both flagella and intracellular forms with a concentration of 0.9 μM and 0.6 μM, respectively (Brenzan et al. 2008). Investigating the antiprotozoal activity of 4 derivatives (**28–31**) (Fig. 17.3) synthesized from 4-arylcoumarins via the Suzuki-Miyaura cross-coupling reaction, amastigote susceptibilities were 5.4 μM, 1.1 μM, 2.6 μM, and 2.4 μM, respectively (Pierson et al. 2010). Coincidentally, the most active, **29** doubled as the most selective to the parasite (Pierson et al. 2010). Evaluating the influence of mono- and di-chlorination on the coumarin-triazolothiadiazine hybrid, the activity of the para-chloro substituted hybrid, **32** displayed a half inhibitory concentration of 2.86 μM, three times higher than the dichloro-substituted derivative **33** (IC_{50} of 0.89 μM) while the positive control amphotericin B was 0.47 μM (Ibrar et al. 2016). In silico studies revealed that **32** and **33** (Fig. 17.3) exhibit their antileishmanial and anticancer properties via inhibition of alkaline phosphatase, a non-specify phosphomonoester hydrolase required for regulating the functions of many biological systems (Ibrar et al. 2016).

Assessing the antileishmanial activity of Vitecetin **34**, a polyhydroxylated aglycone isolated from *Vitex peduncularis*, revealed that **34** pulled down half of *L. donovani* promastigotes and amastigotes at 2.4 μM and 0.93 μM with a selectivity index of 52 and 133, respectively (Rudrapaul et al. 2014). Vitecetin, **34** was found to induce potent host-protective response by activating nitric oxide (NO) and inducible nitric oxide synthase 2 (iNOS2) expressions in infected macrophages, helping to prevent progression of *Leishmania* parasite (Rudrapaul et al. 2014). Due to wide applications as anti-oxidative, anti-inflammatory, antimutagenic, and their ability to modulate key cellular enzyme functions, flavanoids are considered indispensable agents to the medicinal and synthetic chemists. To improve upon the leishmanicidal strength of flavanoids, a series of structurally related flavonoid dimers were synthesized and characterized to assess their antileishmanial activity as well as their toxicity toward cell lines (Wong et al. 2012). Structure activity relationship (SAR) findings revealed that the insertion of a pyridine ring between polyethylene glycol (PEG) linker groups boosted antipromastigotes activity (Wong et al. 2012). Additionally, modifications via linker length shortening, changes in attachment position of flavones to the PEG linker, different substitutions on the flavone core, and further tuning of pyridine ring at the amino PEG linker led to the discovery of one potent compound **35** (Fig. 17.3) (**Wong et al.** 2012). Though its mode of action is yet to be elucidated, **35** showed very consistent and formidable antipromastigotes activity at IC_{50}, ranging from 0.13 to 0.21 μM, while its antiamastigotes showed IC_{50} 0.63 μM (Wong et al. 2012). A strong selective antipromastigotes activity was found with no toxic effect on macrophage RAW 264.7 cell line (Wong et al. 2012).

Based on the close structural similarity between aurones and chalcones, both are expected to share equivalent activities. In 2002, the first series of synthetic aurones were tested in vitro (Kayser et al. 2002). Findings from that study postulated that **36** and **37** have pronounced activity at 6.6 μM and 0.1 μM against *L. donovani* amastigotes (Kayser et al. 2002). Apart from aurones known for disrupting fungal cell walls, **36** and **37** inhibited parasite growths by suppressing mitochondrion fumarate reductase (Kayser et al. 2002).

17.3.2 Alkaloids

From their use in culinary practices to their prominence in the clinics, alkaloids have played and still play an essential role in human medicine and animal defense. As of October 2020, the Dictionary of Natural Product repository contained a total of 27,683 alkaloids (Heinrich et al. 2021). However, yearly trend analysis indicates a decline in the number of marketed alkaloids since 2014 with the biological activity of a large majority of these characterized alkaloids not known (Heinrich et al. 2021). This shortage with rippling effect all across the drug design hub has prompted the search of novel alkaloids and their semisynthetic analogs to meet medical needs. After the first β-Carboline (pyrido indole) was discovered in 1841, numerous analogs within the family have been characterized (Laine et al. 2014). Overall, β-carbolines display a fascinating diversity in structure and pharmacological potential with examples including the harmine, annomontine, and manzamine families. Besides their strong antitumor activity, natural β-carbolines have also demonstrated potent antiparasitic activity against different *Leishmania* strains. In a survey of the manzamine family, pioneering isolates, including manzamine A, **38** and the likes of manzamine E, **39**, manzamine X, **40**, 8-hydroxymanzamine A, **41**, 6-deoxymanzamine X, **42**, and manzamine F, **43** (Fig. 17.4) from Indonesian marine sponges, demonstrated excellent antileishmanial potency at half inhibitory concentrations of 0.9, 3.8, 5.7, 6.2, 3.2, and 4.2 μM, respectively, against *L. donovani* promastigotes (Rao et al. 2003). Apart from inhibiting trypanothione reductase, manzamine and its derivatives also suppress DNA synthesis via intercalation of DNA base pairing (Ashok et al. 2015). In addition, mitochondria dysfunction resulting in cell death via apoptotic pathways is also postulated (Banoth et al. 2020). Exploration of how nature selectively engineers pharmacophoric groups toward particular proteins led to the characterization and identification as leads, manzamine A N-oxide, **44**, 6-hydroxymanzamine E, **45**, neo-kauluamine, **46**, manzamine J, **47**, Ircinal A, **48**, and Ircinol A, **49** (Fig. 17.4) from *Acanthostrongylophora* sp. with strong antiproliferative effect against *L. donovani*. Presented activities were 2.5, 4.2, 25, 4.6, 0.9, and 4.2 μM, respectively (Rao et al. 2006). Some other more alkaloids of same manzamine group with good prospects include 12,28-oxamanzamine A, **50** and 12,28-oxa-8-hydroxy-manzamine A, **51**, which unleashed a moderate antileishmanial activity at 7.8 and 24 μM, respectively (Rao et al. 2006).

Leveraging the structural attributes of natural products to increase potency led to the investigation to find out the influence of peptides on both β-carboline and its

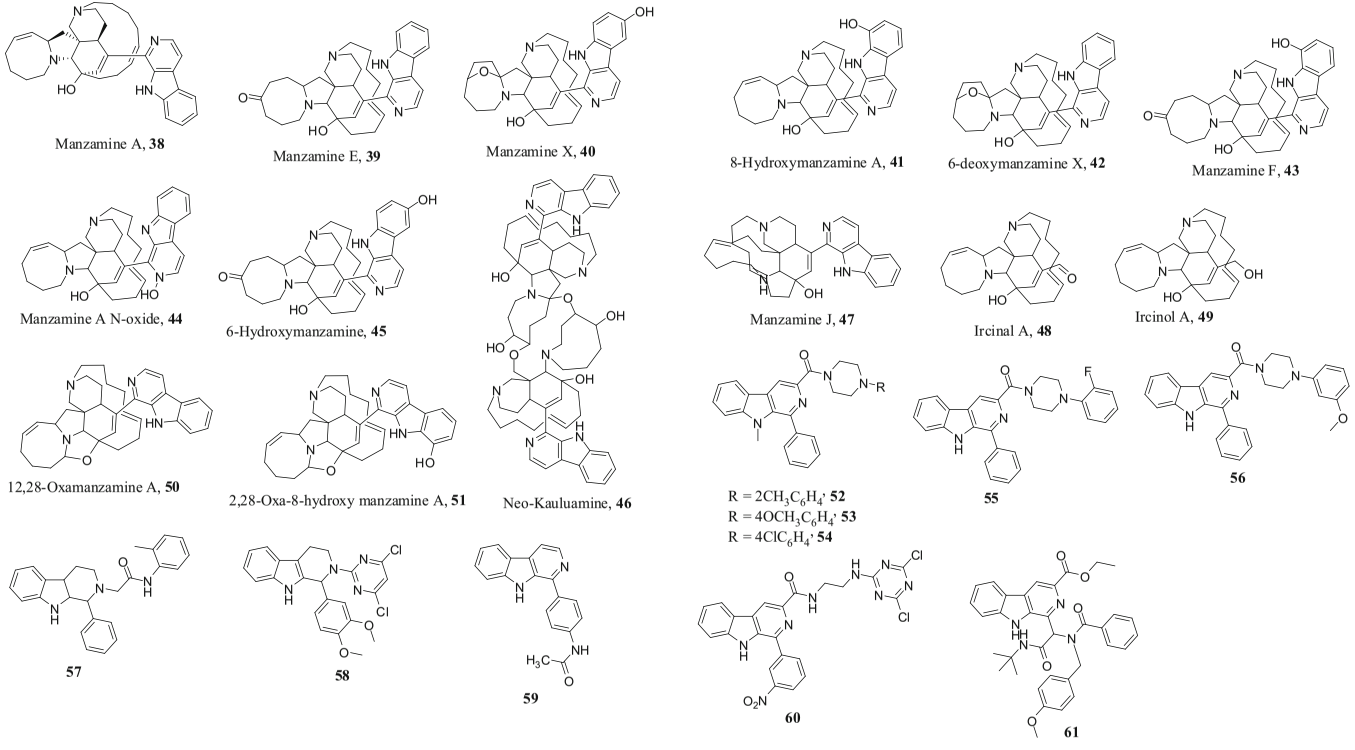


Fig. 17.4 Structurally diverse natural products alkaloids and their semisynthetic analogs as multitarget antileishmanial agents. In a series of synthetic β-carbolines synthesized, only three sets of piperazinyl-β-carboline-3-carboxamide derivatives (**52**–**54**) significantly inhibited the proliferation of *Leishmania infantum* (Ashok et al. 2019). Their EC_{50} values were 1.59, 1.47, and 3.73 μM, for promastigotes and 1.4, 1.9, and 2.6 μM, respectively, for the amastigotes (Ashok et al. 2019). Evaluating the antileishmanial potential of (1-phenyl-9H-pyrido[3,4-b] indol-3-yl) (4-phenylpiperazine-1-yl) methanone derivatives against

tetrahydro derivative via molecular hybridization approach (Khan et al. 2019). Report from the studies disclosed **61** among the potent compounds to possess significant antileishmanial activity against intracellular amastigotes of *L. donovani* at IC_{50} 2.43 μM. Elucidating the possible targets inhibited upon β-carboline treatment, results depict morphological and ultrastructural alterations, depolarization of the mitochondrial membrane, as well as loss of membrane integrity, and an increase in the formation of mitochondrial radical species leading to apoptosis (Baréa et al. 2018; Chauhan et al. 2015).

Apart from the obese β-carbolines, lower molecular weight indole alkaloids like harmaline and coronaridine have also been shown to possess strong antileishmanial activity. Among the aforementioned metabolites, harmaline, **62** (Fig. 17.5), the most potent, showed amastigote-specific activity at IC_{50} of 1.16 μM, while Coronaridine, **63**, which is postulated to possibly affect the energy metabolism of *Leishmania*, terminated 97% of the *Leishmania* parasite at 35.5 μM (Di Giorgio et al. 2004; Delorenzi et al. 2001). Several isoquinoline as leads for antiparasitic drug discovery has been published in literature, and one notable member of this group whose medicinal properties have been widely explored is berberine (Alamzeb et al. 2021). Evaluation of the efficacy of Berberine, **64** and its semisynthetic analog, **65** against in vitro models of leishmaniasis discovered that while **64** suppressed growth of *L. amazonensis* at 17 μM, **65** effectively eliminated the parasite at 0.18 μM, reiterating the potency of analogs (Bahar et al. 2011). Similarly, assessing the potency of **64** and its natural derivatives (palmatine **66**, columbamine **67**, 8-trichloromethyldihydroberberine **68**, and jatrorrhizine, **69**) (Fig. 17.5) against *L. tropica* based on their promising antiparasitic prospects, they all displayed excellent activities at IC_{50} values of 1.50, 2.31, 2.56, 1.40, and 2.44 μM with reported cell death induced through a caspase-independent apoptosis (Alamzeb et al. 2021).

Ramiflorine A, **70** and B, **71**, monoterpenoid indole alkaloids isolated from *Aspidosperma ramiflorum* stem bark, also showed significant antileishmanial activity against promastigotes of *L. amazonensis* with LD_{50} of 35 μM and 10.5 μM (Cunha et al. 2012). Like the isoquinolines, numerous naphthylisoquinolines from nature and the synthetic bench have been evaluated against *Leishmania* parasites (Ibrahim and Mohamed 2015). Employing a recyclable Fe-pillared interlayered clay

Fig. 17.4 (continued) the two forms of *L. infantum* showed 2 promising candidates (**55** and **56**) with EC_{50} values of 3.47 μM, 2.89 μM (promastigotes) and 2.8 μM, 2.80 μM (axenic amastigotes) (Ashok et al. 2018). Similar studies identified a new lead from the tetrahydro-β-carboline derivative **57** whose antileishmanial activity was at 0.67 μM and selectivity index >298.5, comparable with that of amphotericin B, which was used as a positive control (Ashok et al. 2016). With the same tetrahydro-1H-β-carboline precursor was reported a derivative **58** to exhibit significant antileishmanial activity with an IC_{50} value of 4.23 mM against amastigotes (Kumar et al. 2010). Replacing the alpha hydrogen of β-carboline with an aryl amide afforded **59** with notable activity (IC_{50} of 2.16 μM) more than that of miltefosine 2.07 μM (Gohil et al. 2012). In vitro antileishmanial screening reported compound **60** out of the novel β-carboline-1,3,5-triazinehybrids to exhibit potent activity against both amastigotes and promastigotes of *L. amazonensis*. IC_{50} values were 1.1 μM and 5.1 μM for both forms (Baréa et al. 2018)

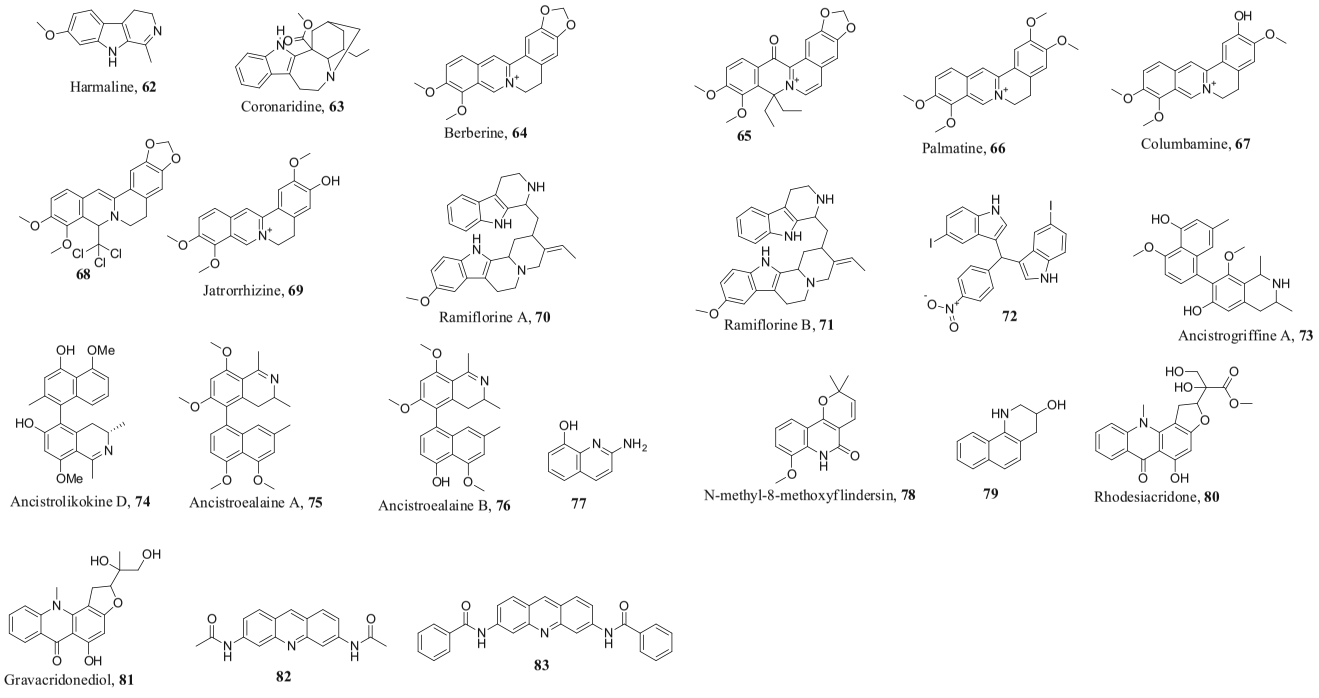


Fig. 17.5 Carboline, indole, isoquinoline, and naphthylisoquinoline alkaloids and their derivatives with multitargeting effects against *Leishmania* parasites efficacy against the parasite with EC_{50} of 1.0 and 1.6 μM and 5.2 and 3.4 μM for promastigote and amastigote forms, respectively (Suarez et al. 2020)

(Fe-PILC) as catalyst for the synthesis of 3,3′-diindolymethanes derivatives afforded 3,3′-((4-nitrophenyl) methylene) bis(5-iodo-1H-indole), **72** to be the most potent compound with IC_{50} values of 7.88 and 8.37 μM against *L. donovani* promastigote and amastigote forms, respectively (Bharate et al. 2013).

Ancistrogriffine A, **73** isolated from the leaves and twigs of *Ancistrocladus griffithii* revealed its in vitro activity against *L. donovani* with IC_{50} of 7.9 μM (Bringmann et al. 2002). Similar studies elucidated the structure of ancistrolikokine D, **74** from the roots of *A. likoko* of same *Ancistrocladus* genus (Bringmann et al. 2003). Its antileishmanial activity against promastigote forms of *L. donovani* was 15.1 μM, as pentamidine displayed IC_{50} of 16.2 μM (Bringmann et al. 2003). Other naphthylisoquinoline alkaloids from the same genus were ancistroealaine A, **75** and ancistrotanzanine B, **76** with the former inhibiting promastigotes of *L. donovani* at an IC_{50} of 9.8 μM in comparison to the highly active latter at IC_{50} of 3.9 μM (Bringmann et al. 2000). SAR studies of quinoline alkaloids with different groups attached to the α-C indicated amino derivative, **77** as the most promising compound against both CL and VL forms. Among the synthetic derivatives of the quinone alkaloid, N-methyl-8-methoxyflindersin, **78**, isolated from the leaves of *Raputia heptaphylla* with activity of 7.4 μM against *L. panamensis,* only two compounds, **77** and **79** from the series had improved antiparasitic.

Within the alkaloid family, one group with underreported antileishmanial activity is the acridones. Efforts to evaluate the in vitro potential of rhodesiacridone, **80** and gravacridonediol, **81** against promastigotes and amastigotes of *L. major* displayed activity at (30.7 and 96 μg/mL) and (54 and 97.2 μM), respectively (Ahua et al. 2004). Among the few synthesized cluster of acridones reported, two promising chemotypes were disclosed (Di Giorgio et al. 2007). The most active compound **82** demonstrated a strong affinity for both parasite forms at half inhibitory values of 1.1 μM and 4.3 μM for amastigotes and promastigotes, respectively, while **83** (Fig. 17.5) prepared via the Ullman reaction displayed a very selective antileishmanial activity at IC_{50} values of 20.1 μM against promastigote forms, and 4.3 μM against the amastigote form (Di Giorgio et al. 2007). Acridines and their semisynthetic derivatives exhibit their mechanism of action against *Leishmania* parasites by inhibiting thymidine, attenuation alterations at the ultrastructural level in the mitochondria, and also DNA intercalation to induce death (Mesa-Valle et al. 1996).

17.3.3 Terpene and Sterols

Synthesized from the mevalonic pathway and in some instances via the methylerythritol phosphate pathway, terpenes are classified based on the number of isoprene makeup. Pharmacological active terpenes were first noticed in the 1960s; since then the rising pace of terpene research and its contribution to disease treatment has speedily accelerated (Yang et al. 2020). Due to their distinctive structural

features and their wide anti-infective activity, terpenoids have been of interest to many medicinal and synthetic chemists.

Screening extracted metabolites from *Taxus baccata*, 10-Deacetylbaccatin III, **84** (Fig. 17.6) killed the intracellular form of *L. donovani* at 70 nM with no signs of toxicity against human macrophages up to 5 μM (Georgopoulou et al. 2007). In vitro antileishmanial activity evaluation of artemisinin-derived trioxanes and synthetic trioxolane derivatives against *L. infantum* promastigotes posited **85** as the most effective among the series at IC_{50} of 3.51 μM (Cortes et al. 2015). Similarly, a SAR study of artemisinin derivatives to improve upon efficacy via the modification of the carbonyl group at C-10 afforded the fluoro-artemisinin derivative, **86** with a better efficacy of IC_{50} 0.38 μM compared to the other 19 derivatives (Chollet et al. 2008). To fully characterize the chemotype behind the antileishmanial effect of *Tridax procumbens* methanol extract, findings from the isolate screening assay indicated the efficacy from oxylipin, **87**, which exhibited significant in vitro activity against intracellular amastigotes of *L. mexicana* at 0.48 μM (Martín-Quintal et al. 2010).

In vitro antileishmanial activity of eupomatenoid-5, **88** from leaves of *Piper regnellii* displayed a dose- and time-dependent inhibition against promastigote, axenic amastigote, and intracellular amastigote forms of *L. amazonensis* with equivalent IC_{50} values of 30.5 μM, 44.2 μM, and 17 μM, respectively (Vendrametto et al. 2010). Investigating the antileishmanicidal activity of the chloroform extract from *Plumeria bicolor* led to the isolation of plumericin, **89** and isoplumericin, **90** (Fig. 17.6) (Singh et al. 2011). Activity studies of the isolates portrayed significant antileishmanial activity against *L. donovani* promastigotes and amastigotes by 8**9** at IC_{50} of 3.17 μM and 1.41 μM, respectively (Singh et al. 2011). Its analog, **90** exhibited a moderate activity at IC_{50} of 7.2 μM and 4.1 μM (Singh et al. 2011). Extensive efforts to discover new saponins with clinical use against leishmaniasis led to the discovery of Maesabalides III, **91** and IV, **92** from *Maesa balansae*, whose in vitro antileishmanial activity was found at IC_{50} values of 4.6 nM for **91** and 9.4 nM for **92**, respectively (Germonprez et al. 2005). Oral administration of purified Maesabalides revealed 95% parasite reduction in mouse livers after a day of treatment with 0.4 mg/kg/day (Germonprez et al. 2005). Another plant-derived saponin, Racemoside A, **93** was found to be effective against *L. donovani* promastigotes with an IC_{50} value of 1.25 μM (Dutta et al. 2007). However, toxicity studies revealed **93** to be toxic to human macrophages when administered above 9.5 μM (Dutta et al. 2007). In an in silico examination to evaluate the antileishmanial effects, pathenolide, **94** demonstrated anti-promastigotes and anti-amastigote activities at 1.49 μM and 3.26 μM (Tiuman et al. 2005). Validating the widespread use of iridoid in traditional medicine, Amarogentin, **95** from *Swertia chirata* was reported to inhibit the activity of topoisomerase I of *L. donovani* in tested hamster animal models at a dose of 2.5 mg/kg/day for 3 days (Medda et al. 1999).

Taking into consideration the fact that *Cistus* sp. are widespread in the Mediterranean basin, investigating the antileishmanial activities of secondary metabolites and their synthetic derivatives from *Cistus creticus* disclosed three compounds, **99**, **100**, and **101** (Fig. 17.6) to be active and selective against *L. donovani* at IC_{50} values

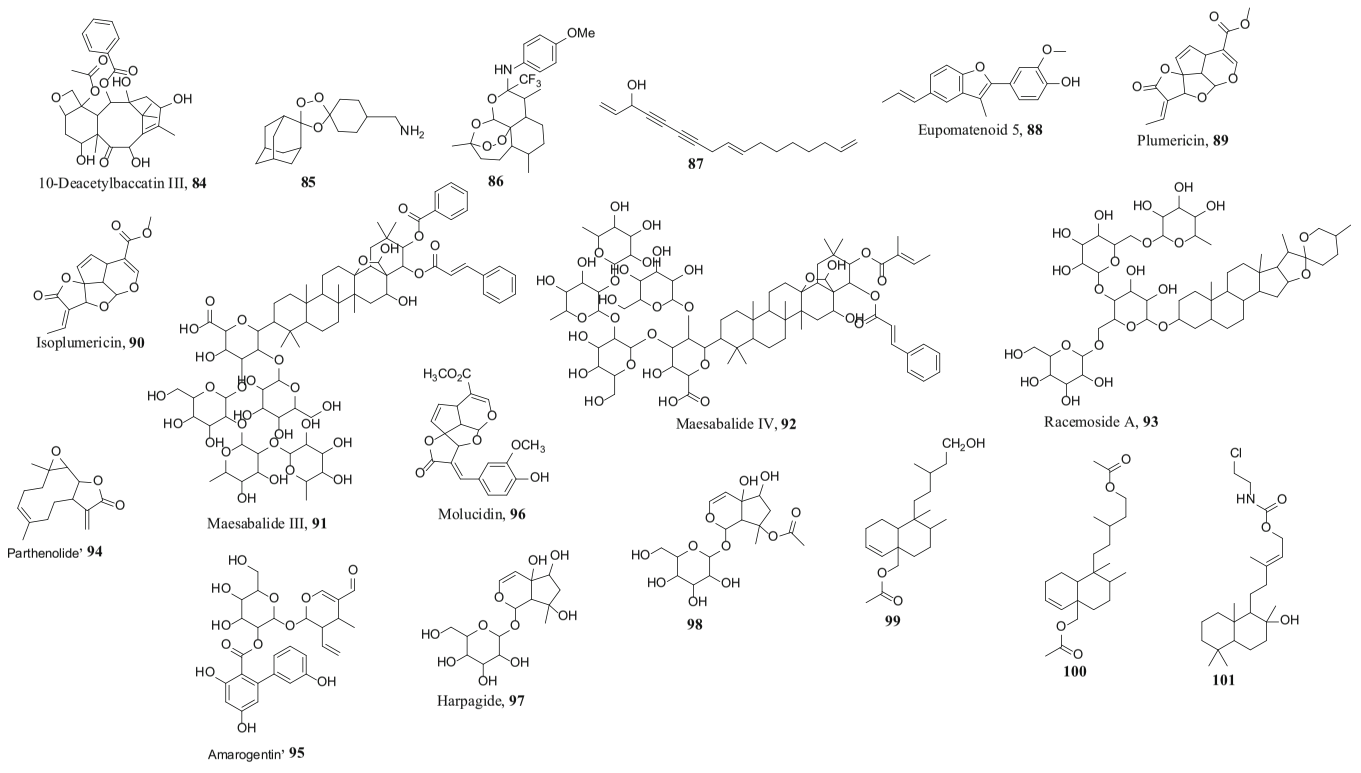


Fig. 17.6 Versatile and structurally diverse natural products terpenes and sterols with potential against multiple targets of *Leishmania* parasites. Past studies have reported the antiprotozoal potential of *Morinda lucida*. Among the three tetracyclic iridoids isolate from its stem, only Molucidin, **96**, the methyl derivative, demonstrated promising activity against promastigotes and amastigotes of *L. major* and *L. donovani* at (1.85 μM and 1.77 μM) and (2.94 μM and 0.9 μM), respectively (Azerigyik et al. 2018). In an effort to explore the Turkish flora, antileishmanial activities of plant extracts from the *Scrophularia* species posited strong potentials. Harpagide, **97** and its acetylated derivative, **98** was isolated from *S. cryptophila* and assayed against *L. donovani* axenic amastigotes. Observed IC_{50} values were 13.4 μM and 17 μM for **97** and **98**, respectively (Tasdemir et al. 2008). Toxicity estimation against L6 cell lines depicted zero cytotoxicity on the cells (Tasdemir et al. 2008)

of 9.8 μM, 9.0 μM, and 8.5 μM, respectively (Fokialakis et al. 2006). All together, intense swelling of the mitochondrion and multiple cytoplasmatic vacuolization, which were visualized in morphological studies, as well intercalation of DNA observed among most isolates appear to be responsible to parasite death (Fokialakis et al. 2006).

17.4 Antileishmanial Multitarget Metallodrugs Involving Versatile Natural Products and Their Derivatives in Coordination with Transition Metals

Despite their demonstrated application in catalysis process, the usefulness of organometallic compounds in medicine has been well documented and can be traced as far back to the start of the eighteenth century when bismuth was used in the treatment of diseases, including fever, rheumatism, and syphilis among others (Keogan and Griffith 2014). Since then, new therapeutic agents needed in treating various diseases, infectious and noninfectious, have involved metallodrugs. Antimony complexes such as sodium stibogluconate, **1** and meglumine antimoniate, **2** are the first-line treatment for leishmaniasis (Kumar et al. 2018). Aside from these, other organometallic complexes involving transition metals such as V, Cu, Co, Mn, Ni, and Au have all been evaluated for the leishmaniasis treatment amid improved efficacy (Aripirala et al. 2014; Christensen et al. 2016; Ong et al. 2019). This notwithstanding, there is still lack of a metallodrug with a multitarget potential against *Leishmania* parasite. With multitargeting as the new paradigm in drug design, and the appreciable level of efficacy of metallodrugs, coordinating these aforementioned versatile natural products and their semisynthetic derivatives with transition metals will most possibly be the game changer in combating leishmaniasis.

17.5 Conclusion

Despite the advances in research on various species of *Leishmania* as well as host–parasite interactions, the available treatment options still suffer drawbacks such as inefficiencies, resistance, and toxicity. A versatile new drug, which would allow a safer, shorter, and cheaper treatment with easy oral administration, is urgently needed. Natural products by their structural complexity, large chemical space, and safety provide secure means of finding multitarget activity drugs against *Leishmania* parasites. This book chapter, therefore, highlighted structurally diverse secondary metabolites and their semisynthetic derivatives with multimodality properties evaluated as potential chemotherapeutic agents against leishmaniasis. The mechanism of action exhibited by these compounds includes inhibition of parasite through interacting with parasite's specific growth, thymidine, topoisomerase II, trypanothione, and glutathione reductase. Some of the compounds also induce death via attenuation alteration of the mitochondrion and DNA intercalation. Collectively, integrating active motifs from natural products into semisynthetic analogs

would create drugs with potent multitarget, resistance proof, low toxicity to host cell, and cost-effective natural therapies.

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Promising Compounds of Plant Origin and Their Synthetic Analogs Against Trypanosomes

18

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Abstract

There are about 20 species of the genus *Trypanosoma*, but among them only a few subspecies—*Trypanosoma brucei rhodesiense, Trypanosoma brucei gambiense*, and *Trypanosoma cruzi* mainly cause disease in humans. The main causative agent of human African trypanosomiasis (HAT) or sleeping sickness is *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*. HAT disease is a parasitic disease spread by vectors and has serious health and financial issues in rural sub-Saharan Africa. Infections by *Trypanosoma* sp. are prevalent in many countries of Mexico, Africa, and Central and South America, which can cause serious public health and epizootic problems. Several control programs pertaining to decreasing the disease risk are carried out in infection-prone areas; for example, vector eradication and treatment of infected people by drug are in operation in these regions. These control strategies are not that successful in solving the problem of trypanosomiasis due to several reasons which stand in the way of effective control. Chemoprophylaxis is an effective way of treatment in

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_18

these areas, but it is not an effective preventive measure. Several drugs that were discovered for the treatment in the early twentieth century, like pentamidine, tryparsamide, atoxyl, and suramin, were incapable of crossing the blood–brain barrier in substantial amount to avoid the recurrence of trypanosomiasis at an advanced stage, which primarily concerned with parasite gradual invasion in the central nervous system. Till today, no vaccines are available in order to prevent the transmission of trypanosomes. Therefore, treatment of trypanosomiasis is unsatisfactory in the current scenario. Failure of chemical-based allopathic drugs and their serious side effects have increased the awareness among the people about health concerns, which leads to a shift in their preferences for traditional plant-based drugs for curing this disease. For ages, knowledge of traditional plant-based drugs has been explored due to the existence of pharmacologically active compounds. These pharmacologically active natural compounds offer a lot of potential for novel medication development, and their changed derivatives can be more effective against pathology and have lower toxicity. Therefore, there is a pressing need to study natural compounds for the treatment of trypanosomiasis. This book chapter focuses on a number of promising natural anti-trypanosomiasis substances that might be investigated further for medication development.

Keywords

Human African trypanosomiasis · Chemoprophylaxis · Trypanosomiasis · Pentamidine · Suramin

18.1 Introduction

Human African trypanosomiasis (HAT), also known as sleeping sickness, is a vector-borne parasite illness. The causal agents of this infectious illness in humans are protozoans of the genus *Trypanosoma*, which are transmitted by the bite of the tsetse fly (*Glossina* sp.) (Franco et al. 2014). There are two types of infectious agents—*Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense,* respectively, cause west African human trypanosomiasis and east African trypanosomiasis. An additional infectious disease is *Trypanosoma cruzi,* a distinct species of *Trypanosoma,* which causes American trypanosomiasis, often known as Chagas disease. (Bolivar-Mejia et al. 2014). This American trypanosomiasis is an endemic disease in Latin America, and it is not that problematic as, in most cases, it is asymptomatic and chronic (Rassi Jr et al. 2010). Aside from them, another protozoan species, *T. rangeli,* infects humans, but they are nonpathogenic (Truc et al. 2013). The disease is endemic to Africa, and distribution is based on the range of tsetse flies. *Glossina* spp. is usually located between 14°N and 20°S latitude (Molyneux et al. 1996). This zoonotic disease impacts animals more in comparison to humans, and

affected animals act as reservoirs (Franco et al. 2014). It is a progressive and acute disease, and therefore in affected areas, it has a negative effect on economic and sociocultural developments. Many people died in the twentieth century, but due to improvements in medical facilities, the disease's spread has been largely restricted, but in sub-Saharan Africa, it still causes significant morbidity (Büscher et al. 2017). There are some nonclassical *Trypanosoma* species or subspecies that are also reported to cause atypical infections; some of them are *T. b. brucei*, *T. evansi*, *T. lewisi*, *T. vivax, T. congolense*, and *T. lewisi*-like (Truc et al. 2013). Although HAT may be lethal if left untreated, it is still generally a neglected condition for a variety of reasons. In Africa, 37 countries suffer from the impact of this disease, with over 70 million individuals at risk (Simarro et al. 2012, 2012). About 95% of reported cases of trypanosomiasis are caused by *Trypanosoma brucei gambiense* (WHO 2022). WHO reported in the year 2022, in 50 years for the first time, the cases were comparatively lower in the year 2009; the number dropped below 10,000, but the disease incidence dropped further in 2019 and 2020, with 992 and 663 cases being reported, respectively (WHO 2022).

This disease has a negative impact on people's quality of life and causes many deaths; it is important to look out for ways to combat it. Researchers have been trying to produce a vaccine against trypanosomiasis for decades, but nothing has succeeded so far. The development of an effective vaccine could be an effective tool to combat this parasite, but at the same time parasites evolve sophisticated immunoprotective systems. Therefore, vaccination appears to be insurmountable (Magez et al. 2010; Autheman et al. 2021). Even therapy for the disease does not provide much relief, and both forms of sleeping sickness are noxious if not treated in a timely manner or if it is left untreated. The drug used to treat the final stage, on the other hand, is rather dangerous (Steverding 2010). Pathogenic trypanosomes also show resistance to drugs (Brun et al. 2001). Due to availability issues and treatment failure, there is an urgent need for alternate options, and throughout the ages, natural products have provided medicine to humans without any side effects. Natural compounds derived from plants and microbes have the potential to be used for disease treatment (Salem and Werbovetz 2006). Because of the affordability and efficacy, these natural compounds are always explored for treatment, and in this chapter, the potential compounds will be discussed.

18.2 *Trypanosoma* sp.: The Parasite

HAT is caused by the hemoflagellate *Trypanosoma brucei,* which belongs to the genus *Trypanosoma*. Many species are present, but only two subspecies, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*, are the causative of sleeping HAT. *T. b. brucei* is another pathogenic subspecies for wild animals that are exploited in experimental studies. The extracellular protozoan parasites can be identified using a microscope; both the pathogenic species are morphologically difficult to differentiate. Serum resistance-associated gene (SRA) is a molecular marker that is used to differentiate them. In *T. b. rhodesiense* isolates,

the SRA gene is present, and another molecular marker, *Tgs*GP gene, is used to characterize *T. b. brucei* (Koffi et al. 2009). *Glossina* (tsetse flies) is the vector of the disease as it helps in the transmission of this *Trypanosoma* sp. When the parasite enters the tsetse fly, the protozoan parasites in trypomastigote forms are ingested into the bloodstream and then move into the midgut. Trypomastigote forms transform into procyclic forms, which cross through the peritrophic membrane and multiply in the host and become monocyclic trypomastigotes; on reaching proventriculus, they later transform into epimastigote form (Dyer et al. 2013; Rotureau and Van Den Abbeele 2013). Epimastigote form migrates from the esophagus, proboscis, and hypopharynx and then reaches the salivary gland and multiplies into infectious forms called metacyclic forms, which are infective to vertebrates. The cycle duration in the vector takes 18–35 days. During tsetse fly meal, the infective metacyclic form is injected subdermally into mammals, where they proliferate and transform into slender forms and replicate in the bloodstream (MacGregor and Matthews 2010). Trypomastigote form is found in the bloodstream. This form can enter into lymph nodes and cerebrospinal fluid and even cross the placenta (Brun et al. 2010; WHO 2013).

18.3 *Glossina* spp.: Vector

The disease trypanosomiasis is caused by infected *Glossina* sp. (tsetse fly). There are 31 species and subspecies, which are categorized into three groups based on different habitats (Rogers and Robinson 2004). *Nemorhina* subgenus, which is found in western and central Africa, belongs to the palpalis group that lives close to riverbanks, lakes, swamps, forests, towns of the periurban area, and areas where there is intensive agriculture. *G. p. gambiensis, G. fuscipes, G. palpalis palpalis,*. *Glossina* sensu stricto subgenus belongs to the Morsitans group; woodland savannah has the presence of cattle and wild fauna. *G. pallidipes*, *G. morsitans*, and *G. swynnertoni* are located in Eastern Africa and might transmit *T. b. rhodesiense. Austenina* subgenus belonging to *fusca* group lives in savannah, coastal forests, and rainforests; they are not the vector of HAT (Cecchi et al. 2008; Rogers and Robinson 2004; Moloo 1993). For growth, flies need a temperature range between 16 °C and 38 °C, and 50% to 80% relative humidity is required (Molyneux 1980). They are mainly present in humid places. *Glossina* has a peculiar life cycle, as there is no oviposition taking place; only a single larva develops in a female uterus. At a particular time, each female can only produce a single offspring, and within the uterus, the third stage larva develops and gets larviposited into a mature larva in a shady place and in humid soil. The larva travels around and eventually buries itself a few centimeters deep in the soil (sandy or clay) to pupate. There in the soil, the pup survives on the food reserves, and after 20–80 days, the adult emerges. The development of the adult depends on the humidity and temperature. After emergence, the female is mated. For the female to be fertile for over 200 days, only one insemination is required (Maudlin and Welburn 1989). The spermatheca accumulates the spermatozoid for 200 days (Musundi 2021). Lifespan is highly variable, and it is dependent

on the season. It can last from 3–5 months during the rainy season to 1–2 months during the dry season, with a maximum of 7 months. As compared to males, females have a longer lifespan. Females and males are hematophagous and can cause infection. Teneral flies, which emerge, immediately find and feed on the host as they do not discriminate against the host. There are high chances that tsetse flies get infected in their first meal by the trypanosomes, but it can happen at any point in their life (Welburn and Maudlin 1992). Meals can be taken up to 10 days when the conditions become adverse. For vasodilation and to avoid blood coagulation, the tsetse fly is injected at the time of blood meal. The saliva containing infected trypanosomes is transferred to the host during the meal. In case the host already has trypanosome infection, the parasite gets ingested by the fly, but they fail to develop. Not much is known about the feeding preferences of the tsetse flies, but for finding a host suitable for feeding, visual factors such as color, size, contrast, and movement along with the odor stimuli that can be produced by secretions from cutaneous, urine, feces, and breath might play a role (Torr 1989; Tirados et al. 2011). These aspects help in designing different methods to control the vector species (Rayaisse et al. 2011).

18.4 Life Cycle Biological Process of Illness

The life cycle and the occurrence of infections depend mainly on the following elements:

- Human host suffering from the disease.
- Animal reservoir.
- Vector of disease—Tsetse flies *Glossina* sp.
- *Pathogenic Trypanosoma.*

Trypanosoma parasite infection occurs via tsetse flies, which are morphologically similar to and often mistaken for houseflies. Although they have some similarities, they can be distinguished when appropriately observed. This vector, after taking a blood meal from a person suffering from trypanosomiasis, transmits the infection to a healthy person. When the parasite is injected, it enters the bloodstream, reaches lymph and spinal fluid, and replicates via binary fission in the extracellular space (Hirumi and Hirumi 1994). After taking a blood meal from an infected host, the tsetse fly is infected by trypomastigotes, which can be detected in the bloodstream. Protozoa proliferate by binary fission in the midgut of the tsetse fly and then leave the midgut to change into epimastigotes that reach the fly's salivary gland. The epimastigotes enter the salivary glands of the fly, and from there, they can be transmitted again (MacNeal 1904; Alfituri et al. 2020) (Fig. 18.1).

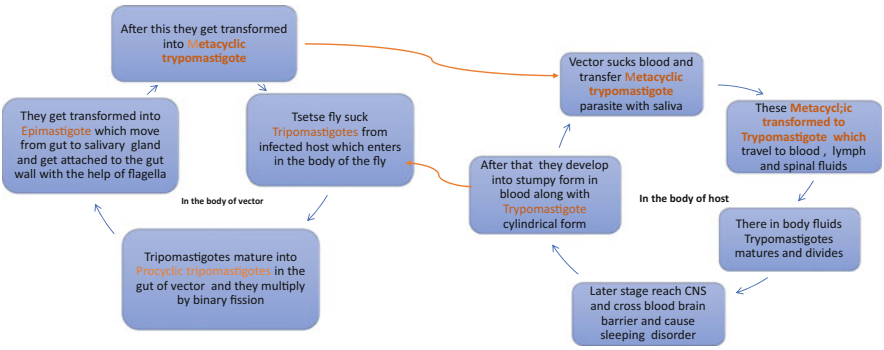

Fig. 18.1 The life cycle of *Trypanosoma brucei* (MacNeal 1904; Alfituri et al. 2020)

18.5 Drugs Development

Over 10.000 molecules are screened for potential drug development. Preclinical trials, which include the pharmacokinetics, efficacy, and toxicity of the drugs, are performed. The clinical development of drugs is divided into four phases (Keiser et al. 2001). The compound that fulfills all the criteria for a potential drug is carried further for the phase I trial, in which the assessment of the tolerability, pharmacokinetics, and pharmacodynamics of the drug is carried out. Phase II of drug testing in a patient in a controlled environment is done to study the adverse effects of the drug and the determination of drug safety (Dickie et al. 2020; Wilkinson and Kelly 2009; Keiser et al. 2001). Phase III studies include the large-scale trials of the selected drug, which includes the testing of the subpopulation suffering from comorbidities such as renal and hepatic problems. In phase IV, which takes place after registration of the drug, the safety, possible drug interactions, and dosage can be refined (Dickie et al. 2020; Wilkinson and Kelly 2009; Keiser et al. 2001). Trypanocidal drug DL-α-difluoro-methyl ornithine (DFMO) is an excellent example that illustrates the drug development stages (Bacchi 2009; Keiser et al. 2001).

18.6 Screening of Active Compounds

18.6.1 Single Synthetic Compounds

The in vitro screening of directly or randomly selected compounds: This approach is cost-effective and rapid, and no prior information is needed about the drug interactions of the selected compound. But its effect on the immune system and its metabolism is ignored in this approach (Romanha et al. 2010).

18.6.2 Combination of Drugs

Infectious infections are treated using drug combinations. The synergies between the two compounds were investigated for African trypanosomiasis treatment. Diminazene aceturate is used with nonsteroidal anti-inflammatory medications (NSAIDs) and lithium chloride (LiCl) to treat pain (Jennings 1993).

18.6.3 Natural Compounds

Half of the drugs that are considered to be best-selling are derived from natural sources. Libraries of active natural products are made because of technological advances along with automated high-throughput drug screening (Rosell 1997). *Allium sativum* and *Cannabis sativum* have shown in vivo activity against trypanosomiasis (Nok et al. 1994; Nok et al. 1996). The plant products such as gallic acid (Koide et al. 1998) and curcumin (Nose et al. 1998) and *Coptis japonica* (Yabu et al. 1998) and *Scutellaria baicalensis* (Mamadalieva et al. 2011) have shown in vitro activity, but in vivo studies are yet to be done.

18.6.4 Drugs Used for Treating HAT

After the infection with both the forms of *Trypanosoma* in the host, the disease presents in two stages: hemato-lymphatic stage and the meningo-encephalitic (Jamonneau et al. 2002). Parasites infiltrate the central nervous system (CNS) in the second stage, causing damage that can be deadly if left untreated (WHO 2013). Both forms have similar symptoms; they only differ in severity. *T. rhodesiense* causes a more severe condition that may result in death within 6 months, whereas *T. gambiense* infection is chronic, and disease progression usually occurs in 3 years (Cecchi et al. 2008; Morrison 2011). These have some common symptoms like fever, headache, cardiac disorders, and skin problems also occur along with pains, weakness, anemia, enlargement of the spleen and liver, etc. initially and second stage place initially, later stages are characterized by sleep disorder (Blum et al. 2020; Kennedy 2013). Overlapping of symptoms and misdiagnosis usually occur (Lejon et al. 2003; Malvy and Chappuis 2011). Now the primary concern is disease therapy and staging. There are several drugs used for the treatment, but none of them is entirely successful. Pentamidine, suramin, melarsoprol, nifurtimox, and eflornithine are important medications for sleeping sickness (HAT) treatment (Fairlamb 2003; Rodgers 2009). Melarsoprol, arsenical-based drug, is one of the oldest drugs developed by Friedheim for the treatment of sleeping sickness in the year 1949 and is one of the oldest and most significant sleeping sickness treatments. It has adverse effects on the nervous system, such as neuropathies and encephalopathies, and on the skin, such as dermatitis (Hannaert 2011; Keiser et al. 2000). Until 1990 for both west and east African origin trypanosomiasis, this was only the drug available there for the treatment of late-stage diseases. Toxicity is a very significant concern with

melarsoprol. Melarsoprol, which is exclusively useful for central nervous system disorders, is so toxic that 5% of patients die from the treatment (Kennedy 2008).

In the 1920s, intravenous suramin was used for the treatment of *T. b. rhodesiense* infections (Legros et al. 2002; Gutteridge 1985; Voogd et al. 1993). This drug hampers uptake of LDL (low-density lipoprotein) through receptor-mediated endocytosis. This LDL is the carrier of cholesterol required for the growth of parasite by forming a complex with LDL (Nok 2003). This drug is usually used in the early stage of HAT, especially when *T. b. rhodesiense* causes infection (Nok 2003). But there are many side effects of this drug, which include renal failure, cutaneous lesions, anaphylactic shock, neurotoxicity, and hematologic toxicity (Rodgers 2009; Legros et al. 2002; Tisdale et al. 1996). Nephropathy, toxicity to bone marrow, and hypersensitive reactions are other major side effects (Burri 2010; Docampo and Moreno 2003).

Another important medicine, pentamidine, operates by interacting with a variety of cellular anions and binding securely to the minor groove of DNA, preventing the replication of nucleic acid (Nun and Neide 1995). It is well tolerated but may cause side effects, including tachycardia, irritation of the skin, low blood pressure, pain in the injection site, and vomiting (White 2005; de Atouguia and Kennedy 2000). Some treatment failures and severe consequences have been recorded, including diabetes mellitus and nephrotoxicity (Sun and Zhang 2008). Pentamidine, which is responsible for hypotension and some other side effects, is due to its good avidity with imidazole receptors (Wood et al. 1998).

