Journal of Pharmaceutical Analysis 15 (2025) 101084

Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

journal homepage: www.elsevier.com/locate/jpa

Review paper

Exploration of innovative drug repurposing strategies for combating human protozoan diseases: Advances, challenges, and opportunities

ShanShan Hu, Zahra Batool, Xin Zheng, Yin Yang, Amin Ullah **, Bairong Shen *

Institutes for Systems Genetics, West China Hospital, Sichuan University, Chengdu, 610213, China

ARTICLE INFO

Article history:
Received 9 May 2024
Received in revised form
12 August 2024
Accepted 22 August 2024
Available online 27 August 2024

Keywords:
Drug repurposing
Human protozoan diseases
Antiprotozoal drugs
Combination therapy
Natural products

ABSTRACT

Protozoan infections (e.g., malaria, trypanosomiasis, and toxoplasmosis) pose a considerable global burden on public health and socioeconomic problems, leading to high rates of morbidity and mortality. Due to the limited arsenal of effective drugs for these diseases, which are associated with devastating side effects and escalating drug resistance, there is an urgent need for innovative antiprotozoal drugs. The emergence of drug repurposing offers a low-cost approach to discovering new therapies for protozoan diseases. In this review, we summarize recent advances in drug repurposing for various human protozoan diseases and explore cost-effective strategies to identify viable new treatments. We highlight the cross-applicability of repurposed drugs across diverse diseases and harness common chemical motifs to provide new insights into drug design, facilitating the discovery of new antiprotozoal drugs. Challenges and opportunities in the field are discussed, delineating novel directions for ongoing and future research.

1. Introduction

Pathogenic protozoa, a diverse polyphyletic group of single-celled eukaryotes, pose a serious threat to public health, contributing to high morbidity and mortality rates. Protozoan infections are predominantly epidemic in tropical and subtropical regions, affecting over a billion people worldwide [1]. There is an increasing incidence of this phenomenon in developed countries because of climate change events, global migration, and travel [2]. Unfortunately, several protozoan diseases have become so-called "neglected tropical diseases", for which experts, pharmaceutical firms, and financiers are less motivated to develop new drugs for prevention, diagnosis, or treatment [3]. Most protozoal pathogens have intricate life cycles, exhibit genetic diversity with multiple genes expressed in different phases, and can substantially avoid the immune systems of various eukaryotic or insect hosts [4]. Furthermore, the infection may persist through several stages, even in various host species, making it difficult to discover druggable antiprotozoal targets and, in some cases, requiring the evaluation of more potential drug candidates for inhibiting different pathogenic strains [5]. Hence, few drugs are available as first-line treatment regimens against these infections. Moreover, authorized drugs have been used for decades, often lacking effective activity. These drugs are associated with high toxicity, extensive side effects, and rapid development of resistance, all of which represent hurdles in terms of accessible therapy for patients [6].

Drug repurposing (also known as drug repositioning, reprofiling, or re-tasking) has attracted remarkable attention for its conspicuous advances over de novo drug discovery [7]. This approach aims to employ approved or investigational drugs that are outside the field of reference of the original disease or target [8] (Fig. 1). Regarding drug repurposing, leveraging the well-established pharmacokinetics and safety profiles of existing drugs is beneficial and effective in overcoming bottlenecks and expediting the development of new therapies compared to conventional pharmacological treatments [9]. Computational methods, biological experimental approaches, or a combination of both are frequently employed in systematic drug repurposing efforts [10]. Screening potential hit or lead compounds from vast libraries using computational methods and subsequently confirming their activity through a series of biological experiments has proven to be cost-effective and of significant value in the discovery of novel therapeutic agents [11].

Two well-described reviews previously explored advances in the discovery of agents against major human protozoan diseases or

* Corresponding author.
** Corresponding author.
E-mail addresses: bairong.shen@scu.edu.cn (B. Shen), amindawar225@gmail.com (A. Ullah).

https://doi.org/10.1016/j.jpha.2024.101084

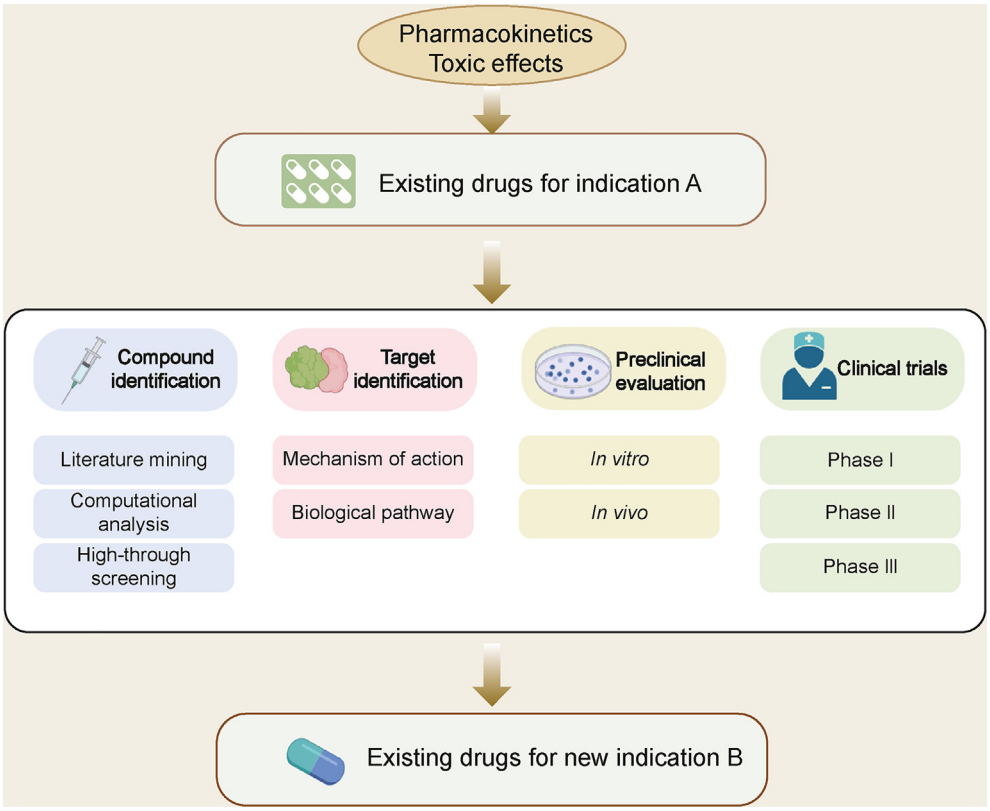

Fig. 1. The process of drug repurposing strategies. Figure created with www.BioRender.com.

antiprotozoal drugs under investigation that are repurposed for other disorders. However, neither of these approaches offers new insights into the mechanism by which these drugs act on protozoan diseases [12,13]. Due to advancements in biological experimental techniques, the tremendous accessibility of data resources, and the broad applications of computational approaches, substantial progress has been made in the development of new therapeutics for combating human protozoan diseases in recent years. In this review, we focused on exploring diverse new solutions to combat human protozoan diseases through drug repurposing, along with existing antiprotozoal drugs repurposed for other clinical manifestations. Nine typical protozoan diseases are reviewed, including Chagas disease, malaria, human African trypanosomiasis (HAT), leishmaniasis, cryptosporidiosis, giardiasis, babesiosis, toxoplasmosis, and amebiasis, spanning in severity from mild to life-threatening. Furthermore, challenges and opportunities in the field are briefly discussed, highlighting the pivotal role of artificial intelligence (AI) in drug repurposing strategies for developing innovative therapeutics against human protozoan diseases.

2. Overview of pathogenic protozoan diseases: impact, transmission, and clinical manifestations

Apicomplexans, a large phylum of single-cell, obligate intracellular protozoa, possess unique organelles for host cell invasion during their parasitic life cycle [14]. The protozoa included in this review, belonging to nine distinct types within this phylum, are responsible for zoonotic parasitic diseases and are closely associated with risk factors such as poor hygiene, contaminated water or food resources, and low educational levels [15]. For instance, cryptosporidiosis, giardiasis, and amebiasis infections via food ingestion or contaminated water intake with their respective parasites cause gastrointestinal symptoms such as diarrhea, abdominal cramps, and nausea. Diseases like babesiosis, cryptosporidiosis, giardiasis, amebiasis, and toxoplasmosis typically manifest as opportunistic infections in humans, in which people with healthy immune systems are usually asymptomatic. In contrast, such infections can be particularly devastating for immunocompromised individuals, such as infants, children under five years old, pregnant women, and those with viral-borne disorders [16].

Protozoan diseases frequently affect various organs and systems of the human body. Unfortunately, effective treatments or vaccines for their management are scarce. The symptoms of infected individuals can range from mild to moderate or even severe, depending on the characteristics of the parasitic protozoa (Table 1) [17].

3. Advances in repurposed drugs for protozoan diseases

3.1. Advances in severe protozoan diseases

Pathogenic protozoa, including *Plasmodium*, *Leishmania*, and *Trypanosoma*, have complex life cycles, causing severe clinical manifestations and extremely high morbidity and mortality rates [18]. Malaria is caused by *Plasmodium* parasites, and artemisinin derivatives are used as front-line drugs. However, emerging resistance poses a significant challenge to ongoing global malaria elimination efforts [19,20]. Chagas disease ranks closely behind malaria in terms of its global morbidity impact [21]. Chagas disease generally presents in both acute and chronic stages, with the chronic stage commonly linked to severe organ damage. Approximately 30% of infected patients progress to the chronic phase, culminating in lethal cardiac and digestive complications [9]. Only two nitro heterocyclic compounds, benznidazole and nitroimidazole, are currently licensed to treat Chagas disease in the acute phase [22]. HAT, commonly known as sleeping sickness, has seen a decreasing incidence of new

Table 1
The summary of main global burden diseases caused by pathogens, drugs, high prevalence regions, and organs involved for their managements [17].

