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Repurposing of Human Kinase Inhibitors in Neglected Protozoan Diseases

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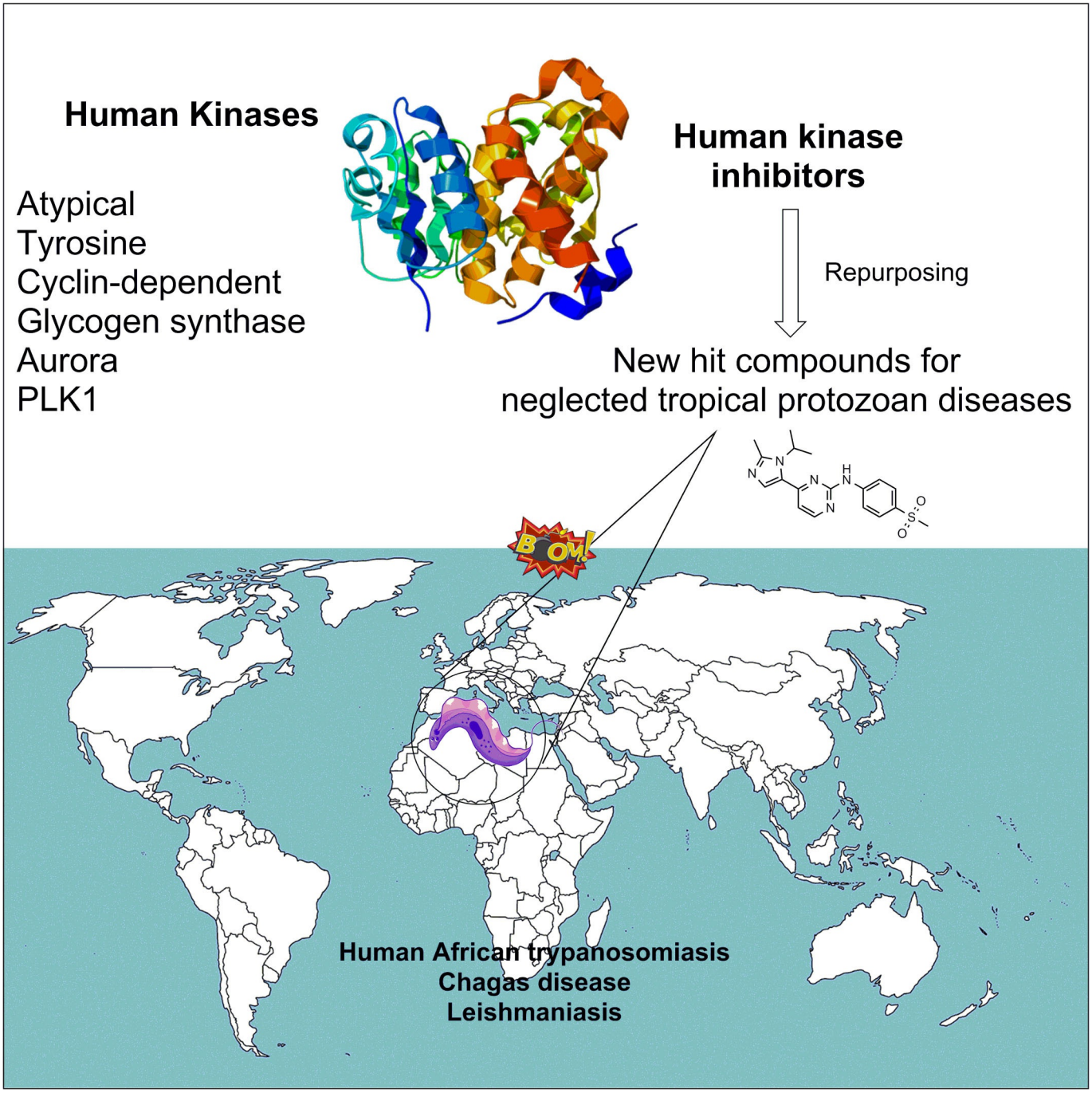

Human African trypanosomiasis (HAT), Chagas disease, and leishmaniasis belong to a group of infectious diseases known as neglected tropical diseases and are induced by infection with protozoan parasites named trypanosomatids. Drugs in current use have several limitations, and therefore new candidate drugs are required. The majority of current therapeutic trypanosomatid targets are enzymes or cell-surface receptors. Among these, eukaryotic protein kinases are a major group of protein targets whose modulation may be beneficial for the treatment of neglected tropical protozoan diseases. This review summarizes the finding of new hit compounds for neglected tropical protozoan diseases, by repurposing known human kinase inhibitors on trypanosomatids. Kinase inhibitors are grouped by human kinase family and discussed according to the screening (target-based or phenotypic) reported for these compounds on trypanosomatids. This collection aims to provide insight into repurposed human kinase inhibitors and their importance in the development of new chemical entities with potential beneficial effects on the diseases caused by trypanosomatids.

1. Introduction

Neglected tropical diseases (NTD) are a group of seventeen communicable diseases mainly found in tropical and sub-tropical conditions in about 150 countries, affecting more than one billion people worldwide.[1] Three NTDs are induced by infection with flagellated protozoan parasites named trypanosomatids: human African trypanosomiasis (HAT), Chagas disease and leishmaniasis. The majority of the current therapeutic trypanosomatid targets are represented by enzymes or cell surface receptors. Among these, eukaryotic protein kinases represent a major group of protein targets whose modulation may be beneficial for the treatment of protozoan NTD.

2. Neglected Tropical Protozoan Diseases

2.1. HAT

HAT, also known as sleeping sickness, is a vector-borne disease transmitted by the bite of tsetse fly (*Glossina* genus).[1] It is caused by the infection with protozoan parasites *Trypanosoma brucei gambiense* (*T. b. gambiense*) and *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*). In the last decade, *T. b. gambiense* has been responsible for 98% of HAT reported cases; this parasite subspecies is found in Western and Central Africa causing a chronic infection, characterized by a slow onset of major signs and symptoms.[2] *T. b. rhodesiense* is responsible for HAT in Eastern and Southern Africa, causing a fast onset, acute and rapidly progressive HAT infection, which accounts for the remaining 2% of reported cases.[2a] Another parasite subspecies, *Trypanosoma brucei brucei* (*T. b. brucei*), is responsible for animal trypanosomiasis; however, being non-infective to humans, is widely employed for in vitro and in vivo studies.[3] In 2013, the estimated number of HAT cases was about 6228.[4] In recent times, the number of infections has dropped to 2804 new cases in 2015, with an overall estimated number of actual cases below 20000. However, the population at risk of infection still remains a major issue in the geographical areas where the disease is endemic, with 65 million people at risk.[1,2]

The HAT cycle is peculiar and depends on the human organs affected by the parasite. After the insect bite occurs, the infective form of the parasite is transferred to the host bloodstream. This parasite form multiplies in the bloodstream and lymphatic system, causing febrile episodes with headaches and enlarged lymph nodes. This is called the hemolymphatic stage. From here, the parasite invades several tissues including the spleen, liver, and heart. When the parasite crosses the blood–brain barrier (BBB) to infect the central nervous system (CNS), the disease reaches the second stage, also called the neurological or meningoencephalic stage. At this stage the typical signs and symptoms of the disease are evident and these include: neurological disorders such as sleep disturbance, ataxia, psychiatric disorders, coma and ultimately, if untreated, death.[5]

2.2. Chagas disease

Chagas disease or American trypanosomiasis is caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*). Around eight million people are estimated to be infected, primarily in 21 Latin American countries where the disease is endemic, with 10600 deaths estimated in 2013 and 25 million people at risk of infection.[1,6]

The protozoan responsible for the infection is transmitted by the bite of the infected triatomine bug, also known as the kissing bug.[7] The disease is characterized by two phases. During the first acute phase, which lasts for 4–8 weeks, the parasite multiplies in the bloodstream and no specific symptoms are evident. The second chronic phase persists for the host's lifespan and is characterized by cardiac and digestive alterations caused by inflammation and parasite accumulation in the heart and other relevant organs.[8] If untreated, the disease is fatal as result of severe myocarditis and, less commonly, meningoencephalitis.[9]

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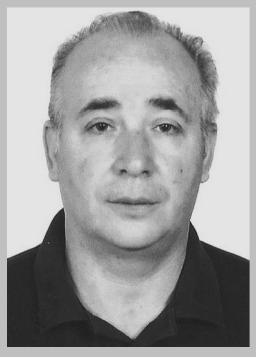
2.3. Leishmaniasis

Leishmaniasis is a vector-borne disease caused by protozoans belonging to the genus *Leishmania* and transmitted by the bite of infected female sandflies. According to the World Health Organization (WHO), the disease is responsible for 20 000 to 30 000 deaths annually, mainly in areas of the tropics, subtropics, and southern Europe, with 900 000–1.3 million new cases per year.[1]

There are three main forms of the disease: cutaneous leishmaniasis (CL), mucosal leishmaniasis (ML) and visceral leishmaniasis (VL). CL, mainly diffused in South America, is characterized by skin ulcers leaving life-long scars and serious disability; ML, common in Bolivia, Brazil, and Peru, causes the destruction of mucous membranes of the nose, mouth, and throat; and VL, highly endemic in the Indian subcontinent and in East Africa, represents a common opportunistic infection in patients with HIV/AIDS.[1,10]

Dr. Maria Dichiara graduated in Pharmacy from the University of Catania (Italy) in 2016. During her undergraduate studies, she attended Paris Descartes University, focusing on the development of a new series of aucubin derivatives. She is currently working at the Department of Drug Sciences, University of Catania, as a volunteer researcher. Her research interests include the synthesis and biological evaluation of anticancer drugs.

Prof. Simona Collina is affiliated with the Department of Drug Sciences at the University of Pavia, Italy. Her research interests include the design and synthesis of new chemical entities effective against infection, cancer, pain, and neurodegenerative diseases. The development of reactions suitable for the preparation of focused compound libraries is another key interest. Among the different research topics, the discovery of new modulators of sigma receptors as well as of small molecules able to counteract Leishmaniasis are the most challenging ones.



Prof. Agostino Marrazzo graduated in Pharmaceutical Chemistry and Technology from the University of Camerino (Italy) in 1990. From May 1990 to December 1991, he served at the Department of Chemistry, University of Parma, as winner of a Scholarship Searches ENI. From 1992 to 2000 he was Assistant Professor, and since 2000 he has been Associate Professor at the Department of Drug Sciences, University of Catania. His research interests are in the design, synthesis, and pharmacological evaluation of new ligands for the treatment of neurodegenerative and malignant processes.

Prof. Antonio Rescifina received his PhD in Chemical Science from the University of Catania, Italy. He is currently a Professor of Organic Chemistry. His research interests include the design of bioactive molecules of pharmacological interest, the study of targeting biological systems by molecular modeling, and the study of reaction mechanisms by computational chemistry.

Prof. Orazio Prezzavento obtained his PhD in Pharmaceutical Science in 1994 at the University of Catania (Italy). In 1994 he was Honorary Research Assistant at the Department of Chemistry, University College London, focusing on the synthesis of H3 antagonists. From 1997 to 2005 he was Assistant Professor, and since 2005 he has been Associate Professor at the Department of Drug Sciences, University of Catania. His research is focused on the design, synthesis and binding affinity evaluation of small molecules.

Dr. Emanuele Amata received his PhD in Pharmaceutical Sciences at the University of Catania (Italy). He has served at Northeastern University (Boston, MA, USA) as postdoctoral research associate in medicinal chemistry of neglected tropical diseases, and as visiting scientist at the University of Mississippi (Oxford, MS, USA). He is now affiliated with the Department of Drug Sciences, University of Catania, as a researcher, working on design and synthesis of novel therapeutic agents for cancer and infectious diseases.

