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Synthesis and antileishmanial activity of novel 5-(5-nitrofuran-2-y1)-1,3,4-thiadiazoles with piperazinyl-linked benzamidine substituents

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ABSTRACT

In order to optimize the antileishmanial activity of piperazinyl-linked 5-(5-nitrofuran-2-yl)-1,3,4-thiadiazoles, we synthesized a series of 5-(5-nitrofuran-2-y1)-1,3,4-thiadiazoles with piperazinyl-linked benzamidine substituent as scaffold found in pentamidine related antiprotozoals. The structure of target compounds was confirmed by IR, ^{1}H NMR, ^{13}C NMR and Mass spectral data. All compounds were tested for *in vitro* activity against the promastigote and amastigote forms of *Leishmania major*. From the results, we found that the substitution on amidine nitrogen has profound role in the biological activity of these compounds. The 5-nitrofuran-2-yl-1,3,4-thiadiazoles having *n*-propyl, *n*-butyl and benzyl side chain on benzamidine (as in compounds **2d**, **2e** and **2g**, respectively) showed very good activity in both forms of promastigote and amastigote. The most active compound was *N*-propyl-4-(4-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)piperazin-1-yl) benzamidine hydrochloride (**2d**) with IC_{50} value of 0.08 μM in promastigote model. This compound showed a very low level of toxicity against macrophages (CC_{50} = 785 μM), with the highest selectivity index (SI = 78.5) among the tested compounds.

1. Introduction

Leishmaniasis is caused by protozoan parasites belonging to the genus *Leishmania*. Official data show that there are 12 million infected people around the world, 350 million at risk of acquiring the disease, and 1.5–2 million that will be infected annually [1]. Leishmaniasis showed a complex and diverse clinical manifestations and epidemiology [2]. In spite of the socioeconomic importance of this tropical infection, efforts directed toward the discovery of new drugs and/or vaccines against it are underdeveloped [3,4]. Drugs currently in use as the antimony derivative glucantine, the bis-amidines, stilbamidine or the glycomacrolide amphotericin B are quite toxic and cause severe side effects such as pancreatitis and cardiac toxicity. In addition, the development of the clinical resistance and the increase of co-infections leishmaniasis AIDS, in some countries, have worried the authorities [5,6]. In addition, most of the drugs currently in use are expensive and require long-term treatment [7]. Thus, the development of new, cheap, efficient, and safe drugs for the treatment of this disease is imperative.

Aromatic diamidine compounds based on pentamidine (Fig. 1) have shown excellent activity against *Leishmania* parasites. The antileishmanial activity of several such diamidines was reported in 1990 [8]. Since that time, many new aromatic diamidines have been synthesized and improved assay systems have been developed for the *in vitro* testing of candidate drugs as antiprotozoal agents. For example, diminazene (Fig. 1), is an aromatic diamidine antiprotozoal related to pentamidine that has been used in veterinary practice in the treatment of trypanosomiasis and babesiosis. Furthermore, imidocarb bearing cyclic diamidine (imidazoline) has antiprotozoal and antibacterial activity [9–11]. On the other hand, the antiparasitic property of 5-(5-nitrofuran-2-yl)-1,3,4-thiadiazoles is well established and their linkage with other heterocycles often modulates their biological activities, depending upon the

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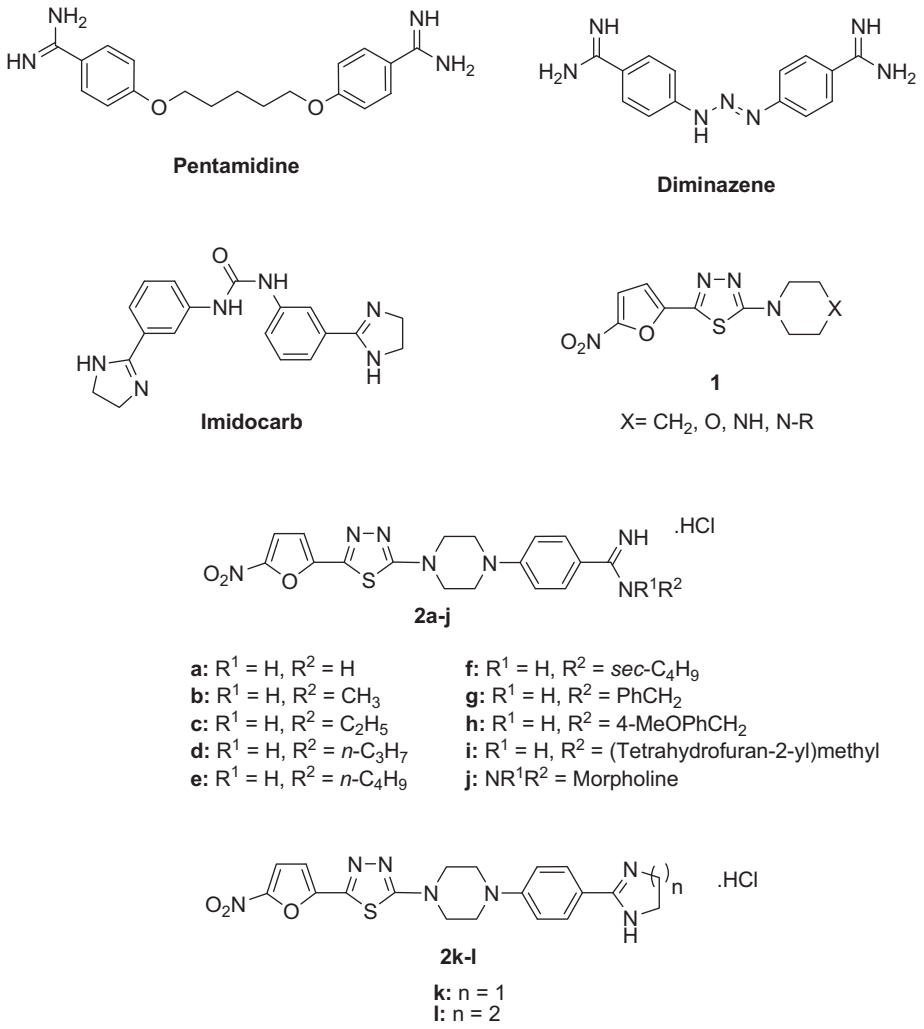


Fig. 1. Structures of diamidine antiprotozoal agents (pentamidine, diminazene and imidocarb), 2-(5-nitrofuran-2-yl)-5-substituted-1,3,4-thiadiazoles (**1**) and designed compounds (**2a**–**l**).

type of substituent and position of attachment [12–15]. In our previous papers [16–18], we described the synthesis and *in vitro* antileishmanial activity of a series of 2-(5-nitrofuran-2-yl)-5-substituted-1,3,4-thiadiazoles (**1**), which several of them showed promising antileishmanial properties (Fig. 1). In view of the biological importance of pentamidine analogs [19–21], it was of our interest to combine the structural features of 2-(5-nitrofuran-2-yl)-5-substituted-1,3,4-thiadiazoles (**1**) with the amidines, we synthesized and evaluated twelve analogs of 1-[5-(5-nitrofuran-2-y1)-1,3,4-thiadiazol-2-y1] piperazine–linked benzamidines **2a**–**j** and related derivatives **2k**,**l** (Fig. 1) as potential new antileishmanial agents.

