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Challenges and Promises for Obtaining New Antiprotozoal Drugs: What's Going Wrong?

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Contents

Abstract Infections by protozoa can cause some of the most serious human diseases, particularly in tropical regions. However, the number of available drugs used to treat such diseases tends to be limited with relatively high toxicity, and the vast majority of such drugs were developed in the 1920s to 1970s. The development of antiprotozoal drugs has been hindered owing in part to: (1) the highly complicated life cycles of such organisms and their ability to avoid innate immune defences; (2) challenges associated with culturing such organisms particularly in different phases of their growth and amplification; and (3) a lack of investment in biomedical research aimed at developing treatments for tropical diseases that do not tend to affect more affluent countries. Indeed, only three new drugs have entered into

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clinical trials in recent times, highlighting the tremendous gap in knowledge that should be bridged to more effectively treat protozoal infections.

Keywords Antiprotozoal drug, Drug resistance, Leishmania, Malaria, Parasitic protozoans

1 Introduction

Protozoans are microscopic, nonfilamentous protists widespread in most aquatic/soil habitats worldwide, being now considered as a paraphyletic group, with a complex evolutionary history [1, 2]. Most protozoans are heterotrophic organisms that acquire their nutrients from the environment, but there are examples of mixotrophic protozoans, such as *Paramecium* spp., which can also perform photosynthesis for their metabolic needs [1, 2]. Although such organisms are fascinating due to their multitude of phyla, genera, and species, many of which possess ecological and industrial importance, we will discuss here only the parasitic protozoans that infect humans and animals. They produce diseases which range from mild to moderate, such as those induced by *Toxoplasma gondii* or *Entamoeba histolytica*, to more serious conditions (e.g., infections due to *Cryptosporidium parvum*, *Giardia lamblia*, *Trichomonas vaginalis, Babesia* spp.) or very serious and widespread ones, such as malaria (infection due to at least five different species of *Plasmodium*), leishmaniasis (infectious protozoans are various species of *Leishmania*), Chagas disease (produced by *Trypanosoma cruzi*), and African sleeping disease (infection due to *Trypanosoma brucei*) [3–11], etc. Although rare, there are also several fatal protozoal diseases, such as those induced by amoebae belonging to the following three genera/species: *Naegleria fowleri* [12] *Acanthamoeba* spp. [13], or *Balamuthia mandrillaris* [13], for which few effective therapeutic approaches are available so far. Although the 12 protozoans genera which produce human disease are now well studied, there are still few available drugs for effectively treating these conditions. Furthermore, the drugs that are used have been available for decades, with high toxicity and low therapeutic indexes, and more concerning, extensive resistance to these treatment options has developed [3–5, 14–18]. Thus, what's going wrong? Why don't we have effective drugs for diseases that affect hundreds of millions of people worldwide, considering, for example, that for malaria alone there is an estimate of 229 million infections in 2019 linked to 409,000 fatalities [19]? We will attempt to answer these questions here.

2 Antiprotozoal Drugs in Clinical Use

One of the complications encountered when studying protozoans and approaches to inhibit their growth is related to the fact that most of them have quite complicated life cycles, with many different stages and also more than one host, with the vertebrate (human) being generally just one component in this intricate cycle [4–6, 16–18, 20, 21]. Taking as example again for malaria, which is provoked by at least five *Plasmodium* species which infect humans (i.e., *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae,* and *P. knowlesi*), the parasite is transmitted by mosquito bites (usually provoked by *Anopheles* mosquitoes) to vertebrates, infecting them, in a two-stage process. The first, pre-erythrocytic stage includes the following phases: (1) the plasmodial sporozoites are inoculated from mosquito salivary glands into the host through the bite; (2) the sporozoites reach the hepatocytes in the host's liver; (3) in the hepatocytes the parasite continues its development leading to schizonts which release into the bloodstream the merozoites, the final pathogenic form of the pre-erythrocytic stage. The second, asexual reproduction cycle (also known as blood-stage infection) includes: (1) the asexual multiplication of merozoites in red blood cells; (2) formation of immature ring stage trophozoites which consume the entire content of the invaded erythrocyte; (3) mature schizonts are formed in this way, which burst the red blood cell and release new merozoites into the bloodstream, which continue and enhance the infection of many other red blood cells. As it can easily be seen from this simplified description, *Plasmodia* have at least six different stages/phases during their life cycle, with the various forms of the pathogen present in different organs and tissues, but also with many different genes which are expressed in the different phases, and with a substantial capability to evade the host immune defences [4–6, 16–18, 20, 21]. This situation is also generally complicated for other pathogenic protozoans, which have complex life cycles and different secondary hosts (which can be different species of insects, for *T. cruzi, T. brucei,* and *Leishmania* spp.), or even other mammals, for example, *Toxoplasma gondii* (usually Felidae) [4, 5, 10]. In the case of *Cryptosporidium* spp., *Giardia lamblia*, *Entamoeba* spp., or *Trichomonas vaginalis* infections it seems that there is not an intermediate host, although these protozoans also have rather complex life cycles [6–9]. Overall, the complicated life cycles make it challenging to clearly identify promising druggable targets.