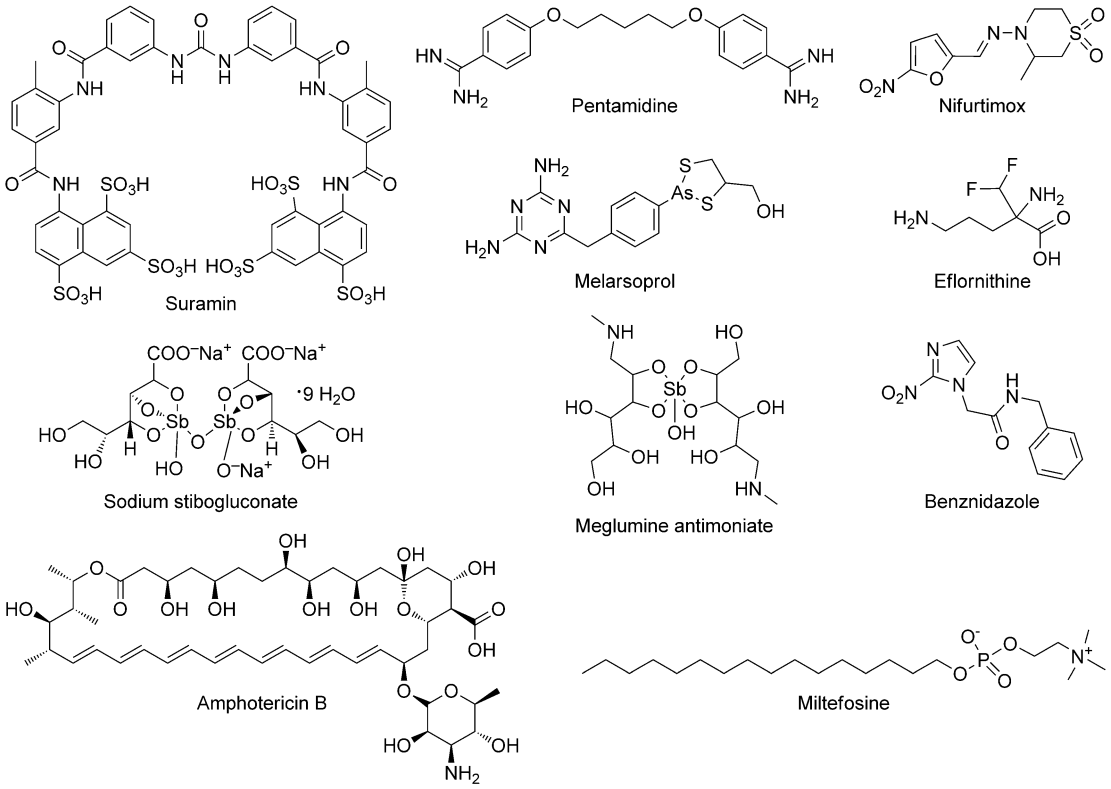


Figure 1. Structures of approved drugs for neglected tropical protozoan diseases.

3. Current Treatments and Drug Development

3.1. Approved therapies, drug candidates, and vaccines

Many of the drugs used to treat protozoan NTDs are agents with a certain level of toxicity, mixed efficacy, complex administration, and, lately, emergence of resistance.[11] Five drugs are available for the treatment of HAT: pentamidine and suramin for the early and peripheral stage of the disease, melarsoprol, and eflornithine alone or in combination with nifurtimox (NECT), for the CNS stage (Figure 1).

Two novel chemical entities are under clinical evaluation for the treatment of HAT. Fexinidazole is in clinical phase II/III trials to prove efficacy and safety as an oral treatment in advanced-stage sleeping sickness.[12] Oxaborole (SCYX-7158), a benzoxaborole derivative, was taken into phase IIb/III trials in 2016 (Figure 2).[13]

Benznidazole and nifurtimox (Figure 1) are the two drugs available for the treatment of Chagas disease, but both are limited in their capacity to completely eradicate the infection, showing also severe adverse effects including being mutagens.[7,14] A 60-day treatment regimen is required for benznidazole, while a 60–90-day treatment regimen is required for nifurtimox. Overall, it must be noted that nifurtimox and benznidazole may eradicate the parasite in the host during the acute stage, however there is questionable efficacy of these compounds in the chronic phase of the disease.[15]

Posaconazole (Figure 2) has been taken into phase II trials for the treatment of chronic Chagas disease. The purpose of the study was to determine whether posaconazole alone or in combination with benznidazole was superior to benznidazole monotherapy in eliminating *T. cruzi*; however recent results have shown no advantages.[16]

The traditional treatment for leishmaniasis is a combination of pentavalent antimony compounds, sodium stibogluconate, and meglumine antimoniate, that can only be given by intravenous injection (antimony compounds) and by intravenous or intramuscular injection (sodium stibogluconate, and meglumine antimoniate). The liposomal amphotericin B (Figure 1) was approved by the FDA for VL having a shorter course and lower toxicity, whereas the oral miltefosine (Figure 1) is an effective FDA-approved treatment against both VL and CL. Some other drugs such as pentamidine and azoles (ketoconazole, itraconazole, and fluconazole) are efficient to treat selected cases of leishmaniasis, but they are not FDA indicated.[17] Two drug candidates, the topical WR-279,396 (15% paromomycin + 0.5% gentamicin) and 18-methoxycoronaridine (Figure 2), are under clinical investigation to evaluate the efficacy and tolerability for CL treatment.[18] Pentoxifylline and imiquimod (Figure 2) have been taken into clinical studies to determine their antitrypanosomal activity when associated to pentavalent antimony compounds for the treatment of CL.[18b,19] Daylight-activated photodynamic therapy (DA-PDT) is also under clinical investigation in treating CL caused by *L. major* and *L. tropical*, as it is a self-administered procedure that does not require medical assistance.[20]

One of the most remarkable features of HAT trypanosomes is their ability to regularly change their surface coat and hence evade the immune system.[21] This mechanism is known as an-

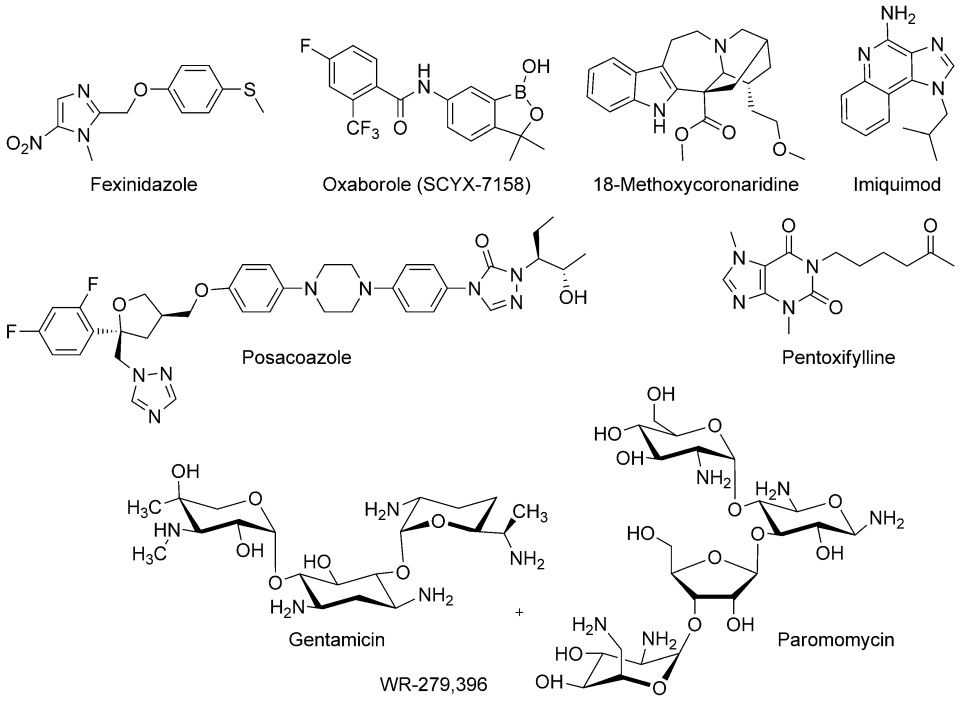


Figure 2. Structures of drug candidates for neglected tropical protozoan diseases.

tigenic variation.[22] In other words, the parasite greatly increases the variation of the expressed protein by creating a puzzle made of fragments of several genes.[23] Thus, no vaccines are available or designed for HAT.[23] Some candidate vaccines are currently under development for both leishmaniasis and Chagas disease.[24] However, the transition from animal models to human clinical evaluation is one of the major limits in protozoan vaccine development and as a consequence, to date, no vaccines are available.[25]

3.2. New drug development

Development of new drugs and vaccines is needed for eliminating protozoan NTDs.[26] However, finding new safe, effective and easy to administer drugs seems to be far away, due to the lack of scientific data and low return on investment making these diseases unattractive from a market perspective.

Indeed, by analyzing the number of active research projects (title and abstract search) in the United States National Institute of Health (NIH) research portfolio tool, for the three protozoan NTDs, the insufficient financial incentives for these diseases are more evident, especially if compared with other diseases. Currently, active research projects returned by the NIH research portfolio online tool search for "Chagas disease" and "leishmaniasis" are 78 and 75, respectively, followed by "human African trypanosomiasis" with 30 active projects returned to the query; search for "malaria" returned 644 hits. At the same time, looking at the number of original articles (topic search; all years) reported in the Web of Science platform, the predominance of original research articles is for "leishmaniasis", with 15435 returned hits to the query, followed by "Chagas disease" (8801) and "human African trypanosomiasis" (1555); the search for "malaria" returned 51476 hits.[27]

In this scenario, it becomes clear that the low economic incentive may account for the lack of scientific data and for finding new drugs. Lately, this gap has been partially covered by private funding institutions and pharmaceutical companies who have contributed to helping the scientific community to improve the knowledge about these diseases and find novel therapies. The Bill & Melinda Gates Foundation supports the development of safe and effective treatments to eliminate HAT in poor and hard-to-reach communities having little access to healthcare.[28] The Drugs for Neglected Diseases initiative (DNDi), launched in 2003, is a collaborative nonprofit research and development organization focused on the development of new treatments for several NTDs, including HAT, Chagas disease, and leishmaniasis.[29] The ChEMBL—Neglected Tropical Disease archive is a repository for Open Access screening and medicinal chemistry data, with the purpose to facilitate the primary screening and medicinal chemistry for NTD data sharing.[30] Pharmaceutical companies are also accelerating research and development efforts in this way. In 2010, GlaxoSmithKline established a not-for-profit group to develop new drugs and vaccines to eradicate NTDs.[31] The NTD Data Sharing Project has been created by combining academic and industrial experts with the common goal to advance drug development for protozoan NTDs.[31,32]

Recently, nanotechnologies have provided progress in the development of alternative strategies for the treatment of NTDs.[33] In addition, macrophage-mediated targeted therapy is also emerging as a possible alternative for therapy of leishmaniasis, as the physiology and role of macrophages in the pathology begins to be better understood.[34]

3.3. Biological evaluation for neglected tropical protozoan diseases

The first step in determining the antiparasitic activity of a chemical entity is its evaluation in a biological assay. This is recognized as a major bottleneck during the drug effectiveness validation process. Indeed, there may be a different activity/efficacy profile when moving from a low complexity biological model as target-based assays to medium complexity biological model like cellular assays, tissue assays or even high complexity system like organs or animal models. With the biological assay complexity scale-up from in vitro to in vivo, often the chemical compound nature has to be refined to fit the new system criteria. This flow process is here defined as "biological scale-up", and represents one of the major issues related to lead identification, and is certainly a primary issue in the medicinal chemistry field, especially when target-based high-throughput screenings (HTSs) are employed. If the lead generation process has to cross all these validation steps, it is understandable that an extremely large number of compounds have to be taken into consideration and modified for testing, in order for the information generated to be used in the design process. To overcome this problem, and to decrease the impact of biological scale-up, the best approach would be to test compounds directly in the most complex biological system available. In the case of a first screening, except for zebrafish, the use of animal models is usually excluded for practical, economic and ethical reasons.

