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Short communication

Nitroimidazolyl-1,3,4-thiadiazole-based anti-leishmanial agents: Synthesis and in vitro biological evaluation

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Abstract

A series of 1-[5-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]-4-aroylpiperazines were synthesized and evaluated in vitro against *Leishmania major*. Most of the target compounds exhibited good anti-leishmanial activity against the promastigote form of *L. major* at non-cytotoxic concentrations. The most active compound was 1-[(5-chloro-2-thienyl)carbonyl]-4-[5-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]piperazine (**5f**) with an IC_{50} value of 9.35 ± 0.67 μM against *L. major* promastigotes. In addition, this compound was effective against intracellular *L. major* and significantly decreased the infectivity index.

Keywords: 1,3,4-Thiadiazole; Nitroimidazole; *Leishmania major*; Anti-leishmanial activity

1. Introduction

Leishmaniasis is a spectrum of diseases, ranging from self-limiting cutaneous infections to more serious disseminating diffuse cutaneous, mucocutaneous and visceral forms of the disease caused by intracellular protozoan parasites belonging to the genus Leishmania [1,2]. Among all leishmaniasis, visceral leishmaniasis is a highly morbid and incapacitating infection, which usually presents with prolonged fever, weight loss and hepatosplenomegaly. Despite the availability of effective treatment, the disease can have a high mortality even at referral centers [3]. 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 [4]. Disease progression is dependent on both the species of leishmania involved (as many as 17 sub-species may infect humans) and the genetics and immune status of the host. The recent increase in the spread of leishmaniasis is due in part to co-infections with the HIV/AIDS virus [5].

There are no vaccines against leishmaniasis and, as with other trypanosomatid diseases; treatment is dependent on a limited range of drugs. Front line drugs include pentavalent antimonials, amphotericin B and, in the case of visceral leishmaniasis, the only orally administered drug, miltefosine. All these drugs are limited to some extent by their toxicity, lack of efficacy, requirement for hospitalisation and/or cost [6]. The problem is further aggravated by the surge of antimonial resistance in some areas where the disease is endemic. While a number of second line drugs including pentamidine, paromomycin and the azoles are being tested or in the process of being introduced into the clinic, there is clearly a need for developing new, effective, cheap and safe drugs in the field of leishmaniasis chemotherapy [6,7].

For the last decade, new potential therapies have been introduced for leishmaniasis including paromomycin and sitamaquine. The latter has completed phase II trials in India, Kenya and Brazil [8]. Moreover, a great number of both natural and synthetic compounds have been evaluated in recent

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years in anti-leishmanial assays [9–13]. In this context, the use of nitroimidazole derivatives as antiprotozoal agents is well established. Although there is a large amount of experimental work on this heterocyclic system, it still remains an area of active research. On the other hand, 1,3,4-thiadiazoles have long been the subject of pharmaceutical interest as a result of their potent biological activities. Indeed, the 1,3,4-thiadiazole derivatives have been reported to possess antiparasitic properties [14] and their attachment with other heterocycles often ameliorates or diminishes the bioresponses, depending upon the type of substituent and position of attachment. As part of our efforts to develop new compounds aimed at the therapy of parasitic infection especially leishmaniasis [12,13], we have synthesized and evaluated some 1-[5-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]-4-aroylpiperazines against *Leishmania major*.

2. Chemistry

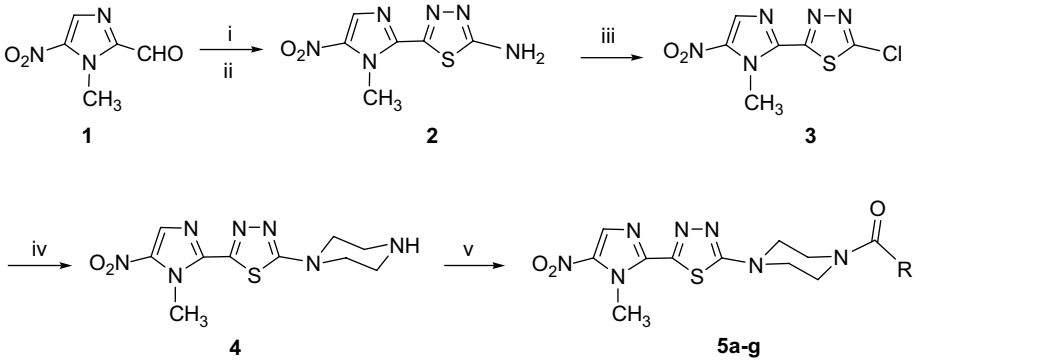
The synthetic pathway to target compounds **5a–g** is shown in Scheme 1. The intermediate 2-chloro-1,3,4-thiadiazole **3** was obtained from 1-methyl-5-nitroimidazole-5-carboxaldehyde according to the previously described methods [15,16]. Thus, treatment of **1** with thiosemicarbazide in the presence of HCl afforded the corresponding thiosemicarbazone which upon cyclization with ammonium ferric sulfate gave 2-amino-1,3,4-thiadiazole **2**. Diazotization of amine **2** in HCl solution, in the presence of copper powder, gave 2-chloro-1,3,4-thiadiazole **3**. The reaction of compound **3** with piperazine in refluxing ethanol gave *N*-piperazinyl compound **4**. *N*-aroylation of the piperazine analog **4** with appropriate benzoyl chlorides or thiophen-2-carbonyl chlorides afforded target compounds **5a–g** [12,13].