A second factor which is associated with difficulties in finding new/effective drugs for these pathogens is related to the fact that some of these organisms are difficult/impossible to grow in culture (e.g., *Cryptosporidium* spp.) [22] or their diverse stages/forms respond differently to drugs. Furthermore, some of these parasite stages are not at all prone to be grown in culture in order to allow a detailed study of the effectiveness of a drug during various stages of their life cycle [22–26].

Last but not least, many of the protozoan diseases are considered tropical diseases which affect a relatively low number of patients from poor countries. This is a very distorted reality, since as already mentioned, only malaria provokes a huge number of infections and many casualties each year. Furthermore, owing to climate change,

Table 1 Diseases provoked by pathogenic protozoans and the therapeutic agents used for their treatment

Disease	Pathogen	Drug
Malaria	*Plasmodium falciparum*	Quinine, chloroquine, primaquine;
	P. vivax, P. ovale, P. malariae, P. knowlesi	Artemisinin-based combination therapies
		Atovaquone; proguanil; clindamycin;
		Sulfadoxine, pyrimethamine, piperaquine
Leishmaniasis	*Leishmania* spp.	Antimonials (Sb(V) and Sb(III) derivatives)
		Amphotericin B; pentamidine; paromomycin;
		Miltefosine; antifungal azoles
Chagas disease	*Trypanosoma cruzi*	Nifurtimox; benznidazole
Sleeping sickness	*Trypanosoma brucei*	Pentamidine; suramin; nifurtimox; eflornithine
		Fexinidazole
Trichomoniasis	*Trichomonas vaginalis*	Metronidazole; tinidazole
Giardiasis	*Giardia lamblia*	Metronidazole; tinidazole; furazolidone
Entamoebiasis	*Entamoeba histolytica*	Metronidazole; tinidazole; paromomycin
Toxoplasmosis	*Toxoplasma gondii*	Pyrimethamine; sulfadiazine; clindamycin
Cryptosporidiosis	*Cryptosporidium* spp.	*Nitazoxanide*
Babesiosis	*Babesia* spp.	Clindamycin; quinine; diminazene

the enhance of temperatures in parts of Europe, North America, and Australia may soon create conditions for some of these "tropical" diseases to also appear (or reappear in some cases) in these parts of the world.

Table 1 shows the diseases provoked by protozoans in humans (and farm animals, in the case of *Babesia*) and the currently used drugs for their treatment. Only the two drugs shown in italics characters in Table 1, Fexinidazole and Nitazoxanide have been released for clinical use in the last 3 years [27, 28]. All other drugs shown in Table 1 were in fact discovered in the '30–'70s (except artemisinin and its derivatives, discovered in the '80s) and are characterized by rather high toxicity, low therapeutic index, and many side effects, although they are effective, especially in early phases of infection [4, 14–18]. Furthermore, just a limited number of chemotypes are present in the armamentarium of the antiprotozoal drugs, with the nitroazoles being predominant, followed by the dihydrofolate reductase and dihydropteroate synthase inhibitors [29] (Fig. 1). Although the recent approval of the two new agents Fexinidazole and Nitazoxanide is remarkable and demonstrates that relevant achievements can be obtained, both belong to the same class of nitroazoles.