In protozoan drug discovery, primarily target-based (protein screening) or cell-based (phenotypic assay) methods are employed for initial screening and hit recognition. The choice between target-based or phenotypic approaches is of crucial importance.[35] Phenotypic screening, bearing a certain biological complexity, may be beneficial in finding active and effective compounds because inhibitors may act on unknown and multiple proteins or pathways unlike biochemical screens, which rely on known single therapeutic pathways. Moreover, in phenotypic screening, compounds must usually cross biological barriers, giving also additional information about their pharmacokinetic properties and overall raising the number of attributes that a compound must possess in order for it to be effective. This will allow identification of the lead compound, as it incorporates other specifications besides the target association. In addition, there are still very few fully validated drug targets in protozoan NTD, and thus the use of target-based approaches may hinder the lead compound finding process for HAT, leishmaniasis and Chagas disease.[35c] By analyzing the FDA approved drugs between 1999 and 2008, 37% resulted from projects that used phenotypic screening whereas target based screening identified 23%.[35a] Overall, phenotypic screening has resulted in the identification of more suitable compounds for effective parasitic infection than target-based assays.[6b,31,36]

3.4. Target and drug repurposing for neglected tropical protozoan diseases

Target and drug repurposing may represent an effective method to identify novel treatments for neglected or rare diseases.[37] Target repurposing is the use of drugs associated with a specific human target, to hit an homologous parasite target. While drug repurposing is the process of finding new indications for existing drugs. Considering the paucity of financial incentive dispensed for protozoan NTDs, drug and target repurposing may be beneficial in discovering new drugs, by looking among established inhibitors of human targets that share homologies with parasite targets even in the absence of information about the parasite target.[38]

Trypanosomatid genome sequencing and the following bioinformatic studies have proven the existence of parasite protein sequences which are similar to humans and can be used as putative targets.[39] Drugs approved for human diseases or clinical candidates represent a starting point to develop parasitic inhibitors, but not having the desired potency and selectivity against the parasitic targets, further optimizations may be needed. As discussed earlier, common approaches in repurposing programs consist of target-based or phenotypic screens, even if the latter has resulted to be more successful in the majority of new molecular entities discovered.[35c,40]

However, repositioning is not a new approach and several drugs currently used for the treatment of NTDs are being repurposed from other FDA-approved therapeutic indications. The originally anticancer drugs miltefosine and eflornithine, the antifungal amphotericin B and the aminoglycoside antibiotic paromomycin (Figure 1) represent successfully repurposed drugs for sleeping sickness.[38]

In this review, we comment on the repurposing of acknowledged human kinase inhibitors in trypanosomatids. We will consider the human kinase inhibitor by kinase family and discuss them in terms of both phenotypic or target-based screenings performed.

4. Human Kinase Inhibitor Repurposing in Trypanosomatids

4.1. Trypanosomatid kinomes versus human kinome

The majority of the current therapeutic NTD targets are represented by enzymes or cell surface receptors. Among these, eukaryotic protein kinases represent a major group of protein targets whose modulation may be beneficial for the treatment of protozoan NTD. By adding phosphate groups to specific substrates, protein kinases are important regulators of several protozoan cellular processes.[39] Analysis of the protein kinase complement in *L. major*, *T. brucei* and *T. cruzi* genomes showed an expression of 179, 156 and 171 conventional eukaryotic protein kinases (ePK) that are catalytically active, as well as 17, 20 and 19 atypical protein kinases (aPK) respectively.[39,41] Most of these ePKs are orthologous among the three parasites, even if each species contains distinctive protein kinases. The trypanosomatid kinomes, which represent about one-third of the

human complement, encoding 491 ePK and 40 aPK, differ in several aspects from that of their mammalian host.

A key difference between mammalian and parasite kinomes is the lack, within trypanosomatids, of ePK mapping tyrosine kinase (TK) and tyrosine kinase-like (TKL). Nevertheless, tyrosine phosphorylation is well documented in trypanosomatids, and may be due to the action of atypical tyrosine kinase phosphorylating serine, threonine, and tyrosine.[39] Another important difference is a low representation, within trypanosomatid genomes, of CAMK and AGC groups that belong to the family of serine-threonine protein kinases. On the other side, the CMGC and STE group are over-represented within trypanosomatid genomes if compared with humans.[39] The trypanosomatid kinomes have a number of gene products which, lacking one or more essential amino acid residues for catalytic activity, are predicted to be catalytically inactive ePKs. Moreover, whereas over 50% of human ePK are characterized by accessory domains needed for protein-protein interactions, trypanosomatid kinomes showed a lack of additional domains. All these specific differences between parasite and human kinomes may be prospectively used to design parasite selective compounds for mammalian targets.

4.2. Human CMGC inhibitors

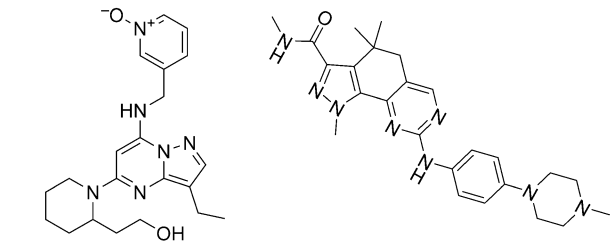
The family name CMGC is an acronym that stands for the initials of some members including cyclin-dependent kinases (CDKs), mitogen-activated protein kinases (MAPKs), glycogen synthase kinases (GSKs) and CDK-like kinases (CDKL. The human CMGC protein kinases have critical roles in a variety of cellular processes including the control of human tumor suppressor activity.[42] Trypanosomatids express a large number of CMGC kinases which are involved in the control of the parasitic life cycle, differentiation and segregation of organelles.[37] Among these, CDKs, MAPKs, and dual specificity CLK and DYRK kinases are well represented relative to the human kinome.[39] The trypanosomatid CDK family is composed of several members named cdc2-related kinases (CRKs), which need an activating cyclin partner to be functional. Despite the large number, only two CRKs, CRK1 and CRK3, have been shown to be particularly essential for parasite cell cycle G1/S transition. Moreover, CRK3 in complex with the CYC6 mitotic cyclin has been shown to regulate G2/M cell cycle progression, whereas in complex with CYC2, it is essential for G1 progression.[39,43]

MAPKs are involved in the coordination of cellular responses to different stimuli in many eukaryotes. In mammals, three major types of MAPKs (ERK, p38, and JNK) have been identified and their malfunction has been related to cancer.[44] A large number of MAPKs have also been identified in trypanosomatids, even if a complete MAP kinase signaling pathway has been impossible to predict on the basis of genomic sequence analysis alone.[39] Among these, MAPK and MAPK-like families have been distinguished. These latter are very similar to CDK-like and RCK families, which have characteristic residues involved in the regulation of MAPKs, and are considered part of a MAPK superfamily.[39] Two MAPKs, *Lmx*MPK1, and *Lmx*MPK9, have shown an essential role in *L. mexicana*.[45] Specifically, *Lmx*MPK1 has proven critical for amastigote proliferation, whereas *Lmx*MPK9 is implicated in flagella length regulation.[45,46] In *T. brucei*, KFR1 and *Tb*MAPK2 have been shown to be involved in bloodstream form (BSF) parasite proliferation and trypanosome differentiation, respectively. A third *T. brucei* MAPK, *Tb*ECK1, having characteristics of both MAPKs and CDKs, is essential in all parasite life cycle.[39]

The serine/threonine kinase GSK3, with the two human isoforms GSK3α and GSK3β, is an important target in several human diseases, as it is involved in different cell signaling pathways, including energy metabolism and cell proliferation. *T. brucei* expresses two homologous proteins to the human GSK3 (*Hs*GSK3), *Tb*GSK3 short (*Tb*GSK3s) and *Tb*GSK3 long (*Tb*GSK3l).[47] It has been shown that *Tb*GSK3s is critical for parasite cell growth having a role in mitosis control and this protein has been validated as a potential drug target for the treatment of HAT.[48] Two GSK3 isoforms, GSK3 short and GSK3 long, are also present in *Leishmania*, with the short form (*Ld*GSK3s) critical for parasite cell growth.[48,49]

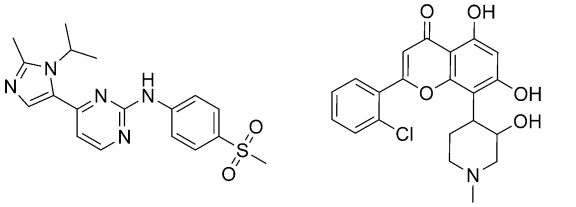
Several human CMGC protein kinase inhibitors have been evaluated in protozoan NTD. Compounds grouped by similarity of the main human kinase targets are reported in Figures 3–7. Some of them have been indicated as potential *T. brucei* growth inhibitors according to a statistical analysis driven approach performed on a high throughput screening released by GlaxoSmithKline.[31,36e] In this study, among over 224 human kinases, 29 kinases, defined as Preferred Human Kinases, were statistically selected, and inhibitors of these kinases are indicated to have a better likelihood of activity in the parasite. Being designed for *T. brucei* growth inhibition, compounds were evaluated in *T. b. brucei* BSF phenotypic screening (strain Lister 427). Due to the similarity in kinomes of the three related kinetoplastids, compounds were also evaluated for activity in *L. major* promastigote and amastigote forms, and *T. cruzi* whole cell phenotypic screening. No information has been reported regarding the parasite targets involved for observed growth inhibition. The most potent compounds in the series resulted in the repurposed human CDK inhibitors (Figure 3). Milciclib and dinaciclib have shown potency in the low nanomolar range against both *T. brucei* parasites (Lister 427 strain) and *T. cruzi* intracellular amastigote (Tulahuén strain), with EC_{50} of 0.025 (*T. brucei*) and 0.040 μM (*T. cruzi*) for milciclib and 0.040 (*T. brucei*) and 0.10 μM (*T. cruzi*) for dinaciclib. *L. major* activity was lower for both compounds in both parasite forms, with the only considerable activity of milciclib for the promastigote form with EC_{50} of 0.32 μM. Alvocidib (Flavopiridol) and AZD5438 have shown a slightly inferior growth inhibition against the three trypanosomatids. Both compounds displayed elevated potency against *T. brucei* parasites (Lister 427 strain) with EC_{50} of 0.032 and 0.16 μM respectively for AZD5438 and alvocidib, but lower activity against *L. major* amastigote and *T. cruzi* intracellular amastigote (Tulahuén strain).

Moderate activity has been shown against *L. major* promastigote form with EC_{50} of 0.39 μM for both compounds.[36e] Evaluated on *Tb*ERK8, milciclib and AZD5438 have inhibited the kinase activity up to 20% or less, but no selectivity over mammalian cells has been observed.[50]



Dinaciclib
T. brucei EC_{50}: 0.040 μM
L. major prom EC_{50}: 1.58 μM
L. major amast EC_{50}: 2.51 μM
T. cruzi EC_{50}: 0.10 μM
3T3 TC_{50}: >100 μM
HepG2 TC_{50}: >39,81 μM

Milciclib
T. brucei EC_{50}: 0.025 μM
L. major prom EC_{50}: 0.32 μM
L. major amast EC_{50}: 5.01 μM
T. cruzi EC_{50}: 0.04 μM
3T3 TC_{50}: >100 μM
HepG2 TC_{50}: 10 μM

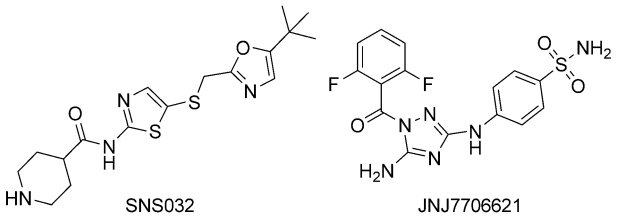


AZD5438
T. brucei EC_{50}: 0.032 μM
L. major prom EC_{50}: 0.39 μM
L. major amast EC_{50}: 2.51 μM
T. cruzi EC_{50}: 1.58 μM
3T3 TC_{50}: >100 μM
HepG2 TC_{50}: 12.59 μM

Alvocidib
*Lmx*CRK3his IC_{50}: 0.1 μM
CRK3his/CYCAhis IC_{50}: 0.102 μM
*Tc*CRK3 IC_{50}: 0.146 μM
T. brucei EC_{50}: 0.16 μM
L. major prom EC_{50}: 0.39 μM
L. mexicana prom EC_{50}: 1.0 μM
L. major amast EC_{50}: 3.16 μM
T. cruzi EC_{50}: 12.59 μM
3T3 TC_{50}: >100 μM
HepG2 TC_{50}: >39,81 μM

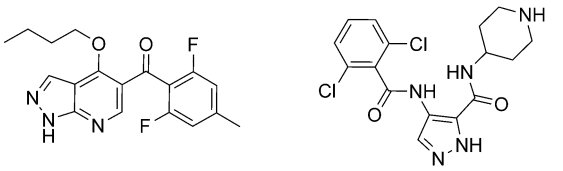
Figure 3. Structures and biological activity of dinaciclib, AZD5438, milciclib, and alvocidib.

