Chapter

Toward New Antileishmanial Compounds: Molecular Targets for Leishmaniasis Treatment

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Abstract

The leishmaniases are a group of diseases caused by protozoan parasites—*Leishmania* sp. Leishmaniasis is classified among the 20 neglected diseases by WHO. Although the disease has been known for more than 120 years, the number of drugs used for the treatment is still limited to 5–6. The first-line drugs against leishmaniasis are pentavalent antimonials, which were introduced to the treatment 70 years ago—despite all their side effects. Molecular targets are becoming increasingly important for efficacy and selectivity in postgenomic drug research studies. In this chapter, we have discussed potential therapeutic targets of antileishmanial drug discovery such as pteridine reductase (PTR1), trypanothione reductase (TR), N-myristoyltransferase (NMT), trypanothione synthetase (TryS), IU-nucleoside hydrolase, and topoisomerases, enzymes and their inhibitors reported in the literature.

Keywords: antileishmanial compounds, molecular target, pteridine reductase, N-myristoyltransferase, inhibitors

1. Introduction

Leishmaniasis is a parasitic disease that occurs in the tropic and subtropics regions, and the parts of southern Europe. The disease is classified among neglected tropical diseases (NTDs) [1]. Leishmaniasis is spread by the bite of phlebotomine sand flies that causes the infection with *Leishmania* parasites. There are three main forms of the disease—cutaneous leishmaniasis (CL) known as the most common form, that causes skin sores; visceral leishmaniasis (VL; kala-azar) is the most severe form, that affects several internal organs; and mucocutaneous leishmaniasis (MCL) that has a chronic and metastatic behavior [2, 3].

Although the disease has been known for more than 120 years, the number of drugs used for the treatment is still limited to 5–6. The first-line drugs used against leishmaniasis are pentavalent antimony (Sb^V) compounds namely sodium stibogluconate (Pentostam®) and meglumine antimonate (Glucantime®), which was introduced into treatment more than 70 years ago, despite all their side effects. Neither their mechanism of action nor their chemical structures have been clarified/verified yet in spite of their wide use for a long time. Other drugs used in *Leishmania* infections are liposomal amphotericin B (L-AmB), miltefosine, paromomycin (aminosidine), and azole-derived antifungals; ketoconazole, itraconazole, and fluconazole.

The need for effective, safe, and selective chemotherapeutics against leishmaniasis increases every day. Targeting distinct molecular pathways is a widely used strategy in rational drug design and discovery for developing such agents to treat leishmaniasis. In this chapter, we would like to focus on enzymes which being targeted by the researcher for antileishmanial studies.

2. Potential molecular targets for the treatment of leishmaniasis

2.1 Pteridine reductase (PTR1, Pteridine reductase 1, EC 1.5.1.33)

PTR1 enzyme is an NADPH-dependent, short-chained reductase enzyme family member [4]. It is broadly active and can reduce a variety of unconjugated pteridines, as well as folates [5]. This enzyme has been investigated in studies of resistance to the dihydrofolate reductase inhibitor methotrexate (MTX) [6, 7]. After finding the missing link of resistance, researchers have suggested that inhibition of PTR1 may be a rational target for chemotherapy [4]. Since trypanosomatids are auxotrophic for folates and pterins, the inhibition of the PTR1 enzyme may also lead to selectivity. Therefore, PTR1 appears to be a rational target for antileishmanial drug development.

The first reported PTR1 inhibitors are pteridine analogs (diaminopteridines and quinazolines) and their activity was tested against purified *Leishmania major* pteridine reductase (*Lm*PTR1) [8]. The structure of *Lm*PTR1 in complex with NADPH and the inhibitor 2,4,6-triaminoquinazoline (TAQ) were reported in 2004 [9]. Based on its crystal structure, Cavazzutti *et al.* analyzed a library of 440 synthetic folate-like compounds and tested selected compounds on *Lm*PTR1 among other enzymes such as DHFR [10]. In this study compound, 6b was found to be the most promising compound with a Ki value of 37 nM toward LmPTR1. Then, the crystal structure of the *Lm*PTR1:NADPH:6b ternary complex revealed a substrate-like binding mode (**Figure 1**) [10].

It was reported that pteridine, pyrrolopyrimidine, and 2,4-diaminopyrimidine scaffold as PTR1 inhibitors with a structure-based approach by Tulloch et al. [11]. Among the tested compounds, compounds 11 and 13 bearings pyrrolopyrimidine core were reported with a modest ED50 value and a good lethality to the parasites. Additionally, a combination of MTX and compound 13 resulted in an improvement in efficacy [11]. Based on these hit molecules, TbPTR1 inhibitors were developed for the treatment of human African trypanosomiasis (**Figure 1**) [12].

Also, nonfolate scaffolds with *Lm*PTR1 inhibition activity were reported. After three rounds of election considering computational and experimental results, 18 compounds were selected, and among them, compound 28b and compound 5c known CNS active drug, showed promising activity with their IC50 values of 93 μM and 50 μM, Ki values of 7 μM and 4 μM, respectively (**Figure 1**) [13]. Moreover, 5c in combination with pyrimethamine showed antileishmanial activity on promastigotes with no hDHFR inhibition [14]. Another nonfolate scaffold, hexahydro pyrimido pyrimidinone, was introduced with potential antileishmanial activity in a virtual screening study. Compound 7 was reported as a potent *Ld*PTR1 enzyme inhibitor (Ki of 0.72 μM) and showed promising *Leishmania donovani* amastigote and *Labrus donovani promastigote* activity with the IC50 value of 3 μM and 29 μM, respectively [15].

Apart from the compounds summed up so far, thianthrene [16], dihydropyrimidines [17], benzothiazoles [18], thiazolidinedione [19, 20], thienopyrimidine [21], thiazolopyrimidine [22], and natural products such as flavanone derivatives