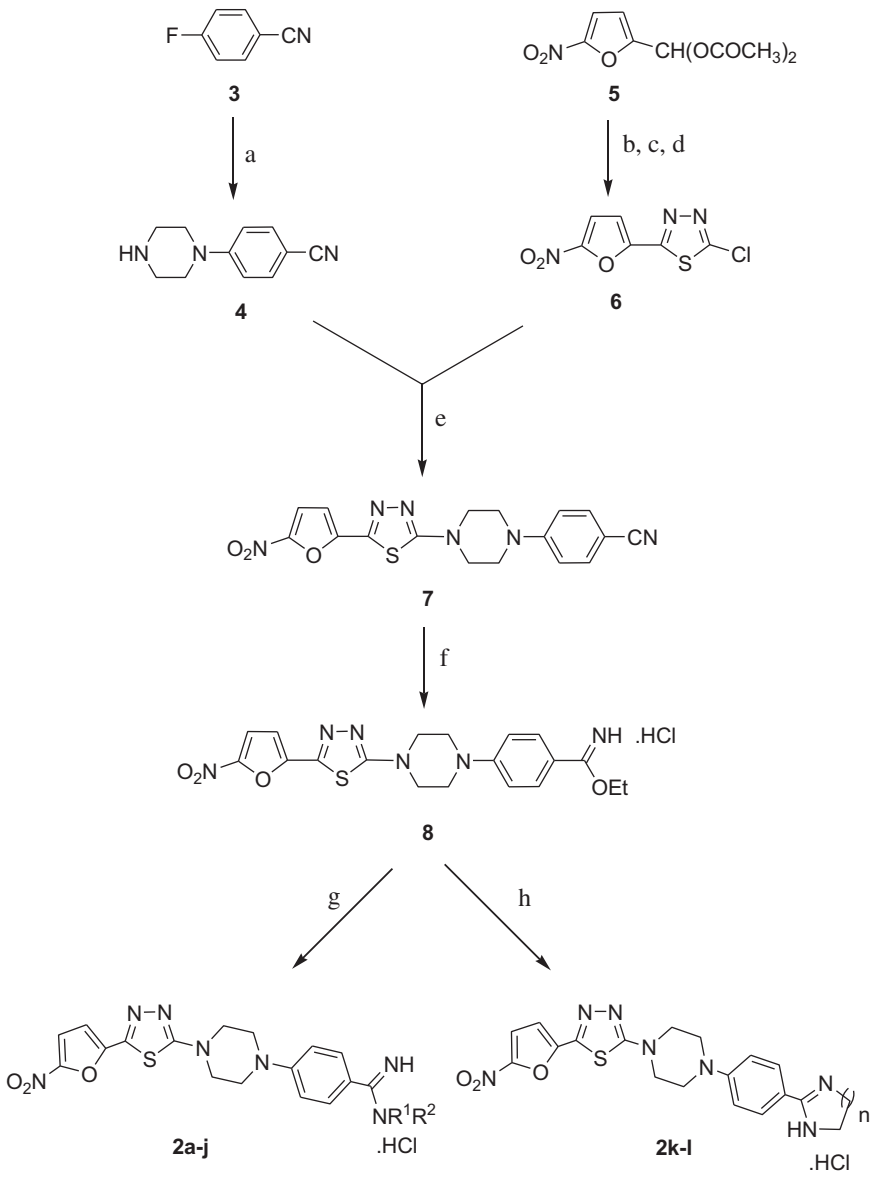
2. Chemistry

The pathway for the synthesis of compounds **2a**–**l** is shown in Scheme 1. 4-(Piperazin-1-yl)benzonitrile (**4**) was obtained from the reaction of excess piperazine with 4-fluorobenzonitrile (**3**). The intermediate 2-chloro-1,3,4-thiadiazole **6** was obtained from 5-nitrofurfurylidine diacetate according to the previously described method [16]. The reaction of chloro-thiadiazole **6** with piperazine **4** in refluxing ethylmethylketone gave compound **7**. Conversion of the nitrile **7** into the amidines 2**a**–**l** was effected by the Pinner reaction [22,23]. Treatment of a solution of compound **7** in dichloromethane with ethanol and gaseous hydrochloric acid afforded the intermediate benzeneimidate **8** which was subsequently reacted with ammonia or appropriate amines to afford benzamidines **2a**–**j**. Reaction of diamine (ethylenediamine or propylenediamine) with imidate **8** enabled to prepare compounds **2k**,**l** bearing an imidazoline or a tetrahydropyrimidine system as cyclic amidine groups (Scheme 1).

3. Pharmacology

3.1. Parasite and culture

The strain of *Leishmania major* used in this study was the vaccine strain (MRHO/IR/75/ER), obtained from Pasteur Institute, Tehran (Iran) [24]. 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 of 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 glutamine (Sigma), pH ~7.2, 100 U/mL penicillin (Sigma) and 100 μg/mL streptomycin (Sigma).



Scheme 1. Synthesis of compounds **2a**–**l**. Reagents and conditions: (a) piperazine, ethylmethylketone, K_2CO_3 reflux; (b) thiosemicarbazide, EtOH, reflux; (c) $NH_4Fe(SO_4)_2{\cdot}12H_2O$, H_2O, reflux; (d) $NaNO_2$, HCl, Cu, 0 °C → r.t, (e) ethylmethylketone, K_2CO_3; (f) CH_2Cl_2, EtOH, gaseous HCl, r.t; (g) appropriate amine, EtOH, reflux; (h) appropriate diamine, EtOH, reflux.

