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REVIEW

Current drug targets for helminthic diseases

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Abstract More than 2 billion people are infected with helminth parasites across the globe. The burgeoning drug resistance against current anthelmintics in parasitic worms of humans and livestock requires urgent attention to tackle these recalcitrant worms. This review focuses on the advancements made in the area of helminth drug target discovery especially from the last few couple of decades. It highlights various approaches made in this field and enlists the potential drug targets currently being pursued to target economically important helminth species both from human as well as livestock to combat disease pathology of schistosomiasis, onchocerciasis, lymphatic filariasis, and other important macroparasitic diseases. Research in the helminths study is trending to identify potential and druggable targets through genomic, proteomic, biochemical, biophysical, in vitro experiments, and in vivo experiments in animal models. The availability of major helminths genome sequences and the subsequent availability of genome-scale functional datasets through in silico search and prioritization are expected to guide the experimental work necessary for target-based drug discovery. Organized and documented list of drug targets from various helminths of economic importance have been systematically covered in this review for further exploring their use and applications, which can give physicians and veterinarians effective drugs in hand to enable them control worm infections.

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Introduction

Helminths (the word is derived from the Greek meaning "worms") are macroparasitic worms. They are the most common infectious agents of humans in tropical countries and produce a global burden of disease that exceeds malaria and tuberculosis. Helminth parasites are a significant cause of economic loss in livestock and crop industries around the world. Today, it is estimated that approximately one-third of the humanity (more than 2 billion people) especially those living on less than two US dollars per day in developing regions of sub-Saharan Africa, East Africa, Asia, and the Americas are infected with one or more helminths (Brooker et al. 2009; de Silva et al. 2003; Hotez et al. 2008; Hotez et al. 2007; Hotez et al. 2006). More than one helminth may thrive in a single host due to their synergistic interactions and non-specific parasite-induced immunosuppression of the host (Christensen et al. 1987). In the USA alone, nematodes cost the livestock industry an estimated $2 billion per year in lost productivity and increased operating expenses. In cattle, buffaloes, sheep, and goats, most emphasis is seen in the field of losses as a result of gastrointestinal nematode infections in livestock as well as liver condemnation (Gasbarre 1997; Rapsch et al. 2008). Liver condemnations as a result of helminth infections by liverflukes, schistosomes, and cestodes have been described quantitatively. Helminth infection for a very long time-period (several years!) is responsible for a huge health burden and causes productivity loss in developing countries across tropical regions. They are considered the masters of immunoregulations capable of escaping host defence system through suppression of either humoral or cellular or both arms of the host immunity and establish chronic infections (Aranzamendi et al. 2013). These worms compromise nutritional status, affect cognitive processes, induce tissue reactions, such as granuloma, and provoke intestinal obstruction or rectal prolapse. These are notably manifested

through major parasitic diseases such as schistosomiasis, onchocerciasis, and lymphatic filariasis. These long-term chronic diseases rarely results into death, but their morbid manifestations in humans are involved in worsening the cases of HIV/AIDS, TB, malaria, and allergy (Hotez et al. 2006). Helminths have been primarily divided into two major phyla. The first one called nematodes (known as roundworms) include the major intestinal worms (also known as soil-transmitted helminths) and the filarial worms that cause lymphatic filariasis (LF) and onchocerciasis, whereas the platyhelminths (also known as flatworms, the second phyla) include the flukes (also known as trematodes), such as the schistosomes, and the tapeworms (also known as the cestodes), such as the pork tapeworm that causes cysticercosis (Wang et al. 2008). The most prevalent helminthiases are those caused by infection with intestinal helminths, ascariasis, trichuriasis, and hookworm, followed by schistosomiasis and LF. Control of helminthiasis is based on drug treatment, improved sanitation and health education. Current efforts for the control of helminthiases have been devised and funded, mainly by international donor agencies, major funding bodies, and academic institutions from the developed world, contributing to the creation of usually North–south "partnerships". All that is required is to shift this paradigm in disease-endemic countries (DECs) by refocusing political will and harnessing commitment by the countries' governments, towards health research and capacity building policies to ensure long-term investment in combating and sustaining the control and eventual elimination of helminthiasis from developing countries (Osei-Atweneboana et al. 2012). To completely eliminate helminth parasites, constant research on major drug targets has to go on in DECs and mass drug administration (MDA) of the available anthelmintic has to be merged with monitoring, education, sanitation, and access to health services (Prichard et al. 2012).

New insights into fundamental helminth biology are accumulating through newly completed genome projects and the nascent application of transgenesis and RNA interference technologies (Chuan et al. 2010; Martin et al. 2011; Pierson et al. 2009; Song et al. 2011; Xu et al. 2010). With the help of second-generation sequencing techniques (454 and Illumina), Welcome Trust Sanger Institute (WTSI) is producing de novo reference quality genomes for parasitic helminth species from cestodes, trematodes, and nematodes (Holroyd and Sanchez-Flores 2012). In the absence of vaccines, control of helminth infections relies mainly on a limited number of drugs, such as the benzimidazoles, the macrocyclic lactones, and the imidazothiazoles (Kaminsky et al. 2008). However, the number of drugs available to treat helminth infections is very limited, and moreover, the mechanism of their action is not fully explored. Almost a century has been lapsed in search of chemotherapeutic drugs for schistosomiasis but in vain (Ribeiro-dos-Santos et al. 2006). Coupled with the threat of impending drug resistance, there is a need to discover new compounds which can feed into the pipeline for drug development (James et al. 2009; Keiser and Utzinger 2010). The current vistas for the future hope is to approach, discover, and study the helminthic drug targets and anthelmintics for rational designing and inventing of compounds which can waive off the drug resistance compounding for the existing drugs (James et al. 2009). Successful drug development has to be stymied by a general focus on target selection rather than clinical safety and efficacy. The very process of validating the targets themselves is inefficient, and in many cases, leads to drugs having poor efficacy and undesirable side effects. The numbers of potential drug targets so far characterized are few in numbers and enlisted in Table 2. The current time and scenario therefore entails further research and development to identify, expand, and select the appropriate drugs and finally deploy the next-generation anthelmintic drugs to combat helminthiasis from humans, livestock, and crops (Keiser and Utzinger 2010).

