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Synthesis and In Vitro Anti-Leishmanial Evaluation of 1-(5-Halo-2-Thienyl)-2-[5-(5-Nitroheteroaryl)]-1,3,4-Thiadiazol-2-Ylthio)Ethanone Derivatives

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A B S T R A C T

The Leishmaniasis is a zoonotic disease that remains a severe public health problem and its burden is increasing. By now there is no anti-leishmaniasis vaccine and as other tripanosomial disease, is treated by limited drugs. First line drug includes pentavalent antimony such as sodium stibogluconat and meglumine antimoniate that use of these drugs is limited due to side effects, long term treatment, high cost and incidence of drug resistance. It is clear that developing new, effective, cheap and safe drugs is necessary for treating Leishmaniasis. In this study new derivatives of 1-(5-halo-2-thienyl)-2-[5-(5-nitroheteroaryl)]-1,3,4-thiadiazol-2-ylthio)ethanone **8a-8d** were synthesized and evaluated against the promastigote form of *Leishmania major* using MTT assay in comparison with control drug (Fluconazole). Concentrations (5-150 µg/mL) of synthesized compounds and control drug were prepared and IC_{50} for each compound was measured. IC_{50} for **8a-8d** was 114.98 µM (44.59±8.15 µg/mL), 118.05 µM (51.25±9.08 µg/mL), 130.7 µM (52.78±12.97 µg/mL), and 252.7 µM (113.3±5.92 µg/mL) respectively that was less than of fluconazole 980 µM (300 µg/mL). The results of this study showed that four synthesized compounds had a significant effect on *Leishmania major* and have a stronger effect than control drug. It seems that these compounds can be used as an alternative in future.

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Introduction

The Leishmaniasis is a zoonotic disease that remains a severe public health problem and its burden is increasing [1, 2]. Annual incidence is estimated at 1–1.5 million cases of CL and 500,000 cases of VL. Overall prevalence is 12 million people and the population at risk is 350 million [3]. The management of leishmaniasis relies entirely on chemotherapy and so far no vaccine approved for human use is available [4]. The drugs available for the treatment of *Leishmania* infections is limited and includes pentavalent antimonials such as sodium stibogluconate (Pentostam®) and meglumine antimonite (Glucantime®), pentamidine, amphotericin B and miltefosine (Figure 1) [5]. These drugs are expensive and potentially toxic and require long-term treatment. In addition the development of drug resistance by the pathogens especially in HIV *Leishmania* coinfected patients, has aggravated public health risks [6]. The use of nitroheterocycles such as 5-nitrofurans and 5-nitrothiophenes as antibacterial and antiprotozoal is well established [7, 8]. On the other hand, the antiphrastic 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 [9,10]. Various 2,5-disubstituted-1,3,4-thiadiazole analogues have been previously synthesized and some of them showed excellent leishmanicidal activity [11-13]. All of these compounds had a nitrogen heterocycle linked to the C-5 position of 1,3,4-thiadiazole ring through the heterocyclic nitrogen. Previous results demonstrated that C-5 substituent is the most adaptable site for chemical change and is an area where it determines the potency and physicochemical properties of 2-(nitroaryl)-1,3,4-thiadiazoles. As part of our efforts to develop new compounds with more selectivity and efficacy aimed at the therapy of parasitic infection especially leishmaniasis [11-13] we have synthesized and evaluated a number of 2-(nitrohetroaryl)-1,3,4-thiadiazoles bearing a 1-(5-halothiophen-2-yl)-2-mercaptoethanone at the C-5 position of 1,3,4-thiadiazole nucleus against *Leishmania major*.