3. Results and discussion

The life cycle of leishmania microorganism consists of two developmental stages: promastigotes, flagellated extracellular parasites of the digestive tract of sand flies, and amastigotes, non-flagellated, non-motile stages that are more sensitive and live in macrophages [17]. In this study we described the anti-leishmanial assay of title compounds against both promastigote and amastigote forms of *L. major* strain (MRHO/IR/75/ER).

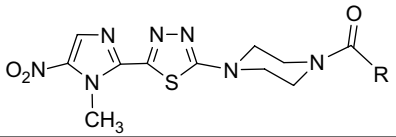
Compounds **5a–g** were tested for in vitro activity against the promastigote form of the *L. major* strain (MRHO/IR/75/ER) along with meglumine antimonate (Glucantime®), using MTT assay [18]. The IC_{50} values are presented in Table 1. The most potent compounds against the promastigote form of *L. major* were found to be *N*-(5-chloro-thiophen-2-yl)carbonyl derivative **5f** and *N*-benzoyl analog **5a** with IC_{50} values of 9.35 ± 0.67 and 10.39 ± 0.95 μM, respectively. The remaining compounds showed IC_{50} values between 15.96 ± 0.77 and 42.91 ± 2.38 μM, whereby the halogen substitution (chloro- or bromo-) on thiophen-2-carbonyl moiety improves the activity against promastigotes but chloro- substitution on benzoyl containing compound (**5a**) decreases the overall anti-leishmanial activity. The effect of positional isomerism of chloro- substitution was investigated by preparing all three possible regioisomers on benzoylpiperazine moiety. The order of activity in chlorobenzoyl series was as follows: 3-Cl > 2-Cl > 4-Cl. A comparison between IC_{50} values of the unsubstituted benzoyl analog **5a** and its unsubstituted thiophen-2-carbonyl counterpart **5e**, against promastigotes, revealed that benzoyl compound possessed better activity with respect to corresponding thiophene derivative. In contract, in halogenated compounds, 5-halo-thiophenes **5f**,**g** exhibited more potent activity than their corresponding chlorophenyl derivatives **5b–d**.

The compounds **5a** and **f** which exhibited potent activity against promastigotes ($IC_{50} \leq 10.39$ μM) were also evaluated for their activity against the amastigote form of *L. major* in peritoneal macrophages (Fig. 1) [19]. As can be deducted from Fig. 1, compounds **5a** and **f** have significantly decreased the number of amastigotes per macrophage and



Scheme 1. Reagents and conditions: (i) thiosemicarbazide, EtOH, HCl, reflux; (ii) ammonium ferric sulfate, H_2O, reflux; (iii) $NaNO_2$, HCl, Cu; (iv) piperazine, EtOH, reflux; (v) appropriate thiophen-2-carbonyl chlorides or benzoyl chlorides, benzene, pyridine, rt.

Table 1
In vitro anti-leishmanial activity of compounds **5a–g** against promastigote form of *L. major*



Compound	R	MW	IC_{50} (μM)[a]
5a	Phenyl	399.43	10.39 ± 0.95
5b	2-Cl–phenyl	433.87	20.26 ± 0.61
5c	3-Cl–phenyl	433.87	17.88 ± 0.72
5d	4-Cl–phenyl	433.87	42.91 ± 2.38
5e	Thiophen-2-yl	405.45	25.58 ± 1.03
5f	5-Cl–thiophen-2-yl	439.9	9.35 ± 0.67
5g	5-Br–thiophen-2-yl	484.35	15.96 ± 0.77
Meglumine antimonate			81.97 ± 3.85[b]

[a] The values represent mean ± SD.
[b] IC_{50} in mM.

both the percentage of macrophage infectivity and infectivity index.

