

Molecular Docking and in Vitro Antileishmanial Evaluation of Chromene-2-thione Analogues

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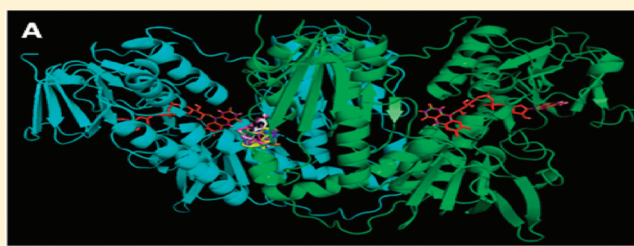
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S Supporting Information

ABSTRACT: Leishmaniasis are an epidemic in various countries, and the parasite is developing resistance against available drugs. Thus, development of new drugs against *Leishmania* is an open area of investigation for synthetic organic chemists. To meet this challenge, a series of chromene-2-thione derivatives have been synthesized and docked into the active site of trypanothione reductase (TryR) enzyme required for redox balance of the parasite. These were screened on promastigote, axenic amastigote, and intracellular amastigote stages of *Leishmania donovani* and found to show high levels of antileishmanial activity together with minimal toxicity to human peripheral blood mononuclear cells. Compounds **3b** and **3k** were found to be the most active among the tested compounds. Although the compounds show moderate antileishmanial activity, they identify a chemical space to design and develop drugs based on these chromene-2-thione derivatives against the *Leishmania* parasite.

KEYWORDS: chromene-2-thione, visceral leishmaniasis, molecular docking, trypanothione reductase, Lipinsky rule



The Leishmaniasis are a spectrum of neglected parasitic diseases caused by different species of the genus *Leishmania* protozoan, which is transmitted to humans by the bite of female phlebotomine sand fly. In the human body, the parasites survive and multiply within phagolysosomes of macrophages as intracellular amastigotes. Leishmaniasis is endemic in 88 countries, and there are 500 000 new cases of its visceral form each year, leading to 50 000 deaths.¹

It is mainly manifested in three forms viz. visceral leishmaniasis (VL), also known as kala-azar caused by *Leishmania donovani*; cutaneous leishmaniasis (CL) caused by *Leishmania major*, *L. donovani*, *Leishmania tropica*, and *Leishmania aethiopica*; and muco-cutaneous leishmaniasis (MCL) caused by *Leishmania braziliensis*. Among all leishmaniasis, the visceral form is the most severe.² In the absence of proper medication, it has a mortality rate of almost 100% independent of the immunological status of the patient.^{3–5}

At the beginning of the VL epidemic in India in early 1970s, sodium stibogluconate (SSG) was the most effective antileishmanial drug; however, over the years, its efficacy declined, and currently, more than two-thirds of VL patients in the Bihar state of India do not respond to SSG.⁶ Furthermore, the long course of treatment of pentavalent antimony (Sb^V) often causes side effects such as myalgia, pancreatitis, cardiac arrhythmia, and hepatitis, leading to the reduction or cessation of treatment. Presently, amphotericin B and the oral anticancer drug

miltefosine are considered the best therapeutic solutions for the treatment of VL.⁷ Considering the high toxicity and incomplete efficacy of the current clinical drugs, the search for new targets showing antileishmanial activity is very much desirable and would be of great relevance to both synthetic and medicinal chemists.

Coumarins (2*H*-chromene-2-ones) are among the best known oxygenated heterocycles and are present as a structural motif in numerous natural products. Interest in their chemistry continues unabated because of the broad range of biological activities displayed by this class of compounds.^{8–11} There are several examples of coumarin derivatives isolated from various families of plants, which have been tested and found to be effective against the promastigote form of *Leishmania* parasite within a range of 17–50 $\mu\text{g}/\text{mL}$ for IC₅₀ determination.^{12–15} Along with these naturally occurring coumarins, some synthetic coumarins such as 4-(3, 4-dimethoxyphenyl)-6,7-dimethoxy-2*H*-chromene-2-one have been shown to exhibit potent activity against *L. donovani* amastigotes (IC₅₀, 1.1 μM).¹⁵