Molecular approaches to drug targets identification and characterization

Target-based drug discovery starts with the identification and elucidation of the function of a potential therapeutic drug target and understanding its essentiality and its role in the disease process. Drug targets are basically molecular structures (chemically definable by at least a molecular mass) that will undergo a specific interaction with chemicals (drugs) because they are administered to treat or diagnose a disease. Most of the times, they are enzymes or proteins involved in biological activity which are produced by expression of active genes in a cell. In helminthes, drug targets are primarily identified through genomic (Abubucker et al. 2011; Chuan et al. 2010; Hagen et al. 2011; Holroyd and Sanchez-Flores 2012; Martin et al. 2011), proteomic (Brophy et al. 2012; Chuan et al. 2010; Mutapi 2012), or through in silico approaches (Abubucker et al. 2011; Martin et al. 2011) and are then structurally and functionally characterized through various approaches viz. biochemical enzymatic reactions, biophysical methods such as x-ray crystallography or NMR technique and gene knockout or RNA interference using model worm *Caenorhabditis elegans* (Chuan et al. 2010; Crowther et al. 2010; Kumar et al. 2007; Lee et al. 2011; Modis 2012; Yadav et al. 2010). This is typically depicted through a simplified picture in Fig. 1, and the details of the contemporary techniques associated with them have been enlisted in Table 1. When the drug target has been evaluated and characterized from one or more methods, then screening of the hits to leads and finally leads to potential drug candidates can be carried out conventionally using 3-(4,5-dimethylthiazol-2-*yl*)-2,5-diphenyltetrazolium bromide (MTT) reduction assay routinely used to assess the viability of the worm (Misra et al. 2011; Srinivasan et al. 2009). Recently, two more in vitro inhibitors screening techniques have been developed viz.

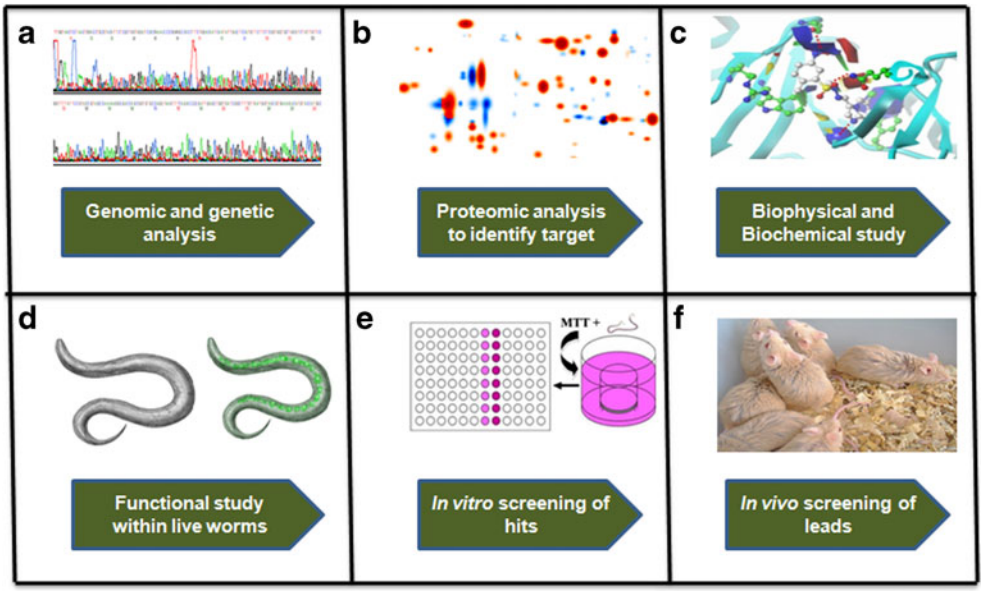

Fig. 1 Various contemporary approaches to identify and characterize drug targets in helminth parasites. **a** Rapid sequencing of parasites genome using next-generation sequencing machines like 454 and Illumina to quickly spot and identify unique/divergent genes. **b** Proteomic analysis (2D, mass spectrometry, SILAC, etc) of the parasites or part of the parasites. **c** Biophysical and biochemical characterization (X-ray crystallography, UV–Visible spectroscopy, circular dichroism spectroscopy, isothermal titration calorimetry, neurobiology, enzymology, etc.) of drug targets. **d** Localization study and RNA interference of genes to know the essentiality and functions of unknown genes in nematode model organism called *C. elegans*. **e** In vitro screening of hits against drug targets in culture plates using MTT assay, NTS assay, etc. **f** In vivo screening of leads (obtained after multiple screening and selecting the best hits) in appropriate animal model for specific helminthiasis

redeployment based drug screening and newly transformed schistosomula (NTS) assay (Khanim et al. 2011; Marxer et al. 2012). Genetically modified *C. elegans* are also used to increase throughput in early discovery of drugs (Kaletta and Hengartner 2006). The last step of preclinical study is to use primary model organisms for in vivo characterization of anthelmintics against drug targets when the drug target has been identified and characterized in vitro. For example, *Mastomys coucha* and BALB/c are successfully being used for *Schistosoma mansoni*, *Brugia malayi,* and *Onchocerca volvulus* infection respectively to test the action of anthelmintics on different stages of worms (Allen et al. 2008; Dangi et al. 2009; Hanelt et al. 2010). Tropical Disease Research (TDR, an special program for research and training in tropical diseases) has already supported four centres focused on anthelmintic drug screening: the Theodor Bilharz Research Institute (TBRI, Cairo) and London School of Hygiene and Tropical Medicine (LSHTM) for schistosomiasis; the Northwick Park Institute for Medical Research (NPIMR, London) for onchocerciasis/LF as well as the CSIR-Central Drug Research Institute for LF.

