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Short communication

Synthesis and in vitro leishmanicidal activity of 2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-5-substituted-1,3,4-thiadiazole derivatives

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Abstract

A series of 2-(1-methyl-5-nitroimidazol-2-yl)-5-(1-piperazinyl, 1-piperidinyl and 1-morpholinyl)-1,3,4–thiadiazoles (**3a**–**g**) were synthesized and evaluated for in vitro leishmanicidal activity against *Leishmania major* promastigotes. The leishmanicidal data revealed that compounds **3a**–**g** had strong and much better leishmanicidal activity than the reference drug pentostam. Compound **3c** (piperazine analog) was the most active compound (IC_{50} = 0.19 μM).

Keywords: 5-Nitro-1*H*-imidazole; 1,3,4-Thiadiazole; *Leishmania major*; Anti-promastigote activity

1. Introduction

The World Health Organization (WHO) has identified leishmaniasis as major and increasing public health problem [1]. In spite of the socioeconomic importance of this tropical infectious, efforts directed towards the discovery of new drugs and/or vaccines against it are underdeveloped [2,3]. Drugs currently in use as the antimony derivative glucantine, the bis-amidines, pentamidine and stilbamidine or the glycomacrolide amphotericin B, display high liver and heart toxicities, develop clinical resistance after a few weeks of treatment and currently contribute to increase co-infections leishmaniasis-AIDS in some countries [4,5]. In addition, most of the drugs currently in use are expensive and require long-term treatment [6]. Given the prospect that antileishmanial vaccines may not become available in the near future [7], the development new effective, cheap, and safe drugs for the treatment of leishmaniasis is there fore an urgent task.

The pharmacological interest of the imidazole ring has been established, nitroimidazoles being extensively used in therapy against amoebic, trichomonal, giardial and anaerobic infections, or as hypoxic cell radiosensitizers [8]. Metronidazole and *N*-substituted imidazoles (ketoconazole, fluconazole and itraconazole) are well-tolerated drugs that are potentially active against *Leishmania*, but their use in the treatment of cutaneous and visceral leishmaniasis has produced conflicting results [9]. On the other hand the antiparasitic property of 1,3,4-thiadiazoles is well documented and their attachment with other heterocycles often ameliorates or diminishes the bioresponses, depending upon the type of substituent and position of attachment [10].

In our previous paper [11], 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)**, and 2-(5-nitro-2-thienyl)-5-substituted-1,3,4-thiadiazoles **(2)**, which several of them showed promising antileishmanial properties. In view of the biological importance of nitroimidazoles [12–14], it was of our interest to prepare 2-(1-methyl-5-nitro-1*H*-

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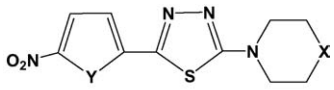
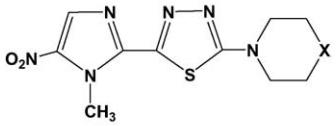
imidazol-2-yl)-1,3,4-thiadiazoles **(3)**, as potential new antileishmanial agents.



X= CH_2, O, NH, N-R

1, Y= O
2, Y = S



X= CH_2, O, NH, N-R

3

2. Chemistry

The synthesis of 2-(1-methyl-5-nitro-1*H*-2-imidazolyl)-5-substituted-1,3,4-thiadiazoles **(3a–g)** was achieved with a versatile and efficient synthetic route outline in Fig. 1. The required 2-amino-5-(1-methyl-5-nitro-1*H*-2-imidazolyl)-1,3,4-thiadiazole **(8)** [15] was prepared according to the newly synthetic route suitable for multigrams scale [13]. The reaction of 1-methylimidazole **(4)** with n-butyllitium at –30 °C and subsequent addition of dimethyl disulfide gave 1-methyl-2-(methylthio)-1*H*-imidazole. Nitration of the latter compound with concentrated nitric acid at 70 °C afforded the corresponding 5-nithroimidazole **(5)**. Oxidation of **5** by hydrogen peroxide in trifluoroacetic acid leads to the sulfone **(6)** where a nucleophilic substation by cyanide anion produces the corresponding carbonitrile **(7)** in good yield. Compound **8** was obtained by treatment of **7** with thiosemicarbazide in an acid catalyzed reaction followed by a ring closure process.