Severity categories of protozoan diseases	Disease	Pathogens	Drugs	High prevalence regions	Human organs involved	Transmission/vector
Mild	Amebiasis	*Entamoeba histolytica*	Metronidazole, tinidazole, and paromomycin	Worldwide	Large intestine and colon, liver	Fecal-oral route and water or food contamination
	Toxoplasmosis	*T. gondii*	Pyrimethamine, sulfadiazine, and clindamycin		Brain, eyes, and muscles	
Moderate	Cryptosporidiosis	*C. parvum*	Nitazoxanide		Intestine and stomach	
	Giardiasis	*G. lamblia*	Metronidazole, tinidazole, and furazolidone		Intestine	
	Babesiosis	*Babesia* spp.	Azithromycin, clindamycin, atovaquone, quinine, and diminazene	Northeastern and upper Midwestern United States	Blood, bone marrow, spleen, and liver	Ticks bites, blood transfusion, and organ transplantation/Ixodes
Severe	Malaria	*P. falciparum*, *P. vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi*	Quinine, chloroquine, primaquine, artemisinin-based derivatives, atovaquone, proguanil, and clindamycin	Sub-Saharan Africa, Asia, South and Latin America, Middle East, South America, and Pacific Islands	Blood, spleen, liver, and brain	Mosquito bites/ Anopheles
	Leishmaniasis	*Leishmania* spp.	Sodium stibogluconate, meglumine antimoniate, amphotericin B, pentamidine, paromomycin, and miltefosine	South Asia, Africa, South America, and Middle East	Skin, spleen, liver, bone marrow, and lymph nodes	Sand fly bites/ Phlebotomus and Lutzomyia
	Chagas disease	*T. cruzi*	Nifurtimox and benznidazole	Latin American and Caribbean	Heart, esophagus, and colon	Triatomine bug bites/ Triatoma and Rhodnius
	HAT	*Trypanosoma brucei*	Pentamidine, suramin, nifurtimox, eflornithine, and fexinidazole	Sub-Saharan Africa	Brain, peripheral nerves, heart, liver, spleen, kidneys, skin, and eyes	Tse-tse fly bites/Tse-tse fly

T. gondii: *Toxoplasma gondii*; *C. parvum*: *Cryptosporidium parvum*; *G. lamblia*: *Giardia lamblia*; *P. falciparum*: *Plasmodium falciparum*; *P. vivax*: *Plasmodium vivax*; *T. cruzi*: *Trypanosoma cruzi*; HAT: human African trypanosomiasis.

cases in the last two decades and is now considered a controllable disease [23]. Leishmaniasis presents a broad spectrum of clinical manifestations, from cutaneous mucosal lesions to visceral lesions, with visceral leishmaniasis being the most severe and potentially fatal if untreated [24]. Despite numerous attempts to develop a universal treatment for leishmaniasis, currently available drugs are limited by severe adverse effects and the emergence of parasite resistance.

3.1.1. Malaria

Artemisinin derivatives are front-line drugs for malaria; however, emerging resistance poses a significant challenge to ongoing global malaria elimination efforts [20]. Some studies have focused on eliminating resistance to approved drugs, providing insights into the anti-malarial mode of action. Based on two specific strains, chloroquine-resistant and artemisinin-sensitive, a previous study systematically assessed three diverse sources of repurposed drugs. The results have shown that two anticancer drugs (idelalisib and 5-fluorouracil (5-FU)), an immunomodulator (imiquimod), and the antibiotic drug moxifloxacin displayed moderate-to-better inhibitory activity against malaria compared to other repurposed drug candidates [25]. However, the intrinsic mechanism underlying the activity of these drugs remains ambiguous. One important mechanism underlying these repurposed drugs, which exhibit anti-malarial activity and therapeutic efficacy against both artemisinin-sensitive and artemisinin-resistant parasites, is the inhibition of hemozoin formation, which has the potential to reduce the toxicity caused by the accumulation of free heme [26,27]. Disrupting the redox balance plays a crucial role in inducing parasite death by enhancing oxidative stress [28]. Another study utilized polypharmacology approaches, such as virtual screening and predictive computational tools, to reveal the effect of drugs approved by the U.S. Food and Drug Administration (FDA) on phosphatidylinositol-3-kinase, a target-inducing artemisinin resistance [29]. These results demonstrate that numerous repurposed drugs hold potential as adjunctive partners to existing anti-malarial regimens, offering a strategy to mitigate drug resistance.

Agents that target specific biological processes that are crucial to parasite functions can reduce the overall infection burden and disease severity. These processes include hindering parasite liver stage development [30] and affecting the metabolism of isoprenoids [31]. A study from the USA showed that tamoxifen can reduce parasitemia levels in treated mice by interfering with the biosynthetic pathway of *Plasmodium falciparum* (*P. falciparum*) [32].

3.1.2. Chagas disease

Most methods concentrate on one or two established targets for antiprotozoal agents, employing computational approaches to screen large amounts of potential drugs from U.S. FDA-approved libraries. These drugs are then further evaluated to determine their activity and efficiency *in vitro* and *in vivo*, which effectively expedites the drug discovery process. Ciprofloxacin and naproxen, chosen from over 130,000 3D protein structures involving a set of 20 targets in *Trypanosoma cruzi* (*T. cruzi*) survival, have demonstrated inhibitory efficacy both *in vitro* and *in vivo* [33]. Similarly, several drugs targeting dihydrofolate reductase-thymidylate synthase and nucleoside diphosphate kinases have been screened, including several antidiabetics [34] and two antihypertensive drugs

[35]. Another study used molecular docking to target triosephosphate isomerase, with the results demonstrating that several chemical compounds exhibited greater potency as trypanocidal molecules than conventional drugs such as nifurtimox and benznidazole [36].

Several studies have demonstrated high trypanocidal activity by altering or inhibiting the energy metabolism of *T. cruzi*. Proline permease participates in crucial processes of *T. cruzi*, such as intracellular differentiation and host cell infection. The crystal violet structural analogues, identified via similarity-based virtual screening, exhibit trypanocidal action in epimastigotes, trypomastigotes, and amastigotes of three *T. cruzi* strains by inhibiting proline uptake. Furthermore, it has been demonstrated that the combination of benznidazole and the crystal violet chemical analogue work synergistically to suppress the action of *T. cruzi* [37]. Two repurposed drugs, isotretinoin [38,39] and carvedilol [40], appear to affect the autophagic and apoptotic processes of *T. cruzi* to alleviate the parasite burden, thereby impeding the proliferation of amastigotes, epimastigotes, and trypomastigotes. Manidipine has displayed an attractive selectivity index with a half maximal drug inhibitory concentration (IC_{50}) of 0.1 μM in amastigotes and 3 μM in trypomastigotes, which disrupts mitochondrial function in *T. cruzi*, possibly constituting its most crucial lethal effect [41].

Broad-spectrum antiparasitic drugs represent another effective approach to ramping up drug discovery. For instance, nitazoxanide disrupts the anaerobic metabolism of protozoa by preventing pyruvate from binding with pyrophosphate, rendering it effective against various protozoa and helminths. Experimental evidence has shown its efficacy against *T. cruzi*, indirectly inducing the generation of immunoglobulin G antibodies and partially mitigating histopathological damage [42].

3.1.3. HAT

A novel and environment-friendly approach to ongoing programs addressing antiprotozoal diseases involves intervening in the vector metabolism pathway. Nitisinone substantially decreases the transmission of HAT by targeting the tyrosine metabolism pathway in tsetse flies, whether administered orally or topically [43].

3.1.4. Leishmaniasis

Inhibiting parasite growth is fundamentally linked to energy metabolism, particularly mitochondrial function. Cyclobenzaprine reduces intracellular adenosine triphosphate (ATP) levels in *Leishmania infantum* (*L. infantum*) promastigotes, exhibiting high antileishmanial activity *in vitro* [44]. The thiocarbamate derivative tolnaftate has also been demonstrated to possess leishmanicidal activity by inducing mitochondrial and cell membrane damage, ultimately leading to parasite death [45]. Pioglitazone and mupirocin were identified from an U.S. FDA-approved drug library as targeting mitochondrial primase, a critical enzyme for parasite replication and growth initiation [46]. Additionally, a mitochondrial primase that controls the DNA replication of *Leishmania donovani* has been identified as a crucial target for anti-leishmanial drugs. Based on *in silico* and *in vitro* drug repurposing approaches, capecitabine has shown promising activity against leishmaniasis [47]. Escitalopram has been documented to exhibit selective antileishmanial activity, with a half maximal effective concentration (EC_{50}) of 25 μM, causing disruption of a single mitochondrion and interference in the cell cycle [48]. Amodiaquine has also been shown to have significant and selective activity by activating oxidative mechanisms in *L. infantum* macrophages [49].

Alternative strategies employed in recent studies involve identifying pharmacologically active compounds capable of disrupting the metabolic pathways of the parasites. Acarbose, a specific inhibitor of glucosidase-like proteins associated with the *Leishmania* N-glycan biosynthesis metabolic pathway, has shown promising results in both *in vitro* and *in vivo* studies [50]. Through a high-throughput screening approach, clemastine fumarate has been demonstrated to significantly disrupt the inositol phosphoryl ceramide synthase of the metabolic pathway, exhibiting high activity and selectivity [51]. Pamidronate has also been shown to inhibit the enzyme farnesyl-pyrophosphate synthase, a crucial regulator of the mevalonate pathway, thereby reducing the number of infected *L. infantum* in monocyte subpopulations [52]. By regulating key pro-inflammatory cytokines to balance immune responses, chloroquine may serve as an effective anti-leishmanial agent for controlling infection [53]. Two experiments have shown that thioridazine, which has relatively safe and effective outcomes, inhibits parasitic growth by promoting the intracellular accumulation of waste products [54,55].

3.2. Advances in moderate protozoan diseases

Moderate symptoms are observed following infection with *Cryptosporidium parvum* (*C. parvum*), *Giardia lamblia* (*G. lamblia*), and different species of the genus *Babesia*, frequently involving gastrointestinal issues that require extended periods of treatment and recovery. The protozoan parasite *Cryptosporidium* (*C. parvum* and *Cryptosporidium hominis* (*C. hominis*)) is a leading cause of diarrheal disease [56]. *C. hominis* simply infects humans, whereas *C. parvum* is zoonotic and affects various vertebrate hosts [57]. Cryptosporidiosis, especially when associated with acquired immunodeficiency syndrome, results in a severe and prolonged illness characterized by persistent diarrhea and other debilitating symptoms [58]. *G. lamblia* (synonyms: *Giardia duodenalis* and *Giardia intestinalis*), which is responsible for human giardiasis, has developed a broad range of mechanisms to escape detection by the host immune system [59]. Despite its simple life cycle, the pathophysiology of giardiasis remains to be fully elucidated. Out of over 100 *Babesia* species, only four recognized species (*Babesia microti*, *Babesia divergens*, *Babesia duncani*, and *Babesia venatorum*) are known to cause human infections [60].