We have selected our title compounds 2*H*-chromene-2-thione and benzo[*f*]chromene-2-thione for development and assessment against *L. donovani*. The compounds have already

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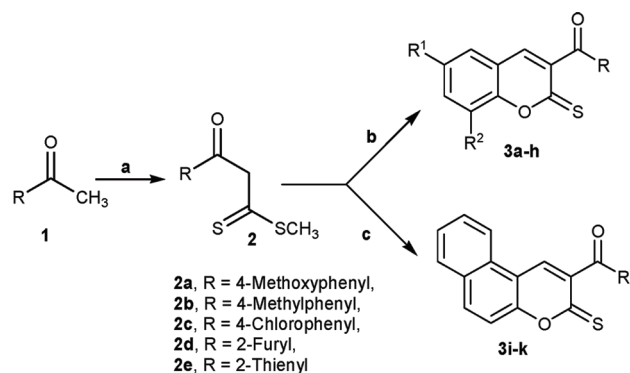
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been reported to exhibit profound antioxidant activities¹⁶ but have not previously been tested against any *Leishmania* species.

Initially, the reaction of *S,S*-dimethyl trithiocarbonate with substituted acetophenone **1** in the presence of NaH afforded the β -oxodithioesters **2** in good yields (Scheme 1). Typical synthetic strategies to obtain the title compounds in excellent yields have been depicted in Scheme 1.

Scheme 1. Typical Synthetic Strategy for the Synthesis of 2*H*-Chromene-2-thione and Benzo[*f*]chromene-2-thione



- a) *S,S*-dimethyltrithiocarbonate, NaH, DMF : Hexane (1:4), stir at RT, 2-4 h
 b) Salicylaldehydes, urea, InCl₃ (20 mol%), solvent-free, 100 °C, 2-4 h
 c) 2-Hydroxy-1-naphthaldehyde, urea, InCl₃ (20 mol%), solvent-free, 100 °C, 2-4 h

The β -oxodithioesters **2** were subjected to cyclocondensation with various 2-hydroxy aromatic aldehydes in the presence of InCl₃ and urea to give chromene-2-thione in excellent yields (Scheme 1 and Figure 1).¹⁷

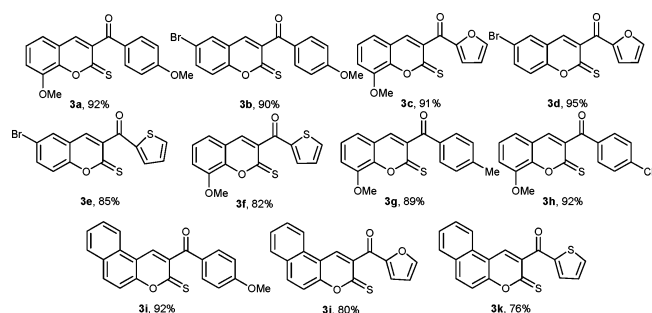


Figure 1. Various synthesized potential conjugates against *L. donovani*.

Trypanothione reductase (TryR) is a key drug target enzyme involved in the redox metabolism of the parasite, and inhibition of TryR may disrupt the redox balance of the parasite leading to parasite death.^{18–21} Thus, organic molecules may be designed, which can bind the active site of TryR leading to its inhibition.

AutoDock 4.2 was used to perform molecular docking of small molecules on macromolecular protein by treating the ligand as conformationally flexible. Autodock 4.2 also has a free-energy scoring function, which uses an AMBER force field to estimate the free energy of binding of a ligand to its target. Our *in silico* analysis suggests TryR as a possible target of these compounds. These compounds show satisfactory docking with acceptable statistics near the active site of TryR.²² Detailed results of analysis (free energy of binding values for each ligand along with the description of interacting residues) are presented in Table 1.

The enzyme (TryR) is a dimer consisting of two active sites. Active sites are formed by the interaction of both chains. The following is the description of residues around one of the active sites.

