European Journal of Medicinal Chemistry 50 (2012) 124–128

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and anti-leishmanial activity of 5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amines containing *N*-[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl] moieties

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ARTICLE INFO

Article history:
Received 28 November 2011
Received in revised form
22 January 2012
Accepted 24 January 2012
Available online 30 January 2012

Keywords:
Antileishmanial activity
5-Nitrofuran
1,3,4-Thiadiazole
1*H*-1,2,3-Triazole

ABSTRACT

A novel series of 5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amines were synthesized by introducing *N*-[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl] moiety as a new functionality on the C-2 amine of thiadiazole ring via click chemistry. The title compounds namely, *N*-[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amines (**3a**–**n**) were characterized by IR, NMR and MS spectra. These compounds were evaluated for their *in vitro* anti-leishmanial activity against promostigote form of the *Leishmania major*. Most compounds exhibited good anti-leishmanial activity against the promastigote form of *L. major*. The most active compound against promostigotes was found to be 4-methylbenzyl analog **3i**, which significantly decreases the number of intracellular amastigotes per macrophage, percentage of macrophage infectivity and infectivity index.

1. Introduction

Leishmaniasis is a major public health problem in new world and causing morbidity and mortality in tropical and subtropical regions of the world. According to WHO reports, it is estimated that 15–20 million infected and annually about three million people will threaten worldwide [1]. The disease is caused by different species of *Leishmania sp.* and transmitted by phelbotomine sandfly. It is manifested in three forms; visceral leishmaniasis, cutaneous leishmaniasis, muco-cutaneous leishmaniasis. In spite of the importance of these tropical and subtropical infections, an effective vaccine is not yet available. Some currently used drugs such as, sodium stibogluconate (pentostam), meglumine antimonate (glucantime), miltefosine, pentamidine and amphotericin B are toxic and cause severe side effects such as pancreatitis and cardiac toxicity. Moreover, they are expensive and required long-term treatment [2–4]. In addition, the development of the clinical resistance and increase of co-infected leishmaniasis with AIDS in some regions is a serious problem [5]. Thus, the development of new, efficient, cheap and safe drug for the treatment of leishmaniasis is imperative. Substituted five-membered heterocyclic rings have diverse antimicrobial activities. Recently, several furanyl and thiophenyl azoles were synthesized and evaluated for their *in vitro* anti-leishmanial activity [6].

In recent years, significant attention have been aroused to 'click chemistry' for their easy and efficient synthesis of 1,2,3-triazole [7,8] that has occupied an important position in medicinal chemistry [9] owing to its chemotherapeutic effect such as antineoplasm [10], antibacterial [11], antifungal [12], antitubercular [13], anti-HIV [14] activities. On the other hand, 1,3,4-thiadiazole is well established to have the excellent antiparasitic property and its attachment to other heterocycles often changes the bioresponses, depending upon the type of substituent and position of attachment [15]. In our previous papers [16–18], we described the synthesis and *in vitro* anti-leishmanial activity of a series of 5-(5-nitrofuran-2-yl)-1,3,4-thiadiazoles **1** containing a cyclic amine at C-2 position of thiadiazole (Fig. 1). Recently, we have focused our attention on modification of the C-2 cyclic amine of the 5-(5-nitrofuran-2-yl)-

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doi:10.1016/j.ejmech.2012.01.046

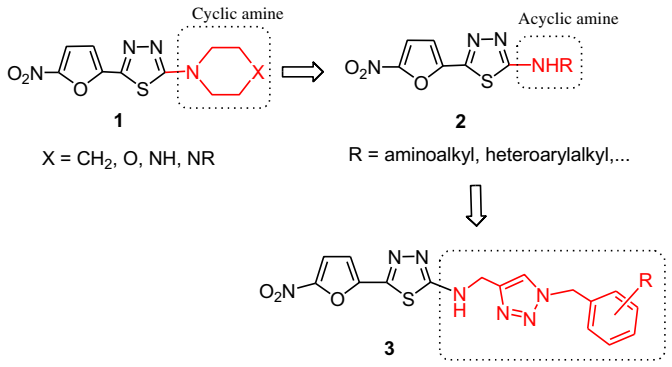
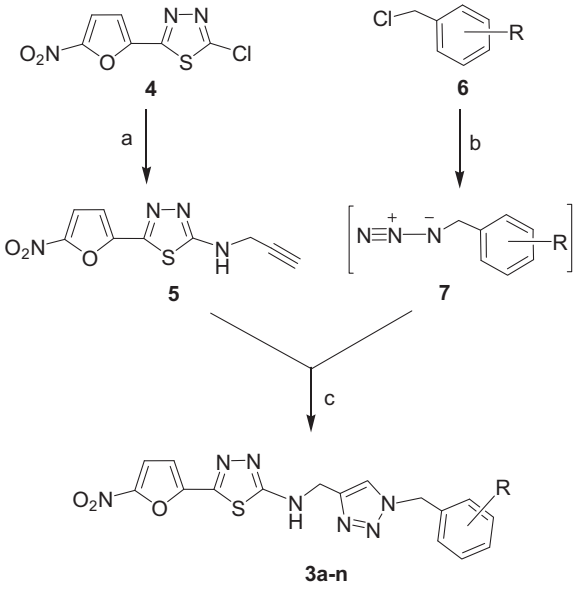


Fig. 1. General structures of previously described compounds **1** and **2**, and new designed compounds **3**.