The cytotoxic property of the title compounds against macrophage cells were also assessed using MTT colorimetric assay [20]. Macrophage cells were treated with compounds in the IC_{50} concentrations for 24 h, side by side the standard drug meglumine antimonate. The results showed that these compounds display anti-leishmanial activity at non-cytotoxic concentrations. Whereas, the reference drug meglumine antimonate decreased viability of macrophages up to 40%.

In conclusion, we have synthesized a series of 1-[5-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]-4-aroylpiperazines via a versatile synthetic route and evaluated them against *L. major*. Most of the target compounds exhibited good anti-leishmanial activity against the promastigote form of *L. major* at non-cytotoxic concentrations. The most active compound was 1-[(5-chloro-2-thienyl)carbonyl]-4-[5-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]piperazine **5f** which also showed good activity against intracellular form of *L. major*.

4. Experimental

4.1. Chemistry

Chemical reagents and all solvents used in this study were purchased from Merck AG Chemical. The key intermediate 2-chloro-5-(1-methyl-5-nitroimidazol-2-yl)-1,3,4-thiadiazole was prepared according to the literature method [13–16]. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide dicks). ^{1}H NMR spectra were recorded on a Varian unity 80 spectrometer and chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (TMS) as an internal standard. Elemental analyses were carried out on a CHN–O rapid elemental analyzer (GmbH-Germany) for C, H and N, and the results are within ±0.4% of the theoretical values. Merck silica gel 60 F_{254} plates were used for analytical TLC.

4.1.1. General procedure for the synthesis of compounds *5a–g*

To a mixture of compound **4** (1 mmol) in dry benzene–pyridine (3:1 mL), appropriate thiophen-2-carbonylchloride or benzoyl chloride (1.1 mmol) was added and the mixture was stirred at room temperature for three days. The solvents were removed under reduced pressure and resulting solid was washed with methanol–water (80%) and crystallized from ethanol to give **5a–g**.

*4.1.1.1. 1-(2-Chlorobenzoyl)-4-[5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazol-2-yl] piperazine (**5b**).* Yield 80%; mp 275–277 °C; IR (KBr) 3140 (H–C imidazole), 1629 (C=O), 1537 and 1358 cm^{-1} (NO_2); ^{1}H NMR (80 MHz, DMSO-d_6) δ 8.15 (s, 1H, H-4 imidazole), 7.40–7.55 (m, 4H, phenyl), 4.35 (s, 3H, NCH_3), 3.60–3.85 (m, 8H, piperazine). MS (*m/e*, %): 435 ([$M^+ + 2$], 5), 433 (M^+, 15), 400 (45), 309 (100), 196 (58), 150 (18), 56 (48).

*4.1.1.2. 1-(3-Chlorobenzoyl)-4-[5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]piperazine (**5c**).* Yield 84%; mp 242–244 °C; IR (KBr) 3140 (H–C imidazole), 1624 (C=O), 1516 and 1363 cm^{-1} (NO_2); ^{1}H NMR (80 MHz, DMSO-d_6) δ 8.2 (s, 1H, H-4 imidazole), 7.3–7.65 (m, 4H, phenyl), 4.35 (s, 3H, NCH_3), 3.69 (br s, 8H, piperazine). MS (*m/e*, %): 435 ([$M^+ + 2$], 6), 433 (M^+, 15), 400 (45), 309 (100), 252 (20), 196 (58), 150 (18), 56 (48).

*4.1.1.3. 1-(4-Chlorobenzoyl)-4-[5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]piperazine (**5d**).* Yield 85%; mp 245–247 °C; IR (KBr) 3129 (H–C imidazole), 1639 (C=O), 1520 and 1340 cm^{-1} (NO_2); ^{1}H NMR (80 MHz, DMSO-d_6) δ 8.20 (s, 1H, H-4 imidazole), 7.50–7.53 (m, 4H, phenyl), 4.34 (s, 3H, NCH_3), 3.68 (br s, 8H, piperazine). MS (*m/e*, %): 435 ([$M^+ + 2$], 8), 433 (M^+, 18), 308 (20), 252 (42), 238 (30), 180 (20), 139 (100), 111 (40), 56 (54).