Another earlier medication, eflornithine, is a polyamine production inhibitor (Milord et al. 1992). Eflornithine or difluoro-methyl ornithine (DFMO) is an ornithine analog with a fluorine group. DFMO is used for treating the late CNS stage of infection caused by *T. gambiense* but cannot be used for the treatment of *T. rodhesiense* HAT. It is also used against cancer (LoGiudice et al. 2018). Poor bioavailability and administered intravenously. Adverse side effects include seizures, diarrhea, headaches, and dizziness. Thrombocytopenia, leukopenia, and anemia are the more adverse symptoms (Sharma and Zunt 2019). Eflornithine is an enzyme-activated, irreversible inhibitor of ornithine decarboxylase (ODC), which is an early enzyme in the polyamine synthesis pathway. ODC is responsible for breaking down ornithine into its component amino acids. The inhibition of this ODC leads to the blockage of cell division. But in the case of *T. b. rhodesiense,* eflornithine becomes ineffective because the comparatively rapid turnover rate of ODC makes *T. b. rhodesiense* parasites survive, whereas *T. b. gambiense* cannot proliferate because of the longer ODC half-life (Babokhov et al. 2013a, 2013b). The regimen for taking eflornithine is quite specific and might be challenging to carry out, which is the most significant disadvantage associated with this substance. Frequent adverse responses, anemia, leucopenia, gastrointestinal issues, and thrombocytopenia are only some of the prevalent adverse effects that patients report experiencing (Hannaert 2011).

NECT is regarded as one of the most highly developed and sophisticated therapies for the treatment of gambiense HAT is a combination of eflornithine and the trypanocidal nitroheterocyclic family of medication nifurtimox (Zhou et al.

2013). Treatment duration with NECT is cut down to 50%. Although it is more tolerable than melarsoprol, it can induce pancytopenia, seizures, diarrhea, and hallucinations. If the therapy is stopped, all adverse responses disappear (Pepin and Milford 1994). Fexinidazole, used for oral treatment against HAT, is a derivative of 5-nitroimidazole that inhibits the synthesis of DNA (Deeks 2019). This could be used to treat the early as well as advanced stages of the HAT (Deeks 2019). The entire current drug sale faces a serious challenge, and their sales can get stopped as it does not add to the profit. Megazol, a nitroimidazole that is able to cross the blood–brain barrier partially, was quite effective against *T. b. gambiense,* but it is discarded due to the risk of mutagenicity. Trybizine-HCl is a triazine derivative of SIPI 1029 against the *Trypanosoma evansi* species, and this is also effective against the *T. b. rhodesiense* and *T. b. gambiense,* but sufficient levels of the drug are not detected in cerebrospinal fluid of animal model as revealed by the pharmacokinetic analysis (Zhou et al. 1996). But based on studies, if the drug is combined with DFMO, a consistent cure can be achieved (Zhou et al. 1996).

All the drugs used for the treatment have side effects; therefore, other prerequisites for the new drugs include the stability of the compound, safety free from major side effects and affordability, and should have a safety profile. To date, many compounds are screened, but none of them has given an effective result for the control and treatment of the disease. The most common problems encountered in the development of drugs are the cost and side effects. Several compounds should be screened to be used as potential drugs. Preclinical trials should be done. More focus should be diverted toward the selection of compounds to be used in traditional medicine as they are easily available and cost-effective as well. A combination of drugs can be used, possibly having synergistic effects and also cost-effectiveness. Financing for drug development should be improved in poor countries by collaborating with academic institutions and research companies (Whitebread et al. 2005).

18.7 Natural Products

Most of the drugs against *Trypanosoma* available to date have some major side effects. The most important drawbacks are the toxicity to the host and the parasite develops resistance against drugs (Lawal et al. 2013). The drugs are costly also, and availability in remote areas is also not good. Therefore, the use of some herbs, plant materials, and extracts thought to be historically effective in the prevention of trypanosomiasis has produced better and less expensive options (Nok 2005). Trypanosomiasis is quite prevalent in Africa, and plants and their derivatives have been utilized for ages in Africa, particularly in rural areas, yet are still commonly used to cure sleeping sickness. The use of plant extracts and herbs is allegedly historically useful in the treatment of trypanosomiasis, and these products do not have major side effects and produce more effective and affordable substitutes. For HAT plant-based products, algae and fungi are a source of medicines from ancient times (Table 18.1)**.** Secondary metabolites of these plants have diverse capabilities; they can kill

protozoa (Brahmachari 2012). Many of the natural plant-based compounds isolated have the potential to inhibit trypanosome growth in vitro, and the EC50 values for a few of the compounds are quite selective (Kayser et al. 2002). Phenolic derivatives like Flavinoids have been noted for their trypanocidal properties (Sülsen et al. 2007). Flavinoid derivatives like 7,8-dihydroxyflavone, rhamnetin, catechol3-hydroxy-flavone, and 7,8,3′,4′-tetra-hydroxy-flavone showed antitrypanosomal activity for *T. brucei rhodesiense* (Tasdemir et al. 2006). Diarylheptanoids Curcumin, a compound present in turmeric, has also been identified as the major trypanocidal compound (Changtam et al. 2010; Jonah and Enoh 2020). Primin a Benzoquinone (Quinones) in vitro showed antiprotozoal activities *Trypanosoma brucei rhodesiense* (Tasdemir et al. 2006). West African Holarrhena extracts and alkaloid-enriched fractions from bark and leaves show remarkable in vitro efficacy against *T. brucei rhodesiense* bloodstream forms (Nnadi et al. 2017).

Nowadays, about one-third of the drugs are based on natural products (Newman and Cragg 2016). Natural products like herbal compounds and plant extracts are biochemical scaffolding used for obtaining novel compounds that have the same drug-like pharmacodynamic and pharmacokinetic properties (DeCorte 2016; Meyer 2016). Secondary metabolites, which are often synthesized by plants in response to external stimuli like insect assaults and environmental changes, are widely recognized in the pharmacy sector for their incredible structural variety and a broad spectrum of pharmacological activities. Natural products such as secondary metabolites quinones, lignins, and terpenes can be used for the synthesis of semi-synthetic analogs and can be used as new derivatives, which have enhanced biological activities (Izumi et al. 2011). Plants are diverse, which makes them a common source of new bioactive compounds. Novel compounds with antimicrobial activity are identified and isolated from plants and can be used as a prototype for the discovery of drugs against HAT disease. Many plant extracts have an IC50 value smaller than 2 μg/mL (Jain et al. 2016). Plant-like *Alnus rubra, Salvia spathacea*, and *Sabal minor* ethanol extract extracts have IC50 values less than 2 μg/mL, and therefore they can be used as potential drugs against HAT (Jain et al. 2016). Alkaloids extracted from different plants source like berberine, digitonin, chelerythrine, homoharringtoninepiperine, and vinblastine are the potential compound that can be used against HAT (Krstin et al. 2015). Natural chemicals or extracts of plants often alter several molecular targets such as DNA intercalation, cell cycle disruption, morphological distortion, apoptosis induction, inhibition of microtubule assembly, suppression of protein production, and membrane permeabilization (Hoet et al. 2004; Rosenkranz and Wink 2008; Sanchez et al. 2013; Krstin et al. 2015; Zulfiqar et al. 2017). Quinones are both oxidants and electrophiles, and the relative contribution of these traits to their therapeutic actions is regulated via respective chemical structures, specifically quinone nucleus features (Ventura and Lisboa de Castro 2009). The primary mechanisms of quinone cytotoxicity are oxidative stress activation and cellular nucleophiles alkylation, which spans a wide spectrum of biomolecules where ROS may directly interact with proteins, lipids, and DNA, resulting in cellular damage (Ventura and Lisboa de Castro 2009).The *Nectandra oppositifolia* (Lauraceae) leaves are used to isolate Licarin A is a

Table 18.1 Compounds having the potential to impact *Trypanosoma* sp. and their source

S. No.	Plant name	Compound	Effective against *Trypanosoma* sp.	Reference
1.	*Phaedranassa dubia*	Alkaloids ungeremine, pseudolycorine, and haemanthamine	*Trypanosoma brucei rhodesiense* and *Trypanosoma cruzi*	Osorio et al. (2010)
2.	*Polyalthia suaveolens*	Alkaloid (polysin)	*Trypanosoma brucei*	Ngantchou et al. (2010)
3.	*Ageratum conyzoides*	Flavonoids, Ageconyflavone C	*Trypanosoma brucei rhodesiense*	Nour et al. (2006), Nour et al. (2010)
4.	*Lychnophora staavioides* Mart.	Flavonoids, quercetin 3-methyl ether	*T. cruzi*	Takeara et al. (2003)
5.	*Lychnophora passerina*	Goyazensolide, sesquiterpene lactone	*T. cruzi*	de Oliveira et al. (1996)
6.	*Achillea fragrantissima,*	Pellitorine, alkamides	*T. b. rhodesiense*	Althaus et al. (2014)
7.	*Tabebuia* sp.	Quinones (lapachol, α and β-lapachone)	*Trypanosoma cruzi*	Ventura and Lisboa de Castro (2009)
8.	*Centaurea salmantica L*	Cynaropicrin (guajanolide sesquiterpene lactone)	*T. b. gambiense, T. b. rhodesiense*	Zimmermann et al. (2012)
9.	*Senna occidentalis*	Alkaloid, tannins, flavonoids, and steroids b	*Trypanosoma brucei brucei.*	Lawal et al. (2013)
10.	*Liverwort Marchantia polymorpha*	Marchantin A, plagiochin A and 2(*R*)-2-isopropenyl-6,7-dihydroxy-4-(2-phenylethyl) dihydrobenzofuran (bibenzyl bis(bibenzyls)	*Trypanosoma brucei brucei*	Otoguro et al. (2012)
11.	*Zingiber officinale*	Methanolic extract presence of alkaloids, phlobatannins, steroids, flavonoids, saponins, tannins, glycosides, and terpenoids.	*Trypanosoma brucei brucei*	Kobo et al. (2014)
12.	*Calophyllum brasiliense*	Coumarin soulamarin	*Trypanosoma cruzi*	Rea et al. (2013)
13.	*Schkuhria pinnata*	Germacranolide sesquiterpene lactones schkuhrin I and II	*Trypanosoma brucei rhodesiense*	Mokoka et al. (2013)

(continued)

Table 18.1 (continued)

S. No.	Plant name	Compound	Effective against *Trypanosoma* sp.	Reference
14.	*Vernonia mespilifolia*	Cynaropicrin	*Trypanosoma brucei rhodesiense.*	Mokoka et al. (2013)
15.	*Waltheria indica L.*	Alkaloids, pentacyclic triterpene derivatives	*T. cruzi*	Cretton et al. (2015)
16.	*C. penicillata, Loranthus regularis, Leucas virgata, and V. bottae*	Methanolic extracts	*Trypanosoma brucei*	
17.	*Anogeissus leiocarpus*	Methanolic root extract	*T. b. brucei*	Atawodi (2003)
18.	*Artemisia annu*	Artemisinin, artemether artesunate, and further novel artemisinin derivatives	*T. brucei, T. cruzi*	Naß and Efferth (2018)
19.	*Guiera senegalensis*	Aqueous extracts	*Trypanosoma brucei brucei.*	Andre et al. (2017)
20.	*Brillantaisia owariensis*	Methanol and aqueous plant extract	*T. brucei brucei*	Ayawa et al. (2021)
21.	*Terminalia catappa*	Ethylacetate fraction	*Trypanosoma brucei brucei*	Ojeleye et al. (2020)
22.	*Bidens pilosa*	Dichloromethane and methanol extract	*Trypanosoma brucei*	Dofuor et al. (2022)
23.	*Aframomum letestuianum*	Diarylheptanoids	*Trypanosoma brucei*	Kamnaing et al. (2003)
24.	*Unonopsis buchtienii*	Aporphine alkaloids	*Trypanosoma brucei*	Waechter et al. (1999)

neolignan and active against *Trypanosoma cruzi* along with *Mycobacterium tuberculosis Leishmania major*, and *Schistosoma mansoni* (Morais et al.2020). It was isolated in the pure form, and analogs were designed by using a molecular simplification approach. *B. pilosa* whole plant extract alters the shape of *T. brucei* and also causes apoptosis-like and necrosis-like cell death, disrupting the cell cycle (Ohashi et al. 2018).

In this chapter, it is shown how important natural resources are to both pharmaceutical chemistry and medicine. Some ethnic medicinal plants used to treat the disease have been shown to display powerful trypanocidal properties, making the use of herbal treatments in the treatment of trypanosomiasis potentially promising. According to Rodrigues et al. 2016, nonconservative strategies, including bioisosterism, are used for the synthesis of natural products. The development of effective drugs using natural compounds for the treatment of HAT is dependent

directly upon the identification of new compounds (Cavalli et al. 2010). The development in computational biology, molecular biology, and the development of biochemical techniques opened doors for the development of many new heterocycle compound analogs based on molecular natural diversity. Crassiflorone is a natural plant product with antigonorrheal and antimycobacterial activity extracted African ebony tree *Diospyros crassiflora* is a pentacyclic furocoumarin naphthoquinone exhibit inhibitory activity against *T. brucei* (Uliassi et al. 2017). Based on the basic concept of chemical nature, a small library of crassiflorone derivatives was prepared, and their potential against *Trypanosoma* was investigated based on docking studies; it was concluded that analogs have potential inhibitory activities against *Trypanosoma* (Uliassi et al. 2017). An analog of the fungal metabolite antiprotozoal apicidin was scanned and compared to the natural product; it shows potent and selective activity in vitro against the parasite *Trypanosoma brucei* along with low mammalian cell toxicity (Murray et al. 2001).

However, relatively few naturally derived trypanocidal pharmacological molecule is now employed to treat *T. brucei* infections, and plants-based medicines are not widely used to treat HAT. Nowadays, with advancements in research and extraction procedures and computational biology to prepare medications, a number of substances with anti-*T. brucei* properties that have been identified from natural sources are being evaluated for their potential. The main problem is the isolation of potential biological compounds due to their low yield and complexity; therefore, in the approved drug list of the Food and Drug Administration, they are represented lower in the hierarchy (Kingston 2011). Seasonal availability is another major concern related to these products, and side effects of many drugs are seen, and in the chronic phase of the infection, it is not that effective (Tasdemir et al. 2020; Tagboto and Townson 2001; Babokhov et al. 2013a, 2013b).

18.8 Conclusion

Infectious illnesses are becoming an increasingly significant worldwide concern, particularly in the tropical and subtropical regions of the globe. This trend is expected to continue. Protozoan diseases are responsible for a significant portion of the yearly mortality toll which include the likes Chagas disease, malaria, Leishmaniasis, and Chagas disease. All these diseases are highly prevalent in low-income countries and are transmitted via vectors. Malaria has gotten considerable attention from the scientific communities and a lot of investment in the development of new drugs has been done, but a lot of research is yet to be carried out in trypanosomiasis and leishmaniasis. Because of this, the World Health Organization (WHO) considers it to be a Neglected Tropical Disease (NTD) (World Health Organization 2015, 2017). The parasites have complex biology which makes the treatment more difficult as these organisms are resistant to many drugs that are already in use. There is a serious urge for the search for an adequate cure for the treatment of the disease. It is a challenge to find an adequate cure as the existing drugs have lower efficacy, are

extremely resistant, have major side effects, are complex to administer, and are successful in the treatment of many diseases.

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Pathogenesis and Immune Response in *T. cruzi* Infection: Quest for Natural Compound-Based Drugs

19

Utpreksha Vaish

Abstract

Trypanosoma cruzi, a pathogenic protozoan, is known to cause chronic and systemic infection in humans called as Chagas disease, which imposes serious public health issues in Latin America, Australia, Europe, and North America. During the past 10 years, vector control initiatives have been found to significantly lower the incidence of Chagas disease in various endemic regions. However, contact with contaminated excrement from infected triatomine insects is a major source of illness. According to the WHO, more than 10,000 people pass away annualy from Chagas disease, and more than 25 million people are still at risk. As a result of coevolution, *T. cruzi* interacts with the host's innate and adaptive immune systems, it escapes the immune response and begins a chronic infection from an oligosymptomatic or asymptomatic acute phase if left untreated, and may manifest as severe cardiac, gastrointestinal, or neurological malfunctions. Benznidazole and nifurtimox are only two drugs to which available treatments are limited. These medications are deadly, and the effectiveness of the available treatment is greatly increased if given during the acute rather than chronic phases of illness, and moreover they are inadvisable in pregnant patients, patients having severe hepatic or renal insufficiency, and advanced Chagas heart disease. Therefore, it is an utmost necessity to discover or repurpose efficacious therapeutic modalities with fewer side effects, preferably natural compound-based therapies, for effective management of chronic *Trypanosoma cruzi* infection.

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_19

Keywords

Trypanosoma cruzi · Chagas disease · Pathogenesis · Immune response · Natural compounds

Abbreviations

DCs	Dendritic cells
IFNγ	Interferon gamma
IL-12	Interleukin 12
iNOS	Inducible nitric oxide synthase
NK cells	Natural killer cells
NO	Nitric oxide
TLRs	Toll-like receptors
TNFα	Tumor necrosis factor alpha
WHO	World Health Organization

19.1 Introduction to Chagas Disease

American trypanosomiasis, often known as Chagas disease, is a persistent and systemic illness. *Trypanosoma cruzi* (*T. cruzi*), a protozoan parasite, was identified as the causative agent in 1909 by Brazilian physician Carlos Chagas (Coura and De Castro 2002). The World Health Organization (WHO) classifies it as one of the 13 neglected tropical diseases (NTDs) (Hotez et al. 2007) and it is estimated to infect seven to eight million individuals worldwide with around 10,000 individuals dying yearly from its clinical complications. Although it is only endemic in Latin America, due to migrating populations it has also been expanded to regions of North America (Montgomery et al. 2014), Europe (Navarro et al. 2012; Gascon et al. 2010), and Australia (Pinto et al. 2014).

T. cruzi is known to be transmitted among animals by various species of Triatoma. When humans first entered the wild, an enzootic disease that had existed for almost ten million years in the Panstrongylus and Rhodnius genera began to spread to humans as an anthropozoonosis (Coura and Viñas 2010). *T. cruzi* DNA was found in human mummies excavated from southern Peru and northern Chile and paleoparasitological research revealed that this parasite started afflicting humans atleast 9000 years ago (Aufderheide et al. 2004). Indigenous Chagas disease got recognized as a zoonosis only in last 200–300 years when the vector triatomine adapted to domestic environments (Coura and Dias 2009). The primary route of transmission in humans is through the excrement of infected triatomine bugs (also known as kissing bugs or reduviid bugs) at the bite site, or an adjacent intact mucosa serves as the portal of entry. *T. cruzi* interacts with the innate and adaptive immune systems in the host in a very complex manner. Since the parasite and host have an old

history of coevolution, the parasite has developed methods to resist and escape host's immune system. Vector control programs in endemic regions have significantly reduced the incidence of this disease, but *T. cruzi* transmission can also occur by non-vectorial means, such as from infected mother to her baby, by organ transplantation or blood transfusion from an infected person, or by consumption of food and drinks contaminated with parasite (Rassi Jr et al. 2010). Acute phase of Chagas disease begins once the incubation period of parasite is completed in the host, and lack of effective treatment leads to infection for a lifetime either as asymptomatic form or as chronic Chagas disease with serious manifestations like cardiomyopathy in 20% to 30% and gastrointestinal or neurological malfunctions in 10% of infected persons (Bern 2015).

The use of two medications, nifurtimox and benznidazole, which were developed 40 years ago but were shown to be only effective in treating the acute stage of Chagas disease, as well as having a number of side effects, is restricted for treating the chronic disease (Fleau et al. 2019). Most of the patients remain asymptomatic in early stage and are generally diagnosed in chronic phase of Chagas disease, wherein these drugs are almost ineffective. Recent efforts to repurpose posaconazole, ravuconazole, and the prodrug of ravuconazole seemed promising at prima facie but proved to be futile (Buckner and Urbina 2012; Torrico et al. 2018).

Consequently, it is a prerequisite to discover an effective and safe treatment for chronic Chagas disease or repurpose already known therapeutic agents that could directly target the parasite, without harming the patient wherein the exploration into the natural compound-based drugs seems to be promising considering the fact that plants and lower organisms already produce metabolites to protect themselves from natural threats.

19.2 Life Cycle of *Trypanosoma cruzi*

Trypanosoma cruzi (*T. cruzi*) is a member of the Kinetoplastida order and the Trypanosomatidae family (Hoare 1971), and it has a complex life cycle in both invertebrate (vector insect) and mammalian host, including man. In general, it appears that the infection has little impact on the insect host. Triatomine consumes trypomastigotes found in the blood of an infected mammalian host to begin the parasite's life cycle (Tyler and Engman 2001); these trypomastigotes transform into epimastigotes, which migrate to the hindgut of triatomine, differentiate into metacyclic form, and get excreted out with the feces. These metacyclic trypomastigotes invade nucleated cells, after entering via a bite or intact mucosa of the mammalian host. Trypomastigotes divide into the intracellular amastigote form in the cytoplasm, which then duplicates and changes into trypomastigotes before being discharged into the bloodstream by rupturing host cells. New replicative cycles are initiated when circulating parasites invade new cells and infect vectors that feed on the host.

19.3 Epidemiology

Chagas disease' epidemiological profile is a result of vector-borne transmission in endemic nations along with the movement of populations from endemic to non-endemic regions over the decades. Vector distribution and pools of *Trypanosoma cruzi* in the American continent ranges from Argentina to the USA and Chile (Coura and Viñas 2010). This disease was formerly restricted to rural areas of Central and Southern America wherein the vectors lived in mud houses. However, the prevalence of new infections in Latin America has been considerably reduced by 70% due to screening blood donors and international vector control measures, and notably transmission has been halted in Chile in 1999, Uruguay in 1997, and Brazil in 2006 (Moncayo 2003; Moncayo and Silveira 2017). Chagas disease is nonetheless becoming a global health problem because of population migration from endemic nations as a consequence of globalization, and as a result, the disease is spreading to the USA, Canada, Europe, and Australia (Montgomery et al. 2014; Navarro et al. 2012; Gascon et al. 2010; Pinto et al. 2014; Schmunis 2007). The most common destination for migrants is the USA where people migrate from Latin America (Bern and Montgomery 2009), followed by Spain, where most of the migrants are coming from Bolivia, Argentina, Peru, and Ecuador (Gascon et al. 2010). Based on the size of the immigrant population and prevalence of *Trypanosoma cruzi* in their native countries, approximately 300,000 infected immigrants reside in USA (Bern and Montgomery 2009), 80,000 in Europe plus western pacific region, 5500 in Canada, etc. (Coura and Viñas 2010). Cumulatively, in the Western world, Chagas disease continues to be the most significant parasite disease that, if ignored, has the potential to have very catastrophic long-term effects on human health (Fig. 19.1).

19.4 Diagnosis

Acute phase of Chagas disease can be diagnosed by microscopic detection of trypomastigotes in blood or by detection of T. cruzi DNA via polymerase chain reaction (PCR) (Gomes et al. 2009). Microhematocrit method has heightened sensitivity and therefore, is employed to diagnose congenital infection; additionally serological test is also recommended after 9 months of age (Freilij and Altcheh 1995). During chronic phase, to confirm the existence of IgG antibodies developed against *Trypanosoma cruzi* antigens, more than one serological method like enzyme-linked immunosorbent assay (ELISA) is employed (Pereira et al. 2015). PCR could be used in the event when a serology test is inconclusive (Pereira et al. 2015).

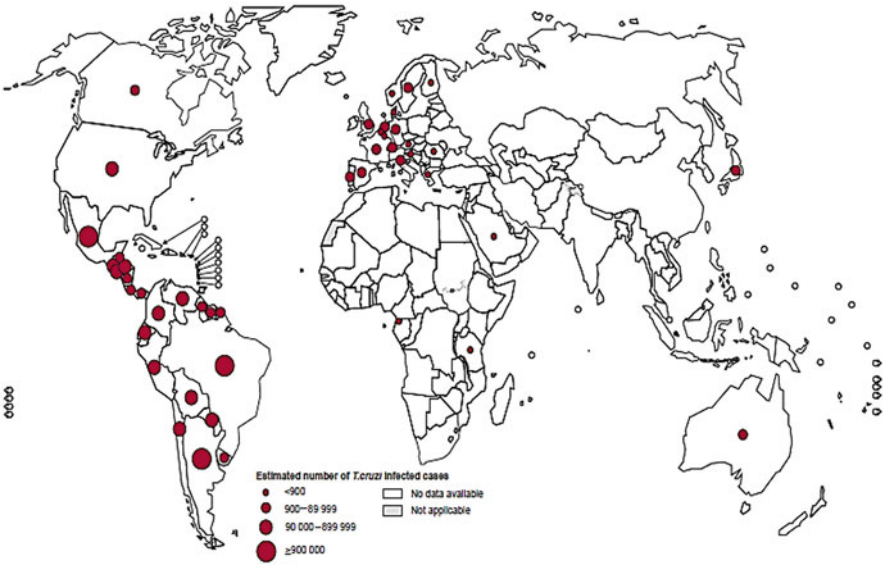

Fig. 19.1 Chagas disease geographical distribution: Global distribution of Chagas disease cases based on official World Health Organization estimates (WHO), 2018

19.5 Clinical Manifestations and Different Phases of Chagas Disease

When the parasite enters human body via vectorial transmission, the incubation period lasts from 1–2 weeks which may vary to few months when infection occurs via blood transfusion and thereafter acute phase of Chagas Disease begins, generally marked by fever, malaise, hepato-splenomegaly, and lymphocytosis (Fig. 19.2) (Rassi Jr et al. 2010). Rarely, chagoma, a skin nodule, or Romaña sign, an edema of eyelid, can develop at the site of inoculation (Bern 2015). Most of the acute infections remain undetected, wherein the immune response reduces parasitemia in 4–8 weeks (Rassi and de Rezende 2012), and less than 10% infection causes mortality because of meningoencephalitis or myocarditis or both (Bern et al. 2011). Patients who survive the acute phase of the disease may develop the undetermined type of chronic Chagas disease, remain asymptomatic, and carry the infection throughout the life, also reported to have antibodies in serum against *Trypanosoma cruzi*, or they may develop determinate form of chronic disease, which is a chronic phase of infection wherein 30–40% of patients are reported with cardiac, cardiodigestive, or digestive abnormalities (Rassi et al. 2009). Patients with Chagasic cardiomyopathy may have rhythmic abnormalities, strokes, or thromboembolism (Acquatella 2007; Paixão et al. 2009). One of the major risk factors for stroke in endemic nations is Chagas disease, which is responsible for about two-thirds of all fatalities (Oliveira et al. 1983; Carod-Artal et al. 2005).

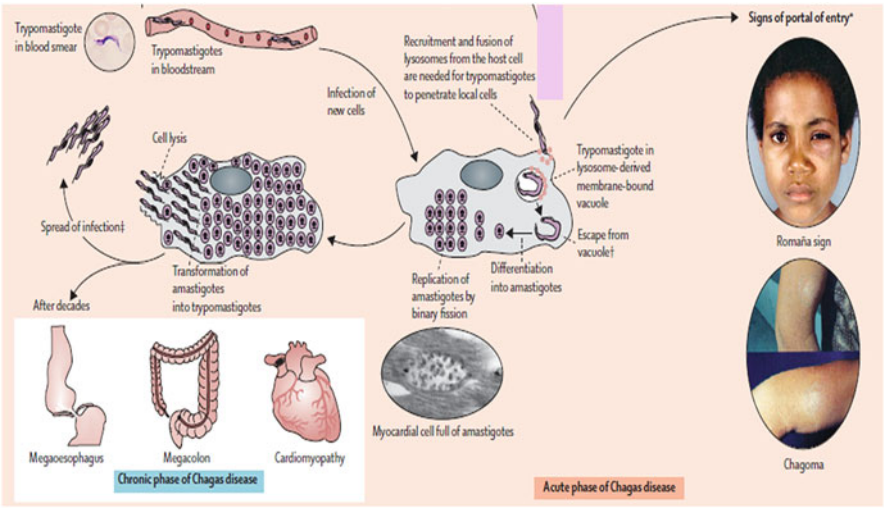

Fig. 19.2 Phase of Chagas disease: Chagas disease is broadly categorized into acute chronic and acute phase. Symptoms can last approximately 2 months and can occur immediately in acute infection, whereas chronic infections may last for years and may develop into cardiac, gastrointestinal malfunctions or both. Figure referred from (Rassi Jr et al. 2010)

The main cause of gastrointestinal Chagas disease is the impairment to the enteric nervous system brought about by *T. cruzi* infection, which predominantly affects esophagus, colon, or both (Matsuda et al. 2009). The manifestations may include asymptomatic motility disorders, mild achalasia, megaesophagus, and megacolon characterized by prolonged constipation and bowel ischemia (Matsuda et al. 2009). Chagasic megaesophagus-associated carcinoma (CMAC) shows increased prevalence ranging from 3.9% to 10% in patients with megaesophagus (Martins et al. 2019). Patients with Chagasic megacolon are reported to have an increased frequency of colorectal cancer (Garcia et al. 2003). Compared to Chagasic cardiomyopathy, gastrointestinal Chagas disease is more prevalent in southern South America than northern South America or Central America, somewhat attributable to a different predominant *T. cruzi* genotypes in a particular geographical region (Miles et al. 1981; Miles et al. 2003). Those women who are infected with *T. cruzi*, can give birth to a child with congenital Chagas disease; child is characterized by low birthweight, hypotonicity, fever, and anemia (Freilij and Altcheh 1995; Bittencourt 1976). Patients having chronic infections can experience reactivation of Chagas disease referred to as Reactivation of Chagas disease, who become immunologically compromised due to administration of immunosuppressive drugs in an organ transplant setting or those who are coinfected with HIV (Braz et al. 2008). Such patients experience elevated parasitemia due to enhanced intracellular parasite replication and have higher mortality (Fiorelli et al. 2005; Sartori et al. 2007).

19.6 *Trypanosoma cruzi* (*T. cruzi*) Infection Pathogenesis and Immune Reaction

During the course of *Trypanosoma cruzi (T. cruzi)* infection, disease progression depends upon host immune response, innate and acquired both, tissue tropism, and toxicity of *T. cruzi* strain (Andrade 1999; Martins et al. 1999; Aliberti et al. 1996; Silva et al. 1998). In acute phase, cells of innate immune system and their cytokines aid in killing the parasite by orchestrating a cascade of reactions, whereas parasite may evade the immune response and multiply to enhance the parasitemia. Whereas in chronic phase, T cell-mediated immunity tries to reduce the burden of parasite (Machado et al. 2012); however, the balance between inflammation of the host tissues and immune-mediated control of parasite determines the progression of the diseases. Both parasitemia and immunity-induced inflammation of host increases with inefficiency of immunological response, leading to a determinate form like cardiopathy. On the other hand, parasitic burden is significantly lowered down by a strong immune response. However, parasitic persistence is considered to be a major cause for failure to downregulate the inflammatory response (Tarleton 2001), but reasons of tissue damage via directly by parasite, autoimmune mechanisms, or indirectly by parasite-driven immunopathology are still unclear (Tarleton and Zhang 1999; Soares et al. 2001). Moreover, polyclonal activation, molecular mimicry by parasite antigen (Cunha-Neto et al. 2006), or shared epitopes of host and parasites (Gironès et al. 2001) lead to autoimmunity that is hypothesized for pathogenesis of Chagas disease (Minoprio 2001).

The pathogenesis of Chagasic cardiomyopathy is incompletely understood, but many reports suggest that a slowly progressive myocarditis leads to impairment of contractile function, known to be caused by fibrosis, myocardial cell death, and invasion of mononuclear cells in myocardium (Andrade 1983), which inclines the patient to arrhythmia and cardiac infarction (Rassi Jr et al. 2000; Rassi Jr et al. 2009). Chronic Chagas gastrointestinal disease mainly affects the colon, esophagus, or both and is caused by intramural autonomic ganglia destruction (Köberle et al. 1968).

19.6.1 Modulation of Innate Immunity in Chagas Disease

The primary defense mechanisms against pathogens include macrophages, monocytes, dendritic cells (DCs), neutrophils, and natural killer (NK) cells. Since they are the sentinels expressed on macrophages and dendritic cells (DCs), toll-like receptors (TLRs) play a major function in the innate immune system in recognizing pathogens. *T. cruzi* is recognized by TLR2, TLR6, and TLR4 (Junqueira et al. 2010; Oliveira et al. 2004), whereas parasite DNA and RNA are recognized by TLR9 and TLR7, respectively (Bafica et al. 2006; Caetano et al. 2011). Alternatively, parasite possesses an antioxidant metabolic network to deal with oxidant agents secreted by the activated macrophage (Cardoso et al. 2016). Several reports in experimental models of *Trypanosoma cruzi (T. cruzi)* infection propose that tumor necrosis factor alpha (TNFα) and interleukin 12 (IL-12)-activated NK cells produce interferon

gamma (IFNγ) after infection, which directs macrophages to produce nitric oxide (NO) by inducible nitric oxide synthase activation (iNOS) (Ding et al. 1988) and kill the parasite. On the contrary, the generation of transforming growth factor β (TGF-β) and interleukin 10 (IL-10) is linked to parasite replication and therefore, enhanced parasitemia by inhibiting macrophage trypanocidal activity (Silva et al. 1991; Reed et al. 1994). Depletion of NK cells in mouse model causes an increment in interleukin-10 (IL-10) production and abrogation of IFN-γ secretion that makes the immune system tolerant to parasite (Cardillo et al. 1996). As per another report, cardiac patients (Vitelli-Avelar et al. 2006) have reduced frequency of mature natural killer cells compared to asymptomatic patients.

Neutrophils produces fibrous structures called neutrophil extracellular traps (NETs) made up of DNA, elastase, histones, and granular proteins, which contribute to pathogen elimination (Sollberger et al. 2018) by aiding in differentiation of trypomastigotes into amastigotes (Junqueira et al. 2010) and thus reducing infectivity. DCs internalize *T. cruzi* (Bafica et al. 2006) and present them to T cells for downstream effect. However, the parasite can impair the ability of dendritic cells to deliver antigens by reducing CD40 co-receptor and class I and class II MHC molecule expression (Sousa-Rocha et al. 2015).

Complement System also plays important role in Chagas disease as *T. cruzi* are susceptible to bind with complement factors, but only the lectin and alternative pathways have effect on metacyclic trypomastigotes and epimastigotes of certain strains (Cestari and Ramirez 2010; Lidani et al. 2017; Ramírez-Toloza and Ferreira 2017). Conversely, sets of molecules like trypomastigote decay acceleration factor (T-DAF) and calreticulin (TcCRT), present on the surface of parasite, aid it to escape the complement system, which in turn inhibits or weakens complement activation (Ramírez-Toloza and Ferreira 2017; Ferreira et al. 2004). In addition, infected host cells or parasite itself releases plasma membrane-derived vesicles that potentially constrain functions of complement system (Cestari et al. 2012).

19.6.2 Modulation of Adaptive Immunity in Chagas Disease

19.6.2.1 B Lymphocytes

B cells secrete antibodies and cytokine and perform antigen presentation for other immune cells. The primary immune system components to be focused on, in the context of Chagas disease are antibodies because they are present in the sera of infected individuals and serve as the foundation of the serological tests. Since they are present in the sera of infected individuals, these antibodies are the main immune system components to be found. Antibodies that react with galactose epitopes mediate lysis of trypomastigotes and are called as "lytic antibodies" (LA) (Cordeiro et al. 2001). Indeterminate patients have higher levels of serum LA, and patients negative for hemocultures for over 10 years did not have any circulating LA (Galvão et al. 1993). The mutant mouse model, unable to produce antibodies, is incapable to control growth of parasites (Kumar and Tarleton 1998). In spite of this, a persistent infection is established as antibodies against *T. cruzi* cannot always eliminate the

parasite effectively. It may be due to antigenic variability (Cardoso et al. 2016; Pitcovsky et al. 2002; Buscaglia et al. 2006), nonspecific polyclonal B cell activation (Bermejo et al. 2011), or reduction of immature B cells in the bone marrow (Zuniga et al. 2005; Acosta Rodriguez et al. 2007). The frequency of circulating B cell has been reported to increase during the early acute phase, which becomes even higher during late acute phase and at early chronic phase (Sathler-Avelar et al. 2003). The acute phase of infection is controlled by large numbers of nonspecific IgM and IgG antibodies generated by B1 subsets of B cells, which greatly lowers parasitemia (Merino et al. 2010). In the absence of mature B cells, the frequency of IFN-γ+ CD4+ T cells (and TH17 cells) drops and the frequency of TNFα + CD4+ T cells increases, which results in an overall exacerbated pro-inflammatory state (Gorosito Serran et al. 2017). A relatively newer class of B cells, Regulatory B cells (Breg), release IL-35, IL-10, and TGF-β, which prevent the growth of pathogenic T cell clones and other pro-inflammatory lymphocytes while encouraging the development of regulatory T cells (Tregs), which exhibit immuno-suppressive functions (Rosser and Mauri 2015; Dai et al. 2017).

19.6.2.2 T Lymphocytes

During the acute phase of Chagas disease, T cells recognize peptide–MHC complexes with the aid of T-cell receptor (TCR) and develop into effector T cells, and simultaneously upregulate adhesion molecules and chemokine receptors to facilitate migration to lymphoid organs and accelerate the immune response (Laidlaw et al. 2016). T cell response is particularly important for the maintenance of the low parasitemia in the chronic phase of the disease and if impaired, it leads to rapid clinical onset (Dos Santos et al. 2014). Additionally, compared to asymptomatic and moderate cardiac patients, there was a decrease in the frequency of circulating CD8+ T cells in severe cardiac patients, indicating that they may play a role in halting the advancement of the cardiac symptoms (Albareda et al. 2006). Perforin and granzymes B, which are markers of activated CD8+ T lymphocytes, have a direct trypanocidal activity wherein granzymes B enters infected cells by the action of perforin and then granulysin penetrates amastigotes and eliminate them (Dotiwala et al. 2016).

CD4+ T cells are characterized by the secretion of cytokines which modulate the activity of macrophages, DCs and CD8+ T cells. They also generate lifelong memory T cells. In a mouse model of chronic *T. cruzi* infection, CD4+ T lymphocytes are a major component of cardiac lesion infiltrates, which may indicate that they are important for the parasite response (Younès-Chennoufi et al. 1988). According to another report, certain parasite enzymes alter the physiology of CD4+ T cells to prevent them from proliferating and favor a TH2 cytokine profile, which is characterized by decreased IL-2, IFN-γ expression and increased IL-4 expression with decreased TCR signaling. Similarly, TcMuc, a sialoglycoprotein on the surface of T. cruzi, was shown to produce similar effects on the expression of IL-2 and CD25, affecting not only IFN-γ, but also IL-4, IL-10, and TGF-β (Nunes et al. 2013). TSA-1 and Tc24, two antigens that have been investigated as potential vaccination candidates in preclinical animal models, are able to elicit CD4+ T cell responses in

some chronic Chagas patients. KMP-11 (kinetoplastid membrane protein-11) is another molecule that alters T cell activity by increasing IFNγ secretion (Cuellar et al. 2009). However, these antigens triggered response in small number of the patients, which indicates that they contain HLA-restricted epitopes.

TH17, a different subgroup of TH cells, has anti-pathogen capabilities (Bettelli et al. 2008). Their main function is recruitment and activation of neutrophils, macrophages, and tissue-resident cells. By encouraging the generation of oxidative species in infected cells, TH17 cells can also play a significant protective role in the fight against *T. cruzi* infection.

After elimination of the antigen by various mechanisms, clonal contraction takes place mostly by apoptosis of the activated effector T cells, which allows the immune system to maintain homeostasis (Pulendran and Ahmed 2006). The differentiation of memory T cells remain beyond the contraction phase and they have the capacity to self-renew and can acquire effector functions in case of a new event (Laidlaw et al. 2016). Fiuza et al. showed that chronic Chagas patients, regardless of their clinical status, had a higher frequency of circulating central memory T cells than noninfected people do (Fiuza et al. 2009), which may be related to a long-lasting activation state. As a result of prolonged expression of the inhibitory receptor and loss of effector capabilities, memory T cells may become exhausted (Wherry and Kurachi 2015; Anderson et al. 2016).

The regulation of various immunological responses depends heavily on regulatory T cells (Treg). Presence of Tregs at an inflammatory site is generally regarded as a contributing factor to pathogenic persistence, because it maintains an attenuated immune response against infectious agents (Belkaid 2007). The role of Tregs in the infection by *T. cruzi* is controversial. Studies show that patients with no symptoms had more circulating Tregs than those with cardiac or gastrointestinal complaints (Guedes et al. 2012; Vitelli-Avelar et al. 2005; da Silveira et al. 2009). Treg cells were discovered to be more prevalent during the acute phase of an experimental infection in mice and they also migrated to the heart of infected mice. However, treatment with anti-CD25 antibody resulted in increased mortality (Mariano et al. 2008). Furthermore, both asymptomatic and cardiac Chagas disease patients have been shown to have activated Treg cells that produce IL-10 (de Araujo et al. 2011). In summary, Treg cells may be crucial to the immune system's ability to maintain balance throughout a *T. cruzi* infection. More studies are necessary to elucidate their precise roles in *T. cruzi* infection.

19.7 Drug Discovery and Bio Drugs

Chagasic patients are treated to give symptomatic relief and to reduce parasitemia. Only benznidazole and nifurtimox are used to effectively treat Chagas disease, and benznidazole is typically regarded as the first-line of treatment due to its notable safety profile (de Andrade et al. 1996; Sosa-Estani et al. 1998; Viotti et al. 2009). Anti-trypanosomal treatment is contraindicated in pregnant women, patients with cardio or gastrointestinal Chagas disease and hepatic-renal malfunctions. Patients

with Chagasic heart disease showed improved survival with amiodarone treatment (Scanavacca et al. 1990). Implantable cardioverter defibrillators (ICDs) have also been used to treat such patients; however, the results are not particularly encouraging (Rassi Jr 2007; Pereira et al. 2016). Another option for people with end-stage heart failure is cardiac transplantation, but studies suggest that Chagasic heart disease patients may have better survival than transplant recipients since immunosuppressive medications may worsen *T. cruzi* infection (Campos et al. 2008). Palliative treatment is advised for gastrointestinal symptoms, with an emphasis on facilitating food passage via the achalasic esophageal sphincter. Sublingual nitrates and nifedipine, which also relaxes the esophageal sphincter, also used before meals as temporary management (Herbella et al. 2008). Surgical organ resection is needed for patients with megacolon and sigmoid volvulus who cannot respond to conventional methods (Garcia et al. 2008). Recent efforts to repurpose old drugs, i.e., posaconazole and ravuconazole, although first seemed promising but have been proved to be futile (Buckner and Urbina 2012; Molina et al. 2014). The recent drug candidate recommended for management of Chagas disease is a prodrug of ravuconazole, i.e., E1224, which also turned out to be unsuccessful (Torrico et al. 2018). Since, the known treatments are partially ineffective, quest for novel candidates for disease control is necessary.

Natural products derived from plants, fungi, and sponges have safe profile and constitute a promising alternative to conventional treatments for Chagas disease. After literature survey, many natural products, plant extracts, essential oils, etc. have been found to show activity against *T. cruzi* and some important ones are discussed here.

Arginine kinase, an essential enzyme for energy homeostasis in invertebrates, could be a potential target to treat Chagas disease. A recent study screened a group of polyphenolic compounds using in silico approach as a potent inhibitor of arginine kinase, and out of them **Resveratrol** was chosen for further study. It has been reported that 50% of recombinant arginine kinase activity is inhibited by resveratrol at 325 μM and it shows antiparasitic activity against *T. cruzi* trypomastigotes with IC_{50} at 77 μM (Vera et al. 2016). A self-emulsifying drug delivery system was developed for resveratrol which significantly improves its cellular uptake (Le Clanche et al. 2018) and could be tested for trypanocidal effective concentrations. Moreover, numerous studies have showed the beneficial effects of resveratrol in treating cardiovascular diseases in animal models (Zordoky et al. 2015) and therefore it is a logical guess that besides killing the trypomastigotes, resveratrol could prove useful in maintaining cardiac health in Chagasic cardiac patients, although more studies and clinical trials are need to be pursued.

Ghrelin, a peptide hormone with cardioprotective and anti-apoptotic functions, was analyzed for its immunomodulatory properties in male Wister rats infected with *T. cruzi* (de Paula et al. 2019).The infected rats when supplemented with ghrelin showed increased macrophage frequency and nitric oxide production, and T cells and cytokines were reported to have anti-inflammatory response. Inflammatory infiltration in cardiac tissue was also reduced with supplementation of ghrelin. Many reports also suggest role of ghrelin as a cardio-protector (Eid et al. 2018;

Khatib et al. 2014), and therefore it is imperative to clinically evaluate its role in treating acute and cardiac phase of Chagas disease.

Microalgae are rich sources of bioactive metabolites and certain species of red and green algae show activity against protozoans. R. Veas et al. found that methanolic extracts of *Scenedesmus obliquus* and *Tetraselmis suecica* and ethanolic extracts of *T. suecica* and *Chlamydomonas reinhardtii* show trypanosomal killing activity on intracellular amastigotes and infective extracellular trypomastigotes (Veas et al. 2020). In addition, ethanolic extract of *C. reinhardtii* intensifies the action of nifurtimox by reducing its IC_{50} from 14 μM to 5 μM and suggests that drug combination therapies could enhance efficacy, lower down the toxicity and reduce chances of resistance to microorganisms than available therapy.

Phyto-compounds like **Sesquiterpene Lactones**, i.e., incomptine B, Ambrosin, and Glaucolide E, were tested for trypanocidal activity in vitro and showed IC_{50} lower than nifurtimox on epimastigotes (Sepúlveda-Robles et al. 2019) and dehydroleucodine and helenalin induced apoptosis in the epimastigotes and trypomastigote (Jimenez et al. 2014). Red microalgae *Laurencia dendroidea* is reported to be a source of sesquiterpene elatol, which is reported to show trypanocidal activity against epimastigotes, trypomastigote, and amastigote (Dos Santos et al. 2010). Ambrosia species derived terpenoids, particularly cumain and cordilin, were active against *T. cruzi* epimastigotes, and cumanin and psilostachyin were also active against amastigotes (Sülsen et al. 2013). Marine polyketide endoperoxides, isolated from a Brazilian sponge *Plakortis angulospiculatus* have been reported to inhibit *T. cruzi* with an IC_{50} of 6.3 μM (Kossuga et al. 2008). Ethanolic extract of *Physalis angulata* was reported to inhibit the proliferation of the epimastigotes and lyse trypomastigotes (Meira et al. 2015).

Many medicinal plants from family Asteraceae are known to possess ethnopharmacological importance, wherein **essential oils** from *Artemisia annua* and *Artemisia absinthium* are reported to have antimicrobial and antiprotozoal effects against *Trypanosoma cruzi* and *Leishmania* (Martínez-Díaz et al. 2015). **Flavonoids** have also been reported to show trypanosomal killing activity. The simplest flavonol from *Brassica oleracea* has been reported to show pan-trypanocidal activity with EC_{50} values of 33 μM against intracellular amastigote (Cockram and Smith 2018). **Xanthones** are another class of natural compounds which could be utilized for treatment of Chagas disease. The prenylated xanthone α-mangostin derived from pericarp of *Garcinia mangostana* fruits displayed significant pan-trypanocidal activity with 8.9 μM EC50 values for amastigotes (Al-Massarani et al. 2013).

Numerous **Quinones** exhibit trypanocidal activity too, preferably by generating reactive oxygen species (ROS) and preventing mitochondrial activity of the parasites (Ventura Pinto and Lisboa de Castro 2009). Komaroviquinone (Tyler and Engman 2001), a potential inhibitor of *T. cruzi* trypomastigotes, exhibits a EC_{50} value of 0.25 μM. Abietane-derived product isolated from the *Salvia cuspidate* is another tetracyclic product that exhibits activity against *T. cruzi* epimastigotes with EC_{50}value of 16.6 μM.

These natural products are non-mutagenic, less toxic, and more stable and hold a great potential for the pursuit of potent trypanocidal compounds. Various natural compounds discussed above possessed micromolar or even sub-micromolar activities against trypomastigotes or epimastigotes or both. Some of them are part of already available therapeutic modalities and need to be explored for their repurposing in Chagasic treatment. Therefore, further research with priority should be done to validate the already known leads which are available in the vast ocean of literature.

19.8 Conclusions

Chagas disease, being a neglected tropical disease, remains to be a significant source of comorbidity as well as mortality in Latin America which is also becoming a global health issue. Conventional treatment is limited to two drugs, and randomized drug trials and better drug regimens with less side effects and enhanced trypanocidal activity are immediately required for effective management of chronic Chagas disease. Although vector control programme and screening of blood donors reduced the incidence of disease, still those suffering from cardiac and gastrointestinal malfunctions need better therapeutic approaches besides palliative care.