1,3,4-thiadiazole-2-amines by introducing acyclic amines (Fig. 1, structure **2**) [19]. Accordingly, our strategy to achieve a novel anti-leishmanial agent has focused on introducing *N*-[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl] moiety as a new functionality on the C-2 amine of thiadiazole ring via click chemistry. Thus, we report here the synthesis and anti-leishmanial activity of *N*-[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amines (**3a**–**n**) (Fig. 1).

2. Chemistry

The synthetic pathway to achieve title compounds **3a**–**n** is shown in Scheme 1. The alkyne derivative **5** was synthesized in 76% yield by the reaction of 2-chloro-1,3,4-thiadiazole **4** with propargyl amine in absolute ethanol. The benzyl azide intermediates **7** were prepared in situ by the reaction of benzyl chlorides **6** with excess NaN_3 (1.5 equiv) in the presence of triethylamine in *t*-BuOH/water. The key step was 1,3-dipolar cycloaddition of alkyne **5** with benzyl azides **7** using catalytic amount of copper iodide in *t*-BuOH/H_2O at room temperature. All of the compounds were characterized by IR, NMR and MS spectra.



Scheme 1. Synthesis of compounds **3a**–**n**. *Reagents and conditions*: (a) propargyl amine, EtOH, reflux; (b) sodium azide, triethyl amine, *t*-BuOH, H_2O; (c) CuI.

3. Pharmacology

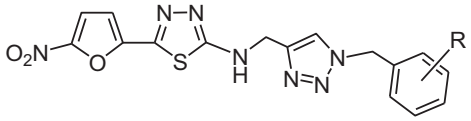
Primarily, the anti-promastigote activity of target compounds **3a**–**n** was evaluated by direct counting and MTT assay according to the literature method [20]. The IC_{50}s (50% inhibitory concentrations) of compounds against promastigote form of *Leishmania major* (vaccine strain MRHO/IR/75/ER, obtained from Pasteur institute, Tehran, Iran) was determined. Two or more independent experiments in triplicate were performed for each compound. The IC_{50}s were calculated by linear regression analysis, expressed in mean values and presented in Table 1. Meglumine antimonate (Glucantime®) was used as a standard drug.

Also, the *in vitro* anti-amastigote activity of compounds was determined in mouse peritoneal macrophages. Briefly, macrophages were placed on sterile glass cover slips in 24-well plates (1×10^6/well). Then, the stationary phase promastigotes in RPMI were added (2×10^6 parasites/well, three parasites/macrophage) to macrophage monolayer and the plates were incubated for 2 h. After removal of extracellular parasites by washing, new media containing IC_{50} concentration of the compounds were added. Two sets of experiments were carried out for each compound at 24 h. Following these procedures, cells were fixed with methanol and stained with Giemsa stain (Sigma). 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) [21]. The results of *in vitro* activity of selected compounds against intramacrophage amastigotes of *L. major* are depicted in Fig. 2.

4. Results and discussion

For evaluation of anti-leishmanial properties of target compounds, the *in vitro* activity was assessed against promastigote (extracellular parasite) and amastigote (intramacrophage parasite) forms of *L. major*. The IC_{50} values of compounds against promastigotes are presented in Table 1. Generally, compounds **3a**–**d**, **3g**–**i**, **3k,** and **3m** showed good activity (IC_{50} values <26 μM), followed by compound **3j** with IC_{50} value of ≈33 μM. The remaining compounds exhibited weak or no activity against promastigotes

Table 1
Anti-promastigote activity (IC_{50}, μM) of compounds **3a**–**n.**



Compound	*R*	IC_{50} (μM)
3a	H	19. 6 ± 0.56
3b	2-F	19.0 ± 0.35
3c	4-F	21.4 ± 1.86
3d	2-Cl	18.9 ± 0.12
3e	3-Cl	107.7 ± 0.57
3f	4-Cl	88.8 ± 0.9
3g	2-Me	25.2 ± 0.54
3h	3-Me	21. 9 ± 0.89
3i	4-Me	12.2 ± 0.66
3j	4-NO_2	32.7 ± 0.31
3k	2-F-6-Cl	17.4 ± 0.76
3l	2,3-Cl_2	103.5 ± 0.58
3m	2,4-Cl_2	19. 9 ± 0.84
3n	3,4-Cl_2	131.8 ± 1.11

The IC_{50} of Glucantime was 68.3 mM.

