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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

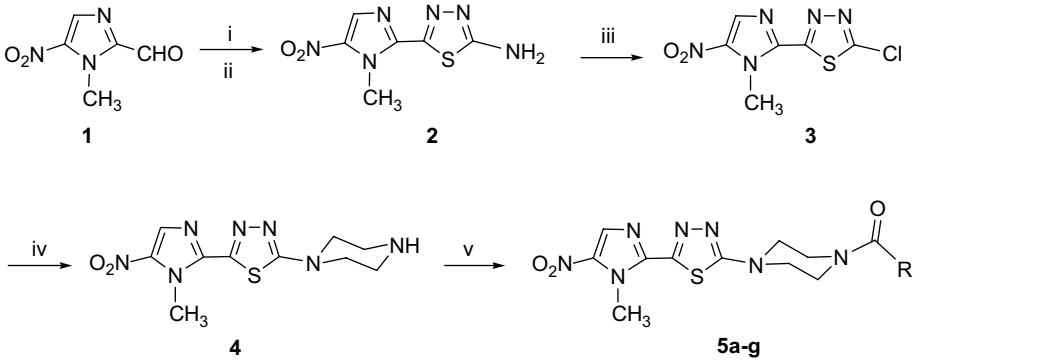
The synthetic pathway to target compounds **5a–g** is shown in Scheme 1. The intermediate 2-chloro-1,3,4-thiadiazole **3** was obtained from 1-methyl-5-nitroimidazole-5-carboxaldehyde according to the previously described methods [15,16]. Thus, treatment of **1** with thiosemicarbazide in the presence 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.