Evaluation of *N*-(2-Thienylidene)amines, *N*-(2-Hydroxybenzylidene)amines and 3-Iminoindolin-2-ones as Antileishmanial Agents

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Abstract: The paper describes the synthesis and antileishmanial activity of *N*-substituted imines, obtained from the reactions of primary amines with three biologically important aldehydes/ketones, thiophene-2-carbaldehyde, 2-hydroxybenzaldehyde (salicylaldehyde) and indoline-2,3-dione. Of the fourteen compounds screened from three classes, five compounds showed significant antileishmanial activity. Among the three classes of imines, the class of *N*-(2-thienylidene)amines showed much better activity than the other two classes. *N*-(2-Thienylidene)benzhydrylamine showed IC_{50} value of 0.51 μg/ml. The effect of substituents on the bioactivity is discussed.

Keywords: Antileishmanial, Imines, Indoline-2,3-dione, Leishmaniasis, Thiophene-2- carbaldehyde, Salicylaldehyde.

1. INTRODUCTION

Leishmaniasis is a tropical disease caused by infection of protozoa, *Leishmania*. It has been recognized by the World Health Organization (WHO) as an increasing health problem [1]. Many parts of Pakistan, India and other developing nations are vulnerable to leishmaniasis. Most of the medicines available currently in the market for treatment of leishmaniasis are costly, have side–effects, and get resistant to pathogen after treatment for several weeks. Recent years have drawn considerable interest in design and development of antileishmanial compounds. Many compounds containing carbon-nitrogen double bond in different structural environments have shown promising results [2,3]. We recently launched a program to synthesize diverse types of nitrogen and sulfur containing compounds and to evaluate them as antileishmanial agents. We reported the synthesis and antileishmanial activity of some *N*-aromatic imines from benzaldehydes, cinnamaldehyde and furan-2-carbaldehyde; many of them showed significant anti-leishmanial activity [4]. In continuation of this study, we decided to evaluate *N*-alkyl and *N*-aryl imines of thiophene-2-carbaldehyde, 2-hydroxybenzaldehyde (salicylaldehyde) and indoline-2,3-dione. We selected these aldehydes and α-amidoketone because of various biological activities associated with their derivatives [5-10]. Among each class, we selected *N*-aryl imines with both electron-donating and electron-withdrawing groups on the aromatic ring, and *N*-alkyl imine such as *N*-benzhydryl imine. The present paper, thus, reports the synthesis and evaluation of fourteen compounds of three imine classes as antileishmanial agents. To the best of our knowledge this is the first report on antileishmanial activity of *N*-alkyl imines.

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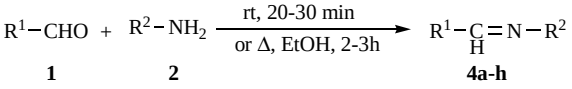
2. RESULTS AND DISCUSSION

N-(2-Thienylidene)benzhydrylamine **4a** and *N*-(2-hydroxybenzylidene)amines **4e-h** were obtained by mixing the equimolar amounts of appropriate aldehyde and amines (Scheme **1**), and allowing the reaction mixture to stand at room temperature for 20-30 min following the method reported for the preparation of the *N*-(benzylidene)benzhydrylamine by Michaelis [11] and used later by our group [10]. Other imines **4b-d** (Scheme **1**) and **4i-n** (Scheme **2**) were obtained by refluxing equimolar amounts of the appropriate amine with thiophene-2-carbaldehyde or indoline-2,3-dione in minimum amount of ethanol for 2-3 hours [12,13]. The compounds, after crystallization from ethanol, were characterized by satisfactory analytical and spectral (IR, ^{1}H NMR and ^{13}C NMR) data (Table **1**, **2**). The IR spectra of the products showed disappearance of the carbonyl absorption and appearance of band in the range of 1616-1625 cm^{-1} corresponding to the azomethine linkage.



Scheme 1.