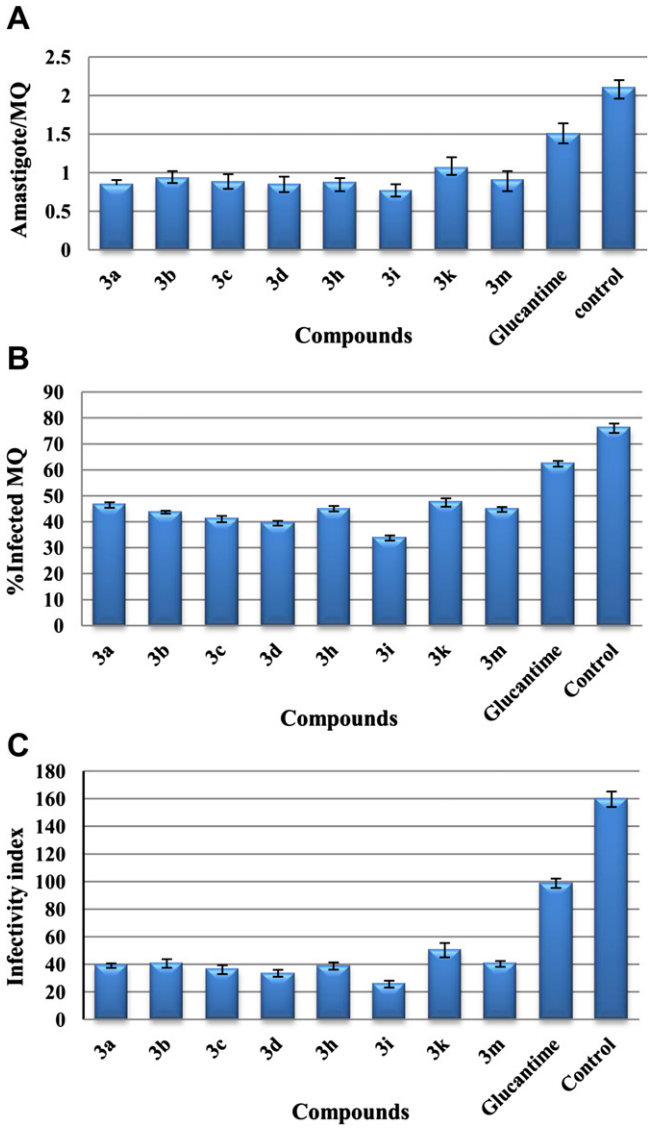


Fig. 2. *In vitro* activity of selected compounds against intramacrophage amastigotes of *L. major*. (A) The mean number of amastigotes per macrophage after treatment with selected compounds for 24 h. (B) The percentage of infected macrophages after treatment. (C) Infectivity index of macrophages cultured 24 h in presence of selected compound. The infectivity index was determined by multiplying the percentage of macrophages that had at least one intracellular parasite by the average number of intracellular parasite per infected macrophage (100 cells were examined/well).

($IC_{50} \geq 88.8$ μM). 4-Methyl analog **3i** was the most potent compound in the terms of anti-promastigote activity. Based on structural points of view, introduction of different substituents on pendent benzyl group could not improve the activity with the exception of 4-methyl substitution which slightly increased the anti-promastigote activity. Among the halogenated compounds, derivatives having 2-halo- substitutions (compounds **3b**, **3d**, **3k** and **3m**) exhibited significant activity. Thus, the introduction of halogen on 3-position of benzyl moiety could be tolerated but 3-chloro substituent decreased anti-promostigote activity (as in compounds **3e**, **3l**, and **3n** with IC_{50} values >100 μM, respectively).

Compounds **3a-d**, **3h**, **3i**, **3k**, and **3m** having good inhibition against promastigotes were also evaluated for their activity against amastigote form of *L. major* in marine peritoneal macrophages (Fig. 2). All tested compounds significantly decreased the number of intracellular amastigotes per macrophage, percentage of macrophage infectivity and infectivity index (Fig. 2A, B and C, respectively). It is notable that 4-Methyl analog **3i** which showed potent activity against promastigotes was the most effective compound against amastigotes as showed in the terms of amastigote number per macrophage, the percentage of macrophage infectivity and infectivity index.