The residues involved in bonding and nonbonding interaction with ligands are as follows: Val-58, Lys-61, Leu-62, and Thr-65 from the B chain and Phe-396, Thr-397, Pro-398, Leu-399, Met-400, His-461, Pro-462, Thr-463, and Ser-464 from the A chain. The residues involved in hydrogen bonding with the ligands are as follows: Lys-61 from the B chain and Leu-399, Met-400, and Ser-464 from the A chain. These residues are found around the active sites Cys-52 and Cys-57 of the B chain. As most of the ligands are surrounded by the above residues, it can be concluded that these residues are highly conserved and play important functional roles. Thus, interaction of the ligands with these residues might be changing the active site structure, which leads to inhibition of the enzyme. Also, these ligands show low free energy of binding against TryR; thus, they bind with high affinity. All of the ligands mostly interact with residues of the A chain and very few residues of the B chain. The interaction of ligands with the enzyme is represented in Figure 2A. The figure shows that the lead molecules dock at a position near the active site of TryR, where the cofactor FAD (shown in red color) is bound. Ligand **3i** shows the least free energy of binding followed by **3k**, **3g**, **3j**, **3h**, **3b**, **3a**, **3d**, **3e**, **3f**, and **3c** in that order.

The protein contact potential was generated for all of the docked conformations (Figure 2B). This depicted the whole surface of the protein. Red and blue colors show negative and positive charges, respectively. In this depiction, all of the ligands were shown to be bound inside a cavity formed at the interface of chain A and chain B, that is, the dimeric interface of the enzyme. Although the docked conformation was different for each ligand, the ligands are positioned at almost the same place, thus showing a consensus pattern of interactions with the residues near the active site. Figures showing interactions of each ligand with the protein are submitted as Supporting Information. It can be predicted that the inhibition modes of all of these ligands could be similar, because of their interactions with almost the same residues.

Our initial testing of these compounds against promastigote and amastigote stages of *L. donovani* showed that the compounds are active in IC₅₀ range of 37–927 μ M against axenic amastigotes, while IC₅₀ was considerably higher (97–4634 μ M) against promastigotes (Table 2). The mean of the IC₅₀ values was found to be approximately five times lower against amastigotes than promastigotes, demonstrating the higher sensitivity of amastigotes to these compounds as compared to promastigotes.

Depending upon the IC₅₀ values of amastigotes and promastigotes, we selected some derivatives for intracellular macrophage susceptibility assay. The intracellular macrophage susceptibility assay of compounds **3b** [6-bromo-3-(4-methoxybenzoyl)-2*H*-chromene-2-thione], **3c** [3-(furan-2-oyl)-8-methoxy-2*H*-chromene-2-thione], **3h** [3-(4-chlorobenzoyl)-8-methoxy-2*H*-chromene-2-thione], and **3k** [3-(thien-2-oyl)-benzo[*f*]2*H*-chromene-2-thione] showed higher potency with IC₅₀ values of 17, 26, 24, and 22 μ M, respectively (Table 2).

The cytotoxicity of all of the synthesized compounds was determined against PBMC (peripheral blood mononuclear cells) at two concentrations (IC₅₀ and twice that of IC₅₀). None of the compounds showed more than 40% killing at a concentration twice that of the IC₅₀ (Table 2). There is

Table 1. Docking Statistics of the Compounds with TryR and Their Interaction with the Protein