3.2. Antileishmanial activity against promastigotes form of L. major

The antileishmanial screening of compounds **2a**–**l**, **7** and **8** was performed using direct counting and MTT assay [25]. 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, 48 h and 72 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, 48 h or 72 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.

3.3. Antileishmanial 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 (100 cells were examined/well) [26].

3.4. Toxicity against macrophages

The toxicity of compounds **7** and **2a**–**j** was assessed against mouse peritoneal macrophages plated in 96-well plates at 2×10^5 cells/well. After cell adherence, the medium was removed and replaced by the media containing different concentrations of each compounds. The plates were incubated for 24 h at 37 °C in a humidified incubator with 5% CO_2. Cell viability was determined by MTT colorimetric assay [27]. Two independent experiments in triplicate were performed for determination of toxicity of each compound, the CC_{50} (cytotoxic concentration for 50% inhibition) were calculated by linear regression analysis.

4. Result and discussion

In the present study, we describe the evaluation of anti-leishmanial activity of target compounds **2a**–**l**, **7** and **8** on both developmental forms of Leishmania consisting promastigotes (extracellular parasites) and amastigotes (that live in macrophages of mammalian hosts). The test compounds were evaluated for anti-promastigote activity along with meglumine antimonate (Glucantime®), using MTT assay [25]. The IC_{50} values (in μM) against promastigotes after 24, 48 or 72 h incubation, in comparison with Glucantime, are listed in Table 1.

The most potent compounds against the promastigote form of *L. major* were found to be compound **2d** followed by compounds **2e**, **2g** and **2h** with IC_{50} values of 0.08 ± 0.01, 0.2 ± 0.1, 0.4 ± 0.2 and 2 ± 0.2 μM recorded at 72 h, respectively. The remaining compounds showed IC_{50} values between 4 and 32 μM after 72 h incubation. These results indicate that the propyl, butyl and benzyl substitutions on the amidine residue improve the activity against promastigotes. The parent amidine **2a**, exhibits low inhibition and it appears that the inhibitory activity can be modulated by the introduction of an alkyl group on a nitrogen atom of the amidine function. Indeed, longer alkyl chains such as *n*-propyl and *n*-butyl (compounds **2d** and **2e**) increased the anti-promastigote activity, whereas a smaller alkyl group such as methyl or ethyl produced moderate activity. The benzyl derivative (compound **2g**) with IC_{50} value of 0.4 ± 0.2 μM has better activity respect to the 4-methoxybenzyl derivative **2h** (IC_{50} = 2 ± 0.2 μM). It should be noted that compounds **2k** and **2l** which substituted by a five-membered ring, namely the imidazoline and six-membered ring, namely tetrahydropyrimidine were devoid of activity (IC_{50} values >200 μM). The lack of activity may be due to the steric hindrance around the nitrogen atoms or to the absence of =NH function.

Some of the compounds were also evaluated for their activity against the amastigote form of *L. major* in peritoneal macrophages (Fig. 2) [25,26]. As can be deducted from Fig. 2, all tested compounds have significantly decreased the number of amastigotes per macrophage and both the percentage of macrophage infectivity and infectivity index. It is notable that non-amidine compounds **7** and **8**, *N*-methylamidine **2b**, *sec*-butyl analog **2f** which showed moderate activity against promastigotes being relatively effective compounds against amastigotes as showed in the terms of amastigote number per macrophage, the percentage of macrophage infectivity and infectivity index.

The *in vitro* cytotoxic activity of compounds **7** and **2a**–**j** against mouse peritoneal macrophages was also determined using MTT assay (Table 1). The CC_{50} values for tested compounds demonstrated that, most of them had low toxicity for macrophages (CC_{50} > 100 μM). The most active antileishmanial compound **2d**, showed very low level of toxicity against macrophages (CC_{50} = 785 μM) with the highest selectivity index (SI = 78.5).

Table 1
In vitro antileishmanial activity of compounds **2a**–**l**, **7** and **8** against promastigote form of *L. major*.

Compound	Anti-promastigote activity IC_{50} (μM)			Cytotoxicity[a] CC_{50} (μM)	Selectivity index (SI) CC_{50}/IC_{50}–24 h
	24 h	48 h	72 h		
7	104 ± 0.7	86 ± 1.3	32 ± 0.45	159	1.53
8	48 ± 0.42	46 ± 0.7	26 ± 0.31	–	–
2a	111 ± 0.8	111 ± 1.4	26 ± 0.3	342	3.08
2b	23 ± 0.25	22 ± 0.5	21 ± 0.14	223	9.69
2c	33 ± 0.62	26 ± 0.4	16 ± 0.7	337	10.21
2d	10 ± 0.65	5 ± 0.5	0.08 ± 0.01	785	78.50
2e	11 ± 1.4	4 ± 1.4	0.2 ± 0.1	123	11.18
2f	33 ± 0.21	18 ± 1.5	13 ± 0.21	770	23.33
2g	9 ± 1.8	1.9 ± 0.7	0.4 ± 0.2	546	60.77
2h	80 ± 1.6	76 ± 1.2	2 ± 0.2	69	0.86
2i	95 ± 0.8	88 ± 0.14	5 ± 0.3	921	9.69
2j	93 ± 0.9	88 ± 1.02	4 ± 0.2	55	0.59
2k	>200	–	–	–	–
2l	>200	–	–	–	–
Glucantime[b]	35 ± 0.2	30 ± 0.18	30 ± 0.167	–	–

[a] Cytotoxicity was evaluated against mouse peritoneal macrophages.
[b] The IC_{50} of Glucantime was in mg/mL.

5. Conclusion

In our earlier studies we have shown that 2-(5-nitrofuran-2-yl)-5-substituted-1,3,4-thiadiazoles showed pronounced *in vitro* anti-leishmanial profile in the terms of activity against promastigote and amastigote forms of *L. major* [16–18]. In view to further optimization of the activity profile, we synthesized a series of 5-(5-nitrofuran-2-y1)-1,3,4-thiadiazoles with piperazinyl-linked benzamidine substituent as scaffold fond in pentamidine related antiprotozoals. From the results we found that the substitution on amidine nitrogen has profound role in the biological activity of these compounds. The 5-nitrofuran-2-yl-1,3,4-thiadiazoles having *n*-propyl, *n*-butyl and benzyl side chain on benzamidine (as in compounds **2d**, **2e** and **2g**, respectively) showed very good activity in promastigote as well as amastigote model. However, benzonitrile substitution as in compound **7** and *O*-ethyl benzimidate substitution as in compound **8** showed lower activity in promastigote model but was found effective in the amastigote model. In summary, we have identified N-substituted benzamidine derivatives of 5-(5-nitrofuran-2-y1)-1,3,4-thiadiazoles as a promising new hit for the antileishmanial chemotherapy.

