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Antileishmanial phytochemical phenolics: Molecular docking to potential protein targets

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ABSTRACT

A molecular docking analysis has been carried out to examine potential *Leishmania* protein targets of antiprotozoal plant-derived polyphenolic compounds. A total of 352 phenolic phytochemicals, including 10 aurones, six cannabinoids, 34 chalcones, 20 chromenes, 52 coumarins, 92 flavonoids, 41 isoflavonoids, 52 lignans, 25 quinones, eight stilbenoids, nine xanthones, and three miscellaneous phenolic compounds, were used in the virtual screening study using 24 *Leishmania* enzymes (52 different protein structures from the Protein Data Bank). Noteworthy protein targets were *Leishmania* dihydroorotate dehydrogenase, *N*-myristoyl transferase, phosphodiesterase B1, pteridine reductase, methionyl-tRNA synthetase, tyrosyl-tRNA synthetase, uridine diphosphate-glucose pyrophosphorylase, nicotinamidase, and glycerol-3-phosphate dehydrogenase. Based on in-silico analysis of antiparasitic polyphenolics in this study, two aurones, one chalcone, five coumarins, six flavonoids, one isoflavonoid, three lignans, and one stilbenoid, can be considered to be promising drug leads worthy of further investigation.

1. Introduction

Leishmaniasis refers to a collection of chronic diseases caused by protozoa of the genus *Leishmania* and naturally transmitted by sandflies (*Phlebotomus* spp. and *Lutzomya* spp.) [1,2]. Pathogenic *Leishmania* can be grouped into viscerotropic leishmaniasis (VL) and cutaneous leishmaniasis (CL) as well as "Old World" and "New World" types. Old World cutaneous leishmaniases ("Oriental sore", caused by *L. tropica*, found in the Middle East, Adriatic, and north Africa; and *L. major*, found in the Middle East, north and sub-Saharan Africa) cause chronic ulcers or nodular lesions of the skin that are slow to heal and generally leave scars. New World cutaneous leishmaniases (caused by *L. braziliensis* complex, found throughout Central and South America; *L. guyanensis* complex, found in Central America and Amazonia; and *L. mexicana* complex, found in Texas, Mexico, the Caribbean, Central America, and tropical South America) are similar to Old World CL, although New World forms of the disease tend to last longer and are more severe. *L. braziliensis* infections sometimes lead to mucocutaneous leishmaniasis with progressive necrotization of the skin and mucosal tissues of the nose, mouth, and pharynx, leading to extreme disfigurement. Visceral leishmaniasis (Old World, "kala azar", caused by *L. donovani*, found in India, China, north and east Africa; and *L. infantum*, found in the Mediterranean region; and New World, caused by *L. chagasi*, found in Brazil and Venezuela) causes chronic fever, liver problems, spleen enlargement, anemia and other blood problems.

Proven chemotherapeutics for leishmaniasis include the antimonials sodium stibogluconate (pentostam) and meglumine antimoniate (glucantime) [3]. There are adverse side effects associated with these compounds and drug resistance is emerging [4]. Alternative antileishmanial chemotherapies include amphotericin B and pentamidine, but these are generally more toxic than the pentavalent antimonials [5]. The alkylphosphocholine miltefosine is currently in clinical use in India and may find more widespread use in the future [6]. There are currently no vaccines available to prevent leishmaniasis, but research toward the development of vaccines is underway [7]. Given the side effects and limitations of current chemotherapies along with the lack of suitable vaccines, there is a pressing need for alternative and readily available chemotherapeutic agents for treatment of leishmaniasis.

Molecular docking is a field of in-silico molecular modeling method that is used to predict the preferred orientation of, generally, a small organic molecule (the ligand) with biological macromolecules (usually proteins) [8]. The preferred orientation of the ligand relative to the protein can be used to predict the binding energy of the ligand to the protein. Molecular docking has become an invaluable tool for high-throughput virtual screening and drug discovery [9].

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Plant-derived phenols constitute a large class of compounds that contain one or more hydroxyl moieties attached to an aromatic ring. There are several phytochemical classes of phenolics including stilbenoids, phenylpropanoids, flavonoids, and quinones [10]. Numerous plant-derived phenolic compounds have exhibited wide-ranging biological activities of medicinal importance. Some notable examples include the stilbenoid resveratrol, which has shown numerous potential health benefits, including anticarcinogenic activity [11], cardioprotective effects [12], and a potential therapeutic agent for neurodegenerative diseases [13]; quercetin, kaempferol, and myricetin, flavonoid components from fruits, vegetable, and green and black teas [14,15], are potent antioxidant and free-radical scavengers [16] and studies have pointed out their possible role in preventing cardiovascular diseases and cancer [17,18]; and genistein and daidzein, isoflavonoids found in soybeans, are phytoestrogens that have shown potential cancer chemopreventive as well as cardiovascular and post-menopausal benefits [19,20]. Numerous phytochemical polyphenolic compounds have also shown activity against parasitic protozoa [21–29]. In this work, we have examined the potential biochemical targets from *Leishmania* spp. of antiparasitic phytochemical polyphenolics using a molecular docking approach in an effort to help elucidate the biochemical mechanisms of activities of these bioactive compounds, as well as to recognize new natural product drug leads.

A total of 24 *Leishmania* enzymes (52 different protein structures from the Protein Data Bank (PDB)) were used as potential protein drug targets using the Molegro Virtual Docking program [30,31]. A total of 352 phytochemical phenolic compounds that had shown antiparasitic activity [21–29], including 10 aurones, six cannabinoids, 34 chalcones, 20 chromenes, 52 coumarins, 92 flavonoids, 41 isoflavonoids, 52 lignans, 25 quinones, eight stilbenoids, nine xanthones, and three miscellaneous phenolic compounds, were used in the virtual screening study (see Supplementary Figures 1–9 and Supplementary Tables 1–9). Additionally, we have considered the drug-likeness of the potential antileishmanial compounds using Lipinski's "rule of five" [32]. Lipinski's rule uses guidelines for molecular descriptors that are important with respect to the adsorption, distribution, metabolism, and excretion (ADME) of chemical compounds. It does not address the pharmacological activity of potential drugs, but rather their molecular properties related to bioavailability. Lipinski's rule states that an orally active drug should: (a) contain no more than five hydrogen-bond donor atoms (N—H or O—H), (b) contain no more than ten hydrogen-bond acceptor atoms (N or O), (c) have a molecular weight less than 500 g/mol, and (d) have an octanol/water partition coefficient (Log *P*) less than 5.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jmgm.2013.12.010.