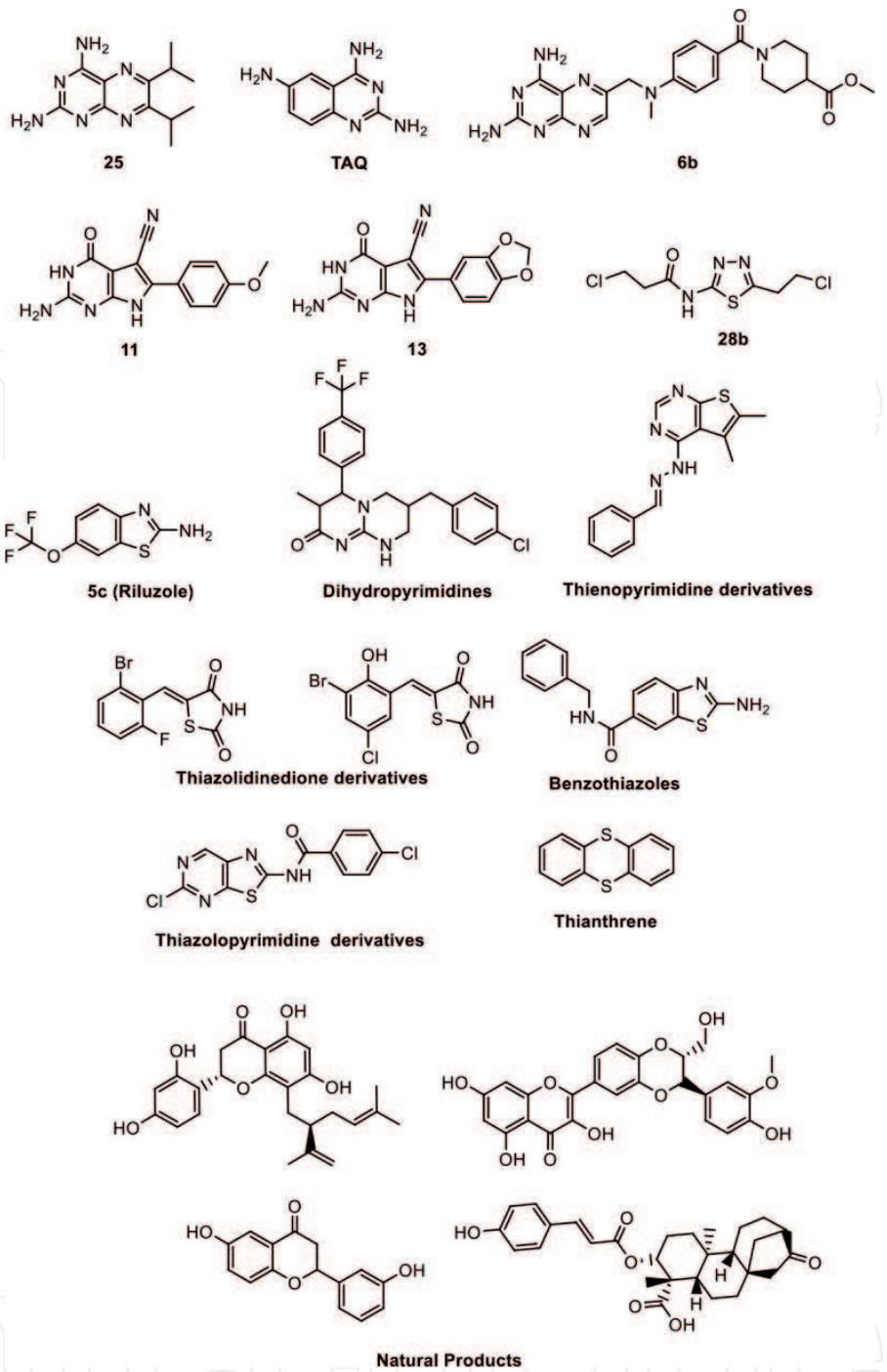

Figure 1.
Examples of PTR1 inhibitor structures with antileishmanial activity.

[23], 2,3-dehydrosilybin A, and sophoraflavanone G [24], kaurane-type diterpenes [25] were reported as PTR1 inhibitors with antileishmanial properties in the literature (**Figure 1**).

2.2 *N*-Myristoyltransferase (glycylpeptide N-tetradecanoyltransferase, NMT; EC 2.3.1.97)

NMT catalyzes the co- and post-translational addition of myristic acid (saturated, 14-carbon fatty acid) onto the N-terminal glycine of specific proteins in

eukaryotes (**Figure 2**). This physiological pathway, *N*-myristoylation, plays an important role in the correct cellular localization and biological functions. NMT enzyme was purified and characterized from yeasts for the first time and it is thought to be a target for development of a new class of antifungal drugs [26]. The presence of NMT in *L. major* was verified in 1997 [27]. Later, NMT enzyme activity was proven essential for viability in *Leishmania sp*. then, it attracted attention as a potential drug target in kinetoplastid parasites [28]. The validation of this enzyme as a target for antitrypanosomal and antileishmanial drug discovery was not until 2010 (**Figure 2**) [29, 30].

A group of antifungal agents was tested to identify the first NMT inhibitors by Panethymitaki et al. in 2006 [31]. Although some of the tested compounds were found to be NMP inhibitors in a low μM concentration range, their antileishmanial activity has not been reported [31].

In an HTS campaign led by Pfizer, around 150.000 compounds from the Pfizer Global Diverse Representative Set were screened against protozoan NMTs. Four different scaffolds, namely aminoacylpyrrolidine (PF-03402623 IC_{50} of 0.093 μM), piperidinylindole (PF-03393842 IC50 of 0.102 μM), thienopyrimidine (PF-00349412 (IC50 of 0.482 μM), and biphenyl (PF-00075634 (IC50 of 0.158 μM) derivatives were identified as novel inhibitors of *Labrus donovani* NMP (**Figure 3**) [32].

Following the previous study, the crystal structures of PF-03393842 and PF-03402623 with the enzyme, the initial hits selected in the HTS campaign, were elucidated. Based on this data, a fused hybrid compound **43** was developed as a highly potent *L. donovani* NMT inhibitor (Ki of 1.6 nM) with good selectivity over the human isoform of the enzyme (Ki 27 nM) (**Figure 3**) [33]. Although the lack of cell activity of 43 attributed to its poor uptake, the HTS campaign, and hybridization of the hit compounds have resulted in the discovery of a new scaffold [33].

Another HTS assay dedicated to identifying novel *Leishmania sp.* NMT inhibitors was focused on a set of 1600 pyrazolyl sulfonamide compounds [34]. Interestingly, no correlation between the enzyme potency of these inhibitors and their cellular activity against *L. donovani* axenic amastigotes was observed. This might be rationalized by the fact that poor cellular uptake considering the basicity of the compounds. The most potent inhibitor of *Lm*NMT (compound 2, Ki of 0.34 nM) exhibited modest activity against *L. donovani* intracellular amastigotes

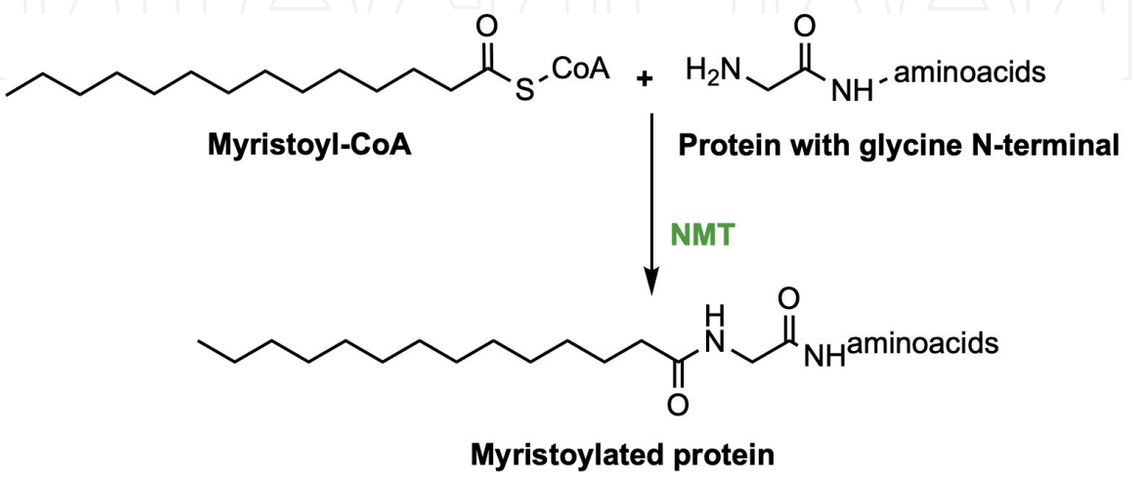

Figure 2.
Myristoylated proteins with NMT.

