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Structure–activity relationship of antileishmanials neolignan analogues

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Abstract—Twenty-two synthetic analogues of neolignans comprising β-ketoethers and β-ketosulfides were obtained from condensation reactions among β-bromoketones and phenols or thiophenols, respectively, in basic solutions, and assayed in vitro for activity against intracellular *Leishmania amazonensis* and *Leishmania donovani* amastigotes, the causative agents of cutaneous and visceral leishmaniasis. The highest selective activity was found for compounds with sulfur bridges, whereas β-ketosulphoxides and β-ketosulphones had significantly less growth inhibitory activity. Compounds 2-[(4-chlorophenyl)thio]propan-1-one and 1-(3,4-dimethoxy)-2-[(4-methylphenyl)thio]propan-1-one were the most potent, inhibiting the growth parasite species by over 90% at microgram/mL, but only compound 1-(3,4-dimethoxy)-2-[(4-methylphenyl)thio]propan-1-one was selectively toxic to the parasites.

1. Introduction

Leishmaniasis is a spectrum of diseases caused by intracellular protozoan parasites belonging to the genus *Leishmania*. Depending on the immune status of the host and the parasite species, the disease may manifest itself in specific or disseminated cutaneous areas (e.g *Leishmania amazonensis*), disfiguring mucocutaneous (*Leishmania braziliensis*) or fatal visceral leishmaniasis (e.g. *Leishmania donovani*).[1,2] The disease is endemic in 88 tropical and subtropical countries, where 350 million people are at risk. There is an estimate of 12 million already contaminated people, as well as an annual incidence of 2 million cases.[3]

So far no vaccine approved for human use is available.[4] Despite the recent advances in new antileishmanial compounds, the first-line therapy to all forms of leishmaniasis still requires multiple, potentially toxic and painful injections with pentavalent antimonials.[5] The problem is further aggravated by the surge of antimonial resistance in some areas where the disease is endemic.[6] Therefore, the development of new therapeutic agents is highly expected.[7–10]

In search of new drugs against leishmaniasis, potentially active natural products and their derivatives have been described, including chalcones present in *Glycirrhyza glabra* and *Piper aduncum*[11,12] and quercitrin present in *Kalanchoe pinnata*.[13] Neolignans form another potential class of new antileishmanials, which are normally found in Myristicaceae and other primitive plant families. They are usually dimers of the oxidative coupling of allyl and propenyl phenols[14,15] while a considerable number of biological properties, namely antifungal,[16] antitrypanosomal,[17] antibacterial,[18] anti-PAF,[19] antitumoral[20] and anti-schistosomal[21] activities have been ascribed to them.

We have previously described that a sulfur analogue of a natural neolignan from the *Virola surinamensis* (Rol.) Warb. was very active against *L. donovani* in vitro.[22] In the present work, we evaluate the antileishmanial activity of novel sulfur and oxygen synthetic analogues. Since the sensitivity of different parasite species to drugs

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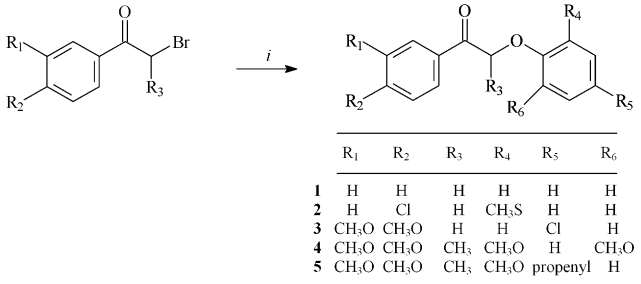
may vary considerably,[23–25] we proposed to evaluate the activity of the analogues against parasite species that cause cutaneous and visceral leishmaniasis.

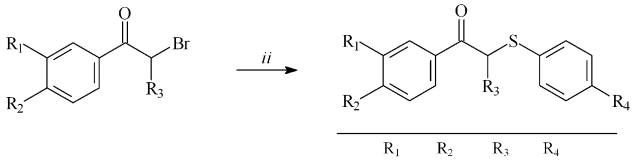
2. Results and discussion

In this work, 22 compounds (18 new) oxygenated or sulphurated neolignan analogues (Schemes 1 and 2) were synthesized, while the activities against both intracellular amastigotes of *L. amazonensis* and *L. donovani* were compared. Synthetic procedures for neolignans were adapted from our previous work[22] through the condensation reaction of α-bromoketones with a phenol or thiophenol derivative, leading to a keto-neolignan analogue (β-ketoethers or β-ketosulphides), compounds **1**–**15** (Scheme 1), in 72–91% yield. Compounds were purified by PTLC and purity done by GC–MS. For the determination of the structures, spectroscopic methods were used, such as: mass spectrometry, high resolution mass spectrometry, ^{1}H and ^{13}C nuclear magnetic resonance and infrared spectroscopy. ^{1}H NMR and IR data of the compounds **11**, **12**, **13** and **15** are in concordance with the literature.[22] ^{13}C NMR data of these compounds are described for the first time in this work.

Sulfur analogues presented greater activity when compared to oxygenated compounds of the same series.[22] Based on those previous results, we intended to associate the structure with biological activity (SAR), to find new active molecules. Several physicochemical properties were calculated for a group of synthetic substances for which the biological activities against *Leishmania* are known.[26] The methodology used was very helpful in providing information to the parasite target of the synthetic neolignans, but did not reveal the degree of activity on the entire cells which may vary according to cell membrane permeability or intracellular drug metabolism.