All the synthesized compounds were screened for their antileishmanial activity using a pre-established culture of *L. major*. According to results shown in the last column of Table **1**, five compounds **4a-c, 4f and 4m** showed significant antileishmanial activity. Among the three classes of imines, the class of *N*-(2-thienylidene)amines **4a-d** was observed as the most active showing IC_{50} in the range of 0.51 to 0.68 μg/ml followed by the class of *N*-(2-hydroxybenzylidene) amines **4e-h** showing IC_{50} in the range of 0.58 to 0.83 μg/ml. *N*-3-Aryl-, *N*-3-alkyl- and *N*-3-cyclohexyliminoindolin-2-ones **4i-n** were the least active showing IC_{50} value in the range of 0.59 to 0.96 μg/ml. Among the class of *N*-(2-thienylidene)amines, *N*-(2-thienylidene)benzhydrylamine was the most potent with the IC_{50} value of 0.51 μg/ml, better than the standard drug. Among the *N*-(2-hydroxybenzylidene)amines **4e-h**, the *N*-(2-hydroxybenzylidene)ben-

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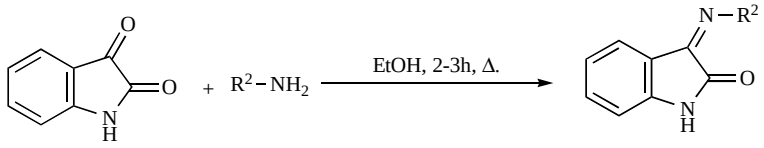
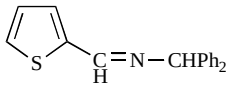
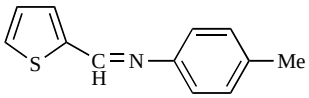
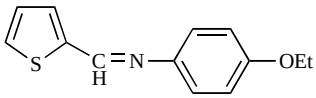
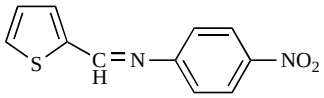
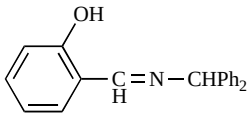
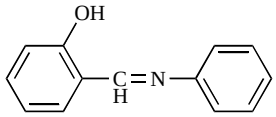
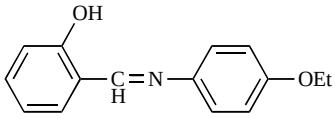
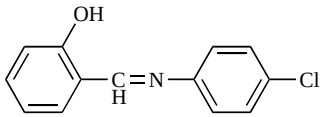
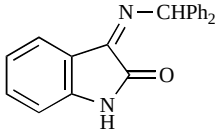
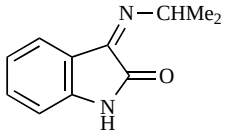
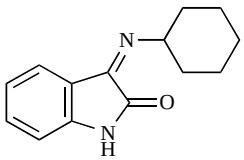


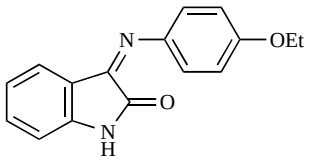
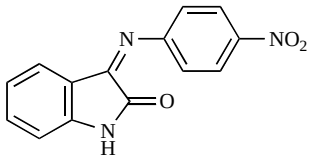
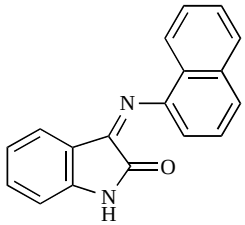
3 **2** **4i-n**

Scheme 2.

Table 1. Physical Data and Antileishmanial Activity of Imines 4a-n

No.	Compound	mp (°C)	Yield (%)	Mol. Formula*	Antileishmanial activity on *L. major*** IC_{50} (μg/ml)
4a		100	90	$C_{18}H_{15}NS$	0.51±0.02
4b		62-64	80	$C_{12}H_{11}NS$	0.57±0.05
4c		70-72	84	$C_{13}H_{13}NOS$	0.55±0.02
4d		130-133 (Lit. 135-137) [4]	58	$C_{11}H_8N_2O_2S$	0.68±0.06
4e		134-136	88	$C_{20}H_{17}NO$	0.63±0.19
4f		48-50	70	$C_{13}H_{11}NO$	0.58±0.04
4g		91-93	78	$C_{15}H_{15}NO_2$	0.83±0.50
4h		94-95	79	$C_{13}H_{10}ClNO$	0.74±0.02
4i		200-204	73	$C_{21}H_{16}N_2O$	0.71±0.19
4j		158-160	68	$C_{11}H_{12}N_2O$	0.62+0.62
4k		140	65	$C_{14}H_{16}N_2O$	0.64±0.10

(Table 1). Contd…..

No.	Compound	mp (°C)	Yield (%)	Mol. Formula*	Antileishmanial activity on *L. major*** IC_{50} (µg/ml)
4l		218-220	88	$C_{16}H_{14}N_2O_2$	0.96±0.03
4m		140-142	57	$C_{16}H_9N_3O_3$	0.59±0.19
4n		240-242	75	$C_{18}H_{12}N_2O$	0.81±0.10
	Standard drug IC_{50} (µg/ml± s.d.): Amphotericin B				0.56±0.20

*All compounds showed satisfactory elemental analysis in the range of ±0.40.
**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).