Classification of current drug targets

We have specifically selected approximately 35 drug targets (Table 2) from various infectious helminths, which have been studied so far from the standpoint of huge loss of health of community as well economy of the developing countries. The most prominent targets among them include the mitochondrial enzyme complex I and II, cathepsin B, voltage gated Ca^{2+} ion channels, receptors such as acetylcholine and DAF-2, β-tubulin, FMRFamide-like signaling pathway, and endosymbiotic bacteria called *Wolbachia* in nematodes. The identification of a target in this count is a result of thorough and extensive study of the up to date research in the field of helminth, the different types of helminths and nature of the helminthiasis that affect the tropical world, the format of assays (chemotherapy of compounds, *C. elegans* being used as an alternative model along with in vitro and in vivo study), and lastly the drug targets insight under each category of classification.

Enzymes

Since enzymes are highly specific for their substrate, the difference in specificity between human and helminth enzyme for their substrate analogs/inhibitors can be utilized to generate target suitability. The enzymes from helminths have now been pursued using molecular approach to hit the target enzymes with highly specific inhibitors against them. A decade ago, mitochondrial complex I and II (NADH-fumarate reductase system, a unique respiratory system in parasitic helminths and the terminal step of the phosphoenolpyruvate carboxykinase-succinate pathway found in many anaerobic organisms) had been investigated

Table 1 Molecular approaches to drug targets identification and characterization

S. No	Molecular approach	Techniques	References
1	Genomics	Analysis of genome and druggable genes through Next Generation sequencing of genomes (454, Illumina) and analysis of drug targets through in silico approach	(Abubucker et al. 2011; Chuan et al. 2010; Hagen et al. 2011; Holroyd and Sanchez-Flores 2012; Kaletta and Hengartner 2006; Kumar et al. 2007; Martin et al. 2011)
2	Proteomics	2D Gel electrophoresis, Mass spectrometry, stable isotope labels with amino acids in cell culture (SILAC)	(Brophy et al. 2012; Chuan et al. 2010; Dangi et al. 2009; Mutapi 2012; Robinson et al. 2009)
3	Biophysical and Biochemical	X-ray crystallography, UV–visible spectroscopy, CD spectroscopy, isothermal titration calometry, Enzyme inhibition study, neurological study of ion channels and receptors	(Andrade et al. 2011; Angelucci et al. 2009; Gelmedin et al. 2008; Johnston et al. 2010; Kawasaki et al. 2010; Salvador-Recatala and Greenberg 2010; Tandon et al. 2006; Wang et al. 2011; Wang et al. 2009; Wojtovich et al. 2008; Wu et al. 2009)
4	Functional study	RNA interference, confocal study and target localization, metabolic pathways and cell signaling study	(Chen et al. 2009; Kita et al. 2003; Kumar et al. 2007; Kushwaha et al. 2012; Mizukami et al. 2010; Pierson et al. 2009; Song et al. 2011; Taylor et al. 2011; Xu et al. 2010)
5	In vitro screening of Inhibitors	MTT assay, redeployment-based drug screening, newly transformed schistosomula (NTS) assay	(Khanim et al. 2011; Marxer et al. 2012; Misra et al. 2011; Srinivasan et al. 2009)
6	In vivo screening of Inhibitors	Animal models; pigs for ascariasis, mastomys for filariasis, BALB/c for schistosomiasis, hamster and dogs for ancylostomiasis	(Dangi et al. 2009a; Dold and Holland 2010; Fujiwara et al. 2006; Hemphill et al. 2010; Keiser 2010; Mutapi 2012)