	R_1	R_2	R_3	R_4	R_5	R_6
1	H	H	H	H	H	H
2	H	Cl	H	CH_3S	H	H
3	CH_3O	CH_3O	H	H	Cl	H
4	CH_3O	CH_3O	CH_3	CH_3O	H	CH_3O
5	CH_3O	CH_3O	CH_3	CH_3O	propenyl	H



	R_1	R_2	R_3	R_4
6	H	H	H	Cl
7	H	Cl	H	Cl
8	H	H	CH_3	H
9	H	H	CH_3	CH_3
10	H	H	CH_3	NH_2
11	H	H	CH_3	Cl
12	CH_3O	CH_3O	CH_3	H
13	CH_3O	CH_3O	CH_3	CH_3
14	CH_3O	CH_3O	H	NH_2
15	CH_3O	CH_3O	CH_3	Cl

Scheme 1. Synthesis of β-ketoethers **1**–**5** and β-ketosulfides **6**–**15**. Reagents and conditions: (*i*) phenol derivatives, K_2CO_3, MeCOEt, Δ; (*ii*) thiophenolderivatives, K_2CO_3, MeCOEt, Δ.

We have previously shown that general compounds with a sulfur bond in carbon 8 have higher activity as compared to compounds bearing oxygen bond, as according to previous studies on *L. donovani*.[22] The synthesis of those analogues was conducted for substitutions in the C-8, whose bonding in the C-7 can be rigid or present free rotation. In the sulfur atom case, it forms bonds where the free capacity of rotation is greater than oxygen, even considering the size of the atom and electronegativity. This may explain the differential biological activity between sulfurated and oxygenated analogues **1**–**5**.

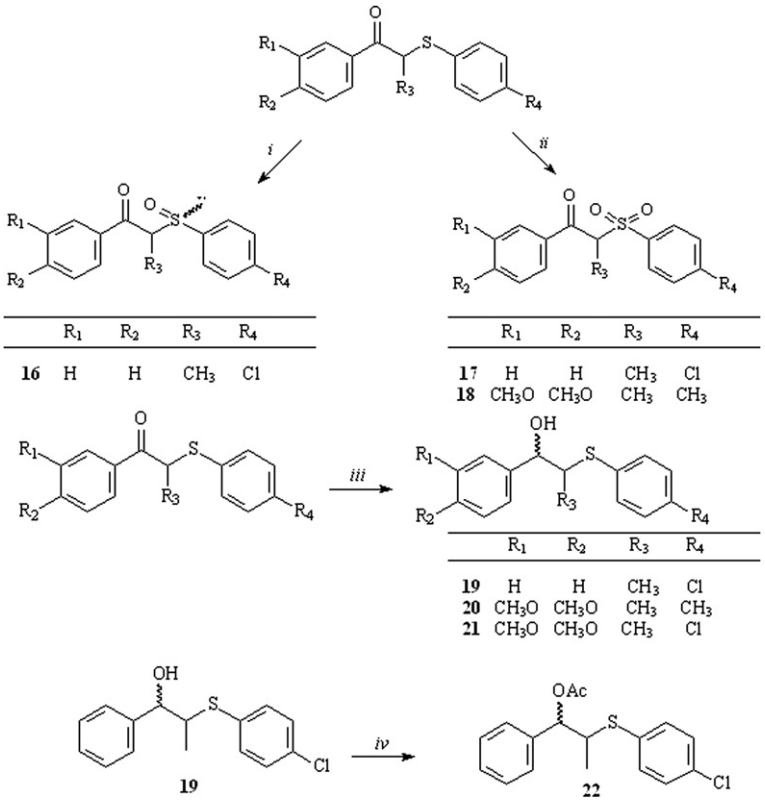
We found that, in general, *L. donovani* and *L. amazonensis* were differently sensitive to most of the synthetic neolignans, and that *L. amazonensis* was more susceptible than *L. donovani* to compounds **3**, **7**, **15**, **16**, **20** and **21**, while compounds **1**, **2**, **6**, **8**, **11**, **13** and **17**–**19** showed similar activity (Table 1). The reference drug meglumine antimoniate (Glucantime) was equally active against both species (26–28%). The differential drug sensitivity among *Leishmania* species may be due to differential metabolism or biosynthetic pathways.[23]

Table 1 also shows that compounds **3**, **16**–**18**, **20** and **22** did not inhibit the growth of *L. donovani*, that the compounds **1**, **4**–**8**, **15** and **19**–**21** were potentially more active than the control drug against *L. amazonensis*, and that sulfurated compounds **11** and **13** were the most active, inhibiting the growth of both parasites in 94.1–100% at 80 μg/mL.

Compound **11** has a group Cl in position 4′ (ring B), whereas compound **13** has CH_3 in position 4′ (ring B) and OCH_3 in positions 3 and 4 (ring A). Compounds **9**, **12** and **14** were extremely toxic to macrophages, probably due to the presence of the OCH_3group in positions 3 and 4 (ring A) (e.g. **12** and **14**) and NH_2 in position 4′ (e.g. **9**). The presence of chlorine in 4′ was associated with low toxicity to mammalian cells, as Cl-containing compounds **6**, **11**, **15**–**17**, **19** and **21** were very well tolerated by the macrophages. Interestingly, compound **15** containing both OCH_3 in positions 3 and 4 and Cl in position 4′ had significantly less activity against *L. donovani* (22% inhibition) although some activity was maintained against *L. amazonensis* (63% inhibition).

