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# Drug repositioning approaches to parasitic diseases: a medicinal chemistry perspective

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**Identifying new indications for clinically useful drugs is a worthwhile approach for neglected tropical diseases. The number of successful repurposing cases in the field is growing as not-for-profit organizations, in association with academia and pharmaceutical companies, enable screening campaigns for the identification of new repositioning candidates. Current programs have delivered encouraging results as the use of state-of-the-art technologies, such as genomic and structural biology tools, and high-throughput screening platforms have become increasingly common in infectious disease research. Drug repositioning has played a key part in improving the lives of those suffering from these conditions, as evidenced by successful precedents and recent studies on preeminent parasitic disorders.**

## Neglected tropical diseases and drug discovery

Neglected tropical diseases (NTDs) are a group of 17 conditions mostly caused by parasitic organisms that affect nearly one billion people, mainly in impoverished environments [1]. As a common feature, NTDs flourish in settings where substandard housing and precarious sanitary conditions promote the proliferation of insects and other vectors that are responsible for their transmission. The impact caused by these disorders in terms of irreversible injuries and premature deaths is translated into an index called Disability-Adjusted Life Years (DALYs), an indicator that now totals 26 million (http://www.who.int/neglected_diseases/9789241564861/en/).

Although NTDs prevail in low-income countries and have traditionally been overlooked by global health agendas, population mobility has broadened the horizon of such diseases. Currently, NTDs can be found in several wealthy nations in North America and Europe, a scenario that has contributed to an improved surveillance system worldwide and has triggered a significant growth in drug discovery funding, specifically through public–private partnerships (PPPs) [2]. These initiatives have been implemented in the past 10 years as global collaborative networks between pharmaceutical companies, not-for-profit agencies and research institutions, consisting of the state-of-the-art executive model for drug discovery and development in NTDs [3]. Notwithstanding these timely efforts, current chemotherapy is restricted to a few options that are known for their limited efficacy and safety issues, and the R&D pipeline has not been satisfactorily filled [4]. Therefore, finding novel, effective and safe drugs remains an urgent demand for these global health problems.

However, addressing this need is not a trivial enterprise, especially considering the history of drug discovery in the field, which until recently was hampered by factors such as limited access to high-quality compounds, the wide predominance of low-technology methods and a lack of robust *in vitro* assay techniques [5,6]. Likewise, a poor understanding of the metabolism of the disease-causing parasites and the absence of molecular biology tools to manipulate these organisms are among the major bottlenecks that have delayed the development of knowledge-based and molecular drug discovery approaches [6]. This scenario began to change a decade ago when pioneering publications on the genome of these pathogens started to reveal the molecular machinery implicated in their biological processes [7–10]. These breakthrough discoveries have since been vital to the identification and characterization of several potential pharmacological targets and have set the foundation for the development of hypothesis-driven methods.

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In a timely and symbiotic way, the newly established PPPs have significantly enhanced the R&D conditions by providing cutting-edge technologies that were previously not accessible. Small molecule screening, for example, evolved from low- to high-throughput procedures, making the evaluation of large compound libraries a feasible task [4,11]. Additionally, sophisticated R&D facilities equipped with forefront resources became available to perform formerly unavailable robust preclinical testing. In fact, several collaborations have been established to connect the fundamental knowledge coming from academia with the expertise and infrastructure provided by industry. One such example is the agreement between GlaxoSmithKline (GSK) and the Drugs for Neglected Diseases Initiative (DND*i*) to develop novel drugs for three preeminent NTDs; namely, leishmaniasis, human African trypanosomiasis (HAT) and Chagas disease (http://partnerships.ifpma.org/partnership/gsk-dndi-collaboration). Under this partnership, hits identified through high-throughput screens on GSK's proprietary libraries undergo optimization programs and comprehensive preclinical testing for efficacy and safety in animal models of the corresponding diseases. In another joint effort, Sanofi and DND*i* have co-developed fexinidazole, an investigational compound for HAT that is currently in clinical development (http://www.dndi.org/diseases-projects/portfolio/fexinidazole/).

## Drug repositioning in NTDs

Taking advantage of the scientific and funding advances achieved over the past 10 years, drug repositioning emerged as a valuable approach for NTDs [12]. Also referred to as drug repurposing, reprofiling, redirecting or rescue, this strategy consists of finding novel indications for marketed therapeutics or highly characterized investigational compounds [13]. Repurposing programs in the field have predominantly relied on bioinformatics and structure-guided approaches based on genomic and macromolecular structural data and on target-agnostic phenotypic screens using whole-cell assays. The first approach is based on the identification of parasitic protein orthologs that correspond to validated targets in other organisms, allowing the use of target-focused compound collections and series of analogs; the strategy also offers opportunities for medicinal chemistry work on structure-based scaffold optimization [14]. However, the latter approach provides the advantages of examining drug-induced effects on cells, evaluating drug uptake issues and identifying synergic events in complex biological networks [15,16]. Owing to the small number of fully validated targets in NTDs, most of the repurposed compounds have arisen from phenotypic campaigns rather than target-based strategies [17].

The appeal of drug repositioning is readily recognized because candidates have previously been through several R&D preclinical and clinical phases, such as hit identification, hit-to-lead and lead optimization, as well as toxicology and pharmacokinetics evaluations. Additionally, the stability, large-scale synthesis and manufacturing issues are already known [18]. Hence, compared with *de novo* drug discovery, the repurposing of pharmaceutical assets can reduce R&D risks, costs and time-lines to the market, providing strategic advantages for NTDs, an area that suffers chronically from limited resources and a serious shortage of effective therapies [12,18].

This strategy already has some success stories that support its use. Table 1 lists the drugs that reached the clinic for a new indication in NTDs along with those in clinical development. Inspired by these cases, several robust initiatives are underway to search the available pharmacopeia for repositioning candidates. WIPO Re:Search, for instance, is a public–private consortium managed by BIO Ventures for Global Health (BVGH) which catalyzes the sharing of intellectual property (IP) assets on investigational and marketed compounds and provides knowhow from pharmaceutical companies with the view to promote collaborative research on NTDs [19]. Successful precedents and recent advances in drug repositioning for prominent NTDs, namely Chagas disease, schistosomiasis, HAT and leishmaniasis, will be highlighted in the following sections.