6. Experimental protocols

6.1. Chemistry

Chemical reagents and all solvents used in this study were purchased from Merck AG Chemical. The key intermediate 2-chloro-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazole (**6**) was prepared according to the literature method [16–18]. 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 500 spectrometer and chemical shifts (δ) are

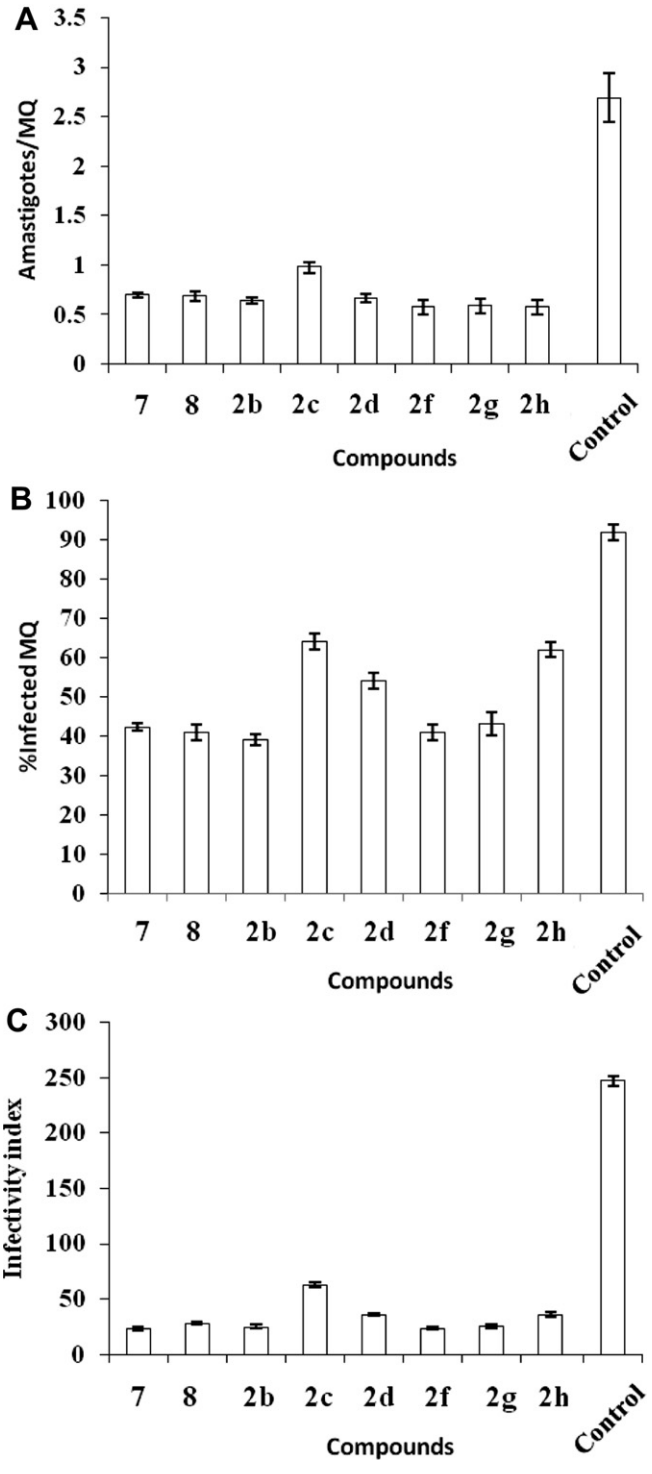


Fig. 2. *In vitro* activity of selected compounds against intramacrophage amastigotes of *L. major*. (A) The mean number of amastigotes per macrophage after treatment with drug for 24 h. (B) The percentage of infected macrophages after treatment. (C) Infectivity index of macrophages cultured 24 h in 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 (100 cells were examined/well).

reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard. The mass spectra were run on an Agilent 6410. Merck silica gel 60 F254 plates were used for analytical TLC. Compound **2a** [28] has been described in the literature.

*6.1.1. 4-(Piperazin-1-yl)benzonitrile (**4**)*

A mixture of 4-fluorobenzonitrile (**3**, 0.36 g, 3 mmol), piperazine (0.64 g, 7.4 mmol) and K_2CO_3 (0.85 g, 6.1 mmol) in ethylmethylketone (50 mL) was refluxed for 4 days. The completion of reaction was detected by TLC. The solvent was evaporated under reduced pressure and then chloroform and water was added. The organic layer was extracted. After evaporation of chloroform, the residue was crystallized from chloroform to give compound **4** as a cream solid. Yield: 95%; m.p 82–85 °C; IR (KBr, cm^{-1}): 3325, 2215, 1606. ^1H NMR (500 MHz, $CDCl_3$): 7.50 (d, 2H, $J = 9.2$ Hz, phenyl), 6.86 (d, 2H, $J = 9.2$ Hz, phenyl), 3.29 (t, 4H, $J = 5.2$ Hz, piperazine), 3.02 (t, 4H, $J = 5.2$ Hz, piperazine). MS (ESI): 188 [$M + H^+$]. Anal. Calcd for $C_{11}H_{13}N_3$: C, 70.56; H, 7.0; N, 22.44. Found: C, 70.35; H, 6.82; N, 22.73.

*6.1.2. 4-(4-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)piperazin-1-yl)benzonitrile (**7**)*

A mixture of compound **4** (0.13 g, 0.7 mmol), compound **6** (0.16 g, 0.7 mmol) and K_2CO_3 (0.145 g, 1.05 mmol) in ethylmethylketone (30 mL) was refluxed for 8 h. After completion of the reaction, the solvent was removed under reduced pressure to obtain a yellow solid (yield 90%). The crude product was purified by column chromatography (silica gel, chloroform). Yield: 78%; m.p > 300 °C; IR (KBr, cm^{-1}): 2217, 1608, 1355, 1254. ^1H NMR (500 MHz, $CDCl_3$): 7.50 (d, 2H, $J = 8.4$ Hz, phenyl), 7.44 (d, 1H, $J = 4$ Hz, furan), 7.19 (d, 1H, $J = 4$ Hz, furan), 6.92 (d, 2H, $J = 8.4$ Hz, phenyl), 3.82 (m, 4H, piperazine), 3.54 (m, 4H, piperazine). MS (ESI): 382.9 [$M + H^+$]. Anal. Calcd for $C_{17}H_{14}N_6O_3S$: C, 53.40; H, 3.69; N, 21.98. Found: C, 53.54; H, 3.82; N, 21.76.