Chagas disease reacts to the immune system of host in a very complex manner and can even re-program it for its own survival. Now, it is believed that the therapy for Chagas disease is buried somewhere in nature, and natural compounds with immunomodulatory and anti-inflammatory properties could become successful therapeutic candidates. Several type of natural compounds such as polyphenols, quinones, terpenes, sesquiterpene lactones, etc. were portrayed in this chapter, which have the potential to be developed as successful Chagasic therapy. Moreover, it is also indicated that a combination of therapeutic modalities should be advised to enhance efficacy and to reduce toxicity, to deal with this age-old ailment.

Acknowledgments I would like to thank my husband, Dinesh, who was always there to lend his ears and hands during the preparation of this book chapter. I thank my family members who have always encouraged me to do better. I would like to thank Dr. A. K. Verma for giving me this opportunity and my friend Nagarjuna for asking me to write this book chapter. Thanks to my PhD mentor, Dr. Rajni Rani, under whom I have improved my writing skills, which helped me during preparation of this chapter. My sincere thanks to the team of editors. Big thanks to everyone on the publishing team.

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Limitations of Current Drugs and Prospects of Plant-Based Compounds and Their Constructed Analogs as Therapeutics for Treatment of Malaria

20

Yogesh Kumar, Akanksha Jain, and Ravindra Kumar

Abstract

Antimalarial drug resistance is persistently posing a major risk for eradicating malaria and is the key challenge for radically curing malaria. Multipronged approach is utmost needed for eliminating malaria. It is promising that several novel strategies to antimalarial drug discovery are presently under assessment, including plant-based drugs. Safety and efficacy aspects of novel drug candidates also require specific focus and are a matter of prime concern for the discovery of antimalarial drugs. The challenge of drug resistance and other limitations yet needs to be overcome and more potent drugs including phyto-products and their synthetic analogs are needed to be further investigated that can plausibly be proved to be potent enough for inhibiting the parasitic transmission at the erythrocytic phase.

Keywords

Malaria · Antimalarial drugs · Plant-based antimalarial products

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_20

20.1 Introduction

Malaria is a fatal disease, which is caused majorly by *Plasmodium* parasite species. Although there have been advancements with respect to the interventions related to its management/control and strategies to manage the disease, malaria-linked lethality, morbidity, and mortality are still soaring. Malaria is an enormously critical disease with devastating health hazards across different parts of the world.

In the year 2019, approximately 229 million malaria cases were estimated globally while total mortality was 4,09,000. It is notable that children below the age of 5 years are the utmost susceptible section greatly affected by malaria accounting for nearly 67% (274000) of global malaria-related mortality in 2019. Of the total malaria burden across the world, the African Region of WHO has the highest share and was home to 94% malarial incidence and associated mortality. Other most affected regions include India, China, and East Asian nations. In human beings, malaria is caused by five variants of parasite, of which the most fatal ones are *P. falciparum* and *P. vivax*. The initial symptoms of malaria that usually appear within 10–15 days post infective mosquito bite include fever, headache, chills, etc. and that the symptoms may be mild and not easy to be identified as malaria. Further, if the early symptoms are not timely treated, within 24-h span, *P. falciparum* malaria can be advanced to serious morbidity and even mortality. The major reasons attributable for the revival of malaria are inappropriate implementation of malaria control actions, insecticidal failure, and advent of drug-resistant parasite strains (WHO 2021).

20.2 Malaria Prevention

Since last 20 years, expansion of WHO suggested tools and strategies related to malaria prevention comprising efficient vector control as well as the usage of preventive malarial drugs had conferred a major impact in curtailing the disease burden globally.

In controlling and eliminating malaria, **vector control** is an important strategy as it not only prevents the infection but also curbs transmission of the disease. The major interventions being mosquito nets treated with insecticides and interior residual sprays.

Preventive chemotherapy implies using single or combination medicines for the prevention of malarial infections as well as their related consequences. It involves therapies like chemoprophylaxis, recurrent preventive treatment of small children and pregnant women, chemoprevention of malaria caused seasonally, and administering drugs on large scale. These strategies are safe, economic, and can effectively be blended with the existing malaria control activities, such as vector control approaches, immediate and timely diagnosis of malaria coupled along with appropriate treatment of identified cases with the antimalarial drugs.

Vaccine: Recently in October 2021, WHO has recommended wide usage of the RTS,S/AS01 malarial vaccine to children residing in areas with moderately high

intensity transmission of *P. falciparum* species. The vaccine has been reported to curb lethality of severe malaria significantly among small children (WHO 2021).

Earlier, malaria was conventionally treated with chloroquine single therapy (D'Alessandro 2009). Malaria has emerged as the most common tropical disease globally owing to the resistance of *Plasmodium* species to chloroquine and the vector mosquito resistance to the insecticides. Over the years, quinine has been utilized as an antimalarial drug. Lately, doctor community have been persistently using quinine owing to the poor resistance ability of *Plasmodium* species in contrast with the other drugs. Usually, quinine is administered in the concentration of 10 mg per kg thrice/day for a period of 7 days. Primaquine is administered to eradicate the latent phase of parasite known as hepatic hypnozoites (Baird and Hoffman 2004). Sulfadoxine and pyrimethamine are administered as individual doses in the concentration of 30 mg per kg and 2.5 mg per kg, respectively. Mefloquine is either administered singly in the concentration of 15 mg per kg or along with drugs like sulfadoxine and pyrimethamine. Three doses of halofantrine are administered in the concentration of 8 mg per kg at a gap of 6 h along with fat-rich meals. But as yet oral dose of chloroquine (4-aminoquinoline) is considered the best while few relapses occur in *Plasmodium vivax* and *Plasmodium ovale* strains. Plausibly, 4-aminoquinoline acts when dosage of chloroquine enters the food vacuoles of the malaria-causing parasite, which inhibits the development of hemozoin (food for parasite) from heme. This in turn will inhibit the food source of the parasite, resulting in accretion of free form of heme in the parasite to the stage of toxicity and ultimately resulting in the death of parasite (Alvarez et al. 2006). Artemisinin, a China-based antimalarial drug, is a sesquiterpene lactone having a unique characteristic in a natural compound; i.e., it comprises endoperoxide moiety Fig. 20.1 depicts the different factors that may lead to drug resistance of malaria).

In this chapter, efficiency of plant-based drugs over the currently used drugs for treating malaria has been documented. Plant-related drugs have been reported to possess in vivo and in vitro activity of antimalaria (Willcox and Bodeker 2004). Extensive body of work on plant-based drugs helps and elevates the sense of effectiveness of these drugs on several diseases and disorders (Ankrah et al. 2003). Since conventional times, traditional medicines are utilized the world over for treating malaria—rather, herbs/plants have been utilized for treating and managing various diseases including malaria, gastrointestinal tract, and central nervous system diseases/disorders. Malaria is among the topmost infectious diseases globally. Conventional healers have been suggesting natural plant-based drugs for treating acute malarial symptoms like fever, etc. Plant-based drugs are vital due to the emergence and widespread resistance of chloroquine. Large body of evidence on plant-based antimalarials can act as a foundation stone for developing novel facility-oriented strategies for treating malaria, particularly the vulnerable or at-risk populations and poor sections of the society in developing nations residing in endemic tropical regions.

Current malarial allopathy drugs are undoubtedly quite effective but they also pose adverse health effects:

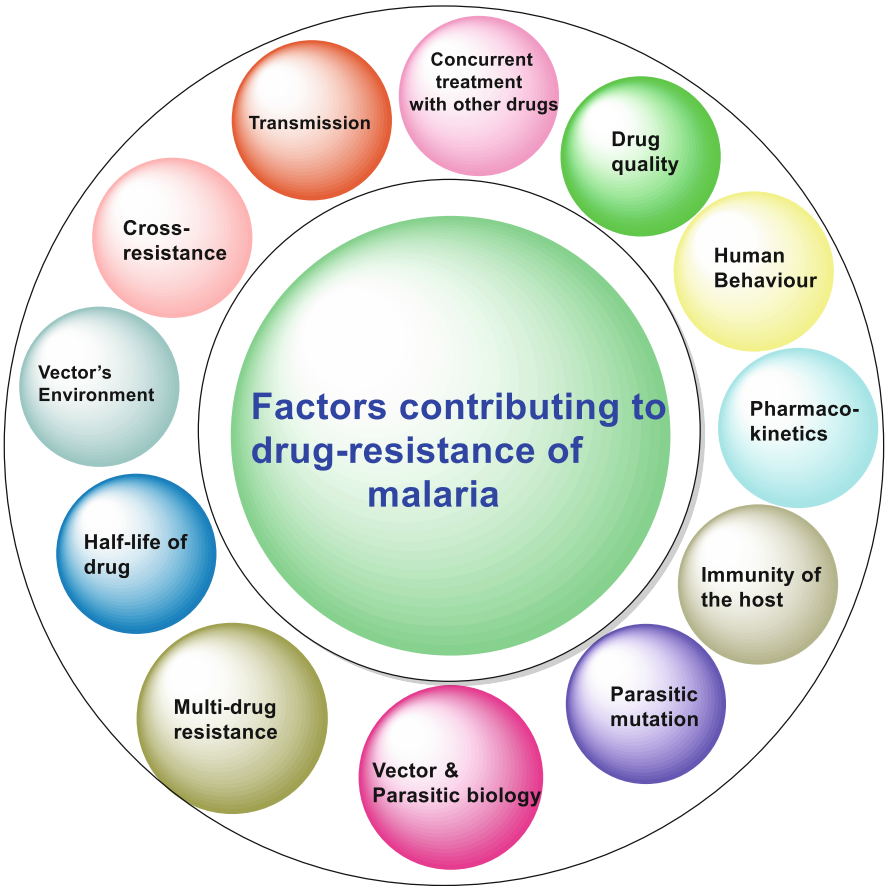

Fig. 20.1 Determinants of antimalarial resistance

- Chloroquine: Head neuralgia, nausea, vomiting, retinal damage of the eyes, corneal opacity, retina impairment or loss of vision, lower than normal levels of neutrophils in blood, chronic itchy skin, interface dermatitis, hair loss, cessation of heart functioning, apnea/respiratory dysfunction, and certain other disorders associated with conduction.
- Primaquine: Abdominal pain and cramps, low white blood cell count, production of abnormal amount of methemoglobin and a blood condition in which destruction of red blood cells is quicker than their production.
- Amodiaquine: Headache neuralgia, nausea, vomiting, lower than normal levels of neutrophils in blood, chronic itchy skin, cessation of heart functioning and breathing, and numerous disorders linked to conduction.
- Quinine sulfate and quinine dihydrochloride: Nausea, headache, gastrointestinal disorders, ringing in ears, antibody-mediated blood platelet deficiency, abnormal heartbeats originating in ventricles, low blood pressure, fever, lack of

granulocytes in the blood, skin rashes, fatal allergic reactions, hyper immunological responses, and visual distortions.
- Pyrimethamine: Seizures, production of abnormal sized RBCs in bone marrow, and GI disorders.
- Mefloquine: Allergic skin, GIT disturbances, blood-related disorder/disease, and acute liver failure.
- Pyrimethamine and sulfadoxine (Fansidar): GIT disorders and skin allergies.
- Halofantrine: Abdominal pain, loose and frequent bowel movement, skin allergies, and abnormal heartbeats originating in ventricles.
- Artemether: Minor increase in glutamicoxalacetic transaminase (SGOT) and glutamicpyruvic transaminase (SGPT), abnormally reduced reticulocytes (Akram et al. 2020).

Malaria is vulnerable for all age groups, but because of increased levels of fetal hemoglobin in neonates and infants, they are specifically resistant to *P. falciparum*. The disease is also quite unusual in individuals with sickle cell hemoglobin as compared to individuals with normal adult hemoglobin. People with sickle cell characteristics have a slightly lesser extent of disease. For instance, individuals with sickle cell attributes are immune to infection caused by falciparum species. Though, it is rather common in the underdeveloped countries. For the development of malarial parasite in the infected vector, temperature between 20 and 30 degree Celsius and 60% relative humidity is considered to be optimum. Mosquito breeding may be due to various unwarranted activities, including burrow pits, excavations during construction work of barrage, railways, roads, irrigation canals, etc. (Patz et al. 2000). Malaria is frequently transmitted by the mosquito bite of infected female *Anopheles* species; however, other uncommon transmitters can be transfusing blood, usage of contaminated syringes/needles, and via mother to fetus (Trung et al. 2004). The incubation period from introducing sporozoites to the arrival of clinical symptom ranges between 7 and 12 days in *P. falciparum* while 12 and 16 days in case of *Plasmodium vivax* and *Plasmodium ovale* species. On the other hand, in the temperate regions, protected incubation span in case of *P. vivax* can range from 8 to 10 months. For *Plasmodium ovale* strain infection via blood transfusion, the incubation span differs with the transfusion of total parasites, which is generally less. In case of *Plasmodium malariae*, incubation period ranges from 7 to 30 days. The incubation span can be extended if antimalarial drug dosage is administered suboptimally. Extrinsically, incubation phase (8–55 days) differs from gamete sucking to the formation of sporozoites, which greatly depends on malarial parasite strains and other factors like relative humidity, temperature, etc. (Brasil et al. 2011). The parasitic asexual erythrocytic phase may result in the arrival of the clinical symptoms, which may be apparent when fully developed infected erythrocytes by the schizont break up and release endogenic pyrogens and certain toxins in the blood. Deficiency of iron, malnutrition, hemolysis, and defective formation of red blood cells (dyserythropoiesis) cause anemia. In addition, malarial pigment (hemozoin) in the hypertrophied Kupffer cells result in hepatomegaly. Further, splenomegaly may be caused due to accretion of massive numbers of monocytes

and macrophages consisting of erythrocytes and malarial pigment (Brasil et al. 2011).

The prevailing antimalarial drugs may be categorized into quinoline derivative, antifolate, and artemisinin derivatives. Till date, none of the drugs alone has been recognized or formulated that can destroy all the species of *Plasmodium*. Therefore, to combat malaria infection effectively, combination drugs are regularly given concurrently. Prevention and control of malaria requires coordinated approaches as already highlighted in the chapter, including vector control, and safe and effective drugs and vaccines. Keeping in view the ever-increasing morbidity and death rate because of malaria, the emergent condition, drug resistance and ineffective existing antimalarial agents against non-erythrocyte and sexual phases, it is highly imperative to recognize innovative plant-based antimalarial drugs by comprehending the actual metabolic signaling channel of the parasite. For fulfilling this goal, investigations on antimalarial agents should address scientifically proven innovative targets for developing novel steer drug/s (Belete 2020; Comer et al. 2014; Fidock et al. 2004). Figure 20.2 demonstrates the recently used antimalarial drugs, their limitations and novel antimalarial targets

Recent developments for preventing and controlling malaria are jeopardized by ever-increasing parasitic resistance for *Anopheles* mosquitoes. Numerous factors may result in increasing resistance. Malarial parasite resistance starts at two stages:

- First stage: In this, a preliminary hereditary event develops a resistant mutant—also referred as de novo mutation in which a novel genetic characteristic provides the parasite an existence benefit against the agent.
- Second stage: Here, the parasites that are resistant are then chosen, which begin to proliferate ultimately leaving the parasitic population non-susceptible for treatment (Bloland 2001).

As per the recent World Malaria Report (2010–2019), 73 nations have indicated resistance to mosquitoes for at least one out of the four commonly referred classes of insecticides. While reportedly in 28 nations, resistance to mosquitoes was prevailing for all the major insecticidal categories.

On the Essential Medicines Model List released by World Health Organisation (2021), for cure-based treatment of malaria, medicines for the treatment of *Plasmodium falciparum* malaria cases should be used in combination. The list currently recommends combinations according to treatment guidelines. In the WHO malaria treatment guidelines, not all the fixed dose combinations are present and fosters their development and meticulous testing procedures. WHO also promotes development and analysis of rectal dosage preparations (Table 20.1).

Artemisinin and its derivatives: In the year 1971, Tu Youyou was the pioneer in isolating Artemisinin from a herb plant called *Artemisia annua*—which has generally been used in Chinese conventional medicine (Qinghaosu Antimalaria Coordinating Research Group 1979). Owing to the enormous favorable effect of artemisinin for the prevention of malaria, Tu Youyou in 2015 was conferred with joint Nobel Prize in Physiology/Medicine for her findings regarding a new treatment

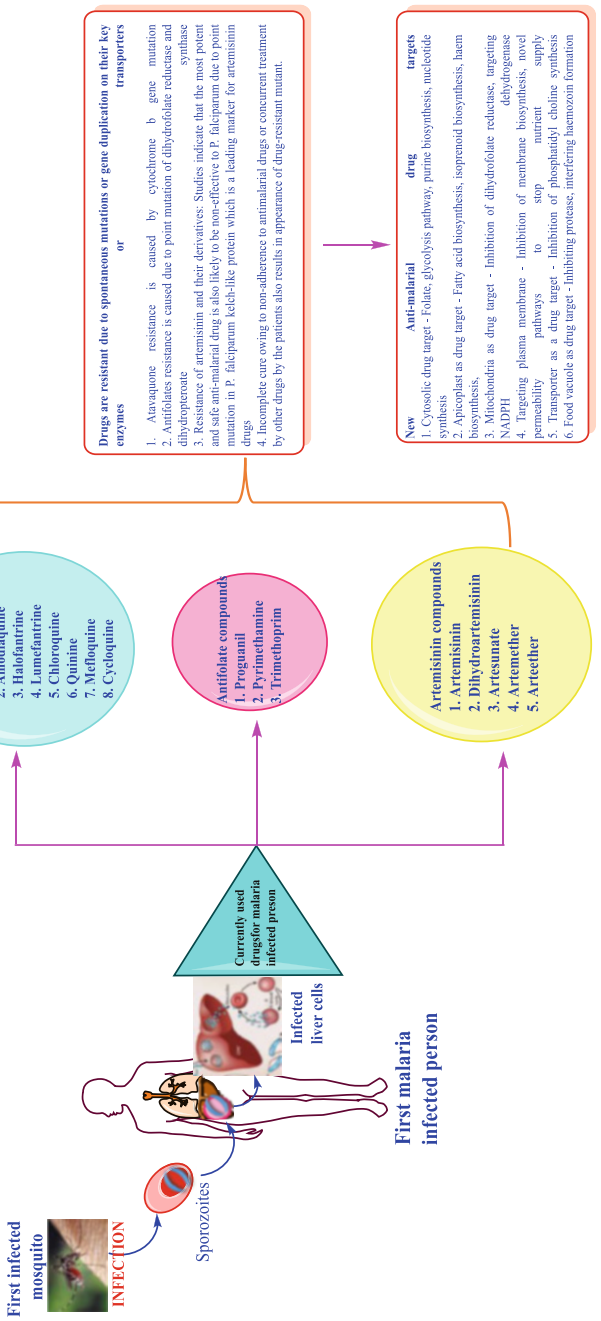

Fig. 20.2 Currently used antimalarial drugs, their limitations, and novel antimalarial targets

Table 20.1 Name of antimalarial drug and dosage

S. no	Name of antimalarial drug	Dosage
1	**Amodiaquine** (For use along with artesunate 50 mg)	Tablet: 153 mg or 200 mg (as hydrochloride)
2	**Artemether** (For treating severe malaria)	Oily injection: 80 mg/mL in 1 mL ampoule
3	**Artemether + lumefantrine** (Not advised during1sttrimester of pregnancy or children weighing <5 kg)	Tablet: 20 mg + 120 mg Tablet (dispersible): 20 mg + 120 mg (specific indication for restricting its use to children)
4	**Artesunate** (To be used in combination with either amodiaquine, mefloquine or sulfadoxine + pyrimethamine)	Injection: Ampoules, containing 60 mg anhydrous artesunic acid with a separate ampoule of 5% sodium bicarbonate solution For treating severe malaria Rectal dosage form: 50 mg†; 100 mg†; 200 mg capsules (for pre-referred management of severe malaria only; patients should be referred to a suitable health-care facility for follow-up)† Tablet: 50 mg
5	**Artesunate + amodiaquine** Other combinations that provide the target dosages required such as 153 mg/200 mg (in the form of hydrochloride) + 50 mg artesunate	Tablet: 25 mg + 67.5 mg; 50 mg + 135 mg; 100 mg + 270 mg
6	**Artesunate + mefloquine**	Tablet: 25 mg + 55 mg; 100 mg + 220 mg
7	**Artesunate + pyronaridine tetraphosphate** (Age or weight restriction on use of the medicine)	Granules: 20 mg + 60 mg (specific indication for limiting restricting its dosage for children) Tablet: 60 mg + 180 mg
8	**Chloroquine**	Oral liquid: 50 mg/5 mL (as phosphate or sulfate) Tablet: 100 mg; 150 mg (as phosphate or sulfate) *For use only for the treatment of *Plasmodium vivax* infection
9	**Dihydroartemisinin + piperaquine phosphate** (Age or weight restriction on use of the medicine)	Tablet: 20 mg + 160 mg; 40 mg + 320 mg
10	**Doxycycline***	Capsule: 100 mg (as hydrochloride or hyclate) Tablet (dispersible): 100 mg (as monohydrate) *To be used only along with quinine

11	**Mefloquine** (For use along with artesunate 50 mg)	Tablet: 250 mg (as hydrochloride)
12	**Primaquine** (Only for use to attain radical cure of *P. vivax* and P. ovale infections administered for the period of 14 days)	Tablet: 7.5 mg; 15 mg (as diphosphate)
13	**Quinine** (Only for managing severe malaria and needs to be used along with doxycycline)	Injection: 300 mg/mL (hydrochloride) in 2 mL ampoule Tablet: 300 mg (sulfate) or 300 mg (bisulfate)
14	**Sulfadoxine + pyrimethamine** (For usage along with artesunate 50 mg)	Tablet: 500 mg + 25 mg

† The medicine(s) require(s) specialist diagnostic or monitoring facilities, and/or specialist medical care, and/or specialist training for their use in children.
* Use in children <8 years only for life-threatening infections when no alternative exists and to be used along with quinine.

against malaria (https://www.nobelprize.org/nobel_prizes/medicine/laureates/2015/). It has been documented that Artemisinin is effective against all multidrug-resistant variants of *P. falciparum*. **Artemether, artesunate, and arteether** are the utmost popular derivative components of artemisinin.

There have been equivocal mechanisms of action of artemisinin (O'Neill et al. 2010)—the most widely acknowledged theory is that heme activates the molecules and forms free radicals, thereby destructing proteins needed for the parasitic existence (Wang et al. 2015; Tilley et al. 2016). However, data have reported numerous other plausible mechanisms. In the year 2013, a computational strategy was adopted for assessing the mechanism of action based on earlier researches that linked heme and PfATP6 ($Ca2^+$ transporter) (Shandilya et al. 2013). Later in the year 2015, artemisinin has demonstrated to be associated with regulation of the signaling pathways of unfolded protein response (UPR) that can be related to reduced growth of parasites (Mok et al. 2015). Another investigation documented that artemisinin effectively inhibits *P. falciparum* phosphatidylinositol-3-kinase (PfPI3K) (Mbengue et al. 2015).

In the year 1948, the synthesis of **Amodiaquine** occurred for the first time (Berliner et al. 1948). It is generally utilized for treating *P. falciparum* malaria without any complications when consumed along with artesunate and is usually marketed as Camoquine/Coarsucam (Bompart et al. 2011). Like chloroquine, mechanism of action of amodiaquines is considered to form complex with heme and prevent development of hemozoin (Combrinck et al. 2013).

In 1960s, **Piperaquine** was formulated under the aegis of Chinese National Malaria Elimination Programme (Chen et al. 1982). In the beginning, it was in usage all across China as a substitute of chloroquine, and its resistance results in its lesser usage in the form of monotherapy. Though, the mechanism of action relating to piperaquine have been still equivocal, researches have proposed that it comes into effect through accumulating in the vacuole of the digestive system and constraining heme component from getting detoxified via binding of species comprising heme (Eastman and Fidock 2009; Vennerstrom et al. 1992). Recently, piperaquine is being utilized as a partner drug along with dihydroartemisinin (generally marketed as Eurartesim).

Under the Chinese antimalarial research investigation—"Project 523," the synthesis of **Lumefantrine**, commonly referred as benflumetol, first took place in the year 1976, which also led to the artemisinin's discovery (Cui and Su 2009). It is presently marketed as Coartem®. The actual mechanism of action of lumefantrine is not clear, though researches have proposed that it hinders nucleic acid and synthesis of protein through hampering the development of beta-hematin by forming hemin complex (Combrinck et al. 2013). Currently, lumefantrine is being utilized only along with artemether.

Proguanil and atovaquone: In the year 1945, proguanil was first documented to be the pioneer antifolate antimalarial drugs (Curd et al. 1945), whereas in 1991, atovaquone was first revealed for treating infections caused due to protozoa (Hudson and Randall 1991). Together, proguanil and atovaquone have been reportedly marketed as Malarone; and ever since 2000, it has been sold by GlaxoSmithKline.

Evidence indicates that it is a highly potent antimalarial drug owing to the synergistic effect developed by the combination of the two compounds. This is mainly because of distinct mechanism of actions of the two components. Further as a cytochrome bc1 complex inhibitor, atovaquone inhibits the electron transport chain of the mitochondria (Fry and Pudney 1992). On the other hand, proguanil when used in isolation as a dihydrofolate reductase inhibitor via cycloguanil (metabolite) which interrupts the synthesis of deoxythymidylate. However, proguanil, when used along with atovaquone, does not act as a dihydrofolate reductase inhibitor; instead, it has been reported to lessen the quantity of atovaquone needed for the treatment of malaria (Srivastava and Vaidya 1999). Generic dose of atovaquone and proguanil is currently existing in market for treating malaria specifically resistant to chloroquine.

Pyrimethamine and sulfadoxine: During the early 1050s, pyrimethamine was developed by the researchers Gertrude Elion and George Hitchings and is presently being marketed as Daraprim. In the year 1988, Elion, Hitchings, and Black were awarded with the joint Nobel Prize in Physiology/Medicine partially for the formulation of pyrimethamine for "their discoveries of important principles for drug treatment" (https://www.nobelprize.org/nobel_prizes/medicine/laureates/2015/). While in the beginning of 1960s, sulfadoxine was developed (Laing 1965). However, owing to extreme resistance levels, it is currently not being used as a drug for preventing malaria. Subsequently in 1981, pyrimethamine along with sulfadoxine was reportedly accepted for usage for treating malaria and is generally sold as Fansidar (trade name). Pyrimethamine and sulfadoxine are documented for targeting the folate biosynthesis pathway of the parasite (Lumb et al. 2011). The former drug inhibits the enzyme dihydrofolate reductase and the latter drug inhibits the enzyme dihydropteroate synthetase.

Pyronaridine: During 1970s, pyronaridine synthesis took place for the first time at the Institute of Chinese Parasitic Disease (Zheng et al. 1979; Chang et al. 1992). Over four decades, pyronaridine has been used in the name of Pyramax along with artesunate, and researches have clearly indicated the efficacy of pyronaridine against the variants that are chloroquine resistant. Similar to lumefantrine, pyronaridine has clearly shown to act by inhibiting the formation of beta-hematin (Croft et al. 2012).

Tafenoquine: In the year 1978, tafenoquine was first discovered. In 2018, it was approved by the US FDA for usage as the first novel single dose for radically treating malaria due to *P. vivax* since past six decades (Food and Drug Administration 2018). Studies have indicated tafenoquines a prodrug that is metabolized to the active quinone tafenoquine, although the mechanism of action is still equivocal (Ebstie et al. 2016). Tafenoquine is presently sold as Krintafel.

Since past few years, though the mortality caused due to malaria has dropped, the improvement pace for eliminating the disease has been rather slow. Owing to the resistance to the present frontline artemisinin-based combination drug approach, there is a dire need discovering novel antimalarial drugs involving new mechanisms of action and which is being considered as the utmost priority developmental agenda currently. Investigations have revealed the major limitations of adopting the traditional chemotherapy for malaria, including multidrug resistance development as well

as the uncertain targeting to intracellular parasites, which leads to need of heavy dosage and subsequently intolerable toxicity.

In view of the developing resistance of the malarial parasites against several existing treatments, it is highly imperative to recognize novel antimalarial plant-based chemotherapeutic drugs, specifically herbal/medicinal plants to plausibly prevent issues associated with resistance to drugs. This may be because of vast usage of plant-based compounds for treating malaria in several conventional clinical practices. Though it has been quite evident that plants were the major sources of the leading antimalarials—quinine and artemisinin (Akram et al. 2020).

Owing to various categories of phytoconstituents, certain plants possess antimalarial activity such as alkaloids, terpenes, steroids, and flavonoids. Alkaloids are a significant class of phytoconstituents possessing varied biological activities, including antimalarial activity. Phytochemical constituents of the below-discussed plants reportedly exhibit antimalarial activities.

***Ageratum conyzoides* (Asteraceae):** The utilizable portion of the plant is bark. It comprises components like tannins, terpenoids, benzofurans, chromenes, essential oils, coumarins, alkaloids, and flavonoids. In an investigation conducted by Ukwe et al. (2010) on mice infected with *Plasmodium berghei* for studying the antimalarial activity of the aqueous extract and *Ageratum conyzoides* leaf fractions, *Ageratum conyzoides* was reportedly found to be effective for treating malaria.

***Bridelia ferruginea* Benth (Euphorbiaceae):** The portion utilized here too is bark. It consists of D-glucopyranoside and biflavanolgallocatechin-[4-O-7]-epigallocatechin. The phytoconstituents present in the plant are not only beneficial for curing bacterial, fungal infections and malaria but it is considered a diuretic, anti-ulcerative, potent inhibitor of xanthine oxidase, antioxidant and anti-plasmodic. Research conducted by Kolawole and Adesoye (2010) reported that bark of *Bridelia ferruginea* Benth possess antimalarial activity. Another study was carried out in which 100 to 400 mg extract per kg of *Bridelia ferruginea* was fed to study its effect against *P. bergheie*-infected mice. The study documented that the *Bridelia ferruginea* extract showed significant anti-plasmodial activity, which was quite equivalent to 100 mg extracted from 1 kg chloroquine (Mbah et al. 2012).

Azadirachta indica (Meliaceae): The portions utilized are bark, root bark, young fruit, nut, seeds, leaves, and flowers. The plant comprises phytoconstituents, including diterpenoids, margolone, nimbonolone, nimbolinin, methyl gallate, margosinone, margosinolone, nimbinasulfur, nimbidin, and oreophenol-nimbiol. These phytoconstituents are highly beneficial for the treatment of different illnesses, including skin-related issues and blood disorders, and is considered a diuretic antimalarial (Zirihi et al. 2005). In an investigation conducted to assess the in vivo anti-plasmodial activity of ethanolic extract of *Azadirachta indica* (neem) via intraperitoneal inoculation of *Plasmodium berghei* ANKA and subsequently intraperitoneally feeding the extract at a daily dosage of 300 mg, 500 mg, and 1000 mg per kg for the duration of 5 days. The standard drugs used were intraperitoneal chloroquine and artemether. No improvement was reported with the use of extract. On the other hand, in standard drug (chloroquine and artemether) users, symptoms of cerebral malaria were absent and no mortality was reported. In case of mice who were

administered neem, edema, cerebral hemorrhage, and Purkinje cell apoptosis were reported. On comparing with the control who were not administered any treatment, it was found that there was a change in the manifestation of neurological symptom, which was rather severe in case of control. Thus, neem was not found to be effective in protecting mice against malarial signs/symptoms but possess lesser neurological protective efficacy (Farahna et al. 2010).

***Ochna integerrima (Ochnaceae)*:** The portion utilized here is usually stem bark comprising flavonoids and glycosides. A study reported that bioflavonoids from *Ochna integerrima* possess antimalarial activity (Ichino et al. 2006).

***Artemisia annua* L. *(Compositae)*:** The utilizable portion here are leaves comprising chemical compounds such as sesquiterpenes and artemisinin; it is effective for treating malaria, cough, and diarrhea (Shedayi and Gulshan 2012) and is considered antimalarial and cytotoxic. *Artemisia annua* consists of a compound artemisinin, which is well known for treating malaria. An investigation was carried out by Mueller et al. (2009) for assessing the antimalarial activity of *Artemisia annua* in which 48 parasitemia-infected patients were selected. The study reported that within 2 to 4 days of administration of *A. annua*, a significant reduction in parasitemia was observed. In a 4-day treatment with *A. annua* on 44 patients, 92% of parasitemia got disappeared, and the malarial symptoms were reduced significantly. Thus, it was concluded that *Artemisia annua* was found to be effective for treating malaria.

***Artemisia nilagirica (Asteraceae)*:** The utilizable portion of this plant are leaves (Parameswari and Devika 2017). *A. nilagirica* consists of phytoconstituents, including flavonoids, alkaloids, terpenoids, tannins, and glycosides (Arokiyaraj et al. 2012). The researches have assessed the efficacy of *A. nilagirica* leaf extracts formulated using various solvents, including aqueous, methanol, ethanol, n-hexane, petroleum ether, and chloroform, against malarial parasite *P. falciparum*. The study reported that the leaf extracts of *A. nilagirica* exhibit antiplasmodial activity, which can be utilized as a potent antimalarial drug owing to the presence of bioactive phytoconstituents (Panda et al. 2018).

***Esenbeckia febrifuga (Rutaceae)*:** The utilizable portion of this plant are stems and consists of isopimpinellin, bergaptene, limonoid, skimmiamine, kokusaginine, and flindersiamine. *E. febrifuga* is found to be effective for treating malaria and fever and is considered antimalarial. In a study, antiplasmodial activity of ethanol extract was assessed against *P. falciparum* W-2 and 3D7 variants. The phytoconstituents of the plant were also isolated and the activity was noted against different variants of *Plasmodium*. It was found that fluoroquinolone 5 and fluoroquinolone 6 exhibited the highest activity at IC50 of less than 50 mg per mL; the activity of findersiamin was found to be comparatively lesser while rutaevine 8 and alkaloid 7 were not active at all (Dolabela et al. 2008).

***Remijia ferruginea (Rubiaceae)*:** The utilizable portion of the plant is bark, which is utilized for treating malaria and herpes, and therefore it is considered antimalarial. In a study, it was reported that *Remijia ferruginea* possess antimalarial activity (Brandão et al. 1997). Animal-based research was carried out on mice infected with *Plasmodium berghei* in which crude extract of plant formulated in

ethanol was assessed. The study reported significant antimalarial activity, and further phytochemical analysis reported the presence of two alkaloids while quinine was completely absent (Andrade-Neto et al. 2003).

***Amaranthus spinosus (Amaranthaceae)*:** The utilizable portion of the plant are roots and leaves consisting of β-carotene, rutin, linoleic acid, stigmasterol, sitosterol, betanin, amaranthine, and glycosides and is beneficial for treating wounds, abscesses, and piles and also possess laxative and emollient effects. In an animal-based study conducted by Hilou et al. (2006) on antimalarial activity of *A. spinous*, its extract was found to be effective on the fourth day of treatment. Further, a significant suppressant effect on antimalarial investigation in mice that were inoculated with red blood cells infected with *P. berghei* and the concentrations for *Amaranthus spinous* were ED (50) of 789. *Amaranthus spinosus* have demonstrated lesser toxic effects at 1450 mg per kg.

***Ticodendron incognitum (Ticodendraceae)*:** The portions utilized in this plant are leaves, bark, and roots comprising phytoconstituents, including phenols, flavonoids, terpenes, triterpenes, reducing compounds, anthocyanins, coumarins, tannins, and sterols. Alpízar-Cordero et al. (2018) assessed the antimalarial activity of the *T. incognitum* plant fractions against *P. berghei* NK65 and it was found that antiplasmodial activity is due to the bioactive metabolites present in the plant.

In addition, numerous other phytoconstituents present in plants exhibit antimalarial activities including the following:

- The flavonoids present in the isolated leaves of *Friesodielsia discolor* (Annonaceae Family) plant including 8-formyl7-hydroxy-5-methoxyflavanone and 30-formyl-20,40-dihydroxy-60-methoxychalcone have demonstrated antiplasmodial activity (Prawat et al. 2012).
- The leaves and twigs extract of Miliusacuneatas Miliusacunines A and B plant formulated in acetonic solvent are phytochemicals possessing antimalarial activity (Promchai et al. 2016).
- Phytoconstituent Miliusacunines A have been found to show antimalarial activity through inhibition of TM4 malarial variant (IC50 19.3 mM) (Mueller et al. 2009).
- Miliusacunines B have also reportedly demonstrated antimalarial activity by preventing the K1 malarial strain (IC50 10.8 mM) (Mueller et al. 2009).
- Phytoconstituent present in an Australian plant species *Mitrephora diversifolia* alkaloid 5-hydroxy-6-methoxy onychia have been found to exhibit antiplasmodial activity with IC50 concentrations of 11.4 (against Dd2 clone of *P. falciparum*) and 9.9 mM (against 3D7 clone of *P. falciparum*) (Mueller et al. 2009).
- In the *Rhaphidophora decursiva* plant (Araceae family), there are seven phytoconstituents in the leaves and stem extracts of the plant formulated in methanolic solvent and are found to demonstrate antimicrobial activity against W2 and D6 clones (Zhang et al. 2002).
- Phytoconstituents including rhaphidecursinols A and B, epigrandisin, grandisin, and decursivine have been found to show antiplasmodial activity against *Plasmodium falciparum* (Zhang et al. 2002).

- A novel steroid-based glycoside has been identified from *Gongronema nepalense* (Asclepiadaceae family) known as gongroneside-A, which was isolated employing antimalarial bioassay. The researchers have documented the antimalarial activity of *G. nepalense* plant species (Libman et al. 2008).
- Polysyphorin and rhaphidecurperoxin have also found to possess antimalarial activity against D6 and W2 variants (Zhang et al. 2002).
- In addition, flavonoid glycosides, luteolin 7-O-glucoside, and Apigenin 7-O-glucoside extracted from *Achillea millefolium* have also reportedly demonstrated antimalarial activity against W2 and D10 variants (Vitalini et al. 2011).
- A bioactive constituent named 2-isopropenyl-6-acetyl-8-methoxy-1,3 benzodioxin-4-one extracted from *Carpesium divaricatum* (a perennial herb) have been reportedly found to show antimalarial activity inhibiting the D10 strains (Chung et al. 2010).
- Animal-based research carried out on Swiss albino mice by Adigo Shibeshi et al. (2021) assessed the antimalarial activity of in vivo assays of *Combretum molle* extract against *P. berghei*. In this study, *P. berghei* was reportedly inoculated to healthy mice and were daily fed with 100, 200, and 400 mg per kg methanol-based crude extract and solvent fractions of *Combretum molle*. The researchers documented that crude extract of leaves of *Combretum molle* have been found to demonstrate potent antimalarial activity. The results of the investigation are envisaged to support the use of *Combretum molle* leaves for treating malaria.
- Plant-based alkaloids derived medicinal plants of Africa have been reportedly shown to possess immense potential for formulation of antimalarial drugs (Titanji et al. 2008).

20.3 Conclusion

It is a matter of grave concern that the recent advent of malarial parasitic resistance of currently marketed drugs is persistently increasing, thereby constraining the capability to manage malaria. Although, it is encouraging that several novel strategies for antimalarial drug formulation are presently being evaluated including plant-based drugs. In addition to new antimalarial drug development, effective vaccine discovery is also crucial for controlling the malaria menace. Vaccines targeting the pre-erythrocytic sporozoite stage still remain the utmost promising approach and is to be dealt on priority basis Katie et al. 2015).

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An Analytical Approach to Progression in Malaria Therapeutics

21

Satyam R. Dwivedi, Lokesh Chandra Mishra, and Gauri Mishra

Abstract

Malaria is one of the leading causes of deaths around the globe, with WHO reporting 435 thousand deaths in 2017. However, great progress has been made over the years to combat malaria and reduce the level of suffering caused by it. India being a tropical country has been affected by malaria since ancient times. One of the earliest descriptions of antimalarial treatment using plant-based extracts is found in Sushruta samhita. In modern world, isolation of quinine from Cinchona tree in the year 1820 proved to be a milestone event in malaria drug development. Since then a number of natural and synthetic compound such as Chloroquine, Mefloquine, and Halofantrine have been developed. On the current list of WHO essential medicines, Artemisinin-based combination therapy (ACT) is found to be most effective in treatment of the disease. These along with the use of insecticide-treated mosquito nets for malaria prevention have made a significant impact in devising an effective strategy against the parasite. However, with passing time, development of resistance by the parasite emphasizes the need for further research and development of new compound to fight the disease.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/978-981-19-9605-4_21.

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_21

Malaria has been a cause of great suffering throughout human history. This chapter describes the efforts made to effectively encounter the threat of malaria over the years and discusses the antimalarial drugs under development. Moreover, potential therapies and novel mechanism of future antimalarial drugs are also outlined toward the end.

Keywords

Malaria · Mosquito · *Anopheles* · Vector-borne disease · Artemisinin · Malaria vaccine

21.1 Introduction

Malaria, a vector-borne disease, is prevalent in tropical conditions with existence in at least 97 countries. It is a disease of global importance as it accounts for 200 million cases and around 6, 00000 deaths annually with mortality rate of 0.3-2.2%. It is one of the most common disease in Africa and several countries in Asia with severe form of infections in tropical climates of the region. *Plasmodium*, a small amoeboid protozoon of intracellular nature, is the causative agent of this disease. Six species of this genus present a significant threat to humans. *Plasmodium falciparum* is considered most lethal of six. *P. vivax* is major cause of illness across different parts of world and is now believed be to a lot more lethal than previously reported. *P. ovale* curtisi, *P. ovale* wallikeri, and *P. malariae* are less common but dangerous nonetheless. *P. knowlesi*, a simian parasite, has recently emerged as a local but important cause of disease in Southeast Asia, especially in Malaysia. Female mosquitoes of *Anopheles* genus serves as vector for the disease as malaria transmission in human is caused by a bite of *Plasmodium* spp. infected female *Anopheles*.

21.2 A Brief History of Malaria

History of malaria goes back to earliest human civilizations. It has long been one of the most widespread diseases among humans leading to large number of deaths, high economic losses, disappearance of nations, and even military defeats. The first descriptions of malaria are found in ancient Chinese medical records of 2700 BC, and 1200 years later in the Ebers Papyrus. Alexander the great, one of the greatest military leaders, died because of malaria at borders of Indian subcontinent on his return to his homeland. The fact that this disease is responsible for deaths of likes of Alexander the great, Christopher Columbus, Medici, Pope Urban VII and George Washington, shows how it has affected all classes of human society equally. Quick transmission of disease, short though complex life cycle of causative agent (*Plasmodium* spp.), feeble immune response, and lack of an effective drug remained major causes behind the prevalence of disease for centuries.

Symptoms of malaria were highly common among inhabitants of major civilizations in ancient world but in the absence of scientific diagnostic tools and techniques, the fever that developed in patients was often associated to supernatural forces and anger of deities. In great Assyrian-Babylonian civilization, deity Nergal was portrayed as a two-winged insect, presumably the supernatural force behind deadly illness caused by high fever. In the fourth century BC, Hippocrates rejected the supernatural basis and proposed the drying of swamps as the cause behind the disease. He speculated that drying of swamps releases a mixture of maleficent gases or Mal-air (hence the name Malaria), which when inhaled caused the disease. This explanation stood for centuries and spread of malaria was linked with foul air. Residents of European towns and cities often kept windows and doors closed to keep this disease carrying foul air away from their houses. However, in 1880, French scientist Alphonse Laveran discovered the cause of disease in his laboratory and rejected the age-old theory of propagation of malaria by foul air. He was a military surgeon and first observed the parasite while working on blood of malaria-affected patients. He was awarded Nobel Prize for this remarkable discovery in 1907.

21.3 Biology of the Disease and Its Transmission

Discovery of *Anopheles* as a vector of malarial infection is attributed to British Surgeon-Major Ronald Ross of British Indian Medical service who discovered it while treating malaria patients in India. He was utterly convinced that *Anopheles* is the vector of the disease. He published his findings in the British Medical Journal in 1897. In 1902, Ross won the Nobel Prize for his successful discovery of the mosquito stages of malaria. August 20, 1897, is when Ross discovered the first signs of such a cycle in a female *Anopheles* mosquito and thus aptly became "Malaria Day." By 1948, the complete malaria cycle with two hosts was worked out (Fig.21.1)

The biological characterization of malaria transmission includes cyclical infection of humans and female *Anopheles* mosquitoes. In humans, the parasites develop at two places. They firstly multiply in the liver cells and finally in the red cells of the blood. After development in RBCs, successive broods of parasites grow inside the red cells and destroy them, which release a large number of merozoites that proceed the cycle by infiltrating other red cells.

Symptoms of the disease are caused by the blood stage parasites. When certain forms of blood stage parasites (gametocytes, which occur in male and female forms) are ingested during blood feeding by a female *Anopheles* mosquito, they mate in the gut of the mosquito and begin a cycle of growth and multiplication in the mosquito. After 10-18 days, a form of the parasite called a sporozoite migrates to the mosquito's salivary glands. When the *Anopheles* mosquito takes a blood meal on human, anticoagulant saliva is injected together with the sporozoites, which migrate to the liver, thereby beginning a new cycle. Thus, the infected mosquito carries the disease from one human to another (acting as a "vector"), while infected humans

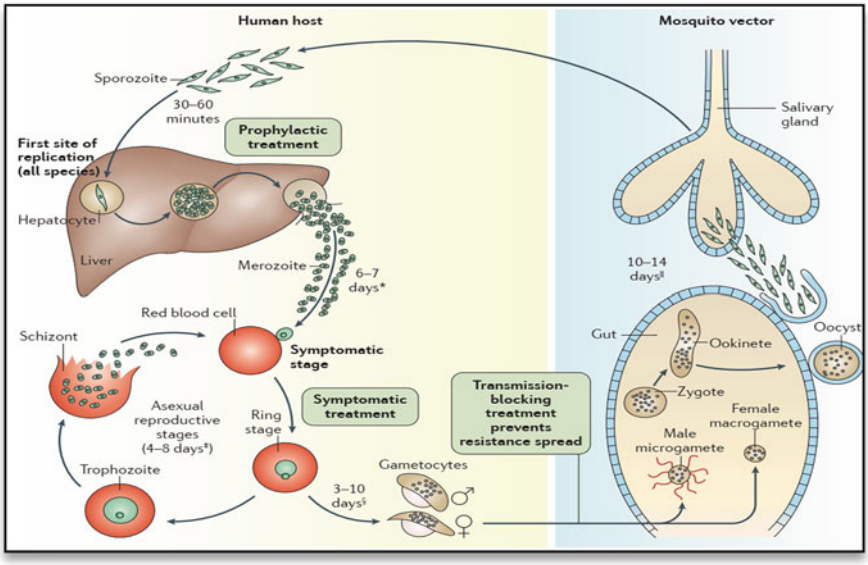

Fig. 21.1 The life cycle of *Plasmodium* spp. comprising humans and mosquitoes. (Source: Phillips et al. 2017)

transmit the parasite to the mosquito. In contrast to the human host, the mosquito vector does not suffer from the presence of the parasites.

21.4 Global Distribution of Malaria

Global distribution of malaria is mainly influenced by climatic factors such as temperature, humidity, and rainfall. Since *Anopheles* mosquitoes thrive in tropical and subtropical climates, malaria is widespread in regions that experience hot and humid conditions. Temperature is critical for malaria transmission as parasite fails to complete its cycle if temperature falls below the optimum. For instance, *P. falciparum* cannot complete its cycle if room temperature falls below 20°C.

Therefore, occurrence of malaria is common in tropical-subtropical regions and regions that lie closer to equator and experience warmer climates (Fig.21.2). In countries with tropical-subtropical climate, such as India, Pakistan, Bangladesh, some parts of China, Japan and Mexico, disease is limited to certain endemic regions as transmission fails to occur in regions which lie at high altitude, experience colder seasons or are hot or cold deserts. However, transmission occurs throughout the year in equatorial regions that lie closer to equator. The transmission is often more profound and varied with a wide variety of parasite. The transmission rate is highest in Africa, South of the Sahara, and in parts of Oceania such as Papua New Guinea.