for drug discovery against *Ascaris suum* which is still a hot concern for lead optimization (Omura et al. 2001; Sakai et al. 2011). Nafuredin competes for quinone binding site and inhibits NADH-fumarate reductase and NADH-rhodoquinone oxidoreductase, a unique anaerobic electron transport system in helminth mitochondria, at nanomolar concentration (Omura et al. 2001). Moreover, nafuredin exerts anthelmintic activity against *Haemonchus contortus* in in vivo trials with sheep. Specific inhibition by nafuredin indicates that the structure of the domain in helminth complex I differs somewhat from that of its mammalian counterparts (Kita et al. 2003; Omura et al. 2001). The intestinal human helminth *S. mansoni* possesses several eukaryotic protein kinases (ePKs) and one important and essential is polo-like-kinases 1(SmPlk1) involved in mitosis and/or meiosis in schistosomes (Long et al. 2010). This has been supported by the in situ detection of SmPlk1 transcripts in female vitelline cells and oocytes as well as in male spermatocytes. Several Plk inhibitors have been found to inhibit SmPlk1 activity in *Xenopus* oocytes and the first-in-class prototype Plk1 inhibitor (BI 2536) induced in vitro dramatic alterations in schistosome gonads, which affected oogenesis and spermatogenesis, indicating a major role for SmPlk1 in parasite reproduction and suggest its importance as a potential new target against schistosomiasis (Long et al. 2010; Long et al. 2012). Another drug target Cathepsin B (CB), a cysteine protease present in a number of helminths like *Fasciola hepatica*, *Clonorchis sinensis,* and *S. mansoni* which has attracted much attention for its essential roles in parasite physiology as well as in host–parasite interactions through their modulation of various pathobiological events, including host tissue invasion, nutrient uptake, host immune evasion, and molting (Chen et al. 2011; Horn et al. 2011; Jilkova et al. 2011). Two vinyl sulfones K11017 and K11777 as inhibitors of cathepsin B have been crystallized with SmCB1 at 1.3 A^{o} resolution and insight of interaction and inhibition specificity has been worked out with the enzyme (Jilkova et al. 2011). The Chinese liver fluke *C. sinensis* and *Taenia asiatica* has been studied to possess lactate dehydrogenase as drug target (Huang et al. 2009; Yang et al. 2006), with gossypol as potential lead compound. Recently, glutathione-*S*-transferase (GST) of filarial worms has been found to be a good target molecule for a number of inhibitors identified through molecular docking study (Awasthi et al. 2009; Srinivasan et al. 2009; Yadav et al. 2010). Topoisomerase II (gyrase) is an another emerging target from a number of study and recently methanolic extract from *Micrococcus luteus BI252* has been shown to inhibit the enzyme and the growth of *Setaria cervi* (Kumar et al. 2008; Misra-Bhattacharya et al. 2004; Sivasamy et al. 2011). The active ingredient was found to be a fatty acid methyl ester derivatives (Z) 15-tetracosenoic acid, such derivatives can be used for development of anti-virulence drug lead compounds (Sivasamy et al. 2011). Another enzyme which also changes the topological properties

Table 2 Drug targets, their known inhibitors, and the helminths under study

Enzymes	Known drugs/inhibitors	Helminths under target/study
ePKs, Polo like kinase (SmPlk1) (Long et al. 2010; Long et al. 2012)	BI 2536	*S. mansoni*
Cathepsin B1(Chen et al. 2011; Horn et al. 2011; Jilkova et al. 2011)	Vinyl sulfones K11017 and K11777	*S. mansoni*
Glutathione-S-Transferase (Awasthi et al. 2009; Srinivasan et al. 2009; Yadav et al. 2010)	Plumbagin, curcumin, ethacrynic acid, 1,3-diarylpropen-1-one	*Setaria digitata*, *B. malayi*, *Wuchereriai*
Mitochondrial complex I & II (Kita et al. 2003; Omura et al. 2001; Sakai et al. 2011)	Nafuredin, Bithionol	*A. suum*, *H. contortus*
Topoisomerase II (Sivasamy et al. 2011)	15-tetracosenoic acid,	*B.malayi*, *W. bancrofti*
Chitinase (Garner et al. 2011)	Closantel	*Onchocerca volvulus*
Trehalose-6-phosphate phosphatase (Kushwaha et al. 2011; Kushwaha et al. 2012)	?	*B. malayi*, *W. bancrofti*
Asparagine tRNA synthetase (Yu et al. 2011)	Tirandamycins	*B. malayi*, *W. bancrofti*
Lactate dehydogenase (Huang et al. 2009; Lu et al. 2006; Yang et al. 2006)	Gossypol	*Clonorchis sinensis*, *Taenia asiatica*
Beta-tubulin (microtubule) (Beech et al. 2011; Chambers et al. 2010; Kaminsky et al. 2008)	Benzimidazole derivatives (Albendazole)	All Helminths
Receptors and Ion channels		
nAChRs, DEG-3 (Robertson and Martin 2007; Rufener et al. 2009; Williamson et al. 2009)	Levamjsole, tribendimidine and Derquantel, Amino-acetonitrile derivatives AADs (Monepantel)	*Strongyloides*, *stercoralis*, *H. contortus*,
GPCR receptors, Latrophilin receptors (Keiser and Utzinger 2010; Kruger et al. 2009; Muhlfeld et al. 2009)	Thiadiazoles, Cyclic depsipeptides, Emodepside	*A. suum*, *H. contortus*
Nuclear receptor DAF-12, DAF-16 (Kawasaki et al. 2010; Senti and Swoboda 2008; Wang et al. 2009)	Dafachronic acid, Apigenin	*Ancylostoma* spp., *and Necator americanus*
Ligand gated Ca^{2+} & K^{+} ion channels (Doenhoff et al. 2008; Kasinathan et al. 2009; Nogi et al. 2009; Zhang and Zhou 2008)	Praziquantel	Cestodes, Trematodes, and Nematodes
Calcium-activated potassium channel, SLO-1(Carre-Pierrat et al. 2006; Crisford et al. 2011; Guest et al. 2007; Welz et al. 2011)	Emodepside	*A. suum*, *Cooperia oncophora*, and *H. contortus*
Cys-loop ligand gated ion channels, Glutamate-gated chloride channels (Glendinning et al. 2011; Hibbs and Gouaux 2011; McCavera et al. 2009; Tandon et al. 2006; Wolstenholme 2011)	Macrocyclic lactones (Ivermectin, Moxidectin), Nicotinic agonists	All Helminths
Serotonin transporter (Crisford et al. 2011; Fontana et al. 2009)	Imipramine, Citalopram, Fluoxetine, fluvoxamine, Aroxetine and Sertraline	*S. mansoni*
Signaling pathways		
Wnt/Frizzled Signaling (Chen et al. 2009; Li et al. 2010; Ouelaa-Benslama and Emami 2011)	Niclosamide,	*Schistosomes*, *tapeworms*,
FaRPergic signaling (Lee et al. 2011; Robertson and Martin 2007)	Piperazine	*A. suum*
FMRFamide like peptide signaling, Allatostatins-like neuropeptide (Marks and Maule 2010; McVeigh et al. 2009; Mousley et al. 2010; Muhlfeld et al. 2009; Novozhilova et al. 2010)	FMRFamide-related peptides	*Heterodera glycines*, *Meloidogyne incognita* and *Panagrellus redivivus*
Metabolic pathways		
Chitin and trehalose metabolism, Nucleic acid metabolism, amine metabolism, Respiratory Pathway (Li et al. 2011a)	DEC, Suramin, Mebendazole,	Nematodes
		S. mansoni, *T. crassiceps*