3.2.1. Cryptosporidiosis

Assessments of two new leads, vorinostat and docetaxel, which can target *Cryptosporidium* histone deacetylase, have demonstrated their potential for repurposing in the development of innovative anticryptosporidial drugs [61]. Pitavastatin, a human 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitor, can reduce the growth of *Cryptosporidium* via on-target inhibition of host HMG-CoA reductase [62].

3.2.2. Giardiasis

Acting on a specific enzyme in *G. lamblia* has been assigned a critical role in preventing infections. Two repurposed drugs, namely rabeprazole and omeprazole, have been shown to reduce trophozoite survival by hindering endogenous *Giardia* arginine deiminase [63]. Disulfiram specifically inactivates *G. lamblia* by modifying its cysteine 222, with no effect on the homologue enzyme [64]. Utilizing a structure-based virtual screening approach targeting the glycolytic enzyme triosephosphate isomerase, tolcapone and imatinib, with the highest average docking scores, were selected and found to exhibit a high level of inhibition against the growth of *G. lamblia* trophozoites [65].

3.2.3. Babesiosis

Tafenoquine received approval from the U.S. FDA in 2018 for treating *Plasmodium vivax* (*P. vivax*) infection and displayed activity against *Babesia* infection related to drug-induced oxidative stress [66].

3.3. Advances in mild protozoan diseases

Toxoplasma gondii (*T. gondii*) or *Entamoeba histolytica* (*E. histolytica*) can induce mild symptoms, such as mild gastrointestinal discomfort, fatigue, or other non-severe manifestations, with easy treatment and quick recovery. *T. gondii* affects approximately one-third of the global population [67]. Regarding human amebiasis, the primary challenge is that the illness is rarely detected in its early stages and only emerges in clinical settings when it becomes severe [68].

3.3.1. Toxoplasmosis

Two approved drugs, clofazimine and triclabendazole, targeting spermidine transport and II-type the reduced form of nicotinamide adenine dinucleotide (NAD) dehydrogenase, respectively, have demonstrated high selectivity against the growth of *T. gondii* with a reduction in replication [69].

The influence of cell apoptosis within the parasite has been primarily investigated in mouse models, with some evidence suggesting the inhibitory effects of parasites. Experiments in mice have revealed that mefloquine can induce cell apoptosis and may be used to treat acute and chronic toxoplasmosis [70]. The impact of auranofin on the early and late stages of chronic *T. gondii* infection revealed its ability to alter membrane permeability and induce apoptosis, which significantly decreased brain cyst burden and inflammatory reactions [71]. Bedaquilin disturbs the oxidative phosphorylation pathway, which is critical for the production of ATP by apicomplexans, leading to elevated reactive oxidative stress and autophagy within the parasite [72]. Guanabenz, which interferes with the translational control of *Toxoplasma*, has the potential to be repurposed as an effective anti-*Toxoplasma* agent, particularly because of its distinctive ability to reduce tissue cysts in the brain [73].

3.3.2. Amebiasis

Recently, two studies reported significant inhibitory effects against the *E. histolytica* parasite via drug repurposing strategies. Zinc ditiocarb offers insights into the inhibition of the constitutive photomorphogenesis 9 signalosome, a pivotal upstream regulator of parasite protein degradation, showing a high level of effective anti-amebic activity even at low nanomolar concentrations in both *in vitro* and *in vivo* experiments [74]. Terfenadine, once withdrawn from the market because of its risk of cardiac arrhythmia, has now been shown to interfere with trophozoite adhesion to Caco-2 cells and erythrophagocytosis, thus altering the molecular mechanisms associated with the virulence of *E. histolytica* [75].

3.4. Strategic insights into protozoan disease drug development: from drug repurposing to chemical structure

3.4.1. Comprehensive exploration of repurposed drugs: efficacy, mechanisms, and strategic directions

For this review, we analyzed and discussed the outcomes, mechanisms, and chemical structures of these repurposed drugs based on the literature we collected (Tables 2 [25–55,61–66,69–75] and S1). These identified drugs were found to primarily inhibit crucial functions of pathogenic protozoa, including energy metabolism, oxidative stress enhancement, and cell apoptosis induction.

Most experimental results were derived from *in vitro* studies, whereas only half were determined to have efficacy *in vivo*. The significant inhibitory effects observed *in vitro* and *in vivo* highlight the potency of these repurposed drugs. For instance, although the experiments were conducted on different cell lines, several repurposed drugs, including moxifloxacin, manidipine, cyclobenzaprine, and thioridazine, achieved IC_{50} values below 10 μM across various life cycle stages of parasitic protozoans (Table S1). In experiments in which infected mice were treated with these drugs, a notable reduction in parasite load was observed, along with a potent inhibition of parasite growth and a higher survival rate than that in the control groups.

Within this array of drugs, certain repurposed drugs may exhibit activity against multiple pathogenic protozoa (Table S2 [27,34,36,37,65,69,76–79]). For example, clofazimine, initially developed for the treatment of leprosy, has shown broad-spectrum efficacy against various pathogenic protozoa, including *T. cruzi* [37], *T. gondii* [69], *Babesia* [76], and *Cryptosporidium* [77,78]. Similarly, the tyrosine kinase inhibitor nilotinib demonstrated efficacy against malaria [27] and *T. cruzi* [34,36] parasites, whereas lapatinib exhibited inhibitory activity against both malaria [27] and HAT parasites [79]. Moreover, chlorhexidine, with halogen atoms and several hydrogen-bond donors in its structure, has shown inhibitory activity against *T. cruzi* [36] and *G. lamblia* [65].

Among the repurposed drugs mentioned, only clofazimine is currently under investigation in a Phase IIa clinical trial to evaluate its efficacy and safety in adults with human immunodeficiency virus (HIV) who present with cryptosporidiosis (clinical trials registration No.: NCT03341767) [77,78]. Although the therapeutic results did not demonstrate efficacy in severely affected HIV populations, this study underscores the potential of clofazimine for treating cryptosporidiosis.

To investigate the possible prioritization of these repurposed drugs, we conducted an analysis based on two key aspects. The first aspect considers the results of both *in vitro* and *in vivo* experiments. Five drugs, including bazedoxifene, 5-FU, moxifloxacin, thioridazine, and chloroquine, not only significantly inhibited the growth of the parasites but also demonstrated IC_{50} values below 10 μM. The second aspect evaluates the broad-spectrum applications of antiprotozoal drugs. In particular, anti-malarial drugs have underlying potential for treating various diseases. For example, chloroquine exhibits favorable characteristics in both aspects, suggesting its prioritization. In addition to chloroquine, another priority candidate, clofazimine, has demonstrated rapid progress in antiprotozoal drug development and has advanced into a series of clinical trials against cryptosporidiosis.

Translating these repurposed drugs from the bench to the bedside is a fundamental issue that requires careful consideration. Further, *in vivo* experiments are essential before progressing to clinical trials under government incentives and guidelines. We emphasize the potential of anti-malarial drugs as sources of insight to repurpose them against other protozoan infections. Furthermore, understanding the mechanisms underlying the bioactivation of repurposed drugs can provide insights into identifying potential targets. Although virtual screening has made significant strides in predicting biological activity and discovering new chemical entities targeting one or two specific molecular targets of interest, computational approaches in this field are still limited. AI algorithms, particularly deep learning approaches, have shown considerable success in drug development. Combining AI techniques can shed light on bioactive chemical compounds and pave the way for the discovery of additional innovative drugs. Consequently, we believe that AI-driven drug repurposing has enormous potential to advance antiprotozoal drug discovery.

3.4.2. Evolution of repurposed drugs against human protozoan diseases

In recent years, significant efforts have been made to discover repurposed drugs, which delineates their evolution within this review (Tables 2 and S2). The burgeoning studies of repurposed drugs for mild or moderate protozoan diseases highlight a pivotal role in exploring the intrinsic relationships among these diseases,

Table 2
The summary of repurposed drug for protozoan diseases.