*6.1.3. O-Ethyl-4-(4-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl) piperazin-1-yl)benzimidate hydrochloride (**8**)*

A mixture of compound **7** (0.3 g, 0.78 mmol) in dry dichloromethane (90 mL) and dry ethanol (3.5 mL) was saturated with HCl gas and the reaction mixture was left at room temperature for 5 days. The precipitate **8** was filtered and washed with acetone. Yield: 75%; m.p > 300 °C; IR (KBr, cm^{-1}): 3448, 1601, 1357, 1242; ^1H NMR (500 MHz, DMSO-d_6): 7.85 (d, 2H, $J = 9$ Hz, phenyl), 7.87 (d, 1H, $J = 4$ Hz, furan), 7.41 (d, 1H, $J = 4$ Hz, furan), 7.10 (d, 2H, $J = 9$ Hz, phenyl), 4.48 (q, 2H, $J = 7$ Hz, $-CH_2-$), 3.67 (m, 8H, piperazine), 1.43 (t, 3H, $J = 7$ Hz, methyl). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 13.7 ($-CH_3$), 45.3 (C-8, piperazine), 46.4 ($-CH_2-$), 48.7 (C-7, piperazine), 112.1 (C-9, phenyl), 113.3 (C-10, phenyl), 114 (C-3, furan), 115.1 (C-2, furan), 130.4 (C-11, phenyl), 145.5 (C-12, phenyl), 147.2 (C-4, furan), 151.5 (C-1, furan), 153.8 (C-6, thiadiazole), 168.6 ($-C{=}N$), 172.3 (C-5, thiadiazole). MS (ESI): 429 [$M + H^+$]. Anal. Calcd for $C_{19}H_{21}ClN_6O_4S$: C, 49.08; H, 4.55; N, 18.08. Found: C, 48.93; H, 4.84; N, 17.77.

*6.1.4. Synthesis of compounds **2a**–**l***

*6.1.4.1. 4-(4-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)piperazin-1-yl)benzamidine hydrochloride (**2a**) [28].* This compound was prepared according to the literature method. The crude product was washed with propanol and crystallized from methanol–diethylether. Yield: 35%; m.p > 300 °C; IR (KBr, cm^{-1}): 3456, 3354, 1608, 1355, 1234; ^1H NMR (500 MHz, DMSO-d_6): 9.01 (br s, 2H), 8.64 (br s, 2H), 7.87 (d, 1H, $J = 4$ Hz, furan), 7.78 (d, 2H, $J = 9.2$ Hz, phenyl), 7.41 (d, 1H, $J = 4$ Hz, furan), 7.13 (d, 2H, $J = 9.2$ Hz, phenyl),3.74 (br s, 4H, piperazine), 3.64 (br s, 4H, piperazine). MS (ESI): 400 [$M + H^+$]. Anal. Calcd for $C_{17}H_{18}ClN_7O_3S$: C, 46.84; H, 4.16; N, 22.49. Found: C, 47.10; H, 3.97; N, 22.18.

*6.1.4.2. N-Methyl-4-(4-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl) piperazin-1-yl)benzamidine hydrochloride (**2b**).* The compound was obtained by treatment of the crude imidate **8** (0.1 g, 0.21 mmol) with methylamine 40% (0.31 mL, 4 mmol) in ethanol (5 mL) at

reflux for 90 min. The reaction mixture was concentrated under reduced pressure and the residue was washed with acetone and crystallized from ethanol–dioxan .Yield: 30%; m.p > 300 °C; IR (KBr, cm^{-1}): 3487, 1610, 1351, 1242; ^1H NMR (500 MHz, DMSO-d_6): 9.14 (br s, 3H), 7.85 (d, 1H, J = 3.8 Hz, furan), 7.71 (d, 2H, J = 8.6 Hz, phenyl), 7.39 (d, 1H, J = 3.8 Hz, furan), 7.13 (d, 2H, J = 8.6 Hz, phenyl), 3.74 (br s, 4H, piperazine), 3.60 (br s, 4H, piperazine), 2.96 (s, 3H, methyl). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 29.4 (−CH_3), 45.7 (C-8, piperazine), 48.9 (C-7, piperazine), 112.1 (C-9, phenyl), 113.8 (C-10, phenyl), 115.1 (C-3, furan), 116.6 (C-2, furan), 129.3 (C-11, phenyl), 145.5 (C-12, phenyl), 147.2 (C-4, furan), 151.5 (C-1, furan), 153.3 (C-6, thiadiazole), 162.3 (−C═N), 172.3 (C-5, thiadiazole). MS (ESI): 414 [M + H^+]. Anal. Calcd for $C_{18}H_{20}ClN_7O_3S$: C, 48.05; H, 4.48; N, 21.79. Found: C, 47.86; H, 4.38; N, 22.13.

*6.1.4.3. N-Ethyl-4-(4-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl) piperazin-1-yl)benzamidine hydrochloride (**2c**).* The compound was obtained by treatment of the crude imidate **8** (0.1 g, 0.21 mmol) with ethylamine 70% (0.17 mL, 3 mmol) in ethanol (5 mL) at reflux for 60 min. The reaction mixture was concentrated under reduced pressure and the residue was crystallized from methanol–diethylether. Yield: 25%; m.p > 300 °C; IR (KBr, cm^{-1}): 3406, 1606, 1360, 1231; ^1H NMR (500 MHz, DMSO-d_6): 7.87 (d, 1H, J = 4 Hz, furan), 7.67 (d, 2H, J = 8.8 Hz, phenyl), 7.41 (d, 1H, J = 4 Hz, furan), 7.07 (d, 2H, J = 8.8 Hz, phenyl), 3.74 (t, 4H, piperazine), 3.53 (t, 4H, piperazine), 3.3 (q, 2H, J = 7.2 Hz, CH_2), 1.19 (t, 3H, J = 7.2 Hz, methyl). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 14.0 (−CH_3), 37.7 (−CH_2−), 46.2 (C-8, piperazine), 49 (C-7, piperazine), 112.1 (C-9, phenyl), 114.1 (C-10, phenyl), 115.1 (C-3, furan), (C-3, furan), 116.8, 128.7 (C-11, phenyl), 145.5 (C-12, phenyl), 147.2 (C-4, furan), 151.5 (C-1, furan), 152.5 (C-6, thiadiazole), 160.2 (−C═N), 172.4 (C-5, thiadiazole). MS (ESI): 428 [M + H^+]. Anal. Calcd for $C_{19}H_{22}ClN_7O_3S$: C, 49.19; H, 4.78; N, 21.13. Found: C, 48.87; H, 4.92; N, 21.22.