As an attempt to improve the permeation through macrophage and parasite membranes, the alcohols **19**–**21** and acetate **22** were synthesized. The ^{1}H NMR spectra of **19**–**21**, presented multiplets (δ 3.22, 3.17 and 3.22) in reference to the coupling between H-8 with H-7 and H-9.

The acetate **22** was evidenced through the absorption band in the IR spectrum at 1736 cm^{-1} corresponding to the stretch of the carbonyl esther group (O—C=O), typical of saturated aliphatic esthers. The ^{1}H NMR



Scheme 2. Synthesis of β-ketosulphoxide 16, β-ketosulphones **17** and **18**, alcohols **19**–**21**, and acetate **22**. Reagents and conditions: (*i*) *m*-CPA, anhydrous THF, RT,1 h; (*ii*) HOOAc, anhydrous THF, RT, 24 h; (*iii*) $NaBH_4$, MeOH/THF, RT, 30 min; (*iv*) Ac_2O, MeCOEt, pyridine Δ, 24 h.

spectrum of acetate **22** presented a singlet (δ 1.93) corresponding to the methyl protons of OAc group, and also a doublet in low field (δ 5.67) referent to H-7, adjacent to the OAc group, resulting from the coupling between H-7 and H-8. The ^{1}H NMR spectrum of the starting compound **19** presented a doublet (δ 4.32) referent to H-7, resulting from the coupling between H-7 and H-8.

We found that the replacement of ketone group in the position 7 by OH (e.g. **20** and **21**) or acetate (e.g. **22**) significantly decreased the activity against both *Leishmania* species, as compared to **13**, **15** and **19**, respectively. This is much more drastic when comparing **13**–**20**.

It is worth mentioning that in the same concentration compounds **11** and **13** were more active than Glucantime (meglumine antimoniate), which is the first-line drug for the treatment of all forms of leishmaniasis; nevertheless, **13** was toxic to macrophages.

3. Conclusions

The results shown here demonstrated that despite the lower toxicity of the oxygenated analogues of the natural neolignans surinamensin and virolin against mammalian cells,[14] they were much less active against leishmania parasites than the sulfurated analogues. The guided synthesis based on biological activity of sulfur analogues produced compounds with high antileishmanial activity. Seven (**5**, **7**, **8**, **11**, **13**, **15** and **19**) out of the 22 synthesized substances were active against at least one of the parasite species (growth inhibition $\geqslant$ 50% at 80 μg/mL). The most active compounds were **11** and **13**, which presented Cl or CH_3 in position 4′ of the ring B. The presence of Cl, but not CH_3, is crucial for the selective toxicity. The presence of the group OCH_3 as in **9** and/or the removal of the group Cl resulting in molecule **12** generated an extremely toxic substance for the macrophages. These results extend our previous observations on the selective and potent activity of sulfur neolignan analogues seen on *L. donovani*, the causative agent of visceral leishmaniasis to *L. amazonensis*, which causes disseminated cutaneous leishmaniasis, a very difficult disease to treat, in men. The wide action spectrum of compound **11**within *Leishmania* species suggests that it may act on a common parasite target, and may serve as a new lead compound for the treatment of all forms of leishmaniasis.

4. Experiment

4.1. General methods

^{1}H NMR (300 and 500 MHz) and ^{13}C NMR (75 and 125 MHz) spectra were recorded on either a Varian Gemini 300BB or Varian INOVA-500 spectrometer. Chemical shifts were reported in ppm from tetramethylsilane on the δ scale. IR spectra were recorded in chloroform solution and measured with a Bomen model MB series II spectrophotometer. HRMS were measured

Table 1. Activity of neolignan analogues against intracellular amastigotes of *L. amazonensis and L. donovani* (80μg/mL)

Compound	Toxicity to MØs	*L. donovani*	*L. amazonensis*
1	—	40.0 ± 0.0	44.0 ± 0.4
2	—	5.8 ± 0.5	8.8 ± 2.0
3	—	0 ± 0	17.7 ± 0.8
4	—	49.9 ± 0.8	37.9 ± 0.0
5	—	50.0 ± 0.5	34.2 ± 0.3
6	—	37.2 ± 0.4	38.8 ± 0.0
7	—	0.0 ± 0.4	50.5 ± 2.5
8	—	70.2 ± 2.5	78.1 ± 0.4
9	+++	nd	nd
10	—	28.8 ± 2.1	0 ± 0.6
11	—	94.1 ± 3.0	100 ± 0
12	+++	nd	nd
13	++	100 ± 0	100 ± 0
14	+++	nd	nd
15	—	22.0 ± 0	63.3 ± 0
16	—	0 ± 0	20.7 ± 0.5
17	—	0 ± 0	0.0 ± 0.2
18	—	0 ± 0	7.0 ± 0.3
19	—	45.2 ± 3.8	50.6 ± 2.0
20	—	0 ± 0.6	40.6 ± 0.7
21	—	0 ± 0.9	36.1 ± 0.6
22	—	0 ± 0	0 ± 0
Meglumine antimoniate*	—	26.0 ± 0.0	28.2 ± 0.5

All compounds were tested in quadruplicate at 80 μg/mL. The results are expressed as inhibition of parasite growth (100 minus % controls cultured in medium alone). Controls were always ⩾ 1500 amastigotes per 100 total macrophages. Means ± SD. nd, not determined. *Glucantime, control antileishmanial drug. Toxicity to macrophages were evaluated under light microscopy:

Key:

—, more than 80% macrophages (MØs) healthy and spreaded.