Diseases	Drugs	Types	Repurposed progress	Targets/main functions	Year	Refs.
Malaria	Idelalisib	Antitumor	*In vitro* and *in vivo*	Unknown	2021	[25]
	5-FU	Antitumor				
	Imiquimod	Antiviral				
	Moxifloxacin	Antibacterial/antifungal				
	Bazedoxifene	Others	*In vitro* and *in vivo*	Inhibits hemozoin formation	2022	[26]
	Lomitapide	Cholesterol-lowering	*In silico* and *in vitro*		2020	[27]
	Nitrofurantoin	Antibacterial/antifungal	*In vitro* and *in vivo*	Enhances oxidative stress and induces parasite death	2023	[28]
	Oxetacaine	Others	*In silico*	PI3K/target	2021	[29]
	Simvastatin	Cholesterol-lowering				
	Repaglinide	Antidiabetic				
	Aclidinium	Others				
	Propafenone	Heart condition				
	Lovastatin	Cholesterol-lowering				
	Metformin	Antidiabetic	*In vivo*	Hinders the liver stage development of the parasites	2019	[30]
	Epirubicin	Antitumor	*In silico*, *in vitro*, and *in vivo*	Affects metabolism of isoprenoids	2020	[31]
	Tamoxifen	Antitumor	*In vitro* and *in vivo*	Interferes biosynthetic pathway of the parasites	2019	[32]
Chagas disease	Ciprofloxacin	Antibacterial/antifungal	*In silico*, *in vitro*, and *in vivo*	Frans-sialidase/target	2020	[33]
	Naproxen	Anti-inflammatory		Farnesyl pyrophosphate synthase/ target		
	Glipizide	Antidiabetic	*In silico* and *in vitro*	Dihydrofolate reductase-thymidylate synthase/target	2020	[34]
	Glyburide					
	Gliquidone					
	Nebivolol	Heart condition	*In silico* and *in vitro*	Nucleoside diphosphate kinases/target	2023	[35]
	Telmisartan					
	Protriptyline	Central nervous system	*In silico* and *in vitro*	Tim/target	2024	[36]
	Montelukast	Anti-inflammatory				
	Cyproheptadine	Antihistaminic	*In silico* and *in vitro*	Inhibits proline uptake	2020	[37]
	Loratadine	Antihistaminic				
	Olanzapine	Central nervous system				
	Isotretinoin	Anti-inflammatory	*In silico* and *in vitro*	Impacts autophagic and apoptotic	2017	[38]
			In vivo	processes	2023	[39]
	Carvedilol	Heart condition	*In silico*, *in vitro*, and *in vivo*		2021	[40]
	Manidipine	Heart condition	*In vitro*	Disrupts mitochondrial functions	2021	[41]
	Nitazoxanide	Antiparasitic	*In vivo*	Disrupts the anaerobic metabolism of protozoa	2023	[42]
HAT	Nitisinone	Others	*In vivo*	Intervenes the metabolism pathway of tsetse flies	2021	[43]
Leishmaniasis	Cyclobenzaprine	Central nervous system	*In vitro*	Reduces intracellular ATP level	2022	[44]
	Tolnaftate	Antibacterial/antifungal	*In vitro*	Induces mitochondria and cell membrane damage	2020	[45]
	Pioglitazone	Antidiabetic	*In silico* and *in vitro*	Mitochondrial primase/target	2022	[46]
	Mupirocin	Antibacterial/antifungal				
	Capecitabine	Antitumor	*In silico* and *in vitro*	LdmtPRI1/target	2024	[47]
	Escitalopram	Central nervous system	*In vitro*	Disrupts single mitochondrion and interfere in the cell cycle	2022	[48]
	Amodiaquine	Antiparasitic	*In vitro*	Activates oxidative mechanisms in *L. infantum*	2023	[49]
	Acarbose	Antidiabetic	*In vitro* and *in vivo*	Inhibits *Leishmania* N-glycan biosynthesis metabolic pathway	2021	[50]
	Clemastine fumarate	Antihistaminic	*In silico*, *in vitro*, and *in vivo*	Disrupts the inositol phosphorylceramide synthase of metabolic pathway	2021	[51]
	Pamidronate	Others	*In vitro*	Inhibits the enzyme farnesyl-pyrophosphate synthase	2022	[52]
	Chloroquine	Antiparasitic	*In vitro* and *in vivo*	Regulates key cytokines to balance immune responses, aids infection control, and parasite elimination	2024	[53]
	Thioridazine	Central nervous system	*In vitro* and *in vivo*	Promotes the intracellular	2024	[54]
			In vitro	accumulation of waste products	2024	[55]
Cryptosporidiosis	Docetaxel	Antitumor	*In vitro* and *in vivo*	*Cryptosporidium* histone deacetylases	2023	[61]
	Vorinostat	Antitumor		vorinostat/target		
	Pitavastatin	Cholesterol-lowering	*In silico* and *in vitro*	Inhibits host HMG-CoA reductase	2013	[62]
Giardiasis	Rabeprazole	Others	*In silico* and *in vitro*	Hinders endogenous *Giardia* arginine deiminase	2021	[63]
	Omeprazole	Others				
	Disulfiram	Others	*In vitro*	Modifies cysteine 222 of *Giardia lamblia*	2017	[64]
	Tolcapone	Central nervous system	*In silico* and *in vitro*	Triosephosphate isomerase/target	2021	[65]
	Imatinib	Antitumor				
Babesiosis	Tafenoquine	Antiparasitic	*In vivo*	Induces oxidative stress	2021	[66]
Toxoplasmosis	Triclabendazole	Antiparasitic	*In vitro*	II-type NADH dehydrogenase/target	2023	[69]
	Mefloquine	Antiparasitic	*In vivo*	Induces cells apoptosis	2023	[70]
	Auranofin	Anti-inflammatory	*In vivo*		2021	[71]

Table 2 (*continued*)

Diseases	Drugs	Types	Repurposed progress	Targets/main functions	Year	Refs.
	Bedaquiline	Antibacterial/antifungal	*In vitro* and *in vivo*	Disturbs oxidative phosphorylation pathway	2023	[72]
	Guanabenz	Heart condition	*In vitro* and *in vivo*	Interferes with translational control of Toxoplasma	2015	[73]
Amebiasis	Ditiocarb	Others	*In vitro* and *in vivo*	Inhibits upstream regulation of parasite protein degradation	2020	[74]
	Terfenadine	Antihistaminic	*In vitro*	Interferes with trophozoite adhesion to Caco-2 cells and erythrophagocytosis	2022	[75]

5-FU: 5-fluorouracil; PI3K: phosphatidylinositol-3-kinase; TIM: triosephosphate isomerase; HAT: human African trypanosomiasis; ATP: adenosine triphosphate; LdmtPRI1: mitochondrial primase 1 of *Leishmania donovani*; *L. infantum*: *Leishmania infantum*; HMG-CoA: 3-hydroxy-3-methyl-glutaryl-coenzyme A; NADH: nicotinamide adenine dinucleotide (NAD) in its reduced form.

as they offer the potential for discovering repurposed drugs to combat human protozoan diseases. Over time, diseases such as Chagas and leishmaniasis have garnered increasing attention, promoting a sustained escalation of related investigations given their life-threatening impact on humans, the scarcity of available drugs, and their classification as neglected tropical diseases. In the case of 2024, five and three repurposed drugs were found to display potential activities against *T. cruzi* and *L. infantum*, respectively.

3.4.3. Harnessing common chemical motifs for breakthroughs in antiprotozoal drug development

Most of these repurposed drugs feature heterocyclic chemotypes, which are vital in the core structures of numerous pharmaceuticals. Collectively, research into these diverse heterocyclic chemotypes may lead to innovative pharmaceutical development with promising biological activities in the future.

Two common chemical motifs, quinoline and imidazole, are recognized as indisputable pharmacophores, opening up new possibilities for medicinal chemists in the design, synthesis, and biological profiling of existing biomolecular compounds. Quinoline and imidazole have been granted considerable attention for their broad spectrum of anti-infectious activities. Quinoline, which is composed of benzene fused with N-heterocyclic pyridine, and its derivatives, has excellent potential for cross-fertilization among protozoal infections [80]. For example, four quinoline analogues, including amodiaquine [49] and chloroquine [53] against *Leishmania*, and tafenoquine [66] and mefloquine [70] against *Babesia* and *T. gondii*, have demonstrated antiprotozoal activities (Fig. 2). Moreover, the results indicate that anti-malarial drugs are frequently repurposed for treating other protozoal infections. Imidazole motifs have also been leveraged for future drug development against human protozoan diseases because of their substantial efficacy. On the basis of their chemical similarity, four imidazole-containing drugs (nitazoxanide, rabeprazole, omeprazole, and triclabendazole) exert valuable functions in combating human protozoan diseases (Fig. 2). Of note, there are many exciting opportunities for these common chemical motifs to provide novel insights into drug design, inform drug repurposing strategies, and even advance the understanding of the mechanisms of drug action. Using these two chemical groups as foundational scaffolds will lead to the design of vast arrays of new chemical entities, thereby spurring further in-depth investigations to identify additional antiprotozoal drug potentials.

4. Exploring additional cost-effective solutions for repurposing drugs for protozoan diseases

4.1. Natural products repurposed for protozoan diseases

Natural products derived from plants, animals, and microorganisms have been recognized as valuable sources of drug regimens, garnering increasing attention from researchers in recent decades. Compared to synthetic substances, natural medicines have made crucial and unique contributions to human health due to their easy accessibility and comparatively lower cost. Nevertheless, their efficacy is often limited by factors such as poor action, rapid metabolism, and difficulty in isolation. Exploring the repurposed therapeutic potential of natural products, particularly those with regulatory approval, represents a promising avenue in drug discovery for protozoan diseases, despite the limited availability of suitable sources.

Approved natural products have been used for the treatment of human protozoan diseases. Indeed, the well-known quinine and artemisinin and their derivatives, which are natural products used as anti-malarial agents, have successfully saved millions of lives [81]. Approved drugs obtained from natural products may show more potential, be attractive as alternative drug regimens, or exert synergistic functions for antiprotozoal infections. β-acetyl-digitoxin, a cardenolide isolated from *digitalis lanata* leaves, showed promising antileishmanial activity with low toxicity to healthy human cells and deserves further investigation to establish its potential to treat visceral leishmaniasis [82]. Moreover, rapamycin, also called sirolimus or rapamune, has been shown to exhibit high inhibition against *Leishmania* in a murine model at clinically relevant doses [83]. Furthermore, atropine sulfate, a salt form of atropine derived from nightshades, has shown anti-Cryptosporidium activity, with an EC_{50} value of 253.5 ± 30.3 nM and confirmed efficacy *in vivo* [84]. These natural products repurposed as antiprotozoal drugs were summarized in Table S3 [82–84].

4.2. Combinatorial therapy: repurposing and synergistic strategies for protozoan diseases

Combinatorial therapy is a complementary approach aimed at enhancing therapeutic efficacy through synergistic or additive chemopreventive effects against protozoan diseases. The combination of repurposed drugs and conventional agents, each with distinct mechanisms of action, might provide a valuable contribution to overcoming the toxicity associated with conventional drugs and serve to alleviate the collective impact of protozoal infections. Furthermore, combinatorial therapies could notably reduce the emergence of drug resistance through multiple mechanisms [85]. Although implementing such a strategy significantly broadens the spectrum of available drugs for clinical applications, careful assessment of the risk of adverse drug-drug interactions is imperative. Such interactions can profoundly impact drug efficacy and contribute to side effects or toxicity. Additionally, the use of multiple drugs may lead to more complex chemical mechanisms and potential uncertainties in drug pharmacokinetics or stability.