Table 2 (continued)

Enzymes	Known drugs/inhibitors	Helminths under target/study
Thioredoxin and glutathione systems (Angelucci et al. 2009; Bonilla et al. 2011; Eweas et al. 2012; Hong et al. 2012; Martinez-Gonzalez et al. 2010)	Auranofin, 8-hydroxyquinoline-5-sufonyl 1,4-diazepine derivatives	
Endosymbiotic bacteria		
Enzymes from heme biosynthetic pathway, Enzymes from FAD syntheisis (Wu et al. 2009)	*N*-methyl mesoporphyrin (NMMP)	*B. malayi*, *W. banchrofti*, *O. convuvulus*
Lipoprotein biosynthesis (Johnston et al. 2010)	Globomycin	*S. mansoni*, *Echinococcus granulosus*
FtsZ (Li et al. 2011b)	Beberine	*B. malayi*, *W. banchrofti*, *O. convuvulus*

of nucleic acid called RNA helicase has been found to be drug target in *Brugia malayi* and *S. mansoni* (Singh et al. 2009; Skinner et al. 2012), and further research is along the way on to screen specific inhibitors against this drug target. In trehalose biosynthesis, the Trehalose phosphate phosphatase (TPP) seems to be a novel drug target as silencing of *tpp* gene in *C. elegans* and *B. malayi* caused the arrested growth of larvae; however, the lethality was not correlated with the accumulation of toxic levels of trehalose-6-phoshate while *tps* gene could not produce any lethal phenotype (Kushwaha et al. 2012). In the absence of known TPP inhibitors, commonly available phosphatase inhibitors, e.g., sodium meta vanadate and sodium azide were used; however, these could not inhibit Bm-TPP enzyme activity, thereby warranting designing of specific *tpp* inhibitors (Kushwaha et al. 2011). Another nematode enzyme, asparaginyl-tRNA synthetase (AsnRS) has been recently considered as an excellent antifilarial target (Yu et al. 2011). There are five tirandamycins (TAMs), TAM E (1), F (2), and G (3), TAM A (4) and B (5), from *Streptomyces* sp. 17944 out of which five selectively inhibit the *B. malayi* AsnRS and efficiently kill the adult *B. malayi* parasite, representing a new lead scaffold to discover and develop antifilarial drugs. Although not an enzyme, the microtubule of parasitic worms has been a shown to be a unique dynamic structure within the cells which is involved in shaping multicellular parasites. There are at least six β-tubulins present in case of *F. hepatica*, which associate with ∝-tubulins to form 13-stranded staggered polymeric structure called microtubule. Several benzimidazoles derivatives are now known to inhibit microtubule assembly in various helminths. Albendazole has been found to disrupt the microtubule structure by tightly binding to the β-tubulin isotype 2 (Chambers et al. 2010), and therefore is still a potent drug to combat helminthic diseases.

Receptors and ion channels

A receptor is a structure on the surface of a cell (or inside a cell) that selectively receives and binds a specific substance such as its canonical ligands, hormones, neurotransmitters, or neuromodulators. They are coupled to various signal transduction systems located both accross the membrane and intracellularly, which ultimately regulate responses to the cellular/tissue microenvironment. The definition of a receptor in pharmacological or physiological terms requires that there is specificity in its interaction with ligands that belong to a given pharmacological class. Receptors are highly selective for its ligand and accordingly react for its biological function. Cholinergic anthelmintics such as levamisole and pyrantel which cause ion channels to open act on Nicotinic Acetylcholine Receptor (nAChR) of nerve and muscle leading to prolonged muscle contraction and spastic paralysis of the parasite (Williamson et al. 2009). A novel chemical class of synthetic anthelmintics, the Amino-

Acetonitrile Derivatives (AADs), has been recently discovered which specifically acts on nAChR (Kaminsky et al. 2008). nAChR and related nAChRs in other species of parasitic nematode can be used to predict the effectiveness of AADs like monepantel against those species, which could be useful when deciding on a course of treatment for worm-infected animals. In case of *C. elegans* and *H. contortus*, AAD-1566 (monepantel) acts via nicotinic acetylcholine receptors (nAChR) of the nematode-specific DEG-3 subfamily (Rufener et al. 2009). New combinatorial approach such as when agonists of Cry5B and nAChR are combined, their activities are strongly synergistic, producing combination index values as good or better than seen with antitumor, anti-HIV, and insecticide combinations (Hu et al. 2010; Hu et al. 2009). Cry5B is the crystal (Cry) proteins made by *Bacillus thuringiensis*. Combination drug therapies are considered the ideal treatment for infectious diseases. G-protein-coupled receptors (GPCRs) are often revealed to play a key role in the signalling cascade. More than 40 % of all prescription pharmaceuticals target GPCRs. The latrophilin-like receptor HC110-R of the parasitic nematode *H. contortus* has been identified as a target for the novel anthelmintic drug emodepside (Kruger et al. 2009). FMRFamide-like neuropeptides as putative ligands have been also investigated as novel anthelmintics when used against latrophilin receptor of *H. contortus* (Muhlfeld et al. 2009).