++, more than 80% macrophages rounded up.

+++, more than 80% macrophages disrupted or detached.

using a direct inlet system VG AUTO SPEC spectrometer. Electrothermal melting point apparatus are uncorrected. Preparative thin-layer chromatography (PTLC) used silica gel plates (Merck, 60 PF-254).

4.2. General procedure for β-ketoethers and β-ketosulphides (1–15)

A solution of 1.03 equivalent of phenol or thiophenol derivatives and 1.80 equivalents of anhydrous K_2CO_3 in anhydrous ethyl methyl ketone (4.5 mL of solvent/mmol of phenol or thiophenol derivative) was stirred for 10 min at room temperature. After this period, a solution of α-bromoketone in anhydrous ethyl methyl ketone (1.5 mL of solvent/mmol of ketone) was added dropwise and the mixture was stirred and refluxed for 3–6 h. The solution was cooled at room temperature, filtered, and the residue washed with CH_2Cl_2. The solution was concentrated in vacuum (to eliminate the ethyl methyl ketone), diluted with H_2O, and extracted thoroughly with CH_2Cl_2 (4×). Organic extracts were combined, washed with water, 5% $NaHCO_3$ solution, NaCl saturated solution, dried over Na_2SO_4 and then filtered and concentrated in vacuum. The β-ketoethers and β-ketosulphides were purified by preparative thin-layer chromatography (PTLC) or crystallization with MeOH, affording a 72–91% yield.

4.2.1. 2-Phenoxy-1-phenylethanone (1). Yield: 75%; colourless crystals; mp 72–74 °C; IR ($CHCl_3$): 3058, 2899, 1706 (C=O), 1600, 1301 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 5.29 (s, 2H, H-8), 6.79–8.19 (m, 10H, H-Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ 70.7, 114.7, 121.6, 128.1, 128.8, 129.5, 133.8, 134.5, 157.9, 194.5. MS *m/z* (+EI): 212 (M^+, 26), 105 (100), 91 (5), 77 (35); HRMS (+EI) $(M)^+$; found: 212.08369; $(M)^+$ calcd for $C_{14}H_{12}O_2$ 212.08373.

4.2.2. 1-(4-Chlorophenyl)-2-[2-(methylthio)phenoxy]ethanone (2). Yield: 75%; colourless crystals; mp 104–106 °C; IR ($CHCl_3$): 3063, 2920, 1702 (C=O), 1589, 1475, 1445, 1400, 1280 cm^{-1}; ^1H NMR (300 MHz , $CDCl_3$): δ 2.44 (s, 3H, H-2′), 5.25 (s, 2H, H-8), 6.75–8.02 (m, 8H, H-Ar). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.7, 111.8, 122.4, 125.8, 126.5, 127.9, 129.1, 129.9, 132.8, 140.3, 154.6, 193.6. MS *m/z* (+EI): 292 (M^+,82), 153 (85), 139 (100) 111 (20), 77 (7); HRMS (+EI) $(M)^+$; found: 292.03287; $(M)^+$ calcd for $C_{15}H_{13}ClSO_2$ 292.03259.

4.2.3. 2-(4-Chlorophenoxy)-1-(3,4-dimethoxyphenyl)ethanone (3). Yield: 78%; colourless crystals; mp 94–97 °C; IR ($CHCl_3$): 3015, 2907, 2835, 1686 (C=O), 1594, 1516, 1262, 1152 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 3.92 (s, 3H, CH_3O), 3.95 (s, 3H, CH_3O), 5.20 (s, 2H, H-8), 6.75–7.67 (m, 7H, H-Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ 55.9, 70.7, 110.1, 110.2, 116.0, 122.6, 126.4, 127.5, 129.3, 149.2, 154.0, 156.6, 192.6. MS *m/z* (+EI): 306 (M^+, 10), 165 (100), 151 (43); HRMS (+EI) $(M)^+$; found: 306.06599; $(M)^+$ calcd for $C_{16}H_{15}ClO_4$ 306.06589.

4.2.4. 2-(2,6-Dimethoxyphenoxy)-1-(3,4-dimethoxyphenyl)propan-1-one (4). Yield: 87%; colourless crystals; mp 93–95 °C; IR ($CHCl_3$): 2939, 2839, 1673 (C=O), 1593, 1514, 1480, 1421 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.87 (d, 3H, J = 6.84 Hz, H-8), 4.03 (s, 6H, $2CH_3O$), 4.22 (s, 3H, CH_3O), 4.24 (s, 3H, CH_3O), 5.60 (q, 1H, J = 6.84 Hz, H-8), 6.75–7.67 (m, 6H, H-Ar); ^{13}C NMR (125 MHz, $CDCl_3$): δ 18.2, 55.7, 55.8, 55.9, 80.7,105.2, 109.8, 111.5, 123.7, 124.0, 128.3, 136.0, 148.8, 153.1, 153.4, 197.4. MS *m/z* (+EI): 346 (M^+, 39), 165 (100), 181 (57), 143 (2), 77 (8); HRMS (+EI) $(M)^+$; found: 346.14157; (M^+) calcd for $C_{19}H_{22}O_6$346.14164.