Conventional benznidazole combined with repurposed chloroquine can potentiate trypanocidal outcomes, reducing the required dose of benznidazole to achieve an effective response. Indeed,

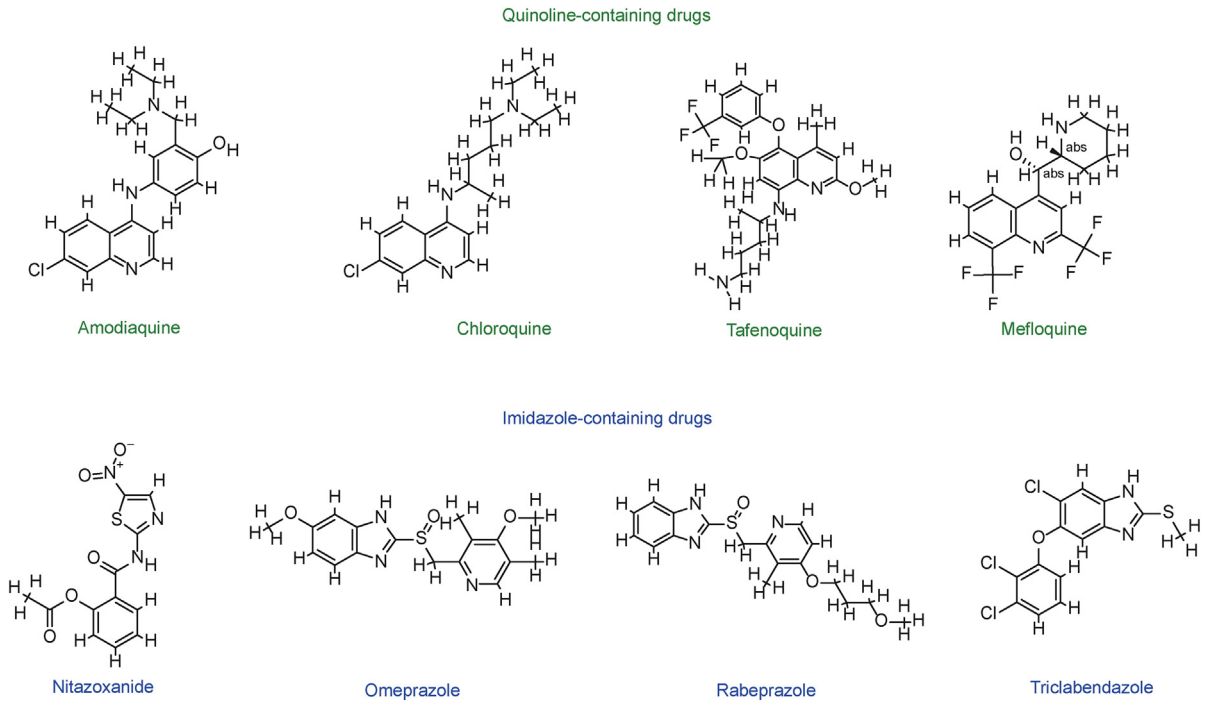

Fig. 2. The chemical structures of repurposed drugs with common chemical motifs are discussed in this article. Four quinoline-bearing chemical compounds (amodiaquine, chloroquine, tafenoquine, and mefloquine) are shown in green and four imidazole-containing drugs (nitazoxanide, rabeprazole, omeprazole, and triclabendazole) are shown in blue.

in vivo experiments demonstrated high treatment efficacy while mitigating the adverse effects of high-dose benznidazole [86]. Amiodarone, an antiarrhythmic agent for patients with chronic Chagas disease, exhibits trypanocidal activity. Well-established *in vivo* and *in vitro* experiments have demonstrated that benznidazole plus amiodarone therapy can attenuate cytoskeleton damage and induce cytotoxic effects [87]. The concomitant administration of benznidazole and miltefosine in both *in vitro* and *in vivo* models based on previous guidelines is a promising alternative in drug repurposing and combinatory therapy approaches for Chagas disease treatment [88]. The oral combination of miltefosine and nitrofurantoin has been revealed as a potential synergistic treatment of visceral leishmaniasis in both axenic and intracellular amastigotes from infected mouse spleen macrophages [89]. Triclabendazole, which can be administered orally, is a benzimidazole used to treat fasciolosis in adults and children. The combined administration of triclabendazole and amphotericin B demonstrated a synergistic effect *in vitro* against intracellular amastigote forms [90]. A recent study discovered that the combination of clofazimine and atovaquone is highly effective, resulting in a radical cure for babesiosis infections in immunocompromised mice. However, the use of clofazimine alone or clofazimine plus azithromycin, as well as atovaquone plus azithromycin, was ineffective and failed to completely eliminate the parasites under the same conditions [91].

5. Therapeutic potency of repurposed antiprotozoal drugs for various diseases

Repurposed existing antiprotozoal drugs, especially antimalarial drugs, have garnered attention for offering new therapeutic opportunities to treat diverse diseases over the past few years. Indeed, the non-profit organization Medicines for Malaria Venture (MMV) has launched Malaria Box and Pathogen Box, each comprising 400 diverse drug-like compounds with activity against malaria [92]. These open-access libraries have been successfully exploited as powerful tools for repurposing novel compounds as starting points for broad-spectrum diseases such as schistosomiasis [93], tuberculosis [94], fungal/viral infections [95], and other protozoan diseases [96–98].

Here, we present some recent instances of the application of antiprotozoal drugs beyond their canonical role (Fig. 3), including oncological diseases, autoimmune diseases, viral infections, and fungal/bacterial infections. In the future, with concerted efforts in preclinical studies and early clinical investigations, these drugs could show promise as viable treatments for a broad spectrum of human diseases.

5.1. Oncological diseases

Amodiaquine, an analogue of chloroquine, is a promising candidate for repurposing efforts that aim to block autophagic-lysosomal and proliferative processes in melanoma cells [99]. The widely used antimalarial drug mefloquine induces growth arrest and apoptosis of colorectal cancer cells in mouse culture [100]. Experiments *in vivo* demonstrated that pentamidine effectively mitigated tumor growth in clear-cell renal cell carcinoma mouse models [101]. Furthermore, a novel combination of antineoplastic agents (doxorubicin and paclitaxel) and antimalarial drugs (artesunate and chloroquine) demonstrated higher cytotoxicity and reduced cell viability in breast cancer cells compared with the use of antineoplastic agents alone [102,103]. These results demonstrate that antimalarial drugs can be repurposed for clinical cancer therapy as a single or synergistic agent.

5.2. Autoimmune diseases

Artemisinin and its derivatives can interfere with the lipid accumulation of orbital fibroblasts, which play a crucial role in differentiating into adipocytes in thyroid eye disease, indicating

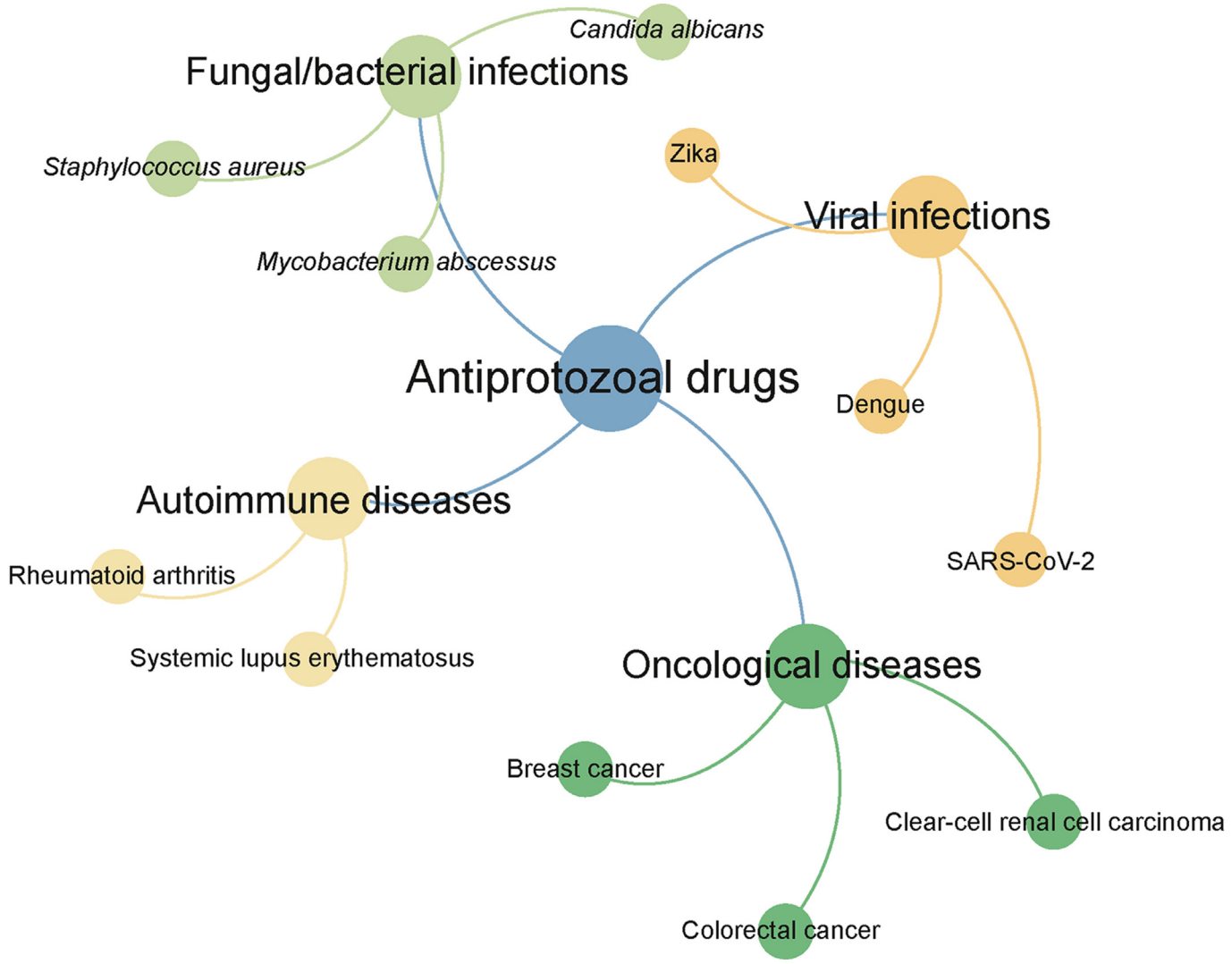

Fig. 3. Cases of applications of antiprotozoal drugs. SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

their potential as therapeutic agents [104]. Chloroquine and hydrochloroquine have immunomodulatory properties, and are reported as the current treatment guidelines for autoimmune diseases, including rheumatoid arthritis [105] and systemic lupus erythematosus [106].

