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Synthesis of 2-methoxybenzoylhydrazone and evaluation of their antileishmanial activity

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ABSTRACT

Compounds **1**–**25** showed varying degree of antileishmanial activities with IC_{50} values ranging between 1.95 and 88.56 μM. Compounds **2**, **10**, and **11** (IC_{50} = 3.29 ± 0.07 μM, 1.95 ± 0.04 μM, and 2.49 ± 0.03 μM, respectively) were found to be more active than standard pentamidine (IC_{50} = 5.09 ± 0.04 μM). Compounds **7** (IC_{50} = 7.64 ± 0.1 μM), **8** (IC_{50} = 13.17 ± 0.46 μM), **18** (IC_{50} = 13.15 ± 0.02 μM), and **24** (IC_{50} = 15.65 ± 0.41 μM) exhibited good activities. Compounds **1**, **3**, **4**, **5**, **9**, **12**, **15**, **18**, and **19** were found to be moderately active. Compounds **13**, **14**, **16**, **17**, **20**–**25** showed weak activities with IC_{50} values ranging between 57 and 88 μM.

Applications of Schiff bases are reported in a broad range of fields, for example, inorganic, biological and analytical chemistry.[1–3] A number of heterocyclic hydrazones were reported to possess antimicrobial,[4] antiglycation,[5] and antifungal, anti-HIV, antibacterial,[6,7] and thymidine phosphorylase inhibitory activities.[8] Metal complexes of Schiff bases have also shown various biological activities.[9–12] Numerous hydrazones have shown interesting bioactivities, such as antiinflammatory, anticancer, antiplatelets, antibacterial, cytotoxic, antifungal, anticonvulsant, and analgesic activities.[13–20] *N*-Cyanoethyl hydrazide analogues were reported to be β-glucuronidase inhibitory agents.[21] In addition, these have also been reported to have a range of bioactivities, including antibacterial, antiviral, antifungal,[22] and GSK-3 inhibitory activities.[23,24] Substituted hydrazines have also found significant commercial applications.[25] A variety of heterocycles can easily be synthesized by hydrazide derivatives.[26] For example diacylhydrazides can be cyclized to 1,3-oxa- and thiadiazoles, and 1,2,4-triazoles.[27]

Leishmaniasis is a protozoan disease with large global distribution which causes a huge number of mortalities every year. Leishmaniasis is classified as cutaneous, visceral (Kala Azar), mucosal or mucocutaneous and diffused cutaneous on the basis of cellular immune systems of the patients and parasite.[28–30]

Cutaneous leishmaniasis produces permanent wounds on the face, arms and feet.[31,32] Due to its high occurrence in many countries, the discovery of potential drugs is highly desirable.[33,34] Drugs currently used include antimonial, glucantine, pentamidine, *bis*-amidines, and stilbamidine. Many of these drugs show high toxicities, produce clinical resistance and contribute to an increase in co-infections such as leishmaniasis–AIDS.[34,35] In addition, these drugs are costly and require long-term treatment.[36] Therefore, there is an urgent need to work and discover molecules having potential antileishmanial properties.

We previously reported the antileishmanial activities of acylhydrazides and disulfides[37] and in continuation of our research on acylhdrazides and leishmaniasis, we have synthesized a library of Schiff bases of 2-methoxybenzoyl hydrazide by condensing it with twenty five (25) aromatic aldehydes and screening them for their antileishmanial activities.

The results of the current study are promising and deserve to be investigated further to develop more active, safe and cost-effective antileishmanial agents.

2-Methoxybenzoylhydrazones **1**–**25** were synthesized from 2-methoxybenzoylhydrazide which was obtained from methyl-2-methoxybenzoate by refluxing with hydrazine hydrate for 2 h which was then crystallized from methanol. 2-Methoxybenzoylhydrazones were prepared by condensing 2-methoxybenzoylhydrazide with different aromatic aldehydes in refluxing ethanol for 3–4 h (Scheme 1) in high yield. The products so obtained were crystallized from methanol and most were obtained as needle-like crystals. The

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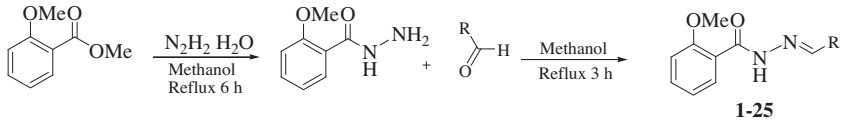


Scheme 1. Synthesis of 2-methoxybenzoylhydrazones **1–25**.