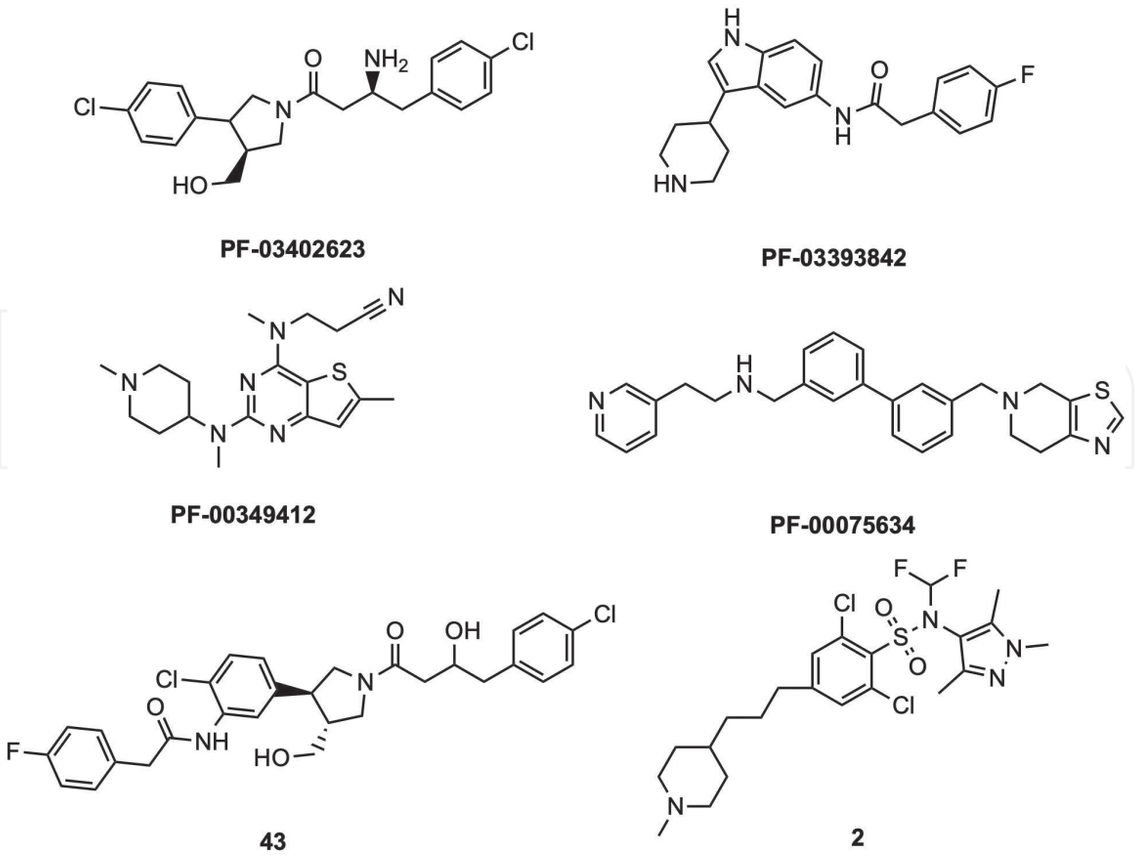

Figure 3.
Examples of NMT inhibitor structures with antileishmanial activity.

(EC50 of 2.4 μM). Yet, advanced studies on compound 2 confirmed the on-target mechanism. Moreover, oral use of compound 2 resulted in a 52% reduction in parasite burden in the mouse model of VL (**Figure 3**) [34].

Other NMT inhibitors as potential antileishmanial compounds were reported in a few publications and patents. In these studies, pyrrolidines, piperidinylindoles, azetidinopyrimidines, aminomethylindazoles, benzimidazoles, thienopyrimidines, biphenyl derivatives, benzofuranes, benzothiophenes, oxadiazoles, (pyrazolomethyl)-1,3,4-oxadiazoles and thienopyrimidine scaffolds, and peptidomimetic inhibitors were reported with their NMT inhibitory properties [35–38].

2.3 Inosine-uridine (IU) nucleoside hydrolase (IU-NH, EC:3.2.2.2)

The nucleoside hydrolase enzyme is an important target for the development of antiparasitic drugs due to its role in the purine salvage pathway. The amino acid sequence and X-ray structure of the enzyme from *L. major* were revealed in 1999 [39]. IU-NH enzyme establishes a homolog in *Leishmania* species.

In contrast to these facts, there is no study on IU-NH enzyme inhibitors possessing *in vitro/in vivo* antileishmanial activity up to our knowledge. Yet, few inhibitors of *Leishmania* IU-nucleoside hydrolase were reported.

Fuernaux et al. reported transition state analogs of nucleosides with IU-NH inhibitory activity [40]. Later, Berg et al. reported iminoribitol derivatives and evaluated their not only *Tabanus vivax*-NH activity but also human purine nucleoside phosphorylase to determine selectivity [41]. In other studies, two ribose-quinolone derivatives were tested against *Ld*NH [42] and Casanova et al. reported proanthocyanidins with *Ld*NH activity [43].

2.4 Enzymes Involved in Polyamine metabolism in *Leishmania*

In *Leishmania* parasites (and other members of the trypanosomatids), polyamine pathways can be considered as a unique pathway; most enzymes are essential for parasitic survival and infectivity (**Figure 4**).

2.4.1 Arginase (L-arginine amidinohydrolase, ARG, E.C. 3.5.3.1)

Arg is an enzyme that catalyzes the conversion of L-arginine amino acid to L-ornithine and urea.

The expression of the *Leishmania amazonensis* ARG in a bacterial host was done [44]. da Silva et al. expressed the recombinant enzyme in *E. coli* and performed biochemical and biophysical characterization studies [45].