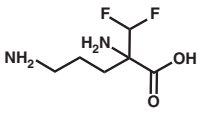
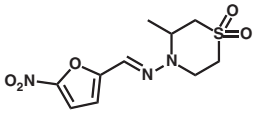
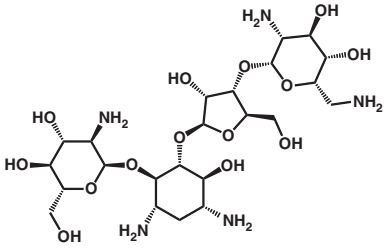
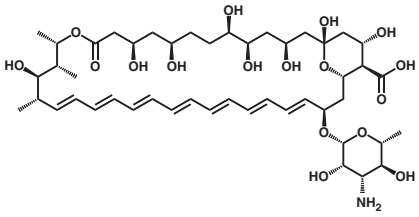
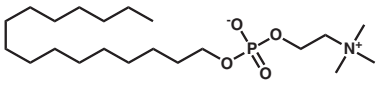
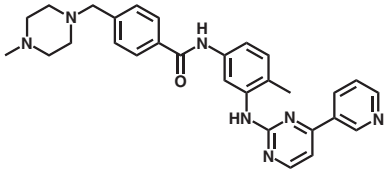
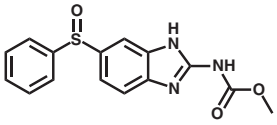
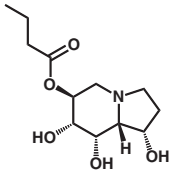
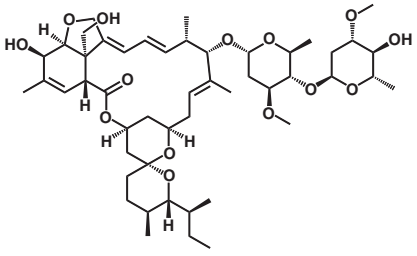
## Chagas disease

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, affects approximately 7 million people worldwide and causes more than 7000 deaths per year (http://www.who.int/neglected_diseases/9789241564861/en/). Although endemic in Latin America, the disease has advanced into North America, Europe and several Western Pacific countries. Outside the endemic regions, the highest burden occurs in the USA, where 300,000 people live with the disease [20]. Chemotherapy relies on benznidazole and nifurtimox, two obsolete nitroheterocyclic derivatives that have serious drawbacks and are effective only if administered during the acute phase of the disease, which corresponds to the first weeks after infection. Furthermore, their adverse effects lead to poor treatment adhesion, especially in adults, which combined with an emerging drug resistance precludes the vast majority of patients from obtaining effective treatment [21,22]. Fexinidazole represents an important advance in Chagas disease drug discovery. This compound is currently being evaluated by DND*i* in clinical trials as a treatment for the chronic phase of the disease (https://clinicaltrials.gov/ct2/show/NCT02498782). *In vivo* studies on several *T. cruzi* strains (CL, Y and the resistant Colombian and VL-10) employing fexinidazole induced a significant reduction in parasite burden and prevented death of infected mice. Cure rates ranging from 77.8% to 88.9% in animals treated during the acute phase (300 mg/kg of body weight for 20 days) were achieved; in the chronic phase, a cure rate of 70% was obtained for the VL-10 strain (300 mg/kg of body weight for 20 days). Moreover, animals infected with the Colombian and VL-10 strains showed reduced myocarditis, although parasite eradication was not attained in all treated mice [23].

DND*i* has adopted well-defined decision matrices – the target product profiles (TPPs) – to guide and manage each R&D project in its portfolio, according to the desired outcome for each disease. The ideal TPP for Chagas disease includes: (i) efficacy in the chronic and acute phases of the disease, in adult and children populations, and over all endemic areas; (ii) safety and efficacy superior to benznidazole against all *T. cruzi* strains, and no contraindications; (iii) activity against nitrofuran- and nitroimidazole-resistant strains; (iv) no drug interactions; (v) no significant proarrythmic potential; and (vi) oral treatment given once daily for 30 days (http://www.dndi.org/diseases-projects/chagas/chagas-target-product-profile).

**TABLE 1**

**Successfully repurposed drugs and repositioning candidates in clinical trials for neglected tropical diseases**

| Drug | Structure | Original use | New use | Refs |
|---|---|---|---|---|
| **Approved** | | | | |
| Eflornithine |  | Cancer | HAT | [54] |
| Nifurtimox |  | Chagas disease | HAT | [55] |
| Paromomycin |  | Antibiotic<br>Amebiasis<br>Cryptosporidiosis | Leishmaniasis | [63] |
| Amphotericin B |  | Fungal infections | Leishmaniasis | [64] |
| Miltefosine |  | Cancer | Leishmaniasis | [65] |
| **Clinical development** | | | | |
| Imatinib |  | Cancer | Lymphatic filariasis | (https://clinicaltrials.gov/ct2/show/NCT02644525) |
| Oxfendazole |  | Veterinary anthelmintic | Cysticercosis | (https://clinicaltrials.gov/ct2/show/NCT02234570) |
| Celgosivir |  | Hepatitis C | Dengue | (https://clinicaltrials.gov/ct2/show/NCT02569827) |
| Ivermectin |  | Strongyloidiasis<br>Onchocerciasis<br>Ascariasis | Dengue | (https://clinicaltrials.gov/ct2/show/NCT02045069) |

### *CYP51 inhibitors have reached clinical development for Chagas disease*

Cytochrome P450 sterol 14α-demethylases, also referred to as CYP51 (EC 1.14.13.70), have been a cornerstone in many Chagas disease drug repositioning programs [24]. The marketed antifungal posaconazole, an azole-bearing *T. cruzi* CYP51 inhibitor, is an emblematic repurposing candidate because it has reached the first clinical trials for Chagas disease in 40 years, which by itself represents a landmark in the field (Fig. 1a) (https://clinicaltrials.gov/ct2/results?term=NCT01377480). In addition to posaconazole, other antifungals, including the prodrug of ravuconazole (E1224) and derivatives of the agrochemical fenarimol, have been extensively explored as *Tc*CYP51 inhibitors [5,24]. The remarkable trypanocidal activity of such compounds derives from their distinguished ability to inhibit the enzyme by establishing a strong interaction between their nitrogen-bearing aromatic rings and the iron atom of the heme group situated at the enzyme catalytic site. The triazole and fluorinated rings of posaconazole and the proximal portion of its arm are enclosed by a deep hydrophobic cavity at the enzyme active site. The middle part of the inhibitor's arm interacts with 12 residues located at the access channel to the catalytic site; and the distal fragment of the drug, which is composed of an *N*-substituted triazolone ring, projects out of the protein surface toward the solvent. Given the structural similarity