Table 2. Spectral Data of Imines 4a-n

No.	IR (KBr, cm^{-1})	^1H NMR ($CDCl_3$, δ ppm)	^{13}C NMR ($CDCl_3$, δ ppm)
4a	1620 (C=N)	8.43 (s, 1H, CH), 7.28 – 7.24 (m, 13H, arom), 5.60 (s, 1H, benzhydryl).	155.5, 144.2, 143.5, 129.3, 128.2, 127.4, 127.1, 126.0, 125.5, 68.2.
4b	1615 (C=N)	8.70 (s, 1H, CH), 7.52 – 7.48 (m, 2H, arom), 7.27 – 7.13 (m, 5H, arom), 2.40 (s, 3H, methyl).	154.8, 146.5, 144.7, 137.5, 137.0, 130.3, 127.5, 127.0, 122.1, 23.8.
4c	1620 (C=N)	8.20 (s, 1H, CH), 7.75 – 7.23 (m, 5H, arom), 6.96 (dd, 2H, arom), 3. 95 (q, 2H, methylene), 1.33 (t, 3H, methyl)	155.8, 152.9, 144.0, 140.5, 127.5, 127.0, 125.8, 122.6, 115.8, 63.5, 14.4.
4d	1610 (C=N) (Lit. 1612) [4]	8.56 (s, 1H, CH), 7.63 – 7.58 (m, 2H, arom), 7.27 – 7.17 (m, 5H, arom).	157.6, 155.7, 145.9, 142.3, 135.5, 134.4, 128.7, 125.4, 121.9.
4e	3418 (O-H), 1621 (C=N)	10.20 (s, 1H, OH, D_2O exchangeable), 8.50 (s, 1H, CH), 7.38-6.85 (m, 14H, arom), 5.66 (s, 1H, benzhydryl).	159.5, 157.6, 141.9, 132.2, 130.5, 129.0, 128.6, 126.0, 124.2, 121.8, 115.9, 67.8
4f	3416 (O-H), 1616 (C=N)	9.68 (s, 1H, OH, D_2O exchangeable), 8.65 (s, 1H, CH), 7.48-6.94 (m, 9H, arom).	159.3, 157.5, 149.9, 132.5, 130.2, 130.5, 127.4, 122.1, 121.4, 119.5, 116.0.
4g	3479 (O-H), 1619 (C=N)	10.62 (s, 1H, OH, D_2O exchangeable), 8.64 (s, 1H, CH), 7.41-6.92 (m, 8H, arom), 4.09 (q, 2H, OCH2), 1.46 (t, 3H, methyl).	159.3, 155.5, 153.2, 144.6, 130.3, 128.6, 123.4, 121.5, 118.6, 116.7, 115.4, 64.2, 14.6.
4h	3417 (O-H), 1611 (C=N)	10.35 (s, 1H, OH, D_2O exchangeable), 8.62 (s, 1H, CH), 7.45-6.95 (m, 8H, arom).	159.0, 157.1, 150.1, 133.4, 132.6, 130.6, 130.1, 123.5, 121.5, 118.5, 114.2.
4i	3310 (N-H), 1710 (C=O), 1615 (C=N)	9.30 (s, 1H, NH, D_2O exchangeable), 7.85, (s, 1H, benzhydryl), 7.78-6.65 (m, 14H, arom),	163.5, 158.9, 152.0, 145.6, 132.7, 128.7, 127.6, 126.9, 123.1, 122.4, 121.8, 108.2, 65.5.
4j	3310 (N-H), 1710 (C=O), 1610 (C=N)	9.68 (s, 1H, NH, D_2O exchangeable), 7.70-6.80 (m, 4H, arom), 5.55 (sept, 1H, methine), 1.45 (d, 6H, methyl).	163.2, 158.6, 150.9, 132.90, 126.6, 122.9, 121.9, 116.3, 50.8, 26.2, 25.6.
4k	3305 (N-H), 1710 (C=O), 1610 (C=N)	10.32, (bs, 1H, NH, D_2O exchangeable), 7.22-7.65 (m, 4H, arom), 4.20 (s, 1H, N-CH), 2.0-1.05 (m, 10H, C-hex).	164.2, 158.9, 150.5, 131.4, 129.2, 124.3, 123.5, 121.5, 55.5, 32.2, 27.6, 23.0.

(Table 2). Contd…..