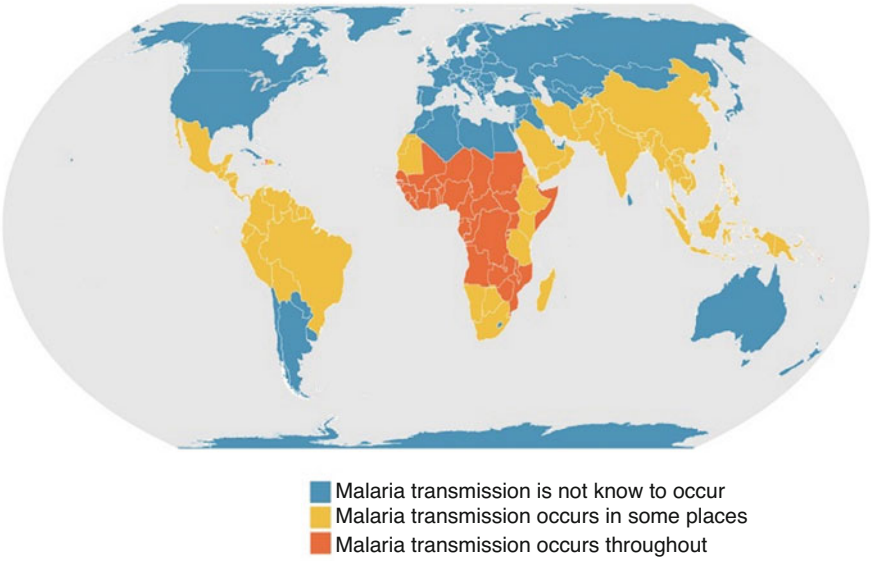

Fig. 21.2 Status of malaria transmission across the world. (Source: CDC, USA)

21.5 Current Status of Malaria: Its Global Impact

Impact of malaria can be understood in terms of deaths caused by the disease. According to World Health Organization's (WHO) malaria report 2017, nearly half of the world's population lives in areas at risk of transmission of malaria in 91 countries.

In several areas of high transmission, malaria is the leading cause of death. The young children who have yet to develop immunity for disease are the most vulnerable ones. Pregnant women also face a serious threat due to natural decrease in immunity as a result of pregnancy. An estimated 6,20,000 people died of malaria in 2017 according to Institute of Health Metrics and Evaluation (IHME), Washington (Fig.21.3). Majority of deaths were caused in underdeveloped countries of tropical and subtropical regions of the world with African regions accounting for about 90% of all the deaths, as depicted in the chart above. Apart from high incidences of deaths, malaria also imposes a high cost of prevention and therapy to both individuals and governments as it may include costs involved in diagnostic tests, purchase of drugs, loss of days at work, and in severe cases hospitalization. Therefore, malaria imposes enormous sufferings on the individuals, families, and countries, struggling with it.

However, a substantial scale-up in joint anti-malaria initiatives among nations and development of a comprehensive approach to curb the disease has rapidly increased malaria control efforts. The focus has been primarily on strengthening

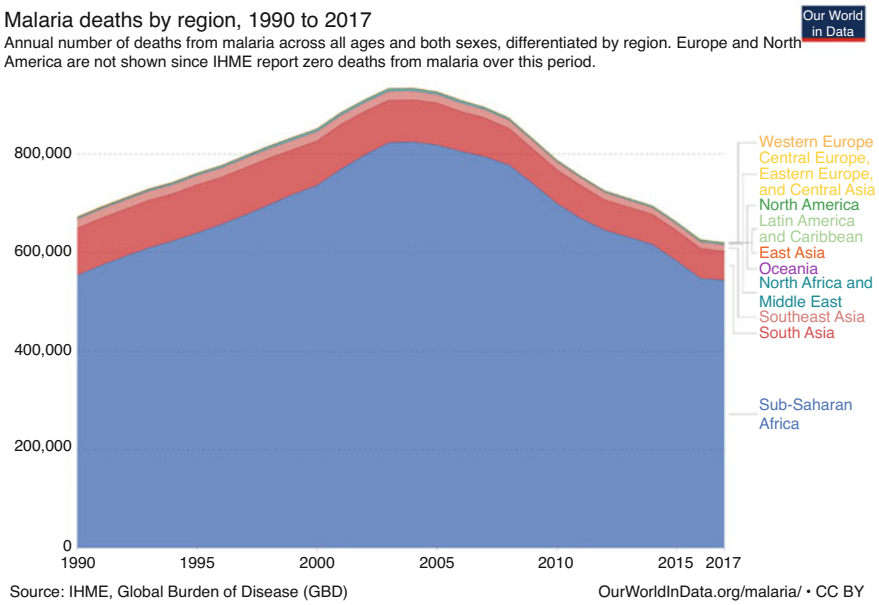

Fig. 21.3 Malaria deaths by region, 1990 to 2017. (Source: Roser, M. and Ritchie, H. Our World in Data)

the preventive efforts and development of better diagnostic techniques and lifesaving drugs, which are also cost-effective. This first optimism is also reflected in the data, with malaria mortality decreasing by 25% between 2010 and 2016. It has led to hopes and plans for the ultimate eradication of this deadly disease by 2030 as targeted by WHO in its Global Malaria Program.

21.6 Antimalarial Drugs and Therapies

Drugs and therapies employed to combat malaria has a history as ancient as the disease itself. Literature is riddled with examples of different plant-based products historically used by people to treat the disease, all across the world. Bark from Cinchona tree, which is rich in alkaloids with antimalarial properties, is perhaps one of the most important instances of such plant-based remedy. This effective compound appears in Western therapeutic literature in the late seventeenth century, and by 1820, quinine (one of the alkaloids) was isolated from the bark and became drug of choice for malaria until World War II. However, since then huge strides have been made in research and development of new and better antimalarial drugs. These advancements are mainly driven by need to find better alternatives as more and more species of malaria parasite became resistant to prevailing drugs, leaving them ineffective. Malarial drug resistance became an enormous challenge with time as parasite kept on evolving in order to combat the effect of drugs. In an effort to

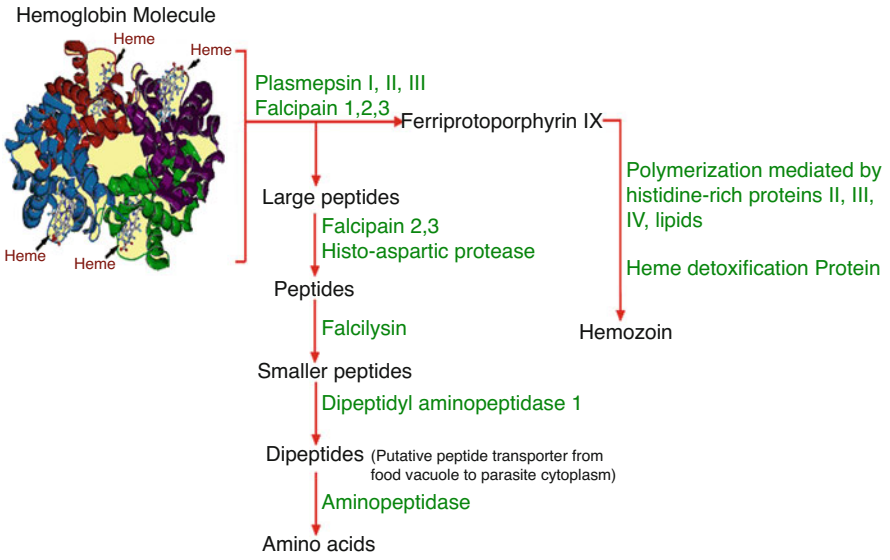

Fig. 21.4 Role of different enzymes in hemoglobin degradation, which is an attractive target for antimalarial chemotherapeutics

counter drug-resistant strains, various alternative synthetic compounds have been deployed since development of first such drug, Chloroquine in the 1930s. Structurally, there are different classes of antimalarial compounds:

- Quinoline derivatives, e.g., 8-aminoquinolines (primaquine), 4-aminoquinolines (chloroquine), Cinchona alkaloid (quinine), Quinoline methanol (mefloquine).
- Non-cyclic derivatives, e.g., Sulfonamides (sulfadoxine).
- Pyrimidine derivatives, e.g., pyrimethamine.
- Sesquiterpene trioxane lactone, e.g., artemisinin and its derivatives.

Sulfadoxine–pyrimethamine and Mefloquine are important examples of such synthetically developed compounds. The structurally similar quinoline drugs (such as quinine, chloroquine, and mefloquine) are thought to work by disrupting the digestion of hemoglobin throughout the blood stage of the parasite (Fig.21.4).

These drugs are recommended for use in combination with other complementary drugs (e.g., mefloquine and artesunate) to reduce the likelihood of developing resistance to quinoline compounds. Although varying degrees of resistance to the replacement treatment quickly emerged, it was found the combination of these drugs may still be effective against various malaria strains, their relatively higher cost and adverse side-effects prevented them from being recommended as first-line of treatment. Search for a better and safer antimalarials, which can form first-line of treatment, led to emergence of artemisinin-based drugs in China. Artemisinin had been recommended by Chinese herbal doctors for centuries before its rediscovery in the 1970s in China and was not introduced to rest of the world until as late as the

1990s. Their improved success over prevailing antimalarials, especially when utilized in combination with other drugs (Artemisinin combination therapy or ACT), has made them the drug of choice for treating falciparum malaria. However, development of resistance against ACT has been a long-standing fear among scientists; therefore, despite its effectiveness against falciparum malaria, WHO looked up to limit its usage only for treatment of serious cases of disease. Besides the presence of traditional drugs and newer ACT-based remedies, identification and development of new antimalarials remains a primary objective. Medicines for Malaria Venture (MMV) is one of the independent bodies which has been at the leading edge of developing new, safe, stable, and effective drugs for the malaria treatment, coming up with better alternatives. In light of above arguments, we will have a detailed look on different types of antimalarial drugs employed to treat the disease in the past and the ones currently employed. Research and development currently in progress for future therapies is also discussed toward the end.

21.6.1 Antimalarials of Past

21.6.1.1 Quinine

Quinine, the pharmaceutical compound that has served as a wonder drug for malaria, originally derived from the bitter bark of a Peruvian tree found at high altitudes. According to the legends, Spanish Countess of Chinchon was treated with this tree's bark for the illness in Peru and got cured. By the sixteenth century, the tree and its remedies found their way into Spain from Peru and Europe was introduced to its first reliable antimalarial in the form of Cinchona tree (*Cinchona succirubra*) bark. In 1820, French chemist Pierre Joseph Pelletie and Jean Biename Caventou isolated the active alkaloid "Quinine" from the Cinchona tree bark, which quickly became the drug of choice for treating malaria all throughout the nineteenth century. Quinine has manifested to be the most effective antimalarial agent to date and still remains an important malaria treatment drug. However, with passage of time, strains of parasite begin to exhibit signs of resistance against the drug. Even though reports of sporadic incidences of quinine-resistant strains go back to 1844 and 1910, the earliest appearance of lack of quinine sensitivity in large numbers was reported in Southeast Asia in the 1980s. It was also observed that lack of quinine sensitivity lead to treatment failures, which ultimately resulted in death of patients. In 2006, quinine was no longer advised as front-line treatment drug but continued to feature in Model List of Essential Medicines (MLEM) by WHO in the approach for the treatment of malaria. It must be noted that to this day, high-level quinine resistance has not been convincingly documented in any case. Usage of quinine is also recommended against *Plasmodium* strains previously found resistant to other antimalarials such as Chloroquine.

21.6.1.2 Chloroquine

Discovery of Chloroquine is attributed to German scientists of the Bayer Dye works who were working on developing a synthetic alternative to Quinine as an outcome of

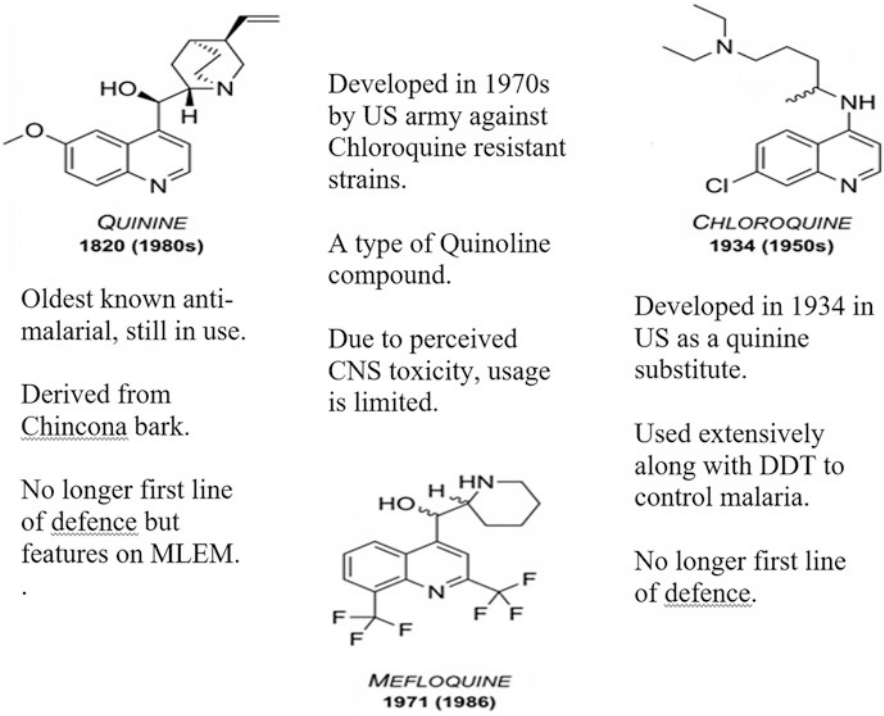

Fig. 21.5 Well-known antimalarial medicines discovered between 1820 and the 1980s. Some are still used today while some have been rendered ineffective due to the development of resistant strains or the emergence of undesirable side effects. Dates of first reported resistance are shown in brackets. (Source: Tse et al. 2019)

World War I. In 1934, Resochin (chloroquine) and Sontochin (3 methyl chloroquine) were developed by Germans. These compounds belonged to new type of antimalarials, the four-amino quinolones (Fig.21.5). However, due to fear of compound's toxicity, the research was halted and formula was passed on to the US sister company at Withrop Stearns. After outbreak of World War II, due to lack of quinine, Withrop researchers slightly altered the formula for German manufactured Sontochin and called the resulting compound Chloroquine, only to realize that resulting compound is identical to existing Resochin. Following the war, Chloroquine, along with DDT, was used extensively to control and eradicate malaria in large parts of the world. WHO deployed Chloroquine in affected regions in substantial amount with favorable results until reports of chloroquine-resistant strains started to emerge. Between 1960 and 1978, new strains of chloroquine-resistant *P. falciparum* (CRPF) emerged at four separate locations along the equatorial and tropical zones. MLEM featured chloroquine against *P. vivax* strains in specific regions that exhibit least or no resistance to the drug, even when Chloroquine is no longer the first-line of defense against malaria.

21.6.1.3 Mefloquine

Mefloquine sold under the trade name Lariam was developed by United States Army in the 1970s and continues to be prescribed by physicians. Its development was a collaborative effort of US Army Medical Research and Development Command, WHO, and Hoffman-La Rosche, Germany, after World War II. Initial idea was to develop a synthetic substitute for quinine. However, by the time preclinical trials were undertaken, chloroquine-resistant strains started to appear in Southeast Asia and South America. Ultimately, the compound (mefloquine) was developed as a 4-quinoline methanol, which showed promising result against chloroquine-resistant strains. Its efficacy against deadly falciparum malaria was first showed in 1974, as it subsequently became a successful treatment agent working both as a prophylactic and curative drug for the disease. Unfortunately, drug resistance against mefloquine was first reported in 1986 in Asia, after the drug became available in general markets. Mefloquine is no longer widely used as prescription drug due to the perception of CNS toxicity experienced by wide number of patients, although it is one of the medicines on the MLEM.

21.6.2 Antimalarials of Present

While abovementioned drugs have been used for decades to treat the disease, development of resistance against these medicines or adverse side effects have rendered them less effective in present scenario. The current generation of antimalarials listed on WHO MLEM include 14 medicines in the curative treatment and 4 medicines in the prophylactic treatment, usually in the form of single compounds or in combinations. Out of these, Artemisinin-based combination therapies, which include short-acting artemisinin derivative paired with one or more complementary compounds that are longer lasting, have proven to be most effective. Some of these prominent drugs of current generation are discussed below in detail while the information is summarized in Table 21.1.

21.6.2.1 Artemisinin and Its Derivatives

Artemisinin, better known as qing-hao to Chinese herbalists, is a sesquiterpene lactone peroxide extracted from the leaves of the shrub *Artemisia annua* (qinghao). Among all the available antimalarials, artemisinin and its derivatives are effective at killing widest range of asexual stages of the parasite, from medium-sized rings to early schizonts. They are also capable of producing a rapid therapeutic response by clearance of ring-staged parasites.

From a historical perspective, artemisinin or qing-hao has been used by Chinese herbalists to treat malaria for over 2000 years. Earliest account, dating to 168 B.C, describes it as a medicine with aromatic nature and bitter taste that was used for treatment of hemorrhoids. Ge Hang in 340 AD recommended artemisinin for treating high-grade fever and sever chills in the book Zhon Hon Bei ji Jang (Handbook of Prescriptions for Emergency Treatments). In spite of being a common household antimalarial remedy in China for centuries, artemisinin was not

Table 21.1 A summary of commonly used antimalarials. (Source: Talapko et al. 2019 with original inputs)

Medication name	Year of discovery/ synthesis	Origin	Usage	Mechanism of action	Side effects
Quinine	1600	Cinchona tree, South America	Resistance to chloroquine, prophylaxis and treatment of malaria	Inhibition of DNA and RNA synthesis	Headache, abortion, or congenital malformations
Chloroquine	1934	Synthesized by German scientist Hans Andersag	Most powerful remedy for the prophylaxis and treatment of malaria	Inhibition of DNA and RNA synthesis	Gastrointestinal disturbances, headache, skin irritation
Primaquine	1953	The 8-aminoquinoline derivative	Infections with *P. vivax* and *P. ovale*, prophylaxis and treatment of malaria	Interferes in transport chain of electrons and destroys parasite mitochondria	Anorexia, nausea, anemia, headaches, contraindicated in pregnancy and children under 4 years of age
Artemisinin	1971	Sesquiterpene lactone peroxide from *Artemisia annua*	Infections with *P. vivax* and treating multi-drug-resistant *P. falciparum* malaria	Generates free radicals, causing lipid peroxidation, which then damage the proteins needed for parasite survival	Headache, vomiting nausea, gastrointestinal disturbances
Mefloquine	1971	USA army and WHO	Multiresistant *P. falciparum* strains, prophylaxis and treatment of malaria	Damage to parasite membrane	Gastrointestinal disorders, CNS disorder, contraindicated in pregnancy and patients with epilepsy
Primethamine	1953	Pyrimidine derivative	For tissue parasites prophylaxis and treatment of malaria	Folic acid antagonist	Gastrointestinal disorders, Neuropathy, in high doses Also megaloblastic anemia
Atovaquone/ proguanil	2000	Ubiquinone analog	For the prophylaxis and treatment of malaria	Inhibition of cytochrome bc1 in *Plasmodium*	Nausea, vomiting, diarrhea, headache, dizziness, anxiety, difficulty falling asleep, rash, fever

considered prescription antimalarial until its rediscovery in the 1970s. Tu Youyou extracted the active compound artemisinin from the plant *Artemisia annua* in the year 1971. Soon after, scientists started testing it on human patients and positive results were published in Chinese Medical Journal 1979. In the year 2015, joint Nobel Prize was awarded to Youyou, in physiology or medicine for her noteworthy discovery. Today, in many areas of Southeast Asia, artemisinin and other artemether-group drugs serve as the foremost line of defense against drug-resistant malaria.

The rediscovery of this potent antimalarial shed light on peculiar nature of the compound itself as its structure was unlike any other compound known at the time. Moreover, antimalarial activity of the compound was extensively studied and several better, more potent derivatives were designed and synthesized in a short span of time. Three of the widely used derivatives of artemisinin are artemether, artesunate, and arteether. Where artemether and arteether are closely related as they are formed of oil-soluble methyl ether, artesunate is a water-soluble hemi-succinate derivative. Dihydroartemisinin (DHA) is utilized to synthesize the three semisynthetic prodrugs, and after administration, they are readily converted back to it within the body.

Mechanism of Action

The mechanism of action of artemisinin has been highly debated in scientific community since its rediscovery. The nature of its structure, which includes an endoperoxide bridge, essential for its antimalarial actions, makes the drug truly unique. The most accepted theory suggests that heme activates the artemisinin molecule to generate free radicals, causing lipid peroxidation, which in turn damage proteins required for parasite survival. However, there are evidences that advocate the presence of other possible MoAs. A computational approach undertaken in 2013 suggests that artemisinin specifically interacts with PfATP6 (Ca2+ transporter) and inhibits its activity. Another study conducted in 2015 reported upregulation of the unfolded protein response (UPR) pathway under the effect of artemisinin derivatives, slowing down the parasite development. Inhibition of *P. falciparum* phosphatidylinositol-3-kinase (PfPI3K) is also reported in some cases as a possible MoAs. It is, however, accepted that early-stage gametocyte mortality is successfully imparted by artemisinin derivatives. Compared to any other antimalarial drug in use, artemisinin derivatives are found to be more effective over a broader range of the parasite life cycle. The drug is also proven to be highly effective against multidrug-resistant forms of *P. falciparum*. Resistance against the drug has been slow to develop but first such report of drug resistance emerged from Cambodia in 2008. In 2018, ten more cases with high resistance, specifically against dihydroartemisinin–piperaquine combination therapy, were reported in Southeast Asia. In order to prevent development of resistance and increase the efficacy of current artemisinin derivatives, Artemisinin-based Combination Therapy (ACT) is recommended and practiced where the drug is combined with another longer lasting antimalarial such as mefloquine to provide desired results.

Artemisinin remains essential in our fight against malaria, with ACT making up majority of modern malaria treatments. They are recommended as main line of defense against the disease and continuous efforts are being made to improve and prolong the effect of the drug. In order to prevent development of resistance against the compound, WHO recommends implementation of ACT wherever possible. So far, no cases of widespread, stable, and clinically relevant ACT-resistant strains have been reported. Studies on drug toxicity have also shown encouraging signs as patients receiving artemisinin derivatives or ACT registered substantially fewer adverse effects compared to other commonly used antimalarial (mefloquine), proving the excellent safety and tolerability of the drug.

21.6.2.2 Proguanil and Atovaquone

During World War II, a pyrimidine derivative called as proguanil (C11H16CIN5) emerged as a successful antimalarial. The drug has been efficient against falciparum and vivax type of malaria. It had a prophylactic action as it stopped the parasite from reproducing by inhibiting its folic acid cycle. Since extensive monotherapeutic usage of proguanil quickly led to the development of resistant strains (one year), combination therapies were being tried to prolong the usage of the drug. Usage of Atovaquone for successful treatment of protozoan infections was initially reported in 1991. Since then, a combination of proguanil and atovaquone has approved to be highly effective due to synergistic effect of both the compounds mainly due to differences in their MoAs (Table 21.1). Atovaquone is an inhibitor of cytochrome bc1 complex that blocks mitochondrial electron transport chain, whereas proguanil acts as an inhibitor of dihydrofolate reductase (DHFR) that disrupts deoxythymidylate synthesis via its metabolite cycloguanil (CG). However, the combination of proguanil and atovaquone does not inhibit DHFR but is proven to reduce the concentration of atovaquone required for the treatment. The combination of these two is sold under the name Malarone™ by GlaxoSmithKline (GSK) since 2000s. However, the combination of proguanil and atovaquone does not inhibit DHFR but is proven to reduce the concentration of atovaquone required for the treatment

21.6.2.3 Pyrimethamine and Sulfadoxine

Development of proguanil paved way for another effective antimalarial, Pyrimethamine (PYR), which was developed in the early 1950s by Gertrude Elion and George Hitchings, now sold under the trade name Daraprim™. The joint Nobel Prize in Physiology or Medicine in 1988 was awarded to Elion, Hitchings and Black for "their discoveries of important principles for drug treatment" as a part of discovering this drug of great importance. However, it became clear that monotherapy dependent on both compounds would not yield desired benefits for long as resistance against both Proguanil and Pyrimethamine soon appeared. As a result, Sulfones and sulfonamides were combined with Proguanil or Pyrimethamine to increase the efficacy. Sulfadoxine was developed in the early 1960 with similar objective. The combination of pyrimethamine and sulfadoxine was found effective and later approved for the treatment of malaria in 1981. Fansidar® is the common

trade name of this combination of drug. The parasite folate biosynthesis pathway is known to be targeted by both these drugs. Dihydrofolate reductase is inhibited by pyrimethamine, while dihydropteroate synthetase is inhibited by sulfadoxine. Resistance to SP was first reported in Thailand in the 1970s and although the parasite resistance to SP spread rapidly throughout Southeast Asia, rates remained low in Africa until a decade ago. Since then, the parasite resistance to SP has rapidly spread across Africa, which is a growing cause of concern.

21.7 Future Prospects

With increase in resistance to prevailing drugs and their long list of adverse effects, research for stable, safer, and sustainable antimalarials is imperative. Countries like China and India along with traditional research hotspots in Europe and the USA have contributed immensely to malaria drug research. Alternatively, global initiatives like Malaria Medicine Venture (MMV) have been at forefront in improving malaria treatments by developing better antimalarials. These newly developed compounds are judged on the basis of their abilities to meet desired requirements, which include:

- No cross-resistance to drugs already in use.
- Novel mode of action.
- Single dose cure.
- Efficacy against both asexual and sexual stages of disease.
- Ability to act as a prophylactic in order to prevent the infection.
- Ability to remove *P. vivax* hypnozoites in the liver to prevent recurrence.

By partnering with various pharmaceutical companies and universities, MMV has been on the front line against malaria that promises a range of better, safer alternatives in future. From drug discovery to product development, various steps are undertaken to ensure the success of the product. A few of these important products and their current status is summarized in Fig. 21.6 in the form of a flowchart.

Exploration of novel combinations of already existing drugs is one of the tried and tested ways to develop new formulations (Pyrimethamine and sulfanide, ACT). This has helped in overcoming drug resistance, ensuring stability of the therapy. Moreover, an existing drug used to treat other ailments may be repurposed and developed as antimalarial if found effective against the parasite. This can be highly advantageous as the MoAs of the drug and its side effects are already studied. Imatinib, a cancer therapy drug, is an example of such a novel antimalarial currently at phase II trials along with dihydroartemisinin-piperaquine as a triple combination drug. Other such compounds include methylene blue, used to treat methemoglobinemia, along with primaquine; Fosmidomycin, an antibiotic in combination with piperaquine and Rosiglitazone, an antidiabetic as an adjunctive therapy for severe malaria. Sevuparin, a potent medicine used to treat sickle cell anemia, is repurposed and under development to fight malaria. Some other important drugs currently under development include Artemisone, a second-generation semisynthetic artemisinin

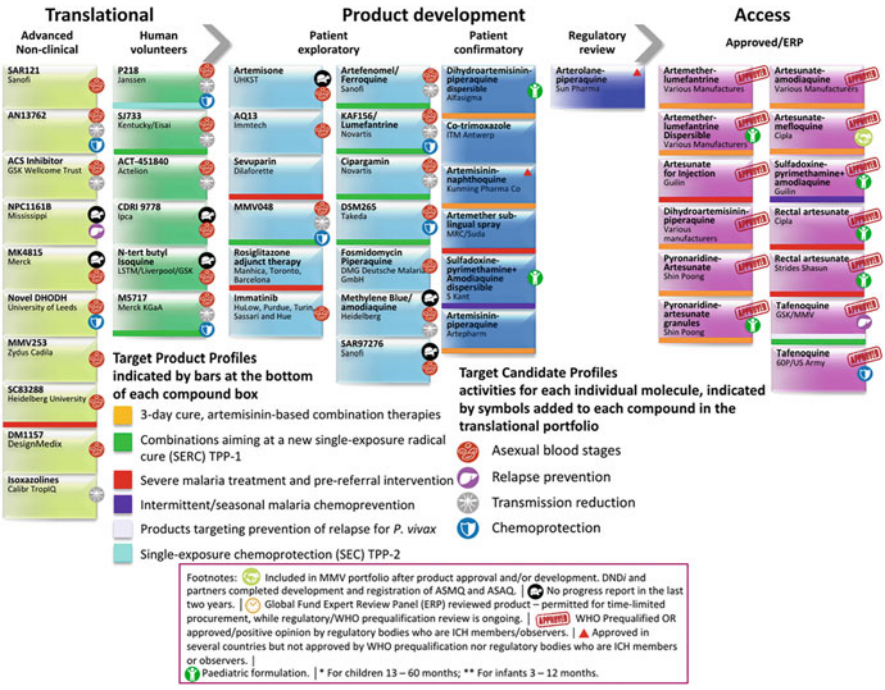

Fig. 21.6 A brief summary of various projects supported by MMV at various stages of production. (Source: Tse et al. 2019)

derivative, N-tert butyl isoquine/GSK369796, developed as an alternative to amodiaquine and MK4815, developed by Merck in 2012, currently in preclinical stages. A special mentioned must be made of compound CDRI 97/78, which is a fast-acting novel trioxane antimalarial, indigenously developed and synthesized in India by a team of scientists at Council of Scientific and Industrial Research (CSIR) and Central Drug Research Institute (CDRI) in the year 2001. The compound has completed all the preclinical trials and was found safe in pharmacology and toxicity studies. Phase-I single studies have also been conducted on patients at PGIMER, Chandigarh, where the drug was found to be well tolerated in normal healthy subjects. Pharmacokinetic (PK) studies in normal healthy subjects have corroborated the preclinical data on the molecule as the drug was found to be absorbed and converted rapidly to its precursor when administered in single dose. The mission is to develop a fast-acting, safer antimalarial to address the existing threat of malaria in Indian subcontinent.

The discovery and development of novel therapies will not only pave way for a series of better and safer antimalarial but also help in achieving the ambitious goal of eradication of malaria by 2030, as set by WHO. Existing therapies are always at a danger of development of resistance against them; therefore, need to design and develop novel compounds cannot be overstated. With advancements in research and

development techniques and global collaboration, the goal of malaria free world can be achieved at a rapid pace.

21.8 Malaria Vaccine: A Great Challenge

Vaccine is a powerful biological preparation, which develops active acquired immunity against diseases. With the help of vaccines, we have eradicated diseases like small pox and polio, either with complete or partial success. Currently, 40 malarial vaccines are able to generate immunity against sporozoites, components of blood stage and liver stage parasite. Many of them are in clinical trial phase, but development of an efficacious, stable, and long-lasting antimalarial vaccine is still a distant dream. The main problem faced in development of malaria vaccine is its complex life cycle and huge collection of polymorphic proteins. Many vaccines work positively in preclinical trials but fail to repeat the results in field trials. It is now believed that a deeper understanding of the mechanism, which aids the parasite to evade host immunity, is necessary to develop an all-round effective vaccine. This was made clear during the development of MSP119, a leading malaria vaccine. The vaccine generated high antibody titers in clinical trials but failed to repeat the same in field, therefore offering incomplete protection against the disease. Further study of MSP119 clarified that malaria parasite caused changes in the host dendritic cells, which decreased their ability to support B cells survival. Such studies provide us with an insight into the possible causes behind inability of memory B cells generated by vaccines to protect the subjects against the disease. Immune signal blocking technique and recombinants proteins are also currently in use to mimic immune signals for the development of vaccine for malaria, but their long-term success is unknown.

21.9 Malaria Drug Research: An Indian Perspective

India has an ancient history of battling with malaria. Descriptions of disease and its symptoms are found in ancient scriptures of the Vedic period (1500–800 BC) where it was referred to as the "king of diseases." Presently, India bears about 4% of total global malaria burden, a drastic reduction since 1950s. National Vector Borne Disease Control Program (NVBDCP) reported 194 death and 0.84 million confirmed malaria cases in 2017 in India. The states of Orissa, Chhattisgarh, Madhya Pradesh, and Jharkhand reported maximum number of malaria cases in India. Similar to WHO Global Malaria Program, India has set an ambitious target of achieving malaria-free India by 2030 by adopting existing tools, new innovations and strategies like novel drug therapies, vector control program, disease surveillance, and mass drug administration. In February 2016, Government of India introduced the National Framework for Malaria Elimination, which describes different strategies to eliminate malaria from India by 2030. Also in 2017, India launched an ambitious five Nation Strategic Plan to eradicate malaria. This has shifted the goal from malaria "management" to "Elimination" in 571 of India's 677 districts by 2022.

After diagnosis (by microscopy or Immuno-chromatography or real-time micro-PCR-based personal digital assistant), treatment is the major step in which discoveries have been recently made. Before independence, quinine and cinchona febrifuge were the major products, which were used for the treatment of malaria. Post-independence antimalarial drugs have evolved at a fast rate since the usage of quinine. First malaria control program came into existence in 1953. The mid of 1960s was the golden period when India seemed firmly on its way of eliminating malaria, as it reported only 99,667 malaria cases in 1965. This was made possible due to chloroquine, which was aptly termed as wonder malaria drug. It was safe and effective against both *Plasmodium falciparum* and *Plasmodium vivax*. But with rapidly developed resistance against chloroquine, this success could not last. Reports of chloroquine resistance started to emerge in 1973, and by 1976, India was again reporting huge number of cases. However, chloroquine was still found to be effective for *P. vivax*. Therefore, management of malaria control program focused on three main objectives:

- Drug pressure reduction (to prevent spread of drug resistance further).
- Drug monitoring and their resistance in different species of *Plasmodium*.
- Investment in research and development (to ensure new and safe drugs for future).

The first antimalarial drug policy comes into existence in 1982. The policy reported chloroquine resistance in different states of India and divided them among chloroquine sensitive area and chloroquine resistance areas. If the patient did not respond to chloroquine, sulfalene-pyrimethamine was recommended in place of chloroquine. In chloroquine-resistant areas, drug amodiaquine was recommended for conjectural treatment, which was detected by active case detection. Sulfalene-pyrimethamine was used as conjectural treatment for the patient detected by passive detection. In radical treatment, sulfalene-pyrimethamine along with primaquine (one dose) was recommended, but this treatment was found to have major adverse effects on the patient health. The drug policy has been modified several times with the introduction of new drugs (Proguanil, amodiaquine, artemisinin derivative, etc.) and reorganization of their doses for the different type of malaria parasite. Currently, India has 14 registered antimalarial drugs, enlisted below (Table 21.2).

An alternative antimalarial Ayurvedic drug named AYUSH-64 is currently developed by Central Council for Research in Ayurvedic Sciences (CCRAS). Although discovered about 38 year ago, this drug was not commercialized as an antimalarial drug due to lack of scientific evidences. Positive responses in clinical trials and double blind patient studies have raised the hopes of having an effective alternative antimalarial developed in India. The drug is also claimed to have no side effects as it is a combination of herbs and herb-based compounds. In 2014, National Research Development Corporation (NRDC) signed an agreement with Dabur India Ltd.to commercialize this drug. On more traditional lines CSIR has come far in development of antilmalarial compound CDRI 97/78, already discussed in previous section. With an impetus on immediate completion of pending projects and timely

Table 21.2 Antimalarial drugs in India. (Source: Anvikar et al. 2014)

Sr. no.	Drug
1	Amodiaquine
2	Artemether + Lumefantrine FDC
3	Arterolane + Piperaquine FDC
4	Artesunate + Amodiaquine FDC
5	Artesunate + Mefloquine blister pack and FDC
6	Artesunate + Sulfadoxine-Pyrimethamine blister pack
7	Chloroquine
8	Injectable artemisinin derivatives
9	Mefloquine
10	Primaquine
11	Proguanil
12	Pyrimethamine
13	Quinine
14	Sulfadoxin-Pyarimethamine

investment in research and development of novel compounds, the future of antimalarial therapies in India can certainly be promising.

Malaria finds an important place in human history. It affected lives of Neolithic villagers, Indian fishermen, Chinese, Greeks, and Americans. It claimed near about 150-300 million lives in the twentieth century alone claiming for 2-5% of total deaths in the period. Human efforts to treat and control malaria have come a long way since identification and production of quinine as an antimalarial in the early twentieth century. Better understanding of disease epidemiology and vector biology has aided pharmacologists and drug researchers. Intervention of insecticides also proved vital in vector control as is evident by the success of DDT after its discovery in 1939. In 1955, WHO proposed the worldwide malaria eradication program which may have not met its anticipatory success but it helped in eradication of malaria in major parts of the world, especially in Europe and the USA. However, in spite of all the efforts, major parts of Africa and Southeast Asia are still battling with the ill effects of the disease. While huge reduction in number of cases is achieved in many of these areas, eradication still remains a distant goal, amplifying the need for a concerted effort to achieve the target. A deeper understanding of complexity of infection and its transmission is of utmost importance for development of better drugs and an effective vaccine. Appearance of resistant strains every decade or so has renewed the challenge of developing effective and stable therapies such as ACT.

Global deaths rate due to malaria has been reduced by 60% and about 66% in Africa, signifying the success of these efforts. However, focus remains on complete eradication of the disease in coming 15 years. This has been a long-term goal of WHO which has seen a renewed interest in recent years. In 2015, a Global Technical Strategy for Malaria developed by WHO was adopted by World Health Assembly (WHO 2015) with the long-term goal of eradication of disease but with a realistic vision and appreciation to overcome threats and barriers such as development of

drug-resistant strains without overlooking the need to substantially bring down morbidity and mortality. Novel drugs, combination therapies, vaccines, and genetically modified vectors for insect control are weapons of future with huge promise, which require immediate attention and investment to be developed in time to reach the ambitious goal of malaria eradication.

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Malaria Drug Discovery: How to Tackle the Problem of Drug Resistance

22

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Abstract

Malaria has been a huge issue in previous years since it is an endemic disease that kills millions of people worldwide, regardless of their age. Chloroquine, quinine, mefloquine, and sulfadoxine-pyrimethamine are among some of the drugs that are used to treat malaria. However, there are multiple *Plasmodium* species, including human-infecting species, that have altered their genome, resulting in drug resistance. They are classified as drug-resistant strains, and the leading cause of their resistance has been linked to mutations and multiple gene alterations, as well as gene suppression or amplification in some cases. The most often affected genes responsible for drug resistance include *PfCRT, PfMDR1, PfDHP, PfDHFR, PfCYTB, PfK13*, and *PfATP6*. Due to the threat caused by drug resistance, certain recommendations for malaria prevention have been provided, ranging from the lowest risk of malaria transmission to the highest risk of drug resistance. Artemisinin combination therapies (ACTs), delaying drug resistance inside the host, combined therapy strategy for antimalaria, and nanomaterial medication

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_22

delivery system are some of the strategies utilized to combat malaria. Moreover, vaccines for malaria are also available in the market. The current chapter focuses on drug resistance, its mechanisms, and various techniques that are used to combat malaria drug resistance in this chapter.

Keywords

Plasmodium species · Drug resistance · Artemisinin combination therapies · Nano material medication · Vaccines

22.1 Introduction

Malaria is an endemic disease that remains a significant factor causing death in adults and children in developing nations, regardless of their age (Tatem et al. 2010). Malaria is prevalent in 40% of the world's population, which is spread among 90 countries where 2400 million people live. According to the statistics, more than 500 million people are infected with the *Plasmodium* species. Female Anopheles mosquito serves as the vector for transmission of this parasite (Garcia 2010). According to a study, due to some scientific and operational considerations, some countries have successfully eradicated malaria. Malaria elimination would have been far more feasible in countries in Asia and America, whereas least viable in countries in Central and West Africa, relative rankings accordingly (Tatem et al. 2010).The five species of *Plasmodium* that can infect humans are *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, and Plasmodium knowelsi*. The parasite *P. falciparum* is the most hazardous, causing the most significant complications and deaths (Zhang et al. 2016).

Due to decreasing medication sensitivity in all *Plasmodium* species, including *P. falciparum*, the treatment of this severe malaria has evolved throughout the last two decades. The diagnosing patient typically exhibits symptoms such as nonspecific and inconsistent fever, cold, headache, and malaise, while almost 20% of persons have vomiting, and many more suffer from paralysis to death (White 1996). Because some *Plasmodium* species cause paralysis and death, the patient is given numerous treatments in combination (Baird 2019).

Artemisinin-based combination therapy (ACTs) is one of the most widely acknowledged treatment. Since introduction in all endemic locations, it became first-line and highly effective antimalarial therapeutic for moderate falciparum malaria in all endemic areas, which provided a much-needed, highly effective antimalarial medication (Antimalarial Drug Combination Therapy: Report of a WHO Technical Consultation, 4–5 April 2001 n.d.; Guidelines for the Treatment of Malaria n.d.). However, as time passes, the combo medicine delivers ACT as an antimalaria drug, although it is resistant. Distinct drugs have various different molecular indicators of resistance, and some of them reveal a linked gene or variant (Ippolito et al. 2021). The proliferation of *Plasmodium* species strains that seem to be resistant to chloroquine as well as other antimalarial medications has complicated the

problem even more (Fidock et al. 2004). Numerous factors contribute to antimalaria drug resistance, and current antimalaria drugs were discovered in response to the parasite's primary metabolic differences from its host which is causing malaria (Fidock et al. 2004).

For the past two decades, Artemisinin has been used frequently to treat malaria. Artemisinin has antibacterial, antifungal, antileishmanial, antioxidant, anticancer, and anti-inflammatory properties (Kim et al. 2015).Corresponding resistance to ACT companion medicines has emerged as an outcome of artemisinin resistance, resulting in dihydroartemisinin-piperaquine significant delayed treatment failure rates (Menard and Dondorp 2017). New strategies and regimens based on currently available antimalarial drugs will need to be introduced in order to ensure effective treatment. Before falciparum malaria becomes untreatable, eliminating artemisinin resistance will mean eliminating all falciparum malaria from the same places (Maude et al. 2009). Antimalarial drug resistance has taken longer to develop in non-falciparum species, presumably because there are fewer mutational events and fewer parasite communities in the host body, whereas the ability of *P. vivax* and *P. ovale* to bypass blood schizonticides by generating hypnozoites in the liver (Ippolito et al. 2021). In this regard, it has been noted that *P. vivax* and *P. ovale* resistance to the drug (such as chloroquine) has increased (Menard and Dondorp 2017).

Quinone and mefloquine, two antimalarial medications, are more susceptible to resistance. However, it requires few genetic events to confer a high level of resistance and have pharmacokinetic properties, which involve a lengthy terminal half-life trying to translate to a longer duration of subtherapeutic drug levels (Menard and Dondorp 2017). A variety of antimalaria drugs are available but as malaria parasites tends to become resistant toward antimalarial drugs, exploration of some new horizons and alternative therapeutics become the need of an hour.

22.2 Malaria Parasites and Anti-malaria Drug

One of five *Plasmodium* species causes human malaria (Fig. 22.1) (Ippolito et al. 2021). There are a total of 5 distinct species of malaria parasites that affect people, and the most lethal *Plasmodium* species is *P. falciparum*; the most prevalent and widely dispersed cause of recurrent malaria, *Plasmodium vivax*; The parasite *P. ovale*, which has two distinct species (*Plasmodium ovale wallickeri* and *Plasmodium ovale curtisi*), is the cause of tertian malaria; One of the several species that causes benign malaria is *Plasmodium malariae*; rare cases of *Plasmodium Knowlesi* in people (Milner 2018). Malaria is the largest cause of sickness and mortality globally, impacting millions of individuals in tropical and temperate Asia, Africa, and the Americas (Battle et al. 2019). The endemic hepatic delay of *Plasmodium vivax* confers a high level of resistance to standard malaria management strategies (Baird 2019). With a few exceptions, malaria is treated with a mixture of various drug compositions (Ippolito et al. 2021).

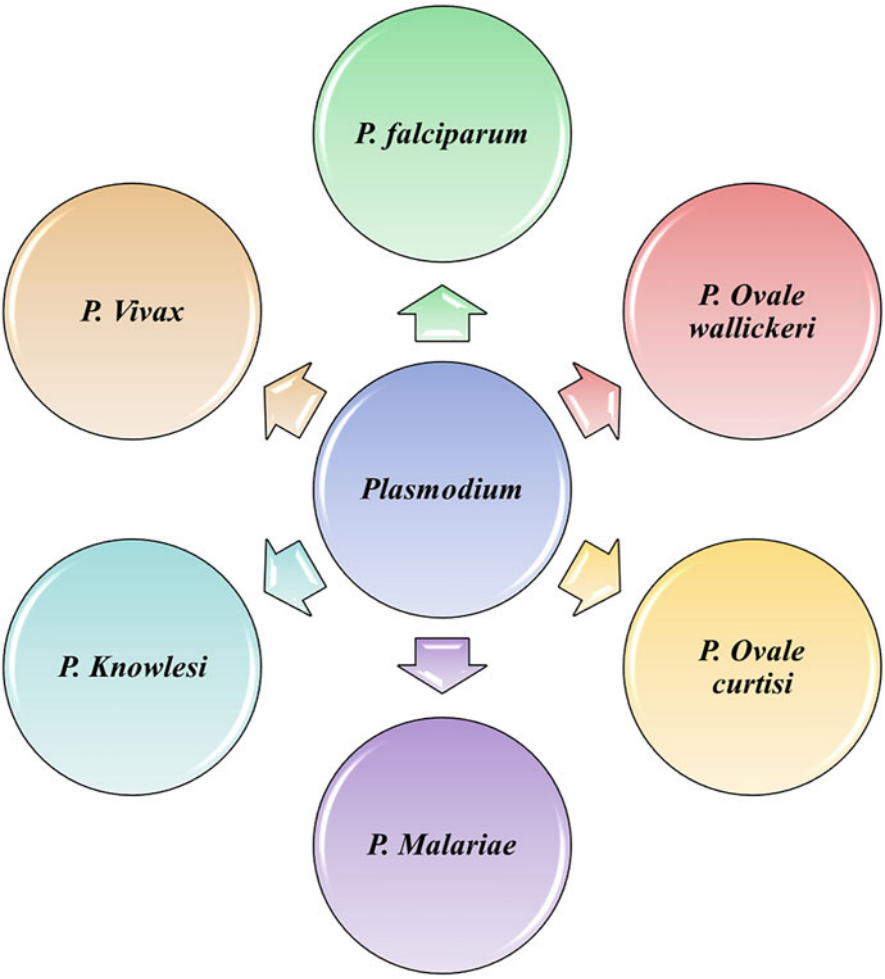

Fig. 22.1 Six different species of malaria parasite (Milner 2018)

Plasmodium species that infect humans follow the same life cycle, with an initial stage of development in the liver continued by further multiplication in the host's blood (Haldar et al. 2018). When mosquitoes transmit *Plasmodium* sporozoites into a human host, the parasites invade and develop inside liver cells, leading to hepatocytic rupture and parasite release into the bloodstream. *P. vivax* and *P. ovale* can remain as hypnozoites in a latent liver stage, resulting in recurrent erythrocytic stage infections that are extended to several months or even years after initial contamination. Merozoite reproducing asexually in red blood cells causes a cycle of parasite development and erythrocytic death, resulting in clinical diagnostic factors including fever, headache, and chills (Poirot et al. 2013).

Life cycle stage influence, molecular targets, and half-life of the parasite are the main factors that are taken into consideration while evaluating antimalarial drugs. Because of stage-specific biology, drug effects vary according to the life cycle stage of the parasite (Daily 2006). They also exhibit comparable resistance and vulnerability toward antimalarial drugs like quinine, chloroquine, and Artemisinin. Precisely, *P. vivax* in the intraerythrocytic stage develops resistance, and the patient condition worsens after chemotherapy, but primaquine, which contains 8-aminoquinolines, is known to successfully prevent this (Baird 2019; Chu and White 2016). The Food and Drug Administration (FDA) approved drug tafenoquine, which is an 8-aminoquinoline, is used for the prevention of all malaria. Tafenoquine produces hemolysis in people with glucose-6-phosphate dehydrogenase (G6PD) deficiency (hemizygous males and homozygous females), as it does with other 8-aminoquinolines, and is therefore contraindicated in the population. Hemolysis is a concern for heterozygous females with intermediate G6PD activity (Baird 2019).

In the clinical aspect, antimalarial medicines are classified into five groups according to their structural foundation and apparent activity (Table 22.1) (Sato 2021).

1. Endoperoxides—Artemisinin and its derivatives
2. 4-aminoquinolines—chloroquine; aryl-amino alcohols-quinine, mefloquine
3. Antifolates— pyrimethamine, proguanil, sulfadoxine
4. Naphthoquinones— atovaquone
5. 8-Aminoquinolines—primaquine, tafenoquine

22.3 Drug Resistance in Human *Plasmodium* Species

The worldwide spread of human-infecting *Plasmodium* species causing endemics has now progressed to the point where *Plasmodium* has developed drug resistance to antimalarial named as chloroquine, sulfadoxine, pyrimethamine, quinine, mefloquine, and others, as listed in the previous table (Mita et al. 2009). According to a recent study, drug resistance is linked to the genes *P. falciparum* chloroquine resistance transporter *(PfCRT)*, *P. falciparum* multidrug drug resistance 1 *(PfMDR1)*, *P. falciparum* kelch 13 *(PfK13)*, (*PfATP6 or PfSERCA)*, *P. falciparum* dihydrofolate reductase *(PfDHFR),* and *P. falciparum* dihydropteroate synthetase *(PfDHPS)* on various codon sites. Resistance is caused by these genes via mutation, variation, amplification, and deamplification (Daily 2006; Menard and Dondorp 2017; Mita et al. 2009).