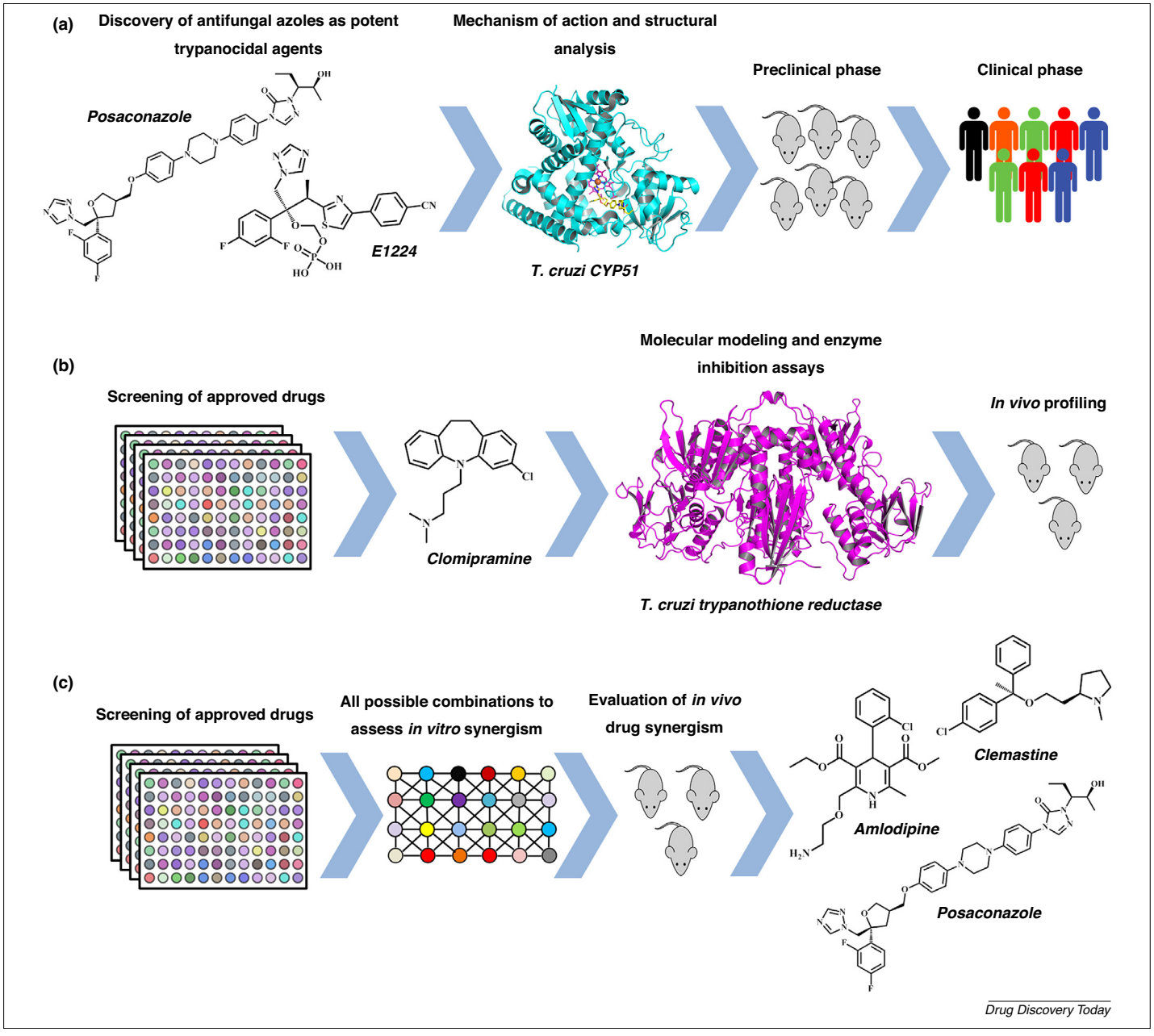



**FIGURE 1**

Strategies used in the identification of repositioning candidates for Chagas disease. **(a)** Azole antifungals are *Trypanosoma cruzi* cytochrome P450 (CYP)51 inhibitors that reached clinical development (PDB ID: 3K1O). **(b)** The antidepressant clomipramine was demonstrated to be an inhibitor of *T. cruzi* trypanothione reductase with the ability to reduce cardiac fibrosis in mice (PDB ID: 1AOG). **(c)** Amlodipine and clemastine, which were identified through phenotypic screens, act synergistically with posaconazole to clear parasite burden *in vivo*.

between the substrate-binding sites of CYP51 and other P450 (CYP) enzymes, off-target effects are a relevant shortcoming of azole-based inhibitors. This drawback can be overcome with the use of structure-based drug design (SBDD) approaches to improve selectivity for the parasite enzyme (with respect to their affinity for other human CYPs) [24].

Posaconazole produces ultrastructural alterations in *T. cruzi* intracellular amastigotes, such as accumulation of vacuoles and vesicles in the cytoplasm and flagellar pocket. In addition, the drug causes shedding of the intracellular content, which is probably a consequence of inhibition of sterol biosynthesis and the resulting destabilization of the plasma membrane [25]. Regardless of the encouraging results in suppressing parasite burden in preclinical *in vivo* models (negative hemocultures in 90% of the treated animals), downstream evaluation of posaconazole and E1224 in clinical trials revealed critical bioavailability problems and a lack of sustained efficacy compared with the standard drug benznidazole [26–28]. These findings ultimately resulted in poor expectations regarding the use of azole-based *Tc*CYP51 inhibitors in the chronic phase of the disease.

### *Clomipramine improves myocardial fibrosis in* T. cruzi*-infected mice*

Clomipramine (Fig. 1b) is a clinically used tricyclic antidepressant with known trypanocidal activity, which, according to enzyme inhibition and molecular modeling studies, is a competitive inhibitor of trypanothione reductase (EC 1.8.1.12) ($K_i$ = 6 μM) [29]. This enzyme is an NADPH-dependent disulfide oxidoreductase unique to trypanosomatids that plays a key part in maintaining the cellular redox balance, thereby avoiding the oxidative stress caused by reactive oxygen species [30]. A recent *in vivo* study performed in Albino Swiss mice infected with *T. cruzi* (Tulahuen strain) showed the efficacy of clomipramine in preventing cardiomyopathy, a typical and serious consequence of Chagas disease [31]. The ability of the antidepressant to reduce myocardial fibrosis is one of the most interesting findings of the study considering the well-known inefficacy of the available antitrypanosomal therapy in blocking such a process in the chronic phase of the disease. Clomipramine also improved the survival rate of mice with chronic cardiac disease compared with control animals at doses of 5 mg/kg administered for 60 days.

### *Amlodipine and clemastine act synergistically with posaconazole*

The use of drug combinations has become an important strategy to combat drug resistance and reduce treatment course in NTDs. The STOPCHAGAS clinical trial, which was supported by Merck, evaluated a combination of posaconazole and benznidazole in the indeterminate asymptomatic phase of Chagas disease (https://clinicaltrials.gov/ct2/show/NCT01377480). The results showed no satisfactory efficacy and indicated some challenges in the use of drug combinations for Chagas disease. One important aspect is to understand how drugs that act by different mechanisms can affect the clinical evolution of the disease; and determine whether clinical candidates need to be active against extra- and intra-cellular stages of the parasite. Benznidazole and nifurtimox are active against both forms of *T. cruzi in vitro*, whereas posaconazole and other azoles act only against the intracellular amastigote stage [5].

In a phenotypic approach by Planer and co-workers, several drug combinations proved effective in reducing parasitemia in animal models of Chagas disease (Fig. 1c) [32]. The study started with ~700 approved compounds, which were screened against *T. cruzi* intracellular amastigotes (Tulahuen strain) in a high-throughput format. Based on several parameters including structural diversity, cytotoxicity, administration route and clinical safety, a subset of 24 active drugs was selected and tested in all possible combinations to assess drug synergism. A few pairings yielded substantial synergistic effects *in vitro* and significant parasite clearance in BALB/c mice. The combination of posaconazole with either the antihistaminic clemastine ($EC_{50}$ = 0.4 μM) or the calcium channel blocker amlodipine used in cardiac conditions ($EC_{50}$ = 1.1 μM) had the most prominent synergistic effect. The $EC_{50}$ values were equivalent to that of benznidazole ($EC_{50}$ = 0.65 μM). Selectivity indexes (SI) of 11.8 and 59.3 were registered for amlodipine and clemastine, respectively, whereas an SI value >40 was reported for benznidazole. Oral administration of amlodipine (10 mg/kg) plus posaconazole (0.04 mg/kg) for 5 days produced the most promising profile, resulting in nearly total suppression of the parasite burden in treated mice and an 80–100% survival rate. In spite of these results, posaconazole did not succeed in clinical trials for chronic Chagas disease. However, the poor understanding of the chronic phase of the disease and the reasons for the failure of posaconazole should motivate additional studies to assess the potential of drug combinations comprehensively.