Table 1
In vitro antileishmanial activities of acylhyrazide and % yield of 2-methoxybenzoylhydrazide1 **1–25**

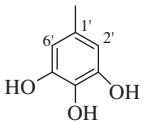
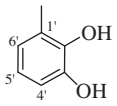
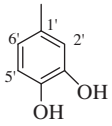
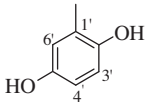
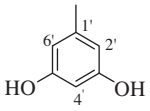
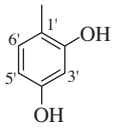
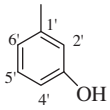
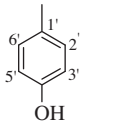
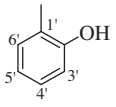
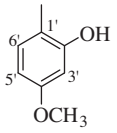
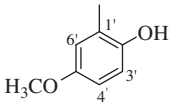
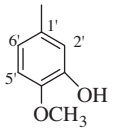
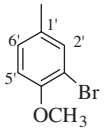
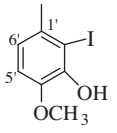
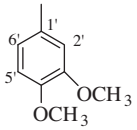
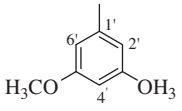
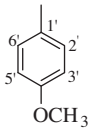
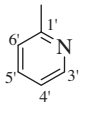
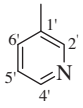
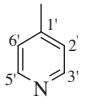
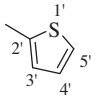
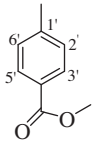
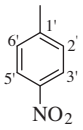
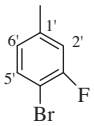
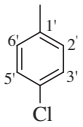
Compd no.	R	Yield (%)	IC_{50} (μM ± SEM[a])	Compd no.	R	Yield (%)	IC_{50} (μM ± SEM[a])
1		82	31.47 ± 0.23	**14**		83	68.05 ± 1.74
2		85	3.29 ± 0.07	**15**		91	34.85 ± 0.48
3		84	28.24 ± 1.44	**16**		92	88.56 ± 2.7
4		87	33.22 ± 1.28	**17**		90	57.41 ± 0.93
5		86	35.41 ± 0.53	**18**		78	13.15 ± 0.02
6		81	>100	**19**		80	45.67 ± 1.09
7		90	7.64 ± 0.10	**20**		82	63.36 ± 2.11
8		91	13.17 ± 0.46	**21**		83	53.13 ± 1.56
9		92	40.07 ± 0.55	**22**		84	59.97 ± 0.6
10		88	1.95 ± 0.04	**23**		86	75.89 ± 0.89
11		84	2.49 ± 0.03	**24**		85	15.65 ± 0.41

Table 1 (*continued*)

Compd no.	R	Yield (%)	IC_{50} (μM ± SEM[a])	Compd no.	R	Yield (%)	IC_{50} (μM ± SEM[a])
12		85	31.56 ± 0.32	**25**		87	77.36 ± 0.58
13		89	68.53 ± 2.30	**Pentamidine[c]**			5.09 ± 0.04

[a] SEM is the standard error of the mean, Pentamidine is the standard drug.

structures of 2-methoxybenzoylhydrazones were elucidated by using NMR spectroscopy. All synthetic compounds gave satisfactory CHN analyses.

Compounds **1**–**25** showed varying degrees of antileishmanial activities with IC_{50} values ranging between 1.95 and 88 μM, as compared to standard pentamidine (IC_{50} = 5.09 ± 0.04 μM) (Table 1). Compounds **10** (IC_{50} = 1.95 ± 0.04 μM), **11** (IC_{50} = 2.49 ± 0.03 μM), and **2** (IC_{50} = 3.29 ± 0.07 μM) were found to be more active than standard pentamidine (IC_{50} = 5.09 ± 0.04 μM). Compounds **7** (IC_{50} = 7.64 ± 0.10 μM), **8** (IC_{50} = 13.17 ± 0.46 μM), **18** (IC_{50} = 13.15 ± 0.02 μM), and **24** (IC_{50} = 15.65 ± 0.41 μM) exhibited good activities. Compounds **3** (IC_{50} = 28.24 ± 1.44 μM), **1** (IC_{50} = 31.47 ± 0.23 μM), **12**, (IC_{50} = 31.56 ± 0.32 μM), **4** (IC_{50} = 33.22 ± 1.28 μM), **15** (IC_{50} = 34.85 ± 0.48 μM), **5** (IC_{50} = 35.41 ± 0.53 μM), **9** (IC_{50} = 40.07 ± 0.53 μM), and **19** (IC_{50} = 45.67 ± 1.09 μM) were found to be moderately active. Compounds **13**, **14**, **16**, **17**, **20**–**23** and **25** showed weak activities with IC_{50} values between 57.41 and 88.56 μM. Only compound **6** was found to be completely inactive Table 1.