The antimalarial drugs mainly act on enzymes of the folate pathway, and due to mutagenic effects of certain chemicals, genes encoding for these enzymes undergo mutations, which lowers the affinity of binding between drug and target enzymes (Cowman 1995), leading to the development of drug resistance. Molecular evolutionary and population genetic tools will significantly benefit our understanding of evolutionary and transmission aspects of resistance to parasite therapy, allowing us to develop more effective malaria control strategies (Mita et al. 2009). There are certain techniques by which we can detect mutated genes, which lead to drug

Table 22.1 Antimalarial drugs: their properties, chemical structure, site of action, molecular signatures of resistance

Drug	Chemical structure	Drug developed	Drug resistance reported	Site of action	Molecular signature of resistance	Reference
Chloroquine phosphate	4-Aminoquinoline	1945	1957	Inhibits intra-parasitic Hgb metabolism in the food vacuole, making it active. It also affects nucleic acid biosynthesis	PfCRT on codon 76; pfmdr-1 on codon 86 with other mutations	Daily (2006), Menard and Dondorp (2017)
Quinine	Aryl-amino alcohol	1820	1908	Inhibits intra-parasitic Hgb metabolism in the feeding vacuole, making it beneficial against big rings and trophozoites	PfCRT on codon 76; pfmdr-1 on codon 1042, 1034 or 1246 and variation in pfnhe1 gene	Daily (2006), Menard and Dondorp (2017)
Mefloquine	4-methanolquinoline	1984	1991	Inhibits intra-parasitic Hgb metabolism in the food vacuole and cytosol endocytosis	Amplified pfmdr-1 gene expression is increase	Daily (2006), Menard and Dondorp (2017)
Sulfadoxine	Sulfonamide	1937	1970	Antifolate biosynthetic pathway and parasite DNA replication are both active by the enzyme inhibitor dihydropteroate synthase (PFDHPS)	Pfdhp on codon 436	Daily (2006), Menard and Dondorp (2017), Shibeshi et al. (2020)
Pyrimethamine	Diaminopyrimidine derivative	1940	1952–1970	By reducing the activity of the dual enzyme dihydrofolate reductase and thymidylate synthase, prevent the synthesis of pyrimidine	Pfdhfr on codon 51	Menard and Dondorp (2017), Shibeshi et al. (2020)

Atovaquone	Hydroxynaphthoquinone	1996	1996	Active by interfering in the electron transport system of cytochrome b and blocking transit of numerous parasite enzymes, which might cause the mitochondrial membrane to separate	Pfcytb on codon 268	Daily (2006), Menard and Dondorp (2017)
Proguanil	Biguanide	1940	1949	By reducing the activity of the bifunctional enzyme dihydrofolate reductase-thymidylate synthase, active triazine metabolites stymie pyrimidine synthesis, and parasite DNA replication	Pfdhfr on codon 16	Daily (2006), Menard and Dondorp (2017)
Artemisinin derivatives-	Sesquiterpene lactone endoperoxide	1980 (monotherapy); 2000(drug in ACT)	2008 (partial resistance)	Through cation-mediated generation of reactive intermediates and peroxide bridge reduction, effective against blood-stage parasites, from ring stages through early schizonts, as well as immature gametocytes	PfK13, PfATP6, PfMDR1	Daily (2006), Menard and Dondorp (2017), Shibeshi et al. (2020)

(continued)

Table 22.1 (continued)

Drug	Chemical structure	Drug developed	Drug resistance reported	Site of action	Molecular signature of resistance	Reference
Primaquine phosphate	8-Aminoquinoline	1950	–	*Plasmodium* mitochondrial metabolic processes are disrupted, ubiquinone's role in electron transporter cause in respiratory chain is disrupted, as well as highly reactive metabolites production, resulting in harmful intracellular oxidative potentials	–	Menard and Dondorp (2017)
Lumefantrine	Aryl-amino alcohol	2000 (combined with artemether)	–	Hinders the mosquito's digestive vacuole's ability to detoxify its own Hgb, as well as parasite endocytosis of the cytosol	Pfmdr-1 on codons 184, 1034 and 1042	Menard and Dondorp (2017)
Piperaquine	Bis-4-aminoquinolin	1960 (monotherapy), 2008 (combined with dihydroartemisinin)	1970	Inhibits intra-parasitic Hgb detoxification in the parasite's digestive vacuole, making it active. Chloroquine may also cause nucleic acid production	PfCRT on codon 350. Deamplification of an 82-kb chromosome 5 region (containing the PfMDR1 gene) and amplification of a 63-kb chromosome 5 region	Menard and Dondorp (2017)

resistance against malaria; some of these methods are Genotyping (qPCR), Immunoblotting, Immunofluorescence assay, and SYBR Green assay (Verzier et al. 2019).

22.3.1 Molecular Mechanisms of Drug Resistance in *P. falciparum* and *P. vivax*

P. falciparum has demonstrated the emergence of mutations causing antimalarial resistance during the asexual blood stage of infection. An infectious mosquito inoculates sporozoites in humans, which causes infection. Sporozoites infect liver cells, while merozoites are released into the bloodstream and infect red blood cells (RBCs). During the asexual blood phase of infection, which is crucial for the development of clinical signs of sickness, the parasites mature and multiply on average at a rate of 109–112 parasites per replication cycle. To begin a new replication cycle, infected RBCs explode, releasing fresh merozoites into the bloodstream. *Plasmodium* infection spreads when a subpopulation of parasites grows into gametocytes and is sucked by another mosquito (Cowell and Winzeler 2019).

Commonly used antimalarial and their discovered genetic mediators to tolerance are:

22.3.1.1 4-Aminoquinolines

Chloroquine, a 4-aminoquinoline compound, has been recognized as acritical essential antimalarial drug that lowers mortality rates (Gabryszewski et al. 2016). Chloroquine enters the parasitic cell and gets stuck in the feeding vacuole, where it stops hematin from bio-crystallizing. Because of the vacuole's acidic environment, chloroquine becomes "imprisoned" in its membrane-impermeable twofold protonated form. Further, chloroquine forms a compound with free heme, causing heme to accumulate and the parasite to die (Lawrenson et al. 2018).

P. falciparum chloroquine resistance transporter *(PfCRT)* is a member of the Drug or Metabolite Transporter superfamily proteins, present on the digestive vacuole (DV) surface of intra-erythrocytic ABS parasites (Fig. 22.2) (Blasco et al. 2017; Fidock et al. 2000). Catabolism of hemoglobin in the mosquito's DV is vital for the parasite since it is a source of the amino acid (Cowell and Winzeler 2019).

The DV (digestive vacuole) is a segment within the parasite where it catabolizes Hgb from the host RBC. When Hgb is broken down, reactive heme is formed, which is then detoxified into hemozoin. The 4-aminoquinoline drug binds to heme and hinders detoxification (Combrinck et al. 2013; Sigala and Goldberg 2014). Pf*CRT* and Pf*MDR1* are membrane proteins found in the DV. *PfCRT* is considered to transport drugs out of the DV, whereas *PfMDR1* is thought to carry them into DV (Martin et al. 2009). *PfCRT*'s T mutation is necessary for CQ resistance, whereas N86Y changes *PfMDR1*'s enhancement. Mutations within those transporters also contribute to sensitivity to aryl-amino alcohols and Artemisinin.

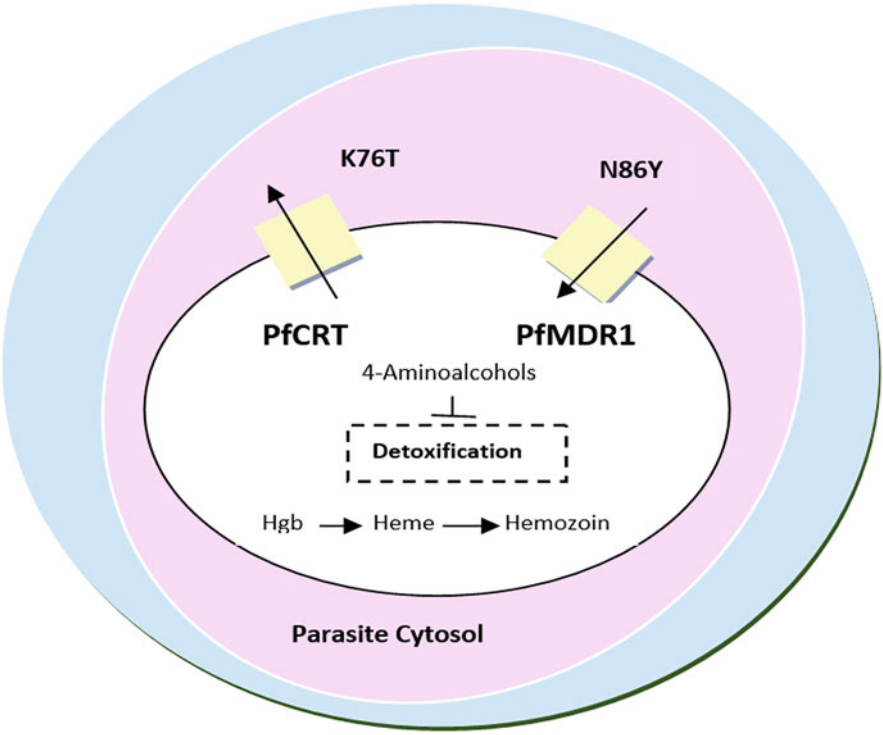

Fig. 22.2 Role of *P. falciparum* chloroquine resistance transport (*PfCRT*) and *P. falciparum* multidrug resistance protein 1 in digestive vacuole (Cowell and Winzeler 2019)

22.3.1.2 8-Aminoquinolines

8AQs are suspected of fighting malaria by interfering with the parasite's electron transport chain and possibly creating reactive oxygen species, while their exact mode of action is uncertain (Hamerly et al. 2019). 8AQ is linked to toxicity of hemolyticin G6PD-deficient individuals, finding a metabolite that can be safely delivered (Nanayakkara et al. 2008). Both primaquine and tafenoquine are antimalarial drugs that are used to treat and prevent malaria. FDA has recently approved tafenoquine as a drug against *P. vivax*; on the other side, surprisingly, primaquine hype the activity of chloroquine to work against chloroquine resistance in *P. falciparum* (Bray et al. 2005). Due to reinfections in malaria-endemic areas, determining *P. vivax* is resistance to primaquine is challenging. However, no genetic indicators for primaquine resistance are currently recognized (Thomas et al. 2016).

22.3.1.3 Antifolate Drugs

Point mutation in dihydrofolate reductase (DHFR) inhibitors, including proguanil, pyrimethamine, and trimethoprim, as well as sulfa medicines like sulfamethoxazole and sulfadoxine, impair parasite folate production (Fig. 22.3). Clinical isolates

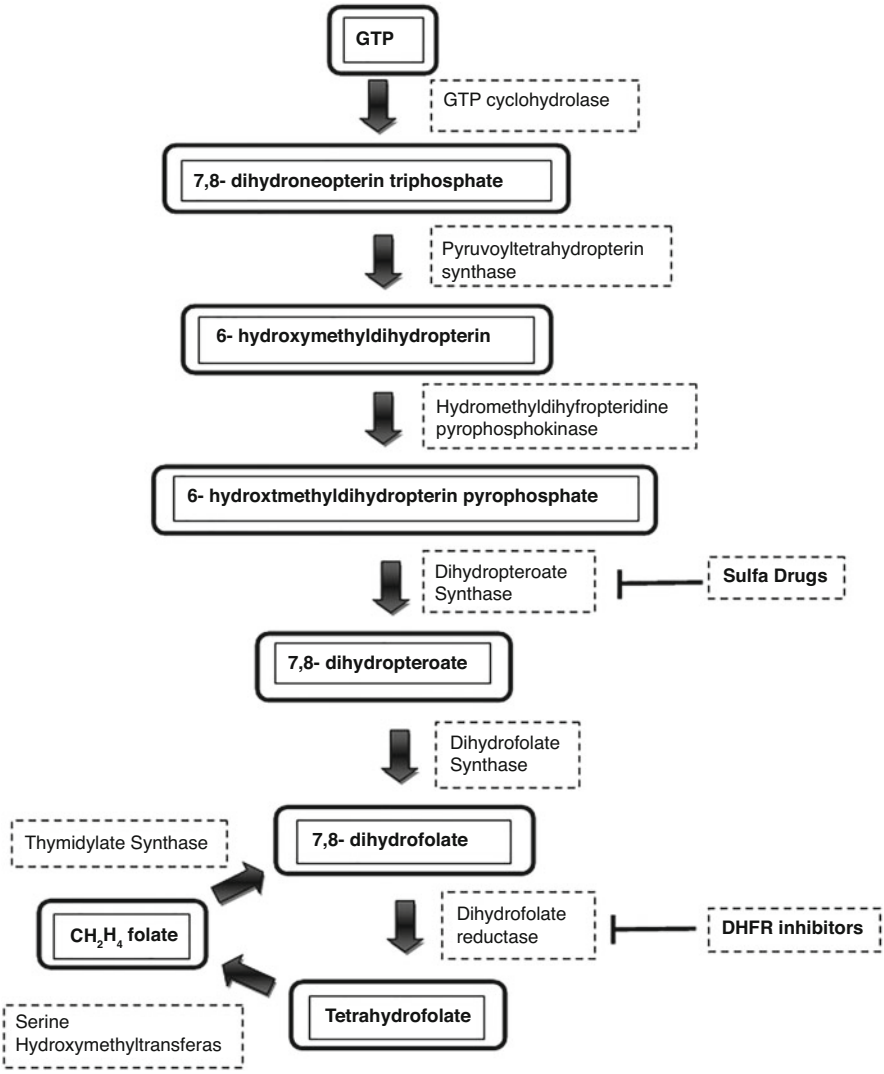

Fig. 22.3 *Plasmodium falciparum* folate biosynthesis route (Hyde et al. 2008)

exhibit more copies of the gch1 gene, which is probably an evolutionary adaptation to the pressure generated by antifolates (Nair et al. 2008).

22.3.1.4 Naphthoquinones

Atovaquone is a brand-new hydroxynaphthoquinone with strong action. The synergistic impact of proguanil is effectively eliminated by the quick appearance of atovaquone-resistant mutations. A fixed combination of atovaquone and proguanil, registered as Malarone, has undergone extensive clinical testing and has proven to be

highly efficient for both preventing and treating malaria (Vaidya 2011). It functions by inhibiting the electron transport chain at the cytochrome bc1 complex. This system supplies electrons to dihydroorotate dehydrogenase (DHODH); an essential enzyme for asexual blood stage parasites is de novo pyrimidine production (Phillips and Rathod 2012).

22.3.1.5 Artemisinin Development

Artemisinin, as well as its variants, are first-line antimalarial medicines with a minimal toxicity profile. For its antimalaria action, it is dependent on its unique peroxide bridge. Since Artemisinin is less soluble in water or oil, scientists engineer to exclude the carbonyl group from the compound to produce dihydroartemisinin (DHA) (Yang et al. 2020). Derivatives of Artemisinin make significant enhancement on antimalarial activity and are used as frontline antimalaria ACTs (Kunkel et al., 2021). In the artemisinin treatment, there has been a solid link with hemoglobin digestion (Xie et al. 2016). Artemisinin is also employed in other treatments, such as cancer therapy, helminths, and the SARs-CoV-2 virus, which is referred to as drug repurposing.

22.3.2 Molecular Mechanisms of Drug Resistance in *P. knowlesi*

There is plenty of information that *P. knowlesi* is primarily a zoonotic illness, even if the theory that Anopheles transmits the parasite from person to person has not yet been proven or disproven (White et al. 2014, Lee et al. 2022). While chloroquine is effective for treating relatively simple knowlesi malaria, ACTs are associated with rapid parasite clearance efficiency and reduce the rate of anemia during join and must be considered the therapy of choice, especially given the risk of misdiagnosing drug-resistant of *P. vivax* or *P. falciparum* malaria as *P. knowlesi* malaria in co-endemic regions (Barber et al. 2021).

Currently, malaria rapid diagnostic tests (RDTs) target three proteins: *Plasmodium falciparum* histidine-rich protein 2 (*PfHRP2*), plasmodial lactate dehydrogenase (pLDH), and plasmodial aldolase. Antibodies that target these antigens are used to identify *P. falciparum* or *P. vivax* specifically. They are also utilized in conjunction with anti-*Plasmodium* antibodies, which attack all *Plasmodium* species (Moody 2002). The ability of these RDTs to detect *P. knowlesi* infection has also been tested (Foster et al. 2014).

Artemisinin and chloroquine sensitivity were found in *P. knowlesi* isolates, although mefloquine sensitivity was lower (Fatih et al. 2013). There was no indication of mefloquine resistance, and it has been approved for the treatment of moderate knowlesi malaria as a monotherapy (Tripathi et al. 2005).

22.3.3 Molecular Mechanisms of Drug Resistance in *P. malariae*

Drug resistance exists against *P. malariae*, despite the fact that it is the parasite that causes quartan malaria, a serious re-emerging parasitic disease that affects people worldwide. Whereas chloroquine treatment for *P. falciparum* and *P. vivax* infections frequently fails, *P malariae* is thought to be chloroquine-sensitive, and oral chloroquine continues to be the treatment of choice for moderate quartan malaria (Maguire et al. 2002). Combination medicines used to treat acute *P. falciparum* malaria can help *P. malariae* survive. *P. falciparum* may then recur after therapy, at which point *P. malariae* may reappear (Fuehrer et al. 2022).

22.3.3.1 Molecular Mechanisms of Drug Resistance in *P. ovale*

Instead of atovaquone/proguanil, *Plasmodium* infection treatment suggestions advocate chloroquine or artemisinin-based combination therapy (ACT) as the first-line treatment for *P. ovale* infections (Bouchaud et al. 2020). On the basis of gene polymorphisms, *P. ovale* has been separated into two different variants, *P. ovale* wallikeri (variant type) and *P. ovale* curtisi (classic type) (Sutherland et al. 2010; Win et al. 2004). RDTs are unsuccessful at detecting *P. ovale*. Due to their subpar sensitivity and accuracy, which causes *P. ovale*. to be improperly identified. Furthermore, due to the genetic heterogeneity of these two species, RDTs frequently fail to identify *Plasmodium Ovale curtisi* when compared to *Plasmodium Ovale wallikeri* (Mahittikorn et al. 2021). Polymerase chain reaction (PCR) has been recognized as the most sensitive method for identifying *P. ovale* spp., even in situations with very low parasite density (Bauffe et al. 2012). *P. ovale* wallikeri has been observed to cause larger levels of parasitemia as higher serious condition than *P. ovale* curtisi with regard of therapeutic manifestations (Shin et al. 2020).

Genetic polymorphisms such as those found in *P. ovale* curtisi and *P. ovale* wallikeri were initially used to discriminate between the many *P. ovale* species include *CYTB b* (cytochrome b), *LDH* (lactate dehydrogenase), *PoRBP2* (*Plasmodium ovale* reticulate binding protein 2), *COX1* (cytochrome oxidase subunit), *PoG3P* (glyceraldehyde-3-phosphate), *PoDHFR-TS* (dihydrofolate reductase-thymidylate synthase), and *K13* gene (Mahittikorn et al. 2021).

22.4 Threats Posed by Drug-Resistant Malaria

To reduce the potential of malaria resistance, the WHO vehemently opposes the usage of ACTs for malaria prophylaxis. Travelers to the United States should take mefloquine, which is particularly effective against chloroquine resistance, together with pyrimethamine and sulfadoxine resistance. It is unclear whether the combination of proguanil and atovaquone and proguanil will eventually overtake mefloquine as the1st malaria chemoprophylactic drug (Nakato et al. 2007).

The suggested malaria prevention procedure for each country is based on the following factors: the risk of undertaking malaria; the prevalent species of malaria parasitic infections in the area; the stage and spread of drug resistance identified from

the country; and the potential risk of adverse side effects from the various prophylactic drugs. The following are some of the malaria prevention measures that are suggested for various regions (Chrubasik and Jacobson 2010):

1. TYPE I—Least risk of malaria transfer: Only mosquito bite protection.
2. TYPE II—Risk of completely *P. falciparum* or *P. vivax* infections or chloroquine sensitivity: Mosquito bite protection with a drug containing chloroquine for chemoprophylaxis.
3. TYPE III—Malaria transmission risk when there is chloroquine resistance: Protection against mosquito bites and chemoprophylaxis with a medication cocktail including chloroquine and proguanil.
4. TYPE IV—*P. falciparum* at extremely high risk for treatment resistance, or *P. falciparum* at medium to low risk for severe drug resistance: Mosquito bite protection with the combination of drug containing either mefloquine and doxycycline or the conjunction of atovaquone and proguanil.

22.5 Strategies Used Against Drug Resistance

In order to combat drug resistance in malaria, a variety of tactics are employed, including the following (Fig. 22.4):

The steps involve in delaying drug resistance inside the host:
In a high-transmission environment, acquired immunity is greater.

1. In high-transmission areas, mixed-strain illnesses are more common.
2. When the prevalence of resistance is 50%, mixed strain infections must be enhanced.
3. Competition between strains affects internal host dynamics.
4. Acquired immunity controls the dynamics of infection.
5. Treatment with antimalaria drug kills sensitive parasites, but it may allow resistance parasites to spread.
6. Host dynamics impact the effectiveness of sensitive and resistant parasitic transmission.

22.5.1 Method 3

In this method Combinational therapy strategies are used for drug resistance against malaria. Poor drug absorption, toxicity, and water solubility are some of the other drawbacks of currently utilized antimalarial medicines. One of the most efficient malaria treatments currently available is combination therapy, which combines a number of medicinal medications (Fig. 22.5) (Alven and Aderibigbe 2019).

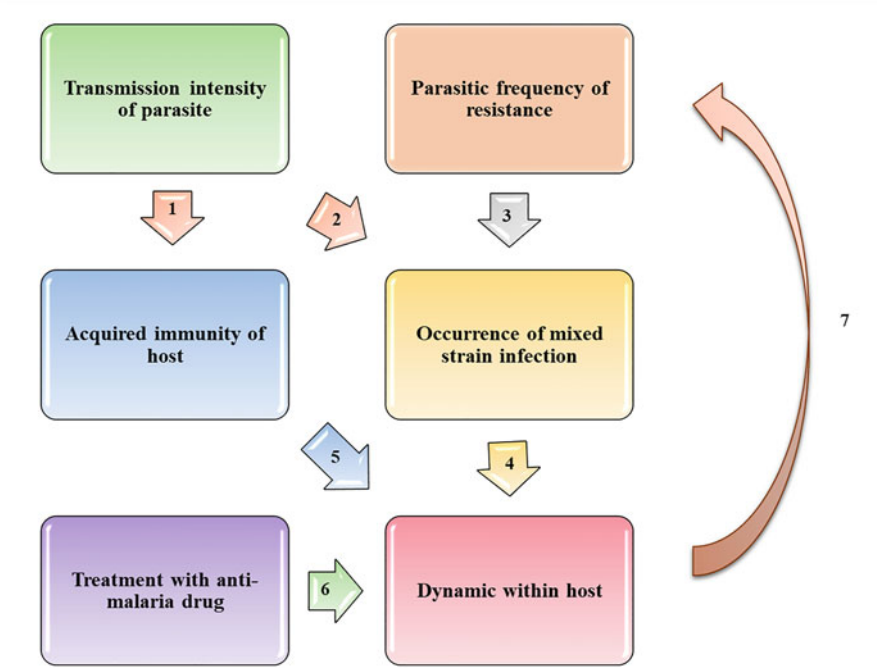

Fig. 22.4 Relationship between host dynamics, parasite resistance frequency, and parasite transmission intensity (Bushman et al. 2018)

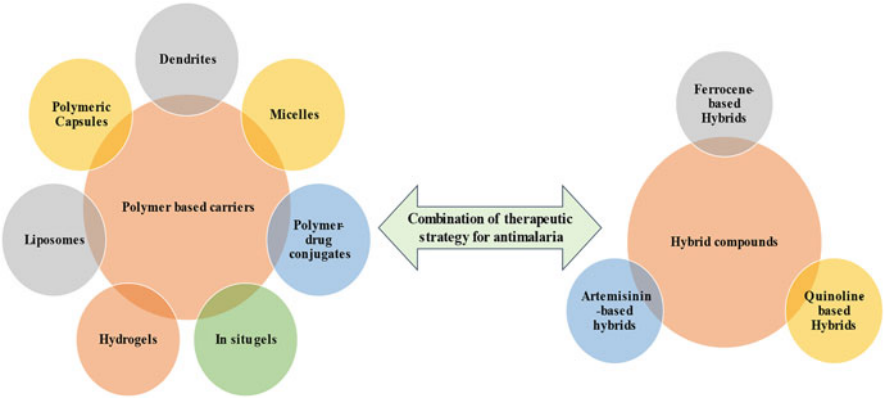

Fig. 22.5 Graphical representation of combination therapeutic strategy for antimalaria (Alven and Aderibigbe 2019)

22.5.2 Method 4

Nanomaterials have been studied in order to successfully deliver antimalarial medications at a local dose, potent enough to kill parasites and limit drug resistance while retaining a lower overall dosage to reduce severe side effects. Several nanostructure systems, including liposomes, polymeric nanoparticles, and dendrimers and have improved antimalaria therapy efficacy in recent years. Nanomaterial is a suitable medication delivery mechanism in this regard, and they can be utilized in therapy methods to fight the parasite in humans as well as the human disease mosquito vector. Chemical investigations of these nanoparticles are critical for the development and proposal of viable malaria prevention strategies (Borgheti-Cardoso et al. 2020).

22.6 Conclusion and Future Prospective

Infections with *Plasmodium* have a significant negative impact on world health. The discovery of new treatments faces enormous challenges as antimalarial drug resistance strains advance at an accelerated rate. Nevertheless, a number of molecular signatures reveal the location of mutation for diverse drug resistance identified for *Plasmodium* spp. and their underlying molecular mechanisms.

The strategies employed to combat antimalarial drug resistance are used to create a new area of research. Various strategies include the proper drug combination, dosage, and drug to postpone the development of drug resistance. Additionally, some nanomaterials have been researched to enhance antimalarial treatment. While in the future, next-generation sequencing for *Plasmodium* species can be used to compare target mutation locations for the treatment of antimalarial drug resistance markers.

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Approaches to Drug Discovery Against Ascariasis: Opportunity and Challenges in Plant-Based Products

23

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Abstract

Current treatment for effective control of ascariasis largely relies on mass use of anthelmintics. Nonetheless, *Ascaris lumbricoides* infection still is a major burden of parasitic diseases, especially in areas with poor sanitation and hygiene. Despite the concerns regarding onset of parasitic genotype resistance to drugs, reduction in drug efficacy, side effects, and cost-effectiveness, there has been slow progress in new drug discovery or no new drug development in pipeline against ascariasis. In this report, we highlighted avenues for soil-transmitted helminthes (STHs) drug candidates based on natural plant products. We give an overview and discussed on the plant-derived compounds having anthelmintic activity and its therapeutic potential. We reviewed some of the opportunities and challenges in the approaches to plant-based drug discovery against ascariasis.

Keywords

Ascariasis · *Ascaris lumbricoides* · Plant product · Drug development · Therapeutic · Anthelmintic · Bioactive compounds

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_23

23.1 Introduction

The giant roundworms, whipworms, and hookworms, commonly grouped as soil-transmitted helminths/nematodes (STHs/STNs), are the intestinal parasites that cause worm infection. These include *Ascaris lumbricoides*, *Ancylostoma ceylanicum*, *Necator americanus*, *Ancylostoma duodenale*, and *Trichuris trichiura.* They cause a group of diseases termed helminthiasis when transmitted through contaminated soil. The helminthic infection has been estimated at 438.9 (hookworms), 819 (giant roundworms), and 646.6 (whipworms) million people throughout the world (Bethony et al. 2006; Horton 2003). Combined, globally they account for at least 4.98 million years lived with disability (Pullan et al. 2014). Morbidity includes respiratory symptoms, intestinal pathology, malnutrition, child development and cognitive impairment and overall significant social, economic, and medical impact (Hotez et al. 2008; Crompton and Nesheim 2002; Loukas et al. 2016). The infections are predominant in endemic areas with suboptimal hygiene. There is a huge economic brunt associated with the incidence of the endemic diseases for the industry due to disease-related sheep livestock or concerns for alleviating poverty for small holder farmers in Africa (Nieuwhof and Bishop 2005; Perry et al. 2002).

Ascaris infection by *A. lumbricoides* has global distribution with more than 1.2 billion infections and 20,000 deaths per year. Ascariasis is endemic in various parts of the Indian subcontinent with an incidence of 25% with females more predisposed than females at1:3 (Khuroo 2001). The reported infection cases are mostly between third and fourth decades of their life (Mukhopadhyay 2009). The jejunum is the usual habitat of adult *Ascaris*, but it has the tendency to explore adjoining ducts and orifices. Migration from the intestine and taking residence in other areas lead to death of the parasites causing transient or specific symptoms in the host (Bahu Mda et al. 2001). Most widely used anthelmintic drug treatments, a broad spectrum STHs such albendazole and mebendazole and pyrantel pamoate are effectively used, yet there are instance of low efficacy of single-dose administration or other concerns of safe use such as environmental problem or teratogenicity in animals. There is an urgent need for or repurposing new drugs for remedy of STNs.

23.1.1 Descriptions of the Condition

Majority of infected individual may not exhibit symptoms but features such as low appetite, abdominal discomfort or recurrent abdominal pain, and usual intermittent diarrhea are prevalent. Fever, dyspnea, chest pain, hemoptysis, and wheezing due to Loffler's syndrome are associated with *Ascaris* pathogenesis. The disease develops as the worm undergoes life cycle and development in the host, broadly as pulmonary, intestinal, and the later complicated stage. Severe infections would result to intestinal obstruction and slow growth in children. Cough may occur because of the worm migration through the body. The worms moving outside from jejunum and getting lodged in the bile or pancreatic ducts result to specific groups of diseases

termed hepatopancreatic ascariasis (HPA). HPA is common in endemic places. Mass of *Ascaris* conglomerate produce obstructions of the ducts, causing hepato-biliary ascariasis (70%), gallbladder ascariasis (16%), and pancreatic ascariasis (14%). Entering of the intestinal worms into the appendix through perforation can cause appendicitis. The worms in the gastrointestine choose linear and wider canal for easy movement. HPA in children are rare due to smaller diameter of the ampulla; however, patients who have a history of sphincterotomy or bilioenteric bypass surgery have high risk due to easy accessibility to wider diameter canal (Robbani et al. 2008; Alam et al. 2007; Javid et al. 1999).

23.1.2 Transmission

Transmission of the embryonated eggs through hand to mouth ingestion from contaminated water and food finds its way in the intestine where it hatches to larvae. The larvae migration to lungs for residence via blood streams and lymphatic system allows maturation. Further growth occurs as they pass to air sac of the lungs, where they travel up the respiratory tract and pass finally to small intestine upon swallowing. The whole cycle to complete usually happens in 4–8 weeks. Matured worms reproduce eggs and these eggs are passed out with feces. Any feco-oral route transmission contaminated with these eggs grows into form of the parasites.

23.1.3 Diagnosis

The diagnosis for infection is best established through stool smears test to check the presence of eggs. Hematology diagnosis has low sensitivity but still has remarkable inference for disease infections by assessing eosinophilia or polymorphonuclear leukocytosis. Bile may aid diagnosis where increased bilirubin up to 3 mg/dL may be indicative of test positive. Imaging techniques such as X-rays or ultrasonography can detect the presence of mass of worms usually in the pancreas and biliary tract. Though limited but minimally invasive endoscopic intervention or surgery may be necessary under circumstances associated with worm infestation complications.

23.1.4 Anthelmintic Drugs Treatment and Mechanism of Action

Infected individual asymptomatic for ascariasis still needed anthelminthic treatment in order to prevent complications from migration of the parasite. Drugs for intestinal worms listed under the model list of essential medicine of World Health Organization include mebendazole, albendazole, levamisole, niclosamide, praziquantel, ivermectin, and pyrantel (WHO 2021). The drugs act as interfering compound to microtubular system (Utzinger and Keiser 2004), as nicotinic acetylcholine receptor agonists, or modulating on neuromuscular system causing paralysis of adult (Delcastillo et al. 1964). It particularly acts on the organisms' target molecules

such as enzymes, ion channels, and structural and transport proteins. Relatively, targets like ion channel are typically quick in response to drug treatment as opposed to slow effect seen in biochemical targets (Kohler et al. 2001). Agonists on binding to excitatory receptor such as nicotinic acetylcholine receptor depolarize and cause spastic muscle paralysis resulting in parasite expulsion or inhibit feeding activity due to flaccidity of musculature and blocking of pharyngeal pumping. This leads to nematocidal exploit. Piperazine, a GABA-gated chloride agonist, upon binding to the channel receptor located on somatic muscle cells induces increased membrane permeability to chloride, which will lead to relaxation of muscle to finally flaccid worm paralysis (Osteux et al. 1971). Most of these drugs are used for mass therapy in endemic places. The most widely used drug is Mebendazole (Vermox 100 mg) twice a day for 3 days, and albendazole (Zentel 400 mg) is marketed for children and nonpregnant individuals while pyrantel pamoate (Antiminth 11 mg/kg) is used to treat ascariasis in pregnant women. Lesser common drugs such as ivermectin, levamisol, nitazoxanide, and piperazine citrate are prescribed. A recent review by Conterno and group reported the comparative efficacy and safety of three commonly used helminthic drugs for treating *Ascaris lumbricoides* infection albendazole, mebendazole, and ivermectin from a 30 multicontinental randomized controlled study conducted between 1981 and 2011 in children and adults infected with *Ascaris* (Conterno et al. 2020). All the three drugs mebendazole, albendazole, and ivermectin reported to be equally effective in reducing eggs excreted and pathological cure of *Ascaris* infection and no differences was found among the drugs nor between single dose and multiple doses. The drugs appear safe with no serious side effects. Beside microtubule and nervous system as the therapeutic targets, other possible drug targets being explored include adenine nucleotide translocators (Hu et al. 2010), which has shown to be associated with energy kinetics of the mitochondria as key regulator in oxidative phosphorylation as well in cell death. Energy route is another interesting area to exploit for anthelminthic therapeutic, particularly due to parasitic adaptations and intrinsic ability to transduce energy source from the hosts. Enzyme NADH-fumarate reductase common in many anaerobic organisms seems to be the probable player. Anthelmintics that aim at recognizing neuropeptide are becoming increasingly examined. One of the roles of the neuropeptide is acting as neurotransmitter on the organism that may cause inhibition of muscle contraction neuromodulator (Maule et al. 1996). The multiple layers of therapeutic targets from biochemical level or receptor to cellular level allow opportunities for target selection, identification of broad spectrum drugs, and development of treatment strategies (Mousley et al. 2005). Details on drug mechanism and pharmacology have been extensively reviewed.

23.2 Opportunity in Antiparasitic Drug Discovery: Plant-Based Anthelminthic Natural Compounds

Plants such as papaya or pineapple known to be effective in curing worms that live in the intestine have been used in traditional practice. Studies on rodents demonstrated that the products from fig and Egyptian milkweed showed to be effective in triggering destruction to *H. polygyrus*. Experimental evidence on the active killing agent both in vivo and in vitro are pointing at cysteine proteinases in the extract that cause attacking on the protective cuticle. The fecal egg count reduction assay two days post administration revealed a count of *A. lumbricoides (98.62%)*, *T. trichiura (99.08%)*, and hookworms (89.88%) with no detectable damage to immune cells or architecture of the mucosa (Chávez et al. 1990; Stepek et al. 2004). Plant-derived compounds known to be anthelmintic are vastly being discovered and information on the nature and nematocidal action mechanism are being described notably. There are numerous studies looking at the potential nematocidal drug candidate derived from natural plants, and yet, considering plant-based extract as alternative approach to drug discovery and development is just beginning to take footing. We discussed some of the important natural compound and its derivate having nematocidal activity.

23.2.1 Plant-Based Anthelmintic Natural Compounds

23.2.1.1 Phenols

Natural phenols from plants extracts are described as anthelmintics based on their nematocidal activities. The most commonly cited phenols with nematocidal properties are carvacrol and thymol along with their respective acetates (Fig. 23.1). As stated earlier, most of the in vitro or in vivo studies are done in *C. elegans*. Lei et al. (2010) demonstrated that carvacrol and thymol induce mortality of *C. elegans* and *Ascaris suum* when exposed to the compounds for 24 h in vitro. In the work of Ferreira et al. (2016), thymol extracted from *Thymus vulgaris* displayed inhibitory properties against egg hatching, larval hatching, and larval mortality in pole worms *H. contortus* at specific concentrations. The acetates of carvacrol and

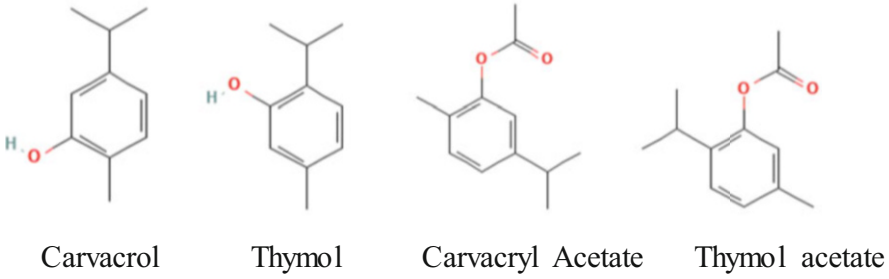

Fig. 23.1 Phenol

thymol also showed a similar mode of mechanism action in *H. contortus* adult as reported by André et al. (2016). However, the level of efficacy was observed to be much lesser as compared to its non-acetylated original compound. Similar result was also reported in another acetylation of thymol study with a tenfold reduction in its potency against egg hatching inhibition assay (André et al. 2017). Due to the lack of metabolite study to determine this result, the active constituent is still not known.

23.2.1.2 Cinnamoyl Derivatives and Polyphenols

The biosynthesis of natural phenolic compounds such as tannins, flavonoids and coumarics, intermediate compound cinnamic acids are produced. Several compounds of cinnamoyl derivatives and polyphenols possess nematocidal properties. Cinnamic acids such as caffeic acid, ferulic acids, and p-coumaric acids (Fig. 23.2) derived from natural extracts of *Acacia cochliacantha* have reported anthelmintic activities. These cinnamic acid derivatives showed egg hatching inhibition in *H. contortus* (Castillo-Mitre et al. 2017) at 1 mg/ml concentration. The bioactivity of inhibition by the three compounds of the extract was confirmed by feeding foliage of *A. cochliacantha* and comparing to goats fed with different feed. Condensed tannins derived from *Salix caprea* leaf and *Tilia* flower such as procyanidins and prodelphinidins (Fig. 23.4) derived from *Ribes rubrum* showed positive result for exsheathment inhibition assay that were similar to flavonoids such as luteolin, quercetin, naringenin (Fig. 23.3).

In this test, L3 stage of *H. contortus* of Juan strain was subjected to different treatment regime. It was found that prodelphinidins were twice more potent than procyanidin fractions. Other tannins exhibiting inhibition of egg hatchings were recorded for tellimagrandin II, pentagalloylglucose and hippophaenin B (Fig. 23.4)

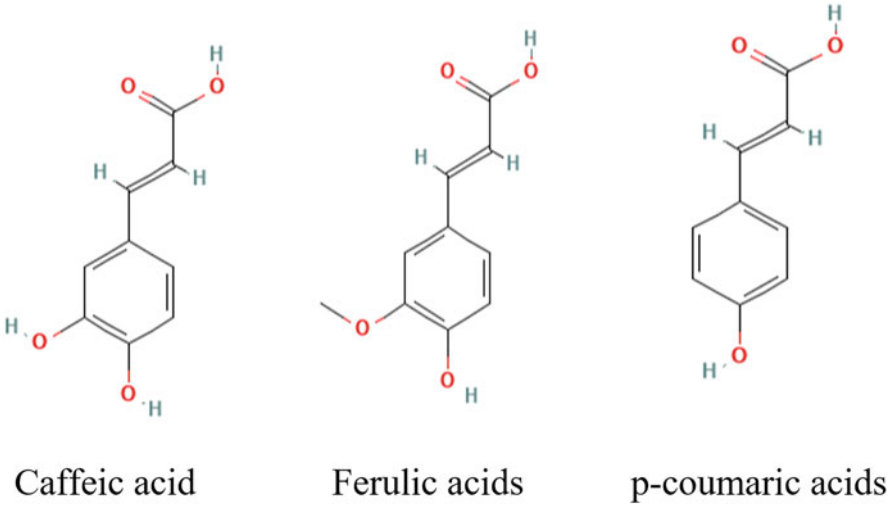

Fig. 23.2 Cinnamic acids

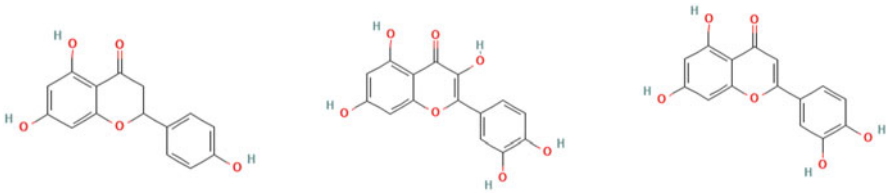

Fig. 23.3 Flavonoids

derived from cold acetone extract of plant from Finland (name undisclosed). In addition, tellimagrandin II, pentagalloylglucose, heptagalloylglucose, and casuarinin (Fig. 23.4) exhibited inhibition of larval motility (Engstrom et al. 2016). The workers suggested that this anthelmintic property was attributed to tannin deposition on the parasite body surfaces and not an antigen–antibody interaction.

Isoflavonoid deguelin (Fig. 23.3) was discovered during screening of natural products library for L3 of nematodes (Dilrukshi Herath et al. 2017). In a motility assay, deguelin could inhibit the larval development from L3 to L4 at 3.2 μM at IC_{50} after 7 days. It was found that the isoflavonoid treatment was linked to differential expression of genes related to mitochondrial respiratory complex-I. Further, in mammals deguelin–respiratory complex inhibition was found to correspond with disturbed PI_3 Kinase Signaling pathway (Bortul et al. 2005). The study yielded an interesting insight on toxicity mechanisms; however, further studies would be needed to understand if deguelin mode of action in nematodes and mammals are same. Analogs of gallic acids isolated from *Anogeissus leiocarpus* demonstrated peak anthelmintic activity at IC_{50} when *C. elegans* and *O. ochengi* were treated at concentrations ranging from 30 to 90 μM (Ndjonka et al. 2014). It was interesting that the potency of the gallic acid analogs ellagic and genstic (Fig. 23.5) were effective against levamisole and ivermectin resistant strain.

Other analogs of gallic acids proanthocyanidins and catechin-O-gallate, namely, Epigallocatechin, gallocatechin, epigallocatechin-3-Ogallate, and epicatechin-3-O-gallate (Fig. 23.5) isolated from Cameroon *Acacia nilotica* fruit had demonstrated anthelmintic activity against *C. elegans* that were ivermectin and albendazole resistant (Dikti Vildina et al. 2017). Reasonably, low concentration at IC_{50} value of 30 μM was observed to be effective against this strain; however, Caco-2 cell line displayed toxicity. This raises the question of its use in clinical level.

Certain polyphenols modified with other synthons have been found effective against nematodes and are traditionally used to treat helminthiasis. Isolates from fern *Dryopteris wallichiana* rhizome and scales obtained unique terpenylated acylphloroglucinols, viz., Wallichin (A, B, C, D), albaspidin (AA and AB), and Filixic acids (Fig. 23.6). Socolsky et al. (2012) observed that these modified polyphenols are more potent than its normal form against rat nematode larvae L4 stage of *Nippostrongylus brasiliensis* at LD50 range of 20–40 μM. They theorized

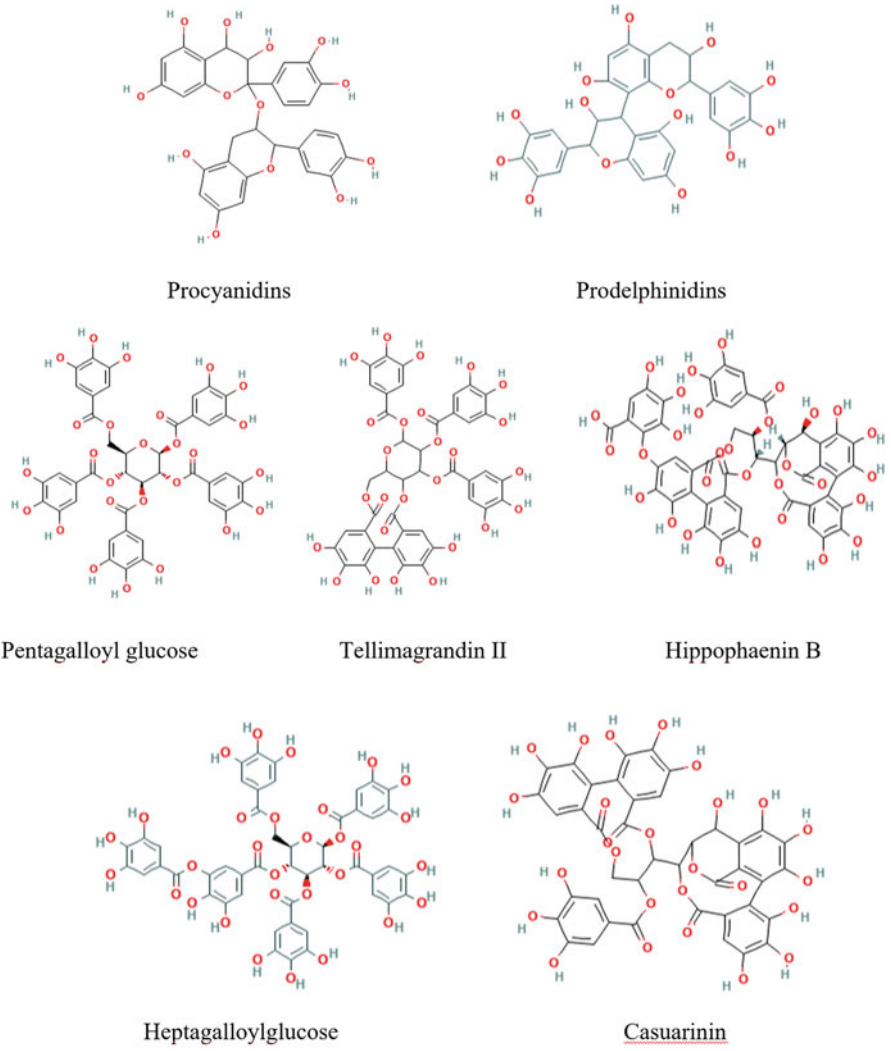

Fig. 23.4 Tannins

that the occurrence of free hydroxyl group at C-4 was the key for the modulating activity of the compounds.