2. Methodology

2.1. Molecular docking

Protein-ligand docking studies were carried out based on the crystal structures of verified *Leishmania* protein drug targets: *L. major* cathepsin B, LmajCatB (prepared by structural homology to *Trypanosoma brucei* cathepsin B, PDB 3hhi [33]), *L. major* dihydroorotate dehydrogenase, LmajDHODH (PDB 3gye [34], PDB 3mhu, and PDB 3mjy [35]), *L. major* methionyl-tRNA synthetase, LmajMetRS (PDB 3kfl [36]), *L. major* nucleoside diphosphate kinase b, LmajNDKb (PDB 3ngs, PDB 3ngt, and PDB 3ngu [37]), *L. major* nucleoside hydrolase, LmajNH (PDB 1ezr [38]), *L. major N*-myristoyltransferase, LmajNMT (PDB 2wsa, PDB 3h5z [39], and PDB 4a30 [40]), *L. major* oligopeptidase B, LmajOPB (PDB 2xe4 [41]), *L. major* phosphodiesterase 1, LmajPDE1 (PDB 2r8q [42]), *L. major* pteridine reductase 1, LmajPTR1 (PDB 1e7w [43], PDB 1w0c [43], PDB 2bf7 [44], and PDB 3h4v [45]), *L. major* tyrosyl-tRNA synthetase, LmajTyrRS (PDB 3p0h and PDB 3p0j [46]), *L. major* uridine diphosphate-glucose pyrophosphorylase, LmajUGPase (PDB 2oef and PDB 2oeg [47]), *L. major* deoxyuridine triphosphate nucleotidohydrolase, LmajdUTPase (PDB 2yay and PDB 2yb0 [48]), *L. donovani* cathepsin B, LdonCatB (prepared by structural homology to *T. brucei* cathepsin B, PDB 3hhi [33]), *L. donovani* cyclophilin, LdonCyp (PDB 2haq [49] and PDB 3eov [50]), *L. donovani* dihydroorotate dehydrogenase, LdonDHODH (PDB 3c61 [51]), *L. donovani N*-myristoyltransferase, LdonNMT (PDB 2wuu [52]), *L. mexicana* glyceraldehyde-3-phosphate dehydrogenase. LmexGAPDH (PDB 1a7k [53] and PDB 1gyp [54]), *L. mexicana* glycerol-3-phosphate dehydrogenase, LmexGPDH (PDB 1evz [55], PDB 1m66, PDB 1n1e and PDB 1n1g [56]), *L. mexicana* phosphoglucose isomerase, LmexPGI (PDB 1q50 and PDB 1t10 [57]), *L. mexicana* phosphomannomutase, LmexPMM (PDB 2i54 and PDB 2i55 [58]), *L. mexicana* pyruvate kinase, LmexPYK (PDB 1pkl [59], PDB 3hqp [60], and PDB 3pp7 [61]), *L. mexicana* triosephosphate isomerase, LmexTIM (PDB 2vxn [62] and PDB 2y61 [63]), *L. infantum* sterol 14α-demethylase, LinfCYP51 (PDB 3l4d [64]), *L. infantum* glyoxalase II, LinfGLO2 (PDB 2p1e and PDB 2p18 [65]), *L. infantum* nicotinamidase, LinfPNC1 (PDB 3r2j [66]), *L. infantum* thiol-dependent reductase I, LinfTDR1 (PDB 4ags [67]), and *L. infantum* trypanothione reductase, LinfTR (PDB 2yau [68], PDB 4adw, and PDB 4apn [69]). Prior to docking all solvent molecules and the co-crystallized ligands were removed from the structures. Molecular docking calculations for all compounds with each of the proteins were undertaken using Molegro Virtual Docker v. 6.0 [30,31], with a sphere (15 Å radius) large enough to accommodate the cavity centered on the binding sites of each protein structure in order to allow each ligand to search. If a co-crystallized inhibitor or substrate was present in the structure, then that site was chosen as the binding site. If no co-crystallized ligand was present, then suitably sized (>50 $Å^3$) cavities were used as potential binding sites. Standard protonation states of the proteins based on neutral pH were used in the docking studies. Each protein was used as a rigid model structure; no relaxation of the protein was performed. Assignments of charges on each protein were based on standard templates as part of the Molegro Virtual Docker program; no other charges were necessary to be set. Each ligand structure was built using Spartan '10 for Windows [70]. The structures were geometry optimized using the MMFF force field [71]. Flexible ligand models were used in the docking and subsequent optimization scheme. As a test of docking accuracy and for docking energy comparison, co-crystallized ligands were re-docked into the protein structures (see Supplementary Table 10). Different orientations of the ligands were searched and ranked based on their energy scores. The RMSD threshold for multiple cluster poses was set at <1.00 Å. The docking algorithm was set at maximum iterations of 1500 with a simplex evolution population size of 50 and a minimum of 30 runs for each ligand. Each binding site of oligomeric structures was searched with each ligand. The lowest-energy (strongest-docking) poses for each ligand in each protein target are summarized in Supplementary Tables 1–9.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jmgm.2013.12.010.

2.2. Homology modeling

The primary sequence of the cathepsin B-like cysteine protease from *T. brucei* (TbCatB, PDB 3hhi [33]) was compared to the

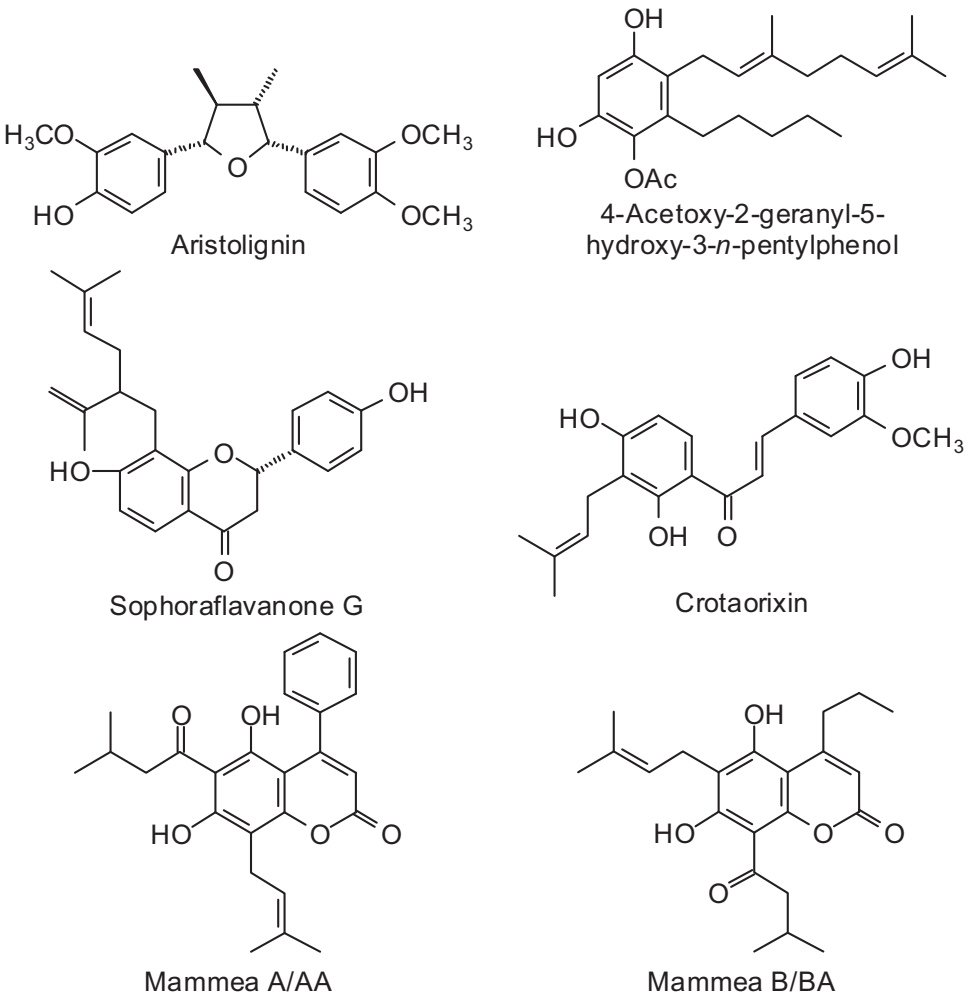

Fig. 1. Polyphenolic compounds with promising docking energies with *Leishmania major* dihydroorotate dehydrogenase.

query sequences of the same functional enzyme from *L. donovani* (LdonCatB) and *L. major* (LmajCatB) using the Protein BLAST (Basic Local Alignment Search Tool). Regions of local similarity and identity were found between the query sequences of LdonCatB and LmajCatB when compared to the model sequence of TbCatB. Both LdonCatB and LmajCatB sequences had 52% and 54% identity with that of TbCatB, respectively. The three-dimensional structure of TbCatB has been determined to 1.6 Å (PDB 3hhi [33]) but there is no structural information available for either LdonCatB or LmajCatB. Calculated models for both LdonCatB and LmajCatB were obtained from combining sequence information of the unknown target structures for LdonCatB and LmajCatB with the known model of TbCatB. The alignment between target and model sequences was used to modify the model PDB TbCatB file by pruning non-conserved residues to the last common atoms using the CCP4 chainsaw molecular replacement utility [72,73] leaving conserved residues unchanged. The resulting models were refined with conjugate gradient minimization with no experimental energy terms used in the crystallographic and NMR System (CNS) program suite [74]. The resulting detailed model was refined with conjugate gradient minimization with no experimental energy terms used. All atoms of the molecules were unrestrained and were minimized for 500 steps with a continuous dielectric constant of one.

3. Results and discussion

3.1. Leishmania cathepsin B

Cysteine proteases are important virulence factors, are essential to parasite survival, and have been identified as potential drug targets [75]. Unfortunately, none of the phenolic compounds examined in this study showed preferential docking to either *L. major* cathepsin B (LmajCatB) or to *L. donovani* cathepsin B (LdonCatB). The ligands with the strongest docking energies were the lignan pseudotsuganol (docking energy, $E_{dock} = -137.7$ kJ/mol) to LmajCatB and the xanthone garcilivin C ($E_{dock} = -129.5$ kJ/mol) to LdonCatB. Both of these ligands docked very strongly to most proteins, however, and also violate Lipinski's rule [32] (see Supplementary Table 11) and so cannot be considered viable drug leads.