Nuclear receptors (NRs) constitute a unique group of transcription factors. They act as sensors for metabolic as well as systemic hormonal signals and regulate a number of cellular processes from growth and differentiation to metabolism. The fact that their activity can be pharmacologically modulated by specific ligands, thereby allowing for agonism, partial agonism, and antagonism, has made them primary therapeutic targets for many years. DAF-12 is an evolutionarily conserved nuclear hormone receptor in several parasitic nematodes including *Strongyloides stercoralis*, *Ancylostoma* spp., and *Necator americanus* involved in signaling pathway which governs development of the stage 3 infective larvae (iL3) (Wang et al. 2009). Administration of dafachronic acid (steroid like molecule and a ligand to DAF-12) has been shown to markedly reduce the pathogenic iL3 population in *S. stercoralis*, indicating the potential use of DAF-12 ligands to treat disseminated strongyloidiasis. Another nuclear receptor of the same family characterized recently is DAF-16 in *C. elegans* which regulates numerous biological activities including larval growth, resistance to oxidative stress, and longevity by activating target genes. Apigenin is a flavone which causes activation of DAF-16 receptor, and its translocation inside nucleus leads to DAF-2/insulin-like signaling which eventually leads to larval growth inhibition (Kawasaki et al. 2010). Here, the target is activated rather than inhibited and is a remarkable and unusual example of receptor induced inhibition of cell growth.

Ion channels are intrinsic membrane proteins which are multimers that act as gated pores (ligand or voltage gated) and regulate the movement of ions across cell membranes. Ion channels present in the nervous system of parasites constitute the majority of targets for current anthelmintics (Wolstenholme 2011). When these ion channels are targeted by therapeutics means, these are either inhibited, preventing the flow of ions, or held constitutively open by the action of agonists, preventing the accumulation of ions on one side of the membrane. The desired activity of the ion channel modulator will depend on its specificity, its nature of action, and the location of the ion cannel in the helminth body. The nervous system of helminth consists of neuronal cells, nerve–nerve innervations, and neuromuscular junctions. Ions channels are present in these cells to control the movement of ions and build a voltage gradient for motor functions. Being the third most targeted molecules for drug discovery after enzymes and GPCRs, ion channels (GABA, glutamate, aspartate, glycine) in helminth are the prioritized target molecules for current anthemintics being applied constantly through FDA approval including those used in mass drug administration (Robertson and Martin 2007). The voltage-gated Ca^{2+} channel (Cav) is an important factor in regulating the intracellular Ca^{2+} level in the neuromuscular system of helminths. Cav from schistosomes and other platyhelminths have several unique properties that make them attractive potential drug targets, and the uniqueness of the subunit structures also provide insights into structure–function relationships as well as evolution of Cav channels (Salvador-Recatala and Greenberg 2010). One of the current drugs used against schistosomiasis is praziquantel (PZQ), which not only affect Ca^{2+} homeostasis in schistosomes, but which has an undefined molecular target and mode of action. PZQ is the only available anti-schistosomal drug in most parts of the world, making reports of PZQ resistance particularly troubling (Doenhoff et al. 2008; Nogi et al. 2009; Salvador-Recatala and Greenberg 2010; Zhang and Zhou 2008). The Ca^{2+} ion concentration when raised inside the cell can activate potassium ion channel. Calcium-activated potassium channel, SLO-1 also called Big Potassium channel (BK channel) belongs to another family of channels that are highly conserved across the animal phyla and regulate neurosecretion, hormone release, muscle contraction, and neuronal network excitability, first characterized in helminth model organism *C. elegans* (Carre-Pierrat et al. 2006; Guest et al. 2007). An emerging molecule called emodepside is a resistance-breaking anthelmintic of a new chemical class, the cyclooctadepsipeptides which is found to interact with SLO-1 channels. Ectopic overexpression of *slo-1* gene in pharyngeal muscle confers

sensitivity of the muscle to emodepside, consistent with a direct interaction of emodepside with the channel (Crisford et al. 2011). Orthologues of *slo-1* are present in *Ancylostoma caninum*, *Cooperia oncophora*, and *H. contortus*, all important parasitic nematodes to be targeted (Welz et al. 2011). Another important ligand gated channel present in nematode is cys-loop ligand gated ion channels (CLGIC) which mediate neurotransmission and are important targets (Williamson et al. 2007). The binding of neurotransmitter (ligand) triggers a conformational change in the receptor, opening an intrinsic chloride channel, and thereby dampening neuronal excitability (Hibbs and Gouaux 2011). The CLGIC superfamily in nematodes comprises ion channels gated by acetylcholine, gamma-amino butyric acid (GABA), glutamate, glycine, and 5-hydroxytryptamine (5-HT). These CLGIC are targets of a number of anthelmintic drugs (Lees et al. 2012). The invertebrate glutamate-gated chloride channels (GluCls) are receptor molecules and targets for the avermectin-milbemycin (AM) group of anthelmintics (McCavera et al. 2009; Tandon et al. 2006). The macrocyclic lactones target the glutamate-gated chloride channels and the nicotinic agonists act on the nicotinic acetylcholine receptors and classified as important category of anthelmintics. Transporters of ions are also classified as under ion channels. Two novel serotonin transporters, SmSERT-A and SmSERT-B from *S. mansoni* are pharmacologically indistinguishable from each other, efflux experiments but they have significantly higher substrate selectivity for serotonin compared with their mammalian counterparts (Fontana et al. 2009). The screening of compounds against the electrogenic SmSERT could result into selective drugs against this essential transporter of *S. mansoni*. Aquaporins (AQPs) are another class of transporter for water molecules which sometimes also transport glycerol and other small solutes as well across biological membranes. The structure, function, and pathology of AQPs have been extensively studied in mammals but data for AQPs from helminths is still limited. An aquaporin from *Fasciola gigantica* has been shown to have altered and lowered water permeability when compared to rat AQP-1 (Geadkaew et al. 2010).