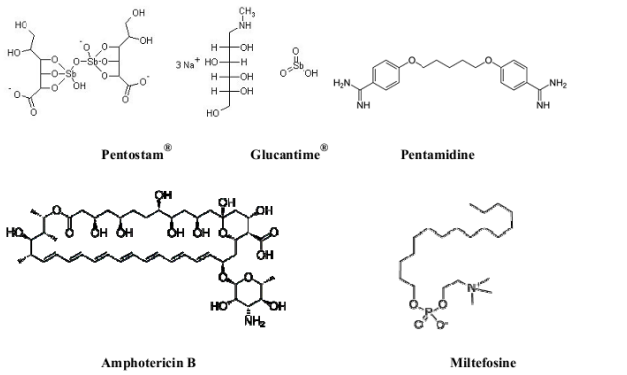


Fig. 1. Structures of drugs available for the treatment of *Leishmania*

Materials and Methods

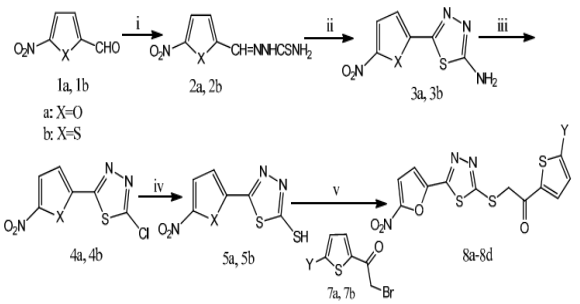
Chemistry

Chemicals and all solvents used in this study were purchased from Merck and Sigma-Aldrich Chemical companies. Intermediates **5a, 5b** and **7a, 7b** were prepared according to the literature [14,15]. Melting points were determined on Electrothermal Capillary apparatus and are uncorrected. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide disks). ^{1}H-NMR spectra were measured using a Bruker FT-200 spectrometer, and chemical shifts are expressed as δ (ppm) with tetramethylsilane as internal standard. The mass spectra were run on a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV. Merck silica gel 60 F254 plates were used for analytical TLC. Yields are of purified product and were not optimized.

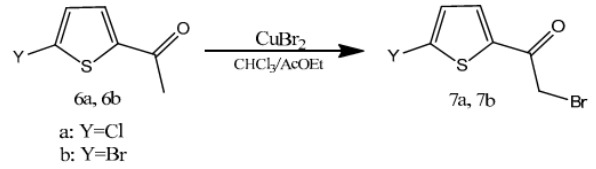
Synthesis of Compounds

Our synthetic pathway to intermediates **7a, 7b** and target compounds **8a-8d** is presented in Schemes 1 and 2. Reaction of nitroaryl aldehyde **1a, 1b** with thiosemicarbazide in refluxing ethanol afforded compounds **2a, 2b**. The 2-amino-5-(nitroaryl)-1,3,4-thiadiazole **3a, 3b** was prepared by oxidative cyclization of 5-nitroaryl carboxaldehyde thiosemicarbazone **2a, 2b**. Diazotation of **3a, 3b** in hydrochloric acid in the presence of copper powder gave 2-chloro-5-(nitroaryl)-1,3,4-thiadiazole **4a, 4b**. The reaction of **4** with thiourea in refluxing ethanol afforded the 2-mercapto-5-(5-nitroaryl)-1,3,4-thiadiazole **5a, 5b** [14]. Ketone **6a, 6b** was brominated with copper (II) bromide in refluxing $CHCl_3$–EtOAc to give corresponding α-bromoketone **7a, 7b** [15]. Reaction of 2-mercapto-5-(5-nitroaryl)-1,3,4-thiadi-

azole **5a, 5b** with α-bromoketone **7a, 7b** in the presence of KOH in refluxing ethanol, afforded target compounds **8a-8d**.



Scheme 1. Synthetic route to target compounds 6a–6d. Reagents and conditions: (i) thiosemicarbazide, EtOH, HCl, reflux; (ii) ammonium ferric sulfate, H_2O, reflux; (iii) $NaNO_2$, HCl, Cu; (iv) thiourea, EtOH, reflux; (v) 2-bromo-1-(5-halothiofen-2-yl)ethanon, KOH, ethanol, reflux.



Scheme 2. Synthesis of α-bromoketone **7a, 7b**

***2-Bromo-1-(5-chlorothiophen-2-yl)ethanone* 7a**

A vigorously stirred solution of compound **6a** (1.6 g, 10 mmol) in $CHCl_3$–EtOAc (1:1, 15 mL) was refluxed and then, pulverized copper (II) bromide (4.46 g, 20.0 mmol) was added portionwise during 4 h. The resulting reaction mixture was refluxed with vigorous stirring for additional 2 h to ensure complete exposure of the copper (II) bromide to the reaction medium. After removal of the copper (I) bromide (white solid) by filtration, the solvents were evaporated from the filtrate under reduced pressure. The residue was crystallized from *n*-heptane to give compound **7a** (1.43 g) (15). Yield 60%; m.p. 57–59 °C; υ_{max} (KBr) 1680 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.59 (d, J = 2 Hz, 1H, $C_3\underline{H}$, sp^2 aromatic), 6.99 (d, J = 2 Hz, 1H, $C_4\underline{H}$, sp^2 aromatic), 4.28 (s, 2H, CH_2, sp^3 aliphatic) [15].