In our previous studies [16–18], we have presented the synthesis and *in vitro* anti-leishmanial activity of 5-(5-nitrofuran-2-yl)-1,3,4-thiadiazoles against promastigote and amastigote forms *L. major* strain, which several of them showed good activity in both forms of promastigote and amastigote. Previous results demonstrated that an amine substituent at C-2 position of 5-(5-nitrofuran-2-yl)-1,3,4-thiadiazoles which connected to a distal hydrophilic atom (O or N) has profound role in the biological activity of these compounds. The distal amine could be a part of cyclic amine (e.g. piperazine), acyclic amine (e.g. aminoalkylamine) or nitrogen containing heteroaromatic linked to C-2 amine of 1,3,4-thiadiazol-2-amines. The former type of pendent amine can be exemplified by pyrimidynyl methyl moiety [19]. In this study, we described the synthesis and anti-leishmanial activity of a series of 5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amines containing *N*-[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl] residue. In this case, 1,2,3-triazole ring plays as nitrogen containing heterocycle in distal position. From the results, it demonstrated that C-2 substituent is the most flexible site for chemical modification and is an area where it determines the potency and physicochemical properties of 5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amines.

5. Conclusion

In summary, a series of *N*-[(1-benzyl-1*H*-1,2,3-triazol-4-yl) methyl]-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amines were synthesized and evaluated for their *in vitro* inhibitory activity against the *Leishmania* parasite. Most of the target compounds exhibited good anti-leishmanial activity against the promastigote form of *L. major*. The most active compound against promostigotes was found to be 4-methylbenzyl analog **3i**, which significantly decreases the number of intracellular amastigotes per macrophage, percentage of macrophage infectivity and infectivity index.

6. Experimental protocols

6.1. Chemistry

All starting materials, reagents and solvents were purchased from Merck AG Chemical. The intermediate 2-chloro-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazole (**4**) was prepared from 5-nitrofurfurylidene diacetate according to the previously described method [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 500 and 400 spectrometer and chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard. The mass spectra were run on an Agilent 6410 LC-MS or a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV. Elemental analyses were carried out on 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 F254 plates were used for analytical TLC.

6.1.1. Synthesis of 5-(5-nitrofuran-2-yl)-N-(prop-2-ynyl)-1,3,4-thiadiazol-2-amine (**5**)

A mixture of compound **4** (0.1 g, 0.43 mmol) with propargyl amine (0.03 mL, 0.43 mmol) in absolute ethanol (7 mL) was

refluxed for 24 h. The completion of reaction was detected by TLC. The solvent was evaporated under reduced pressure and the resulting product was purified using silica gel column chromatography eluting with $CHCl_3$ and then EtOAc. Compound **5** was obtained as a yellow solid. Yield 76%; m.p 204–206 °C; IR (KBr, cm^{-1}) 3299, 3207, 2121, 1566, 1345; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.45 (d, 1H, $J = 4$ Hz, furan), 7.16 (d, 1H, $J = 4$ Hz, furan), 4.27 (br s, 2H, $-CH_2-$), 2.35 (s, 1H, ≡CH). Anal. calcd for $C_9H_6N_4O_3S$: C, 43.20; H, 2.42; N, 22.39. Found: C, 43.53; H, 2.54; N, 22.12.

*6.1.2. General procedure for the synthesis of compounds **3a–n***

A mixture of 1.3 mmol of Et_3N, 0.9 mmol of sodium azide and 1.1 mmol of appropriate benzyl chloride **6** in 4 mL of water and 4 mL of *t*-BuOH was stirred vigorously for 30 min at room temperature. Then, 0.5 mmol of compound **5** and 7% mol of CuI were added into the mixture and stirred for 24–56 h at room temperature. The completion of reaction was detected by TLC. The reaction mixture was diluted with water, cooled in ice. A brown precipitate was formed, filtrated and washed three times with 20 mL of cold water. The resulting product was purified using silica gel column chromatography.

*6.1.2.1. N-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amine (**3a**).* The resulting product was purified using silica gel column chromatography eluting with $CHCl_3$ and then EtOAc containing 2% methanol. Compound **3a** was obtained as a yellow solid. Yield: 76%; m.p 180–182 °C; MS (*m*/*z*, %) 383 (M^+, 69), 368 (26), 353 (7), 339 (7), 313 (16), 292 (10), 264 (28), 236 (13), 212 (9), 201 (9), 183 (13), 144 (27), 104 (7), 91(100), 82 (10), 65 (8), 57 (10), 43(8); IR (KBr, cm^{-1}) 3200, 1542, 1489, 1459, 1358; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.81 (br s, 1H, –NH–), 8.16 (s, 1H, triazole), 7.84 (d, 1H, $J = 3.6$ Hz, furan), 7.38–7.30 (m, 6H, furan and phenyl), 5.59 (s, 2H, $-CH_2$-), 4.63 (d, 2H, $J = 5.2$ Hz, $-CH_2$-); Anal. calcd for $C_{16}H_{13}N_7O_3S$: C, 50.12; H, 3.42; N, 25.57. Found: C, 49.84; H, 3.61; N, 25.82.