Biochemical pathways

1. Cell signaling pathways

Targeting a single molecular mechanism should be sufficient to achieve a significant therapeutic effect; however, a single-target drug would have very little therapeutic impact unless and until it is associated with physiological process of the cell or involved in cell signaling. Anthelmintic niclosamide, a drug used for the treatment of tapeworm, promotes Frizzled1 endocytosis, downregulates Dishevelled-2 protein, and also reported to inhibit Wnt3A-stimulated β-catenin stabilization and LEF/TCF reporter activity (Chen et al. 2009). Additionally, following niclosamide-mediated internalization, the Frizzled1 receptor co-localizes in vesicles containing transferrin and agonist-activated β2-adrenergic receptor. Therefore, niclosamide may serve as a negative modulator of Wnt/Frizzled1 signaling by depleting upstream signaling molecules (i.e., Frizzled and Dishevelled), and moreover, may provide a valuable means of studying the physiological consequences of Wnt signaling. Wnt-4 signaling has been shown to be involved in the development of *Schistosoma japonicum* through canonical pathway (Li et al. 2010). Moreover, in *S. japonicum*, an inhibitor of programmed cell death pathway (apoptosis) has been found to be a potential small molecule that act on a caspase to control the schistosomiasis (Peng et al. 2010b). Besides Wnt/catenin signaling, short amidated neuropeptides such as FMRFamide-like (FLPs), neuropeptide F (NPF)-like, myomodulin-like, buccalin-like, and neuropeptide FF (NPFF)-like peptides are widespread signaling molecules within the nervous systems of all flatworms and roundworms examined (McVeigh et al. 2009) and could therefore represent a starting point for new lead drug compounds with which to combat parasitic helminth infections (Marks and Maule 2010; Mousley et al. 2010). KHEYLRF-NH2 (AF2) is the most abundant FMRFamide-related peptide (FaRP) in *A. suum* and also in many other parasitic and free-living nematodes (Verma et al. 2007). The AF2 abundance in the highly diverse nematodes and its potent and profound effects on the neuromuscular systems make AF2 and its receptors such as G Protein Coupled Receptors (GPCRs) very attractive targets for the discovery of novel broad-spectrum anthelmintics.

2. Metabolic pathways

A clear understanding of the mode of action of anthelmintics awaits greater knowledge of the biochemical pathways operating in helminth parasites. Here, we present the contrasts between helminth and human metabolism so that strategic differences can be harnessed for newer developments in chemotherapy. Platyhelminth parasites have a unique and simplified thiol-based redox system, in which the selenoprotein thioredoxin-glutathione reductase (TGR) (Otero et al. 2010), a fusion of a glutaredoxin domain to canonical thioredoxin reductase domains, is the sole enzyme supplying electrons to oxidized glutathione (GSSG) and thioredoxin. This enzyme has recently been validated as a key drug target for flatworm infections (Bonilla et al. 2011; Boumis et al. 2011; Martinez-Gonzalez et al. 2010). Furthermore, TGR has been characterized in schistosomes through molecular docking and in vitro study with a novel inhibitor (8-hydroxyquinoline-5-sufonyl 1,4-diazepine derivative) and has been found to be a promising anti-schistosomal agent (Eweas et al. 2012). Protein–protein interactions with unique helminth proteins and helminth

proteins with unique features relative to the host, such as indels, have been prioritized as drug targets. The PPIs were scored based on RNAi phenotype and homology to the PDB (Protein DataBank). EST data for the various life stages, GO annotation, and druggability were also taken into consideration. Several PPIs emerged from this study as potential drug targets. A few interactions were supported by co-localization of expression in *Meloidogyne incognita* (plant parasite) and *B. malayi* (*Homo sapiens* parasite), which have extremely different modes of parasitism (Taylor et al. 2011).

Endosymbiotic bacteria

Wolbachia are obligate endosymbiont α-proteobacteria in filarial nematodes and are essential for the female worm survival, reproduction, and fecundity. After coming of the genome data of both *B. malayi* and its endosymbiont *Wolbachia*, the astonishing finding was that *Wolbachia* containing filarial nematodes lacks all heme biosynthetic pathway enzymes except the last step enzyme ferrochelatase (Foster et al. 2005; Wu et al. 2009). Therefore, *Wuchereria bancrofti*, *B. malayi*, and *Onchocerca vulvulus* depend on the α-proteobacterial heme for activating their own heme-containing enzymes. This shows that the heme biosynthetic genes in the *Wolbachia* of *B. malayi* (*wBm*) are essential for the filarial host survival. In addition, the enzymes are likely candidate drug targets based upon significant differences in phylogenetic distance, biochemical properties, and sensitivities to heme biosynthesis inhibitors, as compared to their human homologues. The presumptive transporters, responsible for heme trafficking, could be drug targets as well. Also, since *Wolbachia* are essential for the nematode, so targeting essential genes of *Wolbachia* is another alternative for delving into anti-filarial drug research. *Wolbachia* endosymbionts of filariae are potent inducers of innate and adaptive inflammation, and bacterial lipoproteins have been identified as the ligands that bind toll-like receptors (TLR) 2 and TLR6. Lipoproteins are important structural and functional components of bacteria, and therefore enzymes involved in *Wolbachia* lipoprotein biosynthesis are potential chemotherapeutic targets. Globomycin, a signal peptidase II (LspA) inhibitor, has activity against *Wolbachia,* and a putative lspA gene has been identified from the *Wolbachia* genome of *B. malayi*. Globomycin was screened using this assay, which resulted in a dose-dependent reduction in *Wolbachia* load. Furthermore, globomycin was also effective in reducing the motility and viability of adult *B. malayi* in vitro (Johnston et al. 2010). Phosphoglycerate mutases (PGM) interconvert 2- and 3-phosphoglycerate in the glycolytic and gluconeogenic pathways. A putative cofactor-independent phosphoglycerate mutase gene (iPGM) was identified in the genome sequence of the *Wolbachia* endosymbiont from the filarial nematode, *B. malayi* (wBm). Since iPGM has no sequence or structural similarity to the cofactor-dependent phosphoglycerate mutase (dPGM) found in mammals, it may represent an attractive *Wolbachia* drug target (Foster et al. 2009). A notable wolbachial protein FtsZ, an analog of eukaryotic β-tubulin which is expressed in all developmental stages of *B. malayi,* is a GTPase, thereby making the protein an attractive drug target. Recently, berberine as a small molecule inhibitor and a natural drug identified from a high-throughput screen has been used to inhibit GTPase activity of FtsZ for combating filarial infections (Li et al. 2011).