No.	IR (KBr, cm^{-1})	^1H NMR ($CDCl_3$, δ ppm)	^{13}C NMR ($CDCl_3$, δ ppm)
4l	3295 (N-H), 1705 (C=O), 1620 (C=N)	9.80, (bs, 1H, NH, D_2O exchangeable), 7.45-7.20 (m, 6H, arom), 6.92 (dd, 2H, arom), 4.05 (q, 2H, methylene), 1.45 (t, 3H, methyl).	164.0, 158.5, 155.5, 147.9, 145.2, 131.8, 129.2, 124.5, 122.3, 121.5, 117.4, 115.2, 63.5, 14.2.
4m	3295 (N-H), 1705 (C=O), 1605 (C=N)	9.85, (bs, 1H, NH, D_2O exchangeable), 7.48-7.22 (m, 8H, arom).	164.3, 158.9, 151.9, 148.8, 146.4, 131.2, 129.7, 125.3, 123.4, 122.2, 121.7, 118.2.
4n	3285 (N-H), 1705 (C=O), 1610 (C=N)	10.15, (bs, 1H, NH, D_2O exchangeable), 7.64-7.23 (m, 11H, arom).	164.5, 158.6, 150.2, 147.9, 135.5, 131.3, 129.8, 128.2, 127.8, 127.5, 126.8, 126.3, 124.5, 121.7, 117.8, 115.2

zhydrylamine **4e** was slightly inferior in activity (IC_{50} = 0.63 μg/ml)) than the *N*-aryl imine, *N*-(2-hydroxybenzylidene) aniline **4f** (IC_{50} = 0.58 μg/ml). All three *N*-non-aryl imines of indoline-2,3-diones **4i-k** showed better activity to *N*-aryl imines **4l-n** with exception of 3-(4-nitrophenyl)iminoindolin-2-one **4n** which was the most active in the series (IC_{50} = 0.59 μg/ml). Although the *N*-alkyl imines appeared superior to *N*-aryl imines, the effect of substituents in all three classes was not the same indicating the different factors operating in compound – microorganism interaction of the three classes of imines.

3. EXPERIMENTAL

3.1. Chemistry

Melting points have been recorded on a Stuart Scientific melting point apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer-781 IR spectrophotometer using KBr disc of the sample. The NMR spectra were recorded on a Jeol FX 90Q spectrometer using TMS as an internal standard.

Thiophene-2-carbaldehyde, 2-hydroxybenzaldehyde, indoline-2,3-dione, and all the amines were Aldrich products.

Preparation of Imines: Method A

Salicylaldehyde (1 mmol, 0.12 g) or thiophene-2-carbaldehyde (0.11 g, 1 mmol) was taken in a 50 ml conical flask. An equimolar amount of appropriate amine was added drop-wise to it. A solid was formed within a few minutes that was recrystallized from ethanol. Imines **4a** and **4e-h** were prepared by this method.

Preparation of Imines: Method B

Thiophene-2-carbaldehyde (0.11 g, 1 mmol) or indolin-2,3-dione was refluxed with appropriate amine in minimum amount of ethanol for 2-3 hrs. The completion of the reaction was checked by TLC in each case. After completion of the reaction, the solvent was evaporated under reduced pressure and the solid product obtained was recrystallized from ethanol. Imines **4b-d** and **4i-n** were prepared using this method.

3.2. Pharmacology

The title compounds were screened for their anti-leishmanial activity against the pre-established culture of *L. major*. Parasites were cultured in Medium M199 with 10% foetal bovine serum; 25 mmol HEPES, and 0.22 mg of penicillin and streptomycin, respectively at 24 ^oC in a shaking incubator [14].

Each compound to be tested and amphotericin B (as a positive control) were dissolved in DMSO to a concentration of 1 mg/ml. Parasites at log phase were centrifuged at 3000 rpm for 3 min. Parasites were diluted in fresh culture medium to a final density of 2 $x10^6$ cells/ml. In 96-well plates, 180 μl of medium was added in different wells. Experimental compound (20 μl) was added in medium and serially diluted. Parasite culture (100 μl) was added in all wells. In negative controls, DMSO was serially diluted in medium while the positive control contained varying concentrations of standard antileishmanial compound i.e. amphotericin B. The plates were incubated for 72 hours at 24 ^oC. The culture was examined microscopically on an improved Neubauer counting chamber and IC_{50} values of compounds possessing antileishmanial activity were calculated. All the assays were run in duplicate. IC_{50} of samples was determined by using the Prism software.

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