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3.2. Leishmania dihydroorotate dehydrogenase

Leishmania dihydroorotate dehydrogenase (DHODH) is an enzyme involved in the de novo synthesis of pyrimidine [76]. Several polyphenolic ligands (Fig. 1) showed promising

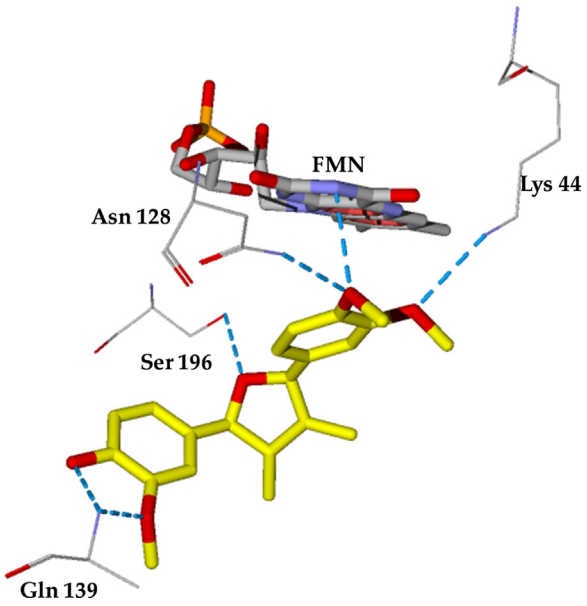


Fig. 2. The lowest-energy pose of aristolignin (yellow and red) interacting with catalytic-site residues Ser 196, Lys 44, Asn 128, Gln 139, and the enzyme cofactor flavin mononucleotide (FMN) of LmajDHODH.

docking with *L. major* dihydroorotate dehydrogenase (LmajDHODH). The lignan aristolignin (E_{dock} = −130.6 kJ/mol), the chalcone crotaorixin (E_{dock} = −133.2 kJ/mol), the coumarins mammea A/AA (E_{dock} = −129.6 kJ/mol) and mammea B/BA (E_{dock} = −124.0 kJ/mol), the cannabinoid 4-acetoxy-2-geranyl-5-hydroxy-3-*n*-pentylphenol (E_{dock} = −128.2 kJ/mol), and the flavonoid sophoraflavanone G (E_{dock} = −131.1 kJ/mol), all had notable docking energies that were less than the co-crystallized ligand (5-nitroorotic acid, E_{dock} = −102.2 kJ/mol, Supplementary Table 10) for this protein. Additionally, both coumarins mammea A/AA and mammea B/BA, as well as aristolignin and crotaorixin obey Lipinski's rule, and 4-acetoxy-2-geranyl-5-hydroxy-3-*n*-pentylphenol and sophoraflavanone G have only one violation each (the calculated partition coefficient, *C* Log *P* = 6.12 and 5.10, respectively). The lowest-energy pose of aristolignin is predicted to interact with residues Ser 196, Lys 44, Asn 128 and Gln 139 in the S1 and S2 catalytic sites of LmajDHODH (Fig. 2). Aristolignin has shown in vitro antiparasitic activity against *Trypanosoma cruzi* [77], while 4-acetoxy-2-geranyl-5-hydroxy-3-*n*-pentylphenol was active against *Leishmania donovani* [78]. Both crotaorixin [79] and sophoraflavanone G [80] have shown inhibitory activity against *Plasmodium falciparum*.

3.3. *Leishmania deoxyuridine triphosphate nucleotidohydrolase*

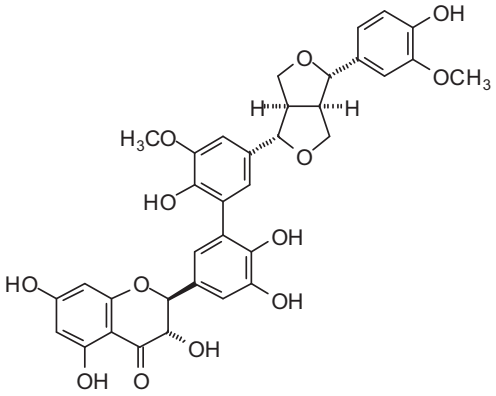
Deoxyuridine triphosphate nucleotidohydrolase (dUTPase), an enzyme involved in controlling intracellular dUTP levels [81], was not a viable target for known antiparasitic polyphenolics. The enzyme has been shown to efficiently hydrolyse both dUTP and dUDP, thus acting as a dual dUTP-dUDPase in *Leishmania*. However, analogs of dUTP have also been shown to be ineffective at inhibiting this enzyme [81]. The strongest-docking ligand was pseudotsuganol (E_{dock} = −149.7 kJ/mol), but this compound may not be a good drug lead (see above).

3.4. *Leishmania nucleoside diphosphate kinase b*

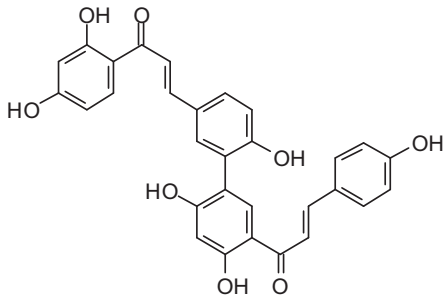
Nucleoside diphosphate kinase b plays a critical role in the purine-salvage pathway of *Leishmania* parasites and has been identified as an attractive drug target [82]. In addition to the role it plays in the salvage pathway, it has also been suggested that it participates in maintaining the integrity of the host cells to the benefit of the parasite by preventing ATP-mediated lysis of macrophages [83]. Human NDK-b activity has been previously shown to be inhibited by the polyphenolic constituents of tea (epigallocatechin gallate, epicatechin gallate, ellagic acid and theaflavins) [84]. Only one ligand showed significant docking preference to *L. major* nucleoside diphosphate kinase b (LmajNDKb), 6,11-dihydroxy-3-methyl-3-(4-methylpent-3-enyl)pyrano[2,3-*c*]xanthen-7(3*H*)-one (E_{dock} = −107.5 kJ/mol), but the docking energy was less exothermic than either of the co-crystallized ligands (AMP, E_{dock} = −122.4 kJ/mol; ADP, E_{dock} = −140.4 kJ/mol; see Supplementary Table 10).

3.5. *Leishmania nucleoside hydrolase*

In contrast to humans, *Leishmania* spp. are not able to synthesize purines de novo and must rely on purine salvage pathways, which nucleoside hydrolases play a key role [85]. A number of nucleoside analogs and bicyclic *N*-arylmethyl-substituted iminoribitol derivatives have been reported to inhibit *Leishmania* nucleoside hydrolase [86,87]. There were no phenolic compounds that preferentially dock to *Leishmania* nucleoside hydrolase, therefore, none could be considered to have any preferential scaffold for nucleoside hydrolase-based drug design. The strongest-docking ligands (Fig. 3) were the lignan pseudotsuganol (E_{dock} = −136.4 kJ/mol) and the chalcone rhuschalcone VI (E_{dock} = −139.7 kJ/mol), but both of



Pseudotsuganol



Rhuschalcone VI

Fig. 3. Pseudotsuganol and rhuschalcone VI, the ligands with the strongest docking energies to *Leishmania major* nucleoside hydrolase.

