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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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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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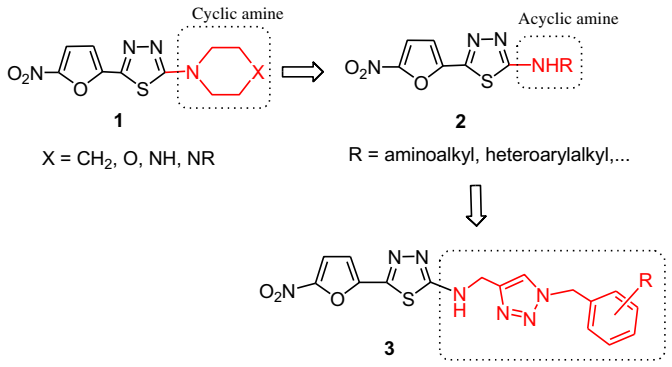
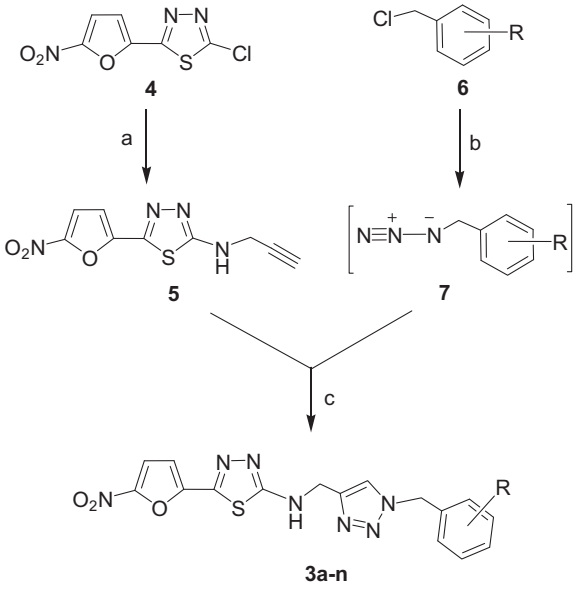


Fig. 1. General structures of previously described compounds **1** and **2**, and new designed compounds **3**.

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

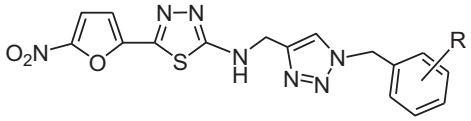
Primarily, the anti-promastigote activity of target compounds **3a**–**n** was evaluated by direct counting and MTT assay according to the literature method [20]. The IC_{50}s (50% inhibitory concentrations) of compounds against promastigote form of *Leishmania major* (vaccine strain MRHO/IR/75/ER, obtained 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.