***2-Bromo-1-(5-bromothiophen-2-yl)ethanone* 7b**

This compound was prepared from its corresponding precursors as described for the compound 7a in 60% yield. m.p.: 88-90 °C; υ_{max} (KBr) 1680 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 7.52 (d, J = 2 Hz, 1H, $C_3\underline{H}$, sp^2 aromatic), 7.13 (d, J = 2 Hz, 1H, $C_4\underline{H}$, sp^2 aromatic), 4.28 (s, 2H, CH_2, sp^3 aliphatic) [15].

***General procedure for the synthesis of compounds* 8a–8d**

A mixture of compound **5a, 5b** (0.5 mmol) and KOH (0.5 mmol) in absolute ethanol (5 mL) was stirred at room temperature for 30 min, then α-bromoketone **7a, 7b** (0.5 mmol) was added and refluxed overnight. The reaction mixture was diluted with HCl (10%) and the formed precipitate was filtered and washed with water and crystallized from ethanol to give target compounds **8a–8d** [14].

***1-(5-Chlorothiophen-2-yl)-2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)ethanone* 8a**

Yield 90%; m.p. 156–158 °C; υ_{max} (KBr) 1670 (stretch C=O), 1500 &1350 (stretch NO_2) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 7.77 (d, J = 2 Hz, 1H, $C_4\underline{H}$ nitrofuran, sp^2 aromatic), 7.5 (d, J = 2 Hz, 1H, $C_3\underline{H}$ nitrofuran, sp^2 aromatic), 7.35 (d, J = 2Hz, 1H, $C_3\underline{H}$ chlorothiophene, sp^2 aromatic), 7.08 (d, J = 2 Hz, 1H, $C_4\underline{H}$ chlorothiophene, sp^2 aromatic), 4.88 (s, 2H, CH_2, sp^3 aliphatic); MS (m/z,%) 388 ([M^++2], 13.5), 3846 (M^+, 11.5), 225 (3), 145 (85), 131 (6), 117 (3), 82 (6), 73 (4).

***1-(5-Bromothiophen-2-yl)-2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)ethanone* 8b**

Yield 85%; m.p. 141–143 °C; υ_{max} (KBr) 1650 (stretch C=O), 1535, 1500 & 1350 (stretch NO_2) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 7.52 (d, J = 2Hz, 1H, $C_4\underline{H}$ nitrofuran, sp^2 aromatic), 7.51 (d, J = 2 Hz, 1H, $C_4\underline{H}$ nitrofuran, sp^2 aromatic), 7.36 (d, J = 2 Hz, 1H, $C_3\underline{H}$ bromothiophene, sp^2 aromatic), 7.23 (d, J = 2 Hz, 1H, $C_3\underline{H}$ bromothiophene, sp^2 aromatic), 4.88 (s, 2H, CH_2, sp^3 aliphatic); MS (m/z, %) 432 ([M^++2], 5), 430 (M^+, 5), 352 (9.5), 191 (60), 96 (8), 82 (21).

***1-(5-Chlorothiophen-2-yl)-2-(5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazol-2-ylthio)ethanone* 8c**

Yield 85%; m.p. 168–170 °C; υ_{max} (KBr) 1665 (stretch C=O), 1340 & 1520 (stretch NO_2) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 7.95 (d, J = 2 Hz, 1H, $C_4\underline{H}$ nitrothiophene, sp^2 aromatic), 7.77 (d, J = 2 Hz, 1H, $C_4\underline{H}$ nitrothiophene, sp^2 aromatic), 7.4 (d, J = 2 Hz, 1H, $C_3\underline{H}$ chlorothiophene, sp^2 aromatic), 7.08 (d, J = 2 Hz, 1H, $C_3\underline{H}$ chlorothiophene, sp^2 aromatic), 4.88 (s, 2H, CH_2, sp^3 aliphatic); MS (m/z, %) 404 ([M^++2], 2), 402 (M^+, 3), 279 (5), 258 (3), 167 (8.5), 145 (40), 131 (4), 112 (4), 83 (9), 69 (27), 57 (23).