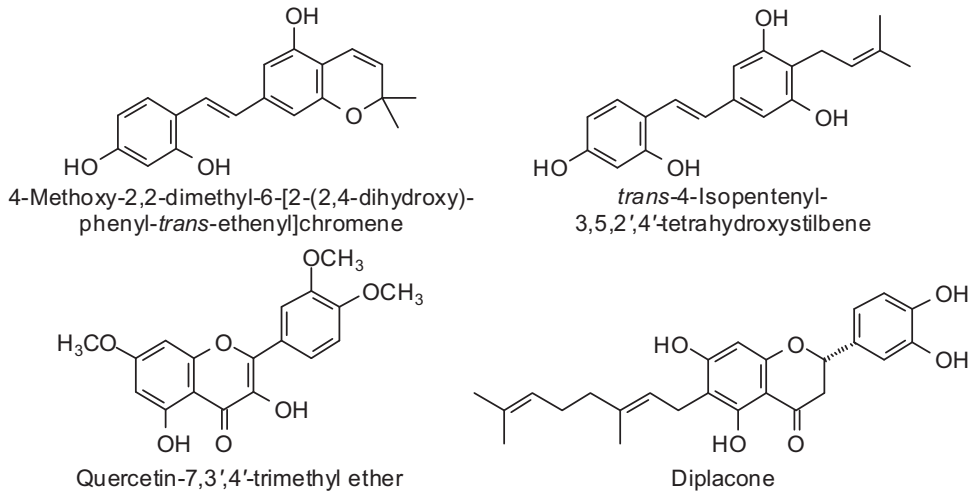


Fig. 4. Polyphenolic compounds that exhibited selective docking to *Leishmania major N*-myristoyltransferase.

these compounds violate Lipinski's rule (Supplementary Table 10) [32].

3.6. *Leishmania N-myristoyltransferase*

Leishmania *N*-myristoyltransferase catalyzes the *N*-myristoylation, a co-translational modification, of *Leishmania* proteins. Gene-silencing approaches have been used to demonstrate that *N*-myristoyltransferase is essential to parasite viability [88]. Chemotypes such as aminoacylpyrrolidines, piperidinylindoles, azetidinopyrimidines, thienopyrimidines have also been identified as selective inhibitors of *L. donovani* *N*-myristoyltransferase. In this work, several polyphenolic compounds (Fig. 4) showed selective docking to *L. major* *N*-myristoyltransferase (LmajNMT) with docking energies comparable to the co-crystallized ligand (myristoyl-CoA, $E_{dock} = -123.8$ kJ/mol). These include the stilbenoids 4-methoxy-2,2-dimethyl-6-[2-(2,4-dihydroxy)phenyl-*trans*-ethenyl]chromene ($E_{dock} = -122.9$ kJ/mol) and *trans*-4-isopentenyl-3,5,2′,4′-tetrahydroxystilbene ($E_{dock} = -127.4$ kJ/mol), both of which have shown antiplasmodial activity on *P. falciparum* [89], and the flavonoids diplacone ($E_{dock} = -135.7$ kJ/mol) and quercetin-7,3′,4′-trimethyl ether ($E_{dock} = -120.5$ kJ/mol). Diplacone has shown moderate in vitro activity against *L. donovani* and *T. brucei brucei* [90]. Diplacone is predicted to form extensive interactions with residues Gly 205, Leu 421, Val 378 and 346, Asn 383, Tyr 345, 217 and 92, and Thr 203 of LmajNMT (Fig. 5). Interestingly, quercetin-7,3′,4′-trimethyl ether docked more strongly to LmajNMT than quercetin itself, the monomethyl ether, the dimethyl ether, or the tetramethyl ether (Supplementary Table 5). Quercetin-7,3′,4′-trimethyl ether has been reported to show trypanocidal activity against trypomastigote forms of *T. cruzi* [91]. The preference for prenylated polyphenolics by LmajNMT, in silico, suggests that potential inhibitors of the enzyme, peptides or small molecules, may require side chain lipidation like the endogenous substrate myristoyl-CoA for them to have good inhibitory activity.

3.7. *Leishmania oligopeptidase B*

The serine protease oligopeptidase B (OPB) has been shown to be a virulence factor in *Trypanosoma cruzi* and *T. brucei*. Although OPB-deficient *L. major* were shown to be less able to infect mice, they were still virulent, suggesting LmajOPB is not likely to be an important virulence factor in *Leishmania* [92]. Nevertheless, molecular docking has revealed that the chalcone 3-methoxycitrunobin-4-methyl ether and the isoflavonoid 4′-*O*-methylglycyrrhisoflavone (Fig. 6) did show significant docking preference for LmajOPB ($E_{dock} = -128.6$ and -127.5 kJ/mol, respectively). 4′-*O*-Methylglycyrrhisoflavone showed antiplasmodial activity against *P. falciparum* [93].

3.8. *Leishmania phosphodiesterase B1*

Leishmania phosphodiesterase B1 catalyzes the hydrolysis of cAMP to AMP, and selective inhibition of the protozoal enzyme has been suggested to be an avenue of antiprotozoal drug development [94]. Several phosphodiesterase B inhibitors have been shown to have relatively low LmajPDEB1-inhibitory activities [95], this suggests, therefore, that a structure-based design approach may help in the development of more selective and potent inhibitors of LmajPDEB1. Four antiplasmodial stilbenoids, machaeriol B, machaeriol C, machaeridiol B, and machaeridiol C [96,97] (Fig. 7), as well as the antiplasmodial flavonoid artonin F [98], showed selective docking to *L. major* phosphodiesterase B1 (LmajPDEB1) with docking energies ($E_{dock} = -121.8$, -116.1, -121.2, -120.7, and -127.4 kJ/mol, respectively) much stronger than the co-crystallized ligand (3-isobutyl-1-methylxanthine, $E_{dock} = -78.1$ kJ/mol). Artonin F does have one Lipinski's rule violation, however (MW = 502.6 amu).

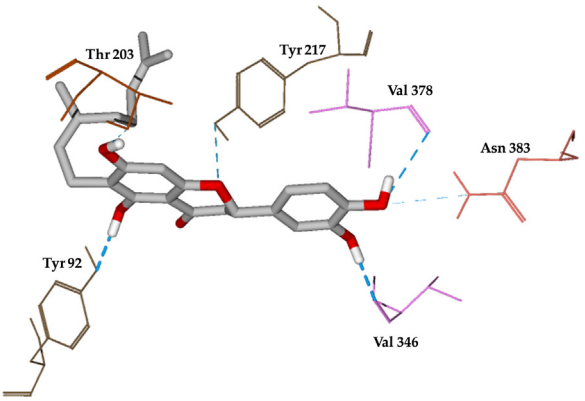


Fig. 5. The lowest energy pose of diplacone (gray and white) and LmajNMT. The blue dash line shows the predicted hydrogen bonding interactions with residues Val 378 and 346, Asn 383, Tyr 217 and 92, as well as Thr 203 of LmajNMT. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

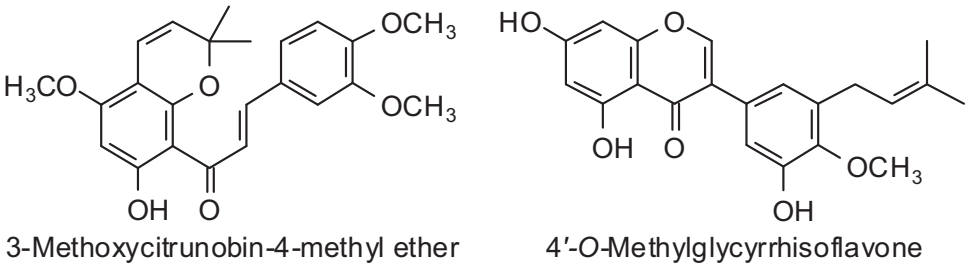


Fig. 6. Phenolic compounds that showed significant docking preference for *Leishmania major* oligopeptidase B.

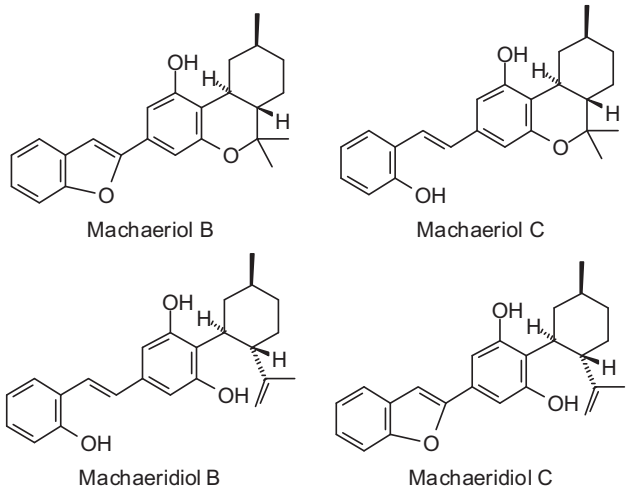


Fig. 7. Antiparasitic stilbenoids with preferential docking to *Leishmania major* phosphodiesterase B1.