5.3. Viral infections

With the emergence of the coronavirus disease 2019 (COVID-19) epidemic, numerous research studies have found that almost all anti-malarial drugs can effectively combat severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. For example, hydroxychloroquine, a hydroxyl analogue of chloroquine, was found to inhibit SARS-CoV-2 infection [107]. A series of experiments demonstrated that artemisinins have potential anti-SARS-CoV-2 activity and can be considered leading candidates for anti-SARS-CoV-2 drug research [108]. The *in silico* approach reported that doxycycline is effectively bound to the spike protein of SARS-CoV-2, representing a promising candidate for repurposing to fight COVID-19 [109]. The anti-malarial compound amodiaquine has also been shown to inhibit both Zika virus and dengue virus infection [110,111].

5.4. Fungal and bacterial infections

Mefloquine and its derivatives exhibit broad-spectrum antifungal activity against pathogenic yeasts and molds [112]. Artemisinin was determined to be a potent and adjunct inhibitor with the capability to target a regulator–dormancy survival regulator system, which can mediate hypoxic signaling in *Mycobacterium abscessus*, providing repurposing opportunities that could promptly translate into meaningful clinical applications [113]. Similarly, tafenoquine and quinacrine have been reported to be clinically active against the growth of *Staphylococcus aureus* [114] and *Candida albicans* [115], respectively.

6. Conclusions and future prospectives

Significant recent advances have been made in exploring potential therapies for combating human protozoan diseases through drug repurposing strategies. These developments highlight the versatility of existing approved drugs, suggesting their cross-applicability to diverse conditions beyond conventional treatments. The emergence of natural products and combination therapy has expanded novel treatment options for protozoan diseases,

whereas common chemical motifs have extended the scope of antiprotozoal drug design. Notably, anti-malarial drugs present a promising avenue for exploring repurposed strategies for diverse ailments.

Despite these achievements, formidable obstacles remain in this area, including inadequate financial incentives for research dedicated to preventing and treating protozoan infections. Moreover, most of the currently available drugs suffer from limitations such as high cost, poor patient compliance, resistance, low efficacy, and safety concerns. Furthermore, most surging studies remain in preclinical phases, where the risk of discontinuation is high due to failures in subsequent clinical trials. Moreover, potential drugs identified in recent studies have demonstrated their efficacy by acting as known targets or molecular mechanisms, which merely represent the tip of the iceberg in the biological processes of these parasitic organisms.

The growing availability of large-scale data repositories and open-access databases, along with advancements in multi-omics technologies and the emergence of computational tools characterizing intelligent algorithms reliant on high-performance computers, provide auspicious opportunities for discovering more effective repurposed drugs. Undoubtedly, the application of AI is poised as a highly effective strategy in drug repurposing, promising to impact the landscape of drug discovery for protozoan diseases in the foreseeable future. AI algorithms have the potential to significantly expedite the identification of novel repurposed drugs, offering renewed hope for tackling these challenging infectious pathogens. Drug repurposing strategies are poised to mark a paradigm shift in the methodology employed in this field.

CRediT authorship contribution statement

ShanShan Hu: Writing – original draft, Conceptualization, Funding acquisition, Writing – review & editing. **Zahra Batool:** Writing – review & editing, Visualization. **Xin Zheng:** Visualization. **Yin Yang:** Visualization. **Amin Ullah:** Writing – review & editing, Supervision. **Bairong Shen:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (Grant Nos.: 32270690 and 32070671) and the Postdoctor Research Fund of West China Hospital, Sichuan University, China (Grant No.: 2024HXBH059). Fig. 1 and Graphical abstract were created by www.BioRender.com. We thank LetPub (www.letpub.com.cn) for its linguistic assistance during the preparation of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpha.2024.101084.

References

[1] H.L.C. Santos, K.M. Rebello, An overview of mucosa-associated protozoa: Challenges in chemotherapy and future perspectives, Front. Cell. Infect. Microbiol. 12 (2022), 860442.
[2] Nisha, V. Mehra, M. Hopper, et al., Design and synthesis of β-amino alcohol based β-lactam–isatin chimeras and preliminary analysis of *in vitro* activity against the protozoal pathogen *Trichomonas vaginalis*, Med. Chem. Commun. 4 (2013) 1018–1024.
[3] S.S. Santos, R.V. de Araújo, J. Giarolla, et al., Searching for drugs for Chagas disease, leishmaniasis and schistosomiasis: A review, Int. J. Antimicrob. Agents 55 (2020), 105906.
[4] S. Consalvi, C. Tammaro, F. Appetecchia, et al., Malaria transmission blocking compounds: A patent review, Expert Opin. Ther. Pat. 32 (2022) 649–666.
[5] M. Vallejo, P.P. Reyes, M. Martinez Garcia, et al., Trypanocidal drugs for late-stage, symptomatic Chagas disease (*Trypanosoma cruzi* infection), Cochrane Database Syst. Rev. 12 (2020), CD004102.
[6] V. Roquini, A.C. Mengarda, R.A. Cajas, et al., The existing drug nifuroxazide as an antischistosomal agent: *In vitro*, *in vivo*, and *in silico* studies of macromolecular targets, Microbiol. Spectr. 11 (2023), e0139323.
[7] E.O.J. Porta, K. Kalesh, P.G. Steel, Navigating drug repurposing for chagas disease: Advances, challenges, and opportunities, Front. Pharmacol. 14 (2023), 1233253.
[8] D. Fontinha, I. Moules, M. Prudêncio, Repurposing drugs to fight hepatic malaria parasites, Molecules 25 (2020), 3409.
[9] L.N. Alberca, M.L. Sbaraglini, D. Balcazar, et al., Discovery of novel polyamine analogs with anti-protozoal activity by computer guided drug repositioning, J. Comput. Aided Mol. Des. 30 (2016) 305–321.
[10] J. Yu, Q. Dai, G. Li, Deep learning in target prediction and drug repositioning: Recent advances and challenges, Drug Discov. Today 27 (2022) 1796–1814.
[11] P.P. Parvatikar, S. Patil, K. Khaparkhuntikar, et al., Artificial intelligence: Machine learning approach for screening large database and drug discovery, Antivir. Res. 220 (2023), 105740.
[12] K.T. Andrews, G. Fisher, T.S. Skinner-Adams, Drug repurposing and human parasitic protozoan diseases, Int. J. Parasitol. Drugs Drug Resist. 4 (2014) 95–111.
[13] S.M. Lee, M.S. Kim, F. Hayat, et al., Recent advances in the discovery of novel antiprotozoal agents, Molecules 24 (2019), 3886.
[14] E. Vannier, B.E. Gewurz, P.J. Krause, Human babesiosis, Infect. Dis. Clin. N Am 22 (2008) 469–488.
[15] A.K. Mitra, A.R. Mawson, Neglected tropical diseases: Epidemiology and global burden, Trop. Med. Infect. Dis. 2 (2017), 36.
[16] N.M. Pazhayam, J. Chhibber-Goel, A. Sharma, New leads for drug repurposing against malaria, Drug Discov. Today 24 (2019) 263–271.
[17] C.T. Supuran, Antiprotozoal drugs: Challenges and opportunities, Expert Opin. Ther. Pat. 33 (2023) 133–136.
[18] M. Parsons, E.A. Worthey, P.N. Ward, et al., Comparative analysis of the kinomes of three pathogenic trypanosomatids: *Leishmania major*, *Trypanosoma brucei* and *Trypanosoma cruzi*, BMC Genomics 6 (2005), 127.
[19] N. Suresh, K. Haldar, Mechanisms of artemisinin resistance in *Plasmodium falciparum* malaria, Curr. Opin. Pharmacol. 42 (2018) 46–54.
[20] B. Hanboonkunupakarn, J. Tarning, S. Pukrittayakamee, et al., Artemisinin resistance and malaria elimination: Where are we now? Front. Pharmacol. 13 (2022), 876282.
[21] S. González, R.J. Wall, J. Thomas, et al., Short-course combination treatment for experimental chronic Chagas disease, Sci. Transl. Med. 15 (2023), eadg8105.
[22] J.A. Pérez-Molina, I. Molina, Chagas disease, Lancet 391 (2018) 82–94.
[23] P. Büscher, G. Cecchi, V. Jamonneau, et al., Human African trypanosomiasis, Lancet 390 (2017) 2397–2409.
[24] P.D. Ready, Epidemiology of visceral leishmaniasis, Clin. Epidemiol. 6 (2014) 147–154.
[25] K. Yadav, R. Shivahare, S.H. Shaham, et al., Repurposing of existing therapeutics to combat drug-resistant malaria, Biomed. Pharmacother. 136 (2021), 111275.
[26] R. Sudhakar, N. Adhikari, S. Pamnani, et al., Bazedoxifene, a postmenopausal drug, acts as an antimalarial and inhibits hemozoin formation, Microbiol. Spectr. 10 (2022), e0278121.
[27] A.C.C. de Sousa, K. Maepa, J.M. Combrinck, et al., Lapatinib, nilotinib and lomitapide inhibit haemozoin formation in malaria parasites, Molecules 25 (2020), 1571.
[28] S. Shafi, S. Gupta, R. Jain, et al., Tackling the emerging Artemisinin-resistant malaria parasite by modulation of defensive oxido-reductive mechanism via nitrofurantoin repurposing, Biochem. Pharmacol. 215 (2023), 115756.
[29] K. Verma, A.K. Lahariya, S. Dubey, et al., An integrated virtual screening and drug repurposing strategy for the discovery of new antimalarial drugs against *Plasmodium falciparum* phosphatidylinositol 3-kinase, J. Cell. Biochem. 122 (2021) 1326–1336.
[30] I.M. Vera, M.T. Grilo Ruivo, L.F. Lemos Rocha, et al., Targeting liver stage malaria with metformin, JCI Insight 4 (2019), e127441.
[31] L.T. Ferreira, J. Rodrigues, G.C. Cassiano, et al., Computational chemogenomics drug repositioning strategy enables the discovery of epirubicin as a new repurposed hit for *Plasmodium falciparum* and *P. vivax*, Antimicrob, Agents Chemother. 64 (2020), e02041-19.
[32] A. Weinstock, J. Gallego-Delgado, C. Gomes, et al., Tamoxifen activity against *Plasmodium in vitro* and in mice, Malar. J. 18 (2019), 378.