*6.1.4.4. N-(1-Propyl)-4-(4-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)piperazin-1-yl) benzamidine hydrochloride (**2d**).* The compound was obtained by treatment of the crude imidate **8** (0.1 g, 0.21 mmol) with propylamine (0.16 mL, 2 mmol) in ethanol (5 mL) at reflux for 60 min. The reaction mixture was concentrated under reduced pressure and the residue was washed with diethylether and crystallized from 2-propanol–dioxan. Yield: 38%; m.p > 300 °C; IR (KBr, cm^{-1}): 3487, 1607, 1359, 1238; ^1H NMR (500 MHz, DMSO-d_6): 9.39 (s, 1H); 9.11 (s, 1H), 8.67 (s, 1H), 7.87 (d, 1H, J = 3.9 Hz, furan), 7.68 (d, 2H, J = 8.7 Hz, phenyl), 7.41 (d, 1H, J = 3.9 Hz, furan), 7.15 (d, 2H, J = 8.7 Hz, phenyl), 3.76 (t, 4H, piperazine), 3.61 (t, 4H, piperazine), 1.66 (m, 2H, J = 7.2 Hz, −CH_2−), 0.96 (t, 3H, J = 7.2 Hz, methyl). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 11.2 (−CH_3), 20.9 (−CH_2−), 44 (−CH_2−), 45.8 (C-8, piperazine), 48.9 (C-7, piperazine), 112.1 (C-9, phenyl), 113.8 (C-10, phenyl), 115.1 (C-3, furan), 116.9 (C-2, furan), 129.5 (C-11, phenyl), 145.5 (C-12, phenyl), 147.2 (C-4, furan), 151.5 (C-1, furan), 153.4 (C-6, thiadiazole), 161.9 (−C═N), 172.4 (C-5, thiadiazole). MS (ESI): 442 [M + H^+]. Anal. Calcd for $C_{20}H_{24}ClN_7O_3S$: C, 50.26; H, 5.06; N, 20.51. Found: C, 49.95; H, 4.87; N, 20.78.

*6.1.4.5. N-(1-Butyl)-4-(4-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)piperazin-1-yl)benzamidine hydrochloride (**2e**).* This compound was prepared by treatment of the crude imidate **8** with butylamine in ethanol at reflux for 45 min and resulting solid was washed with acetone and crystallized from methanol–diethylether. Yield: 40%; m.p > 300 °C; IR (KBr, cm^{-1}): 3432, 1610, 1351, 1233; ^1H NMR (500 MHz, DMSO-d_6): 9.39 (s, 1H), 9.12 (s, 1H); 8.68 (s, 1H), 7.89 (d, 1H, J = 3.35 Hz, furan), 7.68 (d, 2H, J = 8.4 Hz, phenyl), 7.42 (d, 1H, J = 3.35 Hz, furan), 7.15 (d, 2H, J = 8.4 Hz, phenyl), 3.76 (br s, 4H, piperazine), 3.61 (br s, 4H, piperazine), 3.57 (t, 2H, J = 7.1 Hz, −CH_2−), 1.61 (m, 2H, J = 7.1 Hz, −CH_2−), 1.39 (m, 2H, J = 7.1 Hz, −CH_2−), 0.94 (t, 3H, J = 7.1 Hz, methyl). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 13.6 (−CH_3), 19.5 (−CH_2−), 29.5 (−CH_2−), 42.1 (−CH_2−), 45.7 (C-8, piperazine), 48.8 (C-7, piperazine), 112.1 (C-9, phenyl), 113.8 (C-10, phenyl), 115.1 (C-3, furan), 116.9 (C-2, furan), 129.5 (C-11, phenyl), 145.5 (C-12, phenyl), 147.2 (C-4, furan), 151.5 (C-1, furan), 153.4 (C-6, thiadiazole), 161.9 (−C═N), 172.4 (C-5, thiadiazole). MS (ESI): 456 [M + H^+]. Anal. Calcd for $C_{21}H_{26}ClN_7O_3S$: C, 51.27; H, 5.33; N, 19.93. Found: C, 51.49; H, 5.22; N, 19.57.

*6.1.4.6. N-(2-Butyl)-4-(4-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)piperazin-1-yl)benzamidine hydrochloride (**2f**).* This compound was prepared by treatment of the crude imidate **8** with 2-butylamine in ethanol at reflux for 6 h and resulting solid was crystallized from ethanol–diethylether and then from methanol–dioxan. Yield: 25%; m.p > 300 °C; IR (KBr, cm^{-1}): 3433, 1606, 1353, 1238; ^1H NMR (500 MHz, DMSO-d_6): 7.89 (d, 1H, J = 3.85 Hz, furan), 7.68 (d, 2H, J = 8.4 Hz, phenyl), 7.43 (d, 1H, J = 3.85 Hz, furan), 7.15 (d, 2H, J = 8.4 Hz, phenyl), 3.85 (m, 1H); 3.76 (br s, 4H, piperazine), 3.59 (br s, 4H, piperazine), 1.65 (m, 1H, J = 7.1 Hz, −CH−), 1.59 (m, 1H, J = 6.5 Hz, −CH−), 1.22 (d, 3H, J = 6.25 Hz, methyl), 0.92 (t, 3H, J = 7.2, methyl). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 10.4 (−CH_3), 19.3 (−CH_2−), 28.2 (−CH_2−), 30.9 (−CH_2−), 45.9 (C-8, piperazine), 48.9 (C-7, piperazine), 50.2 (−CH_2−), 112.1 (C-9, phenyl), 113.8 (C-10, phenyl), 115.1 (C-3, furan), 116.9 (C-2, furan), 129.6 (C-11, phenyl), 145.5 (C-12, phenyl), 147.2 (C-4, furan), 151.5 (C-1, furan), 153.3 (C-6, thiadiazole), 161.3 (−C═N), 172.4 (C-5, thiadiazole). MS (ESI): 456 [M + H^+]. Anal. Calcd for $C_{21}H_{26}ClN_7O_3S$: C, 51.27; H, 5.33; N, 19.93. Found: C, 51.56; H, 5.45; N, 19.74.