## Schistosomiasis

Schistosomiasis is a highly prevalent NTD caused by trematode flat worms of the genus *Schistosoma*, mainly *Schistosoma mansoni*, *Schistosoma haematobium* and *Schistosoma japonicum*. The disease affects more than 230 million people, mostly children of school age, in tropical and subtropical areas of Africa, the Caribbean, South America, the Middle East and Asia [33,34]. An additional 800 million people are in danger of infection, and the number of deaths attributed to the disease reaches 200,000 annually [35] (http://www.who.int/mediacentre/factsheets/fs115/en/). Pharmacological treatment is based on praziquantel (PZQ), a 30-year-old pyrazinoisoquinoline derivative that is highly effective against adult worms; however it does not act on the immature stages of the parasite [36]. In addition to this drawback, reliance on PZQ as the sole treatment, its contraindication for young children, as well as the reduced drug susceptibility in field isolates have driven the ongoing research on novel antischistosomal compounds [6,36]. The proposed TPP for schistosomiasis includes: (i) chemical class distinct from PZQ; (ii) novel mechanism of action; (iii) activity on mature and immature worms, including eggs, and against all major *Schistosoma* spp.; (iv) safety comparable or superior to PZQ; (v) no contraindication for children and pregnant women; and (vi) oral short-term treatment [37].

### *Statins inhibit* S. mansoni *HMG-CoA reductase and kill immature worms*

Capitalizing on the available genomic data from *Schistosoma*, bioinformatics approaches have identified several putative drug targets, some of which are already validated for known drugs [38].

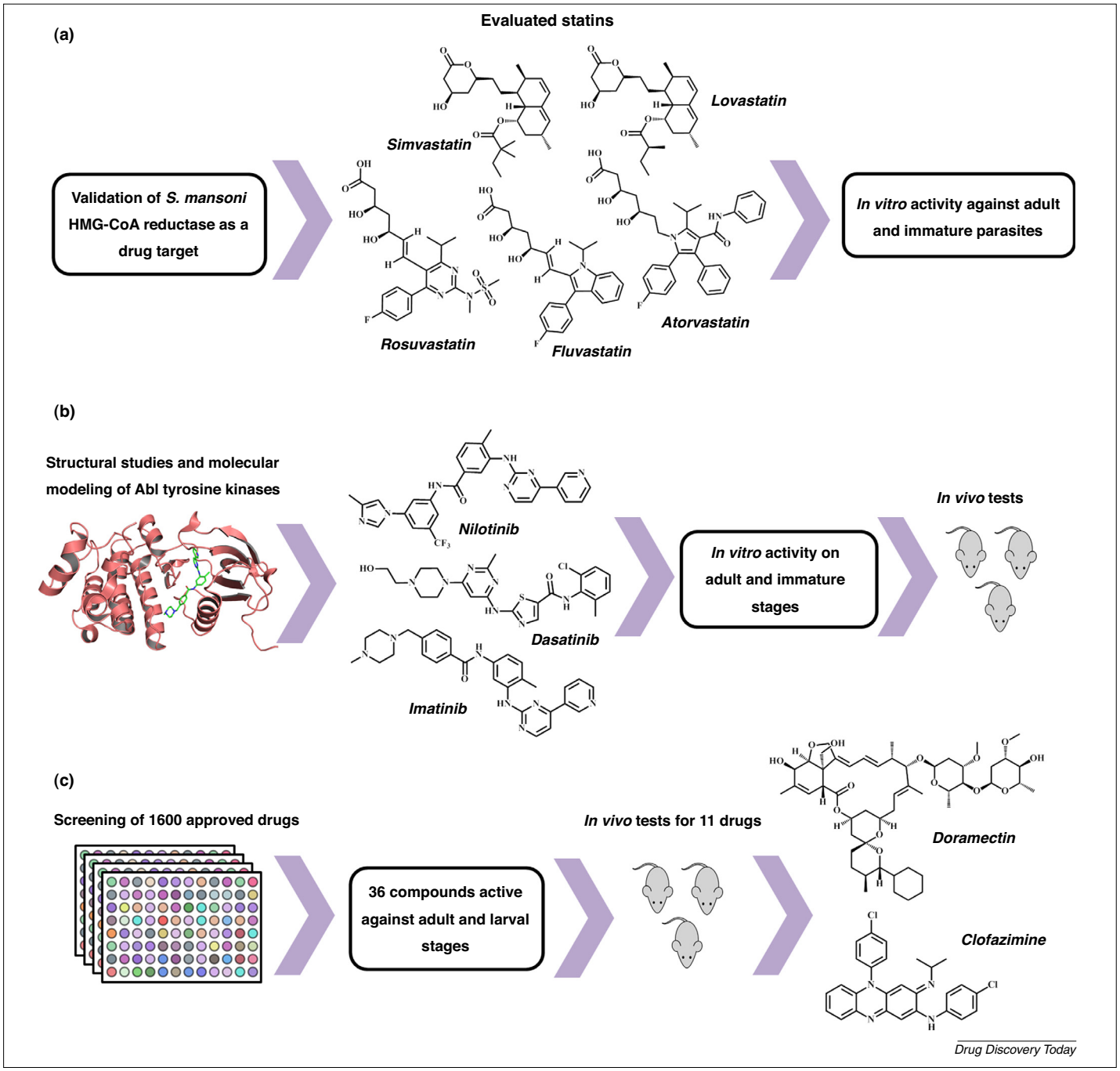



**FIGURE 2**

Approaches explored in repositioning efforts for schistosomiasis. **(a)** Hydroxymethylglutaryl-CoA (HMG-CoA) reductase is a validated target in *Schistosoma mansoni*. Inhibition of the enzyme by selected statins resulted in the death of adult and immature worms. **(b)** Anticancer drugs such as imatinib inhibit schistosomal tyrosine kinases and kill mature and larval stages of the parasite (PDB ID: 3GVU). **(c)** Doramectin and clofazimine were identified in phenotypic screenings and displayed considerable capacity to reduce parasite burden in animal models of schistosomiasis.

One such example is HMG-CoA reductase (EC 1.1.1.34), a key enzyme in the eukaryotic mevalonate pathway and the molecular target of the statin family of cholesterol-lowering drugs. Its essentiality for parasite survival was confirmed in chemical and RNA interference (RNAi) studies, in which the enzyme was validated as a drug target in *S. mansoni* [39,40]. In the same investigation the antischistosomal activity of six statins, namely, atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin, was evaluated by monitoring a series of phenotypic responses in adult and immature *S. mansoni* worms (Fig. 2a). Simvastatin was the most active compound ($ED_{50}$ = 90 nM), followed by lovastatin ($ED_{50}$ = 190 nM), fluvastatin ($ED_{50}$ = 430 nM), rosuvastatin ($ED_{50}$ = 580 nM), atorvastatin ($ED_{50}$ = 1.77 μM) and pravastatin, which displayed negligible activity. This research is still in progress, and selected statin analogs have been supplied by Merck, Sharp & Dohme to the University of San Francisco for additional testing and downstream elucidation of SAR and molecular optimization. The ability of the statins to kill immature parasites contrasts with the well-established poor efficacy of PZQ against somules, a relevant aspect that supports further evaluation of these

drugs as antischistosomal agents. However, considering the requirement for a short-course treatment, the evaluated statins could be at a reasonable distance from the desired TPP. In a previous study, lovastatin showed parasite clearance in a murine model of schistosomiasis when administered in high concentrations (approximately 640 mg/kg/day) for 14–17 days [41]. PZQ, by contrast, eliminates >90% of adult worms in mice at a single oral dose of 500 mg/kg [40].