Alvocidib is particularly noteworthy, as it has been extensively used to understand the biology of trypanosomatids. Hassan et al. have shown that alvocidib inhibited the histidine-tagged CRK3 kinase purified from transgenic *L. Mexicana* (*Lmx*CRK3his), with an IC_{50} of 0.1 μM.[51] *Lmx*CRK3his has been recognized as essential for cell cycle progression of *L. mexicana*. The same authors reported that alvocidib inhibited the growth of *L. mexicana mexicana* (MNYC/BZ/62/M379) promastigotes in a dose-dependent manner in vitro. At 1.0 μM concentration, alvocidib caused a complete arrest of promastigote growth, while a lower concentration resulted in partial growth inhibition.[36e,51] Alvocidib also inhibited the recombinant CRK3his/CYCAhis with an IC_{50} of 0.102 μM, a similar value as compared with IC_{50} of 0.1 μM for *Lmx*CRK3his.[52] Additional evidence has shown that alvocidib is able to inhibit CRK3 from soluble non-infective *T. cruzi* epimastigotes form (*Tc*CRK3) extracts with an apparent IC_{50} of 0.146 μM. An apparent IC_{50} was determined, as the inhibition of CRK3 by alvocidib is reversible. The same authors have evaluated alvocidib for *T. cruzi* epimastigote (Tulahuén strain) growth inhibition, demonstrating a dose-dependent growth inhibition starting at 0.050 μM, although even at 5 μM the cell growth was not completely arrested.[53]



SNS032
T. brucei EC_{50}: 0.13 μM
L. major prom EC_{50}: >25 μM
L. major amast EC_{50}: 15.8 μM
T. cruzi EC_{50}: >50 μM
3T3 TC_{50}: >100 μM
HepG2 TC_{50}: >39.81 μM

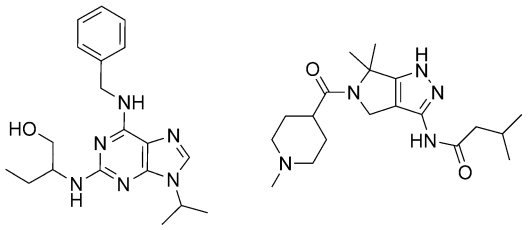
JNJ7706621
T. brucei EC_{50}: 0.32 μM
L. major prom EC_{50}: >25 μM
L. major amast EC_{50}: >15.8 μM
T. cruzi EC_{50}: >50 μM
3T3 TC_{50}: >100 μM
HepG2 TC_{50}: 6,31 μM



BMS265246
T. brucei EC_{50}: 0.50 μM
L. major prom EC_{50}: 0.25 μM
L. major amast EC_{50}: >15.8 μM
T. cruzi EC_{50}: >50 μM
3T3 TC_{50}: >100 μM
HepG2 TC_{50}: >39.81 μM

AT7519
T. brucei EC_{50}: 0.32 μM
L. major prom EC_{50}: >25 μM
L. major amast EC_{50}: >15.8 μM
T. cruzi EC_{50}: 1.26 μM
3T3 TC_{50}: >100 μM
HepG2 TC_{50}: >39.81 μM

Figure 4. Structures and biological activity of repurposed human CDK inhibitors.



Seliciclib
T. brucei EC_{50}: >3.16 μM
L. major prom EC_{50}: 6.31 μM
L. major amast EC_{50}: >15.8 μM
T. cruzi EC_{50}: >50 μM
3T3 TC_{50}: >100 μM
HepG2 TC_{50}: 31.62 μM

PHA793887
T. brucei EC_{50}: 1.99 μM
L. major prom EC_{50}: >25 μM
L. major amast EC_{50}: >15.8 μM
T. cruzi EC_{50}: >50 μM
3T3 TC_{50}: >100 μM
HepG2 TC_{50}: 39.81 μM

Figure 5. Structures and biological activity of repurposed human CDK inhibitors.

Other repurposed human CDK inhibitors include SNS032, JNJ7706621, BMS265246 and AT7519 (Figure 4).[36e] These inhibitors have proven good activity against *T. brucei* parasites (Lister 427 strain) with EC_{50} ranging between 0.13–0.50 μM, but little to no effect on *L. major* and *T. cruzi* intracellular amastigote (Tulahuén strain) with the only exception of BMS265246, which has a reported moderate *L. major* promastigote growth inhibition with EC_{50} of 0.25 μM. PHA793887 and seliciclib (Figure 5), demonstrated high micromolar range activities against the three trypanosomatids.[36e]

Some of the human CMGC inhibitors reported above (SNS-032, AZD5438, milciclib, and dinaciclib), were lately selected

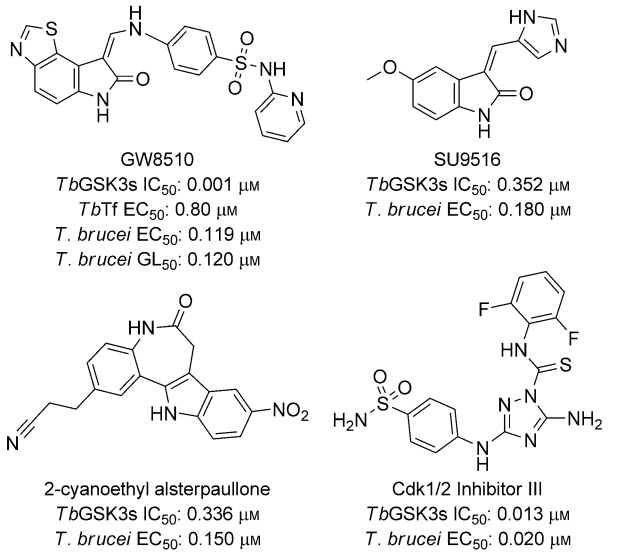


Figure 6. Structures and biological activity of repurposed human CDK inhibitors.

based on their lead and lead-like properties and evaluated again in *T. b. brucei* BSF (strain wild-type-221). Overall, these inhibitors were less potent with respect to the previous evidence, except for SNS-032 and dinaciclib, which demonstrated a similar potency . Indeed, IC_{50} for these four compounds was: 0.12, 0.21, 0.62, and 0.080 μM for SNS-032, AZD5438, milciclib, and dinaciclib, respectively.[50] Ojo et al. evaluated four known human CDK inhibitors for growth inhibition of *Tb*GSK3s and BSF *T. brucei* (Figure 6). *Tb*GSK3s IC_{50} measured on GW8510, Cdk1/2 Inhibitor III, 2-cyanoethyl alsterpaullone, and SU9516 have demonstrated values of 0.001, 0.013, 0.336, and 0.352 μM, respectively. When BSF *T. brucei* growth inhibitions were evaluated, a good correlation between target-based and phenotypic screening was observed, with EC_{50} of 0.119, 0.020, 0.150, and 0.180 μM, respectively.[48] Guyett et al. evaluated GW8510 against *Tb*GSK3s and showed a similar IC_{50} value of 0.001 μM and a GL_{50} of 0.120 μM for *T. brucei* (strain CA427).[47] Moreover, GW8510 has been shown to decrease the receptor-mediated endocytosis of transferrin in *T. brucei* (*Tb*Tf) with an EC_{50} of 0.80 μM (Figure 6).[47]

Human GSK3α and GSK3β inhibitors (SB415286, TWS119, tideglusib, CHIR98014, and SB216763) resulted in lower activity when compared with other human CMGC inhibitors (Figure 7). The most potent compounds were SB415286 and TWS119 for *T. brucei* (Lister 427 strain) growth inhibition with EC_{50} of 0.795 and 1.0 μM, respectively.[36e]

Ojo et al. evaluated SB415286 for inhibition of *Tb*GSK3s and showed an IC_{50} of 1.0 μM and a *T. brucei* BSF growth inhibition of 0.74 μM, similar to the result reported above.[48] Tideglusib has demonstrated a good activity over *L. major* promastigote form with an EC_{50} of 0.32 μM.[36e] Swinney et al., evaluated Tideglusib, TWS119, and compound CHIR99021 (Figure 7) for inhibition of *Tb*GSK3s. IC_{50} values for Tideglusib, TWS119, and CHIR99021were reported as 0.17, 0.60, and 0.20 μM, respectively, demonstrating an analogy between human and parasite inhibitor structural requirements (Figure 6).[54] In the same work

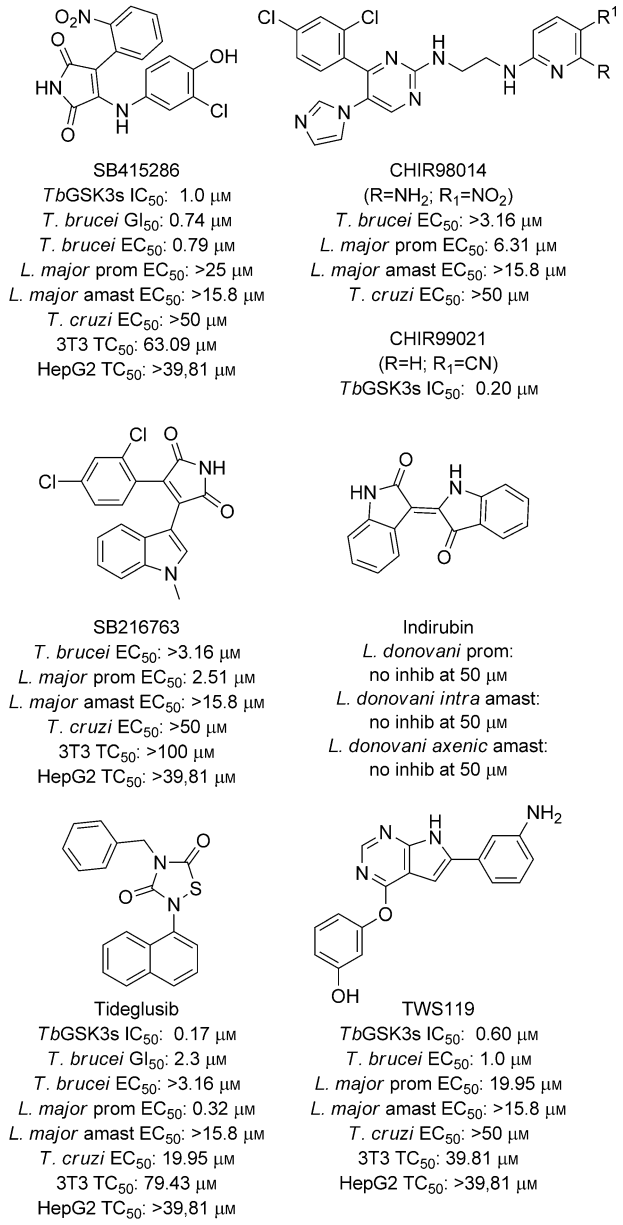


Figure 7. Structures and biological activity of human GSK inhibitors.

the authors also reported that Tideglusib inhibited BSF *T. brucei* growth with a GI_{50} of 2.3 μM.[54] All of these five human GSK3α and GSK3β inhibitors (SB415286, TWS119, Tideglusib, CHIR98014, and SB216763) have shown ability to decrease *T. cruzi* growth, having EC_{50} in the high micromolar range (Figure 7).[36e] Indirubin, a human CMGC family inhibitor with low affinity for GSK3β, and also for CDK2/cycA, p35/CDK5, and CDK1/cycB, has shown no activity against *L. donovani* promastigotes, intracellular amastigotes, and axenic amastigotes having no inhibition at 50 μM (Figure 7).[49]

Harmine (Figure 8), a low-molecular-weight DYRK1B/DYRK1A inhibitor, has shown moderate activity against both forms of *L. major* with EC_{50} of 1.26 μM (*L. major* promastigote and amastigote EC_{50} 1.26 μM), but lower inhibition of both *T. brucei* (Lister 427 strain) and *T. cruzi* intracellular amastigote (Tulahuén strain) (Figure 6).[36e]

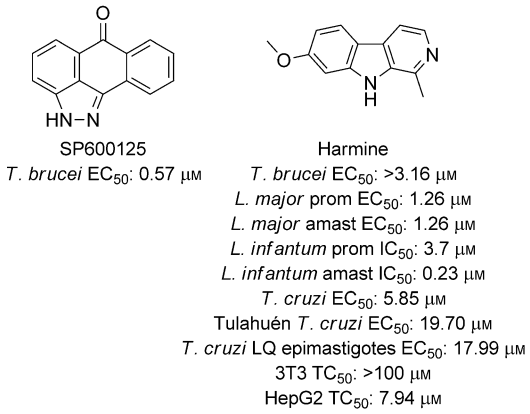


Figure 8. Structures and biological activity of human DYRK and JNK inhibitors.