4.2.5. 1-(3,4-Dimethoxyphenyl)-2-{2-methoxy-4-[(1*E*)-prop-1-en-1-yl]phenoxy}propan-1-one (5). Yield: 81%; colourless crystals; mp 97-99 °C; IR ($CHCl_3$): 2933, 2840, 1683 (C=O), 1594, 1511, 1465, 1511 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.72 (d, 3H, J = 6.85 Hz, H-9), 1.84 (dd; 3H, J = 6.52 Hz and J = 1.56 Hz, H-9′), 3.86 (s, 3H, CH_3O), 3.92 (s, 3H, CH_3O), 3.94 (s, 3H, CH_3O), 5.41 (q, 1H, J = 6.85 Hz, H-8), 6.08 (dq, 1H, J = 15.69 Hz and J = 6.55 Hz, H-8′), 6.19 (dd, 1H, J = 15.72 Hz and J = 1.54 Hz, H-7′), 6.75–7.67 (m, 6H, H-Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ 18.6, 19.5, 56.0, 56.1, 56.3, 109.7, 110.3, 111.5, 116.0, 118.8, 123.9, 124.6, 127.5, 130.7, 132.7, 146.2, 149.2, 153.8, 197.9. MS *m/z* (+EI): 356 (M^+, 53), 191 (2), 165 (100), 77 (2); HRMS (+EI) $(M)^+$; found: 356.16247; (M^+) calcd for $C_{21}H_{24}O_5$ 356.16237.

4.2.6. 2-[(4-Chlorophenyl)thio]-1-phenylethanone (6). Yield: 79%; colourless crystals; mp 74-76 °C; IR ($CHCl_3$): 2944, 2900, 1685 (C=O), 1474, 1389 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 4, 25 (s, 2H, H-8), 7.24–7.99 (m, 8H, H-Ar). ^{13}C NMR (75 MHz, $CDCl_3$): δ 41.2, 129.2, 129.5, 129.9, 130.3, 130.9, 132.4, 132.9, 133.6, 133.8, 140.3, 193.0. MS *m/z* (+EI): 262 (M^+, 17), 157 (2), 143 (2), 105 (100), 77 (43), 51 (11); HRMS (+EI) $(M)^+$; found: 262.02205; (M^+) calcd for $C_{14}H_{11}ClSO$ 262.02191.

4.2.7. 1-(4-Chlorophenyl)-2-[(4-chlorophenyl)thio]ethanone (7). Yield: 88%; colourless crystals; mp 118–119 °C; IR ($CHCl_3$): 2922, 1672 (C=O), 1587, 1389 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 4.20 (s, 2H, CH_2-8), 7.20–7.93 (m, 8H, H-Ar). ^{13}C NMR (75 MHz, $CDCl_3$): δ 40.9, 129.2, 129.5, 129.9, 130.3, 130.9, 132.4, 132.9, 133.6, 133.8, 140.3, 193.0. MS *m/z* (+EI): 296 (M^+, 20), 157 (12), 139 (100), 111 (40), 75 (25); HRMS (+EI) $(M)^+$; found: 295.98296; (M^+) calcd for $C_{14}H_{10}Cl_2SO$ 295.98294.

4.2.8. 1-Phenyl-2-(phenylthio)propan-1-one (8). Yield: 82%; yellow oil; IR ($CHCl_3$): 3059, 2974, 2928, 1680 (C=O), 1596, 1372 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.54 (d, 3H, J = 6.86 Hz, H-9), 4.63 (q, 1H, J = 6.86 Hz, H-8), 7.20–8.00 (10H, m, H-Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.0, 46.1, 127.4, 128.4, 128.6, 128.8, 128.9, 129.0, 129.9, 130.1, 130.3, 131.7, 133.0, 134.6, 135.6, 196.3. MS *m/z* (+EI): 242 (M^+, 34), 137 (100), 105 (57), 77 (52), 65 (10), 51 (20); HRMS (+EI) (M^+); found: 242.07653; (M^+) calcd for $C_{15}H_{14}SO$ 242.07654.

4.2.9. 2-[(4-Methylphenyl)thio]-1-phenylpropan-1-one (9). Yield: 75%; yellow oil; IR ($CHCl_3$): 3061, 3030, 2972, 2925, 1679 (C=O), 1595, 1330 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.55 (d, 3H, J = 6.83 Hz, H-9), 2.38 (s, 3H, H-4′), 4.61 (q, 1H, J = 6.83 Hz, H-8), 7.13–8.03 (9H, m, H-Ar). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.8, 46.0, 128.6, 128.6, 129.1, 129.9, 133.2, 135.1, 135.5, 136.1, 195.9. MS *m/z* (+EI): 256 (M^+, 32), 151 (100), 136 (6), 123 (19), 105 (32), 77 (32), 65 (3), 51 (8); HRMS (+EI) (M^+); found: 256.09229; (M^+) calcd for $C_{16}H_{16}SO$ 256.09219.