[33] M.F. Adasme, S.N. Bolz, L. Adelmann, et al., Repositioned drugs for Chagas disease unveiled via structure-based drug repositioning, Int. J. Mol. Sci. 21 (2020), 8809.
[34] A. Juárez-Saldivar, M. Schroeder, S. Salentin, et al., Computational drug repositioning for chagas disease using protein-ligand interaction profiling, Int. J. Mol. Sci. 21 (2020), 4270.
[35] F. Galceran, F.A. Digirolamo, M. Rengifo, et al., Identifying inhibitors of *Trypanosoma cruzi* nucleoside diphosphate kinase 1 as potential repurposed drugs for Chagas' disease, Biochem. Pharmacol. 216 (2023), 115766.
[36] A. Juarez-Saldivar, R. Gómez-Escobedo, G. Corral-Ruiz, et al., Repositioning FDA-approved drug against Chagas disease and cutaneous leishmaniosis by structure-based virtual screening, Arch. Med. Res. 55 (2024), 102958.
[37] M. Sayé, L. Gauna, E. Valera-Vera, et al., Crystal violet structural analogues identified by *in silico* drug repositioning present anti-*Trypanosoma cruzi* activity through inhibition of proline transporter TcAAAP069, PLoS Negl. Trop. Dis. 14 (2020), e0007481.
[38] C. Reigada, E.A. Valera-Vera, M. Sayé, et al., Trypanocidal effect of isotretinoin through the inhibition of polyamine and amino acid transporters in *Trypanosoma cruzi*, PLoS Negl. Trop. Dis. 11 (2017), e0005472.
[39] M.S. Rial, C. Reigada, N. Prado, et al., Effectiveness of the repurposed drug isotretinoin in an experimental murine model of Chagas disease, Acta Trop. 242 (2023), 106920.
[40] C.V. Rivero, S.J. Martínez, P. Novick, et al., Repurposing carvedilol as a novel inhibitor of the *Trypanosoma cruzi* autophagy flux that affects parasite replication and survival, Front. Cell. Infect. Microbiol. 11 (2021), 657257.
[41] I.T.S. Correa, T.A. da Costa-Silva, A.G. Tempone, Bioenergetics impairment of *Trypanosoma cruzi* by the antihypertensive manidipine: A drug repurposing strategy, Acta Trop. 214 (2021), 105768.
[42] M. Arce-Fonseca, R.A. Gutiérrez-Ocejo, J.L. Rosales-Encina, et al., Nitazoxanide: A drug repositioning compound with potential use in Chagas disease in a murine model, Pharmaceuticals 16 (2023), 826.
[43] M. Sterkel, L.R. Haines, A. Casas-Sánchez, et al., Repurposing the orphan drug nitisinone to control the transmission of African trypanosomiasis, PLoS Biol. 19 (2021), e3000796.
[44] M.L. Lima, M.A. Abengózar, E.C. Torres-Santos, et al., Energy metabolism as a target for cyclobenzaprine: A drug candidate against visceral leishmaniasis, Bioorg. Chem. 127 (2022), 106009.
[45] E.S. Yamamoto, J.A. de Jesus, A. Bezerra-Souza, et al., Tolnaftate inhibits ergosterol production and impacts cell viability of *Leishmania* sp, Bioorg. Chem. 102 (2020), 104056.
[46] P. Rai, H. Arya, S. Saha, et al., Drug repurposing based novel anti-leishmanial drug screening using *in-silico* and *in-vitro* approaches, J. Biomol. Struct. Dyn. 40 (2022) 10812–10820.
[47] M. Nath, D. Bhowmik, S. Saha, et al., Identification of potential inhibitor against *Leishmania donovani* mitochondrial DNA primase through *in-silico* and *in vitro* drug repurposing approaches, Sci. Rep. 14 (2024), 3246.
[48] M.L. Lima, M. Amaral, S.E.T. Borborema, et al., Evaluation of antileishmanial potential of the antidepressant escitalopram in *Leishmania infantum*, J. Pharm. Biomed. Anal. 209 (2022), 114469.
[49] L.M.R. Antinarelli, V. Midlej, E.D.S. da Silva, et al., Exploring the repositioning of the amodiaquine as potential drug against visceral leishmaniasis: The *in vitro* effect against *Leishmania infantum* is associated with multiple mechanisms, involving mitochondria dysfunction, oxidative stress and loss of cell cycle control, Chem. Biol. Interact. 371 (2023), 110333.
[50] R.R. Costa, J.A. Oliveira-da-Silva, T.A.R. Reis, et al., Acarbose presents *in vitro* and *in vivo* antileishmanial activity against *Leishmania infantum* and is a promising therapeutic candidate against visceral leishmaniasis, Med. Microbiol. Immunol. 210 (2021) 133–147.
[51] J.G.M. Mina, R.L. Charlton, E. Alpizar-Sosa, et al., Antileishmanial chemotherapy through clemastine fumarate mediated inhibition of the *Leishmania* inositol phosphorylceramide synthase, ACS Infect. Dis. 7 (2021) 47–63.
[52] J.M. Ribeiro, M.L. Rodrigues-Alves, E. Oliveira, et al., Pamidronate, a promising repositioning drug to treat leishmaniasis, displays antileishmanial and immunomodulatory potential, Int. Immunopharmacol. 110 (2022), 108952.
[53] N.R.D.L.P. Rossi, S.N. Fialho, A.J. Gouveia, et al., Quinine and chloroquine: Potential preclinical candidates for the treatment of tegumentary leishmaniasis, Acta Trop. 252 (2024), 107143.
[54] S. Sifontes-Rodríguez, N. Mollineda-Diogo, L. Monzote-Fidalgo, et al., *In vitro* and *in vivo* antileishmanial activity of thioridazine, Acta Parasitol. 69 (2024) 324–331.
[55] V. Kumar, S. Kumari, R. Ranjan, et al., *In vitro* antileishmanial activity of thioridazine on amphotericin B unresponsive/sensitive *Leishmania donovani* promastigotes and intracellular amastigotes, Exp. Parasitol. 257 (2024), 108688.
[56] F. Lanternier, K. Amazzough, L. Favennec, et al., *Cryptosporidium* spp. infection in solid organ transplantation: The nationwide "TRANSCRYPTO" study, Transplantation 101 (2017) 826–830.
[57] M.G. Nava, F. Calderón, E. Fernández, et al., Tyrosine kinase inhibitors display potent activity against *Cryptosporidium parvum*, Microbiol. Spectr. 11 (2023), e0387422.
[58] A. Diptyanusa, I.P. Sari, Treatment of human intestinal cryptosporidiosis: A review of published clinical trials, Int. J. Parasitol. Drugs Drug Resist. 17 (2021) 128–138.
[59] C. Gaona-López, A.V. Martínez-Vázquez, J.C. Villalobos-Rocha, et al., Analysis of *Giardia lamblia* nucleolus as drug target: A review, Pharmaceuticals (Basel) 16 (2023), 1168.
[60] C.A. Lobo, M. Singh, M. Rodriguez, Human babesiosis: Recent advances and future challenges, Curr. Opin. Hematol. 27 (2020) 399–405.
[61] M. Liu, D. Zhang, D. Wang, et al., Cost-effective *in vivo* and *in vitro* mouse models for evaluating anticryptosporidial drug efficacy: Assessing vorinostat, docetaxel, and baicalein, J. Infect. Dis. 228 (2023) 1430–1440.
[62] K. Bessoff, A. Sateriale, K.K. Lee, et al., Drug repurposing screen reveals FDA-approved inhibitors of human HMG-CoA reductase and isoprenoid synthesis that block *Cryptosporidium parvum* growth, Antimicrob. Agents Chemother. 57 (2013) 1804–1814.
[63] C. Fernández-Lainez, I. de la Mora-de la Mora, I. García-Torres, et al., Multilevel approach for the treatment of giardiasis by targeting arginine deiminase, Int. J. Mol. Sci. 22 (2021), 9491.
[64] A. Castillo-Villanueva, Y. Rufino-González, S.T. Méndez, et al., Disulfiram as a novel inactivator of *Giardia lamblia* triosephosphate isomerase with antigiardial potential, Int. J. Parasitol. Drugs Drug Resist. 7 (2017) 425–432.
[65] A. Juárez-Saldivar, E. Barbosa-Cabrera, E.E. Lara-Ramírez, et al., Virtual screening of FDA-approved drugs against triose phosphate isomerase from *Entamoeba histolytica* and *Giardia lamblia* identifies inhibitors of their trophozoite growth phase, Int. J. Mol. Sci. 22 (2021), 5943.
[66] M. Liu, S. Ji, D. Kondoh, et al., Tafenoquine is a promising drug candidate for the treatment of babesiosis, Antimicrob. Agents Chemother. 65 (2021), e0020421.
[67] M. Montazeri, S. Mehrzadi, M. Sharif, et al., Activities of anti-*Toxoplasma* drugs and compounds against tissue cysts in the last three decades (1987 to 2017), a systematic review, Parasitol. Res. 117 (2018) 3045–3057.
[68] M. Roy, S. Chakraborty, S. Kumar Srivastava, et al., *Entamoeba histolytica* induced NETosis and the dual role of NETs in amoebiasis, Int. Immunopharmacol. 118 (2023), 110100.
[69] M.M. Corvi, F. Rossi, A. Ganuza, et al., Triclabendazole and clofazimine reduce replication and spermine uptake *in vitro* in *Toxoplasma gondii*, Parasitol. Res. 123 (2023), 69.
[70] B.M. El Sharazly, I.A. Aboul Asaad, N.A. Yassen, et al., Mefloquine loaded niosomes as a promising approach for the treatment of acute and chronic toxoplasmosis, Acta Trop. 239 (2023), 106810.
[71] I.F. Abou-El-Naga, N.M.F.H. Mogahed, Repurposing auranofin for treatment of experimental cerebral toxoplasmosis, Acta Parasitol. 66 (2021) 827–836.
[72] Y. Shi, Y. Jiang, H. Qiu, et al., Mitochondrial dysfunction induced by bedaquiline as an anti-*Toxoplasma* alternative, Vet. Res. 54 (2023), 123.
[73] I. Benmerzouga, L.A. Checkley, M.T. Ferdig, et al., Guanabenz repurposed as an antiparasitic with activity against acute and latent toxoplasmosis, Antimicrob. Agents Chemother. 59 (2015) 6939–6945.
[74] S. Ghosh, L. Farr, A. Singh, et al., COP9 signalosome is an essential and druggable parasite target that regulates protein degradation, PLoS Pathog. 16 (2020), e1008952.
[75] I.A. Rangel-Castañeda, A. Castillo-Romero, G. León-ávila, et al., Drug repositioning: Antiprotozoal activity of terfenadine against *Entamoeba histolytica* trophozoites, Parasitol. Res. 121 (2022) 303–309.
[76] B. Tuvshintulga, E. Vannier, D.S. Tayebwa, et al., Clofazimine, a promising drug for the treatment of *Babesia microti* infection in severely immunocompromised hosts, J. Infect. Dis. 222 (2020) 1027–1036.
[77] P.I. Tam, S.L.M. Arnold, L.K. Barrett, et al., Clofazimine for treatment of cryptosporidiosis in human immunodeficiency virus infected adults: An experimental medicine, randomized, double-blind, placebo-controlled phase 2a trial, Clin. Infect. Dis. 73 (2021) 183–191.
[78] C.X. Zhang, M.S. Love, C.W. McNamara, et al., Pharmacokinetics and pharmacodynamics of clofazimine for treatment of cryptosporidiosis, Antimicrob. Agents Chemother. 66 (2022), e0156021.
[79] K.A. Bachovchin, A. Sharma, S. Bag, et al., Improvement of aqueous solubility of lapatinib-derived analogues: Identification of a quinolinimine lead for human African trypanosomiasis drug development, J. Med. Chem. 62 (2019) 665–687.
[80] P. Yadav, K. Shah, Quinolines, a perpetual, multipurpose scaffold in medicinal chemistry, Bioorg. Chem. 109 (2021), 104639.
[81] D.G.I. Kingston, M.B. Cassera, Antimalarial natural products, Prog. Chem. Org. Nat. Prod. 117 (2022) 1–106.
[82] C.S. Freitas, D.P. Lage, J.A. Oliveira-da-Silva, et al., *In vitro* and *in vivo* antileishmanial activity of β-acetyl-digitoxin, a cardenolide of *Digitalis lanata* potentially useful to treat visceral leishmaniasis, Parasite 28 (2021), 38.
[83] F. Khadir, T. Taheri, S. Habibzadeh, et al., Antileishmanial effect of rapamycin as an alternative approach to control *Leishmania tropica* infection, Vet. Parasitol. 276 (2019), 108976.
[84] M.H.B. Kabir, F.C. Recuenco, N.K. Mohd Zin, et al., Identification of potent anti-*Cryptosporidium* new drug leads by screening traditional Chinese medicines, PLoS Negl. Trop. Dis. 16 (2022), e0010947.
[85] W. Sun, P.E. Sanderson, W. Zheng, Drug combination therapy increases successful drug repositioning, Drug Discov. Today 21 (2016) 1189–1195.
[86] R.P. Pandey, M.S. Nascimento, C.H. Franco, et al., Drug repurposing in chagas disease: Chloroquine potentiates benznidazole activity against *Trypanosoma cruzi in vitro* and *in vivo*, Antimicrob. Agents Chemother. 66 (2022), e0028422.
[87] J.M.C. Barbosa, Y. Pedra-Rezende, L.D. Pereira, et al., Benznidazole and amiodarone combined treatment attenuates cytoskeletal damage in *Trypanosoma cruzi*-infected cardiac cells, Front. Cell. Infect. Microbiol. 12 (2022), 975931.
[88] J.E.N. Gulin, M.M.C. Bisio, D. Rocco, et al., Miltefosine and benznidazole combination improve anti-*Trypanosoma cruzi in vitro* and *in vivo* efficacy, Front. Cell. Infect. Microbiol. 12 (2022), 855119.