*4.1.1.4. 4-[5-(1-Methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]-1-(2-thienyl carbonyl) piperazine (**5e**).* Yield 79%; mp 235–237 °C; IR (KBr) 3140 (H–C imidazole), 1603 (C=O), 1521 and 1358 cm^{-1} (NO_2); ^{1}H NMR (80 MHz, DMSO-d_6) δ 8.22 (s, 1H, H-4 imidazole), 7.7–7.9 (m, 1H, H-5 thiophene), 7.45–7.6 (m, 1H, H-3 thiophene), 7.15–7.25 (m, 1H, H-4 thiophene), 4.33 (s, 3H, NCH_3), 3.54–3.96 (m, 8H, piperazine). MS (*m/e*, %): 405 (M^+, 17), 294 (6), 278 (20), 111 (100), 55 (45).

*4.1.1.5. 1-[(5-Chloro-2-thienyl)carbonyl]-4-[5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]piperazine (**5f**).* Yield 85%; mp 234–235 °C; IR (KBr) 3140 (H–C imidazole), 1614 (C=O), 1521 and 1362 cm^{-1} (NO_2); ^{1}H NMR (80 MHz, DMSO-d_6) δ 8.22 (s, 1H, H-4 imidazole), 7.40 (d, 1H, H-3 thiophene, $J = 4.0$ Hz), 7.19 (d, 1H, H-4 thiophene, $J = 4.0$ Hz), 4.33 (s, 3H, NCH_3), 3.54–3.96 (m, 8H, piperazine).