*6.1.2.2. N-((1-(2-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amine (**3b**).* The resulting product was purified using silica gel column chromatography eluting with $CHCl_3$ and then EtOAc. Compound **3b** was obtained as a yellow solid. Yield 72%; m.p 186–187 °C; MS (ESI): 401.7 [M + H^+]. IR (KBr, cm^{-1}) 3221, 1619, 1536, 1495, 1352; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.8 (t, 1H, $J = 4.8$ Hz, –NH–), 8.12 (s, 1H, triazole), 7.83 (d, 1H, $J = 3.6$ Hz, furan), 7.42–7.36 (m, 2H, phenyl), 7.33 (d, 1H, $J = 3.6$ Hz, furan), 7.26–7.19 (m, 2H, phenyl), 5.65 (s, 2H, $-CH_2$-), 4.62 (d, 2H, $J = 4.8$ Hz, $-CH_2$-). Anal. calcd for $C_{16}H_{12}FN_7O_3S$: C, 47.88; H, 3.01; N, 24.43. Found: C, 47.53; H, 2.81; N, 24.16.

*6.1.2.3. N-((1-(4-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amine (**3c**).* The resulting product was extracted with 30 mL of $CHCl_3$ and washed with water. The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure. Compound **3c** was obtained as an orange solid. Yield 70%; m.p 197–200 °C; MS (ESI): 401.7 [M + H^+]. IR (KBr, cm^{-1}) 3243, 1543, 1508, 1490, 1352; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.82 (t, 1H, $J = 5$ Hz, –NH–), 8.17 (s, 1H, triazole), 7.85 (d, 1H, $J = 3.2$ Hz, furan), 7.44–7.37(m, 2H, phenyl), 7.36 (d, 1H, $J = 3.2$ Hz, furan), 7.20 (t, 2H, $J = 8.8$ Hz, phenyl), 5.58 (s, 2H, $-CH_2$-), 4.63 (d, 2H, $J = 5$ Hz, $-CH_2$-). Anal. calcd for $C_{16}H_{12}FN_7O_3S$: C, 47.88; H, 3.01; N, 24.43. Found: C, 48.03; H, 3.01; N, 24.67.

*6.1.2.4. N-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amine (**3d**).* The resulting moist solid was extracted with 40 mL of EtOAc and washed with water. The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure. Compound **3d** was obtained as a yellow solid. Yield 67%; m.p 185–186 °C; MS (*m*/*z*, %) 419 ([M + 2]$^+$, 9), 417 (M^+, 24), 264 (8), 216 (8), 178 (24), 138 (9), 127 (35), 125 (100), 99 (7), 89(20), 82 (15); IR (KBr, cm^{-1}) 3235, 1618, 1533, 1493, 1351; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.84 (br s, 1H, –NH–), 8.14 (s, 1H, triazole), 7.85 (d, 1H, $J = 3.8$ Hz, furan), 7.51 (d, 1H, $J = 7.6$ Hz, phenyl), 7.39–7.34 (m, 3H, furan and phenyl), 7.21 (d, 1H, $J = 7.6$ Hz, phenyl), 5.7 (s, 2H, $-CH_2$-), 4.63 (d, 2H, $J = 5$ Hz, $-CH_2-$); Anal. calcd for $C_{16}H_{12}ClN_7O_3S$: C, 45.99; H, 2.89; N, 23.47. Found: C, 46.14; H, 3.1; N, 23.44.

*6.1.2.5. N-((1-(3-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amine (**3e**).* The resulting product was purified using silica gel column chromatography eluting with $CHCl_3$ and then EtOAc. Compound **3e** was obtained as an orange solid. Yield 71%; m.p 195–196 °C; IR (KBr, cm^{-1}) 3241, 1550, 1510, 1489, 1352; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.82 (t, 1H, $J = 5.6$ Hz, –NH–), 8.2 (s, 1H, triazole), 7.83 (d, 1H, $J = 3.6$ Hz, furan), 7.41–7.38 (m, 3H, phenyl), 7.34 (d, 1H, $J = 3.6$ Hz, furan), 7.28–7.25 (m, 1H, phenyl), 5.61 (s, 2H, $-CH_2$-), 4.63 (d, 2H, $J = 5.6$ Hz, $-CH_2$-). Anal. calcd for $C_{16}H_{12}ClN_7O_3S$: C, 45.99; H, 2.89; N, 23.47. Found: C, 45.80; H, 2.63; N, 23.75.

*6.1.2.6. N-((1-(4-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amine (**3f**).* The resulting product was purified using silica gel short column chromatography eluting with $CHCl_3$ and then EtOAc. Compound **3f** was obtained as an orange solid. Yield 35%; m.p 212–213 °C; IR (KBr, cm^{-1}) 3202, 1566, 1530, 1462, 1353. ^1H NMR (DMSO-d_6, 500 MHz) δ 8.83 (t, 1H, $J = 5.4$ Hz, –NH–), 8.18 (s, 1H, triazole), 7.86 (d, 1H, $J = 4$ Hz, furan), 7.44 (d, 2H, $J = 8.4$ Hz, phenyl), 7.36 (d, 1H, $J = 4$ Hz, furan), 7.34 (d, 2H, $J = 8.4$ Hz, phenyl), 5.6 (s, 2H, $-CH_2$-), 4.63 (d, 2H, $J = 5.4$ Hz, $-CH_2$-). Anal. calcd for $C_{16}H_{12}ClN_7O_3S$: C, 45.99; H, 2.89; N, 23.47. Found: C, 46.72; H, 3.12; N, 23.48.