4.2.10. 2-[(4-Aminophenyl)thio]-1-phenylpropan-1-one (10). Yield: 72%; dark orange oil; IR ($CHCl_3$): 3853 (N—H), 3750 (N—H), 3682 (N—H), 3113, 1673 (C=O), 1595, 1371 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.46 (d, 3H, J = 6.79 Hz, H-9), 3.82 (2H, bs, NH_2), 4.45 (q, 1H, J = 6.84 Hz, H-8), 6.50–7.99 (m, 9H, H-Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.2, 46.1, 115.3, 128.7, 128.8, 128.8, 133.0, 136.2, 137.9, 147.9, 196.5, 200.7. MS *m/z* (+EI): 257 (M^+, 26), 152 (100), 124 (60), 105 (29), 93 (29), 77 (64), 66 (16), 59 (57), 51 (32); HRMS (+EI) (M^+); found: 257.08743; (M^+) calcd for $C_{15}H_{15}NOS$ 257.08695.

4.2.11. 2-[(4-Chlorophenyl)thio]-1-phenylpropan-1-one (11). Yield: 85%; colourless crystals; mp 71 °C; IR and ^1H NMR lit.[22] ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.8, 46.0, 128.2, 128.6, 128.6, 129.0, 129.1, 129.9, 133.2, 135.1, 135.5, 136.1, 195.9. HRMS (+EI) (M^+); found: 276.03747; (M^+) calcd for $C_{15}H_{13}ClSO$ 276.03756.

4.2.12. 1-(3,4-Dimethoxyphenyl)-2-(phenylthio)propan-1-one (12). Yield: 89%; colourless crystals; mp 61–62 °C; IR and ^1H NMR lit.[22] ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.4, 46.0, 55.9, 56.0, 109.9, 110.9, 123.0, 128.4, 128.6, 128.9, 134.1, 149.0, 153.3, 195.2. HRMS (+EI) (M^+); found: 302.09764; (M^+) calcd for $C_{17}H_{18}SO_3$ 302.09767.

4.2.13. 1-(3,4-Dimethoxyphenyl)-2-[(4-methylphenyl)thio]propan-1-one (13). Yield: 90%; colourless crystals; mp 76–77 °C; IR and ^1H NMR lit.[22] ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.1, 21.1, 45.9, 55.8, 56.0, 109.9, 110.9, 123.0, 128.2, 128.6, 129.6, 134.8, 138.7, 148.9, 153.1, 195.0. HRMS (+EI) (M^+); found: 316.11339; (M^+) calcd for $C_{18}H_{20}SO_3$ 316.11332.

4.2.14. 2-[(4-Aminophenyl)thio]-1-(3,4-dimethoxyphenyl)propan-1- one (14). Yield: 91%; dark orange oil; IR ($CHCl_3$): 3455 (N—H), 3371 (N—H), 3233 (N—H), 1654 (C=O), 1594, 1418 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.46 (d, 3H, J = 6.84 Hz, H-9), 3.80 (2H, bs, NH_2), 3.92 (s, 3H, CH_3O), 3.96 (s, 3H, CH_3O), 4.44 (q, 1H, J = 6.84 Hz, H-8), 6.44–7.73 (m, 7H, H-Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.8, 46.1, 55.9, 56.1, 109.9, 111.0, 115.3, 123.07, 125.7, 128.9, 133.9, 134.3, 137.5, 147.1, 148.9, 153.1, 195.1. MS *m/z* (+EI): 317 (M^+, 26), 207 (4), 194 (3), 165 (100), 152 (43), 137 (8), 124 (32), 107 (7), 94 (15), 80 (20), 69 (3), 59 (27), 44 (29).

4.2.15. 2-[(4-Chlorophenyl)thio]-1-(3,4-dimethoxyphenyl)propan-1-one (15). Yield: 87%; colourless crystals; mp 83–84 °C; IR and ^1H NMR lit.[22] ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.0, 45.5, 55.7, 56.0, 110.0, 110.9, 123.1, 128.5, 129.1, 134.9, 135.9, 149.2, 153.5, 195.0. HRMS (+EI) (M^+); found: 336.05871; (M^+) calcd for $C_{17}H_{17}ClSO_3$ 336.05869.

4.3. Procedure to obtain mixture β-ketosulphoxides (16)

To a solution of 100 mg (0.36 mmol) of 2-[(4-chlorophenyl)thio]-1-phenylpropan-1-one **(11)** in anhydrous THF (3.6 mL), 55 mg (0.40 mmol) of *m*-chloroperbenzoic acid (*m*-CPA) in anhydrous THF (1.2 mL) was added dropwise during 10 min. The reaction mixture was stirred for 1 h at room temperature, diluted with distilled water and extracted with diethyl ether (4× 15 mL). The organic layers were collected, washed with a 5% aqueous sodium hydrogen carbonate solution (15 mL), saturated with NaCl solution (15 mL) and then dried over $Na_2SO_4$and evaporated to dryness. The residue was purified by PTLC, with continuous elution in hexane: EtOAc (7:3) and crystallized from MeOH, giving a mixture of two diastereomeric sulphoxides α:β (1:1).