Harmine has also been evaluated for inhibition of *L. infantum* promastigote and amastigote forms and *T. cruzi* Tulahuén and LQ epimastigotes. Results have shown a selective activity against *L. infantum* amastigote as opposed to the promastigote form, with IC_{50} values of 0.23 ad 3.7 μM, respectively.[55] Activity on both *T. cruzi* epimastigote strains was in the range of 18–20 μM.[56]

The pyrazoloanthrone Jun N-terminal kinase (JNK) inhibitor SP600125 (Figure 8) has been evaluated for inhibition of *T. brucei* (strain wild-type-221) growth and it showed a sub-micromolar activity with IC_{50} of 0.57 μM.[50]

Important progress supporting the chemical validation of *Tb*GSK3s as a drug target for HAT was made by Ojo et al., who evaluated a panel of 225 known human GSK3β inhibitors for activity against *Tb*GSK3s.[48] The observed correlation between enzyme and cellular activity supported the chemical validation of *Tb*GSK3s as a drug target (Figure 9).

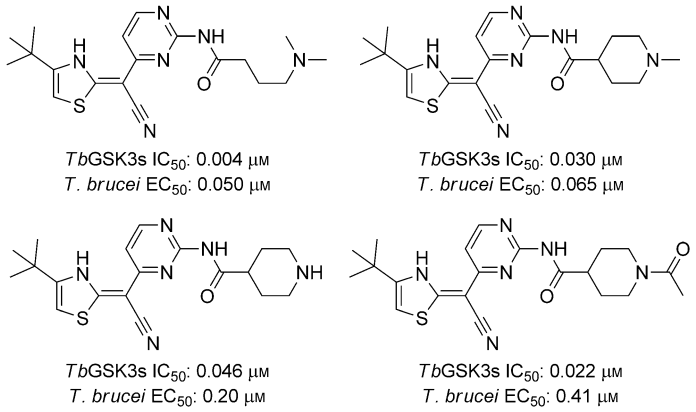


Figure 9. Structures and biological activity of human GSK inhibitors.

4.3. Human tyrosine kinase inhibitors

Tyrosine phosphorylation plays an essential role in controlling growth and differentiation in higher eukaryotes. Indeed, an abnormal level of regulation of these mechanisms has been implicated in cancer and autoimmune disorders.[57] Trypanosomatids lack both TK and TKL families, even if tyrosine phosphorylation has been observed.[39] This activity may be related to atypical protein kinases such as Wee1 and dual specificity kinases acting on serine/threonine and tyrosine residues, such as DYRKs, CLKs, and STE7.[58] In addition, CRK3, the trypanosomatid homologue of human CDK1, contains a conserved tyrosine residue in the same subdomain.[39,59] For these reasons, chemical scaffolds inhibiting human protein TKs could have potential activity when evaluated in trypanosomatids.

One of the first repurposed human TK inhibitors was the quinazolin-4-amine derivative lapatinib (Figure 10), an ErbB2/EGFR TK inhibitor, approved for breast cancer. It has been observed that lapatinib inhibited growth in both Lister 427 and CA427 *T. b. brucei* BSF strains with GI_{50} of 1.5 μM.[60] Lapatinib has also shown significant activity against *L. donovani* amastigote both in vitro, showing an IC_{50} value of 2.48 μM and, in an *L. donovani* BALB/c model, decreasing liver amastigote burden by 35.5%.[61] While against *L. major* amastigotes, lapatinib inhibited the parasite growth with EC_{50} of 11 μM. However, in another study, lapatinib tested against *L. major* was not active at 30 μM. The same authors also reported a lack of activity of lapatinib (30 μM) against other *Leishmania* strains: *L. amazonensis*, *L. mexicana*, *L tropica*.[61] Lapatinib inhibited *T. cruzi* intracellular amastigote growth inhibition with an EC_{50} of 13.0 μM.[62] The chemical structure of lapatinib has been extensively modified in order to improve its parasite efficacy.[62,63]

Two EGFR and HER2/ErbB2 inhibitors, canertinib and AEE788 (Figure 10), inhibited the replication of *T. b. brucei* BSF with GI_{50} values of 2 and 3 μM (strain Lister 427), and 1.4 and 2.5 μM (strain CA427), respectively.[60] Additional evidence has been reported in support of AEE788 *T. brucei* BSF proliferation inhibition. AEE788, evaluated in *T. brucei* (strain RUMP528 or Lister 427), has demonstrated an EC_{50} value of 5 μM between 4 and 9 h of treatment, and beyond 9 h the drug (5 μM) inhibited *Tb*Tf endocytosis.[64]

Because the trypanosome genome lacks receptor tyrosine kinases (RTKs), the activity of lapatinib, canertinib and AEE788 against *T. brucei* could not be related to the inhibition of EGFR and/or VEGFR kinases. Using a chemical proteomic approach, it has been shown that lapatinib, canertinib, and AEE788 associate with different parasite protein kinases named *T. brucei* lapatinib binding protein (*Tb*LBPK). Four kinases have been found to interact with lapatinib: *Tb*LBPK1, *Tb*LBPK2, *Tb*LBPK3 and TbLBPK4 (or TbGSK3s). Canertinib was able to associate with these four kinases and also with *Tb*CBPK1, while AEE788 associated only with *Tb*LBPK1, *Tb*LBPK2, and *Tb*LBPK3.[60b] Further studies conducted on a mouse model of HAT showed that lapatinib was able to cure 25% of infected mice, whereas canertinib and AEE788 extended the survival of treated mice without clearing the parasite.[60a,65]

The EGFR inhibitor, erlotinib (Figure 10) also inhibited the replication of *T. brucei* BSF (strain CA427), with GI_{50} of 1.9 μM.[60a] Erlotinib as well as gefitinib and pazopanib have shown no activity against *L. major*, *L. donovani*, *L. amazonensis*,

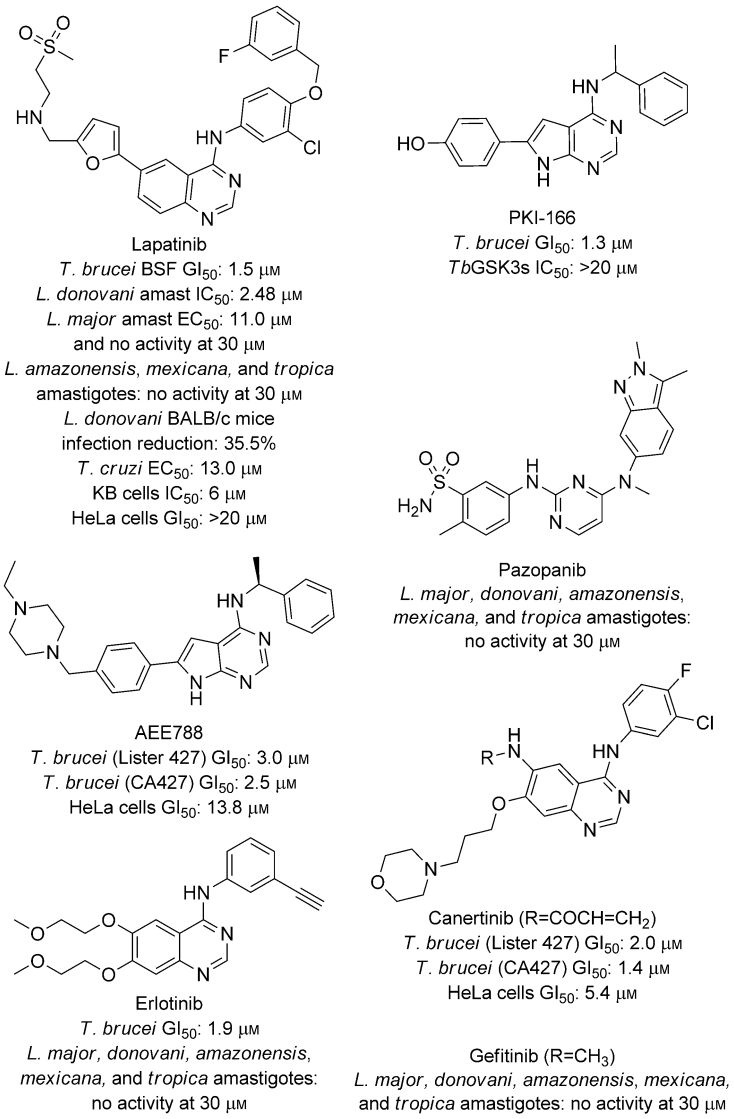


Figure 10. Structures and biological activity of repurposed human EGFR inhibitors.

L. mexicana, *L. tropica* at 30 μM.[61] Gefitinib and pazopanib have shown no inhibitory *Tb*GSK3s activity at 20 μM.[47] Another mammalian EGFR inhibitor, PKI-166 (Figure 10) also inhibited the replication of *T. brucei* BSF (strain CA427), with GI_{50} of 1.3 μM.[60a] Guyett et al. reported that PKI-166 inhibited *Tb*GSK3s with IC_{50} >20 μM, suggesting that PKI-166 could have a different target other than *Tb*GSK3s and be involved in different physiological pathways.[47]

The VEGFR/PDGFR inhibitors axitinib and sunitinib exhibited micromolar growth inhibitory activity over *T. brucei* BSF (strain CA427) with GI_{50} values of 2.0 and 1.3 μM, respectively (Figure 11).[60a] Sunitinib has also shown significant activity against *L. donovani* and *L. mexicana* amastigotes in vitro, showing IC_{50} values of 1.08 and 2.63 μM, respectively.[61] However, sunitinib was not active at 30 μM against other *Leishmania* strains, such as *L. amazonensis*, *L. major*, and *L. tropica*,.[61] In the *L. donovani* BALB/c model, sunitinib decreased liver amastigote burden by 41.15 %.[61]

Several EGFR inhibitors have been evaluated for their *Tb*GSK3s inhibition, however just a few were active against this kinase. In particular, AG-490, and the irreversible mutant-selective EGFR inhibitors, WZ4002, WZ8040 and WZ3146, exhibited *Tb*GSK3s IC_{50} values of 6.3, 3.0, 9.4 and 27 μM, respectively (Figure 12).[47] The multitarget TK inhibitors regorafenib and nintedanib (Figure 12) have been shown to inhibit the recombinant *Tb*GSK3s with IC_{50} values of 2.7 and 2.8 μM, respectively.

Other reversible and irreversible EGFR inhibitors, gefitinib, pelitinib, PD153035, OSI420, afatinib, and sapitinib, as well as the HER2/ErbB2 inhibitors mubritinib, CP724714, and the HER1/HER2 inhibitors AC480, and neratinib have exhibited no inhibitory *Tb*GSK3s activity at 20 μM.[47] Similarly, the human VEGFR inhibitors, lenvatinib, cabozantinib, vandetanib, pazopanib, tivozanib, Ki8751, KRN633, cediranib, motesanib, brivanib, telatinib, and vatalanib, have shown no activity against *Tb*GSK3s at 20 μM concentration.[47]

Mammalian JAK1 and JAK2 inhibitors, momelotinib, ruxolitinib, gandotinib, AZD1480, AZ960 and NVPBSK805 (Figure 13), have been evaluated for inhibition of recombinant *Tb*GSK3s. The most potent compounds, momelotinib and gandotinib, have shown IC_{50} values of 0.6 and 0.5 μM, respectively, while ruxolitinib and AZD1480 showed inadequate inhibition at the tested concentration of 20 μM.[47]

In *T. brucei* BSF (strain CA427) cellular assay, the evaluation of gandotinib, in growth inhibition has shown a value of 1.04 μM. The JAK2 inhibitor, AZ960 has demonstrated low activity over *Tb*GSK3s with IC_{50} of 4.1 μM. However, in a subsequent work of Valenciano et al., AZ960 was identified as one of the most promising compounds in a phenotypic screen against *T. brucei* BSF (strain wild-type-221) with an IC_{50} value of 0.12 μM.[50] In addition, AZ960 showed a K_i of 1.25 μM against an essential protein for parasite survival, the

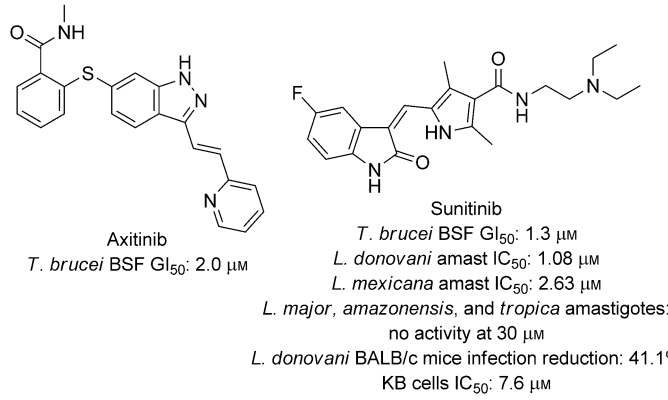


Figure 11. Structures and biological activity of repurposed human VEGFR/PDGFR inhibitors.

