

Evaluation of Some Thiosemicarbazones as Potential Antileishmanial Agents

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Abstract- A series of thiosemicarbazones **1-6** derived from 2-benzoylthiophene, 2-furaldehyde, thiophene-2-carboxaldehyde, 4-dimethylaminobenzaldehyde, 4-methoxybenzaldehyde and 4-hydroxy-3-methoxybenzaldehyde were prepared by condensation of ketones and / or aldehydes with thiosemicarbazide. The antileishmanial activities of these compounds were reported first time *in vitro* against the leishmania major. The results revealed that the thiosemicarbazones **3** has shown significant antileishmanial activity range ($0.58 \pm 0.05 \mu\text{g} / \text{mL}$), towards leishmania major promastigotes.

Keywords- Aryl / heteroaryl thiosemicarbazones, antileishmanial activity

INTRODUCTION

Leishmaniasis is considered a group of diseases caused by a protozoan parasite, genus *Leishmania*, which is transmitted to human being via the bite of phlebotomus sandfly [1]. The transmission of parasite occurs from the invertebrate to the mammalian host during blood feeding. Promastigote and, amastigote are two different forms of leishmania parasite adapted in vertebrate and invertebrate hosts respectively [2]. There are three clinical forms of leishmaniasis according to parasite tropism: cutaneous, mucocutaneous and visceral [3].

According to World Health Organization (WHO), leishmaniasis is arising as a severe public health problem. It is epidemic in 88 countries and 350 million are at risk to be infected world wide [4]. Balochistan and Sindh provinces

of Pakistan are vulnerable to cutaneous leishmaniasis. The appearance of new cases of leishmaniasis is around 2 million annually. Currently, there are no effective drugs available for leishmaniasis. The available drugs to treat the disease are frequently ineffective. The first-line treatment is comprised of highly toxic pentavalent antimonials and stibogluconate [5]. The second line drugs include pentamidine, amphotericin B, and miltefosine which are used in case of antileishmanial resistance [6]. However, these drugs are not widely used because of long term treatment, toxicity, remarkable side effects and inadequate efficacy [7]. Thus, there is a growing interest to investigate inexpensive, low side effect and more potent compounds against leishmaniasis.

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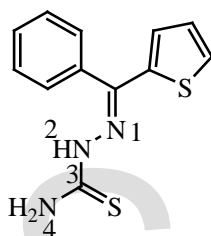
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In this context, thiosemicarbazones, nitrogen and sulfur containing ligands have remained unexplored. Thiosemicarbazones constitute a versatile class of ligands, synthesized by the reaction of thiosemicarbazide with suitable aldehydes or ketones under ambient conditions. They have attracted the medicinal chemists over the past few decades owing to their potential biological activity [8]. Many of the thiosemicarbazones possess a broad spectrum of medicinal properties including antileishmanial [9], antiviral [10], antituberculosis [11], antimalarial [12], anticancer [13], and antifungal [14]. Aromatic groups

bound to azomethine carbon atom make thiosemicarbazone extensively delocalized, which seems essential for biological activity [15]. Thiosemicarbazones have imine (C=N-) moiety, which have shown significant antileishmanial and nematocidal activity [16]-[19]. There is an explosion of interests in designing antileishmanial compounds containing nitrogen atom in different chemical environments because such compounds have exhibited potent antileishmanial activity [20].

In this study, we designed a series of aryl and heteroaryl thiosemicarbazones as potential antileishmanial agents.



compound 1

2. EXPERIMENTAL

2.1 MATERIALS

4-Methoxybenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde, 2-furaldehyde, thiophene-2-carboxaldehyde, 4-dimethylaminobenzaldehyde and thiosemicarbazide were purchased from Aldrich, 2-benzoylthiophene was obtained from Alfa Aesar. Solvents were of reagent grade and used as received.

2.2 GENERAL SYNTHESIS OF THIOSEMICARBAZONES

Equimolar amounts of thiosemicarbazide and the appropriate aldehyde or ketone were dissolved in 35mL of absolute ethanol with the addition of few drops of glacial acetic acid. The resulting mixture was refluxed for Three rows were left for negative and positive controls. In the negative controls, DMSO was serially diluted in medium while the positive control contained varying concentrations of the standard antileishmanial compound Amphotericin B. The plates were incubated for 72 hours at

2-3 hours. The product was separated and recrystallized in a suitable solvent.