23.2.1.3 Terpenes

Terpenes are plant secondary metabolites considered to have antiparasital properties against most of the neglected tropical disease. Classic example is of tea tree oil Terpinen-4-ol extracted from *Melaleuca alternifolia* a conifer tree. Terpinen-4-ol is known to possess a modest anthelmintic activity. However, its potency was

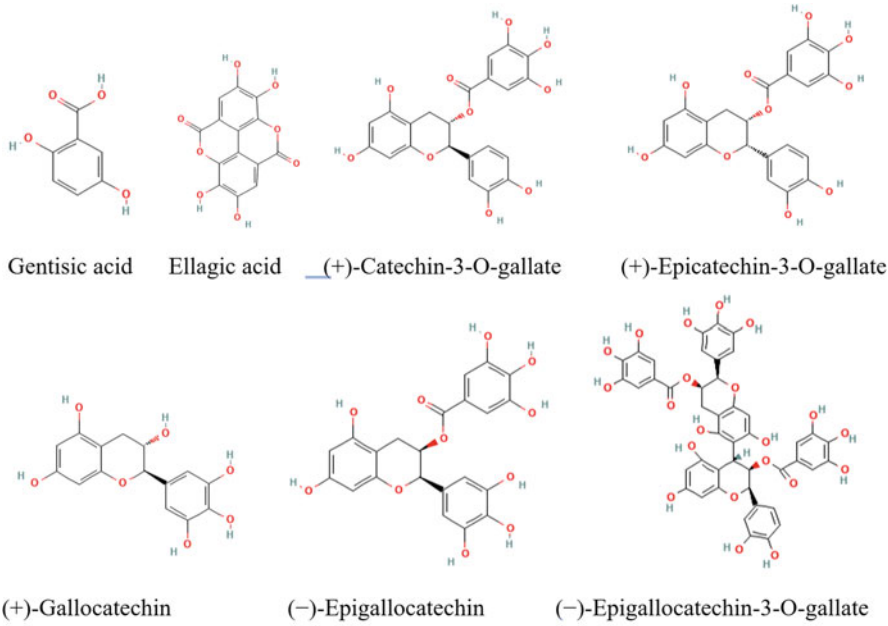

Fig. 23.5 Gallic acids

significantly enhanced when nanostructure formulation of the oil was tested against *H. contortus* (Grando et al. 2016). In their study, a low dose of 4.1 mM at IC_{50} of terpinen-4-ol had demonstrated inhibition of egg hatching and migration of larvae by 83%. Other essential oils that are known to be used in traditional medicines were isolated from prickly shrub *Zanthoxylum simulans* from China and Taiwan and contained important terpene, significantly, borneol (Fig. 23.7). Borneol was demonstrated to exhibit significant inhibition of egg hatching and larval development and suppress 98% larval migration at IC_{50} value 9–130 mM. Aldehyde moieties containing terpenes related to sesquiterpenes structurally were isolated from *Warburgia ugandensis*, a medicinal plant endemic to Southern Africa, which demonstrated anthelmintic properties against drug-resistant *C. elegans* (Liu et al. 2018). The most active constituents were characterized to be warburganal and polygodial at 28 μM and 13 μM IC_{50}, respectively (Fig. 23.7). It was found that the two sesquiterpenes were not affected by the mechanism of drug resistance. Further studies on its chemical structure and activity relation revealed that the presence of at least one aldehyde group in the compound was necessary for this property. In an attempt to elucidate the mechanism of its action, mammalian cells and *S. cerevisiae* were tested against polygodial. The compound was found to be acting as uncoupling agent in oxidative phosphorylation of mitochondria (Castelli et al. 2005). Similar function of polygodial was observed in *C. elegans* as inhibitor of mitochondrial ATP synthesis.

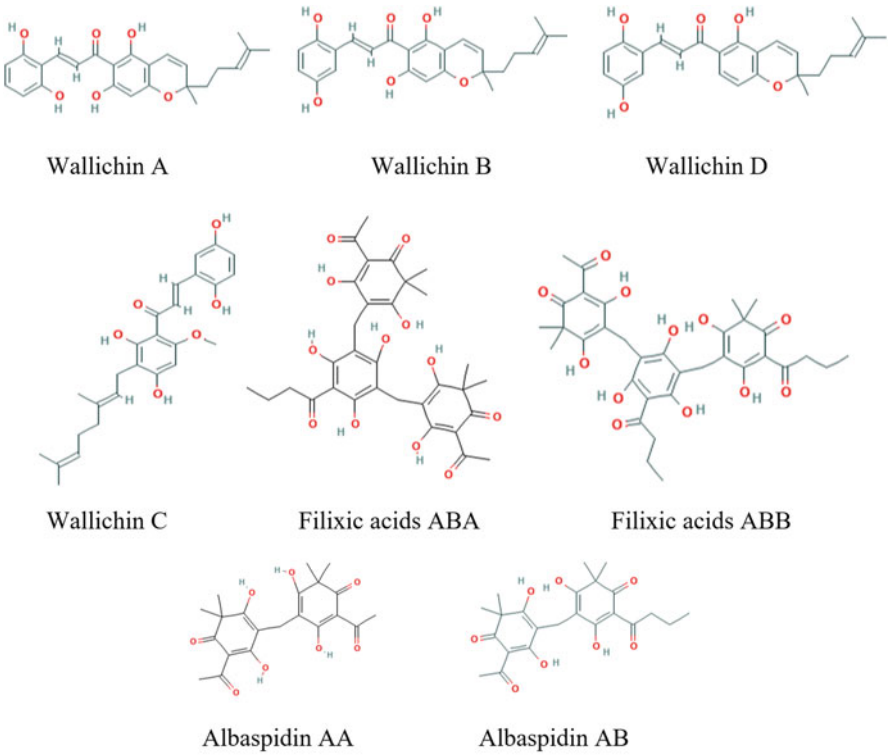

Fig. 23.6 Polyphenols-synthons

23.2.1.4 Prenyl Derivatives

Prenyl derivatives are basically linear mono- and di- sesquiterpenes that are produced during isoprenoid biosynthesis pathway, which are commonly isolated from plants. Large number of plants contains monoterpene alcohol linalol (Fig. 23.8). It is being considered an important compound along with other natural products for anthelmintic drug development (Azeez et al. 2012). This compound showed best hit when screened for binding with glutathione S-transferase model from *Brugia malayi.* The biochemical test of linalool against crude extract of glutathione *S*-transferase from *Dirofilaria immitis* at 1 μg/ml showed 98% inhibition of the enzyme. Though it is imperative that linalool has anthelmintic efficacy, there are interplay factors of the compounds stability and variability of target molecule (isotypes) to represent as anthelmintic drug candidate. Further, it is still not known if enzyme GST works in singularity.

Other prenyl alcohols isolated from *Matricaria chamomilla* extracted essential oils are reported as bioactive anthelmintic compounds in vitro and in vivo. Farnesol, nerolidol and bisabol (Fig. 23.8) isolated from the essential oils demonstrated arrest in motility, tissue injury to worms, and prevented infectivity to rats with *Anisakis simplex* larvae (Romero et al. 2012) at 70–150 μM IC_{50}. This anthelmintic activity of

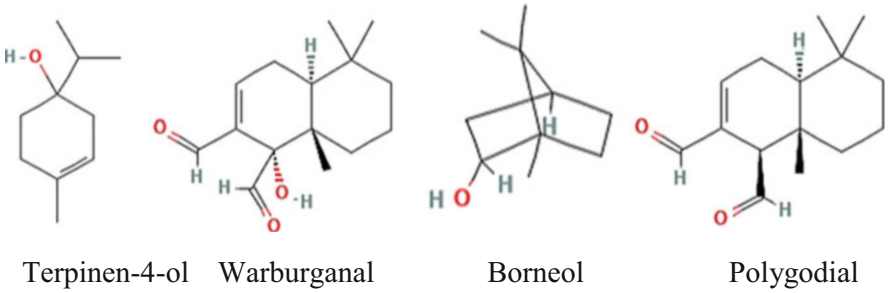

Fig. 23.7 Trepenes

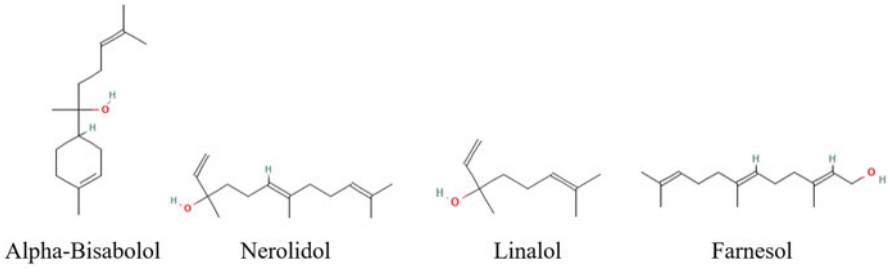

Fig. 23.8 Prenyl derivatives

the compounds was confirmed in *C. elegans* though the potency was to a lower extent (Dichtl et al. 2010). It is reported that prenyl compounds affect cells by the action of interfering with transfer of their functional group to proteins and impeding ecdysis in nematodes.

23.2.1.5 Triterpenoids and Saponins

Triterpenoids are class of natural lipids conjugated with sugar residues present in plants with surfactant properties. They possess large hydrophobic area that imparts in them the affinity to bind with proteins, lipid membrane, and isoprenoid ligands allowing them to be involved with multiple biological activities (Cao et al. 2015; Wei et al. 2009). Triterpenoids extracted from *Calotropis procera* latex have reported potent activity of inhibiting egg hatching, motility in adults, and larval development at EC50 values ranging from 0.05 to 1.6 mg/ml (Cavalcante et al. 2016). Interestingly, individual fractions of the constituents were tested against nematode *H. contortus*; no activities were observed in all the individual fractions. So it is of the opinion that the bioactive compounds in the extract show synergistic mode of action. β-Sitosterol (Fig. 23.9) triterpenoid extracted from edible mushroom *Pleurotus djamor* displayed active ovicidal activity against nematodes (Pineda-Alegria et al. 2017).

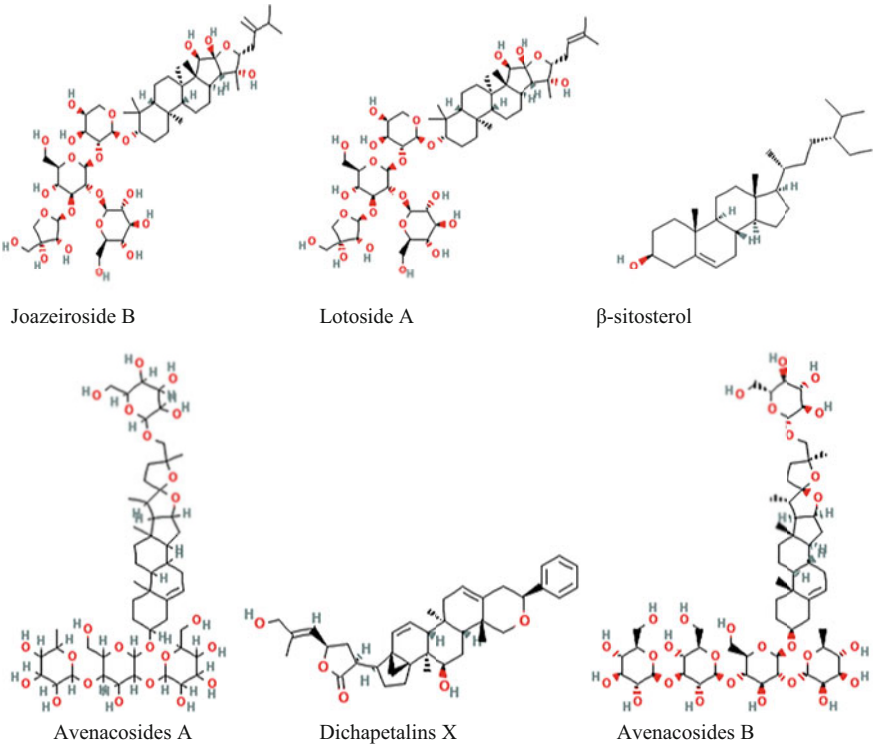

Fig. 23.9 Triterpenoids and saponins

Saponins are glycosides of triterpenes generally toxic and unlike triterpenoids less soluble in water. Though numerous saponins are reported to have anthelmintic activities, its insolubility and cytotoxic nature have been a challenge for oral administration. However, its anthelmintic activity has been very promising for gastrointestinal nematodes. Saponin such as Lotoside A and Joanzeiroside B (Fig. 23.9) derived from *Zizyphus joazeiro* bark extract displayed ovicidal activity against 3 caprine gastrointestinal nematodes (Gomes et al. 2016). Surprisingly, no anthelmintic activity was observed against stage 3 Larvae. They opined that this effect could be due to egg shell and cuticle differential permeability. Traditional use of *Avena sativa* for treating worms in cattle and humans was studied to find the active compounds. Methanolic extract of the Oat plant seedling showed two saponins Avenacosides (A and B) that displayed anthelmintic activity against egg and larvae of *Heligmosomoides bakeri* (Doligalska et al. 2017). Inhibition of egg hatching, ovicidal, and nematocidal activity against human hookworms was identified from *Dichapetalum filicaule,* a plant native to West Africa. Two triterpenoids, dichapetalins (A and X) were identified to be the active nematocidal agent at 160–520 μg/ml EC50.

23.2.1.6 Macrocycles

Macrocycles are a class of compounds containing a cyclic frame of at least 12 atoms. Macrocycles are class of natural products that are active against wide range of biological activities. Macrocycles that are anthelmintic includes avermectins, milbemycins, and their derivatives. Several species of *Streptomyces* produces them. Lately, extract of *Streptomyces microflavus* mycelium with methanol resulted in the purification of compound exhibiting anthelmintic activity against *C. elegans* (Xiang et al. 2010). This bioactive compound resembles the structural make up of Nemadectin, but without lactone ring. Similar mode of bioactivity was also reported for four compounds without lactone ring extracted from *Streptomyces bingchenggenisis,* which earlier was found only in cyclic milbemycin (Xiang et al. 2010). Recently, a semisynthetic macrocycle, oxime of milbemycin, was approved by FDA for human use to treat onchocerciasis (FDA 2018). Though no new anthelmintic macrocycles are reported in the last decade, the options of semisynthesis approach and ease of obtaining in vitro pave a huge advantage for the future possibilities.

23.3 Opportunities for Drug Discovery Through Open Tools

23.3.1 Target Identification

Universally consistent and reliable resource for target identification in vexed parasitic adaptation of nematodes is the genomic resource. WormBase ParaSite (version WBPS 16: WS279) is the primary model organism database containing 157 nematodes species with their genomes, comparative data of genomes, and also RNA sequences. Identification of new drug targets from the available genomic resource was elucidated from a comparative study by international consortium of researchers (IHGC 2019). Their work produces huge open data sets from gene families that are intrinsic to parasitism such as SCP/TAPS (Sperm coating protein/TpX Antigen 5/Pathogenesis related protein-1) genes (Cantacessi et al. 2009), analysis of metabolic pathway across different phyla encompassing metabolic super-pathways underlined in Kyoto Encyclopedia of Genes and Genomes (Kanehisa et al. 2012) for new drugs, to prediction of promising compounds and novel anthelmintic targets. Prediction of protein-coding genes in parasitic helminthes obtained through MAKER software from existing functional genomic resources mainly from *C. elegans* can generate the accurate set of genes (Quinlan 2014). The adoption of CRISPR/Cas9 to gene resources of *C. elegans* Gene Knockout Consortium, which has around 15,000 genes with putative knock out mutants (Stanke et al. 2006), have paved way for the application of RNA interference and CRISPR methodologies. These methodologies can be applied for the target identification and validation of targets for genetic or pharmacological proof of concept. Open Worm Movement Database (Ter-Hovhannisyan et al. 2008) is a platform for analysis and an open source for sharing worm behavioral data. This database allows investigators to obtain clues pertaining to phenotypical movement pattern of worm

strains and can be utilized for target identification and validation. The huge amount of information in the database on nematode DNA sequence and newly added gene function in the collection will serve an exceptional prospect to categorize pre-validated drug upon proficient mining.

23.3.2 Phenotype

Discovery and validating the effect of drugs on the helminths through phenotype analysis or motility quantification has been developed by several workers using the thresholding image/movies approaches. One such method is WormScan that employ flatbed scanner for capturing sequential image of the parasite movement (Mathew et al. 2012). The scanner is equipped with a high-intensity light that could stimulate movement of the worm. This system has been applied for screening *C. elegans* against 26,000 compounds in growth assay. Monitoring motility of worm up to thousand in number simultaneously is possible through Lifespan Machine (Stroustrup et al. 2013). It has can also be used to determine the mortality of individual worm. Motion-based screening of macroscopic parasites for determining motility, called WormAssay, has been used for validating anthelmintic activity against number of nematodes. Another application for monitoring microscopic parasites is Worminator. This computer-based application build on WormAssay platform. Screening work using Worminator has identified drug Auranofin for the treatment of onchocerciasis and lymphatic filariasis (Bulman et al. 2015). Auranofin initially was used for the treatment of rheumatoid arthritis. Identification of hit compounds by determining the motility through ImageJ Macro that uses pixel difference thresholdings is reported for cyclophyllid tapeworm *Echinococcus multilocularis* (Ritler et al. 2017). Similar to ImageJ Macro, Wiggle Index is an extensively used app for screening exsheathing by quantification of motility and difference thresholding. Library-scale screening of *H. contortus* stage 3 larvae exsheathing has been done by several workers (Preston et al. 2016, 2017). Another open source that allows users to quantify phenotype through image data is CellProfiler. Identification and validation of hits from virtual screening approach can be tested in CellProfiler through which viability, motility, exsheathing, and other phenotype scores can be quantified (Neves et al. 2016).

23.3.3 Physiology

To have a better understanding of how anthelmintic compounds works on the physiology of worms, several open-source systems are being developed. Though the app is still in its infancy, these methods could serve to be an important criteria/basis in discovery of anthelmintic compounds.This approach focuses on the aspects of interactions with the host that indicates damage on to the worm. Parameters taken for evaluating such effects are behavioral in nature such as swim pattern, bending, and reversal. The open source CeleST analyzes the swim behavior of nematodes by

quantification of locomotion (Restif et al. 2014). One of the most exciting and potential system that can aid in the discovery of anthelmintic compound is the application of Microfluidic system. This system not only provides the worm's detailed longitudinal microscopy but also enables effective screening of compounds in terms of quantity and economics. Some workers have designed microfluidic chip called Stress-Chip. This chip enables isolation and monitoring of hundreds of worms within a single arena when compounds are allowed to flow over the worms (Banse et al. 2019). For developing microfluidic device, CAD file can be obtained from Figshare (CC BY 4.0).

23.3.4 Therapeutics

Open-source library of compounds can be used to screen against number of organisms for compounds with anthelmintic activities. Such open approach would enable workers in identifying and prioritizing compounds that are active against nematodes. Currently, an open-access project called "The Pathogen Box Project" is available where users are free to access with the condition of sharing the results generated from it. The Pathogen Box project is a collection of more than 400 compounds free to use for a non-profit product development by Medicine for Malaria Venture (158). More than 290 assays against diverse screens related to neglected tropical disease and malarias are available. One leading result from Medicine for Malaria Venture project is the identification of previously developed KDR (Kinetoplastid Dihydriflote Reductase) inhibitor compound MMV690102 that was found to be active against trematodes *S. haematobin*, *F. hepatica*, and *S. mansoni* (Pasche et al. 2019). Another promising result is the identification of compound, tolfenpyrad, exhibiting anthelmintic property of exsheathing in L3 and L4 stage of *H. contortus* and nematode *C. elegans* (Risi et al. 2019).

23.4 Challenges of Plant Products in Drug Development

The potential of developing plant products to a full-scale clinical drug candidate is no less. However, the existing plant-derived bioactive compounds are yet to cross clinical barriers that are described below.

23.4.1 Bioassay

In most in vitro studies, the criteria that are used to define anthelmintic activity are based on the effect on egg hatching, motility, and mortality. Though all the stages of nematodes are being considered in many studies, L3 stage of nematodes especially in the model organism *C. elegans* is the most extensively studied stages (Holden-Dye and Walker 2014). Consequently, it is prudent that one establishes the interaction of drug versus life stages or organism species in order to yield maximum benefit.

Consensus on which parameters would be the best clinical hits is still yet to be defined. Therefore, insights on these critical period/targets could be achieved by broadening the parameters of in vitro assays to come up with novel mechanism of action on phenotype (Abriola et al. 2019). The technique of whole organism assays is advantageous for the fact that it is target blind and may lead to finding new compounds which otherwise would not have showed up in a single target approach. It allows identifying whether the compounds have defined biological effects against related parasites or to know molecular targets of other organisms; however, there are demerits in this approach often being low-throughput, cost and needs trained specialist. It is evident that most anthelmintics in use are neuro-motor antagonist causing paralysis to the worm. However, there is requirement for quantification of the reduced activity to define the bioactive efficacy for development to clinical drug (Arafa et al. 2015). Additionally, it does not show whether the effect exerted by these bioactive compounds are permanent or transient (Holden-Dye and Walker 2014). Not much data for in vivo efficacy assays have surfaced and at the most studies are done using animal model. Though these tests are expensive and burdensome, it is prima facie step to progress for clinical trial (Giordani et al. 2016).

23.4.2 Therapeutic Potency and Toxicity

Existing literature illustrated that the potency of bioactive compounds is dependent on the type of bioassay, parameters of study, and species of nematodes. Taking into account data related to potency of bioactive compounds against wide range of nematodes ranges from 1 μg/ml to 1000 μg/ml at IC_{50} as standard. It has also been observed that these values also vary widely within a single species, taking *C. elagans* as the model organism. Though not extensive data has been reported with *Ascaris* spp., the range of active compounds is observed to overlap with *C. elegans*. The ascribed values of bioactive anthelmintic compounds are similar to compounds from chemical library especially in the lower range at IC_{50} values (Mathew et al. 2016). However, most of the reported anthelminthic bioactive compounds derived from plants are in mM range. Therefore, identification of actual bioactive compound has still not being characterized to its required stage. The information on toxicity trials for bioactive anthelmintic compounds are still not many (Martin and Robertson 2010). Most toxicity test on mammalian cell lines are considered to be not as relevant as they served to be so useful in predicting in vivo toxicity for STHs even though they are essential part for assessment to head way to clinical stage.

23.4.3 Synergism and Spectrum

Synergy is a common phenomenon observed especially in medicinal plants. Though demonstrations of synergy are also observed in other plants, there are not many reports that suggest display of synergy of bioactive compounds isolates from the same plant (Liu et al. 2018a, 2018b; Klongsiriwet et al. 2015). Wagner and

Ulrich-Merzenich (2009) appraised that such synergism could be to increase the phyto-medicinal efficacy due to low potency of individual bioactive compounds in phyto-constituent mixture. Nonetheless, it was opined that such synergy could throw advantages of imparting lower resistance risk. In that perspective, the plausibility of developing new therapies through synergy of bioactive compounds with clinically used drugs is a promising aspect. However, it has rarely been tested to realize its anthelmintic properties (Hu et al. 2010). Another aspect of challenges in anthelmintic drug discovery would be the spectrum of organisms subjected to bioactive compounds trials. In most of the case studies, the target organism are conducted over *C. elegans,* which may not be necessarily parasitic in nature. Though the general consensus takes into confidence that the activities observed in model organism would persist in the parasites, in vitro demonstration of anthelmintic properties of the bioactive compound on the parasite would be essential.

23.4.4 Mechanism and Pharmacokinetics

Investigation on the bioactive compounds have led us to clearer understanding of mechanism action of anthelmintic. Much of the activity of plant-derived bioactive compounds is observed to be through mitochondrial inhibition (Liu et al. 2018a; Sakai et al. 2012), transient receptor potential (TRP) channels (Liu et al. 2018b), acetylcholine receptors (Dubois et al. 2019), and inhibition of glutathione *S*-transferase enzymes (Azeez et al. 2012). It is imperative that the tasks of undertaking such studies are difficult. However, identifying the mechanism of anthelmintic activity would be necessary for development of novel drug. Further, information regarding the fate of the bioactive compounds applied against the Ascaris or nematodes are still at its exploratory stage. Pharmacokinetics of anthelmintic bioactive compounds such as rate of absorption, metabolism, excretion, and distribution are important. These processes are directly related to attaining the required concentration of drugs at the site of action to ensure the efficient pharmacological effect of the compound (Lifschitz et al. 2017).

23.4.5 Other Challenges in Antiparasitic Drug Discovery

Notwithstanding the available anthelmintic treatment effectively used for the control and prevention of the parasitic infections, the impending challenges to entirely treating ascariasis are manifold. Poor sanitation and hygiene in endemic regions and lack of effective vaccines support continuity of the life cycle of these parasites. Broadly used drugs for treating tropical diseases although introduced 10 years are far exceptional but still are using in many practices. As many in the scientific community have asserted that market forces alone is deficient to push for new drug discovery and development, it has been reported that out of 13,000 drugs introduced from 1975 to 1999, tropical diseases drugs amount to 13 only (Pink et al. 2005). A new compound to develop is a long and iterative process involving discrete levels

beginning with molecule identifications, assay development and screening, requiring to undergo tightly controlled procedures of safety and efficacy measure, and validation in models and humans. Further operations including optimizations of cost-effective manufacturing and passing through regulatory body for registration and ongoing pharmacovigilance. The issue concerning why there are limited new compound and how that can be changed was discussed at the eighth Consortium for Anthelmintic Resistance and Susceptibility meeting, Madison 2019. There has been observed a new impetus to anthelmintic drug discovery in the recent times, including the founding of new public private partnerships on tropical disease and publicly funded parasite genome sequencing (Trouiller et al. 2002; Kettler and Marjanovic 2004; Global Forum for Health 2004). Taking opportunity into consideration, here we summarize, in limited, some challenge to the discovery and development of anthelmintic drugs.

23.4.5.1 Suitable Model

The progress in developing new class of drugs is further impaired due to constraints of right model such as ruminant or non-human primates. The challenge is further complicated by the complex immunological host–parasite interactions favoring protections of the parasite. From 2000 up to now, only three drug products have been manufactured for animal use and none of them have reached market for use on humans (Harder and von Samson-Himmelstjerna 2001; Woods et al. 2012). Much of our understanding comes from experimental studies in the model, and therefore more extensive investigations in real terms of host–parasite relationship will give better insights for new therapeutic explorations, efficacy, and safety as well. Few of the interesting areas of investigations include determining right nematode-derived immunomodulatory and antigens, which can be utilized to designing pharmacological drugs. Employing this approach successfully could impede the immunoprotections to the parasites and destroy its evasion strategies as well as use of effective vaccines could confer long-lasting protection to the human host, respectively (Hagel and Giusti 2010).

23.4.5.2 Resistance to Anthelmintics

When the effect of a determined drug concentration is shown decreased and become insensitive to cause resulting effect we can refer to them as resistance to that drug particular administration. These heritable characters of genetic modification which confer protection and impart survival to recommended therapeutic doses of drugs is seen in a given helminths population where mass drugs have been used and repeatedly. It implies evolution of drug resistance in that particular geographic locations (Keiser and Utzinger 2008). Veterinarian believes that repeated large-scale drug treatment will mount heightened drug burden that will promote helminthes resisting to effective drug dose treatment (Vercruysse et al. 2011). One test to evaluate possible drug resistance at the community level in human populations is fecal egg count reduction test (RECRT) where the suggested cutoff values for *T. trichuria* and *A. lumbricoides* are 50% and 70%, respectively. Anthelminthic resistance study based on the "candidate gene studies" has limitations

owing to the source of information generated lacking in explaining whole genetic basis of resistance.

A study conducted in Vietnam reported mebendazole ceased to be effective as compared to drug control groups highlighting a need for close evaluation of reduction in drug efficacy (Flohr et al. 2007; Vercruysse et al. 2011). Exposure to anthelmintics such as albendazole or mebendazole has been reported to cause abnormality in developing fetus. Effort for unified eradication in particular locale requiring mass drug administration raises concerns about the risk of anthelmintic resistance onset (Geerts and Gryseels 2000). Mass administration control program must be avoided in order to maintain efficacy and change of potency of presently used drugs, for which alternative and creation of new approach to eradication is an added challenge. The development of new techniques and more sensitive assays may be important for future studies (McCavera et al. 2009; Lake et al. 2009).

23.4.5.3 Legislature and Regulatory Hurdles

According to Veterinary International Committee on Harmonization (VICH) guidelines, anthelmintics for animal use required efficacy of above 90 percent. Besides efficacy and animals safety, food safety hurdle for livestock such as poultry, dairy or meat industry encompasses regulatory body ensuring the detectable limits of the anthelmintics be below the maximum residue levels and withdrawal duration within a defined period (Delatour et al. 2018). Accordingly, drug development for livestock must be designed to overcome the impediment to food safety. This is evident by the use of ivermectin as undesirable despite its effectiveness due to high lipophilicity content whereby its metabolites require extended withdrawal time from the system and elimination along the milk (Campbell 2016). Environmental issue is becoming an added challenge for drug development in the last decades due to the potential harmful effect of drug residual on biotic life and ecosystem. This complication especially for livestock anthelmintics is serious, inherently, due to mixing of ecotoxicity and persistence of residue in soil which will expose the livestock to a greater risk when they feed on green pastures (Liebig et al. 2010). Any pharmaceutical drug is compelled to pass through environmental risk assessment before made available in the market (Lumaret et al. 2012). Ensuring safety measures for the users through legislature and assessments in the chemistry, quality, control, and manufacturing process seem hurdle and iterative process but is one imperative and essential discreet step for any drug development.

According to estimates, an economic stimulus of approximately $100 million and $2.5 billion for animal health products and human drugs, respectively, are stipulated to be significantly supporting drug discovery (Yarborough 2016; DiMasi et al. 2016). Drug discovery is a costly affair and many may not succeed. The goal of drug development process for the "neglected" diseases are chiefly field, to meet the clinical benefit and disease-control program in the field. Therefore, a collegial effort encompassing scientific industry, government, non-government organizations, academia, and legislatures can provide impetus and help bolster program of discovery (Moffat et al. 2017).

23.5 Concluding Remark

Infection by worm *Ascaris lumbricoides* in the human gastrointestinal is one of the leading causes of STNs. It affects particularly young children and is considered the most neglected tropical diseases. Less attention is given in spite of the debilitating impact on children, their health quality, and the trap cycle of poverty. Despite the current anthelminthic treatments that have been effectively used with average cure of 95%, due to reinfection in treated patients within months, there have been instances of high prevalence and increased incidence rates following single-dose administration besides instances of drug resistance in helminthes populations (Hagel and Giusti 2010). Pharmacological-based eradication programs employ administration of repeated doses of extensive treatment, which may lead to drug resistance. One of the desired prophylactic approaches is vaccination, which can avoid both drug resistance and slow progress of new drug discovery; however, the development of efficient helminthic vaccines poses a far greater challenge than for viruses or bacteria. This stems from the human hosts and intestinal worms association involving complex immunological interactions, which confer protection of the worm parasites. The imperative challenges and seriousness in treating ascariasis highlight for due attention and importance of new drug development. Many available drugs lack full efficacy and lead to side effects. Very limited compounds are being tested and no new drugs in late-phase of clinical trials (Geary et al. 2010). The nematode parasite genome, especially identifying DNA sequences containing new gene function or antigens and immunomodulatory molecules are potential target avenues to unfold strategic therapeutic modalities to identification of new drugs. Awareness on public hygiene and health are other areas of effective long-term preventive measures.

Natural compounds from plants extracts are reported to contain antiparasitic properties based on their nematocidal activity and have been traditionally used against neglected tropical disease such as helminthiasis. Vast information on plant-derived compounds and its derivatives having anthelmintic values began to accumulate and has been the focus for an alternative approach to drug discovery and development in the recent times. New drugs based on natural plant products are reasonable going to be well accepted. In an effort to novel drug development, the desired plants can be evaluated and tested for nematocidal activity by determining the levels of reduction of parasitism as well as validating the results on livestock and humans before their wider acceptance. Validation of side effects notably its possible anti-nutritional is necessary (Capasso et al. 2000). Recently we are observing encouraging progress in the effort to new drug discovery by the re-engaging of the private biopharmaceutical company. Emphasizing on the building and leverage of such partnership between scientific community, industry, and public health leaders in particular from tropical disease prevalent countries could pave way to hindrance to progress. Lastly, drug discovery for the tropical diseases are chiefly field-driven, which means emphasis on low cost of goods and lacking trained medical monitoring of safe use of drugs. It is crucial to highlight drug profile to those officials in the control programs in the affected poor region what is required for safe use (Diagram 23.1).

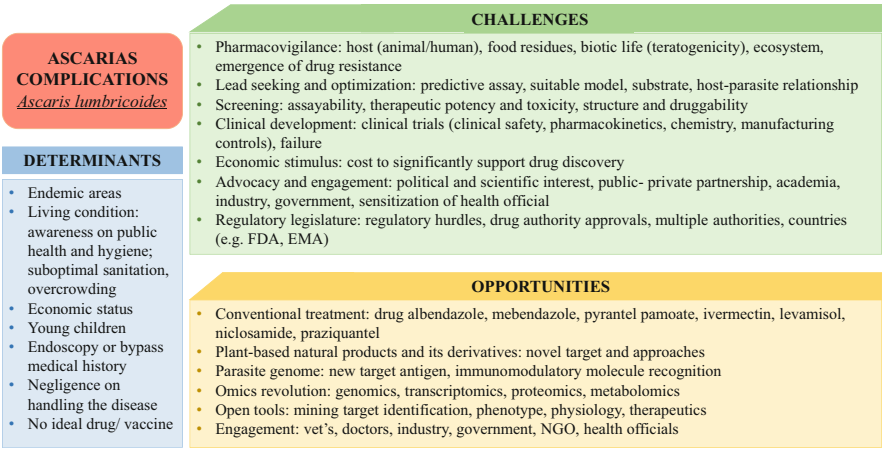

Diagram 23.1 An outline depicting the determinant of risk factors, challenges, and opportunities for therapeutic intervention approaches in *Ascaris lumbricoides* complications in the human host

To conclude, we summarized data providing information about currently available plant-based compounds and its derivatives that are being explored as potential drug candidate for discovery and development against helminths. Some of the challenges of plant products in drug discovery briefly highlighted emphasizing on bioassays, therapeutic efficacy and toxicity, mechanism, and pharmacokinetics.

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The Potential of Plant Secondary Metabolites as Drugs or Leads Against Helminths

24

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Abstract

Helminths are a large parasitic class that is divided into three groups: nematode, cestode, and trematode, all of which cause parasitic sickness in people and animals. One of the most prevalent helminthic illnesses in children is enterobiasis, which is also known as pinworm infection. The World Health Organization also recommends other kinds of medications to combat parasite infections. Albendazole and mebendazole are two common medications used to treat various illnesses. However, these parasites have started developing medication resistance, which has directed recent research to focus on how to address this issue. As an alternative, plant secondary metabolites (PSMs) are also utilized as anthelmintic. Many plants, such as *Nicotiana tabacum, Azadirachta indica, Cannabis sativa,* and *Allium sativum,* possess phytochemicals with anthelmintics properties. Further, nanoparticles manufactured by the green synthesis methods (silver and gold nanoparticles) have also shown tremendous potential for drug delivery. This chapter mainly focuses on the recently discovered drugs and plant-derived

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_24

chemicals that act against helminth infection and how drug repurposing can assist in the discovery of pharmaceuticals with great potential.

Keywords

Helminths · Enterobiasis · Parasitic diseases · Plant-derived drugs · Secondary metabolites

24.1 Introduction

Helminth parasites are multicellular, bilaterally symmetrical parasites with three germ layers. It is derived from the Greek word "helmins," which means "worms." Helminths are multicellular organisms and belong to the kingdom Animalia. In modern classification, these are classified into various phyla: Platyhelminthes, Aschelminthes, Annelida, and Nematoda. Annelida consists of segmented worms, and it is found in various habitats across the world, including deep sea, soil, and freshwater settings. Another helminth class, Nematoda, is made up of worms known as roundworms. It may be found in a variety of environments, including free-living species, plants, and animals as parasites. Infections caused by helminth parasites are among the most common infectious disorders in several countries. In endemic countries, intestinal parasites cause significant sickness and mortality. Common helminth infections exist in the human stomach, but they are incapable of reproducing within the human body. There are several types of intestinal helminths: *Ascaris lumbricoides*, *Trichuris trichiura, Ancylostoma duodenale*, and *Necator americanus*; they are the most common parasites. It is especially prevalent in tropical and subtropical regions of developing countries, where basic water and sanitation services are inadequate (Haque 2007). Helminthic parasites infect over half of the world's population, causing severe illness and disability. In public health terminology, illnesses with whipworm, roundworm, and hookworm are characterized as soil-transmitted helminthiasis (STH) because of soil contamination in their transmission. WHO and its partners assist endemic countries in implementing large-scale anthelmintic treatment for children and women of reproductive age (WRA; except in the first trimester of pregnancy) (Jourdan et al. 2018). Norman Stoll calculated the prevalence of helminth infections in humans in 1947. Recently, *A. lumbricoides* infected a million people, *T. trichiura* infected 795 million, and hookworms infected 740 million. The largest incidence of *Ascaris* infection is found in China and Southeast Asia, as well as the coasts of West and Central Africa. *Trichuris* infection is another kind of illness that is common in central Africa, southern India, and Southeast Asia. Infections with hookworm are common in Sub-Saharan Africa, China, and Southeast Asia (De Silva et al. 2003). The most prevalent tropical illnesses that go unnoticed in the world are soil-transmitted helminth infections.

24.2 Current Situation of Helminths Diseases

The geographical region with high *Trichuris* and *Ascaris* prevalence rates includes Nations such as Malaysia, Myanmar, Vietnam, and Bangladesh. Further examination revealed that the greater prevalence rate of hookworms was found in rural regions (about 19%) as opposed to tribal communities (approximately 15%); however, in the case of *Trichuris*, they showed 38%, and *Ascaris* revealed 32% in tribal communities than 13% and 14% in rural communities (Silver et al. 2018). According to the global illness burden and risk factors research, the prevalence of STH infection is 1.5 billion instances, with roughly 800 million cases of *ascariasis* and more than 400 million cases of *trichuriasis* and hookworm illness (Silver et al. 2018). There are several means by which the infection spreads, such as parasite eggs discharged into the environment through waste material like excrement or hookworm eggs that develop and hatch in soil and produce larvae that can enter human skin when individuals come into contact with contaminated habitat. Moderate- to high-severity infections are associated with diarrhea, anemia, malnutrition, and cognitive impairment. Several programs at the WHO generated guidelines for large-scale preventive chemotherapy using the drugs Mebendazole and Albendazole. The WHO recommends that at least 76% of school-aged children (SAC) who face the most frequent illnesses or people in high-risk employment be targeted (Fig. 24.1) (Silver et al. 2018). The WHO hopes to eliminate soil-transmitted helminth infection as a public health risk in the forthcoming years, especially related to STH infections, which are more common in SAC, Pre-SAC, or women of reproductive age (WRA) (Freeman et al. 2019).

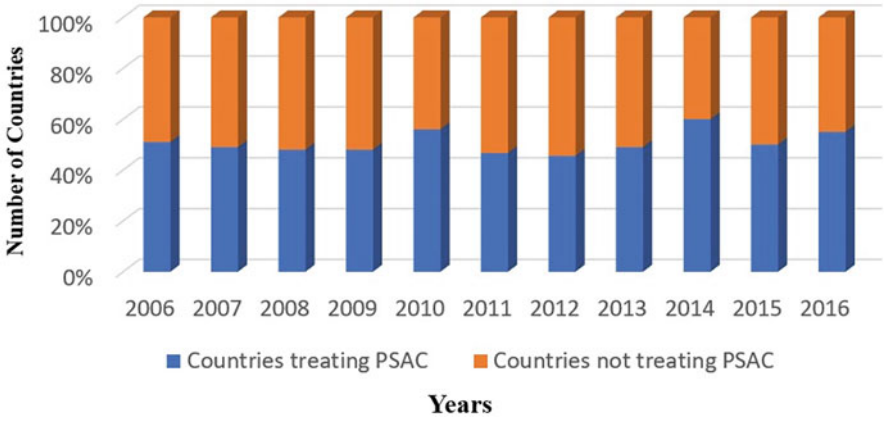

Fig. 24.1 Global PSAC (preschool-age children) Treatment and Coverage, 2006–2016. Source: WHO data, Graph shows the challenges for control and elimination of STDs beyond the age of 2020 (Freeman et al. 2019)

24.3 Classification of Helminths

There are several types of helminth infections triggered by different parasitic worms such as nematodes, trematodes, and cestodes (Table 24.1). Common helminths infections are widespread and transmitted by a number of vectors which are typically seen in various areas. Through different vectors they effect on body, causing helminthiasis.

Cancer has been linked to several helminth parasites such as *Opisthorchis viverrini*, *Clonorchis sinensis*, and *Schistosoma haematobium* (Brindley et al. 2015). Helminth infections can inhibit the host's immune response, reducing vaccination effectiveness and increasing the risk of other infectious illnesses. It also

Table 24.1 Characterization of various species of helminths (Weinstock 2005)

Species	Common name	Site of action	Common infection vector	Cause by vector	Common region
Nematodes (roundworms)					
Trichuris trichiura	Whipworm	Intestine	Oval in soil	Oral	Worldwide
Enterobius vermicularis	Pinworm	Intestine	Ova in feces/ soil encysted larvae in meat	Oral	Worldwide
Ascaris lumbricoides and *Trichinella spiralis*	Pinworm	Intestine	Ova in feces/ soil encysted larvae in meat	Oral	Temperate region
Wuchereria bancrofti and *Brugia malayi*	Filarial worms	Lymphatics	Larvae from insect bites	Skin	Tropics
Necator americanus and *Ancylostoma duodenale*	Hookworm	Intestine	Larvae in soil	Skin	Worldwide
Strongyloides species	Threadworm	Intestine	Larvae in soil	Skin	Tropic, subtropics
Trematodes (flukes)					
Schistosoma species	Blood flukes	Mesenteric veins	Cercariae from snails	Skin	Tropic, subtropic
Fasciolopsis buski	Intestinal fluke	Small intestine, lungs	Cercariae from snails on plants	Oral	Tropics
Clonorchis sinensis and *Opisthorchis species*	Biliary flukes	Biliary ducts	Metacercariae in fish	Oral	Tropic to temperate
Cestodes (tapeworms)					
Taenia saginata, T. solium, and T. latum	Tapeworms	Intestine	Encysted larvae	Oral	Worldwide
Hymenolepis nana	Tapeworm	Intestine	Ova in soil	Oral	Worldwide

lowers the immunological response to SARS-CoV2 and can enhance the morbidity of COVID-19 (Abdoli 2020).

During pandemic period, WHO organized treatment guidelines for helminth infections with the goal of controlling the morbidity of helminths that can be eliminated by mass medicines such as lymphatic filariasis (LF) and onchocerciasis, which are more effective (Wright et al. 2018). Just a few drugs are available to treat zoonotic parasites infections like neurocysticercosis and echinococcosis. Recently, researchers have started working on oxfendazole, an anthelmintic medication that is more effective in treating helminth infection (Gonzalez et al. 2019). Oxfendazole is a member of benzimidazole of anthelmintics drugs. It is widely used to control parasitic infection in meat-producing animals like pigs or lambs. Extensive use of veterinary drugs in food-producing animals causes minor quantities of drug residues in food. The Food and Agriculture Organization (FAO)/WHO was established in 1999 as an expert committee on food additives, which evaluates the toxicological and residual data of certain drugs, such as oxfendazole's toxicological risk to human health (El-Makawy et al. 2006). Niclosamide and artemisinins are two popular anthelmintic medications that function against the activities of helminths. However, many medications are multi-purpose, such as artemisinins, which are also used in the treatment of malaria. Likewise, niclosamide drugs are effective as anticancer as well as antihelminthic. Niclosamide is an antihelminthic medicine that is taken orally and is used to treat parasite infections. It can also be used as an anticancer agent in osteosarcoma (OS) by reducing the proliferative activity of osteosarcoma cells. As a result, niclosamide is a repurposed drug (Liao et al. 2015). Anthelmintic medicines are designed to reduce morbidity by reducing the worm burden. It inhibits the irreversible effects of schistosomiasis and single-dose mebendazole and albendazole for hookworm and trichuriasis therapy (Adams et al. 2016).

24.4 Classification of Anthelmintic Drug

We have discussed several medications that have diverse modes of action for treating helminthic infection. The most commonly used drugs in the therapy of helminth infection against schistosomes and hookworms include oxamniquine, praziquantel, ivermectin, tetrahydropyrimidines. Benzimidazoles, imidazothiazoles, tetrahydropyrimidines, macrocyclic lactones, amino-acetonitrile derivatives, spiroindoles, and cyclooctadepsipeptides are classified anthelmintic drugs. There are several kinds of drugs approved for the treatment of helminth infections, as shown below (Table 24.2).

24.5 Anthelmintic Resistance

Several reports in recent years have highlighted the main issue of anthelmintics medication resistance. Anthelmintic resistance (AR) is a severe hazard to helminth infection, especially when it is difficult to diagnose. We must understand the

Table 24.2 Classification of approved anthelmintic drugs (Abongwa, et al. 2017)

Approved drug	Helminths species	Year	Type of diseases	Mode of action
Benzimidazoles				
Thiabendazole	Threadworm	1964	Larva migrans	Prevent worms growth
Albendazole	Dog tapeworm	1982	Neurocysticercosis	Inhibition worm absorbing sugar of worms
Mebendazole	Pork tapeworm	2016	Cystic echinococcosis (hydatid diseases)	Inhibit the synthesis of microtubule or blocking uptake of glucose of helminths
Flubendazole	Threadworm	1980	Gastrointestinal nematode infection	Helminths Disruption of microtubule structure and function of worm
Triclabendazole	Trematodes and flukes	1980	Fascioliasis	Inhibition of microtubule formation
Imidazothiazoles				
Tetramisole and levamisole	Hookworms or Ascaries	1970	Autoimmune disorder	Change the nicotinic acetylcholine receptor
Tetrahydropyrimidines				
Pyrantel	Pinworm	Mid 1970s	Enterobiasis, oxyuriasis	Paralyze the worm
Oxantel	Hookworm	1970	Enterobiasis, ascariasis	Making worm unable to move Paralyze the worm
Morantel	Parasitic worms	1982	Lactating problem	Affect the nervous system of worms
Macrocyclic lactones (MLs)				
Avermectin Ivermectin	Heartworm	1975	Onchocerciasis (river blindness)	Applied on skin
Milbemycin	Hookworms	1972	Not use in human	Control or removal of some nematode
Milbemycin oxime	Roundworms	1972	Not use in human	Control or removal of some nematode
Praziquantel (PZQ)	Parasitic worms (schistosomiasis)	1973	Infection in urinary tract or bowls	Rapid contraction and paralysis of worm muscles

(continued)

Table 24.2 (continued)

Approved drug	Helminths species	Year	Type of diseases	Mode of action
Diethylcarbamazine (Hetrazan)	Wuchereria bancrofti (roundworm)	1947	Lymphatic filariasis	Unclear mechanism (effect on intestinal nematode)
Moxidectin	Heartworms, hookworms, roundworms	1980	River blindness (onchocerciasis)	Ivermectin-resistant strains

mechanism of action and resistance in order to design new medications and supply all knowledge for overcoming parasite resistance. The primary cause of anthelmintic resistance is the use of extensive and irregular anthelmintic dose, which, when combined with other circumstances, results in drug resistance (DR), which causes a significant helminth infection (Yamson et al. 2019). They raise awareness of the problem of anthelmintic resistance (AR) in the treatment and management of helminths (Geerts and Gryseels 2000). Anthelmintic/drug resistance (DR) is the most severe kind of resistance. Resistance of parasites to one medicine in a chemical class is also resistance to other drugs in the same class. This is true for benzimidazoles (BZ), *Imidazothiazoles,* and avermectin/milbemycin anthelmintics (AMs) resistant in Cooperia oncophora in cattle, also the possibilities in humans (Sangste 1999). According to the present global situation of antihelminthic resistance, South Africa has 79%, 73%, or 23% resistance to BZ, IVM, or LEV, whereas Brazil has 68%, 7%, or 19% resistance (Geerts and Gryseels 2001). As previously stated, the first macrofilaricidal regimen is employed in *Onchocerca volvulus*, praziquantel for schistosomiasis, benzimidazoles as prevention, albendazole or mebendazole of drug resistance development (Geary 2012). The recent success of preventive chemotherapy (PC) in resolving anthelmintic drug resistance or low efficiency of drugs against parasites has been highly appreciated by the scientific community.

24.6 Secondary Metabolites as Anthelmintic

Our natural environment contains a wealth of cures for all human maladies and disorders. Herbal treatments have been utilized since ancient times. Before the Christian period, this kind of herb was utilized medicinally in China, India, Greece, and Egypt. Secondary metabolites have a high economic importance in several places. Secondary metabolites are substances produced by plants in response to their environment. Plant secondary metabolites (PSMs) are utilized to combat parasites. Scientists have focussed on PSMs with antiparasitic action and investigated their structure, intake, and availability. According to recent research, anthelmintics cause toxicity in people, which is why the discovery of novel medications that operate as anthelmintics derived from plants is the greatest source

of bioactive chemicals. Natural anthelmintics include tobacco, walnut, garlic, honey, vinegar, male fern, kalonji seeds, and other natural plants combined with water that work as vermifuges (Yadav and Singh 2011). Numerous ethnoveterinary medications are produced, which appears to be a plant extract suited for treating each parasite illness. Natural herbs such as garlic seeds (*Allium sativum*), onion seeds (*Allium cepa*), and mint (*Mentha* spp.) are used against animals with gastrointestinal parasites, while tobacco plant extract (*Nicotiana tabacum*) is used to cure the skin condition (Athanasiadou and Kyriazakis 2004). Dryopteridaceae, Arecaceae, Santonin, Asteraceae, Rutaceae, Lamiaceae, Fabaceae, Myrsinaceae, Amaranthaceae, and other ancient herbs are used as antihelminthic medications (Wink 2012).