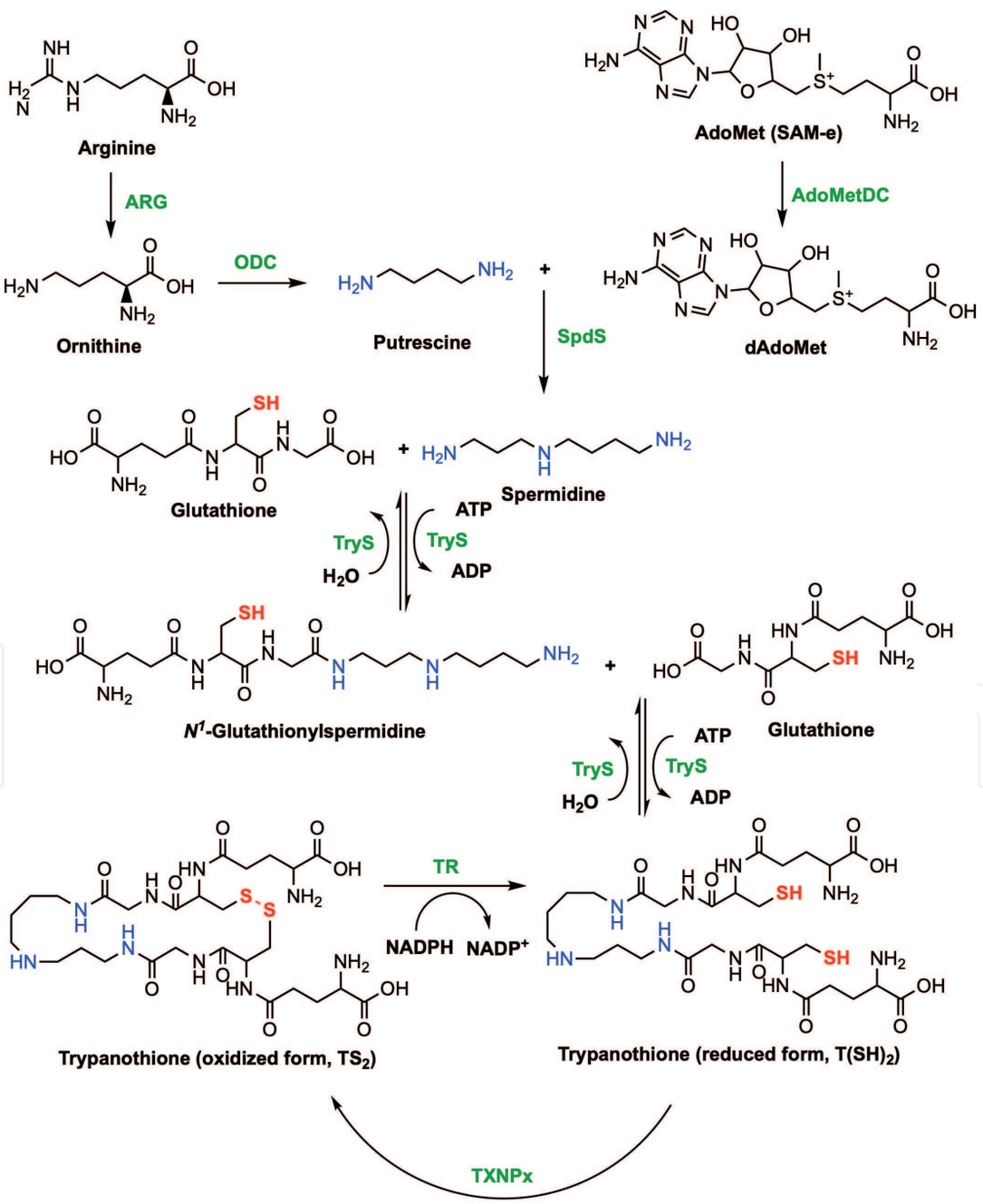

Figure 4.
Polyamine metabolism and enzymes in the pathway.

Reguera et al. suggest that broad inhibition of ARG activity alone will be insufficient to achieve therapeutically useful control of leishmaniasis, but combined inhibition of ARG with downstream enzymes leading to polyamine synthesis could result in improved therapeutic responses [46]. 3′-methoxy-cinnamoyl-1,3,4-thiadiazolium-2-phenylamine, an ARG inhibitory compound, exhibited moderate antileishmanial activity upon amastigotes of *L. amazonensis* [47].

[1,2,4]triazolo[1,5-a]pyrimidine derivatives [48], pyrazolo[3,4-d]pyrimidine derivatives [49], α,α-difluorohydrazide derivatives [50], chalcone derivatives [51], cinnamide derivatives [52], and 7,8-dihydroxyflavone—gold nanoparticles [53] were also studied as antileishmanial compounds with the mechanism of ARG inhibition.

On the other hand, antileishmanial natural products exhibiting ARG inhibitor activity with antileishmanial properties were reported—flavonoid and quercetin derivative [54], orientin and isovitexin [55], verbascoside [56], fisetin [57], rosmarinic acid, and caffeic acid [58].

2.4.2 Ornithine decarboxylase (ODC, EC 4.1.1.17)

ODC metabolizes ornithine to the diamine putrescine by its catalytic action [59]. Although alpha-difluoromethylornithine (DFMO) is an irreversible inhibitor of ODC, DFMO has not shown any antileishmanial activity [60]. Therefore, inhibition of ODC serves as a promising therapeutic paradigm for the treatment of leishmaniasis [61].

3-aminooxy-1-aminopropane was reported as a selective ODC inhibitor with potent antileishmanial activity against *Labrus donovani* (*L. donovani* promastigotes IC50 of 42 μM and *L. donovani* amastigotes IC50 of 5 μM) [62].

Gama-guanidinooxypropylamine [63], diospyrin [64], oxochromen, xanthone, and azaspirodecene derivatives [65] are reported in the literature with their ability to inhibit ODC enzyme and antileishmanial activity.

2.4.3 Spermidine synthase (SpdSyn, SpdS, EC 2.5.1.16)

SpdS catalyzes the conversion of putrescine to spermidine, a crucial polyamine for parasite proliferation. Genetic studies proved that SpdS is an essential gene in *L.donovani* [66]. Additionally, it was demonstrated that *L. donovani* amastigotes require SpdS activity to sustain a robust infection in mice; which is required for virulence [67].

Up to our knowledge, the only reported SpdS inhibitor with antileishmanial properties is natural compound hypericin [68].

2.4.4 S-Adenosylmethionine decarboxylase (AdoMetDC, EC 4.1.1.50)

AdoMetDC is involved in the synthesis of spermidine and spermine, an essential polyamine for *Leishmania*. Therefore, AdoMetDC may be a potential therapeutic target for leishmaniasis [69].

CGP40215A, a specific AdoMetDC inhibitor, was also reported with the antileishmanial effect that verified the potential of AdoMetDC enzyme inhibition strategy [70].

2.4.5 Trypanothione synthetase (Trypanothione synthase, TryS; EC 6.3.1.9)

TryS bifunctionally catalyzes both biosynthesis and hydrolysis of the glutathione-spermidine adduct trypanothione, which is the main regulator in intracellular

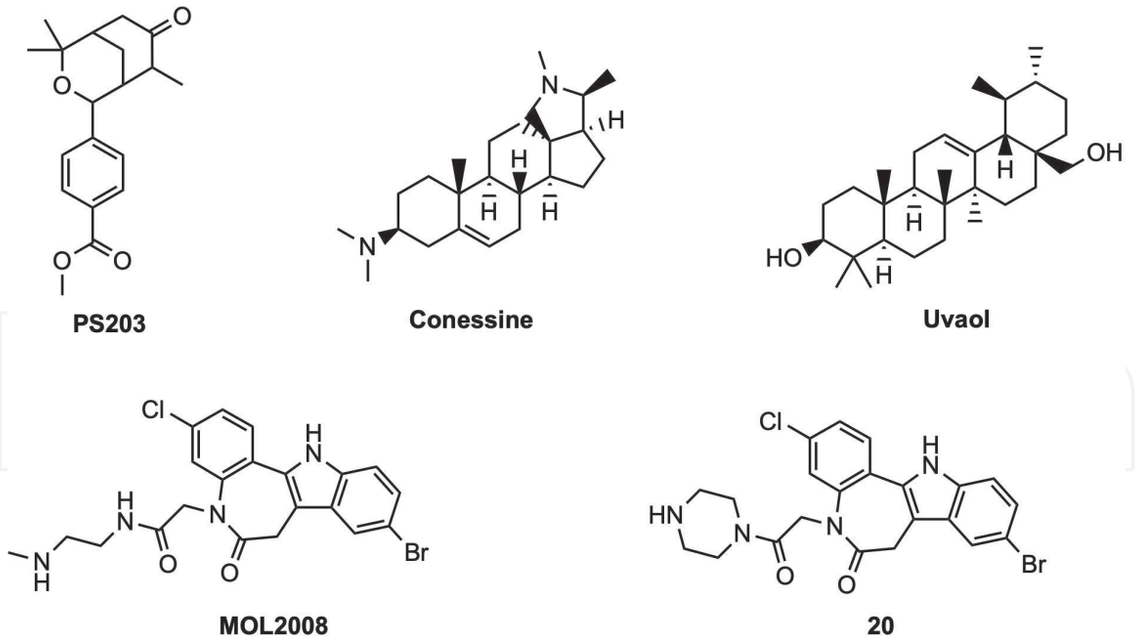

Figure 5.
Examples of TryS inhibitor structures with antileishmanial activity.