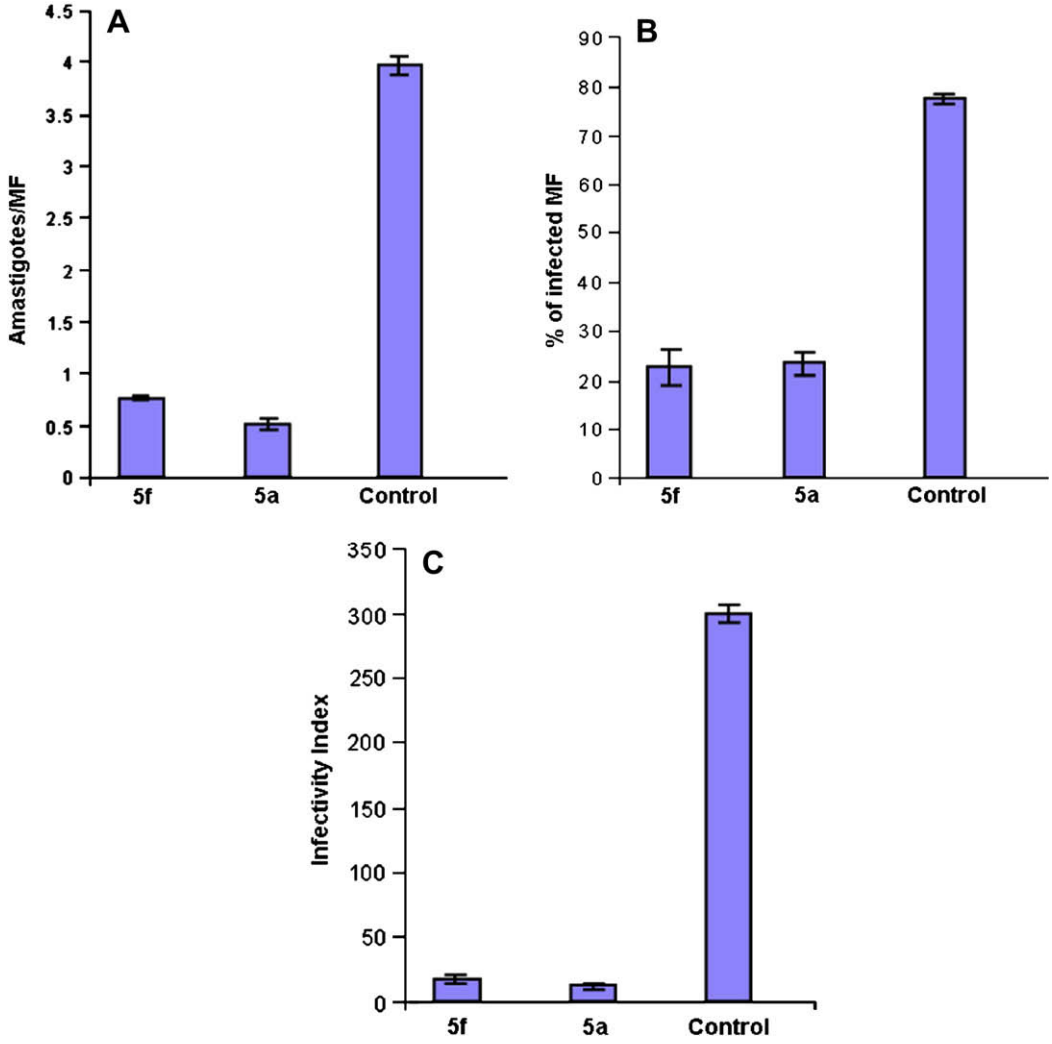


Fig. 1. In vitro activity of compounds **5a** and **f** against intramacrophage amastigotes of *L. major*. (A) The mean number of amastigotes per macrophage after treatment with drug for 24 h. (B) The percentage of infected macrophages after treatment. (C) Infectivity index of macrophages cultured 24 h in the presence of selected drugs. The infectivity index was determined by multiplying the percentage of macrophages that had at least one intracellular parasite by the average number of intracellular parasites per infected macrophage (60 cells were examined/well).

MS (*m/e*, %) 441 ([$M^+ + 2$], 7), 439 (M^+, 25), 276 (60), 251 (95), 178 (76), 143 (100), 99 (45), 54 (60).

*4.1.1.6. 1-[(5-Bromo-2-thienyl)carbonyl]-4-[5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]piperazine (**5g**).* Yield 77%; mp 260–262 °C; IR (KBr) 3138 (H–C imidazole), 1608 (C=O), 1511 and 1362 cm^{-1} (NO_2); ^1H NMR (80 MHz, DMSO-d_6) δ 8.22 (s, 1H, H-4 imidazole), 7.34 (d, 1H, H-3 thiophene, $J = 3.9$ Hz), 7.29 (d, 1H, H-4 thiophene, $J = 3.9$ Hz), 4.33 (s, 3H, NCH_3), 3.58–3.96 (m, 8H, piperazine) MS (*m/e*, %) 485 ([$M^+ + 2$], 38), 483 (M^+, 40), 403 (20), 277 (100), 251 (89), 238 (67), 189 (76), 152 (36), 99 (18), 68 (24).

4.2. Biological activity

4.2.1. Parasite isolates and culture conditions

The strain of *L. major* used in this study was the vaccine strain (MRHO/IR/75/ER), obtained from Pasteur Institute, Tehran (Iran). The infectivity of the parasites was maintained by regular passage in susceptible BALB/c mice. The promastigote form of parasite was grown in blood agar cultures at 25 °C. The stationary parasite inoculation was 2×10^6 cells/mL. For the experiments described here, the stationary phase promastigotes were washed with phosphate buffered saline and recultured in RPMI 1640 medium (Sigma) at 2×10^6 cells/mL density, supplemented with 10% of heat-inactivated fetal bovine serum, 2 mM glutamine (Sigma), pH ~7.2, 100 U/mL penicillin (Sigma) and 100 μg/mL streptomycin (Sigma).

4.2.2. Anti-leishmanial activity against promastigotes form of L. major

The anti-leishmanial screening was performed using direct counting and MTT assay [18]. It should be noted that at first, the growth curve of the *L. major* strain was determined daily under light microscope and counting in a Neubauer's chamber. Then, parasites (2×10^6/mL) in the logarithmic phase were

incubated with a serial range of drug concentrations for 24 h at 25 °C. To determine 50% inhibitory concentrations (IC_{50}), the tetrazolium bromide salt (MTT) assay was used. Briefly, promastigotes from early log phase of growth were seeded in 96-well plastic cell culture trays, containing serial dilution of drug and phenol red free RPMI 1640 medium, supplemented with 10% of FBS, 2 mM glutamine, pH ~7.2 and antibiotics, in a volume of 200 μL. After 24 h of incubation at 25 °C, the media was renewed with 100 μg/well of MTT (0.5 mg/mL) and plates were further incubated for 4 h at 37 °C. The plates were centrifuged (2000 rpm × 5 min), the pellets were dissolved in 200 μL of DMSO. The samples were read using an ELISA plate reader at a wavelength of 492 nm. Two or more independent experiments in triplicate were performed for determination of sensitivity to each drug, the IC_{50} was calculated by linear regression analysis, expressed in mean ± SD. Control cells were incubated with culture medium plus DMSO.