2.3 BIOLOGICAL SCREENING: ANTILEISHMANIASIS ACTIVITY - PREPARATION OF SAMPLES

Each compound (1 mg) was dissolved in DMSO (1 mL) and Amphotericin B (1 mg) was also dissolved in DMSO (1 mL) as positive control. Parasites at log phase were centrifuged at 3,000 rpm for 3 minutes. Parasites were diluted in fresh culture medium to a final density of 2×10^6 cells/mL. In 96-well plates, 180 μ L of medium was added in different wells. Twenty μ L of the compound was added in medium and serially diluted. Parasite culture (100 μ L) was added in all wells.

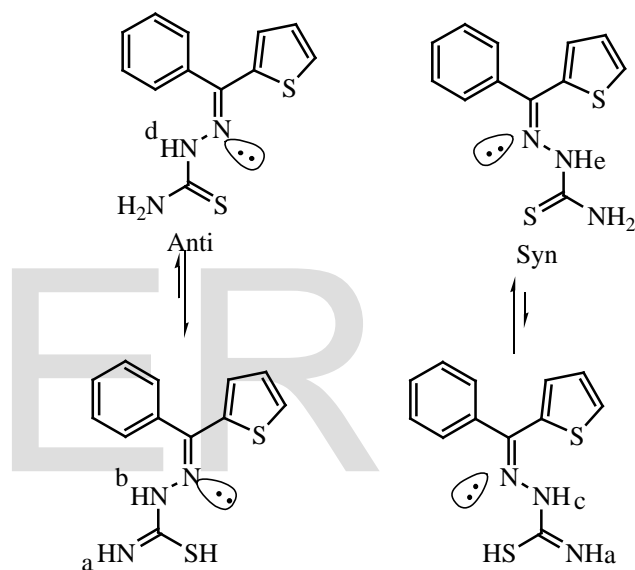
24 °C. The culture was examined microscopically on an improved Neubauer counting chamber and IC₅₀ values of compounds possessing antileishmanial activity were calculated. All assays were run in triplicate. IC₅₀ of samples was determined by using the Prism software.

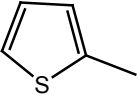
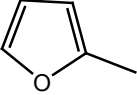
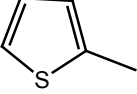
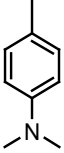
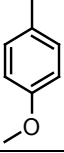
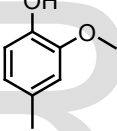
3. RESULTS AND DISCUSSION

The synthesis of thiosemicarbazones **1-6** was depicted in scheme 1. The condensation of appropriate aldehydes or ketones was carried out with thiosemicarbazide in ethanol. All the thiosemicarbazones were obtained in good yield and found stable. The reactions were facile and completed in 2-3 hours of refluxing (TLC monitoring). The physical properties and chemical data of compounds **1-6** are listed in table 1. It has been found in literature review that there is no report on antileishmanial activity of prepared thiosemicarbazones. According to our knowledge, compound **1** has been reported first time. In solutions, all compounds may show iminothione-thiol tautomerism because they contain a proton adjacent to thione group (C=S). On the other hand, the IR spectra of the synthesized semithiocarbazones exhibit the appearance of $\nu(\text{N-H})$ band at 3163 cm^{-1} and the absence of absorption band of $\nu(\text{S-H})$ at $2600\text{-}2500\text{ cm}^{-1}$ and thus suggest that compound **1** exists in solid state in thione form. This was further confirmed by the presence of $\nu(\text{C=S})$ peak at 1232 cm^{-1} . The occurrence of strong band absorption at 1584 cm^{-1} in the IR spectrum was ascribed to $\nu(\text{C=N})$ (C-N) stretching of the imine linkage of compound **1**.