### *Anticancer compounds target schistosomal tyrosine kinases*

Other advances have come from antiproliferative agents that interact with tyrosine kinases, enzymes that are heavily explored in many therapeutic areas because of their vital importance in cell homeostasis [42]. Research on Abelson tyrosine kinases (Abl-TKs) (EC 2.7.10.2) has demonstrated the pleiotropic and fundamental role of these signaling molecules in the survival of schistosomes [43,44]. An approach combining computational and experimental techniques assessed the repositioning potential of imatinib, an inhibitor of Abl-TKs that is widely employed to treat cancer (Fig. 2b) [45]. Homology modeling of *S. mansoni* Abl-TKs revealed a high degree of 3D structural similarity between schistosomal and human enzymes; moreover, molecular docking simulations demonstrated that imatinib assumes a nearly identical conformation and preserves key enzyme–inhibitor interactions in *S. mansoni* and human Abl-TKs. The top-scoring docking pose of imatinib in the homology model showed four interactions of the inhibitor's amide and pyridine groups with the active site residues Asp422, Met331, Met359 and Leu289. In line with these findings, the *S. mansoni* Abl-TK activities were monitored in competitive germinal vesicle breakdown (GVBD) assays, which employed *Xenopus* oocytes transfected with cRNA encoding the catalytic domain of Abl-TKs. The GVBD results demonstrated that imatinib inhibits two *S. mansoni* Abl-TK isoforms (full GVBD suppression at 1 μM for Abl1 and 100 nM for Abl2). Moreover, treatment of adult worms with the drug caused several dose- and time-dependent morphological changes such as body swelling, impaired oogenesis and spermatogenesis, and decreased pairing stability, which ultimately led to the death of the parasites. Imatinib also interfered with the viability of immature schistosome stages. Although the results from the GVBD assay provided evidence for the inhibition of *Sm*Abl-TKs, they did not exclude the possibility that the observed phenotypes are mediated by other mechanisms. Dasatinib and nilotinib, second-generation inhibitors that are highly selective for mutated forms of human Abl-TKs, were less competent in producing lethal effects. These findings suggest that an increased selectivity for human kinases correlates with a decreased activity against the parasite. This indicates that molecular optimization of imatinib toward selective inhibitors of *Sm*Abl-TKs is a feasible task.

Oral administration of imatinib in mouse and hamster models of schistosomiasis was not effective, which contrasts with the high efficacy of PZQ. The high degree of interaction of imatinib with plasma proteins – serum albumin and α-1 acid glycoprotein (AGP) – was raised as a potential cause for the lack of efficacy. Erythromycin, which efficiently binds to AGP, is able to partially recover the lethal activity in cultures of schistosomes containing imatinib and AGP [46]. Imatinib is in clinical development for lymphatic filariasis, which indicates the potential of the drug as a therapy for helminthic infections.

Other anticancer drugs have recently been evaluated as repositioning candidates for the therapy of schistosomiasis. In a study by Cowan and Keiser, 114 compounds from the Developmental Therapeutics Program (DTP) of the National Cancer Institute (NCI) were screened against the larval stage of *S. mansoni* [47]. The mitogen-activated extracellular signal-regulated kinase (MEK) inhibitor trametinib ($IC_{50}$ of 4.6 and 4.1 μM, larval and adult stages, respectively) showed a promising *in vivo* profile. The compound was able to reduce the worm burden by 63.6% at a single oral dose of 400 mg/kg body weight in Naval Medical Research Institute (NMRI) mice infected with *S. mansoni*.

### *Doramectin and clofazimine reduce* S. mansoni *viability* in vivo

By contrast to the above-mentioned target-based approaches, Panic *et al*. reported a phenotypic, whole-cell screening campaign on a library of 1600 marketed compounds (Fig. 2c) [48]. Evaluation of the full collection against the larval stage of *S. mansoni* identified 121 active compounds. When this subset was advanced for testing against adult worms, 36 drugs displayed antischistosomal activity, including the antihypertensive nicardipine and the local anesthetic oxethazaine ($IC_{50}$ of 2.67 and 2.95 μM, respectively). Analyses of these *in vitro* data, in parallel with pharmacokinetics and toxicology information, were conducted to exclude molecules with unsuitable administration routes, poor absorption and high toxicity, a procedure that resulted in the selection of 11 compounds for *in vivo* experimentation. Doramectin and clofazimine were the most effective agents for reducing parasite load in an NMRI murine model of the disease. Doramectin, a drug used to eliminate gastrointestinal parasites in cattle, led to a 60% reduction in worm burden (10 mg/kg given orally), which is superior to the efficacy of the related anthelmintic ivermectin (a compound co-administered with PZQ in specific regions) (http://www.who.int/schistosomiasis/en/). In a recent study, administration of ivermectin as a single dose of 25 mg/kg in BALB/c mice showed minimal efficacy [49]. Clofazimine, originally indicated for leprosy, decreased parasite burden by 82.7% (400 mg/kg given orally). These results support additional investigation of these drugs for the treatment of schistosomiasis.

## Human African trypanosomiasis

HAT, caused by the protozoans *Trypanosoma brucei gambiense* and *T. brucei rhodesiense*, is a deadly NTD that afflicts populations in remote areas of Africa [50]. Although fewer than 10,000 new cases are communicated annually, which corresponds to a decrease of 76% in 15 years, at least 70 million people remain at risk of infection (http://www.who.int/neglected_diseases/9789241564861/en/). Pharmacotherapy consists of suramin, pentamidine, melarsoprol, eflornithine and nifurtimox, a set of compounds that is notorious for narrow efficacy and serious toxicity [51].

Eflornithine, the first-line treatment for *T. brucei gambiense* late-stage HAT, is a successful case of drug repositioning [52,53]. Acting as an inhibitor of the polyamine metabolism enzyme ornithine decarboxylase (EC 4.1.1.17), eflornithine was licensed as an orphan drug for HAT in 1990 after its discontinuation from a cancer clinical development program [54]. Nifurtimox is another repurposing case, this time originating from a treatment for Chagas disease. The compound has been applied since 2009 in combination with

eflornithine to shorten the treatment course of *T. brucei gambiense* late-stage HAT, an approach that was named nifurtimox–eflornithine combination therapy (NECT) [55].

The first priority for a novel therapy is the development of a safe and effective drug against early- and late-stage HAT to replace the current treatments and simplify the disease management. This is detailed in the TPP released by DND*i*, which requires: (i) broad spectrum activity (*T. brucei gambiense* and *T. brucei rhodesiense*) and clinical efficacy >95%; (ii) effectiveness in melarsoprol refractory patients; (iii) safety in pregnancy and lactation and a drug-related mortality rate <0.1%; (iv) adult and pediatric formulations that do not require hospitalization; (v) oral or intramuscular treatment given once daily for up to 7 days; and (vi) multitarget mechanism of action (http://www.dndi.org/diseases-projects/hat/hat-target-product-profile).