thiol-redox metabolite for parasitic trypanosomatids. As TryS is absent in humans, targeting this enzyme provides selectivity. Inhibition of TryS results in controlling relative levels of the critical metabolites, trypanothione, glutathionylspermidine, and spermidine in *Leishmania* [71]. Genetic and chemical analyses reveal that TryS is essential for *Leishmania infantum* [72].

In a computational screening campaign, oxabicyclo[3.3.1]nonanone skeleton was identified not only as a TryS inhibitor but also with TR inhibitory properties. A modest antileishmanial activity was reported for compound PS203 upon *L. donovani* promastigotes (**Figure 5**) [73]. In another study, TryS from *L. donovani* was characterized and inhibition studies with the natural compounds selected from an earlier Micro Source discovery natural product data set were performed [74]. Among the tested natural compounds, conessine and uvaol showed good TryS inhibition (Ki of 3.12 μM and 3.55 μM, respectively) with significant antileishmanial activity on *L. donovani* promastigotes (IC50 of 13,42 μM and 11,23 μM, respectively) (**Figure 5**) [74].

About 144 compounds belonging to seven different scaffolds were tested for TyrS inhibitory properties in a study by Benitez et al. One of the most promising inhibitors (IC50 of 0.15 μM) namely MOL2008, an N^5-substituted paullone derivative was evaluated upon *L. infantum* promastigotes (EC50 of 12.6 μM) (**Figure 5**) [75]. Following these results, 36 different derivatives of MOL2008 were developed by the same group [76]. Based on intriguing TyrS inhibition of compound 20 (IC50 0.3 μM), it was tested on both *L. infantum* promastigotes and *L. infantum* amastigotes. The metabolic changes exerted by 20 in both promastigote form and amastigote form of *L. infantum* are compatible with TryS inhibition (**Figure 5**) [76].

2.4.6 Trypanothione reductase (TR, TryR, Trypanothione-disulfide reductase 1, EC 1.8.1.12)

One of the main strategies of the host organism to overcome the infection is oxidative stress. TR has been purified from *T.cruzi* [77], first, and then from *Labrus donovani* [78]. TR enzyme is responsible for keeping trypanothione in the reduced state that is a variant of glutathione in *Leishmania* parasites. These enzyme

inhibitors have been investigated in antileishmanial drug discovery as the enzyme is essential for the parasite survival and its absence in the host, in which glutathione reductase (GR) is found, provides selectivity [79]. Although both TR and GR are inhibited by trivalent antimonials, TR is considerably more sensitive [80]. TR enzyme is also a target for anti-Chagas compounds and antimalarials. The main limitation of TR becoming a target in antileishmanial drug discovery is that in order to obtain a considerable effect in parasites' redox state, a minimum of *85%* inhibition is required [81]. Additionally, GR should be considered as an off-target for TR inhibitors and the selectivity over TR enzyme of the compounds may be presented. Apart from being an interesting target for antileishmanial drug design, it is also a popular target for antimalarial compounds.

The early discovery of tricyclic inhibitors that are specific for TR over GR led to the design and synthesis of a group of phenothiazine derivatives and their opened-ring analogs.

The first rational drugs with TR inhibitor activity over GR inhibition are tricyclic structures like phenothiazine and imipramine. Based on this, among several of quaternary phenothiazines, [3-(2-chloro-4a,10a-dihydrophenothiazin-10-yl) propyl] - (3,4-dichlorobenzyl) dimethylammonium derivative (Ki 0.12 μM) was reported possessing improved activity up to 2-fold compared to chlorpromazine on *L. donovani* species [82]. Compound **10**, an opened ring analog of phenothiazine, showed antileishmanial activity upon *L. donovani* (IC50 of 3.9 μg/mL). Expectedly, it was one of the most active compounds for TR enzyme with the Ki value of 6.5 μM [83].

A series of bis (2-amino diphenyl sulfides) were designed and synthesized to inhibit TR [84]. Among them, compound **15** was found to be the most active with the IC50 value of 200 nM. Although there was no correlation between TR inhibition and antileishmanial activity, the compounds showed activity upon *L. infantum* amastigotes (**Figure 6**) [84]. Sulfonamide and urea derivatives of quinacrine with varying methylene spacer lengths were designed as TR inhibitors and their antiprotozoal activities were evaluated [85]. Compound **2b** (TR IC50 of 3.3 μM and GR IC50 of 27.2 μM) was also one of the most active compounds upon *L. donovani* among with *Trypanosoma cruzi and Trypanosoma brucei* [85] (**Figure 6**).

In the pursuit of discovering novel lead heteroaromatic frameworks, harmaline, pyrimidobenzothiazine, and aspidospermine scaffolds were tested against TR inhibition (Ki of 35.1 μM, Ki of 26.9 and Ki of 64.6 μM, respectively) and *L. amazonensis* promastigote toxicity. Moreover, compounds have not exhibited any GR inhibitory activity [86]. Interestingly, Blackie et al. has introduced ferrocenic 4-aminoquinoline urea compounds with TR inhibitory and antileishmanial properties to the literature [87]. Although compounds inhibited TR in a low μM range with good selectivity over GR and showed antileishmanial activity on *L. donovani* amastigotes, unfortunately, these compounds were found to be toxic to macrophages (**Figure 6**) [87].

In an HTS campaign, 100,000 lead-like compounds were evaluated for their TR inhibition. As our focus on antileishmanial compounds, 2 series of compounds namely, nitrogenous heterocycles (triazine and pyrimidine derivatives) and conjugated indole derivatives took our interest in their potential on *L. donovani* amastigotes (**Figure 6**) [88].