***1-(5-Bromothiophen-2-yl)-2-(5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazol-2-ylthio)ethanone* 8d**

Yield 85%; m.p. 168–170 °C; υ_{max} (KBr) 1665 (stretch C=O), 1340 & 1520 (stretch NO_2) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 7.95 (d, J = 2 Hz, 1H, $C_4\underline{H}$ nitrothiophene, sp^2 aromatic), 7.71 (d, J = 2 Hz, 1H, $C_4\underline{H}$ nitrothiophene, sp^2 aromatic), 7.4 (d, J = 2 Hz, 1H, $C_3\underline{H}$ bromothiophene, sp^2 aromatic), 7.23 (d, J = 2 Hz, 1H, $C_3\underline{H}$ bromothiophene, sp^2 aromatic), 4.87 (s, 2H, CH_2, sp^3 aliphatic); MS (m/z, %): 448 ([M^++2], 9.5), 446 (M^+, 5), 368 (22), 245 (5), 191 (85), 126 (5), 82(5).

Parasite and culture

The strain of L. *major* used in this study was the vaccine strain (MRHO/IR/75/ER), obtained from Pasteur Institute, Tehran (Iran). 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, 2 mM glutamine (Sigma), pH~7.2, 100 U/mL penicillin (Sigma) and 100 mg/mL streptomycin (Sigma).

In vitro antileishmanial activity

The antileishmanial evaluation of compounds **8a–8d** was performed using direct counting and MTT assay [16]. 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 cells/mL) in the logarithmic phase were incubated with a serial range of drug concentrations for 24 h at 25 °C. To determining 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 FCS, 2 mM glutamin, pH~7.2 and antibiotics, in a volume of 200 µL. After 24 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} were calculated by linear regression analysis, expressed in mean±SD. Control cells were incubated with culture medium plus DMSO.

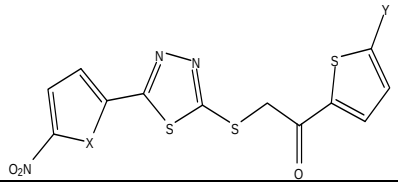
Results and discussion

In our study, the target compounds were subjected to in vitro antileishmanial activity profile against the promastigote form of the *Leishmania major* using MTT assay, side by side the reference drug fluconazole. The inhibitory concentrations for 50% of inhibition (IC_{50}) of *Leishmania* growth, at third day of incubation, were calculated based on a linear regression. All data were reported as the mean ± SD in Table 1.

Generally, the IC_{50} values of the test compounds **8a–8d** indicate that all compounds exhibit high activity against L. *major*. The most potent compound against the promastigote form of L. *major* was found to be nitrofuran analogues with chlorine substitution.

8a with IC_{50}=114.98 µM (44.59±8.15 µg/mL). Comparison between IC_{50} values of nitrofurans **8a, 8b** and nitrothiophene analogues **8c, 8d** against promastigotes revealed that nitrofurans possessed better activity with respect to corresponding nitrothiophenes and Compounds containing chlorine on the thiophene ring have a greater antileishmanial effect than of compounds have bromine.

Table 1. In vitro activities of compounds **8a-8d** against promastigote form of L. *major*.



compound	X	Y	IC_{50} (μM)[a]	IC_{50} (μg/mL)[a]
8a	O	Cl	114.98±21	44.59±18.15
8b	O	Br	118±21	51.25±9.08
8c	S	Cl	130.7±32	52.78±12.97
8d	S	Br	252.7±13	113.3±5.92
Fluconazole			980	300

[a] The values represent mean±SD.

The antileishmanial activity of these new nitroheteroaryl-1,3,4-thiadiazole derivatives **8a-8d** may be due to the reduction potential of the single-electron transfer ArNO2/ArNO2$^{\bullet}$. Nitroheterocylic drugs are generally believed to exert their cytotoxic effects only after activation by single-electron reduction of their corresponding nitro anion radicals [11-15]. Under anaerobic conditions, the radical anion can be transformed into the corresponding nitroso derive- ative. 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 [11-15]. Thus, it seems that the antileishmanial activity of the new nitro- heteroaryl-1,3,4-thiadiazole derivatives are associated with their interference with oxygen metabolism as well as their role as thiol scavenger. Recently, Porrajab et al. reported selective leishmanicidal effect of 1,3,4-thiadiazole derivatives and possible mecha- nism of action against Leishmania species [17]. Their findings showed that at least part of leishma nicidal effect of the compounds could be attributed to disruption DNA-relaxed activities of topoisomerases I and II, and cleavable-complex formation.

Conclusion

In conclusion, 1-(5-halo-2-thienyl)-2-[5-(5-nitroheteroaryl)]-1,3,4-thiadiazol-2-ylthio)ethanone derivatives demonstrate significant effect on *Leishmania major*. These compounds with antileishmanial activeities comparable to standard drug and better IC_{50} values have led to a new lead for further exploration and development of safe and effective antileishmanial drugs.

Conflict of interest

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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