### *Antibiotics and psychoactive agents overcome* T. brucei *growth*

A recent phenotypic screening conducted by DND*i* identified several clinically used compounds as inhibitors of *T. brucei rhodesiense* proliferation (STIB900 strain) (Fig. 3a) [56]. Two nitroheterocyclic antibiotics that are structurally related to nifurtimox, namely nifuroxazide ($IC_{50}$ = 0.03 μM and SI = 410) and nitrofurantoin ($IC_{50}$ = 0.5 μM and SI = 181), are among the most promising compounds, displaying outstanding antitrypanosomal activity. Nifuroxazide exhibited activity comparable to the standard drug pentamidine ($IC_{50}$ = 0.01 μM and SI = 887). Nitroheterocycles are a privileged scaffold in drug discovery for trypanosomatid diseases, as corroborated by the use of benznidazole in Chagas disease, nifurtimox in HAT and Chagas disease and the current development of fexinidazole for HAT and Chagas disease.

Another therapeutic class that exhibited noteworthy activity consists of psychoactive agents that, given their ability to penetrate the central nervous system, hold promising prospects for further evaluation, especially in disease models of late-stage HAT. Among the evaluated phenothiazine antipsychotics, one can highlight the multitarget D2 dopamine receptor antagonists and calcium channel blockers thioridazine ($IC_{50}$ = 0.53 μM and SI = 10), trifluprom azine ($IC_{50}$ = 1.42 μM and SI = 13) and promazine ($IC_{50}$ = 2.16 μM and SI = 14). Among the tricyclic antidepressants, the most active one was nortriptyline ($IC_{50}$ = 1.17 μM and SI = 24), a broadly prescribed norepinephrine reuptake inhibitor also used in several off-label indications, such as chronic pain and migraine prophylaxis. In the class of antidepressants, clomipramine ($IC_{50}$ = 2.06 μM and SI = 10) and amitriptyline ($IC_{50}$ = 3.03 μM and SI = 14) also exhibited interesting activity. Furthermore, noteworthy results were obtained for the selective serotonin reuptake inhibitors sertraline ($IC_{50}$ = 0.77 μM and SI = 11) and paroxetine ($IC_{50}$ = 1.13 μM and SI = 12). Some of these

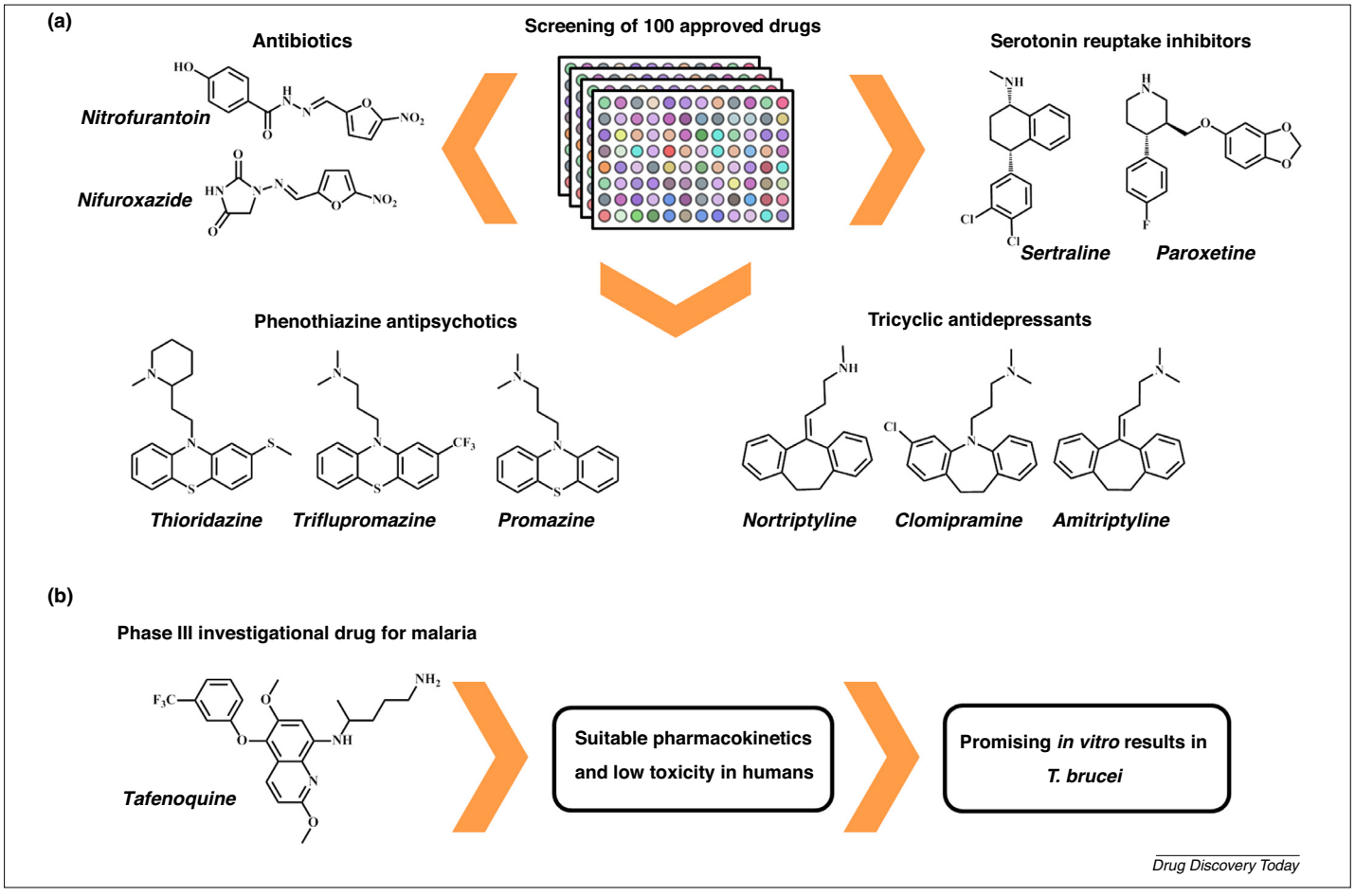



**FIGURE 3**

Drug repurposing approaches in human African trypanosomiasis. **(a)** Screening of 100 marketed drugs resulted in the discovery of highly active antibiotics, antipsychotics and antidepressants as repositioning candidates. **(b)** The antimalarial clinical candidate tafenoquine is a highly characterized compound that presents favorable pharmacokinetics upon oral administration and potent *in vitro* activity against *Trypanosoma brucei*.

drugs showed equivalent or improved *in vitro* activity when compared to nifurtimox ($IC_{50}$ = 1.44 μM and SI = 60) and fexinidazole ($IC_{50}$ of 2.57 and 1.14 μM, *T. brucei rhodesiense* and *T. brucei gambiense*, respectively) (http://www.dndi.org/images/stories/events2008/asmth/4_torreele_fexinidazole_dndihat_astmh2008.pdf). Considering the excellent sensitivity profiles recorded in this campaign, some of the identified compounds are likely to be tested soon in preclinical *in vivo* models of HAT for detailed efficacy and toxicity profiling.