*6.1.2.7. N-((1-(2-Methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amine (**3g**).* The resulting product was purified using silica gel column chromatography eluting with CH_2Cl_2 and then EtOAc. Compound **3g** was obtained as a yellow solid. Yield 46%; m.p 179–183 °C; MS (*m*/*z*, %) 397 (M^+, 38), 292 (15), 279 (23), 264 (20), 226 (9), 212 (20), 203 (9), 185 (10), 167 (39), 158 (35), 149 (98), 118 (8), 105 (100), 79 (13), 57 (9); IR (KBr, cm^{-1}) 3189, 1569, 1529, 1491, 1353; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.81 (br s, 1H, –NH–), 8.1 (s, 1H, triazole), 7.85 (d, 1H, $J = 3.6$ Hz, furan), 7.69 (d, 1H, $J = 7.6$ Hz, phenyl), 7.35 (d, 1H, $J = 3.6$ Hz, furan), 7.24–7.18 (m, 2H, phenyl), 7.08 (d, 1H, $J = 7.6$ Hz, phenyl), 5.6 (s, 2H, $-CH_2$-), 4.63 (br s, 2H, $-CH_2$-), 2.31 (s, 3H, CH_3); Anal. calcd for $C_{17}H_{15}N_7O_3S$: C, 51.38; H, 3.80; N, 24.67. Found: C, 51.09; H, 3.52; N, 24.44.

*6.1.2.8. N-((1-(3-Methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amine (**3h**).* The resulting product was purified using silica gel column chromatography eluting with CH_2Cl_2 and then EtOAc. Compound **3h** was obtained as an orange solid. Yield 55%; m.p 168–169 °C; MS (ESI): 397.7 [M + H^+]. IR (KBr, cm^{-1}) 3180, 1545, 1491, 1352; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.81 (br s, 1H, –NH–), 8.14 (s, 1H, triazole), 7.84 (d, 1H, $J = 4$ Hz, furan), 7.34 (d, 1H, $J = 4$ Hz, furan), 7.25 (t, 1H, $J = 7.6$ Hz, phenyl), 7.14–7.1(m, 3H, phenyl), 5.54 (s, 2H, $-CH_2$-), 4.62 (br s, 2H, $-CH_2$-), 2.28 (s, 3H, CH_3). Anal. calcd for $C_{17}H_{15}N_7O_3S$: C, 51.38; H, 3.80; N, 24.67. Found: C, 51.50; H, 3.87; N, 24.40.

*6.1.2.9. N-((1-(4-Methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amine (**3i**).* The resulting product was purified using silica gel column chromatography

eluting with $CHCl_3$ and then EtOAc. Compound **3i** was obtained as an orange solid. Yield 61%; m.p 183–185 °C; MS (ESI): 397.7 [M + H^+]. IR (KBr, cm^{-1}) 3235, 1618, 1537, 1496, 1350; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.8 (t, 1H, J = 5.35 Hz, −NH−), 8.12 (s, 1H, triazole), 7.84 (d, 1H, J = 4 Hz, furan), 7.36 (d, 1H, J = 4 Hz, furan), 7.22 (d, 2H, J = 8 Hz, phenyl), 7.17 (d, 2H, J = 8 Hz, phenyl), 5.53 (s, 2H, $-CH_2$-), 4.62 (d, 2H, J = 5.35 Hz, $-CH_2$-), 2.28 (s, 3H, CH_3); Anal. calcd for $C_{17}H_{15}N_7O_3S$: C, 51.38; H, 3.80; N, 24.67. Found: C, 51.21; H, 3.58; N, 24.97.

*6.1.2.10. N-((1-(4-Nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amine (**3j**).* The resulting product was purified using silica gel column chromatography eluting with $CHCl_3$ and then EtOAc. Compound **3j** was obtained as an orange solid. Yield 78%; m.p 234–236 °C; MS (ESI): 428.7 [M + H^+]. IR (KBr, cm^{-1}) 3212, 1561, 1517, 1493, 1352; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.84 (t, 1H, J = 5 Hz, −NH−), 8.26–8.2 (m, 3H, triazole and phenyl), 7.83 (d, 1H, J = 3.8 Hz, furan), 7.53 (d, 2H, J = 8.4 Hz, phenyl), 7.34 (d, 1H, J = 3.8 Hz, furan), 5.78 (s, 2H, $-CH_2$-), 4.64 (d, 2H, J = 5 Hz, $-CH_2$-). Anal. calcd for $C_{16}H_{12}N_8O_5S$: C, 44.86; H, 2.82; N, 26.16. Found: C, 44.97; H, 2.63; N, 26.26.