*6.1.4.7. N-Benzyl-4-(4-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl) piperazin-1-yl)benzamidine hydrochloride (**2g**).* This compound was prepared by treatment of the crude imidate **8** with benzylamine in ethanol at reflux for 3 h and resulting solid was washed with THF and crystallized from methanol–diethylether. Yield: 25%; m.p > 300 °C; IR (KBr, cm^{-1}): 3434, 1606, 1350, 1236; ^1H NMR (500 MHz, DMSO-d_6): 7.88 (d, 1H, J = 4 Hz, furan), 7.75 (d, 2H, J = 8 Hz, phenyl), 7.41 (m, 5H, phenyl), 7.34 (d, 1H, J = 4 Hz, furan), 7.15 (d, 2H, J = 8 Hz, phenyl), 4.7 (s, 2H, −CH_2−), 3.75 (br s, 4H, piperazine), 3.61 (br s, 4H, piperazine). MS (ESI): 490 [M + H^+]. Anal. Calcd for $C_{24}H_{24}ClN_7O_3S$: C, 54.80; H, 4.60; N, 18.64. Found: C, 54.47; H, 4.83; N, 18.83.

*6.1.4.8. N-(4-Methoxybenzyl)-4-(4-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)piperazin-1-yl)benzamidine hydrochloride (**2h**).* This compound was prepared by treatment of the crude imidate **8** with *p*-methoxybenzylamine in ethanol at reflux for 60 min. The resulting solid was washed with diethylether and acetone and crystallized from ethanol–diethylether. Yield: 40%; m.p > 300 °C; IR (KBr, cm^{-1}): 3440, 1608, 1351, 1237; ^1H NMR (500 MHz, DMSO-d_6): 8.07 (br s, 3H), 7.88 (d, 1H, J = 3.8 Hz, furan), 7.72 (d, 2H, J = 8.7 Hz, phenyl), 7.42 (d, 1H, J = 3.8 Hz, furan), 7.36 (d, 2H, J = 8.35 Hz, phenyl), 7.15 (d, 2H, J = 8.7 Hz, phenyl), 6.98 (d, 2H, J = 8.35), 3.94 (s, 2H, −CH_2−), 3.76 (br s, 8H, piperazine), 3.62 (s, 3H, methoxy). MS (ESI): 519.9 [M + H^+]. Anal. Calcd for $C_{25}H_{26}ClN_7O_4S$: C, 54.00; H, 4.71; N, 17.63. Found: C, 54.19; H, 4.57; N, 17.92.

*6.1.4.9. N-((Tetrahydrofuran-2-yl)methyl)-4-(4-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)piperazin-1-yl)benzamidine hydrochloride (**2i**).* This compound was prepared by treatment of the crude imidate **8** with 2-tetrahydrofurfurylamine in ethanol at reflux for 60 min. The resulting solid was washed with hot acetone, diethylether and chloroform. Yield: 25%; m.p > 300 °C; IR (KBr, cm^{-1}): 3430, 1607, 1351, 1234; ^1H NMR (500 MHz, DMSO-d_6): 7.85 (d, 1H, J = 4 Hz, furan), 7.66 (d, 2H, J = 8.8 Hz, phenyl), 7.39 (d, 1H, J = 4 Hz, furan), 7.14 (d, 2H, J = 8.8 Hz, phenyl), 4.09 (m, 1H, OCH−), 3.8 (m,

1H, –CH–); 3.75 (m, 4H, piperazine), 3.68 (m, 1H, –CH–), 3.60 (m, 1H, –CH–), 3.47 (m, 5H, piperazine and –CH–), 2.02 (m, 1H, –CH–), 1.87 (m, 2H, $-CH_2-$); 1.59 (m, 1H, –CH–). MS (ESI): 484 [M + H^+]. Anal. Calcd for $C_{22}H_{26}ClN_7O_4S$: C, 50.81; H, 5.04; N, 18.86. Found: C, 51.16; H, 5.13; N, 18.61.

*6.1.4.10. Morpholino(4-(4-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)piperazin-1-yl)phenyl)methanimine hydrochloride (**2j**).* This compound was prepared by treatment of the crude imidate **8** with morpholine in ethanol at reflux for 60 min. The resulting solid was washed with 2-propanol and acetone and crystallized from methanol and dioxan. Yield: 35%; m.p > 300 °C; IR (KBr, cm^{-1}): 3448, 1604, 1353, 1229; ^{1}H NMR (500 MHz, DMSO-d_6): 7.88 (d, 1H, J = 3.75 Hz, furan), 7.47 (d, 2H, J = 8.5 Hz, phenyl), 7.42 (d, 1H, J = 3.75, furan), 7.16 (d, 2H, J = 8.5 Hz, phenyl), 3.75 (br s, 8H, piperazine and morpholine), 3.57 (br s, 8H, piperazine and morpholine). MS (ESI): 469.9 [M + H^+]. Anal. Calcd for $C_{21}H_{24}ClN_7O_4S$: C, 49.85; H, 4.78; N, 19.38. Found: C, 49.72; H, 4.57; N, 19.57.

*6.1.4.11. 1-(4-(4,5-Dihydro-1H-imidazol-2-yl)phenyl)-4-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)piperazine hydrochloride (**2k**).* This compound was prepared by treatment of the crude imidate **8** with 1,2-ethylene diamine in ethanol at reflux for 60 min. The resulting solid was washed with acetone and crystallized from ethanol–diethylether. Yield: 40%; m.p > 300 °C; IR (KBr, cm^{-1}): 3444, 1606, 1357, 1235; ^{1}H NMR (500 MHz, DMSO-d_6): 7.87 (d, 1H, J = 3.55 Hz, furan), 7.41 (d, 2H, J = 8.35 Hz, phenyl), 7.42 (d, 1H, J = 3.55, furan), 7.02 (d, 2H, J = 8.35 Hz, phenyl), 3.74 (br s, 4H, imidazoline), 3.57 (br s, 4H, piperazine), 3.41 (br s, 4H, piperazine). MS (ESI): 426 [M + H^+]. Anal. Calcd for $C_{19}H_{20}ClN_7O_3S$: C, 49.40; H, 4.36; N, 21.23. Found: C, 49.11; H, 4.55; N, 21.51.