Diazotization of amine **(8)** in hydrochloric acid in the presence of copper powder [16] gave 2-chloro-5-(1-methyl-5-nitro-1*H*-2-imidazolyl)-1,3,4-thiadiazole **(9)** [17]. Reaction of compound **9** with piperidine or morpholine in refluxing ethanol gave compounds **3a** or **3b**, respectively. Similarly, the reaction of compound **9** with piperazine, *N*-methylpiperazine and *N*-phenylpiperazine gave the corresponding compounds **3c–e**, respectively. Acetylation of the piperazine analogue **3c** with acetic anhydride gave acetylated compound **3f**. The reaction of compound **3c** with benzoyl chloride yielded benzoylated analogue **3g** in high yield (Fig. 1).

3. Pharmacology

The in vitro leishmanicidal activity of the compounds **(3a–g)** on promastigotes of *Leishmania major* (ATCC J 774, HB-197) was assessed by a previously described method [18]. Promastigotes (3×10^6) were cultured in medium 199 containing 10% heat-inactivated fetal calf serum. Incubation and growth of the parasite were carried out at 26 °C. Promastig-

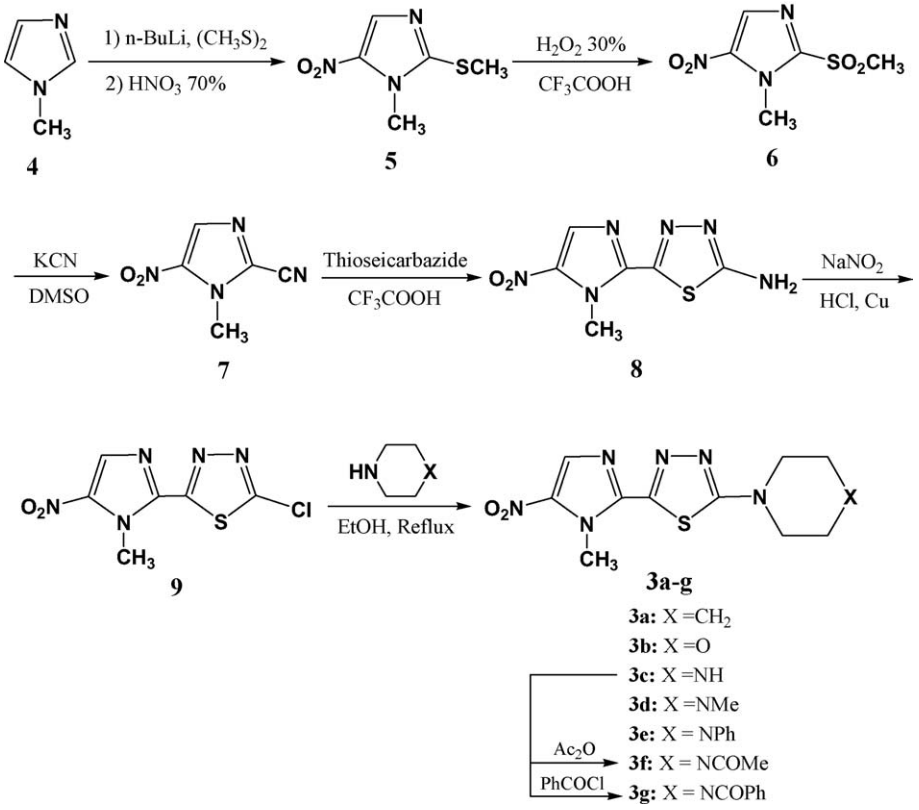


Fig. 1. Synthetic pathway to compounds **3a**–**g**.

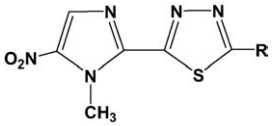
otes were harvested on day 4 of the culture and used. The culture of parasite was diluted with the fresh medium to a final concentration of 5×10^6 parasites per ml. The compounds to be checked were dissolved in DMSO (15 mM) and further diluted to appropriate concentrations. In a 96-well microtiter plates, 160 μl of the promastigotes suspension was added to 40 μl of various concentrations of each compound, medium alone, or pentavalent antimonial Pentostam as positive control. After 2 h, 1 μCi of ^{3}H-thymidine was added to each well. The cultures were incubated for 18 h and ^{3}H-thymidine incorporation was measured. To ensure that the solvent had no effect on parasite growth a control test was performed with test medium and DMSO at the same dilutions as used in the experiment. The results are from three experiments and are given as IC_{50} (mean ± S.D.) as measured by ^{3}H-thymidine incorporation (Table 1).

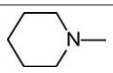
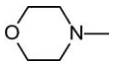
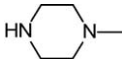
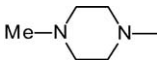
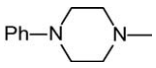
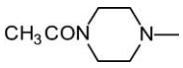
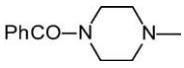
4. Results and discussion

The in vitro leishmanicidal activity of the synthesized compounds was determined by measuring the ^{3}H-thymidine incorporation in promastigotes of *L. major*, as reported in Table 1.