4.3.1. 2-[(4-Chlorophenyl)sulfinyl]-1-phenylpropan-1-one (16). Yield: 45; colourless crystals; mp 114–117 °C; IR ($CHCl_3$): 1675 (C=O), 1578, 1474, 1448, 1048 (S=O), 1593, 1387, 1342 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ α-isomer 1.33 (d, 3H, J = 6.84 Hz, H-9), 4.89 (q, 1H, J = 7.06 Hz, H-8), 7.24–8.02 (m, 9H, H-Ar); β-isomer 1.68 (d, 3H, J = 7.07 Hz, H-9), 4.60 (q, 1H, J = 6.8 Hz, H-8), 7.24–8.02 (m, 9H, H-Ar). ^{13}C NMR (75 MHz,

$CDCl_3$): δ 17.0, 45.5, 55.9, 56.0, 110.0, 111.0, 123.1, 128.5, 129.1, 134.9, 135.9, 149.2, 153.5, 195.0. MS *m/z* (+EI): 292 (M^+, 15), 165 (100), 151 (12), 123 (7); HRMS (+EI) (M^+) found 292.03249; (M^+) calcd for $C_{15}H_{13}ClSO_2$ 292.03248.

4.4. General procedure for β-ketosulphones (17 and 18)

To a β-ketosulphide solution in anhydrous THF (10 mL solvent/mmol of β-ketosulphide), peracetic acid (2.20 equivalent) was added dropwise in anhydrous THF (3 mL of the solvent/mmol of the peracid) during 10 min. The reaction was stirred at room temperature for 24 h. Distilled water was added, extracted with diethyl ether (4×). The organic layers were combined, washed with water, 5% $NaHCO_3$ solution, saturated with NaCl solution and then dried over Na_2SO_4. The solvent was removed in vacuum. The residue was purified by PTLC and crystallization.

4.4.1. 2-[(4-Chlorophenyl)sulfonyl]-1-phenylpropan-1-one (17). Yield: 45%; colourless crystals; mp 119–120 °C; IR ($CHCl_3$): 675, 1681 (C=O), 1582, 1475, 1450, 1320 (O=S=O), 1151 (O=S=O) cm^{-1}; ^1H NMR (500 MHz,$CDCl_3$): δ 1.57 (d, 3H, *J* = 6.93 Hz, H-9), 5.17 (q, 1H, *J* = 6.93 Hz, H-8), 7.24–8.03 (m, 9H, H-Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.3, 53.4, 65.02, 128.9, 129.1, 129.2, 131.3, 136.0, 141.2, 192.4. MS *m/z* (+EI): 308 (M^+,1), 105 (100), 51 (11); HRMS (+EI) (M^+) found 308.02733; (M^+) calcd for $C_{15}H_{13}ClSO_3$308.02739.

4.4.2. 1-(3,4-Dimethoxyphenyl)-2-[(4-methylphenyl)sulfonyl]propan-1-one (18). Yield: 45%; colourless crystals; mp 125–126 °C; IR ($CHCl_3$): 3023, 2940, 1700 (C=O), 1371 (O=S=O), 1162 (O=S=O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.54 (d, 3H, *J* = 6.91 Hz, H-9), 2.42 (s, 3H, H-4′),3.92 (s, 3H, CH_3O), 3.95 (s, 3H, CH_3O), 5.10 (q, 1H, *J* = 6.93 Hz, H-8), 6.87–7.70 (m, 7H, H-Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.4, 21.6, 56.0, 56.1, 64.6, 110.0, 110.8, 124.6, 129.4, 129.4, 129.8, 133.0, 145.2, 149.1, 154.2, 190.7. MS *m/z* (+EI): 348 (M^+, 15), 165 (100), 151 (12), 123 (7); HRMS (+EI) (M^+) found 348.10330; (M^+) calcd for $C_{18}H_{20}SO_5$ 348.10315.

4.5. General procedure for reduction with $NaBH_4$

To a solution of $NaBH_4$ (2.02 equivalent) in MeOH (1 mL solvent/0.13 mmol), the β-ketosulphide in THF (1 mL solvent/0.04 mmol) was slowly added. The mixture was stirred at room temperature for 30 min. Water was added and extracted with ethyl ether (4×). The organic extracts were combined, washed with distilled water, 5% $NaHCO_3$ solution, saturated with NaCl solution and then dried over Na_2SO_4. Filtration and evaporation under reduced pressure afforded a mixture of diastereomeric alcohols *erythro*:*threo*. The major product (*threo*) was obtained by PTLC with continuous elution in hexane:EtOAc (9:1).

4.5.1. *threo*-2-[(4-Chlorophenyl)thio]-1-phenylpropan-1-ol (19). Yield: 95%; colourless oil; IR ($CHCl_3$): 3422 (OH), 3062, 2969, 1475, 1452, 1388 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.02 (d, 3H, *J* = 6.99 Hz, H-9), 3.11 (1H, s, OH), 3.22 (dq, 1H, *J* = 8.47 Hz and *J* = 6.97 Hz, H-8), 4.32 (d, 1H, *J* = 8.52 Hz, H-7), 7.19–7.36 (m, 9H, H-Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.0, 53.1, 125.9, 126.8, 127.4, 128.0, 128.1, 129.1, 129.2, 130.9, 133.7, 134.16, 134.8, 139.1. MS *m/z* (+EI): 278 (M^+, 5) 165 (100), 151 (12), 123 (7); HRMS (+EI) (M^+); found: 278.05321; (M^+) calcd for $C_{15}H_{15}ClSO$ 278.05321.