Various chemical structures were reported with TR inhibitor activity and leishmaniacidal activity to the literature: Ag(0) nanoparticles encapsulated by ferritin molecules [89], Cu(II) diketonates [90], oxabicyclo[3.3.1]nonanones [73], azole-based compounds – e. pyrrole [91], β-carboline–quinazolinone hybrid [92], phenothiazine and phenoxazine derived chloroacetamides [93], selenocyanates and diselenide compounds [94, 95], iminodibenzyl derivatives with ethylenediamine,

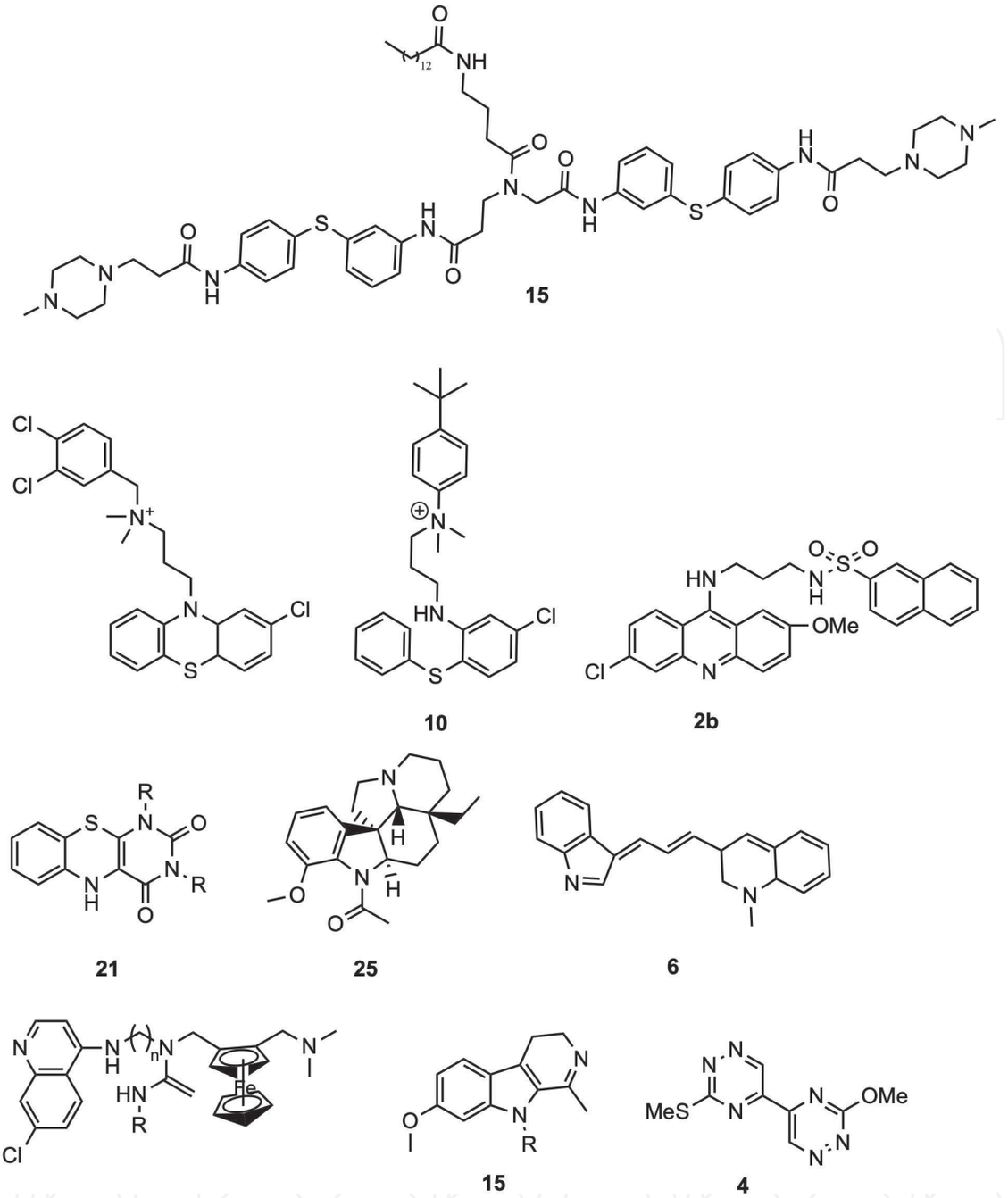

Figure 6.
Examples of TR inhibitor structures with antileishmanial activity.

ethanolamine and diethylenetriamine and their copper(II) complexes [96], diaryl sulfide derivatives [97], ammonium trichloro [1,2-ethanediolato-*O,O′*]-tellurat [98], all-hydrocarbon stapled peptides [99] chalcone derivatives [100], thiophene derivatives [101], imidazole-phenyl-thiazole compounds [102], isothiocyanate derivatives [103], (phenylthio)pyrimidin-4-amine derivatives [104], ferrocenylquinoline derivatives [105], triazole-phenyl-thiazoles derivatives [106], fluorene derivatives [107], adamantan derivatives, and their gold complexes [108] and natural products [109, 110] (**Figure 6**).

2.4.7 Tryparedoxin peroxidase (TryPI, TXNPx, EC 1.11.1.15)

Crystal structures of the tryparedoxin-tryparedoxin (TXN-TXNPx) peroxidase couple were reported but there is no study that targeted this system with antileishmanial activity [111].

2.5 Phosphotidylinositol-3-kinase (PI3K, EC 2.7.1.137)

The discovery of apoptotic pathways regulated by intracellular protozoan parasites and inhibit apoptosis, studies on signaling pathways have accelerated [112–114]. Interestingly, it was reported that there is an *L. major* PI3K mediated negative feedback mechanism for IL-12 production and PI3K/Akt signaling in *Leishmania* promastigotes [115].

Various heterocyclic compounds (quinoline, quinazoline, purine, thiazolopyrimidine scaffolds, etc.) as PI3K inhibitors were reported for treatment of several diseases alongside *Leishmania* [116, 117]. Later, Khadem et al. showed idelalisib—known PI3K inhibitor—and ampB combination therapy resulted in the reduction in parasite burden and moderate immune response [117]. A recent study showed that PI3K/mTOR inhibitor Torin2, Dactolisib, and NVP-BGT226 also possess good antileishmanial activity [118].

Imidazo[1,2-b]pyridazin scaffold was designed to inhibit various eukaryotic kinases by Bendjeddou et al. [119]. In this study, some of the compounds were tested against *L. amazonensis* parasites. The compounds showed antileishmanial activity at rather high concentrations (10 μM) although the compounds have not exhibited any toxicity at cell viability assays regarding concentrations [119].

Because of *Leishmania* parasite has a life cycle in the mammalian host, inhibition of signal transduction protein kinases for antileishmanial activities was investigated. Polyfluoroalkyl sp^2-glycolipid compounds were reported with antileishmanial properties by binding p38a-MAPK [120]. Purine derivatives, benzopyrroles, and benzopyrrolidines exhibited CRK3 cyclin-dependent kinase inhibitory properties and showed antileishmanial activity upon *Labrus donovani* amastigotes [121]. Lastly, a chemical inhibitor of heat shock protein 78 (HSP78), namely Ap5A reported with antileishmanial activity [122].

2.6 Topoisomerase I and II (TOPI, EC 5.6.2.1; TOPII, EC 5.6.2.2)

Topoisomerases are enzymes that modulate DNA topology. Firstly, topoisomerase II and then topoisomerase I enzymes were reported in *Leishmania* species [123, 124].

Different classes of TOP inhibitors show activity against *L. donovani* parasites by the means of DNA TOPI catalytic activity. The most important point is providing selectivity over parasite-human topoisomerase enzymes [125]. Pentostam's one of the proposed modes of action is inhibition of TOPI of *L. donovani* [126]. Werbovetz et al. tested known TOPII inhibitors, acridine derivatives, against *L. chagasi* and *L. donovani*, therefore, it was suggested that TOPII could serve as a useful target for parasite chemotherapy [127].