The IC_{50} values of the test derivatives indicate that all compounds exhibit high activity against *L. major* ($IC_{50} \leq 1.77$ μM) with respect to the Pentostam ($IC_{50} = 243.3$ μM). The overall activity profile of compounds **3a**–**g** demonstrated that there is a small difference in their IC_{50} values. Thus, the biological activity was slightly influenced by the type of cyclic amine attachment at the 5-position of the 1,3,4-thiadiazole nucleus and a structure–activity relationship study was not crucial. However, it is notable to observe that unsubstituted piperazine derivative **(3c)** ($IC_{50} = 0.19 \pm 0.08$ μM) proved to be statistically the most effective in this series (P values < 0.05), followed by piperidine and morpholine derivatives **(3a** and **3b)** with IC_{50} values of 0.42–0.44 μM, and this level of activity could be maintained with a *N*-benzoylated piperazine group (compound **3g**). Comparison of the IC_{50} values of **3c** with N_4-substituted piperazine derivatives (**3d**–**g**) reveals that certain substituents (such as, methyl, phenyl, acetyl and benzoyl) at N_4-position of piperazine ring are permitted and well-tolerated but, not improved the leishmanicidal activity.

Table 1
Effect of compounds **3a**–**g** on the growth of *L. major* promastigotes



Compound	R	IC_{50} (μM)[a]
3a		0.44 ± 0.14
3b		0.42 ± 0.11
3c		0.19 ± 0.08
3d		1.38 ± 0.27
3e		1.63 ± 0.14
3f		1.77 ± 0.32
3g		0.48 ± 0.09
Pentostam		243.3 ± 3.8

[a] Values are expressed as mean ± S.D. for three independent experiments.

From our biological results, it is evident that nitroimidazolyl-1,3,4-thiadiazoles **(3)** exhibited very potent antileishmanial activity. It is worthwhile observing that, in comparison with nitrofuran derivatives **(1)**, previously described by us [11], both of them have a better antileishmanial activity than nitrothiophene derivatives **(2)**. However, the nitroimidazoles **(3a**–**d)** were evidently more active than the corresponding nitrofuran analogs [11]. These results brought to our attention that this pharmacomodulation (replacement of furan with imidazole) in many cases exerted a positive effect.

The antileishmanial activity of these new nitroimidazolyl-1,3,4-thiadiazole derivatives **(3)** may be due to the reduction potential of the single-electron transfer $ArNO_2/ArNO_2^{\bullet -}$. Nitroheterocylic drugs (e.g. nifurtimox, metronidazole, benznidazole and megazole) are generally believed to exert their cytotoxic effects only after activation by single-electron reduction of their corresponding nitro anion radicals [19–21]. Under anaerobic conditions, the radical anion can be transformed into the corresponding nitroso derivative. This nitroso form has been put forward as an efficient scavenger of essential thiols in the cell. Under aerobic conditions, the nitro radical anion reacts with oxygen to form superoxide anion and hydroxyl radical. The resulting oxygen-derived free radicals would damage the enzyme, DNA or important structures in the surrounding cell, and result in a cytotoxic action [22–25]. Thus, it seems that the antileishmanial activity of the new nitroimidazolyl-1,3,4-thiadiazole derivatives **(3)** (structurally related to megazole and other nitroimidazoles) are associated with their interference with oxygen metabolism as well as their role as thiol scavenger.

In conclusion, the high in vitro leishmanicidal activity of compounds **3** makes these compounds a promising lead for the development of an effective therapeutic agent. However, this study will be completed by additional tests in amastigote/macrophage in vitro models or in vivo mouse models.

5. Experimental

5.1. Chemistry

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 disks). ^{1}H-NMR spectra were recorded on a Varian unity 400 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; column chromatography was performed on Merck silica gel (70–230 mesh).

5.1.1. 1-[5-(1-Methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]piperidine (**3a**)

A mixture of compound **9** [17] (491 mg, 2 mmol) and piperidine (340 mg, 2 mmol) in ethanol (5 ml) was refluxed for 30 min. After cooling, water was added and the precipitated solid was washed with water and crystallized from ethanol/water to give **3a** (81%). M.p. 220–222 °C. IR (KBr) ν_{max}: 3135 (CH-imidazole), 1520 and 1363 cm^{-1} (NO_2). ^{1}H-NMR ($CDCl_3$) δ: 8.03 (s, 1H, H_4-imidazole), 4.48 (s, 3H, CH_3), 3.62 (brs, 4H, CH_2NCH_2), 1.74-1.71 ppm (m, 6H, piperidine).