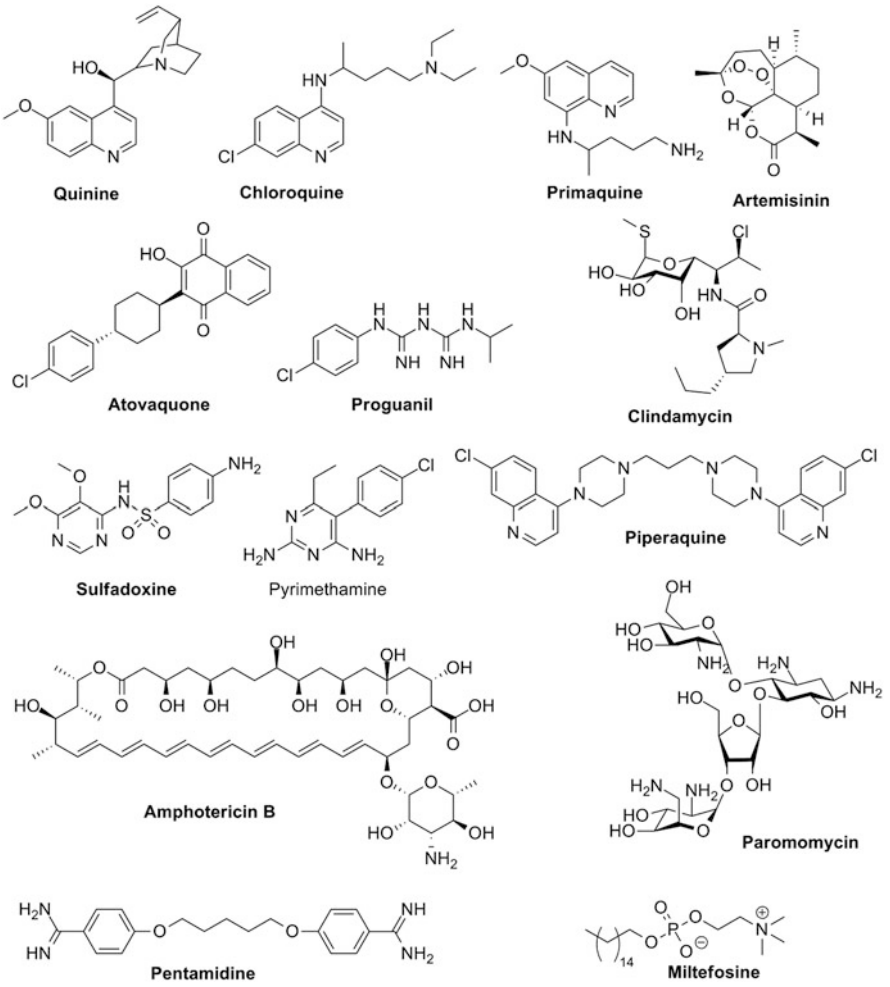


Fig. 1 Chemical structure of drugs currently used in the therapy of human diseases caused by protozoans

3 New Drugs and Compounds in Clinical Trials

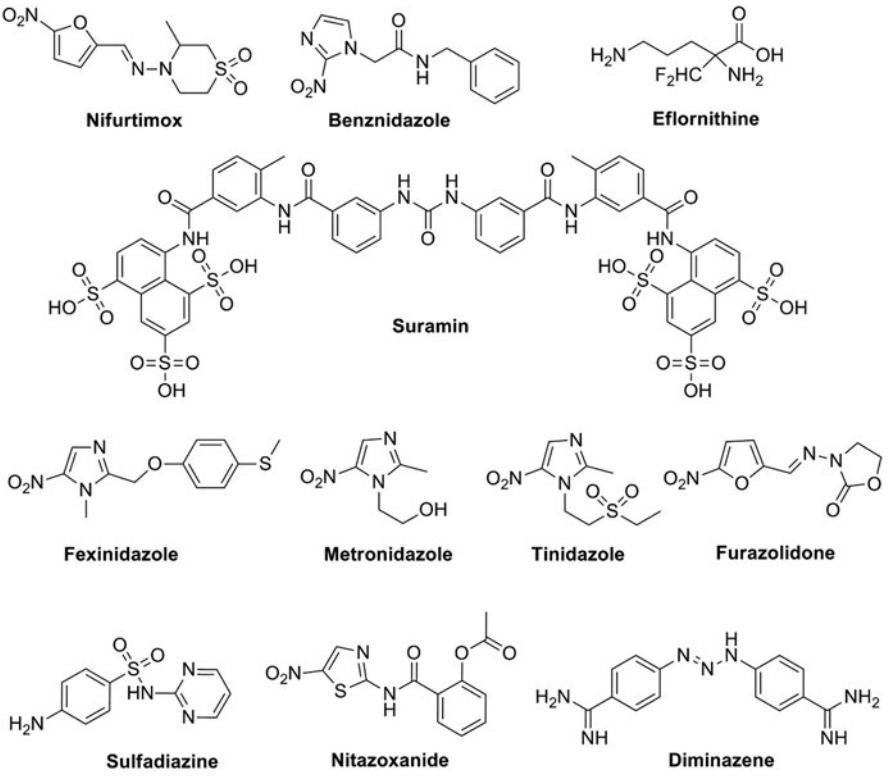
Acoziborole (SCYX-7158) (Fig. 2) is one of the few compounds in Phase III clinical trials for the treatment of *T. brucei* infection, being a benzoxaborole derivative, i.e., a totally new chemotype in the armamentarium of antiprotozoal drugs [30, 31]. Benzoxaboroles possess a range of pharmacological activities [32] in addition to antiprotozoal activity, including anti-bacterial, anti-fungal, antiviral as well as carbonic anhydrase inhibitory action [33–35].