*6.1.4.12. 1,4,5,6-Tetrahydro-2-(4-(4-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)piperazin-1-yl)phenyl)pyrimidine hydrochloride (**2l**).* This compound was prepared by treatment of the crude imidate **8** with 1,3-diaminopropane in ethanol at reflux for 60 min. The resulting solid was washed with acetone and crystallized from ethanol–diethylether. Yield: 32%; m.p > 300 °C; IR (KBr cm^{-1}): 3461, 1605, 1350, 1229; ^{1}H NMR (500 MHz, DMSO-d_6): 9.75 (s, 1H), 8.11 (br s, 2H), 7.87 (br s, 1H, furan), 7.69 (d, 2H, J = 7.6 Hz, phenyl), 7.41 (br s, 1H, furan), 7.15 (d, 2H, J = 7.6 Hz, phenyl), 3.75 (br s, 4H, piperazine), 3.60 (br s, 4H, piperazine), 3.45 (br s, 2H, 6-CH_2 tetrahydropyrimidine), 2.88 (br s, 2H, 4-CH_2 tetrahydropyrimidine), 1.95 (m, 2H, 5-CH_2 tetrahydropyrimidine). MS (ESI): 440 [M + H^+]. Anal. Calcd for $C_{19}H_{20}ClN_7O_3S$: C, 50.47; H, 4.66; N, 20.60. Found: C, 50.11; H, 4.53; N, 20.85.

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References

[1] http://www.who.int/tdr/diseases/leish/diseaseinfo.htm.
[2] P. Desjeux, Comp. Immunol. Microbiol. Infect. Dis. 27 (2004) 305–318.
[3] S.L. Croft, Parasitology 114 (1997) S3–S15.
[4] H.D. Engers, R. Bergquist, F. Moddaber, Dev. Biol. Stand. 87 (1996) 73–84.
[5] M. Ouellette, J. Drummelsmith, B. Papadopoulou, Drug Resist. Update 7 (2004) 257–266.
[6] S.L. Croft, K. Seifert, V. Yardley, Indian J. Med. Res. 123 (2006) 399–410.
[7] H.W. Murray, Am. J. Trop. Med. Hyg. 71 (2004) 787–794.
[8] C. Bell, J. Hall, D. Kyle, M. Grogl, K. Ohemeng, M. Allen, R. Tidwell, Antimicrob. Agents Chemother. 34 (1990) 1381–1386.
[9] S.C. Sweetman (Ed.), Martindale: The Complete Drug Reference, thirty fifth ed. Pharmaceutical Press, London (UK), 2007.
[10] K. Werbovetz, Antimicrob. Agents Chemother. 52 (2008) 4098–4114.
[11] D.A. Patrick, S.A. Bakunov, S.M. Bakunova, E.V.K. Suresh Kumar, H. Chen, S.K. Jones, T. Wenzler, T. Barzcz, K.A. Werbovetz, R. Brun, R.R. Tidwell, Eur. J. Med. Chem. 44 (2009) 3543–3551.
[12] R.F. Rodrigues, E.F. da Silva, A. Echevarria, R. Fajardo-Bonin, V.F. Amaral, L.L. Leon, M.M. Canto-Cavalheiro, Eur. J. Med. Chem. 42 (2007) 1039–1043.
[13] A. Khalaj, M. Nakhjiri, A.S. Negahbani, M. Samadizadeh, L. Firoozpour, S. Rajabalian, N. Samadi, M.A. Faramarzi, N. Adibpour, A. Shafiee, A. Foroumadi, Eur. J. Med. Chem. 46 (2011) 65–70.
[14] E. Alipour, S. Emami, A. Yahya-Meymandi, M. Nakhjiri, F. Johari, S.K. Ardestani, F. Poorrajab, M. Hosseini, A. Shafiee, A. Foroumadi, J. Enzyme Inhib. Med. Chem. 26 (2011) 123–128.
[15] M.H. Moshafi, A. Yahya-Meymandi, S.E. Sadat-Ebrahimi, S. Emami, M. Nakhjiri, F. Siavoshi, M. Omrani, M. Vosooghi, E. Alipour, A. Shafiee, A. Foroumadi, J. Serb, Chem. Soc. 76 (2011) 201–210.
[16] A. Foroumadi, S. Pournourmohammadi, F. Soltani, M.A. Rezaee, S. Dabiri, A. Kharazmi, A. Shafiee, Bioorg. Med. Chem. Lett. 15 (2005) 1983–1985.
[17] F. Poorrajab, S.K. Ardestani, A. Foroumadi, S. Emami, A. Kariminia, M. Behrouzi-Fardmoghadam, A. Shafiee, Exp. Parasitol. 121 (2009) 323–330.
[18] M. Behrouzi-Fardmoghadam, F. Poorrajab, S.K. Ardestani, S. Emami, A. Shafiee, A. Foroumadi, Bioorg. Med. Chem. Lett. 16 (2008) 4509–4515.
[19] V.J. Ram, M. Nath, Curr. Med. Chem. 3 (1996) 303–316.
[20] S.L. Croft, V. Yardley, Curr. Pharm. Des. 8 (2002) 319–342.
[21] A. Mayence, J.J. Vanden Eynde, L. LeCour Jr., L.A. Walker, B.L. Tekwani, T.L. Huang, Eur. J. Med. Chem. 39 (2004) 547–553.
[22] R.L. Shriner, F.W. Neumann, Chem. Rev. 35 (1944) 351–425.
[23] R. Roger, D.G. Neilson, Chem. Rev. 61 (1961) 179–211.
[24] Z. Nazarian, S. Emami, S. Heydari, S.K. Ardestani, M. Nakhjiri, F. Poorrajab, A. Shafiee, A. Foroumadi, Eur. J. Med. Chem. 44 (2010) 1424–1429.
[25] A. Dutta, S. Bandyopadhyay, C. Mandal, M. Chatterjee, Parasitol. Int. 54 (2005) 119–122.
[26] A.K. Tanaka, V.B. Valero, H.K. Takahashi, A.H. Straus, J. Antimicrob. Chemother. 59 (2007) 487–492.
[27] A.F. Kiderlen, P.M. Kaye, J. Immunol. Methods 127 (1990) 11–18.
[28] B. Tao, T.L. Huang, Q. Zhang, L. Jackson, S.F. Queener, I.O. Donkor, Eur. J. Med. Chem. 34 (1999) 531–538.