3.9. Leishmania pteridine reductase 1

Pteridine reductase catalyzes the reduction of pterins/folates such as biopterin to tetrahydrobiopterin in *Leishmania*. *Leishmania* as well as trypanosomes are able to overcome dihydrofolate reductase (DHFR) inhibition by overexpressing pteridine reductase 1. Thus, simultaneous inhibition of DHFR and PTR1 could provide a means to develop an effective antileishmanial therapy [99]. Numerous coumarins, flavonoids, isoflavonoids, and lignans showed selective docking to *L. major* PTR1 with docking energies < −120 kJ/mol (see Supplementary Tables 4–7). However, four of these, *rel*-(7*R*,8*S*,7′*S*,8′*S*)-4,5,4′,5′-dimethylenedioxy-3,3′-dimethoxy-7,7′-epoxylignan (E_{dock} = −146.5 kJ/mol), 5-allyl-3-(4-allyl-2-methoxyphenoxymethyl)-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran (E_{dock} = −140.5 kJ/mol), 3′-*O*-methyldiplacone (E_{dock} = −142.2 kJ/mol), and cannflavin A (E_{dock} = −144.0 kJ/mol), have docking energies comparable to the co-crystallized ligand, methotrexate (E_{dock} = −149.8 kJ/mol) (see Figs. 8 and 9). The lignan *rel*-(7*R*,8*S*,7′*S*,8′*S*)-4,5,4′,5′-dimethylenedioxy-3,3′-dimethoxy-7,7′-epoxylignan demonstrated in vitro trypanocidal activity against epimastigotes of *T. cruzi* [100] while the neolignan 5-allyl-3-(4-allyl-2-methoxyphenoxymethyl)-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran showed antileishmanial activity against the promastigotes of *L. major* [101]. Both cannflavin A and 3′-*O*-methyldiplacone have shown in vitro activity against *L. donovani* and *T. brucei brucei* [90].

3.10. Leishmania methionyl-tRNA synthetase

Methionyl-tRNA synthetase (MetRS), one of the aminoacyl-tRNA synthetases [102], is an essential enzyme in protein translation; it catalyzes the aminoacylation of methionyl-tRNA. Shibata and co-workers have demonstrated the potential of MetRS as a drug target by the selective inhibition of *Trypanosoma brucei* methionyl-tRNA synthetase [103]. Larson and co-workers have also suggested that *L. major* methionyl-tRNA synthetase can also be explored for antileishmanial structure

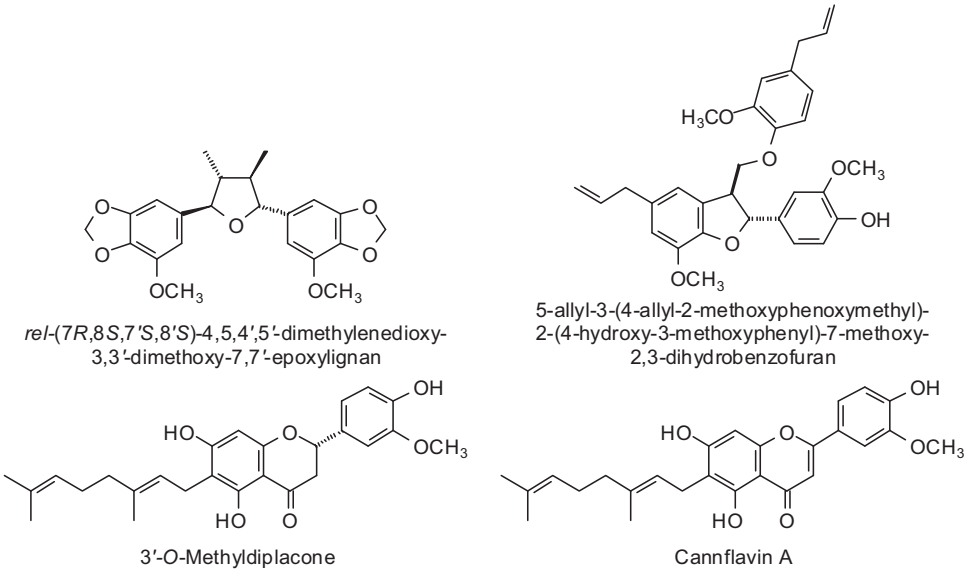


Fig. 8. Phenolic ligands with notably strong docking energies with *Leishmania major* pteridine reductase 1.

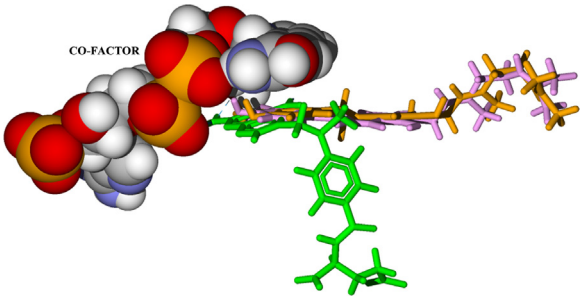


Fig. 9. The lowest energy poses of cannflavin A (lilac) and 3′-*O*-methyldiplacone (orange) with pteridine reductase (PDB 1e7w). The blue dash lines indicate hydrogen bonding interactions between the co-crystallized ligand methotrexate (green) and pteridine reductase's co-factor dihydro-nicotinamide-adenine-dinucleotide phosphate (NADPH) through the ribose ring and phosphate group of the co-factor. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

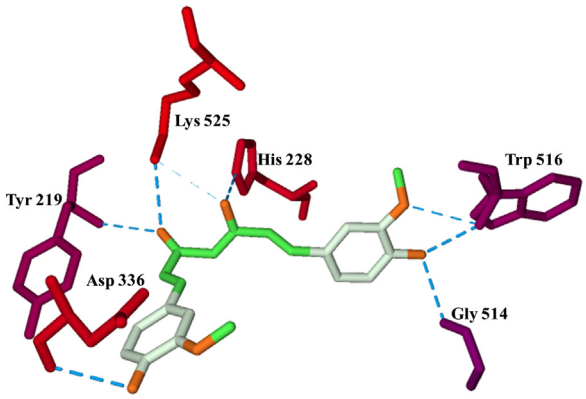


Fig. 11. The lowest energy pose of curcumin and LmajMetRS. Hydrogen bonding interactions is predicted between Tyr 219, Lys 525 and His 228 of LmMetRS and the β-ketol of the curcumin; also, the methoxyphenol group is predicted to have favorable interactions with Tyr 516 and Gly 514.

based design [36]. The ligands with the strongest docking energies to *L. major* MetRS were the flavonoid (2*S*,2″*S*)-7,7″-di-*O*-methyltetrahydroamentoflavone (E_{dock} = −172.5 kJ/mol), the chalcone rhuschalcone VI (E_{dock} = −177.2 kJ/mol), and the lignan pseudotsuganol (E_{dock} = −187.7 kJ/mol) (Fig. 10). These ligands had docking energies comparable to that of the co-crystallized ligand, methionyl-adenylate (E_{dock} = −175.7 kJ/mol), for this protein. Additionally, the three ligands showed selective docking for LmajMetRS. Unfortunately, all three violate Lipinski's rule (Supplementary Table 10) [32]. Curcumin (E_{dock} = −141.8 kJ/mol), 4,6-dibenzoyl-2-[phenylhydroxymethyl]-3(2*H*)-benzofuranone (E_{dock} = −162.4 kJ/mol), bipinnatone A (E_{dock} = −151.1 kJ/mol), 2-geranyl-3-hydroxy-8,9-methylenedioxypterocarpan (E_{dock} = −149.2 kJ/mol), and 3′-*O*-methyldiplacol (E_{dock} = −145.9 kJ/mol) (Fig. 10) all showed significantly selective docking to LmajMetRS, relatively strong docking energies, and no Lipinski's rule violations. Curcumin has shown in vitro antiparasitic activity against *L. donovani* promastigotes [104], but in an in vivo infection model, curcumin exacerbated leishmaniasis [105]. 3′-*O*-Methyldiplacol has demonstrated antiparasitic activity against both *L. donovani* and *T. b. brucei* [90]. 2-Geranyl-3-hydroxy-8,9-methylenedioxypterocarpan has shown antitrypanosomal activity (*T. cruzi* epimastigotes; IC_{50} = 12.2 μg/mL) [106]. Curcumin is predicted to have hydrogen bonding interactions with Tyr 219, Lys 525 and His 228 via its β-ketol group, while one of its methoxyphenol groups is predicted to have favorable interactions with Try 516 and Gly 514 (Fig. 11). Tyr 219, Trp 516 and Lys 525 have been identified as potentially important residues in the catalysis of LmajMetRS [36].