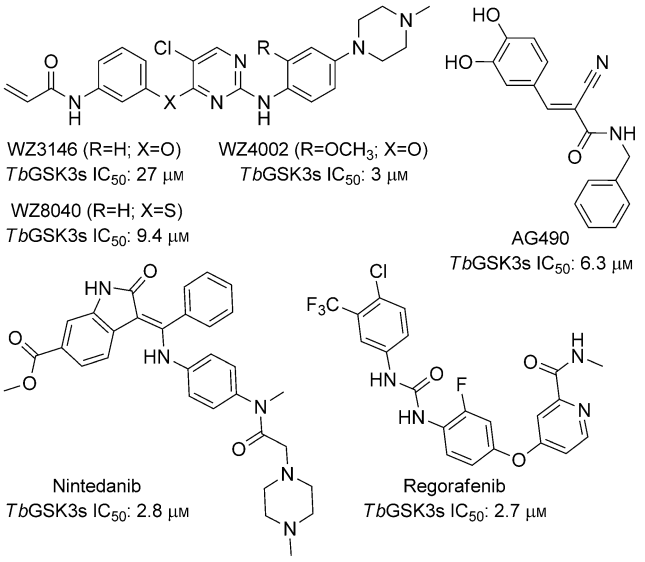


Figure 12. *Tb*GSK3s activity and structures of human reversible and irreversible TK inhibitors.

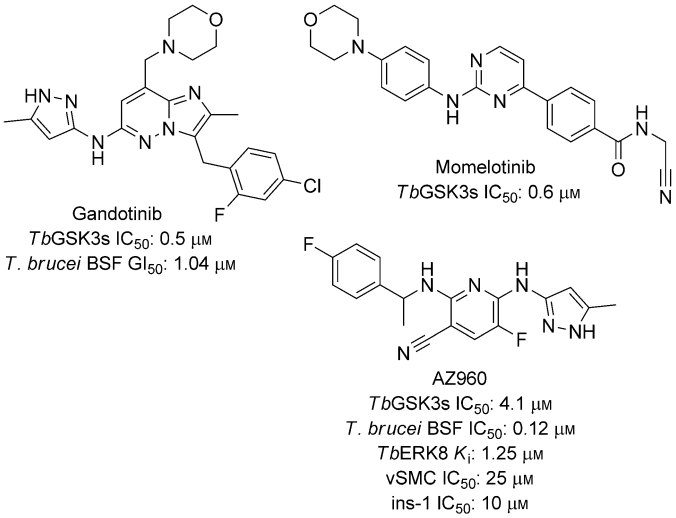


Figure 13. Structures and biological activity of mammalian JAK inhibitors.

T. brucei extracellular signal-regulated kinase 8 (*Tb*ERK8), demonstrating its utility in *T. brucei* growth inhibition (Figure 11).[50] AZ960 has >80-fold selective toxicity against *T. brucei* over mammalian cells.

Crizotinib (Figure 14), a potent inhibitor of human c-Met and ALK, is an anticancer drug approved for the treatment of non-small-cell lung carcinoma (NSCLC). When tested against the three trypanosomatids, it showed low micromolar potency against *T. brucei*, with an EC_{50} of 0.40 μM and moderate activity against *T. cruzi*, with an EC_{50} of 7.94 μM.[36e] Crizotinib has also exhibited significant activity in both *L. major* promastigote and amastigote forms, with EC_{50} values of 0.63 and 1.58 μM, respectively.[36e] In addition, it has been observed that crizotinib moderately inhibits the recombinant *Tb*GSK3s showing an IC_{50} value of 7.6 μM.[47] BMS794833 (Figure 14), another inhibitor of mammalian c-Met and VEGFR2, has shown moderate activity

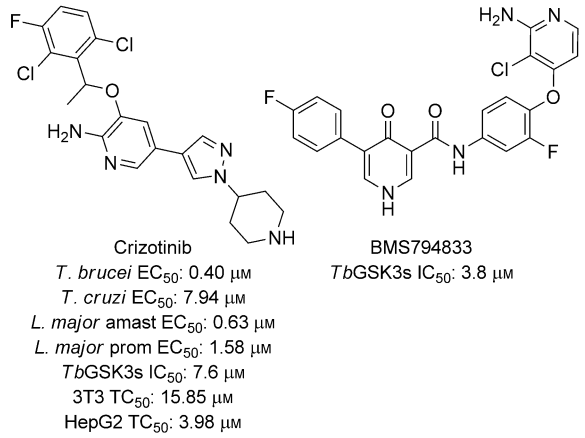


Figure 14. Structures and biological activity of repurposed human TK inhibitors.

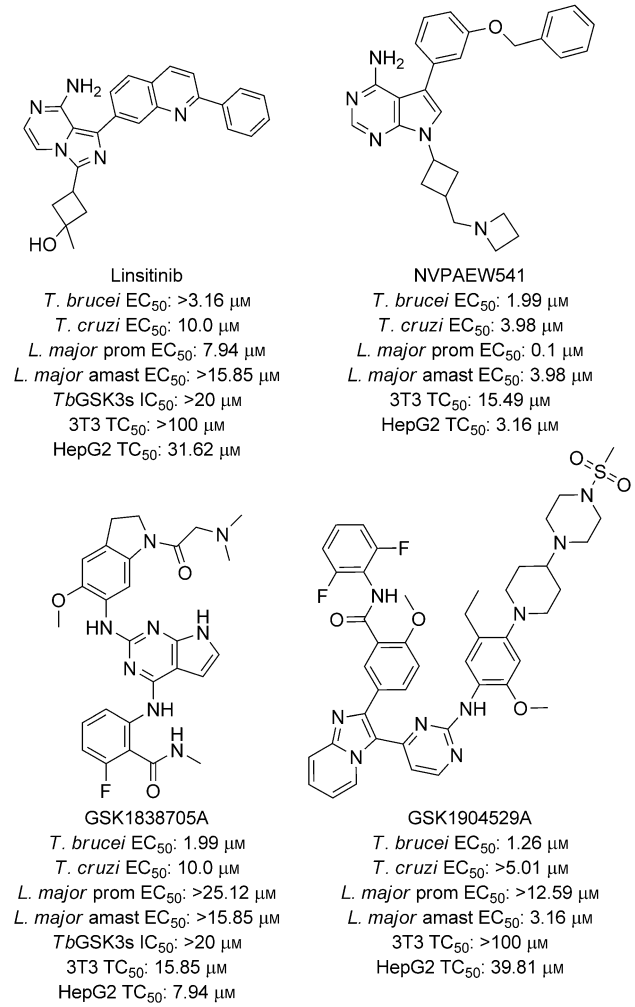


Figure 15. Structures and biological activity of mammalian IGF-1R and IR inhibitors.

against *Tb*GSK3s with IC_{50} of 3.8 μM.[47] Other repurposed human c-Met inhibitors including JNJ38877605, MGCD265, foretinib, BMS777607, and SGX523, have shown no inhibitory *Tb*GSK3s activity at 20 μM.[47]

Linsitinib (Figure 15), a human inhibitor of both insulin-like growth factor 1 receptor (IGF-1R) and insulin receptor (IR), is under clinical investigation for the treatment of various types of cancer. Linsitinib has been identified as a weak inhibitor against both *T. brucei* and *T. cruzi*, with EC_{50} >3.16 and 10.0 μM, respectively. Similar activity has been shown over *L. major* promastigotes and amastigotes, with EC_{50} of 7.94 and >15.85 μM, respectively.[36e] No *Tb*GSK3s inhibitory activity has been proven when linsitinib was used at 20 μM concentration.[47] NVPAEW541 (Figure 15), another potent inhibitor of IGF-1R, has shown good micromolar activity when evaluated against both *T. brucei* and *T. cruzi*, showing EC_{50} of 1.99 and 3.98 μM, respectively. NVPAEW541 has also demonstrated activity over *L. major* promastigote and amastigote forms, with EC_{50} of 0.10 and 3.98 μM.[36e]

The two mammalian IGF-1R and IR inhibitors GSK1904529A and GSK1838705A (Figure 15) showed similar activity over the three kinetoplastids, with EC_{50} values of 1.26 and 1.99 μM against *T. brucei* (Lister 427 strain), and >5.0 and 10 μM for *T. cruzi* intracellular amastigote (Tulahuén strain), respectively. Results have also shown a low activity for *L. major* promastigote and amastigote, with EC_{50} in the range of 12.5–25 μM, except for GSK1904529A, which exhibited moderate activity against the amastigote form with an EC_{50} of 3.16 μM.[36e] GSK1838705A was also evaluated for activity against *Tb*GSK3s, but no inhibition has been observed at the concentration of 20 μM.[47] The same profile against *Tb*GSK3s for NVPADW742, another human IGF-1R inhibitor, has been observed (>20 μM).

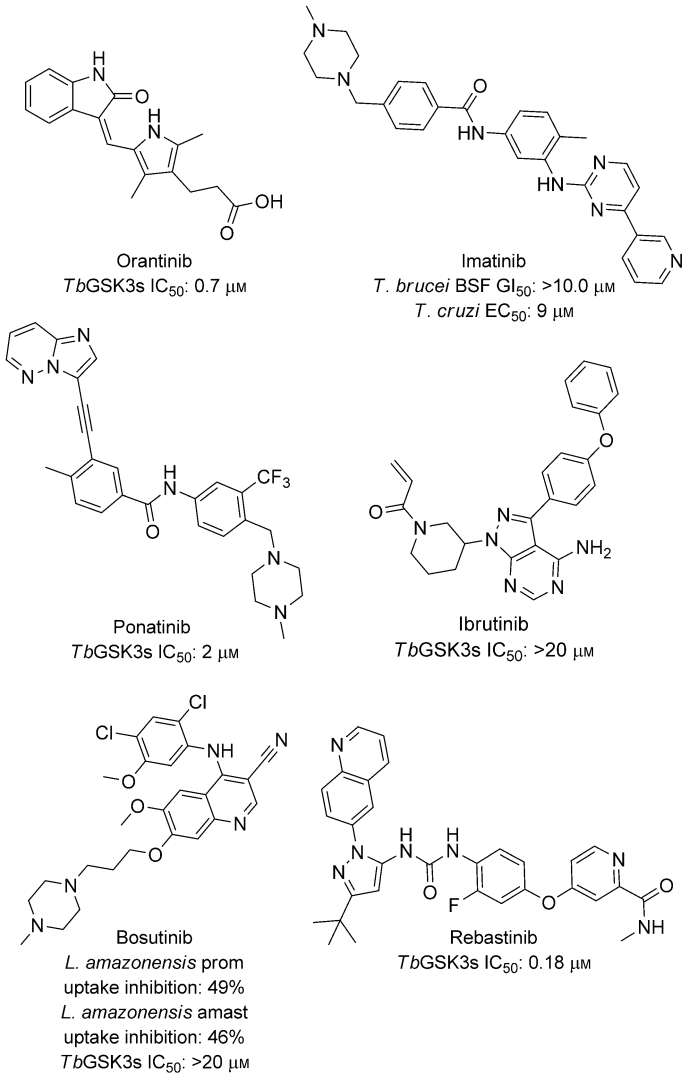


Figure 16. Structures and biological activity of orantinib, imatinib, ponatinib, rebastinib, bosutinib, and ibrutinib.