4.2.3. Anti-leishmanial activity against amastigotes form of L. major

Mouse peritoneal macrophages were plated in RPMI 1640 supplemented with 10% of heat-inactivated fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin (Sigma) and 100 μg/mL streptomycin. Macrophages were placed on sterile glass cover slips in 24-well plates (1×10^6/well). After 1 h non-adherent cells were removed by washing with RPMI, the stationary phase promastigotes in RPMI were added (2×10^6 parasites/well, three parasites/macrophage) to macrophage monolayer and the plates were kept at 37 °C in a CO_2 incubator for 2 h. Extracellular parasites were removed by washing and then new media containing IC_{50} concentration of the drug were added. Two sets of experiments were carried out for each drug at 24 h. Following these procedures, cells were fixed with methanol, stained with Giemsa stain (Sigma) and the infectivity index was determined by multiplying the percentage of macrophages that had at least one intracellular parasite by the average number of intracellular parasites per infected macrophage (60 cells were examined/well) [19].

4.2.4. Toxicity on peritoneal macrophages

In vitro toxicity toward mouse peritoneal macrophages was assessed with cells plated in 96-well plates at 2×10^5 cells/well. After cell adherence, the medium was removed and replaced by the media containing IC_{50} concentration of each compound. The plates were incubated for 24 h at 37 °C in a humidified incubator with 5% CO_2. Control cells were incubated with culture medium plus DMSO. Cell viability was determined by MTT colorimetric assay [20].

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References

[1] B.L. Herwaldt, Lancet 354 (1999) 1191–1199.
[2] S.L. Croft, G.H. Coombs, Trends Parasitol. 19 (2003) 502–508.
[3] G. Malatesha, P. Mathur, K. Madan, P. Samanta, J.C. Samantaray, Trop. Gastroenterol. 28 (2007) 28–31.
[4] P. Desjeux, Comp. Immunol. Microbiol. Infect. Dis. 27 (2004) 305–318.
[5] P.K. Sinha, K.I. Pandey, S.K. Bhattacharya, Indian J. Med. Res. 121 (2005) 407–414.
[6] C.R. Davies, P. Kaye, S.L. Croft, S. Sundar, BMJ 326 (2003) 377–382.
[7] T.K. Jha, Indian J. Med. Res. 123 (2006) 389–398.
[8] S.L. Croft, S. Sundar, A.H. Fairlamb, Clin. Microbiol. Rev. 19 (2006) 111–126.
[9] P. González, C. Marín, I. Rodríguez-González, A. Illana, H. Mateo, S.S. Longoni, M.J. Rosales, A. González-Coloma, M. Reina, M. Sánchez-Moreno, J.G. Díaz, Pharmacology 76 (2006) 123–128.
[10] A.G. Tempone, S.E.T. Borborema, H.F. de Andrade Jr., N.C.de A. Gualda, Á. Yogi, C.S. Carvalho, D. Bachiega, F.N. Lupo, S.V. Bonotto, D.C.H. Fischer, Phytomedicine 12 (2005) 382–390.
[11] K.M. Khan, M. Rasheed, Z. Ullah, S. Hayat, F.K. Kaukab, M.I. Choudhary, Atta-ur-Rahman, S. Perveen, Bioorg. Med. Chem. 11 (2003) 1381–1387.
[12] A. Foroumadi, S. Pournourmohammadi, F. Soltani, M. Asgharian-Rezaee, S. Dabiri, A. Kharazmi, A. Shafiee, Bioorg. Med. Chem. Lett. 15 (2005) 1983–1985.
[13] A. Foroumadi, S. Emami, S. Pournourmohammadi, A. Kharazmi, A. Shafiee, Eur. J. Med. Chem. 40 (2005) 1346–1350.
[14] M. Behrouzi-Fardmoghadam, F. Poorrajab, S.K. Ardestani, S. Emami, A. Shafiee, A. Foroumadi, Bioorg. Med. Chem. 16 (2008) 4509–4515.
[15] A. Foroumadi, M. Mirzaei, A. Shafiee, Il Farmaco 56 (2001) 621–623.
[16] A. Foroumadi, S. Mansouri, Z. Kiani, A. Rahmani, Eur. J. Med. Chem. 38 (2003) 851–854.
[17] S.L. Croft, V. Yardley, Curr. Pharm. Des. 8 (2002) 319–342.
[18] A. Dutta, S. Bandyopadhyay, C. Mandal, M. Chatterjee, Parasitol. Int. 54 (2005) 119–122.
[19] A.K. Tanaka, V.B. Valero, H.K. Takahashi, A.H. Straus, J. Antimicrob. Chemother. 59 (2007) 487–492.
[20] T. Mosmann, J. Immunol. Methods 65 (1983) 55–63.