*6.1.2.11. N-((1-(2-Chloro-6-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amine (**3k**).* The resulting product was purified using silica gel column chromatography eluting with $CHCl_3$ and then EtOAc. Compound **3k** was obtained as a yellow solid. Yield 73%; m.p 207–208 °C; MS (*m*/*z*, %) 437 ([M + 2]$^+$, 20), 435 (M$^+$, 52), 292 (7), 264 (41), 234 (43), 212 (12), 198 (24), 196 (74), 161 (20), 156 (34), 145 (92), 143(100), 107 (21), 82 (15); IR (KBr, cm^{-1}) 3215, 1615, 1534, 1496, 1352; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.8 (t, 1H, J = 5.2 Hz, −NH−), 8.14 (s, 1H, triazole), 7.86 (d, 1H, J = 3.95 Hz, furan), 7.53–7.48 (m, 1H, phenyl), 7.42 (d, 1H, J = 8 Hz, phenyl), 7.36 (d, 1H, J = 3.95 Hz, furan), 7.33 (d, 1H, J = 8 Hz, phenyl), 5.72 (s, 2H, $-CH_2$-), 4.62 (d, 2H, J = 5.2 Hz, $-CH_2$-); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 169.2, 151.4, 147.6, 144.8, 143.3, 134.9, 131.9, 131.8, 125.9, 123.8, 120.8, 115.1, 114.9, 111.9, 44.4, 39.0; Anal. calcd for $C_{16}H_{11}ClFN_7O_3S$: C, 44.09; H, 2.54; N, 22.50. Found: C, 44.42; H, 2.44; N, 22.21.

*6.1.2.12. N-((1-(2,3-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amine (**3l**).* The resulting product was purified using silica gel column chromatography eluting with CH_2Cl_2 and then EtOAc. Compound **3l** was obtained as an orange solid. Yield 69%; m.p 220–222 °C; MS (ESI): 451.6 [M + H^+]. IR (KBr, cm^{-1}) 3195, 1589, 1527, 1498, 1353; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.82 (t, 1H, J = 5 Hz, −NH−), 8.16 (s, 1H, triazole), 7.84 (d, 1H, J = 3.6 Hz, furan), 7.65 (d, 1H, J = 8 Hz, phenyl), 7.38 (t, 1H, J = 8 Hz, phenyl), 7.34 (d, 1H, J = 3.6 Hz, furan), 7.14 (d, 1H, J = 8 Hz, phenyl), 5.75 (s, 2H, $-CH_2$-), 4.62 (d, 2H, J = 5 Hz, $-CH_2$-). Anal. calcd for $C_{16}H_{11}Cl_2N_7O_3S$: C, 42.49; H, 2.45; N, 21.68. Found: C, 42.27; H, 2.31; N, 21.85.

*6.1.2.13. N-((1-(2,4-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amine (**3m**).* The resulting moist solid was heated in hot acetone and filtrated. Then, the organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. Compound **3m** was obtained as a yellow solid. Yield 72%; m.p 154–155 °C; MS (ESI): 451.6 [M + H^+]. IR (KBr, cm^{-1}) 3200, 1619, 1540, 1493, 1356; ^1H NMR (DMSO-d_6, 500 MHz) δ: 8.83 (br s, 1H, −NH−), 8.16 (s, 1H, triazole), 7.85 (d, 1H, J = 3.5 Hz, furan), 7.7 (s, 1H, phenyl), 7.47 (d, 1H, J = 8.25 Hz, phenyl), 7.35 (d, 1H, J = 3.5 Hz, furan), 7.27 (d, 1H, J = 8.25 Hz, phenyl), 5.7 (s, 2H, $-CH_2$-), 4.64 (br s, 2H, $-CH_2$-); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 169.3, 151.4, 147.6, 144.8, 143.6, 133.9, 133.7, 132.5, 131.9, 129.1, 127.9, 124.1, 115.2, 111.9, 50.0, 39.0; Anal. calcd for $C_{16}H_{11}Cl_2N_7O_3S$: C, 42.49; H, 2.45; N, 21.68. Found: C, 42.62; H, 2.63; N, 21.44.