Orantinib (Figure 16), an ATP-competitive inhibitor of mammalian PDGFRβ, FGFR1, and VEGFR2 (Flk1/KDR), is under clinical investigation for the treatment of hepatocellular carcinoma and other solid tumors.[66] Evaluated over the recombinant *Tb*GSK3s, orantinib has shown sub-micromolar inhibitory activity with an IC_{50} of 0.7 μM.[47] Imatinib (Figure 16), a multi-target inhibitor of c-Kit, PDGFR, and Abl/Arg kinases, is an oral anticancer drug approved for the treatment of chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL) and some types of gastrointestinal stromal tumors (GIST).[67] It has been shown that imatinib is able to decrease opsonized polystyrene bead phagocytosis and *Leishmania* uptake, indicating that Abl and Arg are involved in phagocytosis and thus in *Leishmania* infection. Moreover, imatinib-treated mice have exhibited smaller lesions with few parasites in comparison with the control.[68] Evaluated in *T. brucei* BSF and *T. cruzi*, imatinib showed GI_{50} >10 and EC_{50} of 9 μM, respectively.[60a,69] The multi-targeted inhibitors of c-Kit and PDGFR amuvatinib and masitinib, and the selective inhibitor of PDGFRα/β crenolanib, have shown no activity when tested against *Tb*GSK3s at 20 μM.[47]

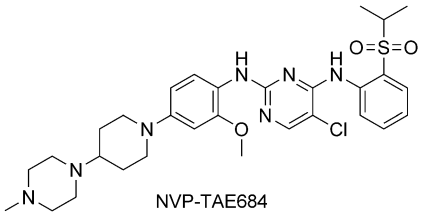
Ponatinib (Figure 16), a potent multi-target inhibitor of Abl, PDGFRα, VEGFR2, FGFR1, and Src kinases, is an oral drug approved for CML and ALL. It has been proven that ponatinib exhibited inhibitory activity against *Tb*GSK3s with an IC_{50} of 2 μM. Rebastinib (Figure 16), a Bcr-Abl inhibitor under clinical phase I investigation for the treatment of the metastatic breast cancer and leukemias, has shown sub-micromolar inhibitory activity against *Tb*GSK3s, with an IC_{50} of 0.18 μM.[47] Other human Abl inhibitors, dasatinib, KX2391, and nilotinib, have shown no activity against *Tb*GSK3s at 20 μM.[47]

Bosutinib (Figure 16), an Abl and Src kinase inhibitor, is an antineoplastic drug approved for CML and other cancers.[70] Bosutinib inhibited *L. amazonensis* promastigote and amastigote uptake by bone marrow-derived macrophages (BMDMs) of 49 and 46%, respectively.[71] In the same work, it has been shown that bosutinib reduces the lesion size in a mouse model of CL. Evaluated for recombinant *Tb*GSK3s inhibition, bosutinib has shown no activity at 20 μM.[47]

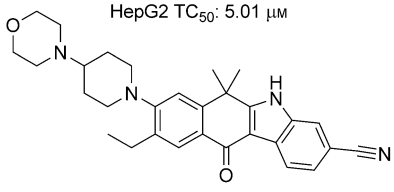
Ibrutinib (Figure 16), a Bruton's TK (BTK) inhibitor in B cells, is an anticancer drug approved for the treatment of mantle cell lymphoma and lymphoid leukemia. Since ibrutinib is also

an irreversible inhibitor of the interleukin-2-inducible T-cell kinase (ITK), it has been evaluated in a mouse model of CL.[72] Indeed, T helper cell 2 (Th2) immunity suppression and T helper cell 1 (Th1) immunity stabilization have been related to improved parasite clearance in ibrutinib-treated mice, although, the drug has not shown direct effects when used in *L. major*.[72] Evaluated for the activity of *Tb*GSK3s, ibrutinib has shown no activity at 20 μM concentration.[47]

NVP-TAE684 (Figure 17), a human ALK inhibitor, has shown micromolar potency against both *T. brucei* and *L. major* promastigote and amastigote forms, with EC_{50} values of 0.1, 0.32 and 1.26 μM, respectively. Moderate activity has also been observed for *T. cruzi*, with an EC_{50} of 5.0 μM.[36e] Evaluated for inhibition of *Tb*GSK3s, NVP-TAE684 showed an IC_{50} of 6.8 μM.[47] Another human ALK inhibitor, alectinib (Figure 17), has proven low micromolar activity over *T. brucei* and sub-micromolar potency against *L. major* promastigote, with EC_{50} values >3.16 and 0.12 μM, respectively. Alectinib has also shown low activity against *L. major* amastigote with an EC_{50} >15 μM. No activity has been observed for *T. cruzi* ($EC_{50}>50$ μM).

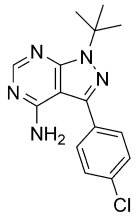


NVP-TAE684
T. brucei EC_{50}: 0.1 μM
L. major amast EC_{50}: 1.26 μM
L. major prom EC_{50}: 0.32 μM
T. cruzi EC_{50}: 5.01 μM
*Tb*GSK3s IC_{50}: 6.8 μM
3T3 TC_{50}: 25.12 μM
HepG2 TC_{50}: 5.01 μM

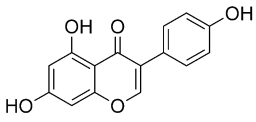


Alectinib
T. brucei EC_{50}: >3.16 μM
L. major amast EC_{50}: 0.12 μM
L. major prom EC_{50}: >15.85 μM
T. cruzi EC_{50}: 50.12 μM

Figure 17. Structures and biological activity of NVP-TAE684, and alectinib.



PP2
*Lm*CK1.2 IC_{50}: 1.60 μM
Leishmania prom EC_{50}: >10 μM
Leishmania amast EC_{50}: >50 μM
Leishmania intrac EC_{50}: 1 μM
L. donovani, *amazonensis*: no activity
KB cells IC_{50}: 42 μM



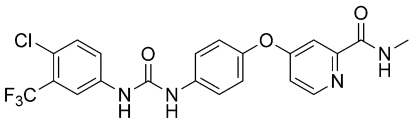
Genistein
T. b. rhodesiense IC_{50}: 4.81 μM (1.3 μg/ml)
L. donovani IC_{50}: 30 μM (8 μg/ml)
T. cruzi IC_{50}: 87 μM (23.4 μg/ml)
TbGSK3s IC_{50}: >20 μM

Figure 18. Structures and biological activity of PP2 and genistein.

PP2 (Figure 18), a proto-oncogene tyrosine protein kinase (Src) inhibitor, has been shown to inhibit *Leishmania* casein kinase 1.2 (*Lm*CK1.2), an essential kinase for intracellular parasite survival and infectivity, with an IC_{50} of 1.60 μM. When evaluated on cultured promastigotes and amastigotes, PP2 has shown low inhibitory activity with EC_{50} values >10 and >50 μM. Estimated EC_{50} on intracellular parasites was ~1 μM.[73] Similar results have also been published by Sanderson et al., where PP2 was inactive on both *L. donovani* species.[61] In the *L. donovani* BALB/c model, PP2 also reduced liver amastigote burdens by 53.4%.[61]

Genistein (Figure 18), an ATP-competitive inhibitor of the human protein tyrosine kinase (PTK) which, in humans, blocks the mitogenic effect mediated by EGF, has been shown to inhibit the anti-CD8a-stimulated NO production responsible for the killing of *L. major*.[74] Genistein exhibited low micromolar activity over *T. b. rhodesiense* with an IC_{50} of 4.81 μM (1.3 μg mL^{-1}) and low potency against *L. donovani* and *T. cruzi* with IC_{50} of 30 (8 μg mL^{-1}) and 87 μM (23.4 μg mL^{-1}), respectively.[75]

Sorafenib (Figure 19), a multikinase inhibitor of Raf-1, B-Raf and VEGFR-2 has been shown to inhibit both *Tb*Tf endocytosis and *Tb*GSK3s, with EC_{50} of 5.9 μM and IC_{50} 1.7 μM, respectively. The antitrypanosomal activity of sorafenib against *T. brucei* BSF has shown a GI_{50} value of 3.1 μM.[47] Sorafenib has also been shown to be active against *L. major* amastigotes showing an IC_{50} of 3.77 μM. Lower activities have been shown over other *Leishmania* amastigote species, with IC_{50} of 3.72, 6.87, and 4.72 μM for *L. donovani*, *L. amazonensis* and *L. mexicana*, respectively.



Sorafenib
*Tb*Tf endocytosis EC_{50}: 5.9 μM
*Tb*GSK3s IC_{50}: 1.7 μM
T. brucei BSF GI_{50}: 3.1 μM
L. major amast IC_{50}: 3.77 μM
L. donovani IC_{50} 3.72 μM
L. amazonensis IC_{50}: 6.87 μM
L. mexicana IC_{50}: 4.72 μM
L. tropica: not active
L. donovani BALB/c burden reduction: 41.1%
KB cells IC_{50}: 7 μM

Figure 19. Structure and biological activity of sorafenib.

No in vitro activity has been shown against *L. tropica*. Evaluated on an *L. donovani* BALB/c model, sorafenib showed a percentage burden reduction of 41.1%.[61]

4.4. Human atypical kinase inhibitors

Atypical trypanosomatids PKs (aPKs) lack the 11 subdomains characterizing ePKs and include the better characterized families of RIO, alpha, PIKK, and PDK. Two RIO proteins, assigned to the RIO1 and RIO2 subfamilies, have been identified and a similarity between human and trypanosomatid RIO2 has been recognized.[39] The alpha kinases, so named because they phosphorylate substrates within alpha helices, are present in all three trypanosomatids, with *L. major* possessing two additional alpha kinase genes.[39] The phosphatidylinositol kinase-related kinase family (PIKK) includes protein kinases with domains structurally resembling those of phosphatidylinositol 3-kinases (3-PKI). The parasites possess specific PIKK kinase homologues involved in genome surveillance and four FRAP family kinases, such as FKBP-12-rapamycin associated protein (FRAP) and the mammalian target of rapamycin (mTOR). TOR is a serine/threonine kinase implicated in the regulation of cell growth in eukaryotes, through the activity of two multiprotein complexes: TOR complex 1 (TORC1) and TOR complex 2 (TORC2).[76] TORC1, sensitive to rapamycin, regulates temporal cell growth, whereas TORC2, rapamycin-resistant, modulates spatial cell growth. Two TOR orthologous, named *Tb*TOR1 and *Tb*TOR2, together with TORC1 and TORC2 partners, have been identified in *T. brucei*. In particular, *Tb*TOR1 has been implicated in cell cycle, nucleolus structure, and protein synthesis regulation, whereas *Tb*TOR2 regulates cell polarization and cytokinesis.[77] The trypanosomatids also have some genes codify for putative pyruvate dehydrogenase kinases (PDK) which lack the domains characterizing ePKs.[39]

Rapamycin (Figure 20) is a specific mTOR inhibitor having immunosuppressant functions and is used to prevent organ transplant rejection. It has been shown that rapamycin has no effects on the growth and proliferation of *L. major* promastigotes, rather exerting a positive effect on parasite survival.[78] This has been related to a tryptophan substitution in the TOR isoform of *L. major*, essential for rapamycin binding to mTOR.[79] Barquilla et al., showed rapamycin inhibited *T. brucei* BSF proliferation with an EC_{50} of 0.15 μM.[77]

Two mammalian atypical kinase inhibitors, idelalisib, a p110δ-specific inhibitor approved for treatment of different B cell malignancies, and IC87114, a mixed PI3K inhibitor, have been evaluated in *Leishmania* (Figure 20).[80] Prophylactic administrations of idelalisib (0.05 mg per mouse) or IC87114 (0.5 mg per mouse), have been shown to significantly ($p < 0.01$) decrease lesion size in CL and parasite burdens in VL and CL in the spleen and liver of *L. donovani*-infected mice, and footpad of *L. major*-infected mice.

Therapeutic administrations (after one or two weeks) of idelalisib (0.05 mg per mouse) have been shown to significantly ($p < 0.05$) lower parasite burden in the spleens and livers of *L. donovani*-infected mice. In addition, treatment of infected mice with idelalisib (0.05 mg per mouse) and amphotericin B

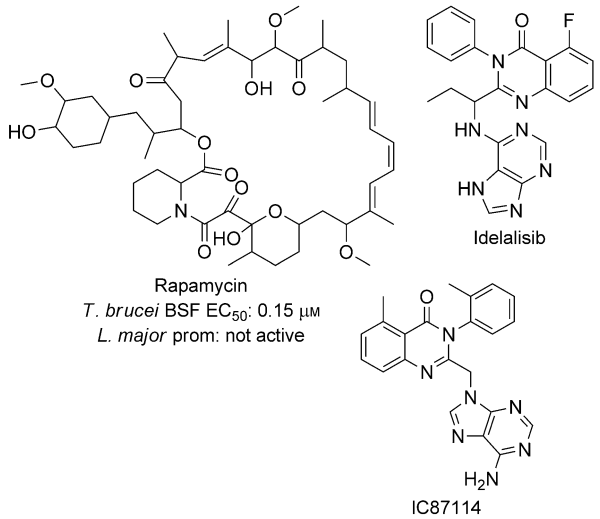


Figure 20. Structures of human atypical kinase inhibitors.