3.11. *Leishmania tyrosyl-tRNA synthetase*

Three ligands (Fig. 12), the lignan 3,3′,4,5-tetramethoxy-4′,5′-methylenedioxy-7,7′-epoxylignan (E_{dock} = −126.7 kJ/mol), the coumarin mammea B/BA cyclo F (E_{dock} = −121.8 kJ/mol), and the

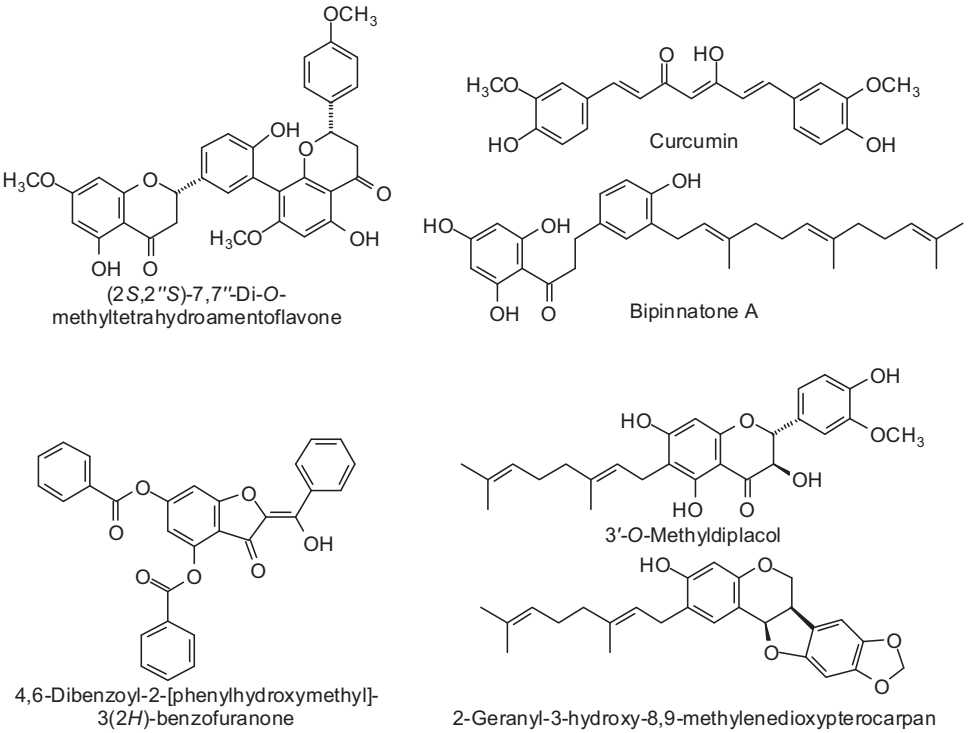


Fig. 10. Phenolic ligands with notably strong and/or selective docking to *Leishmania major* methionyl tRNA synthetase.

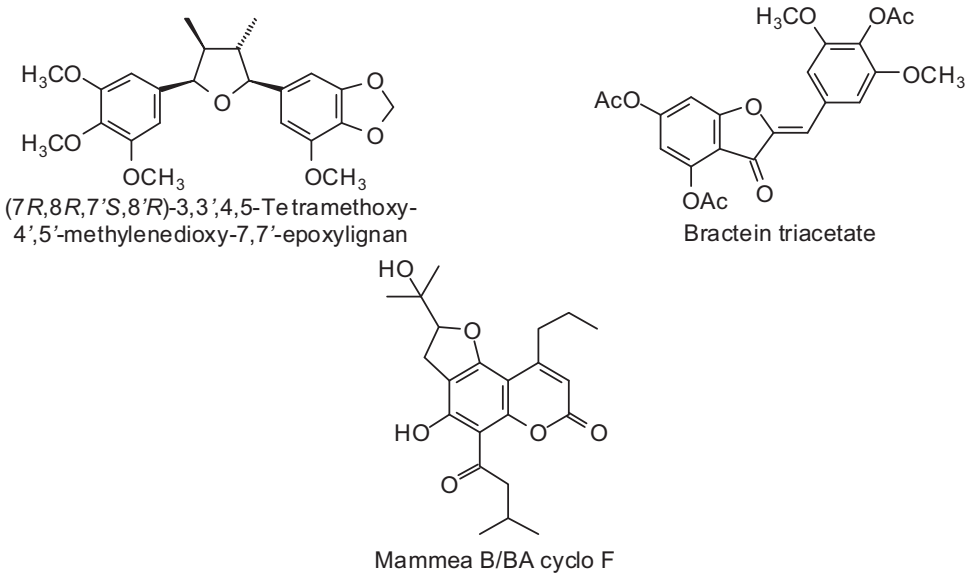


Fig. 12. Phenolic ligands with selective docking to *Leishmania major* tyrosyl tRNA synthetase.

aurone bractein triacetate (E_{dock} = −138.4 kJ/mol), exhibited selective docking to LmajTyrRS with docking energies lower than the co-crystallized flavonoid ligand, 3,7,3′,4′,tetrahydroxyflavone (E_{dock} = −90.3 kJ/mol). Bractein triacetate has shown notable antiplasmodial activity against the drug-resistant K1 strain of *P. falciparum* [107], while 3,3′,4,5-tetramethoxy-4′,5′-methylenedioxy-7,7′-epoxylignan was active against epimastigotes of *T. cruzi* [100].

3.12. Leishmania uridine diphosphate-glucose pyrophosphorylase

Leishmania major uridine diphosphate-glucose pyrophosphorylase (LmajUGPase) is involved in the de novo synthesis of UDP-glucose. UDP-glucose is important for the formation of glycocalyx in *Leishmania*. Inhibition of LmajUGPase could potentially prevent *Leishmania* glycocalyx formation, but a recent study has shown that UGPase-deficient mutants were only modestly affected [108]. The strongest-docking flavonoid ligands (amentoflavone, cissampeloflavone, and styracifolin B) all violate Lipinski's rule and dock less strongly than the enzyme substrate, uridine-5′-diphosphate-glucose (E_{dock} = −143.9 kJ/mol). Likewise, the chalcone rhuschalcone VI and the lignan pseudotsuganol dock strongly to LmajUGPase, but violate Lipinski's rule. The aurone 4,6-dibenzoyl-2-[phenylhydroxymethyl]-3(2*H*)-benzofuranone (see above) did dock with comparable docking energy (−142.0 kJ/mol) and does not violate Lipinski's rule.

3.13. Leishmania cyclophilin

Cyclophilins have peptidyl-prolyl *cis-trans* isomerase activity and are believed to play a role as regulators of protein folding. These proteins are secreted by protozoans such as *Trypanosoma* and *Leishmania* during host cell infection possibly to initiate signaling events in the host cells [109]. Because cyclosporin A binds to *Leishmania* cyclophilin and inhibits parasite proliferation, *Leishmania* cyclophilins have been identified as prime drug targets [110]. None of the flavonoid ligands showed preferential docking to *L. donovani* cyclophilin. 3′-*O*-Methyldiplacone, however, did dock relatively strongly (E_{dock} = −126.5 kJ/mol) and this ligand does not violate Lipinski's rule. Cannabigerolic acid (Fig. 13) was the only ligand that showed both preferential and relatively strong docking to LdonCyp (E_{dock} = −126.4 kJ/mol). Although this compound does have one Lipinski's rule violation (*C* Log *P* = 6.09), it has shown antileishmanial activity against *L. donovani* promastigotes [111].