Several new generation azoles such as ravuconazole and its prodrug (fos-ravuconazole) seem to be promising anti-*T. cruzi* agents [36], but there is

limited information regarding their clinical trials. It is rather disheartening to see that even for malaria, the worst of the documented protozoan diseases, most of the clinical trials that are registered in EU [37] deal with various vaccine candidates or combination therapies of existing drugs, but do not consider novel chemical entities.

4 Challenges for the Future after the COVID-19 Pandemic

The SARS-CoV-2 pandemic that emerged in late 2019 to early 2020 in China and spread all over the world [38, 39] should teach us that neglected diseases are a Sword of Damocles for the entire planet. The unprecedented (at least since 1918) crisis created by the outbreak of this viral disease demonstrated how susceptible the world is and how unprepared we were to tackle such a situation. As shown in this chapter, the number of protozoans is huge and many of them are poorly investigated and understood. Furthermore, such diseases are not restricted to tropical countries as some of the deadliest can be encountered in milder climates, including various *Amoeba* species that can provoke meningoencephalitis, which is difficult or



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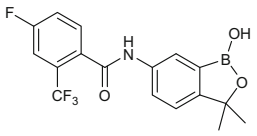

Acoziborole

Fig. 2 Chemical structure of Acoziborole

impossible to treat with currently available drugs [12, 13]. The number of available drugs is limited, restricted to a low number of clinical classes, and only a few stages of the parasite life cycle, which is by itself rather complex. The antiprotozoal drug targets are also quite limited, and although a relevant number of important discoveries have emerged by use of various 'omics methods over the previous two decades, there were essentially no significant translational studies from the lab to the clinic. Specifically, with the exception of two nitroazoles (see above), which were approved in the last 5 years, and the benzoxaborole derivative acoziborole (Fig. 2), no new drugs have emerged to treat protozoan-based diseases. What is going wrong? In addition to the challenges outlined above, there is also the perception that these are tropical diseases that will not affect people in affluent countries in which pharmaceutical research and large companies tend to be highly active. However, the tragic events of the last 18 months should remind us that this is no longer the case. Why do drug companies not invest in developing new antiprotozoal drugs, considering that the available ones are of low effectiveness and can have high toxicity? This situation should change, given the many interesting discoveries from academic researchers based all over the world that have emerged over recent decades, many of which are presented in the chapters of this book. By presenting an update of the state of the art in such diseases for nearly all protozoan infections, the current and broad gap in knowledge that needs to be bridged to develop excellent drugs for the treatment and management of these pathologies will become clearer.

Compliance with Ethical Standards *Conflict of Interest*: The authors declares that they have no conflict of interest.