The $^1\text{H-NMR}$ spectra were recorded in DMSO-d_6 . In the $^1\text{H-NMR}$ spectrum of compound **1**, the aromatic protons

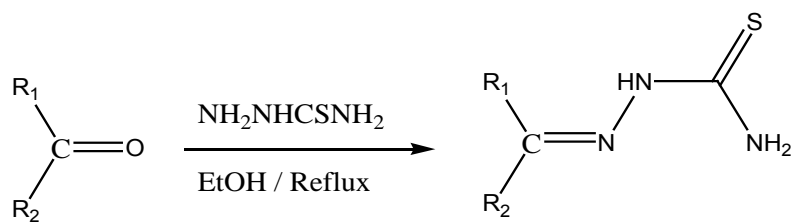
are embedded in protons of thiophene ring and showed a multiplet between 7.68 - 7.10 ppm. $^1\text{H-NMR}$ also reveals that thiosemicarbazone **1** exists in a mixture of syn and anti stereoisomers in a ratio of 50 : 50. Furthermore, each of these isomers exists in solution in iminothione - thiol tautomeric forms in the ratio of 19.00 : 81.00. Other protons have shown broad singlets signals at 8.93 (NHd), 8.67 (NHa), 8.56 (NHb), 8.31 (NHc), 7.98 (NH₂) [21], 7.87 (NHc) and 6.97 (SH). In our speculation, the thiol tautomer is more stabilized due to the hydrogen bonding between S-H and N(2) atom.



Compound	R ₁	R ₂
1		C ₆ H ₅ -
2		H
3		H
4	H	
5	H	
6	H	

The synthesis of

thiosemicarbazones is depicted in scheme 1



Scheme 1. General synthetic route of thiosemicarbazones

Table 1. Physical and analytical data of the thiosemicarbazones (1-6)

Compd. No.	Molecular formula (Mol. Mass) ^a	Colour	MP (°C) (recryst. solvent) ^b	Yield (%)	Elemental analysis, calculated (Found)		
					C (%)	H (%)	N (%)
1	C ₁₂ H ₁₁ N ₃ S ₂ (261.0)	Yellow	141-142 (EtOH)	65	55.1 (55.8)	4.24 (3.97)	16.1 (16.9)
2	C ₆ H ₇ N ₃ OS ^[22] (169.03)	yellow	143-145 (EtOH)	76	42.5 (42.8)	4.17 (4.27)	24.8 (24.4)
3	C ₆ H ₇ N ₃ S ₂ ^[23] (185.00)	Pale yellow	176-177 (EtOH)	63	38.9 (38.8)	3.81 (3.76)	22.6 (22.5)
4	C ₁₀ H ₁₄ N ₄ S ^[24] (222.09)	Pale yellow	211-213 (EtOH)	80	54.0 (54.3)	6.30 (6.52)	25.2 (25.7)
5	C ₉ H ₁₁ N ₃ OS ^[24] (209.06)	White	167-169 (EtOH)	75	51.7 (51.3)	5.30 (5.51)	20.1 (20.7)
6	C ₉ H ₁₁ N ₃ OS ^[25] (225.27)	Pale yellow	210(d) (MeOH)	67	47.9 (47.6)	4.92 (4.97)	18.6 (18.4)

^a References are given for known products, ^b EtOH = Ethanol, MeOH = methanol

3.1 ANTILEISHMANIASIS ACTIVITY.

. Antileishmanial activity of compounds (1-6) was assayed by Zhai's method [26] using a pre-established culture of *L. major* (Table 2). Parasites were cultured in medium M199 with 10% foetal bovine serum; 25 mM of HEPES, and 0.22 µg of penicillin and streptomycin, respectively, at 24 °C in a shaking incubator. Compound 6 has low activity against *L. major*. Compounds 1, 2 and 4 show good activity against

L. major and compounds 3 and 5 show significant activity. The high *in vitro* antileishmaniasis activity of these compounds makes them promising leads for development of effective therapeutic

COMPOUNDS	<i>L. major</i> ^a
1	0.61±0.04
2	0.63 ±0.02
3	0.58 ±0.05
4	0.69 ±0.09
5	0.59 ±0.01
6	0.89 ±0.03
Standard Drug IC50 (µg/mL± S.D) Amphotericin B	0.56 ± 0.03

Table 2

% Inhibition of thiosemicarbazones against *L. major* leishmania

a percentage inhibition activity: 100 = (non-significant; 0.95–0.80 = low; 0.79–0.70 = Moderate;

0.69–0.60 = Good; below 0.59-0.56 = Significant activity).

CONCLUSION

The thiosemicarbazones under investigation have so efficient in vitro antileishmanial activity against L Major that they could be utilized as potent therapeutic agents.

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