entry	binding energy	H-bond	possible hydrophobic interactions
3a	-7.78	2JK6:Met-400(A):NH	Pro-398, Phe-396, Glu-467, Glu-466, Thr-463 from A chain; Leu-62, Lys-61 from B chain
3b	-8.09		Val-58, Lys-61, Leu-62, Thr-65 from B chain; Phe-396, Thr-397, Pro-398, Leu-399, Met-400, His-461 and Pro-462 from A chain
3c	-6.82		Val-58, Lys-61, Leu-62, Thr-65 from B chain; Thr-463, Pro-398, Phe-396, Leu-399, Met-400 and Pro-462 from A chain
3d	-7.63	2JK6:Lys-61(B):HZ2	Val-58, Lys-61, Leu-62 from B chain; Phe-396, Pro-398, Leu-399, Thr-463 and Ser-464 from A chain
3e	-7.54		Val-58, Lys-61, Leu-62, Thr-65 from B chain; Phe-396, Pro-398, Met-400, His-461 and Pro-462 from A chain
3f	-7.35		Lys-61, Leu-62, Thr-65, Phe-396, Pro-398, Met-400 and Pro-462
3g	-8.22	2JK6:Lys-61(B):HZ2	Leu-62, Thr-65 from B chain; Phe-396, Thr-397, Pro-398, Met-400, Pro-462 and Thr-463 from A chain
3h	-8.11		Val-58, Lys-61, Leu-62, Thr-65 from B chain; Phe-396, Pro-398, Met-400, Pro-462 and Thr-463 from A chain
3i	-9.20		Val-58, Lys-61, Leu-62, Thr-65 from B chain; Phe-396, Leu-399, Met-400, Thr-463, Glu-466 and Glu-467 from A chain
3j	-8.17	2JK6:Lys-61(B):HZ2 2JK6:Leu-399(A):HN	Phe-396, Pro-462, Thr-463 and Ser-464 from A chain
3k	-8.61	2JK6:Ser-464(A):SH and OH	Val-58, Lys-61, Leu-62 from B chain; Phe-396, Pro-398, Leu-399 and Met-400 from A chain

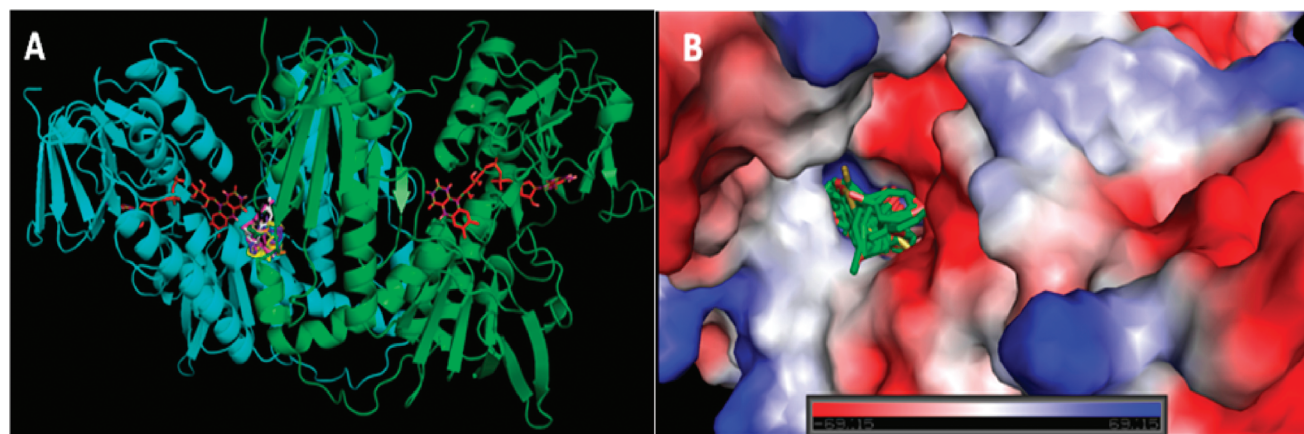


Figure 2. (A) Binding of the 11 ligands to the dimer TryR (PDB ID: 2JK6). (B) A vacuum electrostatics depiction of TryR (PDB ID: 2JK6) bound to all 11 ligands, showing protein contact potential.

Table 2. Antileishmanial Activity and Cytotoxicity of Chromene-2-thione Conjugates

entry	IC ₅₀ (μM)			cytotoxicity (%)	
	axenic amastigotes	promastigotes	intracellular amastigotes	at IC ₅₀	at 2 × IC ₅₀
3a	502	1398		16.63	14.82
3b	198	450	17	32.91	40.51
3c	65	1224	26	12.31	25.96
3d	524	1030		10.72	17.57
3e	83	4634		10.95	6.66
3f	927	4300		11.08	19.52
3g	446	744		9.40	33.14
3h	73	48	24	0.46	20.22
3i	221	335		15.11	26.96
3j	37	2774		12.03	27.79
3k	36	97	22	28.84	24.63
3l ^a	0.061	0.465		16.02	24.40

^aCompound 3l is amphotericin B.

apparently only a weak or no correlation between log IC₅₀ and binding energies. This may be due to differences in the bioavailability of these compounds.