16-phenyl-6-hexadecynoic acid and 16-phenylhexadecanoic acid derivatives were synthesized by Carballeira et al. [128]. Compounds 1 and 2 showed promising activity on *L. donovani* TOPIB (EC50 14 μM and 36 μM, respectively). Moreover, compounds 1 and 2 showed cytotoxicity toward L. infantum amastigotes (IC50 of 3–6 μM) and L. infantum promastigotes (IC50 of 60–70 μM) [128].

In another study, compounds bearing 1,5-naphthyridine scaffold were reported [129]. Compound 22 was found to be one of the promising ones with the IC50 value (0.58 ± 0.03 μM) against L. infantum amastigotes similar to the standard drug amphotericin B (0.32 ± 0.05 μM) and selectivity over host murine splenocytes. Additionally, this compound showed remarkable inhibition on leishmanial TopIB [129].

Three compounds were identified in a very recent virtual screening campaign with a significant *Ld*TopIB activity (IC50of LRL-TP-85: 1.3 μM; LRL-TP-94: 2.9 μM;

and LRL-TP-101: 35.3 μM) [130]. Further studies showed that compounds were selective for *Ld*TopIB over *Homo sapiens* (Hs) TopIB. After that, compounds were evaluated for their in extracellular promastigote (4.9 μM, 1.4 μM, and 27.8 μM, respectively) and intracellular amastigote (34.0 μM, 53.7 μM, and 11.4 μM, respectively) activities [130].

Apart from these recent advances, several scaffolds such as bis-naphtoquinone [131, 132] betulinic acid derivatives [133], bisbenzimidazoles [134] and protoberberine alkaloids [135], and 1,3,4-thiadiazole derivatives [136] were identified with TOP inhibitor activity as potential antileishmanial compounds. Additionally, acetylenic fatty acids, 6-heptadecynoic acid, and 6-icosynoic acid derivatives [137], 2-octadecynoic acid [138], 3,3′-diindolylmethane derivatives [139], bis-lawsone analogs [140], spirooxindole derivatives [141], indeno-1,5-naphthyridines [142], diamidine derivatives [143], and copper salisylaldoxime [144] compounds are other reported topoisomerase inhibitors with antileishmanial activity.

2.7 Cysteine synthase (CS, O-acetylserine sulfhydrylase, OASS, EC 2.5.1.47)

Cysteine biosynthesis is a potential target for antileishmanial drug development. The structure of *L. major* cysteine synthase was revealed in 2012 by Fyfe et al. [145]. Cyclic imide derivatives were identified with a multitarget profile including TOPOI, *N*-myristoyltransferase, cyclophilin, and CS enzymes using *in silico* approach and *L. amazonensis* activity of the compounds were reported [146].

2.8 Oligopeptidase B (OPB, EC 3.4.21.83)

It was found out that a high level of serine protease activity was expressed by *L. donovani*, which was explained by an increase in OPB enzyme activity [147]. The crystal structure of *L. major* OPB was revealed in 2010 by McLuskey et al. [148]. Epoxy-α-lapachone was shown activity on both promastigote and amastigote forms of *L. amazonensis* in a study exploring natural compounds as potential antileishmanial agents. Moreover, this activity was associated with serine proteinase inhibitory activity of epoxy-α-lapachone in the same study [149]. Peptidic structure ShPI-I (Kunitz-type protease inhibitor from the sea anemone *Stichodactyla helianthus*) was shown to be a potent inhibitor of *L. amazonensis* serine proteases [150].

2.9 Superoxide dismutase (SOD, EC 1.15.1.1)

SOD enzyme was found in *L. tropica* by Meshnick and Eaton and it was suggested that the enzyme may be containing iron (Fe) which causes a difference from its host's enzymes which is linked to a copper or zinc atom [151]. Later, molecular isolation and characterization of Fe containing SOD cDNAs of *L. chagasi* were reported in 1997 [152] and the 3D structure of Fe-dependent superoxide dismutases (FeSODs) from *L. major* was reported [153].

In a study, imidazole-containing phthalazine derivatives were found to be potent inhibitors of Fe-SOD with antileishmanial properties. Additionally, the tested compounds were selective toward parasite Fe-SOD over human CuZn-SOD [154]. Arylamine Mannich base derivatives, known to be effective against *Trypanosoma cruzi*, were exhibited remarkable activity against *Leishmania* species. The mechanism of action of these compounds was linked to their potent Fe-SOD inhibition [155].

2-Iminothiazole derivatives [156], scorpiand-like azamacrocycles [157, 158], pyrazole-containing polyamine macrocycles [159], natural product momordicatin [ethyl 2-(4-hydroxybutyl)benzoate] [160], imidazole or pyrazole-based benzo [g]

phthalazine derivatives [161], triphenyl tin salicylanilide thiosemicarbazone [162], Se containing aromatics and heteroaromatic compounds [163], ruthenium complexes with purine analogs [164], fisetin—a flavanoid anolog [57] and dialkyl pyrazole-3,5-dicarboxylates [165] were reported as SOD inhibitors exhibiting antileishmanial activity in the literature.

2.10 Nitroreductases (NTR, EC 1.7.1.16)

Nitroreductase enzymes catalyze the reduction of nitro/nitroaromatic compounds. Based on oxygen sensitivity, NTRs are divided into two groups: NTR1 is oxygen-insensitive and functions via a series of two-electron reductions, NTR2 is oxygen-sensitive and mediated a one-electron reduction [166]. NTR1 enzyme is found mainly in bacteria and absent in most eukaryotes. Keeping this in mind, *L. major* NTR1 (*Lm*NTR) was characterized and identified as a potential drug target for leishmaniasis [167].

It was reported that aziridinyl nitrobenzamide compounds [168], nitroquinolinone derivatives [169], 3-nitro-2-(phenylsulfonylmethyl) imidazo[1,2-a]pyridine derivatives [170], and nitro-heteroaryl nitrone derivatives [172] are NTR inhibitors with antileishmanial effects.

2.11 Nucleoside hydrolases (NH, EC 3.2.2.1)

Koszalka and Krenitsky, separated and purified three nucleoside hydrolases from promastigotes of *L. donovani*—purine 2′-deoxyribonucleosidase, purine ribonucleosidase, and pyrimidine ribonucleosidase [172]. Then, the X-Ray structure and amino acid sequence of nucleoside hydrolase from *L. major* was revealed alongside its several nanomolar transition state inhibitors [39].

Augustyns's research group design and synthesize various compounds and tested against IAG-NH (inosine-adenosine-guanosine nucleoside hydrolase) from *Tabanus vivax*. In contrast to promising enzyme activity of the compounds, antileishmanial activity of the compounds hasn't been investigated [41, 173, 174]. Freitas et al. also tested immucillin derivatives against *L. donovani*, *L. inf. Chagasi* and *L. amazonensis* parasites [175].

It was found out that hydroxychromenone and tetrahydrocyclohexanecarboxylic acid fragments could bind to the enzyme in a fragment-based analysis on *Ld*NH using saturation transfer difference (STD) NMR spectroscopy [176].

In a recent study, a natural product from Brazilian flora, flavonoids, and proanthocyanidins, with antileishmanial activity screened against *Ld*NH and described as an inhibitor of *Ld*NH [43, 177].

Interestingly, *Ld*NH (NH36) is the main area of interest for human recombinant vaccine-based studies and phase I trial of nucleoside hydrolase NH36 of *L. donovani*, the main antigen of the Leishmune® vaccine, and the sterol 24-c-methyltransferase (SMT) from *L. infantum* is in progress [178].