Limited SAR suggests that the activity mainly depends upon the presence of hydroxyl and methoxy groups and their respective positions at ring A. If we compare the activity of the most active member of the series, compound **10** (IC_{50} = 1.95 ± 0.04 μM) with its analogous compounds **11** (IC_{50} = 2.49 ± 0.03 μM), and **12** (IC_{50} = 31.56 ± 0.32 μM) we find that both the positions of methoxy and hydroxyl groups at ring A affect the activities of the compounds.

Compound **10** showed excellent activity having 2-hydroxy-4-methoxy substituents at ring A, however, when the methoxy group shifted to position 5 as in compound **11** its activity decreases slightly. A sharp decline (~16-fold) in the activity of compound **12** was observed when the hydroxyl changed from position 2 to 3. This difference in activity suggested that the 2-hydroxy substitution at ring A is vital for antileishmanial activity for this type of compounds along with methyl group. The other methoxybenzoylhydrazones, compounds **15**, **16**, and **17** showed moderate to weak activities. This difference in activity may be due to a hydroxyl residue along with a methoxy group which play significant role in antileishmanial activity of this type of compounds.

Compound **2** (IC_{50} = 3.29 ± 0.07 μM) having 3,4-dihydroxy substitution at ring A was found to be the third most active compound in the series. The other analogous compounds **3**, **4**, **5**, showed moderate activities but surprisingly, compound **6** was found to be completely inactive.

The difference in activities of dihydroxy-containing compounds is controlled by the intramolecular hydrogen bonding at ring A and methoxy group at position 2 at ring B. 3,4-Dihydroxy substitution at ring A and 2-methoxy at ring B seemingly control the antileishmanial activity of compound **2**. However, changing the position of the dihydroxy from 3, 4 to 2, 3 in compound **3** decreases its activity by about eight times because the intramolecular hydrogen bonding is not as effective as in case of compound **2**. The decline in activities of compounds **4**, **5** and **6** may also be due to the alterations in positions of dihydroxy groups at ring A. The compound **4** has 2,5-dihydroxy, compound **5** has 3,5-dihydroxy and compound **6** has 2,4-dihydroxy and chances of intramolecular bonding are not as effective as in the case of compound **2** and **3**. The monohydroxy substituent show varying degrees of activities and the activity mainly depends upon the position of hydroxyl group at ring A. This suggests that monohydroxy group at ring A along with 2-methoxy at ring B are responsible for activity. Compound **7** (7.64 ± 0.10 μM) is the most active among monohydroxy analogues having a hydroxyl at position 3 of ring A, however, if the hydroxy residue shifts to position 4, its activity decreases as in compound **8** (13.17 ± 0.46 μM). Interestingly, if the hydroxyl shifts to position 2 as in compound **9** (40.07 ± 0.55 μM) the activity drops by almost 5 times.

If ring A is replaced by a pyridine ring then interesting results are observed and the activities of the compounds vary according to the position of nitrogen of the pyridine. The most active among the pyridine derivatives is compound **18** (13.15 ± 0.02 μM), with the nitrogen at position 2. Interestingly, when the nitrogen atom of pyridine goes away from the hydrazine bridge as in compound **19** to position 3 and compound **20** to position 4, the activity decreases sharply almost fourfold and sixfold, respectively. The compounds that have the halogenated analogues of hydrazones along with other substituent's show weak activities. In conclusion, it can be said that the antileishmanial activity of this class of compounds mainly depends upon the suitable substitution on rings A and B. Suitable combinations of substituent's at rings A and B are found to be 2-hydroxy-4-methoxy for ring A and 2-methoxy for ring B as in compound **10**. Several other compounds demonstrated remarkable antileishmanial activities. However, compound **10** is found to be the most active antileishmanial molecule and may serve as a lead compound for further studies. In summary it can be concluded that the 2-methoxybenzohydrazones with the utmost antileishmanial activities bear 2-hydroxy along with methoxy residue. From the present study it is concluded that compounds having 2-hydroxy along with methoxy residue are potential lead compounds for further research

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.03.051.

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