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References

1. Cavalier-Smith T, Chao EE, Snell EA, Berney C, Fiore-Donno AM, Lewis R (2014) Multigene eukaryote phylogeny reveals the likely protozoan ancestors of opisthokonts (animals, fungi, choanozoans) and Amoebozoa. Mol Phylogenet Evol 81:71–85
2. Lax G, Lee WJ, Eglit Y, Simpson A (2019) Ploeotids represent much of the phylogenetic diversity of Euglenids. Protist 170(2):233–257
3. Carolino K, Winzeler EA (2020) The antimalarial resistome - finding new drug targets and their modes of action. Curr Opin Microbiol 57:49–55

4. Vermelho AB, Rodrigues GC, Supuran CT (2020) Why hasn't there been more progress in new Chagas disease drug discovery? Expert Opin Drug Discov 15(2):145–158
5. Vermelho AB, Capaci GR, Rodrigues IA, Cardoso VS, Mazotto AM, Supuran CT (2017) Carbonic anhydrases from *Trypanosoma* and *Leishmania* as anti-protozoan drug targets. Bioorg Med Chem 25(5):1543–1555
6. Mørch K, Hanevik K (2020) Giardiasis treatment: an update with a focus on refractory disease. Curr Opin Infect Dis 33(5):355–364
7. Urbański LJ, Di Fiore A, Azizi L, Hytönen VP, Kuuslahti M, Buonanno M, Monti SM, Angeli A, Zolfaghari Emameh R, Supuran CT, De Simone G, Parkkila S (2020) Biochemical and structural characterisation of a protozoan beta-carbonic anhydrase from *Trichomonas vaginalis*. J Enzyme Inhib Med Chem 35(1):1292–1299
8. Vinayak S (2020) Recent advances in genetic manipulation of *Cryptosporidium*. Curr Opin Microbiol 58:146–152
9. Bua S, Haapanen S, Kuuslahti M, Parkkila S, Supuran CT (2018) Sulfonamide inhibition studies of a new β-carbonic anhydrase from the pathogenic protozoan *Entamoeba histolytica*. Int J Mol Sci 19(12):3946
10. Dubey JP (2021) Outbreaks of clinical toxoplasmosis in humans: five decades of personal experience, perspectives and lessons learned. Parasit Vectors 14(1):263
11. Elsworth B, Duraisingh MT (2021) A framework for signaling throughout the life cycle of *Babesia* species. Mol Microbiol 115(5):882–890
12. Debnath A (2021) Drug discovery for primary amebic meningoencephalitis: from screen to identification of leads. Expert Rev Anti-Infect Ther. https://doi.org/10.1080/14787210.2021.1882302
13. Kofman A, Guarner J (2021) Free living amoebic infections: review. J Clin Microbiol 16: JCM0022821. https://doi.org/10.1128/JCM.00228-21
14. Pessanha de Carvalho L, Kreidenweiss A, Held J (2021) Drug repurposing: a review of old and new antibiotics for the treatment of malaria: identifying antibiotics with a fast onset of antiplasmodial action. Molecules 26(8):2304
15. Vallejo M, Reyes PP, Martinez Garcia M, Gonzalez Garay AG (2020) Trypanocidal drugs for late-stage, symptomatic Chagas disease (*Trypanosoma cruzi* infection). Cochrane Database Syst Rev 12(12):CD004102
16. de Araújo RV, Santos SS, Sanches LM, Giarolla J, El Seoud O, Ferreira EI (2020) Malaria and tuberculosis as diseases of neglected populations: state of the art in chemotherapy and advances in the search for new drugs. Mem Inst Oswaldo Cruz 115:e200229
17. Roatt BM, de Oliveira Cardoso JM, De Brito RCF, Coura-Vital W, de Oliveira Aguiar-Soares RD, Reis AB (2020) Recent advances and new strategies on leishmaniasis treatment. Appl Microbiol Biotechnol 104(21):8965–8977
18. Mansoldo FRP, Carta F, Angeli A, Cardoso VDS, Supuran CT, Vermelho AB (2020) Chagas disease: perspectives on the past and present and challenges in drug discovery. Molecules 25(22):5483
19. World Malaria Report (2020) 20 years of global progress and challenges. Geneva World Health Organization, Geneva
20. Krungkrai J, Krungkrai SR, Supuran CT (2007) Malarial parasite carbonic anhydrase and its inhibitors. Curr Top Med Chem 7(9):909–917
21. Krungkrai J, Krungkrai SR, Supuran CT (2008) Carbonic anhydrase inhibitors: inhibition of plasmodium falciparum carbonic anhydrase with aromatic/heterocyclic sulfonamides-in vitro and in vivo studies. Bioorg Med Chem Lett 18(20):5466–5471
22. Müller J, Hemphill A (2013) In vitro culture systems for the study of apicomplexan parasites in farm animals. Int J Parasitol 43(2):115–124
23. Krungkrai J, Prapunwatana P, Wichitkul C, Reungprapavut S, Krungkrai SR, Horii T (2003) Molecular biology and biochemistry of malarial parasite pyrimidine biosynthetic pathway. Southeast Asian J Trop Med Public Health 34(Suppl 2):32–43