3.14. Leishmania sterol 14α-demethylase (CYP51)

Sterol 14α-demethylase is a cytochrome P450 monooxygenase (CYP51) that catalyzes oxidative removal of the 14α-methyl group from sterols and is an essential enzyme in sterol biosynthesis. There is low sequence similarity between the human enzyme and those of *Leishmania*, which suggests that the parasite enzyme can be a viable drug target for selective inhibition [112]. We have shown in recent in silico studies that steroid-like chemotypes preferentially dock to *Trypanosoma* and *Leishmania* sterol 14α-demethylase [113,114]. Those studies support the suggestion by Hargrove and co-workers that modifications of the substrate analog 14α-methylenecyclopropyl-Δ^7-24,25-dihydrolanosterol (MCP) may provide excellent drug leads against protozoans [115]. Two quinone ligands, cochlioquinone A and isocochlioquinone A (Fig. 14) showed strong selective docking to *L. infantum* CYP51 with docking energies of −122.4 and −113.8 kJ/mol, respectively; stronger than the co-crystallized ligand for this protein (fluconazole, E_{dock} = −91.3 kJ/mol). The two quinone ligands do have one Lipinski's rule violation, however (MW = 532.67 amu). Cochlioquinone A and *epi*-cochlioquinone A have been reported to inhibit diacylglycerol acyltransferase [116] and lecithin cholesterol acyltransferase [117], respectively.

3.15. Leishmania nicotinamidase 1

Nicotinamidase (PNC1) is an essential enzyme for the production of NAD^+ in *Leishmania* and is important for parasite growth and establishment of infection. The enzyme is absent

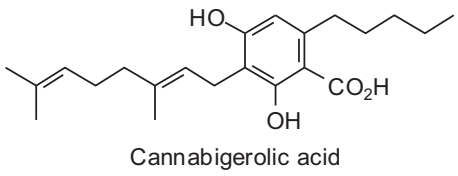


Fig. 13. Cannabigerolic acid, which showed notable docking to *Leishmania donovani* cyclophilin.

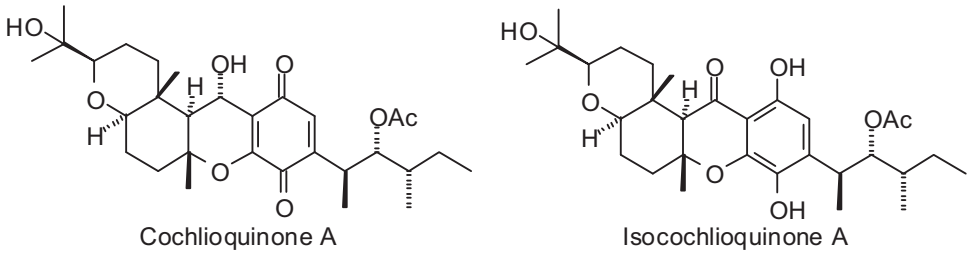


Fig. 14. The quinone ligands that dock strongly to *Leishmania infantum* CYP51.

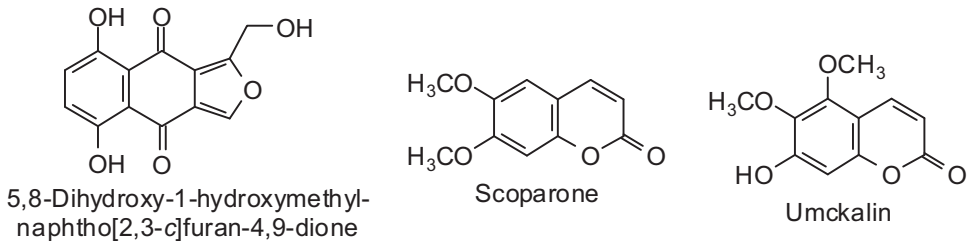


Fig. 15. Ligands showing selective docking to *Leishmania infantum* nicotinamidase.

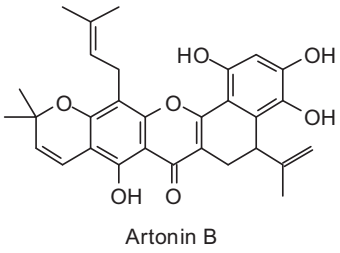


Fig. 16. Artonin B, a flavonoid ligand that selectively docked to *Leishmania infantum* trypanothione reductase.

from human cells and this makes it a potentially attractive chemotherapeutic target [66]. Only a few polyphenolic ligands had negative (exothermic) docking energies with *L. infantum* PNC1, and most of those that did docked only weakly and non-selectively. The antiplasmodial naphthoquinone 5,8-dihydroxy-1-hydroxymethylnaphtho[2,3-*c*]furan-4,9-dione (Fig. 15) [118], however, showed significantly selective docking to LinfPNC1 with a docking energy (E_{dock} = −98.1 kJ/mol) lower than the co-crystallized ligand, nicotinic acid (E_{dock} = −65.6 kJ/mol). Likewise, several lower-molecular-weight coumarins (see Supplementary Table 4), e.g., scoparone (E_{dock} = −91.1 kJ/mol) and umckalin

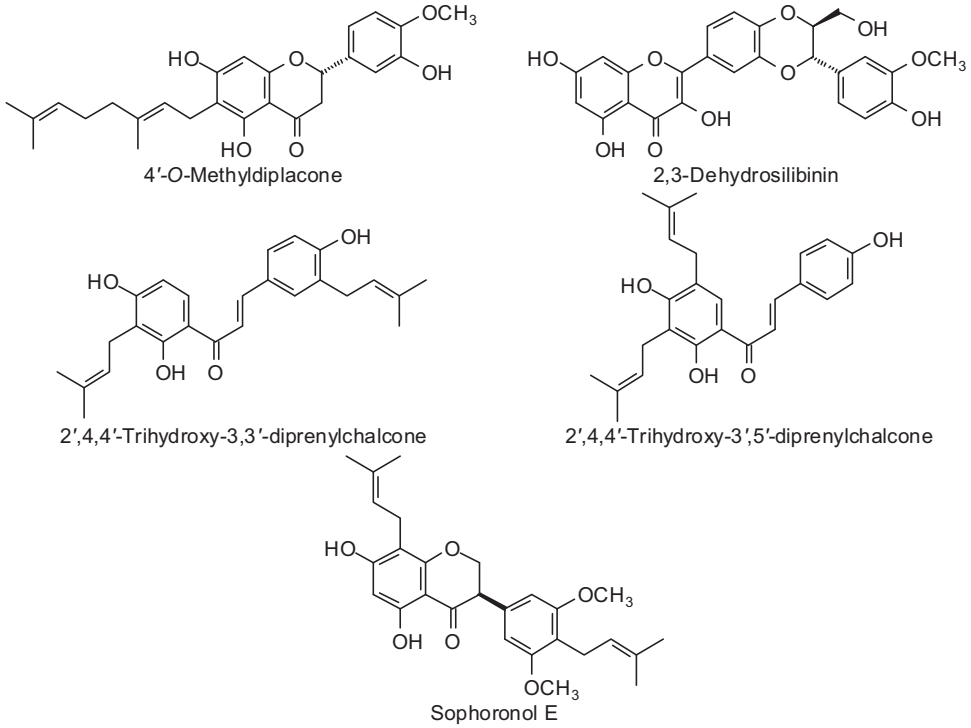


Fig. 17. Polyphenolic ligands that exhibited significantly selective docking to *Leishmania mexicana* glycerol-3-phosphate dehydrogenase.

(E_{dock} = −91.4 kJ/mol) (Fig. 15), showed docking selectivity for LinfPNC1.

3.16. Leishmania trypanothione reductase

Trypanothione reductase converts trypanothione disulfide to the reduced trypanothione dithiol and is the homolog of mammalian glutathione reductase, serving to maintain redox balance and antioxidant defenses [119]. The trypanothione system has been extensively investigated as a target of trypanocidal and leishmanicidal agents [120,121]. The flavonoid artonin B (Fig. 16) showed selective docking to *L. infantum* TR, but the docking energy (−127.4 kJ/mol) was not as strong as the substrate trypanothione (E_{dock} = −144.1 kJ/mol). The only ligand with stronger docking than trypanothione was rhuschalcone VI (E_{dock} = −154.6 kJ/mol), but this ligand violates Lipinski's rule.

3.17. Leishmania glycerol-3-phosphate dehydrogenase

In order to use glycerol as an energy source, it is believed that *Leishmania* utilize glycerol-3-phosphate dehydrogenase (GPDH) to oxidize glycerol-3-phosphate to dihydroxyacetone phosphate [122] and has been suggested to be a viable drug target [54]. A number of the phytochemical polyphenolic ligands showed significantly selective docking to *L. mexicana* GPDH (Fig. 17). These included the flavonoids 3′-*O*-methyldiplacone, 4′-*O*-methyldiplacone, and 2,3-dehydrosilibinin (E_{dock} = −148.3, −143.1, and −141.0 kJ/mol, respectively), the chalcones 2′,4,4′-trihydroxy-3,3′-diprenylchalcone, 2′,4,4′-trihydroxy-3′,5′-diprenylchalcone, bipinnatone A, and bipinnatone B, (E_{dock} = −150.6, −146.7, −151.1, and −152.5 kJ/mol), the isoflavonoid sophoronol E (E_{dock} = −141.8 kJ/mol), the lignan 5-allyl-3-(4-allyl-2-methoxyphenoxymethyl)-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydro-benzofuran (E_{dock} = −151.6 kJ/mol), and the cannabinoid 4-terpenyl cannabinolate (E_{dock} = −150.2 kJ/mol).