PSMs can serve as a significant alternative medicine against parasitic infections which can be utilized as an anthelmintic medicine, especially during scenarios of developing anthelmintic drug resistance. Researchers have investigated natural herbs or weeds that have the ability to kill helminth infections, such as tobacco, which showed a more effective impact at higher concentrations (Bowman and Rand 1980). A wide variety of alkaloids have been isolated from plants, including nicotyrine, nornicotine, anabasine, nicotelline, and myosmine, that have antihelminthic activities (Athanasiadou and Kyriazakis 2004). Herbs as a whole are also utilized as anthelmintic agents (Nadkarni and Nadkarni 2000). As an anthelmintic, we test an aqueous or alcoholic tobacco extract against parasites (Nouri et al. 2016). Tobacco leaf contains nicotine, which is responsible for the anthelmintic properties of the plant. Levamisole is a common drug used to activate the nicotinic receptor in antihelminthic action, and methanol extracts of *Nicotiana tabacum* show activity against sheep intestinal worms (Nouri et al. 2016). Scientists have also found a link between smoking and helminths, as tobacco users have a higher risk of helminthiasis. Currently, modern anthelmintics such as levamisole and tetrahydropyrimidines succeed by targeting the same receptor as nicotine on the nicotinic acetylcholine receptor on the somatic muscle of parasites, which induces spastic paralysis, but only to a certain extent because they have a high potent neurotoxin via smoking and chewing (Yamson et al. 2019). However, due to habitual cigarette use, helminth illnesses, which account for a significant portion of the disease burden, have increased. In a study, the alkaloid content of methanolic extracts of several *Nicotiana* species (*N. glutinosa, N. glauca, N. debneyi,* and *N. tabacum*) was assessed to check the antiparasitic activity against worms (Fig. 24.1). This work successfully analyzed pure alkaloids (nicotine, nornicotine, anabasine, anatabine) discovered in tobacco extracts at high concentrations using ultrahigh-performance liquid chromatography with a mass spectrometer (UHPLC-MS) (Fig. 24.2) (Weber et al. 2019).

24.7 Mechanism of Formation of Nanoparticle

Nanobiotechnology is a branch of research that uses a multidisciplinary approach to create novel products. There are several applications of nanobiotechnology in the form of nanoparticles for diagnostic purposes, disease prevention and treatment, and drug targeting or delivery (Irache et al. 2011). A wide pool of medicinal herbs has

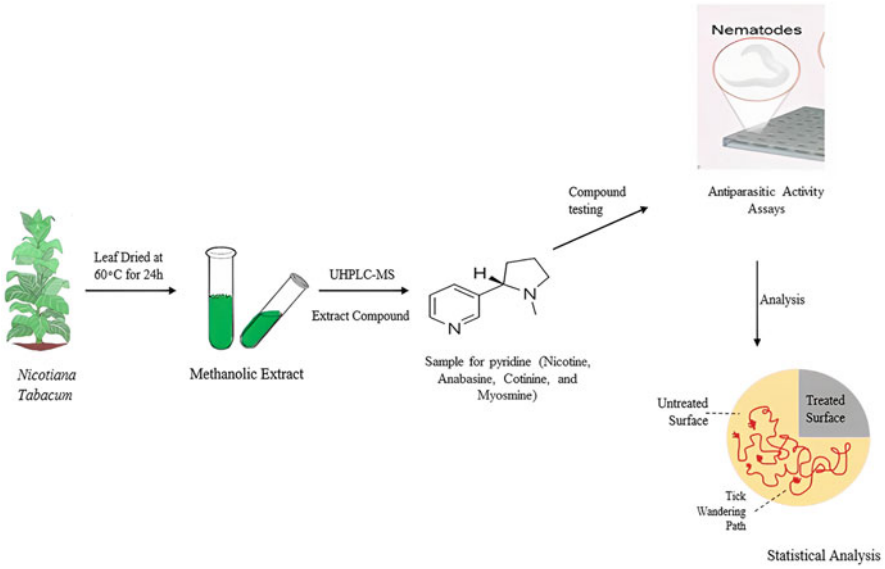

Fig. 24.2 Mechanism of action: tobacco plant

been shown to be effective against helminths (Pathak and Chhabra 2014). Researchers also investigated tobacco leaf extract, which is utilized in the manufacture of silver nanoparticles (AgNPs), a simple and environmentally friendly biosynthesis of silver nanoparticles as a reducing agent (Kuchekar et al. 2015). In helminths parasites, silver nanoparticles (AgNPs) are commonly manufactured with the soil-isolated fungus *Trichoderma harzianum,* which is then bound with the anthelmintic triclabendazole for improving the properties of anti-*Fasciola (F. hepatica)* (Fig. 24.3). Another one is gold nanoparticles (Au NPs) that have anthelmintic efficiency by treating with mycelia-free culture filtrate of the fungus *Nigrospora oryzae* as a drug delivery system tested against parasites (poultry cestode) (Kar et al. 2014). In vitro anthelmintic efficacy of AgNPs against gastrointestinal nematode using synthetic aqueous extracts of *Ziziphus jujuba* and *Azadirachta indica* leaf extract (GIN) to reveal the potency of anthelmintic properties (Preet and Tomar 2017). FTIR spectroscopy data are used to extract numerous metabolites from plants that have reduced the silver ions, such as terpenoids, flavonoids, eugenol, and polyphenols. These compounds are found in plants such as Geranium, Cinnamon, and Sweet Basil and are used in the early stages of AgNP synthesis (Shankar et al. 2003; Sathishkumar et al. 2009).

Recently, researchers tested the aqueous extract of *Azadirachta indica* leaves for anthelmintic efficacy against earthworms, tapeworms, and roundworms. This extract has vermicidal action and is used as an anthelmintic. Piperazine citrate is a pepper plant-derived organic compound. Piperazine is currently being tested on helminths, and preliminary results show that it is particularly effective on parasitic worms. It has

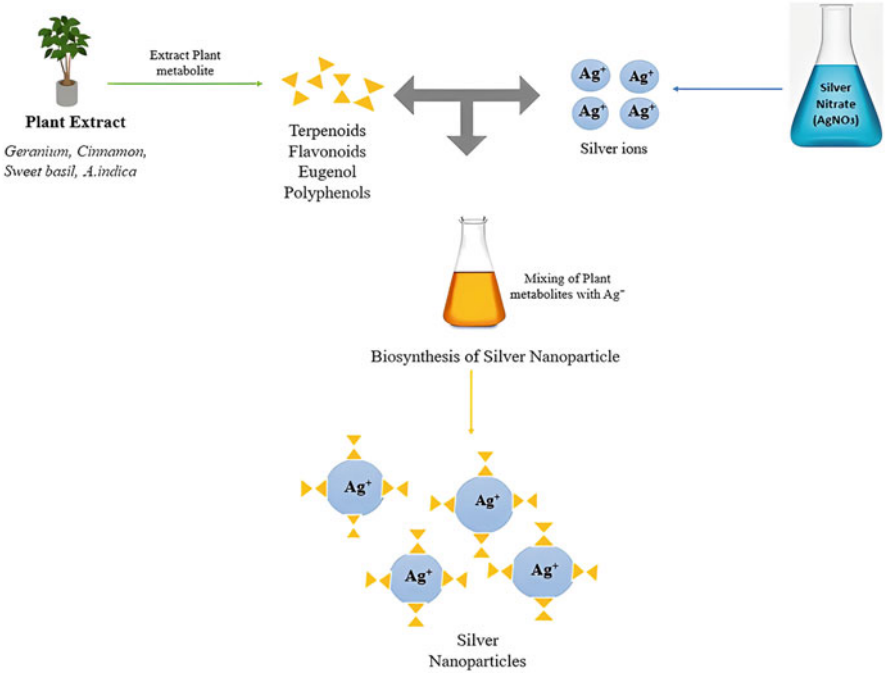

Fig. 24.3 Mechanism of silver nanoparticles (Khatoon et al. 2017)

an immediate action on the nerve, causing worm paralysis (Rabiu and Subhasish 2011).

Cannabis possesses certain habit-forming substance that also has antiparasitic and therapeutic properties. This is the sole medical application for yellow fever therapy. Cannabis includes the anthelmintic component used to defend against parasite infection. It has been used as a herbicide and a companion crop to help minimize plant worms (McPartland 1997). It also has effectiveness against human parasites or infections. Cannabinoids with antibacterial and anthelmintic properties include cannabidiol, cannabichromene, cannabigerol, THC, and cannabinol. It also causes paralysis and death in intestinal trematodes (Appendino et al. 2008). Similarly, tobacco is a source of nicotine and includes bioactive chemicals such as nornicotine and anabasine, which are also utilized as recreational plant drugs (Roulette et al. 2014).

Similarly, neem (*Azadirachta indica)* and tamarind (*Tamarindus indica*) leaves were also evaluated for their anthelmintic properties. For this investigation, dried leaves were collected, and the key components that operate against helminths or parasites were extracted. The neem:tamarind (1:1) is more efficient (68.14%) than the individual neem (29.94%) and tamarind (29.94%) (42.66%). These are more efficient and effective than cattle anthelmintics (Table 24.1.3) (Amin et al. 2022).

Table 24.1.3 Table of various secondary metabolites

Secondary metabolite	Scientific name	Helminths species	Extracted compound	Site of action	Reference
Garlic seed	*Allium sativum*	Nematode	Organosulfur compound Allicin, sulfur-containing products like lachrymatory factor	Inhibit the growth of trypanosomes and leishmanias	Mikaili et al. (2013) Krstin et al. (2018)
Onion	*Allium cepa*	Earthworm, *Leishmania* sp.	Sulfoxide like alliin	Inhibit the growth of trypanosomes and leishmanias	Krstin et al. (2018)
Mint	*Mentha* spp.	Intestinal worm	Essential oil	Use in gastrointestinal problem	Silva (2020)
Tobacco plant	*Nicotiana tabacum*	Nematode	Nicotine	Reduce worm burden during infection	Nouri et al. (2016)
Dried flower	*Chenopodium ambrosioides*	Hookworm	Superoxide, hydrogen peroxide radicals	Used in treatment of anorexia, diarrhea, piles	Poonia and Upadhayay (2015)
Male fern	*Dryopteris filix-mas (Dryopteridaceae)*	Intestinal Cestodes, roundworms	Containing compound aspidin, albaspidin, Filicinic acids	Target on nematode nervous system	Egorova et al. (1900)
Pomegrante	*Punica granatum (Lythraceae)*	Gastrointestinal nematode, *A. caliginosa* worms, hookworm, roundworm, whipworm	Oxalic and tartaric acids, phenolic compound	Neuromuscular paralysis in worms, effects on acetylcholine receptors in muscles, causes spastic paralysis	Amelia et al. (2017)
Areca palm	*Areca catechu (Arecacae)*	Flukes	Arecholine	Against *Fasciola* spp., paralysis of helminths	Yamson et al. (2019)

24.8 Drug Repurposing for Treating Parasitic Infection Caused by Helminths

The process of identifying new uses for medications is known as drug repositioning/repurposing. It is used to locate a substance that was originally designed to treat other diseases. Researchers discovered the possibility of medication repurposing. It has been discovered that certain medications have a more noticeable effect on other disorders. As previously stated, niclosamide works against helminth infection and is utilized as a repurposed medicine against parasites for future prospect as an antiviral, antibacterial, and anthelmintic (Fig. 24.4) (Farha and Brown 2019).

Praziquantel, which has replaced numerous other medications in the treatment of helminth diseases, is the most common example of pharmacological repurposing. It is an effective medication against several *Schistosoma* species (*S. haematobium, S. japonicum, S. mansoni*) and intestinal flukes (Keiser and Utzinger 2004). Tribendimidine is also used as an anthelmintic against soil-transmitted helminths, similar to how albendazole works against *Ascaris lumbricoides* (Xiao et al. 2013) (Panic et al. 2014). Another example of pharmaceutical repurposing is oxyamniquine (OXA), which only acts against *S. mansoni* when paired with PZQ. *S. mansoni* is typically treated with mefloquine (MFQ), ibuprofen, and naproxen (Vale et al. 2020).

24.9 Conclusion and Future Prospective

The above chapter highlighted on a newly defined drug for combating anthelmintic resistance via drug repositioning. Current study suggested that secondary metabolites are also employed as an antiparasitic drug. It will be carried out with the help of secondary metabolites such as tobacco plants and *A. indica*, both of which are less expansive. Plant-derived secondary metabolites have the potential to be used as a parasite-inhibiting drug. As a parasiticidal medicine, a novel clinical trial chemical has been designed. Aside from that, a recent effort in the field of nanotechnology is being made to create metallic nanoparticles in parasitology for

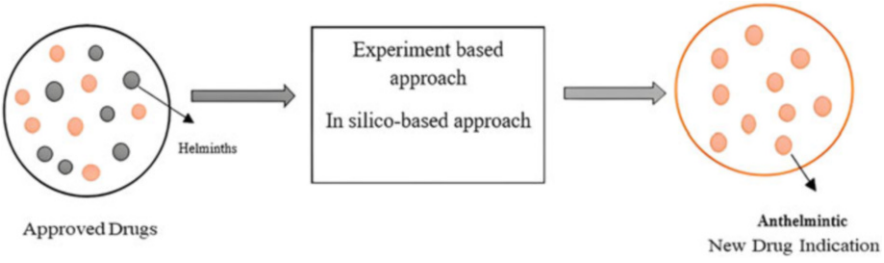

Fig. 24.4 Drug repurposing

parasite detection. Another biologically relevant application for these nanoparticles is drug repurposing or repositioning, which is a promising area for future research.

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Drug Resistance in Helminth Parasites: Role of Plant-Based Natural Therapeutics

25

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Abstract

Neglected tropical diseases (NTD) are considered as the most varied group of diseases caused primarily by parasites. NTDs affect more than a billion people globally. A majority of the neglected tropical diseases like lymphatic filariasis, schistosomiasis, and river blindness were caused by helminthic parasites. The World Health Organization along with local health agencies plays a key role in controlling and eliminating the NTDs. These diseases are endemic in African regions and pose devastating health and economic burden. Generally, in those areas mass drug administration is employed. In general, periodic mass drug administration to the population at risk is employed to control these diseases. The current treatment relies majorly on benzimidazole anthelmintics, levamisole, pyrantel, and praziquantel are the conventional antiparasitic drugs. Relying on single drug administration for such a long time, use of same drugs in livestock, and already emerged resistance to the current known drugs in livestock indicates the necessity of newer novel therapeutics. Studies had already reported reduced efficacy of the human antihelminthic drugs. The extent of this problem of drug resistance is likely to increase. Given the dearth of drugs for treatment of NTDs, there is an urgent need for novel new anthelmintic drugs. Medicinal plants are a great source of effective treatment and majority of the current drugs are based on

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_25

the natural molecules. Many studies were reported on the antihelminthic activity of plant extracts. The natural compounds hold a great promise as drugs and can offer alternative treatment options to handle the drug resistance. This chapter discusses particularly on the drug resistance mechanisms of the current antihelminthic drugs and the possible future plant-based natural therapeutics.

Keywords

Neglected tropical diseases (NTD) · Drug resistance · New anthelmintic drugs · Plant-based natural therapeutics

Abbreviations

BZ	Benzimidazoles
EPG	Egg per gram
GluCl	Glutamate-gated chloride channels
IVM	Ivermectin
MDR	Multidrug resistance
PZQ	Praziquantel

25.1 Introduction

Helminths consists of various groups of parasitic worms, consisting of nematodes, cestodes, and trematodes that are responsible for major hazards of health trouble in individuals and domestic as well as wild animals all over the world (Sutherland and Leathwick 2011; Hotez et al. 2008). The infection caused by helminthic worms in animals causes morbidity and zoonotic infections and influence production of food. Although several measures were implemented to minimize helminthic infections, which included improved sanitation and pasture control in domestic animals, for the eradication of these parasites, the above ideas are too little. In the past few years, largest drive for mass administration of antihelminthic drugs in the people infected from helminthes was done in association of government and NGOs across the world. The drive for antihelminthic drug administration drastically reduced the deaths and morbidity and infection spreading and eventually helped to eradicate parasites. However, exhaustive administration of these drugs in the livestocks as well as drug administration programs at huge levels in humans led to induction of drug resistance in helminthes against currently available drugs (Wolstenholme et al. 2004; Laing et al. 2017). The first report of development of drug resistance in helminthes appeared at late 1970s. With new findings and producing few antihelminthic drugs could not help the humans to win fight against helminthic parasites. The methods of discovery and development of drugs against helminthes is summarized in Fig. 25.1. Helminthic infections are generally treated with several varieties of drugs such as

Fig. 25.1 Summary of antihelminthic drug discovery and development

macrocyclic lactones, praziquantel (PZQ), imidazothiazoles, and benzimidazoles (McKellar and Jackson 2004). The widespread development of drug resistance against helminthes is reported in farm animals and also in humans. The drug albendazole in benzimidazoles group and ivermectin (IVM) in avermectins group are reported to be ineffective against nematode parasites infecting farm animals. Moreover, helminthic parasites also reported to develop resistance against new anthelmintic drug, monepantel. The human parasite *Onchocerca volvulus* responsible for river blindness disease, *Trichuris trichiura*, human whipworm, and *Dirofilaria immitis*, heartworm, were also reported to be resistant against available anthelmintics. Therefore, it is very crucial to understand the mechanism of development of resistance in the helminthic parasites for the successful development of new drugs, maintaining the prolonged efficiency of a drug and development of markers for proper monitoring and understanding mechanism of drug resistance.

There is a substantial argument regarding the term "resistance" and "tolerance," which generally illustrate the phases of success and failure during drug administration. Coles (2006) explained that development of resistance could be considered if susceptible parasites exhibit decreased response against treated drug and is done when the maximum given concentration that can be indulged by the host has zero effect. The decrease in drug response can be evident either as a heritable decline against the susceptible parasitic population or decline in time a drug shows its effect.

The resistance against any drug occurred due to survival of increased proportion of individuals within a population with specific gene linked with resistance (Prichard 2007). The helminths usually found in huge numbers with higher genetic variability lead to faster resistance and selection of inherently resistant individuals in a population.

However, study of drug resistance in cancer cells suggest that due to selection pressure against drug administered, some heritable changes occurred in the cells that lead to drug resistance. These changes that can be transferred to next generation could be any alterations in DNA such as point mutation, deletion, and insertion (genetic changes) or modification of DNA like methylation of promoter of genes (epigenetic changes) that causes modification of gene expression against response to any drug (Fojo 2007).

The mechanism of drug resistance can be understood by analyzing the chemotherapy developed resistant cancer cells. The resistance developed in these cells can either be "inherent" in which cells did not respond to the drug or "acquired" where cancer cells develop resistance through initial treatment with the drug (Zhou 2008). These mechanisms have also been demonstrated against antibiotic resistance of disease causing bacteria (Raghunath 2008) that clearly explains the similar underlying and universal mechanism for cellular drug resistance in prokaryotes as well as eukaryotes. Even though, drug resistance could occur due to mutation in the specific gene that decreases the affinity for drug-binding site and effectively provides resistance to a particular drug. Moreover, there are several other entrenched pathways that minimize the interaction of drug with its target, such as alteration in cell permeability reduces quantity of drug accomplished to its target. On the other hand, the drug also undergoes into detoxification process through different available antioxidant defense enzymes such as cytochrome P_{450} enzymes or glutathione—thioredoxin systems. Any alteration or modification in these defense enzymes leads to multidrug resistance (MDR) in the organism or cellular system. However, excessive and unsystematic administration of synthetic drugs for management of parasitic diseases now became a nightmare for drug resistance in parasites.

Therefore, due to severe side effect of synthetic drugs, it is valuable to include that herbal or plant-derived drugs provide very effective, modest, and more reliable cure against various parasitic diseases in humans.

25.2 Development of Drug Resistance at Genetic Level

It has been clearly demonstrated that development of drug resistance in parasitic helminthes depend upon several factors; among them major contribution of resistance development is the occurrence of resistance alleles present in the early untreated helminthic population. For example, the normal population of *Haemonchus contortus* exhibited the allele frequency for benzimidazoles (BZ) resistance at isotype 1 and 2 β-tubulin loci as 46% and 12%, respectively (Blouin et al. 1995), and also elevated allele frequency (10 ± 20%) against ivermectin (IVM) resistance (Anderson et al. 1998). Moreover, only 4% of the allele frequency was found in the untreated subsets of IVM-resistant cyathostomes (Young et al. 1999).

The genes actively participated in drug resistance, and their dominance or recessiveness governs the impact on the rate of spreading of drug resistance in the helminthic population. However, in *H. contortus* the BZ resistance represented inheritable trait with at least two or three genes involving recessive alleles. One gene or cluster of genes with autosomal recessive nature leads to levamisole resistance, whereas resistance against IVM is mediated by dominant effect of a single of cluster of genes (Anderson et al. 1998). There is a popular concurrence that relapse of resistant to susceptibility is scarce when parasitic helminthic worms become resistant, even when other drugs had different working mechanism used for longer time period (Conder and Campbell 1995).

25.2.1 Nematodes

Genetically heterogeneous populations of these parasites are found in nature. Therefore, these helminthic populations can respond to selective pressure of various anthelmintic drugs (Grant 1994), and, in turn, drug pressures select parasite lines having resistant alleles or tolerance to drug effect. The rate of spreading of drug resistance in the worm population depends upon proportional contribution of surviving Helminthes to the upcoming generation. It has been governed by the timing and frequency of treatment, efficiency of drug, generation time, gene flow, fecundity, total number of genes as well as frequency of resistance alleles and dominance or recessive character of genes. Several mathematical models demonstrated in gastrointestinal helminths drug resistance mechanism (Jabbar et al. 2006) and one of the interesting models was developed by Waller (1997) in grazing sheep for *Trichostrongylus colubriformis*. The model allowed maximum of three genes with two alleles for each gene, against drug resistance that independently combined during random mating and also in the absence of any antihelminthic drug, supposed to be evenly fit. The assumption of first few frequency of very low (0.01%) range of these population-governing alleles had been used. To understand the effect of two drugs combination or single drug with different rotation time, two genes of independent nature for tolerance against two drugs with dissimilar mode of action were imitated, with resistance being codominant and per drug caused 99%, 10%, and 50%

mortality of homozygous susceptible (SS), homozygous resistant (RR), and heterozygous (RS) genotypes, respectively. For the period of 20 years, simulations were done with three treatments per year in female and one treatment per year in the case of male. When combination of two drugs had been used, the result exhibits negligible development of resistance. Whereas, rotation strategies lead to development of considerable resistance, which showed annual rotation strategy of 1, 5, and 10 yearly with slowest drug resistance development.

When assumption was made about equivalent beginning drug capability and resistance of allele frequency to be present in a given population of worms, the development of tolerance occurred more rapidly where involvement of single gene was found in the mechanism rather than involvement of two or more genes. Moreover, evolution of resistance occurred very fast when alleles were dominant and slower in the case of codominant and very slow when it was recessive. However, these models ignored the overdispersed distribution of parasitic as well as free-living helminth stages.

25.2.2 Trematodes

The oxamniquine resistance in schistosomes through genetics is very well understood; however, this is not in the case of praziquantel (PZQ). In classical mechanism of drug resistance in helminthes, the resistance spreads gradually in a population under selection pressure at low frequency; however, development of resistance against hycanthone-oxamniquine occurred universally in F_1progeny of helminthic worms when treated with drug (Brindley 1994). It distinctly indicates that resistance is persuaded at the place of pre-existing population selections (Brindley 1994). Cioli et al. (1993) demonstrated that single autosomal recessive gene possibly developed resistance against oxamniquine that spreads rarely within communities and remains restricted to individual cases. It might occur under lack of drug pressure, which causes selective disadvantage of resistant schistosomes (Cioli et al. 1992). Limited knowledge about biochemical or genetic basis of resistance development to PZQ is known in *Schistosoma mansoni*. Although, it was found that expression of mRNA encoding part of subunit I of cytochrome *c* oxidase was increased 5–10 times in the resistant strain than susceptible strain.

25.3 Factors Causing Resistance in Helminthes

25.3.1 High Frequency of Treatment

It is considered as the important factor in the selection of resistance strain for drug. If the frequency of drug treatment is higher, it results in high pressure of drug, which causes selection at faster rates of resistant helminthes strains. Although, treatment frequencies of 10–15 times per year have been reported in livestock (Dorny et al. 1994), most often it is limited to 1–3 times per year. Even at lower frequency of

treatment, report of drug resistance has been found in sheep and goat helminthes (Burger and Bauer 1994; Boudsocq et al. 1999; Coles et al. 1995). The annual frequency of helminth treatments control programs in human also comes within the range of 1–3 treatments for *Trichuris/Ascaris* (Renganathan et al. 1995) and 1–2 times for *O. volvulus* and *schistosomes* (El Khoby et al. 1998).

25.3.2 Use of Single Drug

When the same drug, which remained effective in the early period of times, is administered, it no longer works. Use of IVM several times in the Onchocercosis Control Program of West-African and PZQ in schistosomiasis control programs in Egypt might be responsible for resistance. Long-term use of single antihelminthic drug, levamisole, in cattle also led to the development of resistance (Geerts 1993). This can be clearly observed in livestocks, where farmers continue to use one drug until it fails (Reinemeyer et al. 1992). Use of computer models also demonstrated that drug resistance development in livestock helminthes is postponed by using combinatorial mechanism of action of different drugs. Two drugs rotating within same year is another option as it delays the resistance for years as compared to the case of drugs rotation at each treatment or rotations after 5- or 10-year intervals.

25.3.3 Prophylactic Mass Treatment

During anthelmintic treatment, the target group offers one of the crucial parameters that governs the drug resistance. Previously, prophylactic mass treatments of livestock were done, importantly the drench-and-move system; during this process, all animals are treated, followed by moving to clean paddock having worm numbers either very less or no in refugia, which is a potent selector for drug resistance. Different models explain delay in the development of drug resistance in non-treated population of around, e.g., 20%, although that reason might cause certain effects on productivity (Barnes et al. 1995).

In human, helminthes control programs, indiscriminate mass treatments are not promoted by medical parasitologists and are restricted to school children as one of their target groups, which reduce selection pressure.

In addition to treated population, the time period of the treatment also influences the growth of resistance in parasitic helminthes. In dry Greek islands, development of resistance in sheep helminthes occurs with higher frequencies than in wetter climate having similar test patterns. This term can be explained using certainty that the helminthes generation forms in dry environments, and resistant worms obtained after treatment, whereas susceptible worm pre-parasitic stages in wetter regions may be lived because of pasture and the resistant genes dilution might occur in the upcoming generation.

25.3.4 Underdosing

Underdose also play an important role in the drug resistance development as subtherapeutic doses did not lead to 100% mortality and heterozygous resistant worms still survived on these doses (Smith 1990). The model of Smith (1990) demonstrated that the impact of drug on helminthic worm is dependent on the frequency of alleles showing resistant nature before and after the treatment of drug in a population. Depending on the effectiveness of drug to induce mortality in the complete or part of susceptible homozygous, heterozygous, and/or resistant homozygote population, the underdose of drug promotes the development of drug resistance, whereas higher doses impeded the resistance. By assuming that resistance development in helminthes is governed by only one gene with single locus pair of alleles with minimal initial repetition, the most effective dose is the one that leads to cause 100% mortality in susceptible homozygotes, but not in the other genotypes. Moreover, when there is increased frequency of the resistant allele, the dose of drug that supports resistance is that which causes death of all susceptible heterozygotes and homozygotes, but resistant homozygotes remain least affected (Smith 1990).

Underdosing of drug in humans can be most commonly found in several developing countries. The drugs generally used are only half of the recommended doses by most of the poor families. Moreover, generic drugs with substandard quality as well as drugs with expiry date are widely distributed in the pharmacies. Also, the mediocre drugs documented in human and livestocks are produced by numerous unlicensed companies across the world and are lacking quality control measures.

25.4 How Drug Resistance Is Examined?

There are several laboratory tests available for the AR detection in livestock infected with helminthes infection (Conder and Campbell 1995). The most commonly used tests to detect drug resistance in helminthes in human and livestock (Table 25.1) are briefly described here.

25.4.1 Fecal Egg Count Reduction Test (FECRT)

It is frequently employed to diagnose the issues of anthelmintic resistance in which egg counts are compared with pre- and posttreatment with drug. In small ruminants,

Table 25.1 List of techniques used for the diagnosis of drug resistance helminthes

Examine	Anthelmintic	Sensitivity
Fecal egg count reduction	All	Low
Egg hatch	Benzimidazoles	Low
Larval development	All	Low
PCR	Benzimidazoles, ivermectin	High

preferably young animals, two groups are used to collect fecal samples with minimum of 15 individuals, which breed on the farm and previously remained untreated in last 8–12 weeks. The animals were distributed randomly during the test and collection of fecal samples were done after 10–14 days of treatment. It has been observed that comparing posttreatment and control in animals exhibit same reliability as comparing pre- and posttreatment samples and counting of eggs commonly performed with standardized McMaster method (Coles et al. 1992). For valid comparison, the eggs per gram (EPG) derived from feces in control group should be higher than 150 and EPG percent reduction is calculated as: ERR = $100(1\text{-}X_t/X_c)$, in which X stand for EPG arithmetic mean, c denotes control, and t denotes treated groups. The resistance in helminthes is considered when there is 95% ERR, and the 95% of lower confidence interval is less than 90%. The resistance can be suspected when at least one of both criteria is met (Coles et al. 1992).

25.4.1.1 Parasitological Methods

An individual egg counting would be determined by standardized egg counting technique. Peters et al. (1980) and Katz et al. (1972) described Kato technique, which is used for the counting method in the case of schistosomes, *Ascaris,* and *Trichuris.* For later reference and quality store purpose, the slides can be stored. For validation and standardization of the Kato technique in the case of hookworms, utmost care is required. On the basis of only a few clinical samples, recommended time period for reading the slide is after 30 min and not more than after 60 min (Martin and Beaver 1968). However, stool transparency and consistency can differ between individuals and communities in the same field. In any case, stool samples slide of Kato technique of more than 25 mg, such as the standard Kato-Katz, can hardly be read after only 1 h (Peters et al. 1980), and due to that reason it not preferable for standardized quantitative hookworm research.

During experimental studies, the results of the fecal egg count reduction test (FECRT) proved that only 25% of the helminth population shows resistance gene carriers. The egg hatch assay are used to detect drug resistance (Martin et al. 1989). The assumption needed for the larval development assays, that slightly better sensitivity, detection of resistance on/after 10% of the worm population being resistant.

Similarly, with slightly manipulated factors could be designed for the drug resistance detection in helminths infecting humans. On the basis of available data that groups of adults or children are commonly less homogeneous than flocks or herds of animals and considering the necessary drop-out rate, in the study group, number of people should be higher (preferably 50 or more) than the animal numbers (Bonhoeffer et al. 1997). The Kato technique applied in the standard way as explained by Katz et al. (1972) for schistosomes, *Ascaris*, and *Trichuris*.

The Kato slides in the case of hookworm must be observed within 30 min after preparation for obtaining reliable results (Martin and Beaver 1968). At least three stool sample collections were done for subsequent days, including robust day-to-day changes in schistosome egg (Engels et al. 1996). The time gap between medication

and egg count was altered by the helminth species; e.g., 2 weeks for hookworms and 5 ± 6 weeks for other schistosomes should be considered (Hotez et al. 2008).

25.4.2 Hatching Test for Eggs

This test was described by Le Jambre (1976). Basis of this test was ovicidal activity of the BZ resistance detection in livestock helminthes. Freshly collected fecal samples or samples stored anaerobically are required to obtain reliable data. Helminth eggs purification was done from feces and incubated in different concentrations of thiabendazole (TBZ). This compound dissolves simply in dimethyl sulfoxide but the data shows BZ group side resistance for other members. The total number of larvae hatched in 24 h is counted. In resistant helminthes, a higher percentage of eggs hatched as compare to susceptible variety. However, the FECRT and the egg hatch test diagnose resistance particularly when the helminth population reach 25% (Martin et al. 1989).

25.4.3 Larva Developmental Methods

Coles et al. (1988) originally described this assay, which was later improved by various scientist (Gill et al. 1995) and is now available at commercial level (DrenchRite; Horizon Technology). This assay is more time-consuming as compared to egg hatch test. However, it allows detecting resistance to the avermectins-mylbemycins and various others drugs of major broad-spectrum anthelmintic category. Anthelmintic drug of different concentrations is poured on microplates having agar wells on which exposure of nematode eggs or L_1 larvae occurs. The result of exposure of the drugs is measured by the development of larvae into L_3 stage. This experiment is considered high delicate than FECRT and egg hatch test and identified the resistant strain when resistance genes are carried by approximately 10% of the worm population (Sangster 1999).

25.4.4 Larval Motility or Paralysis Test

On the basis of motility in larvae, various in vitro assays have been described to identify resistance to BZ, levamisole morantel, or macrocyclic lactones (Conder and Campbell 1995). The divergence of vulnerable and resistant strains is not always promising by the latter group of drugs (Geerts et al. 1989). To find out the sensitivity of ivermectin on *O. volvulus* microfilariae, a motility test based on the above data has been applied (Townson et al. 1994). For a more accurate interpretation to detect drug resistance, micro-motility meter has been developed for *H. contortus* and *T. colubriformis* (Folz et al. 1987).

25.4.5 Polymerase Chain Reaction (PCR)

The first specific primers to detect resistance in helminthes. These primers can distinguish alleles (β-tubulin isotype 1) of heterozygous and homozygous BZ-resistant *H. contortus* and *T. colubriformis*. PCR is used to identify 1% of individuals having resistance capacity within a susceptible population (Roos et al. 1995). Elard et al. (1999) discovered a more systematic and easiest method for detecting BZ-resistant *O. circumcincta* by including four primers (two nonallele-specific and two allele-specific ones) in the same set of PCR. The characteristic of worm is the mutation of residue 200 of isotype 1 β-tubulin.

In most of the parasitic nematodes, as the mutation causes BZ resistance, it might be expected to be present also in nematodes affecting humans, which develop resistance to BZ. *O. volvulus* has two assumed P-glycoprotein-coding genes that provide resistance to helminthes against IVM in livestock. Expressivity of P-glycoproteins is at high level in IVM-tolerant adults and at low level in IVM-sensitive (Huang and Prichard 1999). The RT-PCR was described to detect a subunit 1 cytochrome *c* oxidase fragment overexpression in resistant strains (Pereira et al. 1998), and a primer was discovered for RAPD±PCR to eminent resistant strains from sensitive Egyptian strains of *S. mansoni* (Tsai et al. 2000).

25.5 Molecular Mechanism of Development of Resistance

25.5.1 Genetic Modulations in the Target of Drugs

Maximum efforts done specially confined for identification of drug resistance in helminths to alter the mechanistic action of the drug's cellular target, which comes in light due to the variations in the genome sequence. Various SNPs polynucleotide sequences are unique genetic sequences that differ within a population. Gene mutations after drug treatment may also cause SNPs production. Any alterations in the nucleotide of a drug target protein alter the affinity of the drug toward target and might be the reason of decreased drug effectivity. Several SNPs are constitutively present in the genome of the human genome that contains approximately 12 million SNPs, with very less established association with disease (Voisey and Morris 2008).

Reports obtained from the *Haemonchus contortus* suggested that parasite population was genetically heterogenous, and that was the reason for the variations in drug response that enabled rapid selection of resistant populations (Prichard 2001). Major area of work has been done with resistant populations to understand the specific mutations leading resistance development, including benzimidazole resistance mutations. Benzimidazole binds with tubulin, causing disrupting microtubules, which causes the death of the worms (Fig. 25.2). Several SNPs are involved in resistance development, including β-tubulin isotype1, the most common SNP being a tyrosine substitution at the place of phenylalanine at position 200 in the gene (Phe200Tyr) (Ghisi et al. 2007). The experiment was performed in *C. elegens*, wherein the above genetic changes confirms resistance to benzimidazole (Kwa et

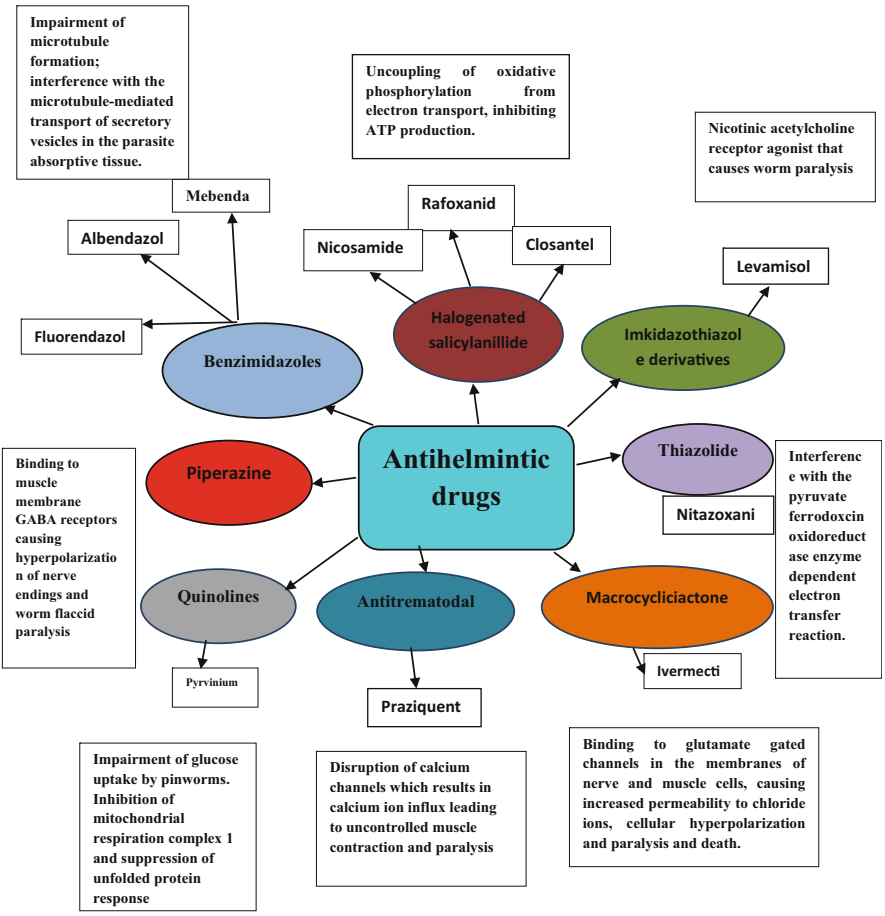

Fig. 25.2 Classification and mechanism of antihelminthic drug

al. 1995). The resistance codon frequency at position 200 differs greatly but the resistance population shows low frequency which point toward SNPs being the primary cause for developing resistance to drugs, and the only way for acquiring benzimidazole resistance in parasite populations.

Macrocyclic lactones bind to rare invertebrate-specific glutamate-gated chloride channels (GluCl) and are responsible for immobility. Resistance development toward drug involves GluCl channel mutation in extracellular domain of *Cooperia oncophora* and *H. contortus* (Wolstenholme and Rogers 2005; Blackhall et al. 1998, 1998). In *C. elegans,* ivermectin and the macrocyclic lactone works again by GluCl channel activation. However, single alteration in the GluCl subunit is not sufficient to develop resistance in *C. elegans*; therefore, multiple mutations are required to confer resistance, which implies that resistance development not only requires single SNP mutations (Ghisi et al. 2007; Dent et al. 2000). Another drug, levamisole,

Table 25.2 Tabular representation of ABC transport proteins in helminthes

Helminthes	ABC subfamily	Gene or EST	Homolog	Resistance	Reference
Fasciola hepatica	P-glycoprotein	Fhmdr1	SMDR2	Not determined	Reed et al. (1998)
	Multidrug-resistance-associated protein	FhMRP	MRP1	Not determined	
Schistosoma mansoni	P-glycoprotein	SMDR1	Cehaf-6	Not determined	
		SMDR2	RnMDR1A	No	
	Multidrug-resistance-associated protein	SMRP			Sato et al. (2004)
Onchocerca volvulus	P-glycoprotein	ABC-1		Not determined	Bourguinat et al. (2008)
		ABC-3		Not determined	
		MRD-1		Not determined	
		PGP-1		Ivermectin	
	Multidrug-resistance-associated protein	?			
Haemonchus contortus	P-glycoprotein	PGPA		Ivermectin	
	Multidrug-resistance-associated protein	HCC06778	CeMRP1		
		HCC04267 HCC06887	CeMRP5 CeMRP5		

nicotinic acetylcholine receptor-targeted drug, has been found to be in association with pig roundworm, *Ascaris suum* drug resistance cases (Martin et al. 2004).

Several SNPs causes resistance in helminths; whereas in other cases it may not enable development resistance which successfully observed in benzimidazole resistance for the Phe200Tyr SNP in *H. contortus* but not in the hookworms (Albonico et al. 2004). A variety of SNPs have been identified and characterized in the case of helminths (Ghisi et al. 2007). The issues regarding helminths resolve, which shows SNPs identification due to different loci has multiple genetic changes that lead to a resistant phenotype, and resistance gradually occurs even when no SNPs are identified (Table 25.2).

25.5.2 Alteration in Transport of Drugs

In addition to genetic selection, several other mechanisms also favor drug resistance. In the case of cancer cells, inherently resistant development occurs in some tissues and posttreatment resistant development occurs in other tissues. This resistance is offered by P-glycoprotein, a membrane protein expression (Zhou 2008). In the case of humans, drugs export involving P-glycoprotein causes decreased intracellular concentration, causing suboptimal dosing. P-glycoprotein head-on interaction with drugs has been demonstrated, but the actual transport mechanism is still debated. Several drugs such as benzimidazoles, ivermectin, and imidazothiazole derivatives are transported via human P-glycoprotein in cels which includes transport protein expression has been found (Naito et al. 1998; Prichard et al. 2012), which clearly indicates that emanation pathway of drug also contributes in drug resistance adverse effects on the transport proteins of helminthic worms. P-glycoprotein comes under ABC transporter family; having ABCB1 family and most ABC transport proteins it provides multidrug resistance for a variety of drugs, including the resistance protein involved in breast cancer (BCRP/ABCG2) and multidrug resistance-associated protein (MRP-1/ABCC1) (Lage 2003).

In helminths, drug resistance involving ABC transport proteins functions is poorly studied, although in a number of cases, resistance development is associated with ABC transport proteins (Table 25.1). In trematode, *Schistosoma mansoni*, praziquantel drug resistance was associated with increased ABC transport protein, which involves MRPs and P-glycoprotein homologs (Sato et al. 2004). Treatment of *Fasciola gigantic* and *Fasciola hepatica* liver flukes using anti-trematodal agent triclabendazole suggests resistance development via increased MRP-1 expression (Reed et al. 1998). In *H. contortus*, resistance against ivermectin has emerged within creased P-glycoprotein mRNA expression (Smith and Prichard (2002). All these studies clearly indicate the importance of ABC transport proteins in drug resistance.

In humans, some specific SNPs have been discovered which causes P-glycoprotein alteration of transport characteristics (Sauna et al. 2007). Interestingly, *Onchocerca volvulus* was found with changes in the allelic frequency of P-glycoprotein with reduced allelic variation. Post treatment with ivermectin, some specific alleles present in the worm populations (Sauna et al. 2007). Likewise, in *H. contortus* condition, the specific alleles of P-glycoprotein were targeted for benzimidazole-resistant parasites (Blackhall et al. 2008). Different drugs select specific phenotypes (alleles) that might be associated with the population survival as they more efficient system for efflux of drugs which further hindered medicines to meet their target.

25.5.3 Drug Consumption

Also, in the case of eukaryotes, harmful xenobiotics are necessarily converted into less toxic compounds by various cellular detoxification pathways (Fig. 25.3). The very initial steps involved conversion of toxic compounds into oxido−/reduction or

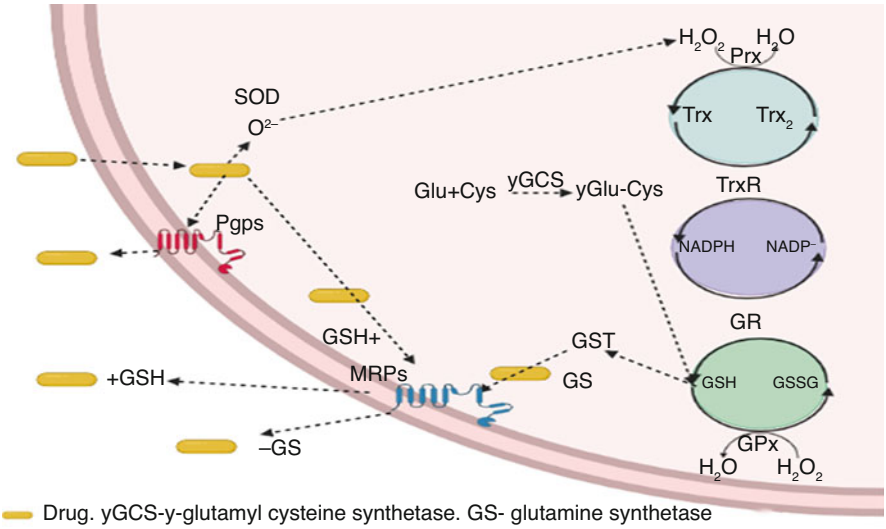

Fig. 25.3 Demonstration of different pathway of a drug when it enters into a cell. When drug enters into the cell, it has options to be eliminated by ABC transport protein P-glycoprotein (Pgps; i) or bind to glutathione (GSH; ii) through (GST; iii); then it is distributed by (MRPs; iv). Communication of cellular molecule with drug causes free radical formations, like superoxide anions (O_2^-). The enzyme superoxide dismutase (SOD; v) absorbs free radicals by conversion to H_2O_2.. It is then metabolized into unharmful product by peroxiredoxin enzyme (Prx; vi) or glutathione peroxidase (GPx; vii). During this pathway, the enzymes work as reducing agents and again reformed by the thioredoxin system or the glutathione system, respectively. Thioredoxin (Trx; viii) and GSH (ii) are the respective electron donors for the peroxidases and are reduced by their reductases thioredoxin reductase (TrxR; ix) and glutathione reductase (GR; x), with NADPH as the electron donor

hydrolyzed form using P_{450}, acytochrome enzymes that function in the form of addition or removal of reactive functional groups. With the help of glutathione S-transferase (GSTs) enzymes, functional groups are attached with glutathione, glucuronic acid, or glucose. Now the toxins are removed from the cell with the help of an ABC transport protein, a type of glutathione conjugate pump. The conjugation process involves foreign compounds that transform toxic to less toxic against cellular targets using hydrophilic properties of new conjugate. This process helps in easy removal by specific membrane transporters from the cell. In helminthes, drugs are metabolized by these cellular enzymes (Cvilink et al. 2009). The ubiquitous thiol-glutamyl–cysteine–glycine, a tripeptide glutathione, is needed for maintaining homeostatic oxidative processes. It has a key functioning in the field of balancing the thiol redox condition of the cell and also protects from any oxidative damage and removes toxins of exogenous and endogenous reactive metals and electrophiles. In cancer cells, resistance against various drugs is associated with overexpressing glutathione-related and/or increased glutathione enzymes (Tew 1994). The increased drug metabolism is also associated with parasites drug resistance. However, many anthelmintic drugs induce cytotoxic activity via increased

production of free radicals. For example, artemisinin and praziquantel in drugs used to treat *Schistosoma* spp. produce more free radicals (James et al. 2009; El-Bassiouni et al. 2007).

The free radical detoxification occurs by enhanced production of glutathione enzymes as well as cellular glutathione. This situation is more toxic for immature schistosomes in comparison with adults having intense peak of antioxidant enzymes. In *H. contortus*, resistance to cambendazole, benzimidazole compound, was found to have elevated GST activity in comparison with sensitive isolates (Kawalek et al. 1984). Kerboeuf and Aycardi (1999), demonstrated that for increasing the sensitivity toward thiabendazole, glutathione-synthesizing inhibitors co-administered with an inhibitor provides a support for the role of glutathione in resistance to benzimidazoles. The thioredoxin system, which runs parallel with it, plays an important role in oxido-reduction regulation and is also applied in resistance development toward cancer drugs (Powis and Kirkpatrick 2007). This is a form of constitutively expressed protein with di-thiol-reducing activity as well as also plays roles in free radicals scavenging and provides oxidative damage related protections. In the helminthic adult worms, enzyme cytochrome P_{450} has little expression (Barrett 1998); therefore, for quenching of free radical, glutathione and thioredoxin systems are needed. Drug resistance development systems linked with these antioxidant systems because of the systems concerned for the ABC transport proteins regulatory expression (James and Davey 2009). Still, much more experimental input is required to conclude the part of drug resistance using drug metabolism in worms.