2.12 Cysteine proteases

There are two cysteine protease genes from *L. major*—one is structurally similar to the cathepsin L (CatL) family and the other is similar to the cathepsin B (CatB) family of cysteine proteases. These cysteine protease enzymes were isolated and sequenced by Sakanari et al. [179].

It is reported that aziridine-2,3-dicarboxylate [180], natural products flavone derivatives [181], trans-aziridine-2,3-dicarboxylate derivatives [182] organotellurane RF07 and palladacycle complex [183–185], and dipeptidyl enoates [186] exhibit antileishmanial effect and inhibit cysteine proteases.

2.13 Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC 1.2.1.12)

GAPDH activity was detected in two cell compartments of *Leishmania mexicana* promastigotes [187]. Then, the crystal structure of *L. mexicana* GAPDH in complex with inhibitors was reported to the literature [188].

Although GAPDH enzyme is found in *Leishmania sp*., it is an attractive target for the development of novel antitrypanosomatid agents rather than antileishmanial compounds.

2.14 Dihydroorotate dehydrogenase (DHODH, EC 1.3.5.2)

DHODH enzyme catalyzes the stereoselective oxidation of (S)-dihydroorotate (DHO) to orotate (ORO) in the *de novo* pyrimidine biosynthetic pathway. The structure of *L. major* DHODH was revealed by X-ray diffraction analysis [189]. It was reported that natural compounds from Asteraceae species could inhibit *Lm*DHODH by Chibli et al., though the antileishmanial effect of the compounds has not been evaluated [190].

2.15 Methionyl-tRNA synthetase (MetRS, EC 6.1.1.10)

Considering the structure of *L. major* MetRS, the difference in human cytosolic and mitochondrial MetRS and near the ATP- and methionine-binding regions of *Lm*MetRS promises selectivity for MetRS inhibitors [191].

DDD806905, a known *Tb*MetRS inhibitor, tested against *Ld*MetRS and showed antileishmanial effect upon *Leishmania* axenic amastigote yet, it has not shown efficacy in an animal model of leishmaniasis due to high protein binding as well as sequestration of this dibasic compound into acidic compartments [192]. Researchers have characterized a new series of *Ld*MetRS inhibitors bearing 4,6-diamino-substituted pyrazolopyrimidine core that target a previously undefined, allosteric binding site in the enzyme recently [193].

2.16 Phosphodiesterases (PDE, EC 3.1.4.17)

Phosphodiesterases control the cellular concentration of the second messengers cAMP and cGMP that are key regulators of several physiological processes.

A correlation between cAMP concentration in *Leishmania* cells and proliferation and transformation is demonstrated. By the addition of phosphodiesterase inhibitors to the culture medium, the intracellular level of cAMP was increased [194].

Crystal structure of the *L. major* phosphodiesterase *Lmj*PDEB1, one of the five PDE encoding genes, was reported in 2007 [195].

Isoxazolo[3,4-d]pyridazinone analogs were reported to inhibit PDE extracted from *L. mexicana* [196]. Later, it was reported that triphenyl-substituted imidazole compound exhibits *in vitro* antileishmanial and PDE inhibitor activity. Moreover, there was a correlation between *in vitro* antileishmanial activity and cAMP content [197].

2.17 Squalene synthase (SQS, SSN, E.C. 2.5.1.21)

SQS enzyme catalyzes the first step in sterol biosynthesis. Cloning, expression, and purification of a catalytically active recombinant squalene synthase of *L. donovani* (*Ld*SSN) [198].

Biphenylazabicyclooctanol, biphenylquiniclidine, and quiniclidine derivatives possessing *Lm*SQS inhibitory activity have shown antileishmanial effects against

L. amazonensis, therefore, SQS might serve as a potential target for antileishmanial drug discovery [199–201].

2.18 Uridinediphosphate-glucose pyrophosphorylase (UGPase, EC 2.7.7.9)

UGPase enzyme catalyzes the reaction of UTP and glucose-1-phosphate to 3-UDP-glucose and PPi in the presence of Mg^2 *in vivo*. It was reported that protozoan UGP differed from its mammalian counterparts which might provide selectivity [202]. *L. major* UGPase three-dimensional structure was reported but there has not been any reported *in vitro/in vivo* inhibitor of the enzyme yet although virtual screening campaigns have been applied to the enzyme [203].

2.19 Deoxyuridine 5′-triphosphate nucleotidohydrolase (dUTPase, EC 3.6.1.23)

The levels of dUTP are kept low by the action of dUTPase, a ubiquitous enzyme that catalyzes the hydrolysis of dUTP to PPi and dUMP, a substrate for thymidylate synthase (TS) [204]. The purification and characterization of *L. major* dUTPase were reported alongside its crystal structure [205, 206].

Deoxyuridine derivatives were shown to inhibit *L. major*, and human dUTPase enzymes exhibited moderate activity against *L. donovani* [207].

2.20 γ-Glutamylcysteine synthetase (Gcs, EC 6.3.2.2)

Gcs is an essential protein of the trypanothione biosynthesis pathway, which catalyzes ATP-dependent ligation of L-cysteine to L-glutamate. Characterization of *L. donovani* Gcs was reported to the literature in 2016 [208]. Agnihotri et al. identified carbamate, urea, and purine derivatives as Gcs inhibitors using *in silico* tools, then antileishmanial effect of the compounds was reported *in vitro* [209].

2.21 Cyclophilin (Cyp, Peptidylprolyl isomerase, EC 5.2.1.8)

Cyclophilins are a ubiquitous class of proteins with peptidylprolyl *cis-trans* isomerase activity. The structure of cyclophilin from *L. donovani* bound to cyclosporin was reported in 2009 [210]. Interestingly, a recent study showed that cyclosporin A, cyclophilin A modulator, does not express any significant inhibitory effect on intracellular *L. donovani* amastigotes, therefore, further studies are needed to validate this enzyme [211].

2.22 Other *Leishmania sp.* enzymes

We have summarized the validated targets for antileishmanial drug discovery and tried to give examples of potential modulators of these targets so far. Up to our knowledge, there are several other enzymes involved in kinetoplastids' physiological pathways which might serve as a potential target and provide selectivity, such as NDKb (nucleoside diphosphate kinase B, C 2.7.4.6), GPD (glycerol-3-phosphate dehydrogenase, EC 1.1.1.8), PGI (glucose-6-phosphate isomerase, EC 5.3.1.9), GspS (glutathionylspermidine synthetase, EC 6.3.1.8), PMM (phosphomannomutase, EC 5.4.2.8), PyK (pyruvate kinase, EC 2.7.1.40), TIM (triosephosphate isomerase, EC 5.3.1.1.), DHS (deoxyhypusine synthase, EC 2.5.1.46), and DOHH (deoxyhypusine hydroxylase, EC 1.14.99.29). Yet, the antileishmanial effect by the modulation of these targets has not been reported therefore further studies on these targets are needed.

3. Conclusion

Leishmaniasis treatment research has long been neglected. In this postgenomic era, work on leishmaniasis has accelerated, but great challenges still remain for medicinal chemists and chemical biologists—selectivity over human enzymes and efficacy over parasite life cycles. This chapter will be useful for researchers who will do *in silico* and *in vitro* studies.

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