Discussion

Currently, we do not have any good vaccines for the majority of the helminths due to complex immunological interactions occurring during helminth infections (Aranzamendi et al. 2013; Kozak and Kolodziej-Sobocinska 2009). As such, drugs are really the only direct intervention currently available. The prospects for specific and effective anthelmintic development are bright through drug target approach. The list of drug targets, its inhibitors, and the helminths in target are summarized in Table 2. Helminths are in vitro uncultivable parasites at defined laboratory conditions and due to the lack of true experimental animal models, the research on drug targets has to go on due to rapid development of resistance with the heavy use of current anthelmintics. It is important to note that the above refer only to the early stages of the drug discovery process. As active compounds emerge against the current drug targets, this will create a demand for chemistry to support hit expansion and lead identification. Further on in the process, lead optimization will require yet more dedicated chemistry supported by resource for ADMET (absorption, distribution, metabolism, excretion, and toxicity) studies at the clinical level (Lin et al. 2003).

It is not the final numbers of targets we have considered here, rather, we have stressed on the important developments going on in target research and how insights into molecular reactivity with their inhibitors is bringing out active pharmaceutical ingredients against pathogenic helminths. We have considered helminthic enzymes such as polo like kinases, cathepsin B, microtubule, GST, ion channels such as K^+ and Ca^{2+}, cys-loop ligand gated; cell surface receptors (nAcR and DEG), latrophilin receptors and nuclear receptors (DAF); cell signaling wnt pathway and neuropeptide signalling; thioredoxin system as well as endosymbiotic bacteria *Wolbachia*. Many of these proteins and processes have been targeted for therapeutic purposes in mammals, which demonstrate at least the theoretical possibility that helminth specific compounds could be developed. Potential targets, in addition to those already discussed, could include the transporters that carry transmitters across plasma and vesicle membranes or the enzymes involved in the biosynthesis, processing, and degradation of

neurotransmitters especially small peptides. Some of these potential drug target candidates which further needed to be explored so as to screen, identify new lead compounds of anti-parasitic importance in near future are myophilin-like protein, lactate dehydrogenase, methionine aminopeptidase 2, thioredoxin peroxidase-2, aldose reductase, etc. (Hong et al. 2012; Huang et al. 2009; Huang et al. 2012; Liu et al. 2012; Lu et al. 2006; Peng et al. 2010a; Yang et al. 2006). There are also many downstream signaling proteins and chaperones that are essential for accurate neurotransmission and disruption of which, by genetic means, have been shown to be lethal or deleterious to *C. elegans*, but these potentially antiparasitic targets remains unexplored.

The current druggable biomolecules are very few, and the available drugs are being nullified in a small period of time very fast by rapid development of drug resistance possibly by section of drug resistance genes of helminths (Beech et al. 2011; James et al. 2009; Jones and George 2005). However, recent developments in genomics data for a number of helminths have paved the way for rapid drug discovery after identifying appropriate drug target and the constant research and development on these targets ultimately leading to potential drugs (Abubucker et al. 2011; Chuan et al. 2010; Hagen et al. 2011; Martin et al. 2011). The endosymbiotic bacteria *Wolbachia* is being approached with substantial amount of interdependent pathway enzymes with its host worm having certain enzymes unique to it (Wu et al. 2009). Therefore, the potential gains to be made from parasitic helminth genome projects are huge. Not only will they directly provide new insights—perhaps revealing novel metabolism to be exploited in drug discovery—but will also provide a welcome boost to pump prime activities throughout the helminth research community.

There is remarkable differences in the physiology of various helminths (Halton 2004; Parker et al. 2003; Von Brand 1948), and therefore target prioritization could be given according to the structural features as well as uniqueness of enzymes, receptors, ion channels, or biochemical pathways and the sensitivity of the these parasites towards small molecules that are potential drugs against these targets. Despite the structural differences between the morphological features of nematodes (the cuticle) and the morphological features of cestodes and trematodes (the tegument), the mechanism of drug entrance into both types of helminth depends on the lipophilicity of the anthelmintic compound which is the major physicochemical determinant for the drug to reach a therapeutic concentration in the body of parasite (Alvarez et al. 2007). There is no denying the fact that humility follows hand in hand with side effects of the drugs or development of drug resistance in medical and pharmaceutical research, and this is the driving force for developing newer drugs against debilitating helminth infections. The search for a novel drug targets against parasitic helminths remains a challenge in developing countries.

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