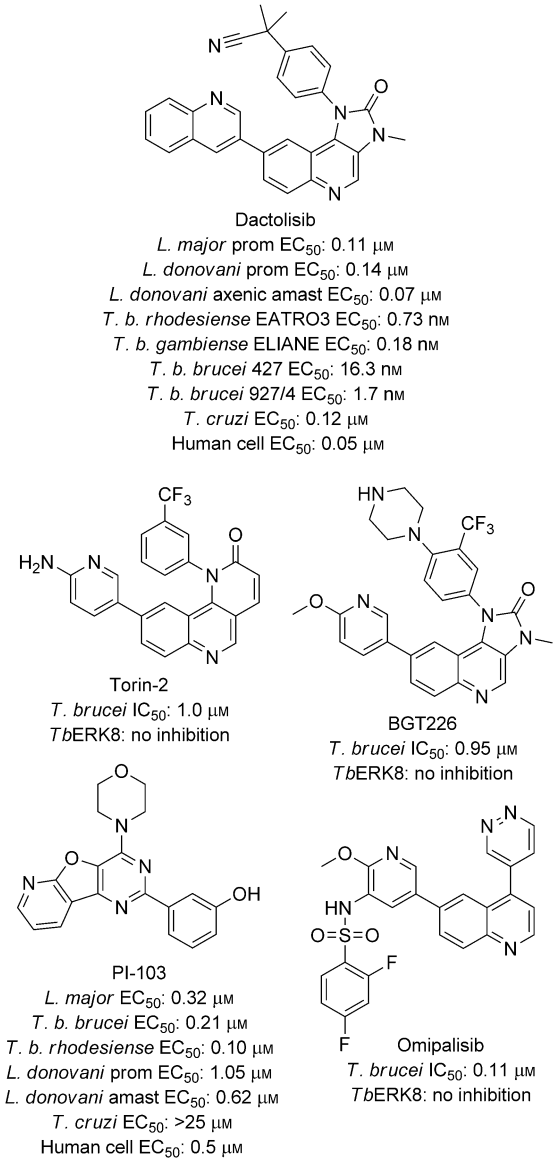


Figure 21. Structures and biological activity of human atypical kinase inhibitors.

(0.1 mg kg^{-1}) also leads to a complete clearance of parasites both in spleen and in the liver.[80] The decrease in parasite burden was associated with a concomitant reduction in regulatory T cell numbers and cytokine production by the liver, spleen and lymph node cells, as the direct growth of *L. donovani* promastigotes in axenic was not affected by any concentration tested of idelalisib or IC87114. Thus, in vivo effects of idelalisib or IC87114 are not mediated by direct parasite killing but through their ability to modulate the host immune response to *Leishmania*.

The imidazoquinoline derivative dactolisib (Figure 21) is a PI3K and mTOR inhibitor under clinical investigation as a possible anticancer drug. Dactolisib has been shown to reduce parasite growth in all species/strains of *Leishmania* and *Trypanosoma* tested. Sub-micromolar activity has been shown on *L. major* and *L. donovani* promastigotes with EC_{50} values of 0.11 and 0.14 μM, respectively and on *L. donovani* axenic amastigotes with an EC_{50} of 0.07 μM.[81]

Dactolisib showed very potent inhibition of *T. b. rhodesiense* EATRO3 growth with EC_{50} values of 0.73 nM, and of *T. b. gambiense* ELIANE with EC_{50} of 0.18 nM. On *T. b. brucei*, dactolisib exhibited optimal growth inhibition with EC_{50} of 16.3 nM and 1.7 nM for strains Lister 427 and 927/4, respectively.[81] The in vitro potency against *T. cruzi* was also in the sub-micromolar range with an EC_{50} of 0.12 μM. Moreover, in an animal model of *T. b. rhodesiense* infection, dactolisib reduced parasitemia, whereas in *L. major*-infected mice showed no therapeutic effect.[81]

The potent p110α/β/δ/γ inhibitor Omipalisib and two mTOR inhibitors BGT226 and Torin-2 (Figure 21), have been shown to reduce *T. brucei* (strain wild-type-221) proliferation with IC_{50} of 0.11, 0.95 and 1.0 μM, respectively. These latter compounds, tested for inhibition of *Tb*ERK8 were not active.[50]

The multitarget PI3K inhibitor (p110α/β/δ/γ) PI-103, has shown sub-micromolar activity against *L. major, T. b. brucei* and *T. b. rhodesiense* with EC_{50} values of 0.32, 0.21 and 0.10 μM, respectively. Good activity was also shown against *L. donovani* promastigotes and amastigotes with EC_{50} values of 1.05 and 0.62 μM, respectively. The in vitro potency against *T. cruzi* was higher than 25 μM.[81]

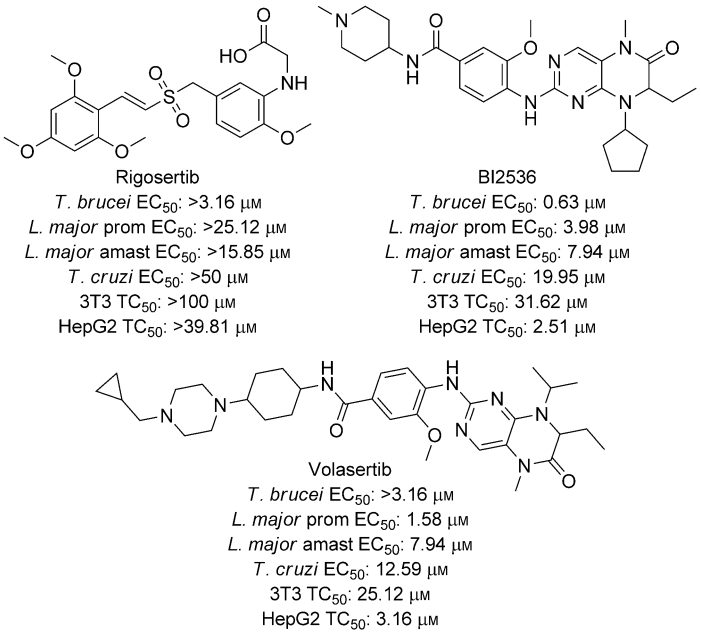


Figure 22. Structures and biological activity of mammalian PLK1 kinase inhibitors.

4.5. Human other kinase inhibitors

The trypanosomatid other protein kinase family is represented by different kinases, and among these Aurora (AUR), and polo-like kinases (PLK), are involved in cell division, DNA replication, and repair.[39]

Rigosertib, volasertib, and BI2536 (Figure 22), three potent polo-like kinase 1 (PLK1) inhibitors in clinical trial for the treatment of different types of cancers, have been evaluated over different species/strains of *Leishmania* and *Trypanosoma*. Low potency has been shown against *T. brucei* (Lister 427) with EC_{50} > 3.16 μM for both rigosertib and volasertib, and 0.63 μM for BI2536. Volasertib and BI2536, have exhibited moderate activity when evaluated against *L. major*, with EC_{50} values of 1.58 and 3.98 μM for promastigotes and 7.94 μM for amastigotes. Low activity has been proven over *T. cruzi*, with EC_{50} values ranging from 13 to 50 μM.[36e]

In humans, Aurora kinases have been proven as essential in chromosome segregation and cytokinesis.[82] It has been shown that *T. brucei* kinome encodes for three Aurora kinases; only the *Tb*AUK1, but neither *Tb*AUK2 nor *Tb*AUK3, is critical for mitotic progression.[83]

The pyrrolopyrazole danusertib (Figure 23), is an inhibitor of Aurora A/B/C kinases in advanced clinical trials for the treatment of multiple myeloma and other types of cancer. Danusertib has been shown to associate with and inhibit *Tb*AUK1.[36d] Moreover, danusertib inhibited cell growth on both *T. b. brucei* (Lister 427) and *T. b. rhodesiense* with EC_{50} values of 0.6 and 0.15 μM, respectively.[36d] Tested against *L. major* promastigote form, danusertib has shown low activity with an EC_{50} > 20 μM.[84]

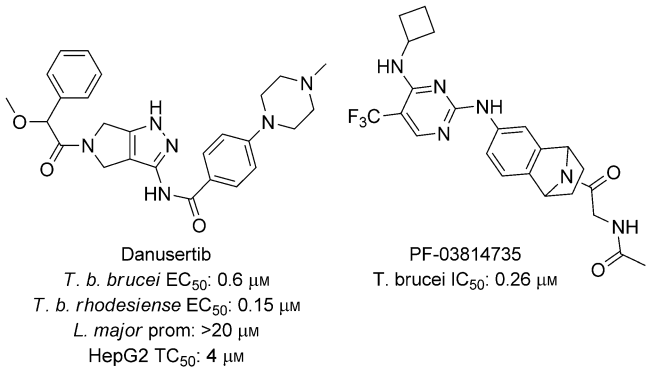


Figure 23. Structures and biological activity of mammalian Aurora kinase inhibitors.

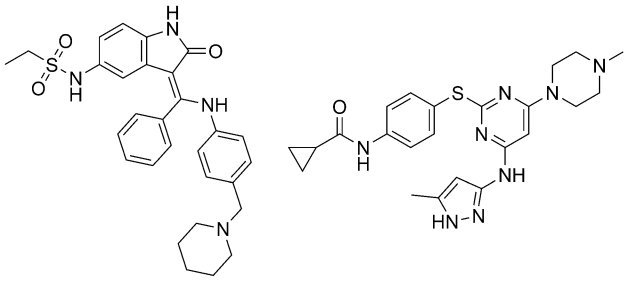


Figure 24. Structures and biological activity of mammalian Aurora kinase inhibitors.

The Aurora kinase inhibitor PF-03814735 (Figure 23), has been evaluated for the growth inhibition of *T. brucei* (strain wild-type-221) showing a sub-micromolar activity of 0.26 μM.[50] At 1 μM, PF-03814735 inhibits almost completely both *Tb*ERK8 and HsERK8.[50]

Hesperadin (Figure 24), a potent inhibitor of Aurora B kinase, has been shown to be highly effective on cell proliferation inhibition for *T. brucei* with an EC_{50} of 0.06 μM. Also tested on other trypanosomatids, hesperadin has exhibited low micromolar activity against *T. cruzi*, with EC_{50} of 3.9 μM, and against both *L. major* promastigote and amastigote forms with EC_{50} of 0.12 and 2.37 μM.[84] Tozasertib (Figure 24), is a pan-Aurora inhibitor, mostly acting against Aurora A kinase, under phase II clinical evaluation for the treatment of cancer. Tozasertib has been evaluated for growth inhibition of both *T. brucei* and *L. major* promastigote, showing no activity (EC_{50} of 10 and >20 μM).[36d,84]

5. Conclusions

Neglected tropical protozoan diseases are a group of three communicable diseases, induced by infection with flagellated protozoan parasites named trypanosomatids: human African trypanosomiasis, Chagas disease, and leishmaniasis. Current drugs are limited by serious side effects, inconvenient route of administration, increasing incidence of drug resistance, and proper efficacy. As a consequence, new, safe, effective, and easier to administer drugs are needed. The majority of the current therapeutic trypanosomatid targets are represented by enzymes or cell surface receptors. Among these, eukaryotic protein kinases represent a major group of protein targets whose modulation may be beneficial for the treatment of neglected tropical protozoan diseases.

This review summarizes the finding of new hit compounds for neglected tropical protozoan diseases, by repurposing the major synthetic human kinase inhibitors on trypanosomatids, using both target-based and phenotypic screening. This collection aims to provide an important insight into the human kinase inhibitors and their importance in the development of new chemical entities with potential beneficial effects on the diseases caused by trypanosomatids.

The repurposing of human kinase inhibitors in NTD has resulted in compounds with potent antiparasitic activities. The vast majority of successfully repurposed compounds have been found through phenotypic screening with most potent inhibitors belonging to the CMCG and atypical human kinase families. Unfortunately, for most of these repurposed inhibitors the parasite targets are unknown. In this regard, additional effort should be made to recognize which specific target is responsible for the antiparasitic activity. The latter is not an essential task in finding new drugs for NTD, although it may help the drug design process.

Conflict of interest

The authors declare no conflict of interest.

Keywords: drug discovery · kinase inhibitors · neglected tropical diseases · parasites · repurposing

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