*6.1.2.14. N-((1-(3,4-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amine (**3n**).* The resulting product was purified using silica gel column chromatography eluting with $CHCl_3$ and then EtOAc. Compound **3n** was obtained as a yellow solid. Yield 70%; m.p 189–191 °C; MS (ESI): 451.6 [M + H^+]. IR (KBr, cm^{-1}) 3199, 1600, 1530, 1498, 1355; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.84 (t, 1H, J = 5 Hz, −NH−), 8.22 (s, 1H, triazole), 7.85 (d, 1H, J = 4 Hz, furan), 7.64 (d, 1H, J = 8 Hz, phenyl), 7.61 (s, 1H, phenyl), 7.35 (d, 1H, J = 4 Hz, furan), 7.3 (d, 1H, J = 8 Hz, phenyl), 5.62 (s, 2H, $-CH_2$-), 4.62 (d, 2H, J = 5 Hz, $-CH_2$-). Anal. calcd for $C_{16}H_{11}Cl_2N_7O_3S$: C, 42.49; H, 2.45; N, 21.68. Found: C, 42.75; H, 2.71; N, 21.37.

Acknowledgment

This work was supported by grants from the Research Council of Tehran University of Medical Sciences and Iran National Science Foundation (INSF).

References

[1] D. Sereno, A.C. da Silva, F. Mathieu-Daude, A. Ouaissi, Parasitol. Int. 56 (2007) 3–7.
[2] F. Chappuis, S. Sundar, A. Hailu, H. Ghalib, S. Rijal, R.W. Peeling, J. Alvar, M. Boelaert, Nat. Rev. Microbiol. 5 (2007) 873–882.
[3] C. Graebin, F.D. Uchoa, L.S.C. Bernardes, V.L. Campo, I. Carvalho, V.L. Eifler-Lima, Anti-Infect. Agents Med. Chem. 8 (2009) 345–366.
[4] R.N. Coler, S.G. Reed, Trends Parasitol. 21 (2005) 244–249.
[5] S.L. Croft, K. Seifert, V. Yardley, Indian J. Med. Res. 123 (2006) 399–410.
[6] V.K. Marrapu, M. Mittal, R. Shivahare, S. Gupta, K. Bhandari, Eur. J. Med. Chem. 46 (2011) 1694–1700.
[7] J.E. Moses, A.D. Moorhouse, Chem. Soc. Rev. 36 (2007) 1249–1262.
[8] C.J. Hawker, K.L. Wooley, Science 309 (2005) 1200–1205.
[9] G.C. Tron, T. Pirali, R.A. Billington, P.L. Canoniico, G. Sorba, A.A. Genazzani, Med. Res. Rev. 28 (2008) 278–308.
[10] J.J. Wu, N. Green, R. Hotchandani, Y.H. Hu, J. Condon, A. Huang, N. Kaila, H.Q. Li, S. Guler, W. Li, S.Y. Tam, Q. Wang, J. Pelker, Bioorg. Med. Chem. Lett. 19 (2009) 3485–3488.
[11] O.A. Phillips, E.E. Udo, M.E. Abdel-Hamid, R. Varghese, Eur. J. Med. Chem. 44 (2009) 3217–3227.
[12] N.G. Aher, V.S. Pore, N.N. Mishra, A. Kumar, P.K. Shukla, A. Sharma, M.K. Bhat, Bioorg. Med. Chem. Lett. 19 (2009) 759–763.
[13] C. Gill, G. Jadhav, M. Shaikh, R. Kale, A. Ghawalkar, D. Nagargoje, M. Shiradkar, Bioorg. Med. Chem. Lett. 18 (2008) 6244–6247.
[14] M.J. Giffin, H. Heaslet, A. Brik, Y.C. Lin, G. Cauvi, C.H. Wong, D.E. McRee, J.H. Elder, C.D. Stout, B.E. Torbett, J. Med. Chem. 51 (2008) 6263–6270.
[15] R.F. Rodrigues, E.F. da Silva, Á Echevarria, R. Fajardo-Bonin, V.F. Amaral, L.L. Leon, M.M. Canto-Cavalheiro, Eur. J. Med. Chem. 42 (2007) 1039–1043.
[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] M. Behrouzi-Fardmoghadam, F. Poorrajab, S. Kaboudanian Ardestani, S. Emami, A. Shafiee, A. Foroumadi, Bioorg. Med. Chem. 6 (2008) 4509–4515.
[18] A. Tahghighi, F. Rezazade Marznaki, F. Kobarfard, S. Dastmalchi, J. Shahbazi Mojarrad, S. Razmi, S. Kaboudanian Ardestani, S. Emami, A. Shafiee, A. Foroumadi, Eur. J. Med. Chem. 46 (2011) 2602–2608.
[19] A. Tahghighi, F. Rezazade Marznaki, S. Emami, S. Kaboudanian Ardestani, S. Dastmalchi, F. Kobarfard, A. Shafiee, A. Foroumadi, J. Enzyme. Inhib. Med. Chem. (2011) Submitted manuscript.
[20] A. Dutta, S. Bandyopadhyay, C. Mandal, M. Chatterjee, Parasitol. Int. 54 (2005) 119–122.
[21] A.K. Tanaka, V.B. Valero, H.K. Takahashi, A.H. Straus, J. Antimicrob. Chemother. 59 (2007) 487–492.