### *Antimalarial tafenoquine has pronounced trypanocidal activity*

Tafenoquine (TFQ) is an 8-aminoquinoline developed by GSK and the Medicines for Malaria Venture (MMV) and is currently in Phase III clinical trials as a novel antimalarial agent [57]. The compound has been referred to as a single-dose radical cure for *Plasmodium vivax* malaria, an infectious disease that affects approximately 16 million people worldwide (http://www.who.int/malaria/publications/atoz/9789241509244/en). After a series of *in vitro* analyses, Carvalho and co-workers reported the remarkable antitrypanosomal activity of TFQ ($EC_{50}$ = 0.17 μM, *T. brucei rhodesiense* STIB900) along with valuable insights into the compound's mechanism of action (Fig. 3b) [58]. The drug also displayed activity in the nanomolar range against *T. brucei brucei* S427 and *T. brucei brucei* S16 ($EC_{50}$ of 0.22 and 0.42 μM, respectively). These results are superior to those previously obtained for nifurtimox ($EC_{50}$ = 2.1 μM) and fexinidazole ($EC_{50}$ = 1.4 μM), but are inferior to $EC_{50}$ values for pentamidine ($EC_{50}$ = 1 nM) and melarsoprol ($EC_{50}$ = 7.8 nM, *T. brucei rhodesiense* Z310) [59,60]. Parasites treated with TFQ underwent depolarization of the mitochondrial membrane potential, elevation of intracellular $Ca^{2+}$ and production of reactive oxygen species. Suggesting a multitarget mechanism of action, these molecular events resulted in complete membrane disintegration and loss of cytoplasmic content. TFQ is a highly characterized investigational drug featuring favorable pharmacokinetics after oral administration, and follow-up *in vivo* studies are expected to evaluate its usefulness in the management of HAT.

## Leishmaniasis

According to WHO, 1.3 million new cases of leishmaniasis emerge annually, and 310 million people are vulnerable to infection in almost 100 tropical and subtropical countries (http://www.who.int/neglected_diseases/9789241564861/en/). The most serious clinical form of the disease, visceral leishmaniasis (VL), which is caused by two protozoan species of the genus *Leishmania* (*Leishmania infantum* and *Leishmania donovani*), is a life-threatening condition that kills 20,000–50,000 people each year [61]. Although a few drugs are available – pentavalent antimonials, amphotericin B, paromomycin and miltefosine – they have several shortcomings, such as severe toxicity, lengthy treatment, need for hospitalization and susceptibility to drug resistance. The high treatment cost is also a major limiting factor that precludes a broader use of these agents [62]. The main goals for a novel VL treatment are the development of safe, effective, oral and short-course drugs. The TPP developed by DND*i* requires: (i) clinical efficacy >95% against all species and over all endemic areas; (ii) efficacy in immunosuppressed patients; (iii) activity against resistant strains; (iv) no drug interactions and suitability for drug combinations; and (v) no adverse effects that require hospitalization (http://www.dndi.org/diseases-projects/leishmaniasis/tpp-vl). Most of the leishmaniasis drug arsenal was repurposed from other indications. Paromomycin is a natural aminoglycoside isolated from *Streptomyces krestomuceticus* that was previously prescribed as a broad-spectrum antibiotic as well as for management of other infections, such as cryptosporidiosis and amebiasis [63]. Amphotericin B is a remarkably toxic macrolide extracted from *Streptomyces noclosus* that was originally used in fungal infections [64]. Miltefosine, the most recent antileishmanial introduced into the clinic, is an alkylphosphocholine developed in the 1980s for cutaneous metastases of breast cancer and solid tumors (it is discontinued for these indications) [65]. The repurposing of miltefosine represents an important advance in the field because it is the only oral drug available to treat all of the clinical forms of leishmaniasis [66].

### *Anticancer kinase inhibitors have antileishmanial properties*

Kinases have recently been explored as druggable targets in leishmaniasis [67]. It has been proposed that miltefosine and other related alkylphospholipids exert their anticancer activity by interfering with signaling pathways that originate in the plasma membrane, such as the protein kinase B (Akt)/phosphoinositide 3 kinase (PI3K)/mammalian target of rapamycin (mTOR) cascade; however, the exact mechanism of action remains unknown [68,69]. In *Leishmania*, the mechanism of action of miltefosine is also not fully understood, but it could involve kinases associated with cell-cycle regulation and apoptosis. Based on this background, chemical and genetic studies on *Leishmania* have suggested that cyclin-dependent kinases (CDKs) and mitogen-activated kinases (MAPKs) are essential for cell viability and proliferation and are therefore potential molecular targets for drug discovery [70]. Following these findings, 10 FDA-approved anticancer kinase inhibitors were evaluated against several *Leishmania* spp. [71]. Of the tested compounds, sunitinib, sorafenib and lapatinib were identified as the most active repositioning candidates against *L. donovani*, yielding $IC_{50}$ values of 1.1, 3.7 and 2.5 μM, respectively (Fig. 4a). This activity range was comparable to that of miltefosine ($IC_{50}$ = 1.03 μM), but far inferior to that of amphotericin B ($IC_{50}$ = 20 nM). No significant toxicity was detected against the host murine macrophages used in the *in vitro* intracellular amastigote assays. Sorafenib also exhibited a broad-spectrum activity profile on the cutaneous leishmaniasis agents, *Leishmania amazonensis*, *Leishmania mexicana* and *Leishmania major* ($IC_{50}$ of 6.9, 4.7 and 3.8 μM, respectively). Sunitinib and sorafenib are used to impair tumor-induced vascular remodeling by inhibiting vascular endothelial growth factor receptors (VEGFRs) and platelet-derived growth factor receptors (PDGFRs). Additionally, such compounds have been reported to be nonselective ligands of several structurally distinct kinases. Lapatinib is a highly selective inhibitor of the epidermal growth factor family of receptors (EGFRs) [72]. Because no orthologs of these targets are known in *Leishmania*, the mechanism of action of these drugs remains unclear. Sunitinib, sorafenib and lapatinib displayed moderate activity in a BALB/c model of infection, reducing the parasite load by 30–40% at an oral dose of 50 mg/kg for 5 days. Miltefosine reduced parasite burden by 66.8% at 5 oral doses of 12 mg/kg/day, whereas liposomal amphotericin B (AmBisome®) achieved a 97.9% reduction at 3 intravenous doses of 1 mg/kg/day. Despite the modest *in vivo* results, these trials provided evidence and