Treatment options for VL are limited. A high level of parasite resistance has been developed for the available drug sodium

antimony gluconate. The single oral drug miltefosine available for VL has limitations of a long half-life and toxicity issues. Paromomycin also shows adverse events during treatment. The antifungal drug amphotericin B deoxycholate requires long hospitalization and adverse effects such as renal toxicity.²³ The lipid formulation of amphotericin B is the best of the available drug options for VL patients, but it is expensive. So, in the effort to develop an antileishmanial drug for the treatment of VL, we have tested these compounds against promastigote and amastigote stages of *L. donovani*, and the experimental observations showed that compounds 3b and 3k were found to be the most active. The increased potency of the compounds in the intracellular macrophage susceptibility assay leaves room for further exploration of the metabolism of the chemical compounds within the macrophage. Inhibition of TryR by compounds may lead to an imbalance in the parasite's redox potential, which weakens the parasite's defense against the oxidative pressure that they experience within the macrophage, and thus, an increased potency is obtained.

The ligands were also checked for compliance to the Lipinsky rule of five, and the results are summarized in Table 3. The rule states that a molecule likely to be developed as an orally active drug candidate should show no more than one violation of the following four criteria: It should not have more than five hydrogen bond donors, it should not have more than

Table 3. Molinspiration Calculation of Properties for the Lipinsky Rule^a

entry	nViol	natoms	miLog P	MW	nON	nOHNH	nrotb
	acceptable range→		<5	<500	<10	<5	
3a	0	23	3.805	326.37	4	0	4
3b	0	22	3.87	379.27	3	0	3
3c	0	20	2.627	290.34	4	0	3
3d	0	19	3.071	339.21	3	0	2
3e	0	19	3.713	355.27	2	0	2
3f	0	20	3.269	306.40	3	0	3
3g	0	22	3.818	314.40	3	0	3
3h	0	22	4.048	334.82	3	0	3
3i	0	25	4.956	346.40	3	0	3
3j	0	22	4.156	306.34	3	0	2
3k	0	22	4.798	322.41	2	0	2

^anViol, no. of violations; natoms, no. of atoms; miLog P, molinspiration predicted Log P; MW, molecular weight; nON, no. of hydrogen bond acceptors; nOHNH, no. of hydrogen bond donors; and nrotb, no. of rotatable bond.

10 hydrogen bond acceptors, it should not have molecular weight greater than 500 Da, and it should not have an octanol–water partition coefficient greater than 5. Molecular properties of all ligands were calculated by molinspiration, and it was found that no ligand showed any violation of the above criteria (Table 3). Therefore, these ligands have a good potential for eventual development as oral agents and can be potentially active drug candidates.

Within this manuscript, we present the efficient synthesis of a series of chromene-2-thiones, which show antileishmanial activity against *L. donovani*. Docking statistics and in vitro analysis against different forms of parasite suggested that compounds **3b** and **3k** were most active among the tested compounds. Although these compounds show a narrow therapeutic margin, there is chemical space to design and develop more selective and potent compounds. The viability of *Leishmania* in this work was determined by the MTT assay, which relies partially on mitochondrial functions. Recently, it was shown that chroman derivatives inhibit the mitochondrial bc1 complex, which influences the MTT reduction.²⁴ The possibility that mitochondrial bc1 complex of the parasite also be an alternative target cannot be ruled out. Ligand docking studies could provide the first evidence for binding of compound in different areas of TryR. Thus, chromene-2-thiones can be a possible lead for the development of novel drug against leishmania.

■ ASSOCIATED CONTENT

Supporting Information

Information on compound synthesis and characterization data, methods for molecular docking and results, and protocols and results for various analyses on *Leishmania* parasite. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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