4.5.2. *threo*-1-(3,4-Dimethoxyphenyl)-2-[(4-methylphenyl)thio]propan-1-ol (20). Yield: 88%; colourless crystals; mp 120–123 °C; IR ($CHCl_3$): 3494 (OH), 2969, 2866, 1592 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.04 (d, 3H, *J* = 6.94 Hz, H-9), 2.37 (3H, s, H-4′) 3.17 (dq, 1H, *J* = 8.90 Hz and *J* = 6.98 Hz, H-8), 3.48 (s, 1H, OH), 3.87 (s, 3H, CH_3O), 3.88 (s, 3H, CH_3O), 4.27 (d, 1H, *J* = 8.99 Hz, H-7), 6.70–7.47 (m, 7H, H-Ar). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.0, 53.1, 125.9, 126.8, 127.4, 128.0, 128.1, 129.1, 129.2, 130.9, 133.7, 134.2, 134.8, 139.1. MS *m/z* (+EI): 318 (M^+,1) 167 (67), 152 (100), 139 (56), 91 (18), 77 (16); HRMS (+EI) (M^+) found 318.12896; (M^+) calcd for $C_{18}H_{22}SO_3$ 318.12897.

4.5.3. *threo*-2-[(4-Chlorophenyl)thio]-1-(3,4-dimethoxyphenyl)propan-1-ol (21). Yield: 96%; colourless crystals; mp 123–125 °C; IR ($CHCl_3$): 3422 (OH), 3062, 2969, 1475, 1452, 1388 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.02 (d, 3H, *J* = 6.95 Hz, H-9), 1.54 (s, 1H, OH), 3.18 (dq, 1H, *J* = 8.54 Hz and *J* = 6.96 Hz, H-8), 3.79 (s, 3H, CH_3O), 3.80 (s, 3H, CH_3O), 4.26 (d, 1H, *J* = 8.61 Hz, H-7), 7.19–7.36 (m, 7H, H-Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.1, 53.1, 125.9, 126.8, 127.4, 128.0, 128.1, 129.1, 129.2, 130.9, 133.7, 134.2, 134.8, 139.1. MS *m/z* (+EI): 338 (M^+,1), 167 (67), 152 (100), 137 (56), 91 (6), 77 (11); HRMS (+EI) (M^+) found 338.07428; (M^+) calcd for $C_{17}H_{19}ClSO_3$ 338.07434.

4.5.4. *threo*-2-[(4-Chlorophenyl)thio]-1-phenylpropyl acetate (22). One-hundred milligrams (0.36 mmol) of *threo*-1-phenyl-2-(4′-chlorothiophenoxy)-1-propanol **(19)**, 59 mg (0.58 mmol) of acetic anhydride and 74 mg (0.94 mmol) of pyridine in anhydrous methyl ethyl ketone (20 mL) were stirred under heating at 80 °C during 24 h. After this period, the solution was washed with 5% HCl solution (3×), water, and then dried over Na_2SO_4. After filtration and evaporation under reduced pressure, the product was purified by PTLC with continuous elution in hexane:EtOAc (9:1), giving the acetylated derivative **(22)**. Yield: 78%; colourless oil; IR ($CHCl_3$): 3063, 2971, 1736 (OAc), 1476, 1371 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.05 (d, 3H, *J* = 6.8 Hz, H-9), 1.93 (s, 3H, OAc) 3.49 (qt, *J* = 7.18 Hz, 1H, H-8), 5.67 (1H, d, *J* = 7.3 Hz, H-7), 7.15–7.39 (9 H, m, H-Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.0, 46.1, 127.1, 127.4, 128.5, 128.6, 128.6, 128.9, 130.1, 130.3, 131.7, 133.0, 134.5, 135.6, 196.2. MS *m/z* (+EI): 320 (M^+, 5), 260 (27), 171 (77), 149 (20), 136 (8), 117 (17), 107 (65), 43 (100), 91 (11), 77 (13), 59 (11). HRMS (+EI) (M^+) found 320.06369; (M^+) calcd for $C_{17}H_{17}ClSO_2$ 320.06378.

5. Antileishmanial activity

The in vitro sensitivity of *L. donovani* (LD-1S/MHOM/SD/00-strain 1S) and *L. amazonensis* (MHOM/BR/75-Josefa) amastigotes to test compounds was determined in a mouse peritoneal macrophage model, using a modification of the method described by Neal & Croft.[27] Mouse peritoneal macrophages were isolated from BALB/c mice and seeded in 16-well tissue-culture chamber slides (Labtek Products, Miles Laboratories) at 2×10^5 cells per 0.1 mL DMEM/chamber. The cells were maintained at 37 °C in a 4% CO_2/air mixture for 2 hours and then infected with 8×10^5 stationary-phase culture promastigotes of *L. donovani* or *L. amazonensis* in 0.1 mL DMEM containing 5% heat-inactivated foetal calf serum, 50μg/mL streptomycin and 5 U/mL penicillin for 4 h.

The cultures were then washed with pre-warmed saline to remove free parasites and non-adherent cells, and 0.2 mL test compounds or Glucantime (Rhodia, Meglumine antimoniate), used as a reference drug, was added in quadruplicate to infected macrophages at 80 μg/mL. After 48 h, the macrophage monolayers were fixed with methanol and stained with Giemsa. The total number of amastigotes per 200 cells was determined and the results were expressed as percentage growth inhibition (100 minus the percentage of parasites in relation to controls cultured in the absence of any drug). The toxicity to macrophages was scored according to the degree of cell disruption in Giemsa-stained cultures.

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