25.6 Plant Botanicals: Alternative Approach to Synthetic Antihelminthics

The drugs referred to as Anthelminthics categories are used for the treatment of parasitic cestodes, nematodes, and trematodes in humans and animals consuming thousands of humans lives and even billions of dollars in every year (King et al. 2015). Helminths cause diseases of chronic nature and debilitating diseases that cause lots of deaths and greater social and economic deprivation among humans and animals in comparison to any other parasites. The helminthiasis data shows that about half of the world's population are suffering from this disease and the count increases day by day. The cosmopolitan nature indicates their prevalance in subtropical and tropical countries as well as endemic in various regions because of malnutrition, poor family hygiene, poor sanitation and crowded living condition. The condition becomes worse in developing countries through misuse and/or overuse of anthelmintics, shared water and land use between farm animals and humans (King et al. 2015). Anthelmintics chemical classes are limited in nature on which prophylaxis and treatment relies. The regular use of anthelmintics required an urgent focus about the anthelmintic resistance development in companion and production animals. Even in the case of humans, the reduced anthelmintic efficacy is also a point of major concern for the resistance development as risk for mass drug

administration efforts (Hotez et al. 2008). Synthetic anthelmintics involvement in biodiversity causes more ecological and economic effects involving reduction in invertebrate diversity found in soils (Spratt 1997). In view of the increasing problem with nematode resistance, it will be difficult to maintain an approach to control based primarily on the use of chemical antiparasitic drugs (Salgado and Santos 2016) as they cause severe side effects. Based on the side effects and resistance produced by the excessive use of drugs urged scientists to adapt alternative nematode control methods to reduce the risk of environmental pollution. The consumer demands "green and clean" by-products that are free from environmental residuals, growth promoters and cost-effective.

So, the challenge for the new era is —from where we can obtain the new drug for anthelmintics? The biodiversity offers the new resource for discovery of anthelmintics including the discovery of ivermectin which was isolated from a strain of bacteria—*Streptomyces avermitilis* in the 1970s. The evolving development in the field of macrocyclic lactone class reorganizes health of human and animal leading to save millions of life forms, which falls in the class of anthelmintics. This breakthrough was received Nobel prize in Physiology or Medicine 2015 being shared with William C. Campbell and Satoshi Omura "for their discoveries concerning a novel therapy against infections caused by roundworm parasites". Following the emodepside precursor, camellia plant leaves having fungal colonies produce PF1022 A (Vinogradov et al. 2019). Excluding microorganisms classes, various animals and plants species manufacture defensive molecules against parasites and predators, even for helminths (Trowbridge 2014), concluding the discovery of large diversified library of probable biopharmaceuticals against helminth. The salient feature of successful natural drug products is mainly one compound obtained from any living source, instead of whole plants, as in the case of bacteria or fungi. These natural products are having highly variable characteristic, making unsuitable for making combinations in strict regulation for the production of drugs that has to follow quality assurance requirements (CMC). The last few years have witnessed a steep rise in the use of natural product that contain various components with biological activity, that provide the tools to identify, select and process natural products destined for medicinal use (Atanasov et al. 2015). Medicinal plants have been used to combat parasitism and other veterinary and human ailments for years and in many parts of the countries for primary health care due to better compatibility with the human body, better cultural acceptability, and few or no side effects. The WHO reported that in developing countries, around 80% of the populations rely on traditional medicine, mostly drugs obtained from plants, for their initial stages of health care needs (WHO 2008). Current situation again fascinates us toward the traditional health practices encompassing whole world using herbal remedies and ethnobotany. The theory behind this is the thought of that "natural is nice," whereas synthetic drugs leave residues behind in the environment and even in food chains, which becomes the concern because of generation of various resistant pest organisms through improper use and overuse of conventional drugs. Two main approaches have traditionally been used in efficacy studies against helminths. The efficacy of plant products is mediated via feeding plants or their parts to animals with

infections (Iqbal et al. 2004). The second one is by testing plants active components and concoctions from plants having medicinal properties through in vivo and in vitro applications (Githiori et al. 2004).

25.6.1 Secondary Metabolites as Active Constituents

The active components mostly of secondary metabolites of the plant exhibit therapeutic properties. The components are formed using pathways as glucosinolate, acetate-mevalonate, phenyl-propanoid pathway, and shikimate pathways. During the past years, secondary metabolites were treated as plant metabolism waste products, but according to the current scenario, normal plant functions include variety of secondary metabolites. Plant-secreted secondary metabolites can possess various functional groups like SH and aldehyde groups, triple bonds and epoxide double bonds with enon configuration. Secondary metabolites showing specific groups bind with either DNA or proteins and change in their normal functioning (Wink 2012). The action of secondary metabolites was reported on specific proteins, such as a neuroreceptor, cytoskeleton elements, ion channels, and an enzyme, hence varying the mechanism of action of these proteins. The secondary metabolites in nature are biochemically defined as polysaccharides, terpenoids, alkaloid, and peptides. Tarpenes monomer units are C-5 isoprene units, which are further classified based on the number of molecules of terpenes as monoterpenes, diterpenes, sesquiterpenes, etc. Lipophilic nature of terpenes makes it reliable to ease in interaction with bio-membranes and related proteins. Mechanism of action of terpenes includes cellular activity via efflux regulation of ions metabolites and cell leakage. Sesquiterpene lactones are more secreted in plant families such as Apiaceae and Magnoliaceae. Naturally present N-SM includes alkaloids which are found to be widely distributed in plant kingdom. In this class of metabolites, nitrogen is either in the rings or in chains. The functions vary in nature, involving physiological functions in living beings. Neuro-receptors are the primary molecular targets for alkaloids. Some mutagenic alkaloids are also reported to function via DNA alkylation. Few of them are also hindered with enzymes like topoisomerases and telomerases, and biosynthesis of protein eventually accumulated in apoptosis. Nitrogen-containing secondary metabolites are inclusively cyanogenic glucosides, which are mostly reported in leaves, roots, and seeds of plant communities involving Juncaginaceae, Euphorbiaceae, Fabaceae, Caprifoliaceae, and Linaceae. These compounds after contact with β-glucosides break into its sugar and nitrile moiety which finally generates the HCN compound that further forms hydrolases. The components of hydrolysis are responsible for mitochondrial respiratory inhibitions. Effect of plant secondary metabolites on various metabolic activities of helminthes is summarized in Table 25.3.

Table 25.3 Effect of plant secondary metabolites on the various metabolic activities of helminthes

Plant secondary metabolite	Activity
Tannins	Energy metabolism inhibition
Adenine, chymopapain, caricain, lutein, malic acid, ascorbic acid, genistein, glycyl endopeptidase, papain	Causes epidermal lesions
Saponins, tannins	Decrease motor activity
Terpenoids	
Caffeic acid	
Tannins	Eggs to larvae transformation inhibition

25.6.2 Plant Extracts as Antihelminthics

The experiment performed with hydro alcoholic extracts of the *Embelia schimperi* fruits showed significantly higher anthelminthic activities against the *Hymenolepis nana* (dwarf tapeworm), in vivo at the dosage of 1000 mg/kg body weight. Further, the dry fruit extracts of *Embelia schimperi* also exhibited antihelminthic activities on *Hymenolepis diminuta* in rats at the dosage of 100 mg/kg (Debebe et al. 2015). Samburu Country also shows a similar study in the pastoral field conditions as FECR of 77% and 90% for *Myrsine africana* and *Albizia anthelmintica*, respectively. This study also reported higher activity against *Moniezia* tapeworms (Gathuma et al. 2004). Moreover, similar study with naturally occurring mixed gastrointestinal parasites in northern Uganda had a FECR of 78%. Further, extracts from the fruits of *Myrsine africana* were reported to have higher efficiency against *Taenia solium and Oesophagostomum columbianum* (Kakrani and Kalyani 1983).

Various families of Euphorbiaceae, Fabaceae, and Asteraceae have been found with antifilarial activity. *Setaria digitata* studies suggested macrofilaricidal activity against *Sida acuta, Oldenlandia herbacea,* and *Cassia occidentalis* (Behera and Bhatnagar 2018). *Butea monosperma* shows antifilarial activity against *Setaria cervi* in combination with antibiotic ciprofloxacin. *Butea monosperma* ethanolic extract has been found to show significant antifilarial effects (Radhika and Sathya 2014). Both anti-leishmanial and antifilarial activities have been reported in *Piper betel* (Salehi et al. 2019).

The n-hexane and methanolic extract fraction of *P. betel* exhibited significant increase in cell-mediated and humoral immunity (Salehi et al. 2019). The extract of methanolic solvent was also showing immune-modulatory properties via type-2 and type-1 cytokine responses. The secondary metabolites like saponin, alkaloid, and flavonoids present in the roots also show antifilarial activity. *Vitex negundo* extract of root parts and *Aegle marmelos* leaf extract at concentration of 100 ng/mL studies of 48 h incubation time shows complete motility loss of *B. malayi* microfilariae (Sahare et al. 2008). Both in vivo and in vitro studies performed in *Streblus asper* show antifilarial activity; it is also efficient in curing filarial lymphedema.

Filacid, *S. asper* decoction, was found very efficient in comparison to other plants such as *Argyreia nervosa, Butea monosperma*, and *Crataeva nurvala* (Romero-Benavides et al. 2017). Aqueous and methanolic leaf extracts of *Senecio nudicaulis* and *Mallotus philippensis* inhibit *Setaria cervi* nerve-muscle preparation movement, and aqueous extracts of both the plants also show blockage of the stimulatory response of AcH (acetylcholine) on worm movement (Singh et al. 1997). Taeniasis, an intestinal infection, is caused by adult tapeworms such as *T. saginata*, *Taenia solium,* and *T. asiatica*. Cysticercosis/taeniasis mainly arises in the countries of Africa, Latin America, and Asia populations (Garcia et al. 2003). The study conducted on *Areca* nut extract and pumpkin seed treatment showed a better prevention for taeniasis-suffering people. Studies performed in China, using the areca nut and pumpkin seed individually, showed a significant outcome in a group of patients with taeniasis. The combination therapy involving above two has found in an average time of 2 h with complete elimination of tapeworm (Li et al. 2012). Later on, experiments conducted using *Hedychium coronarium* root oil *(*Zingiberaceae*)* and *H. spicatum (*Zingiberaceae*)* have shown more promising results than synthetic medicines like piperazine phosphate, antihelminthic drug opposes tapeworms (Arroyo-Lopez et al. 2014).

Another case involving a 43-year-old woman living in Tibetan region was reportedly healed by use of pumpkin seeds for the treatment (Ito et al. 2013). Another experiment designed to test the traditional drug *Glinus lotoides* used in the treatment of taeniasis in Ethopia (Demma et al. 2007) using rats as model organisms, and the results were found with zero toxicity against the plant extract which help in further clinical trials. Roundworm, *Ascaris lumbricoides*, a helminthic parasite, causes small intestine infection. This parasite is widely distributed in subtropical and tropical countries and it is very popular in East Asia, sub-Saharan Africa, China, and Latin America (Haque et al. 2019; Darlington and Anitha 2018), annually causing more than 60,000 deaths. *Zanthoxylum zanthoxyloides, Clausena anisata,* and *Punica granatum* plant extracts were found to show anti-ascariasis activities in significant manner. In vitro study was done for observing anti-ascariasis activity using *Ascaris suum,* which is a swine parasite having similarities with roundworm (Adebayo and Amoo 2019). In vitro experiments were designed to study the anthelmintic action of alcoholic extract of *Lippiano diflora* against human *Ascaris lumbricoides*, which exhibited strong activity against *A. lumbricoides* (Senthilkumar and Ramakrishnan 2018). *Zanthoxylum zanthoxyloides*, *Clausena anisata,* and *Punica granatum* extracts were obtained, which exhibited potent antihelminthic activity at 74, 97, and 164 μg/mL of concentrations for *A. suum*. A Santonin (15 carbon terpene lactone) was successful in exclusion of *A. lumbricoides,* which isolated from *Artemisia santonica*. Although, the compound is also found to be toxic in nature, which has been overcome using synthetics benzimidazole drug derivatives. *Chenopodium ambrosioides* provides secondary metabolite, anti-ascariasis properties were found in Ascoridole (Romero-Benavides et al. 2017). Various categories of Ayurvedic plants have been explored to reduce and eliminate intestinal worms like *Artemisia vulgaris,*

Ricinus communis, *Dryopteris filixmas, Matricaria chamomilla, Syzygium aromaticum,* and *Juglans nigra* (Bahmani et al. 2014).

25.7 Challenge of Resistance and Ongoing Management

Resistance of helminthes against various drugs causes severe problems all over the world in different geographic localizations. There are several soil-transmitted helminthes as well as increasing number of detections of the food-borne helminthes infections that are more concerns about the resistance induction to different antihelminthic drugs (Fairweather et al. 2020). The helminthic parasites are specific to hosts; therefore, some helminthes causing infection to both animals and humans are exposed to different drugs in each group (Nixon et al. 2020). There are also reports of gene transfer of horizontal axis that persist in the middle of hosts and parasites (Wijayawardena et al. 2013). The parasites are not only resistant to antihelminthics; however, there is a serious issue of reduced effectiveness and multidrug resistance in companion animal nematodes (Jimenez Castro et al. 2019). Recent studies have suggested the reduced efficacy of drugs such as praziquantel against human blood fluke worm infections (Deol et al. 2019); *O. volvulus* infections using ivermectin drug (Webster et al. 2014) and benzimidazole against roundworms (Furtado et al. 2019) have been reported. The discovery of novel classes of drugs as well as new target site for the drug would be a key opportunity to prevent development of resistance. The discovery of novel classes as well as new target site for the drug would be a key opportunity to prevent from development of resistance. Moreover, plant-derived chemicals (secondary metabolites) are also found effective against helminthes parasites with more complex mechanism of action and also prevent parasites to develop resistance. These are easily available, cost-effective, environment-friendly, and with least or no side effects to humans. Though the administration of any novel anthelmintic, plant products can also be used in combination to increase effectiveness; however, we also have to evaluate the resistance development for promising results with future sustainability of new drug. More study is further needed to analyze and compare the effectiveness of plant-derived products and also about the mechanism of action before implementing to control helminthes worm.

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Schistosomiasis: Current and Future Strategies to Develop Novel Therapeutics

26

Renu Kathpalia and Tanushri Saxena

Abstract

A chronic human parasitic disease schistosomiasis, commonly known as bilharzia, is caused by trematode flukes belonging to the genus *Schistosoma*. More than 229 million people all over the world are suffering from parasitic invasion of *Schistosoma* spp. The worm evades human immune system by excreting thousands of eggs and colonizes in human blood vessels for years. The systemic pathological disorders are tremendous, ranging from anemia, stunted growth, impaired cognition, hepatosplenism, periportal fibrosis, portal hypertension, urogenital inflammation, and ultimately leading to cancer. The disease was recognized more than 100 years ago but insufficient action led to prevalence of this among people of all ages, especially children under 5 years of age. To suppress morbidity, treatment with isoquinoline drug and praziquantel is the only option available. The apathy in treatment of this disease is mainly due to failure in recognizing morbidities as it is confounded by another co-endemic disease, mainly malaria. There is urgent need to develop sensitive diagnostics tools to understand the cumulative impact of this infection not only in terms of organ infection but at whole body performance of growing child and young adults. Currently, the goal has been focused on the elimination of transmission rather than development and utilization of novel therapeutics in the treatment of this deadly disease. Nonetheless, an integrated environmental management approach is a must to ensure elimination of this neglected disease of poor community all over the world. The aim of this review is to highlight the recent

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A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_26

advances made in treating disease at community level by enforcing strategies to bring changes in morbidity and physical fitness by mass drug administration (MDA). To add further, the advancement made during the past decade using molecular diagnostics to regulate the cycle of pathogen, development of vaccine, boosting immune response and combinational chemotherapy to eradicate this disease.

Keywords

Schistosomiasis · Mass drug administration · Vaccine · Infection · Chemotherapy

Abbreviations

DMT	Divalent metal transporter
FABP	Fatty acid-binding protein
GAGs	Glycosaminoglycans
GST	Glutathione S-transferase
IgE	Immunoglobulin E
MDA	Mass drug administration
SEA	Soluble egg antigens (SEA)
SMase	Sphingomyelinases
TH2	T helper-2 cells
VCAM-1	Vascular cell adhesion molecule
WHO	World Health Organisation

26.1 Introduction

The German physician Theodor Bilharz in 1851 at Egypt discovered a parasitic disease following an autopsy; later on, this disease was commonly known as bilharzia (Di Bella et al. 2018). Schistosomiasis is an acute disease caused by parasitic worms belonging to *Schistosoma* genus; platyhelminth worms live in the bloodstream of animals and human. Three species of *Schistosoma,* mainly *S. mansoni, S. haematobium,* and *S. japonicum,* are responsible for spreading this chronic disease in more than 78 countries and endemic in 51 countries with more than 236.6 million people requiring treatment (WHO 2022). Schistosoma eggs present in contaminated water multiply and develop in certain types of snails (*Biomphalaria* spp.*)* where miracidia (free-swimming ciliated larvae) differentiate into sporocysts (Table 26.1). The sporocysts multiply by asexual reproduction to produce daughter sporocysts, which mature into numerous cercariae around in 30 days in response to sunlight (Fig. 26.1). Even if the infection is not severe, it accelerates infection of other diseases such as hepatitis B, C viruses and human

Table 26.1 The main features of three different species of *Schistosoma* with their intermediate snail host

Schistosoma species	Niche of snail	Host of snail	Snail endurance to dehydration	Organ affected by chronic inflammation
S. japonica	Aquatic	*Bulinus* spp.	Buried in soil and survives for 7 months	Hepatic, intestinal, and ectopic disease
S. haematobium	Amphibious (water and muddy banks)	*Oncomelania* spp.	Decent in mud	Genital, urinary, and ectopic disease
S. mansoni	Aquatic	*Biomphalaria* spp.	Less resistant	Hepatic, intestinal, and ectopic diseases

immunodeficiency and increases susceptibility to pathogens like mycobacteria and protozoa.

Schistosomiasis is considered a neglected tropical disease by WHO (WHO 2009). In the last decade, the prevalence and intensity of schistosomiasis were predicated by the application of remote sensing technologies for determining risk profiling and estimating the need of the treatment. Not much progress has been achieved in controlling and treatment of this disease, and ultimately the most effectual and extensively used drug is praziquantel, an acylated quinoline-pyrazine derivative used for last more than 30 years (Pedrique et al. 2013).

26.2 Mechanisms of Infection

The infection process of schistosomiasis is usually divided in three stages that are overlapping and is greatly influenced by the infection duration. The three stages are.

26.2.1 Acute Stage

This stage is referred as cercarial dermatitis which involves entry of pathogen through skin where a section of larvae die on skin surface, while other penetrate the venous circulation of the host through a miniature blood vascular system directly into lymphatic vessel. The flowing blood takes the larvae to their maturation site in the liver. The immunity response of the host to dead larvae begins with hypersensitivity reaction known as cercarial dermatitis in individuals exposed for the first time. After penetration and maturation of schistosomula, the infectivity may have adverse effect in acute symptomatic stage. This is also known as Katayama syndrome or Katayama fever and occurs in person infected for the first time (Ross et al. 2007). These cercariae are free-swimming larvae stage which moves to its final host within 48 h through the hair follicle in the skin. During penetration through the skin, the tail

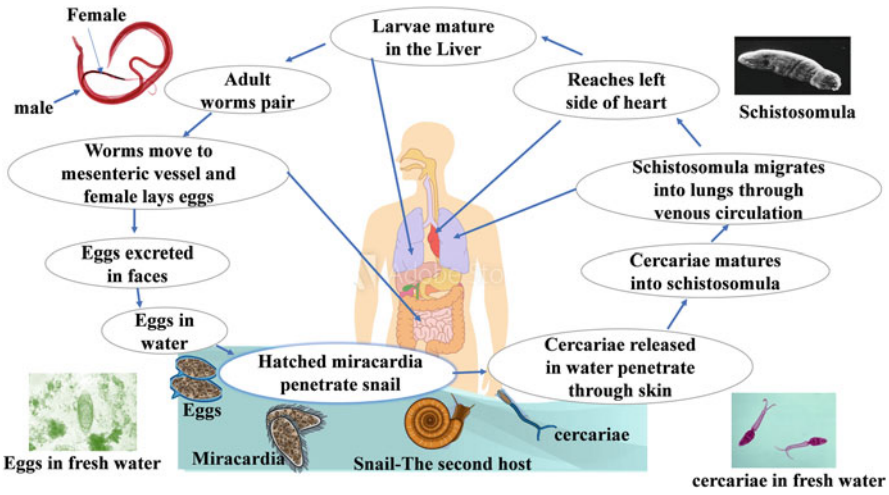

Fig. 26.1 Diagrammatic view of *Schistosoma* spp. life cycle involving two hosts, a snail and human. The feces of human host releases eggs that hatch on coming in contact with water, liberating free-swimming ciliated miracidia larvae. These miracardia penetrates the other host *Biomphalaria* spp. (snails). In snails, miracardia develop into sporocytes, which by asexual reproduction produces free-swimming larval stage known as cercariae. These cercariae release from snail when exposed to light in nearly 30 days and infect the skin of human hosts. The cercariae lose their tail, transforms into schistosomula, penetrates the blood vessels straight invading lymphatic system, and reaches the lungs via right side of the heart and then transported through arterial circulation. Through hepatic portal system, the schistosomula moves toward mesenteric veins of the liver and there it matures into adults. The male worm grasps the female in the gynaecophoral canal and migrates to the mesenteric veins of the bowel. After 5 weeks of infection, the female produces eggs, which are then released into the bloodstream, moving through the intestinal wall released via feces

of cercariae drop off and the larvae transform into schistosomula or schistosoma. Developing schistosomes have covering of tegument, a 2–4-μm-thick syncytium. The basal membrane forms the innermost tegument while the outer membrane is trilaminate in cercariae, which is replaced within 3 h by heptalaminate membrane to protect the pathogen from immune system of the host. The schistosoma directly enters into the venous blood vessels, or through attacking the lymphatic system, where it matures into adult worms in around 72 h after infection. They are taken to right side of the heart and then the lungs, where it stays for 3–16 days and turn into long slender organisms capable of making its way to liver where it actively divides (Mowafy and Abdel-Hafeez 2015; McManus et al. 2018).

26.2.2 Active Infection

Livelihood of individual in endemic regions where symptomatic occurrence of acute schistosomiasis is not prevalent, the disease straightforward attains active infection. After reaching hepatic portal system, the schistosoma travel to mesenteric veins in

liver, where it matures into sexually differentiated adult after 28–35 days of infection (Costain et al. 2018). The male worm being bigger in size grasps along with female to gynaecophoral canal, where it enters in mesenteric veins, hereby providing site to female for laying eggs (almost 5 weeks after infection) (Galanti et al. 2012). The egg-derived antigenic glycoproteins help the passage of eggs from blood vessels to intestinal lumen and urinary bladder. The antigen of the egg forms granulomas consisting of eosinophils, neutrophils, lymphocytes, and macrophages around eggs (Fig. 26.2). The eggs retained in the wall of blood vessel severity lead to bleeding in the intestine and urinary system, enlargement of liver and spleen, and fibrosis of periportal, ultimately causing bladder cancer. This stage involves excretion of living eggs in stool and urine.

26.2.3 Chronic Infection

With repeated infection, the worms are gradually reduced with time due to natural death. New granulomas of smaller size with less inflammation results in reduction in severity of symptoms.

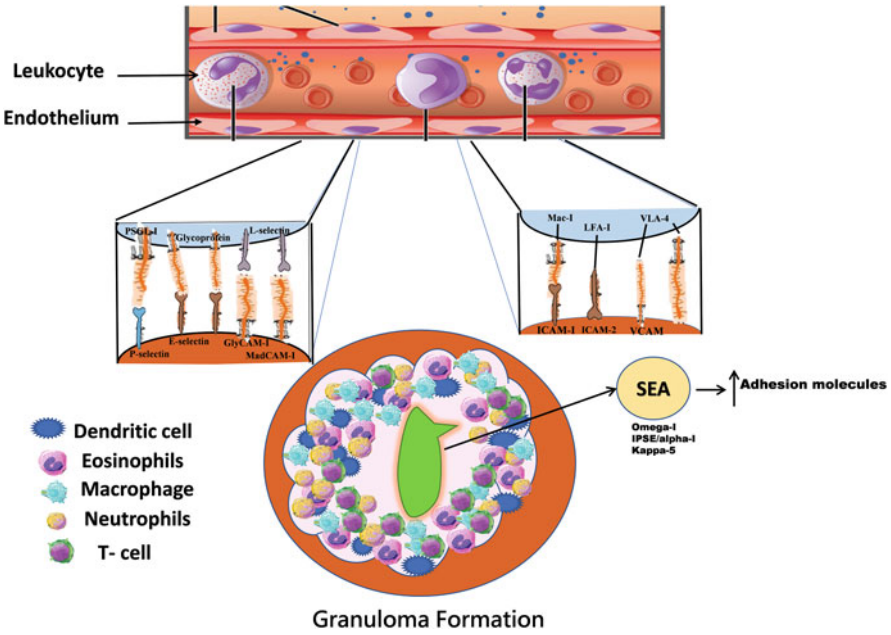

Fig. 26.2 The process of granulomas formation takes place by the accumulation of collagen-bound inflammatory cells encapsulating mature eggs during the infection to protect living eggs from the immune response of host. Schistosome eggs secretes glycoproteins and glycolipids, collectively known as soluble egg antigens (SEA), which induce production of various inflammatory or innate cells such as mast cells, dendrite cells, macrophages, lymphocytes, neutrophils, and eosinophils. These cells accumulate around egg and form granulomas

26.3 Immunology and Host–Parasite Interactions

The host pathogen interaction is quite complex as it involves different stages of parasite. Over the years, protective immune responses against schistosomes develop but children of age less than 10 years are repeatedly infected. The parasite outer lipid bilayer of the membrane has sphingomyelin molecules, which are tightly bound with water molecules, resulting in the formation of barrier concealing its membrane proteins from the antibody of the host. The hydrolysis of sphingomyelin is catalyzed by sphingomyelinases (SMase), a group of enzymes functional at different pH, resulting in the formation of ceramide and phosphorylcholine (Airola and Hannun 2013). Tegument-associated Mg^{2+}-dependent neutral sphingomyelinase (nSMase) hydrolyzes sphingomyelin so that nutrient from host can enter but not the antibodies (Clarke and Hannun 2006). The immune response is linked to the resistance to reinfection which occurs through T helper2 (TH2) cell on release of IgE (immunoglobulin E) and cytokines like IL-4 and IL-5, however the production IgG4 causes susceptibility to reinfection by blocking action of IgE (Fig. 26.3). It has been hypothesized that death of adult worms leads to release of antigens that react with larval antigens and produce IgE antibodies, and more the death of worms, stronger is immunity. The average life of a worm is 3–10 years; therefore, children have few dying adult worms and thus have less resistance (Colley and Secor 2014).

26.4 Organ-Specific Schistosomiasis

Organ-specific morbidity develops due to accumulation of parasite eggs, leading to the development of fibrosis in the infected organs (Colley et al. 2014).

(i) **Intestinal schistosomiasis**: The mature worms present in the mesenteric veins causes intestinal schistosomiasis by inducing inflammation in mucosal granulomatous region along with microulcerations leading to blood loss and pseudo polyposis (Tamer and Gamal 2013).

(ii) **Hepato-splenic schistosomiasis:** The flow of blood from venous blood vessels takes the eggs to miniature portal branches in liver where they get entrapped in pre-sinusoidal periportal tissues. The inflammation takes place due to granulomas around the eggs, which causes enlargement of liver and spleen, known as hepato-splenic schistosomiasis (Warren 1978).

(iii) **Urogenital schistosomiasis***: S. haematobium* adult worms reside in the pelvic venous plexus and lead to urogenital schistosomiasis, where eggs are deposited in bladder and genital organs. This often coincides with sloughing off of the epithelial surface, bleeding, and ulcer, followed by pesuodpolyps formation. It is also linked to squamous cell carcinoma of urinary bladder. It may play an important role in the transmission of HIV (Ishida and Hsieh 2018).

(iv) **Neuroschistosomiasis**: It is caused due to inflammation in cerebral and spinal venous plexus, related with granulomatous lesions in different region of brain

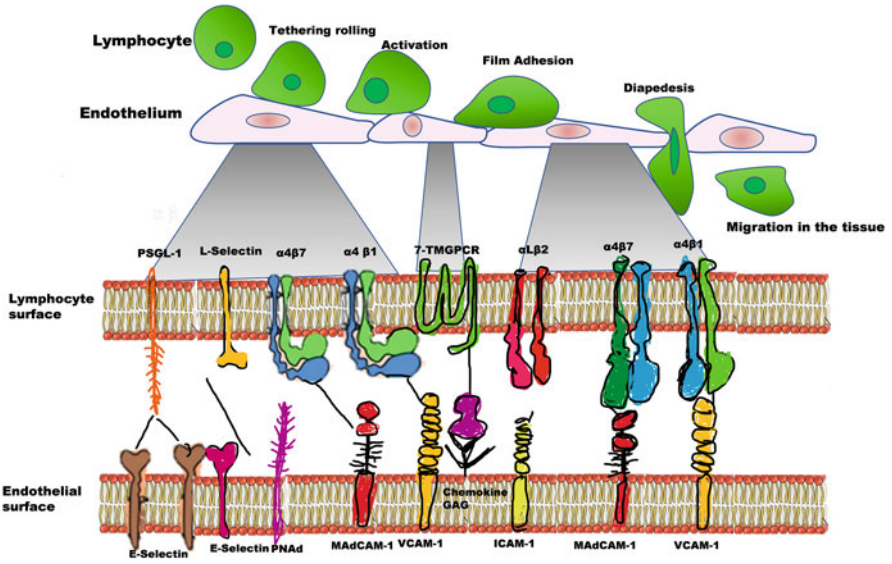

Fig. 26.3 The multistep and multiple homing molecule during the immunity response at the membrane of lymphocyte and endothelial surface of the target tissue. (**a**) Initial process of "tethering" involves PSGL-1, L-selectin, and 4α integrins on the lymphocytes, interacting with E- and P-selectin, PNAd, MAdCAM-1, and vascular cell adhesion molecule (VCAM)-1 on the endothelial cells. (**b**) The lymphocyte is slowed down due to contact and causes the lymphocyte to move along the length of endothelium. (**c**) Lymphocyte here expresses seven trans membrane G protein-coupled receptors; 7-TM GPCR (specific chemokine receptors) that attach with chemokines of the endothelial cells, viz. glycosaminoglycans (GAGs). It results in an intracellular signal activation inducing conformational alterations in the β2 and/or α4 integrins on the surface of lymphocyte. (**d**) These integrins are responsible for sturdy binding of endothelial receptors (ICAM-1, ICAM-2, VCAM-1, and MAdCAM-1), causing strong adhesion and ultimately arresting lymphocyte. (**e**) Here, lymphocyte gets polarized and migrates throughout the vascular wall into the interstitial tissue, commonly known as diapedesis. (**f**) The migrating cell is further guided by different concentrations of chemoattractants

that may cause epileptic seizures, visual injury, and ataxia (Nascimento-Carvalho and Moreno-Carvalho 2005).

(v) **Pulmonary schistosomiasis**: It causes portal-caval shunting, and hence the eggs are delivered into lung capillaries where it induces granuloma formation in perialveolar areas (Papamatheakis et al. 2014).

26.5 Diagnostics of Schistosomia

The standard method of diagnosis is the presence of viable eggs in urine, feces, or tissues biopsy. The procedure recommended by WHO is microscopic examination of remnants of eggs, mainly polycarbonates in urine, urine dipstick assay for heme,

or Kato-katz fecal examination. Molecular techniques are available to detect DNA of the pathogen in fecal,

urine, and serum specimens, but due to irregular distribution of eggs, it is not regularly used (Ajibola et al. 2018). For symptomatic travelers, serological assays to detect antibodies are recommended but not for the people residing in the regions endemic for schistosomiasis as it is difficult to discriminate between the antibodies formed due to present infection or past infection. Pet scans have also been used to detect adult parasites. There is need to develop better diagnostics method which will indeed help not only clinical diagnosis but also for the development of effective drugs and vaccine. An important aspect of monitoring and elimination program is to detect infection in the alternative host, the snail. Snail xenodiagnoses by parasitological techniques such as PCR or loop-mediated isothermal amplification assays enable the identification, control, and elimination programs (Gray et al. 2011). Early diagnosis of schistosomiasis is crucial to control this disease, but there is no reliable biomarker reported for early diagnosis of schistosomiasis. In *S. japonicum* and *S. mansoni*, infected mice showed the presence of miRNA of different species of the pathogen (Hoy et al. 2014) and three of these miRNA can be potentially used biomarker for the diagnosis of schistosomes infection. Circulating miRNAs present in a stable form in serum or plasma of an infected host are being considered as ideal biomarkers for the diagnosis of some cancers. It is possible that such circulating miRNAs could also serve as biomarkers for schistosomiasis diagnosis. Additionally, novel amplification methods such as RAKE assay, rolling-circle, and DNA concatamers-based amplification with internal reflection fluorescence microscopy could be used to enhance sensitivity of detection of miRNA (Zhu et al. 2014).

26.6 Old Age Therapies

In the mid-1970s, praziquantel was discovered as an anti-schistosoma drug, and the efficacy was determined on the basis of cure rate and egg reduction rate (Ojurongbe et al. 2014). It has been reported to be the safest and efficient against all *Schistosoma* spp. adult worms. Usage of praziquantel has been suggested by WHO for the treatment of pregnant women after third trimester and against *S. mansoni*. The drug is metabolized in liver to inactive metabolites and is excreted out via kidney within an hour of ingestion. In the mass drug administration (MDA) control program, the WHO suggests using one dose. Praziquantel therapy is thought to be safe, and the symptoms that manifest are brought on by the release of antigens from dead or dying worms. The medication causes the parasite to contract tetanically, which is followed by the development of tegumental vacuoles. This surface damage causes the adult worm to detach from the venous wall and eventually perish. The exact mechanism of action is still not clear but it is predicted that the drug increases calcium uptake, which induces the anthelmintic effect of praziquantel, although this needs further investigations. Praziquantel has no adverse effects on young schistosomes; therefore, it cannot stop the parasite's life cycle or prevent reinfection. The worm has developed resistance to praziquantel and its incomplete efficacy calls

for the development of a new drug for treating patients infected with schistosomes. The large size tablet with bitter taste due to racemic mixture of (S) and (R) stereoisomers, bitterness is mainly (S) praziquantel, is responsible for not being used for treating schistosomiasis in preschool children. Praziquantel effectiveness against adult schistosomes is well documented, but still it cannot be used as a chemoprophylactic drug due to its short half-life of just 1–1.5 h and 3–21 days old migrating schistosomula are refractory to the drug (Vale et al. 2017).

In chronic infection, the only treatment is chemotherapy of schistosome with antimonial compounds, commonly used for treating leishmaniasis (Ponte-Sucre et al. 2017). These compounds constrain the enzyme schistosome phosphofructokinase (converts fructose-6- phosphate to fructose-1,6-phosphate) at a very low concentration but it produces extreme toxicity and strong side effects, which makes it an outdated therapy (El Ridi and Tallima 2013). Another old practice was to use modified organophosphorus insecticide 2,2,2-trichloro-1- hydroxyethyl dimethyl phosphonate into metrifonate (O, O-dimethyl-2,2,2-trichloro-1-hydroxyethylphosphonate). Even metrifonate was administrated orally to paralyze the worm, resulting in lose attachment of it to blood vessels and sweeping off the pathogen away within the bloodstream. The drug lowers the erythrocyte cholinesterase and host plasma that results in sweating, muscular weakness, diarrhea, vomiting, etc., leaving no other option other than oxamniquine and praziquantel for treating schistosomiasis. The worm has developed resistance to oxamniquine by mutating its gene encoding an esterifying enzyme, so at the end the only medicine left is praziquantel (PQ). It is a pyrazino-isoquinoine derivative with no water solubility but fair solublility in ethanol and readily miscible in dimethylsulfoxide and chloroform and it is used for five species of Schistosoma affecting humans with cure rates of 60–90% but still the exact mode of infection is not clear as yet.

26.7 Novel Therapies

26.7.1 Combinational Chemotherapy with Novel Drugs

Several in vitro testing has been done on a number of endoperoxide and praziquantel conjugates as a potential drug against both adult and schistosomula of the parasite. Omeprazole, a proton pump inhibitor, and Praziquantel show synergistic effect; however, Praziquantel combination with Synriam, an antimalarial, did not enhance the efficiency. Praziquantel derivatives combined with schistosomicides such as benzodiazepine, mefloquine (an antimalarial drug), and artemether (a methoxy derivative of artemisinin) are extensively studied both in vivo and in vitro. Artemether interacts with hemin and acts as schistosomicides; however, mefloquine inhibits hemozoin formation. Trioxolanes, another antimalarial drug, was found to be very effective against *S. mansoni* and *S. japonicum* (Xiao et al. 2007). Adenine (S)- HPMPA ((S)-9-[3-hydroxy-2-(phosphonomethoxy) propyl]) acyclic nucleotide analogs and their derivatives were shown to be actively schistosomicidal in vivo and in vitro as they possess antiviral activity mainly by acting on DNA polymerase as

well as on reverse transcriptase (Botros et al. 2003, 2009). Oxadiazoles are reported to have antimicrobial and anticancer activity, and being constituted of heteroaromatic rings (5 membered) containing two carbons, two nitrogen, and one oxygen atom, they also possess inhibitory activity against *S. japonicum* and *S. mansoni* (Sayed et al. 2008; Prast-Nielsen et al. 2011). A hybrid drug such as Trioxaquines (1,2,4-trioxane and a 4-aminoquinoline) used for treating malaria display dual mode of actions, viz. stacking with heme by aminoquinoline, leading to inhibition of hemozoin formation in vitro and heme alkylation, also show anti-*S. mansoni* activity (Portela et al. 2012). An antimicrobial and antifungal pentagonal heterocyclic compound, imidazolidines, is also potent in vitro schistosomicide (Neves et al. 2011). Compounds that are targeted toward histone acetyltransferase and histone deacetylase (schistosome histone modifying enzymes) result in apoptosis of parasite and show positive results (Pierce et al. 2011). In acute schistosomiasis, corticosteroids, such as prednisone, are recommended in addition to praziquantel. The acute allergic reactions can be prevented by the use of corticosteroids, which also reduces the inflammation caused by schistosome egg granulomas in the central nervous system and intracranial hypertension and hydrocephalus.

Arachidonic acid (ARA), a novel schistosomicidal compound, is an essential unsaturated fatty acid (omega-6 fatty acid), found in animal and human phosphatides as well as in the liver, brain, and other glandular organs synthesized from dietary linoleic acid. ARA has four cis double bonds, which maintain the fluidity of the membrane at physiological as well as at sub-zero temperatures. ARA can react with molecular oxygen through the activity of three enzymes, namely, cytochrome P450, lipoxygenase (LOX), and cyclooxygenase (COX), all being oxygenases. ARA is also the precursor for synthesis of hormones belonging to a group of dienolic prostaglandins (PG). ARA is present mainly in meat, egg yolks, and some fish oil and is consumed in regular diets. Treatment with ARA increases nSMase in *S. mansoni* by several folds and results in the exposure of membrane antigens to the antibodies. Excessive intake of ARA results in inflammation that can be counteracted by eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). ARA-mediated attrition of worms has been reported in 1, 3, 4, 5, and 6 weeks old *S. mansoni* and 9–12 weeks old *S. haematobium* worms within 3–4 h after treatment. Addition of peripheral blood mononuclear cells and serum antibodies along with ARA resulted in adult worms attrition. Studies conducted on humans in vivo and in vitro with ARA found it to be a nontoxic, cost-effective treatment for schistosomiasis.

26.7.2 Plant-Derived Schistosomicidal Compounds

Curcuma longa rhizome containing curcumin has schistosomicidal activity against *S. mansoni* worms (El-Banhawey et al. 2007; Magalhães et al. 2009). Mirazid, a commercial drug licensed by Pharco (Pharmaceutical company of Egypt), is derived from the stem of *Commiphora molmol* containing oleo-gum resin and acting as uncoupler of male and female worms and flushing out worms from liver tissue

(Badria et al. 2001). But efficacy of this drug is doubted for use in treating schistosomiasis (Botros et al. 2005; Osman et al. 2010).

Artemisia spp. are the source of artemisinin, from which potent antimalarial artesunate and artemether are derived. These two compounds are also effective against schistosomula during early infection and if given after every two weeks can kill all immature schistosomula too. The antischistosomal activity has been clinically tested and found to be effective in chemoprophylaxis of groups with high-risk people in endemic areas; however, it is not suggested in the malaria-native areas. Artemisinins and Artementher are suggested to be incorporated as a combined therapy with praziquantel, where cure rate is more than praziquantel alone (Bergquist and Elmorshedy 2018).

26.7.3 Vaccination

There is no vaccine available for schistosomiasis till today because the complexity of life cycle of schistosomas involving two hosts and to identify the stage for targeting with vaccine is another big challenge (Molehin 2020a, 2020b). It is still unclear how host immune effector cells react when interacting with internal and cytosolic proteins such as paramyosin, fatty acid-binding protein, GST, TPI, or SG3PDH to eradicate parasites (El Ridi and Tallima 2013). The main obstacle in the development of a vaccine is the host immune system resistance to the surface membrane antigens of growing larvae. However, the excretory-secretory products (ESP) from viable lung schistosomula can be used for ideal vaccine formation as it induces local primary and memory immune response targeting immune effectors, toxic radicals, and inflammatory cytokines. Irradiated cercariae were injected to "endemic normal" (who does not record of any infection in the past or current) and then they were exposed to schistosome-contaminated water. Repeated stool and urine examination showed no eggs and very low level of reinfection in these endemic normal which were used to isolate first subunit of vaccine. Antibodies from resistant people and mice vaccinated with the irradiation cercariae vaccine were utilized to test cDNA libraries of schistosoma. Numerous antigens have been discovered and evaluated over the past few decades, but only a few number have progressed to human clinical trials. The failure of *S. mansoni* cercariae to mature into adult worms in naive mice in one experiment supports the idea that choosing the right model organism is crucial for the creation of a vaccine. Baboons, a nonhuman primate species, may make an excellent testing model for developing a vaccination since they are a natural host of schistosomes, where cercarial infections result in the maturation of juvenile worms into adult worms. (Molehin 2020a, 2020b). Four main recombinant antigens, viz. *Schistosoma haematobium* (rSh28 GST (glutathione S-transferase)), *S. mansoni* (fatty acid binding protein Sm14), *S. mansonitetraspanin* surface antigen (Sm-TSP-2), and *S. mansoni calpain* (Sm-p80) are under active development (Jia et al. 2014).

The 28-kD glutathione S-transferase catalyzes metabolic process involved in parasite by abrogating the movement of epidermal Langerhans cells to lymph

nodes and binds to testosterone, which is main enzyme of the host involved in parasite detoxification pathway. Clinical trial with patas monkey were successful but trial done on infected children were not rewarding, may be because of counter effect of PZQ lead in preventing IgG4 production instead of inducing IgG3 antibodies.

Many helminths contain the 14-kDa fatty acid-binding protein (FABP), which is necessary for the uptake and transport of sterols of human origin because they lack any oxygen-dependent pathways for the production of organic compounds. Hence, FABPs have very critical role to play; vaccine Sm14 is considered as potential vaccine. Testing of recombinant Sm14 (rSm14) without any adjuvants shows significant reduction in adult worm as well as provides protection in mice and sheep against *Fasciola hepatica* infections. In phase I clinical trial, Sm14 along with glucopyranosyl lipid A (Sm14/GLA-SE) was effective with mild adverse effects but no production of deleterious IgE antibodies.

On the membrane surface, a family of proteins, namely Tetraspanins, made up of four transmembrane domains are abundantly expressed. Tetraspanins have two extracellular loops of different size, which are readily accessible to immune system of the host. The two tetraspanins, namely, TSP-1 and TSP-2 in *S. mansoni*, are termed as Sm-TSP-1 and Sm-TSP-2, respectively (Tran et al. 2010). The extracellular loop of TSP-2 is strongly recognized by IgG1 and IgG3, main protective antibodies in healthy and resistant individuals and not by infected individuals. Therefore, clinical trials are mainly focused on the antigen TSP-2 using Sm-TSP-2/Alhydrogel, but till date no results have been published.

Another component, namely, Calpin, a neutral protease activated by calcium, is present at the tegument of adult as well as in other stages of schistosomes. Sm-p80 is one of the subunits of calpin that possess a major role in the tegument biogenesis and control host immune response. Several studies to develop vaccine using Sm-p80 as antigen showed prophylactic, therapeutic, killing of adult worms and reduced egg load and fecal egg excretion. Clinical trials have produced Sm-p80-specific IgE antibodies, and human phase I clinical trials of the Sm-p80/GLA-SE vaccine have been approved (Wang, and Da'dara AA, Skelly PJ. 2017).

26.8 Novel Preventive Methods

Another data suggests that the pathogen needs iron for growth and reproduction. Heme and hematin, as well as isoforms of Ferritin (Fer), a highly conserved iron-storage protein, bind iron in the gastrodermal lumen. Female worms express 15 times more Fer-1 (present in yolk platelets) than males, and Fer-2 is present in equal amount in somatic tissues of both males and females. Transferrin (Tf), a blood glycoprotein that binds and transports two ferric ions with high affinity, and Non-Tf-bound Iron (NTBI), which encompasses all forms of iron not bound with Tf, have an impact on the proliferation of Schistosomula in vitro. NTBI contains iron that is only weakly complexed with molecules including albumin, citrate, amino acids, and carbohydrates. By adding an iron chelator such as desferroxamine, this complex can be broken. The presence of DMT-1 and DMT-2, two isoforms of a divalent

metal transporter (DMT), on the membrane suggests that iron absorption occurs via nonspecific binding of the host carrier protein transferrin and is surface-mediated. One theory for how iron is acquired is that ferrous ions (Fe^{2+}) are produced when ferric ions (Fe^{3+}) complexed with Tf are broken down by ferric reductase and transported by DMT1. Heme, a byproduct of blood feeding from the lysis of host erythrocytes via the hemoglobinolytic route, is another suggested method of iron acquisition. Heme is taken up by a transporter for hemoglobin that is found in the gastrodermis, where it is broken down by hemoglobin oxygenase to release iron. It is common for metazoan parasites to use host hemoglobin to make hemoprotein; however, it is unclear how schistosomes break down hemoglobin to release iron. The latest therapies are based on drug or vaccine targeting surface located transporter and receptors of iron in pathogen (Glanfield et al. 2007).

Iron chelators have been recognized in chemotherapy that targets iron intake and control because in vitro research demonstrates that they inhibit the growth of protozoan and schistosome parasites, but the mechanism of action in humans is yet unknown. As it is well established, numerous biological processes, including cell metabolism, proliferation, differentiation, death, and signal transduction, are heavily regulated by miRNAs in many different animals. Researchers have seen the role of miRNAs in the regulation of many stages as well in pathogenesis schistosomiasis. Dicer, ago protein and the essential element in miRNA production are all expressed differently at different phases of schistosome development. The role of host miRNAs in regulating pathogenesis of schistosomes has been studied. Studies on the miRNA profiles from various organs of infected mice showed that miRNAs play a role in the synthesis of insulin, TGF-a, and MAPK signaling pathways during infection. However, the exact role of different miRNAs in different stages of pathogenesis needs further investigations so that miRNA-based technologies can alternatively be used for the control of the disease (Chen et al. 2019).

26.9 Conclusion

In this era of highly evolved medicinal and target drug delivery and therapies schistosomiasis is still a major global health problem. The presence of alternate host has further aggravated the problem of both diagnosis and treatment. To inhibit the development of this fatal disease, it is important to create an effective pathogen vaccination and strategies for overcoming environmental dangers. Most of the studies have been done with mice; therefore, it is utmost important to develop a model such as nonhuman primates and need to exercise caution before human clinical trials. Recent studies with miRNAs have provided valuable insight regarding understanding the pathogenesis of schistosomiasis as well novel methods in diagnostics; the molecular mechanisms still remains an enigma. More scientific inputs are the need of hour and with the growing technology and techniques such as RNA interference and CRISPR/Cas9, there is hope of development of vaccine sooner rather than later.

The infected snails should be removed using chemicals such as niclosamide, along with other environmental approaches such as digging water drainage tunnels and burying the snail habitat. The risk of infection from water-related activities should be known by travelers to endemic areas. Preventive measures such as access to clean water and sanitary facilities, as well as refraining from certain activities like fishing should be practiced. The global as well as local community-based control program should be strictly followed.

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