3.18. Leishmania pyruvate kinase

Pyruvate kinase (PYK) is one of the enzymes necessary for glycolysis and energy metabolism in *Leishmania* [123]. It is becoming increasingly clear that several classes of kinases are inhibited by compounds that possess flavonoid-like structural scaffolds. The flavonoid cissampeloflavone selectively docked into the ATP binding site of *L. mexicana* PYK with a docking energy (−157.6 kJ/mol) lower than ATP, the co-crystallized ligand, itself (E_{dock} = −145.3 kJ/mol). Cissampeloflavone does violate Lipinski's rules, however. Likewise, the xanthone garcilivin A also violates Lipinski's rules, but selectively docked to the ATP-binding site of LmexPYK (E_{dock} = −150.3 kJ/mol). The lignan kusokinin (Fig. 18) does not violate Lipinski's rule and this ligand showed selective docking to the ATP-binding site of LmexPYK with a weaker docking energy of −130.5 kJ/mol.

3.19. Molecular docking with other Leishmania protein targets

All of the polyphenolic ligands docked more strongly to *L. infantum* glyoxalase II (LinfGLO2) than the co-crystallized ligand (spermidine, E_{dock} = −55.9 kJ/mol), but none showed selective docking to the protein. None of the polyphenolic ligands showed selective docking to *L. infantum* thiol-dependent reductase 1 (LinfTDR1). None of the polyphenolic ligands showed selective docking to *L. mexicana* glyceraldehyde-3-phosphate dehydrogenase (LmexGAPDH). None of the polyphenolic ligands showed selective docking to *L. mexicana* phosphoglucose isomerase (LmexPGI). Sikokianin C (E_{dock} = −129.1 kJ/mol) showed selective docking to *L. mexicana* phosphomannomutase (LmexPMM) with docking energy comparable to the co-crystallized ligand (glucose-1,6-bisphosphate; E_{dock} = −121.3 kJ/mol), but the flavonoid ligand violates Lipinski's rule. None of the polyphenolic ligands showed selective docking to *L. mexicana* triosephosphate isomerase (LmexTIM). It is not likely that GLO2, TDR1, GAPDH, PGI, PMM, or TIM are important *Leishmania* protein targets for phytochemical polyphenolics.

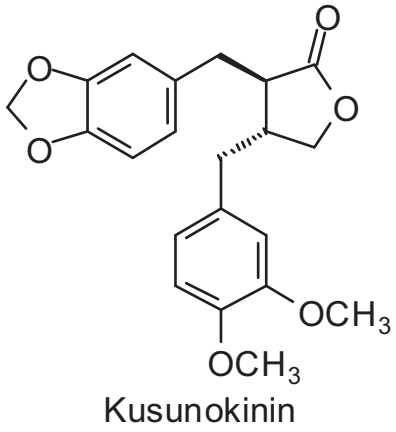


Fig. 18. The lignan kusinokinan, which selectively docked to *Leishmania mexicana* pyruvate kinase.

4. Conclusions

Based on in-silico analysis of antiparasitic phytochemical polyphenolics in this study, several compounds have shown promise as potential ligands affecting *Leishmania* protein targets and that also obey Lipinski's rule (Table 1). In particular, the aurones bractein triacetate and 4,6-dibenzoyl-2-[phenylhydroxymethyl]-3(2*H*)-benzofuranone, the chalcone crotaorixin; the coumarins mammea A/AA, mammea B/BA, mammea B/BA cyclo F, scoparone, and umckalin; the lignans aristolignin, 3,3′,4,5-tetramethoxy-4′,5′-methylenedioxy-7,7′-epoxylignan and 4,5,4′,5′-dimethylenedioxy-3,3′-dimethoxy-7,7′-epoxylignan; the stilbenoid machaeriol B; as well as flavonoids diplacone, quercetin-3′,4′,7-trimethyl ether, cannflavin A, 3′-*O*-methyldiplacone, 3′-*O*-methyldiplacol, 4′-*O*-methyldiplacone, and isoflavonoid sophoronol E, can be considered to be promising drug leads worthy of further investigation. Prenylation provides flexible hydrophobic linkages that complement the hydrogen-bonding hydroxyl and methyoxyl groups of polyphenolic ligands.

Acknowledgments

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Table 1
Summary of promising antiparasitic phytochemical polyphenolic drug leads.

Compound	Target(s)	E_{dock} (kJ/mol)	Lipinski's rule			
			HBD	HBA	MW	*C* Log *P*
3,3′,4,5-Tetramethoxy-4′,5′-methylenedioxy-7,7′-epoxylignan	LmajTyrRS	−126.7	0	7	416.464	3.9
4,5,4′,5′-Dimethylenedioxy-3,3′-dimethoxy-7,7′-epoxylignan	LmajPTR1	−146.5	0	7	400.422	3.93
2,3-Dehydrosilibinin	LmexGPDH	−141.0	5	10	480.42	0.87
3′-*O*-Methyldiplacol	LmajMetRS	−145.9	4	7	454.512	3.97
3′-*O*-Methyldiplacone	LmajPTR1	−142.2	3	6	438.513	4.62
	LdonCyp	−126.5	3	6	438.513	4.62
	LmexGPDH	−148. 3	3	6	438.513	4.62
3-Methoxycitrunobin-4-methyl ether	LmajOPB	−128.6	1	6	396.433	3.61
4,6-Dibenzoyl-2-[phenylhydroxymethyl]-3(2*H*)-benzofuranone	LmajMetRS	−162. 4	1	5	478.449	4.45
4′,6-Dihydroxy-2-[phenylmethylene]-3(2*H*)-benzofuranone	LmajUGPase	−142.0	2	4	254.237	1.79
4′-*O*-Methyldiplacone	LmexGPDH	−143.	3	6	438.513	4.62
4-Methoxy-2,2-dimethyl-6-(2-(2,4-dihydroxy)phenyl-*trans*-ethenyl)chromene	LmajNMT	−122.9	3	4	310.344	3.68
4′-*O*-Methylglycyrrhisoflavone	LmajOPB	−127. 5	3	6	368.38	3.21
4-Terpenylcannabinolate	LmexGPDH	−150.2	1	3	490.673	8.4
5,8-Dihydroxy-1-hydroxymethylnaphtho-[2,3-*c*]furan-4,9-dione	LinfPNC1	−98.1	3	6	260.199	−1.59
Aristolignin	LmajDHODH	−130.6	1	5	358.428	3.86
Bractein triacetate	LmajTyrRS	−138.4	0	7	456.399	1.07
Cannflavin A	LmajPTR1	−144.0	2	6	436.497	4.39
Crotaorixin	LmajDHODH	−133.2	3	5	354.396	4.06
Curcumin	LmajMetRS	−141.8	3	6	368.38	3.43
Diplacone	LmajNMT	−135.7	4	6	424.486	4.36
Kusunokinin	LmexPYK (ATP site)	−130.5	0	5	370.396	3.8
Machaeriol B	LmajPDEB1	−121.2	3	3	362.461	4.84
Mammea A-AA	LmajDHODH	−120. 4	1	4	406.478	2.14
Mammea B/BA	LmajDHODH	−121.7	2	4	372.461	1.79
Mammea B/BA cyclo F	LmajTyrRS	−121.8	2	5	388.46	0.28
Quercetin-7,3′,4′-trimethylether	LmajNMT	−120.5	2	7	344.315	0.72
Scoparone	LinfPNC1	−91.1	0	3	206.197	−1.45
Sophoronol E	LmexGPDH	−141.8	3	7	468.539	4.38
trans-4-Isopentenyl-3,5,2′,4′-tetrahydroxystilbene	LmajNMT	−127.4	4	4	312.36	4.26
Umckalin	LinfPNC1	−91.4	1	4	222.194	1.36

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