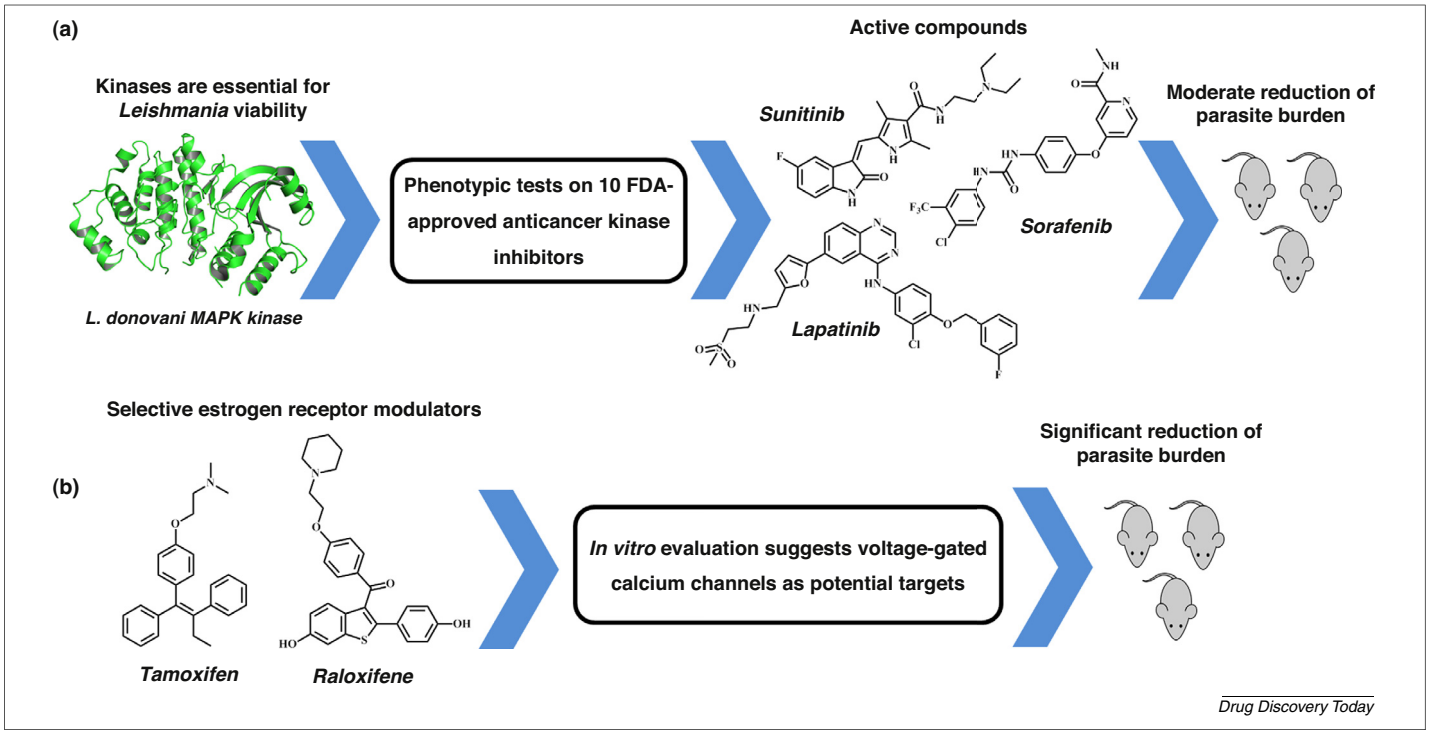



**FIGURE 4**

Drug repurposing strategies for leishmaniasis. **(a)** In line with studies suggesting mitogen-activated kinase (MAPK) as a putative target in *Leishmania* (PDB ID: 4QNY), a series of anticancer kinase inhibitors was tested *in vitro* and in animal models and showed moderate activity in reducing parasite load. **(b)** Selective estrogen receptors tamoxifen and raloxifene displayed important parasite clearance in mouse models of leishmaniasis and might target voltage-gated calcium channels.

contributed to building the case of anticancer kinase inhibitors as novel antileishmanial agents.

### *The estrogen receptor modulator raloxifene is a novel chemotype in leishmaniasis drug discovery*

Another advance coming from anticancer research is raloxifene (originally named keoxifene) (Fig. 4b), a selective estrogen receptor modulator (SERM) that failed as a treatment for tamoxifen-resistant breast tumors and is currently prescribed for the prevention and treatment of osteoporosis in post-menopausal women [73,74]. The triphenylethylene tamoxifen, the most broadly used SERM, was previously demonstrated to be highly effective in murine models of cutaneous (*Leishmania braziliensis*) and visceral (*Leishmania chagasi*) leishmaniasis, reducing parasite burden by 99% in cutaneous lesions and by 95–98% in the liver and spleen (20 mg/kg/day for 15 days) [75]. These findings prompted investigations on structurally diverse estrogen receptor modulators, whereby the antileishmanial properties of the benzothiophene raloxifene were discovered. The compound was profiled *in vitro* and proved to be effective against a broad spectrum of *Leishmania* spp., including intracellular amastigotes of *L. amazonensis* and *L. infantum* ($EC_{50}$ values of 16.2 and 8.8 μM, respectively) [76]. Miltefosine, used as the standard drug, has $EC_{50}$ values of 16.8 and 2.7 μM against *L. amazonensis* promastigotes and intracellular amastigotes, respectively. The SI for raloxifene ranges between 1.76 and 3.24 for *L. amazonensis* and *L. infantum chagasi* intracellular amastigotes, respectively (J774 macrophages). Raloxifene-treated parasites showed functional damage of the plasma membrane, impairment of the mitochondrial energy-coupling system and efflux of the matrix content. The drug also induced the formation of autophagic vacuoles, which ultimately resulted in cell death. The ability of the compound to collapse the membrane ionic gradient in *Leishmania* and mammalian cells, in addition to the presence in the parasite of a voltage-gated calcium channel structurally related to the human receptor, suggests that raloxifene might target these membrane proteins. Treatment of *L. amazonensis*-infected BALB/c mice with the drug (10 oral doses of 100 mg/kg on alternate days) reduced the average lesion size by 54.3% and the parasite load by 89.7%. Considering the previous data on tamoxifen, these findings confirmed that SERMs are practical repositioning candidates for further development.

## Concluding remarks

Drug repurposing is a mainstream approach that has been eagerly pursued by key players involved in drug discovery, which is corroborated by recent numbers showing that repositioned drugs correspond to ~30% of the FDA approvals in recent years [77]. In parasitic diseases, drug repositioning has been explored as a strategy to overcome decades of paucity of newly designed compounds and the historical scarcity of resources. As a discovery strategy, NTD-focused drug repurposing is an evolving approach that needs to surmount important challenges. From a bureaucratic viewpoint, there are legal issues related to IP, out-licensing and patenting, as well as questions concerning the outlining of appropriate decision-making models to address the specificities of these diseases. From a technological standpoint, the field has not yet reached its plenitude and preclinical- and clinical-phase hurdles stand in the path that leads to fully filled repurposing pipelines.

At the current stage, these limitations are to a large extent comprehensible and even expected, because the technical advances required for vigorous and consistent research have become available only in the past 10 years. Therefore, further progress in the area is strongly linked to the optimization and diversification of the methodologies contained in the drug repurposing toolbox. From a medicinal chemistry perspective, drug repositioning demands solutions to important questions such as the manipulation of pharmaceutical properties and the development of better and more tolerable formulations. Repositioning also requires efforts to discover novel drug combinations that can produce synergistic and therefore superior effects compared with single compounds. Also essential are the elucidation of mechanisms of action and validation of novel targets in relevant host–parasite interaction models depending on solid medicinal chemistry knowledge. The critical nature of these aspects is evident in cases where data gathered from *in vitro* or animal models are not confirmed in subsequent clinical trials. Notwithstanding these complex challenges, advances have been achieved in recent years as engaged consortia have taken the responsibility of leading the efforts. It is worth highlighting two recent milestones: posaconazole, the first clinical candidate for Chagas disease in 40 years; and miltefosine, the first oral treatment for leishmaniasis. Considering these and other successful examples, promising advances can be achieved by repurposing candidates currently in clinical development. Based on this scenario, drug repositioning will develop into a robust discovery strategy to minimize the suffering of billions of people affected by NTDs.

## Conflicts of interest

The authors declare no competing financial interests.

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