5.1.2. 4-[5-(1-Methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]morpholine (**3b**)

This compound was prepared by the same method used for **3a** in 80% yield (the reaction time was 1.5 h). M.p. 231–232 °C (EtOH/H_2O). IR (KBr) ν_{max} : 3152 (H-C_4 imidazole), 1510 and 1356 cm^{-1} (NO_2). ^{1}H-NMR ($CDCl_3$) δ: 8.03 (s, 1H, H_4-imidazole), 4.49 (s, 3H, CH_3), 3.82-3.79 (m, 4H, CH_2OCH_2) and 3.70–3.66 ppm (m, 4H, CH_2NCH_2).

5.1.3. 1-[5-(1-Methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]piperazine (**3c**)

Compound **3c** was obtained by the same method used for **3a** in 85% yield. M.p. 238–240 °C. IR (KBr) ν_{max}: 3140 (H-C_4 imidazole) 1520 and 1340 cm^{-1} (NO_2). ^{1}H-NMR ($CDCl_3$) δ: 8.05 (s, 1H, H_4-imidazole), 4.50 (s, 3H, NCH_3), 3.62–3.58 (m, 4H, piperazine), 2.97–2.93 ppm (m, 4H, piperazine).

5.1.4. 4-Methyl-1-[5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]piperazine (**3d**)

This compound was prepared by the same method used for **3a** from the reaction of compound **9** with *N*-methylpiperazine in 80% yield. M.p. 252–254 °C (EtOH). IR (KBr) ν_{max}: 3140 (H-C_4 imidazole), 1520 and 1340 cm^{-1} (NO_2). ^{1}H-NMR ($CDCl_3$) δ: 8.02 (s, 1H, H_4-imidazole), 4.48 (s, 3H, NCH_3 imidazole), 3.66 (m, 4H, piperazine), 2.56 (m, 4H, piperazine) and 2.36 ppm (s, 3H, NCH_3).

5.1.5. 1-[5-(1-Methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]-4-phenylpiperazine (**3e**)

This compound was prepared by the same method used for **3a** from the reaction of compound **9** with *N*-phenylpiperazine. The reaction time was 2 h and the resulting product was purified using silica gel column chromatography eluting with CH_2Cl_2 containing 2% ethanol. Yield 88%. M.p. 257–259 °C (EtOH). IR (KBr) ν_{max}: 3135 (H-C_4 imidazole), 1487 and 1360 cm^{-1} (NO_2). ^{1}H-NMR ($CDCl_3$) δ: 8.04(s, 1H, H_4-imidazole), 7.32–6.90 (m, 5H, phenyl), 4.50 (s, 3H, CH_3), 3.87 (m, 4H, piperazine), and 3.35 ppm (m, 4H, piperazine).

5.1.6. 4-Acetyl-1-[5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]piperazine (**3f**)

A mixture of compound **3c** (885 mg, 3.0 mmol), acetic acid (5 ml) and acetic anhydride (0.5 ml) was refluxed for 20 min. After cooling, the reaction mixture was added to a mixture of ice and water and the precipitated product was filtered and washed with water and crystallized from ethanol to give 890 mg (88%) of **3f**. M.p. 269–270 °C. IR (KBr) ν_{max}: 3120 (H-C_4 imidazole), 1645 (C=O), 1525 and 1355 cm^{-1} (NO_2). ^{1}H-NMR ($CDCl_3$) δ: 8.03 (s, 1H, H_4-imidazole), 4.49 (s, 3H, NCH_3), 3.72–3.67 (m, 8H, piperazine) and 2.16 ppm (s, 3H, CH_3CO).

5.1.7. 4-Benzoyl-1-[5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]piperazine (**3g**)

To a mixture of compound **3c** (590 mg, 2.0 mmol), in dry benzene (5 ml) and pyridine (1 ml) was added benzoyl chloride (281 mg, 2.0 mmol) and the mixture was stirred at room temperature overnight. The solvents were removed under reduced pressure and the resulting solid was washed with water and crystallized from ethanol to give 638 mg (80%) of **3g**. M.p. 225–227 °C. IR (KBr) ν_{max}: 3140 (H-C_4 imidazole), 1640 (C=O), 1520 and 1356 cm^{-1} (NO_2). ^{1}H-NMR ($CDCl_3$) δ: 8.02 (s, 1H, H_4-imidazole), 7.45 (brs, 5H, phenyl), 4.49 (s, 3H, CH_3) and 3.75–3.71 ppm (m, 8H, piperazine).

Acknowledgments

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