24. da Silva Cardoso V, Vermelho AB, Ricci Junior E, Almeida Rodrigues I, Mazotto AM, Supuran CT (2018) Antileishmanial activity of sulphonamide nanoemulsions targeting the β-carbonic anhydrase from leishmania species. J Enzyme Inhib Med Chem 33(1):850–857
25. Vermelho AB, da Silva CV, Ricci Junior E, Dos Santos EP, Supuran CT (2018) Nanoemulsions of sulfonamide carbonic anhydrase inhibitors strongly inhibit the growth of Trypanosoma cruzi. J Enzyme Inhib Med Chem 33(1):139–146
26. Ramirez JL (2020) Trypanosoma cruzi genome 15 years later: what has been accomplished? Trop Med Infect Dis 5(3):129
27. Wang B, Castellanos-Gonzalez A, White Jr AC (2020) Novel drug targets for treatment of cryptosporidiosis. Expert Opin Ther Targets 24(9):915–922
28. Neau P, Hänel H, Lameyre V, Strub-Wourgaft N, Kuykens L (2020) Innovative partnerships for the elimination of human African trypanosomiasis and the development of fexinidazole. Trop Med Infect Dis 5(1):17
29. Capasso C, Supuran CT (2020) Dihydropteroate synthase (sulfonamides) and dihydrofolate reductase inhibitors. In: Bonev BB, Brown NM (eds) Bacterial resistance to antibiotics - from molecules to man. Wiley, pp 163–172
30. Dickie EA, Giordani F, Gould MK, Mäser P, Burri C, Mottram JC, Rao SPS, Barrett MP (2020) New drugs for human African trypanosomiasis: a twenty first century success story. Trop Med Infect Dis 5(1):29
31. Wall RJ, Rico E, Lukac I, Zuccotto F, Elg S, Gilbert IH, Freund Y, Alley MRK, Field MC, Wyllie S, Horn D (2018) Clinical and veterinary trypanocidal benzoxaboroles target CPSF3. Proc Natl Acad Sci U S A 115(38):9616–9621
32. Nocentini A, Supuran CT, Winum JY (2018) Benzoxaborole compounds for therapeutic uses: a patent review (2010-2018). Expert Opin Ther Pat 28(6):493–504
33. Bonardi A, Nocentini A, Cadoni R, Del Prete S, Dumy P, Capasso C, Gratteri P, Supuran CT, Winum JY (2020) Benzoxaboroles: new potent inhibitors of the carbonic anhydrases of the pathogenic bacterium *Vibrio cholerae*. ACS Med Chem Lett 11(11):2277–2284
34. Nocentini A, Cadoni R, Dumy P, Supuran CT, Winum JY (2018) Carbonic anhydrases from Trypanosoma cruzi and Leishmania donovani chagasi are inhibited by benzoxaboroles. J Enzyme Inhib Med Chem 33(1):286–289
35. Alterio V, Cadoni R, Esposito D, Vullo D, Fiore AD, Monti SM, Caporale A, Ruvo M, Sechi M, Dumy P, Supuran CT, De Simone G, Winum JY (2016) Benzoxaborole as a new chemotype for carbonic anhydrase inhibition. Chem Commun (Camb) 52(80):11983–11986
36. Mazzeti AL, Capelari-Oliveira P, Bahia MT, Mosqueira VCF (2021) Review on experimental treatment strategies against Trypanosoma cruzi. J Exp Pharmacol 13:409–432
37. https://www.clinicaltrialsregister.eu/ctr-search/search?query=Malaria. Accessed 28 Jun 2021
38. Supuran CT (2021) Coronaviruses. Expert Opin Ther Pat 31(4):291–294
39. Mori M, Capasso C, Carta F, Donald WA, Supuran CT (2020) A deadly spillover: SARS-CoV-2 outbreak. Expert Opin Ther Pat 30(7):481–485