[89] E. Melcon-Fernandez, G. Galli, C. García-Estrada, et al., Miltefosine and nifuratel combination: A promising therapy for the treatment of *Leishmania donovani* visceral leishmaniasis, Int. J. Mol. Sci. 24 (2023), 1635.
[90] B.S. Borges, G.P. Bueno, F. Tomiotto-Pellissier, et al., *In vitro* anti-*Leishmania* activity of triclabendazole and its synergic effect with amphotericin B, Front. Cell. Infect. Microbiol. 12 (2022), 1044665.
[91] B. Tuvshintulga, T. Sivakumar, A.B. Nugraha, et al., Combination of clofazimine and atovaquone as a potent therapeutic regimen for the radical cure of *Babesia microti* infection in immunocompromised hosts, J. Infect. Dis. 225 (2022) 238–242.
[92] S. Duffy, M.L. Sykes, A.J. Jones, et al., Screening the medicines for malaria venture pathogen box across multiple pathogens reclassifies starting points for open-source drug discovery, Antimicrob. Agents Chemother. 61 (2017), e00379-17.
[93] M. Maccesi, P.H.N. Aguiar, V. Pasche, et al., Multi-center screening of the Pathogen Box collection for schistosomiasis drug discovery, Parasit. Vectors 12 (2019), 493.
[94] D. Cantillon, A. Goff, S. Taylor, et al., Searching for new therapeutic options for the uncommon pathogen *Mycobacterium chimaera*: An open drug discovery approach, Lancet Microbe 3 (2022) e382–e391.
[95] B. Pan, H. Weerasinghe, A. Sezmis, et al., Leveraging the MMV pathogen box to engineer an antifungal compound with improved efficacy and selectivity against *Candida auris*, ACS Infect. Dis. 9 (2023) 1901–1917.
[96] M.F. Canever, L.C. Miletti, Screening and identification of pathogen box® compounds with anti-*Trypanosoma evansi* activity, Acta Trop. 206 (2020), 105421.
[97] F.F. Boyom, P.V. Fokou, L.R. Tchokouaha, et al., Repurposing the open access malaria box to discover potent inhibitors of *Toxoplasma gondii* and *Entamoeba histolytica*, Antimicrob. Agents Chemother. 58 (2014) 5848–5854.
[98] A. López-Arencibia, I. Sifaoui, M. Reyes-Batlle, et al., Discovery of new chemical tools against *Leishmania amazonensis* via the MMV pathogen box, Pharmaceuticals (Basel) 14 (2021), 1219.
[99] S. Qiao, S. Tao, M. Rojo de la Vega, et al., The antimalarial amodiaquine causes autophagic-lysosomal and proliferative blockade sensitizing human melanoma cells to starvation- and chemotherapy-induced cell death, Autophagy 9 (2013) 2087–2102.
[100] X. Xu, J. Wang, K. Han, et al., Antimalarial drug mefloquine inhibits nuclear factor kappa B signaling and induces apoptosis in colorectal cancer cells, Cancer Sci. 109 (2018) 1220–1229.
[101] L.F. Zerbini, M.K. Bhasin, J.F. de Vasconcellos, et al., Computational repositioning and preclinical validation of pentamidine for renal cell cancer, Mol. Cancer Ther. 13 (2014) 1929–1941.
[102] D. Duarte, M. Nunes, S. Ricardo, et al., Combination of antimalarial and CNS drugs with antineoplastic agents in MCF-7 breast and HT-29 colon cancer cells: Biosafety evaluation and mechanism of action, Biomolecules 12 (2022), 1490.
[103] D. Duarte, N. Vale, New trends for antimalarial drugs: Synergism between antineoplastics and antimalarials on breast cancer cells, Biomolecules 10 (2020), 1623.
[104] Y. Guo, Y. Cheng, H. Li, et al., The potential of artemisinins as novel treatment for thyroid eye disease by inhibiting adipogenesis in orbital fibroblasts, Invest. Ophthalmol. Vis. Sci. 64 (2023), 28.
[105] J. Ma, J. Jing, J. Wang, et al., A novel function of artesunate on inhibiting migration and invasion of fibroblast-like synoviocytes from rheumatoid arthritis patients, Arthritis Res. Ther. 21 (2019), 153.
[106] E. Schrezenmeier, T. Dörner, Mechanisms of action of hydroxychloroquine and chloroquine: Implications for rheumatology, Nat. Rev. Rheumatol. 16 (2020) 155–166.
[107] X. Yao, F. Ye, M. Zhang, et al., *In vitro* antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Clin. Infect. Dis. 71 (2020) 732–739.
[108] R. Cao, H. Hu, Y. Li, et al., Anti-SARS-CoV-2 potential of artemisinins *in vitro*, ACS Infect. Dis. 6 (2020) 2524–2531.
[109] C. Sachdeva, A. Wadhwa, A. Kumari, et al., *In silico* potential of approved antimalarial drugs for repurposing against COVID-19, OMICS 24 (2020) 568–580.
[110] Y. Han, H.T. Pham, H. Xu, et al., Antimalarial drugs and their metabolites are potent Zika virus inhibitors, J. Med. Virol. 91 (2019) 1182–1190.
[111] S. Boonyasuppayakorn, E.D. Reichert, M. Manzano, et al., Amodiaquine, an antimalarial drug, inhibits dengue virus type 2 replication and infectivity, Antiviral Res. 106 (2014) 125–134.
[112] M.C. Montoya, S. Beattie, K.M. Alden, et al., Derivatives of the antimalarial drug mefloquine are broad-spectrum antifungal molecules with activity against drug-resistant clinical isolates, Antimicrob. Agents Chemother. 64 (2020), e02331-19.
[113] J.M. Belardinelli, D. Verma, W. Li, et al., Therapeutic efficacy of antimalarial drugs targeting DosRS signaling in *Mycobacterium abscessus*, Sci. Transl. Med. 14 (2022), eabj3860.
[114] P. She, Y. Yang, L. Li, et al., Repurposing of the antimalarial agent tafenoquine to combat MRSA, mSystems 8 (2023), e0102623.
[115] V.V. Kulkarny, A. Chavez-Dozal, H.S. Rane, et al., Quinacrine inhibits *Candida albicans* growth and filamentation at neutral pH, Antimicrob. Agents Chemother. 58 (2014) 7501–7509.