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N-Hydroxythiosemicarbazones: Synthesis and in vitro antitubercular activity

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Abstract—*N*-Hydroxythiosemicarbazide was prepared by two methods starting from 2,4-dimethoxy benzyl amine and hydroxylamine hydrochloride, which in turn was reacted with various aldehydes and ketones to obtain the titled compounds. Eighteen compounds were tested for their in vitro activity against *Mycobacterium tuberculosis* H37Rv using the agar dilution method. Compound **10p** was found to be the most potent compound (MIC: 0.28 μM) and was 2.5 times more active than standard isoniazid.

Tuberculosis (TB) is one of the leading causes of death due to a single infectious organism in the world. Approximately one-third of the world's population has been infected with the causative organism *Mycobacterium tuberculosis* (MTB), eight million become sick with TB every year, and globally it accounts for almost three million deaths annually.[1] One-fifth of all deaths of adults in developing countries are due to TB, which is a re-emergent problem particularly in many industrialized countries. It was reported that around 19–43% of the world's population might get infected with MTB between 2000 and 2020 if control measures are not strengthened further.[2] The increase of TB during recent years was largely due to HIV-1 infection, immigration, increased trade, and globalization.[3] Moreover, the increasing emergence of a drug resistant TB, especially multidrug resistant-TB (MDR-TB), is particularly alarming. Multidrug resistant (MDR)-TB has already caused several fatal outbreaks[4] and poses a significant threat to the treatment and control of the disease in some parts of the world, where the incidence of MDR-TB can be as high as 14%.[3] As a consequence, without more effective treatments, the number of infections caused by MDR-TB will probably increase out of control. Therefore, the development of new antimicrobial drugs with potent anti-TB activity is urgently needed.[5] However, in the last 40 years, only a few drugs have been approved by the Food and Drug Administration (FDA) to treat TB, reflecting the inherent difficulties in discovery and clinical testing of new agents and the lack of pharmaceutical industry research in the area.[6] In particular, in addition to the current drugs approved by the FDA for the treatment of TB and the drugs that commonly are recommended for the treatment of TB but are not FDA approved,[7] a variety of other compounds or classes of compounds are under investigation as potential antimycobacterial drugs. Among them, thiosemicarbazone derivatives were reported by us and other groups[8–10] as antimycobacterial agents. Compound (4-bromophenyl)(phenyl)methanone *N*-(5-cyclobutyl-1,3-oxazol-2-yl)thiosemicarbazone was found to be the most active compound in vitro with MIC of 0.05 μg/mL against MTB and MDR-TB, and was 31 times more potent against MDR-TB when compared to the standard drug INH.[8] In continuation of our work on antimycobacterial thiosemicarbazones, herewith we are reporting synthesis and antimycobacterial evaluation of newer *N*-hydroxythiosemicarbazones.

N-Hydroxythiosemicarbazide was prepared by two methods (Scheme 1), in the first method 2,4-dimethoxy benzyl amine (**1**) was condensed with 1,1-thiocarbonyl diimidazole (**2**) in dry methanol to furnish the thiocarbonyl derivative (**3**). Further reaction with hydroxylamine hydrochloride (**4**) in 75% aqueous methanol

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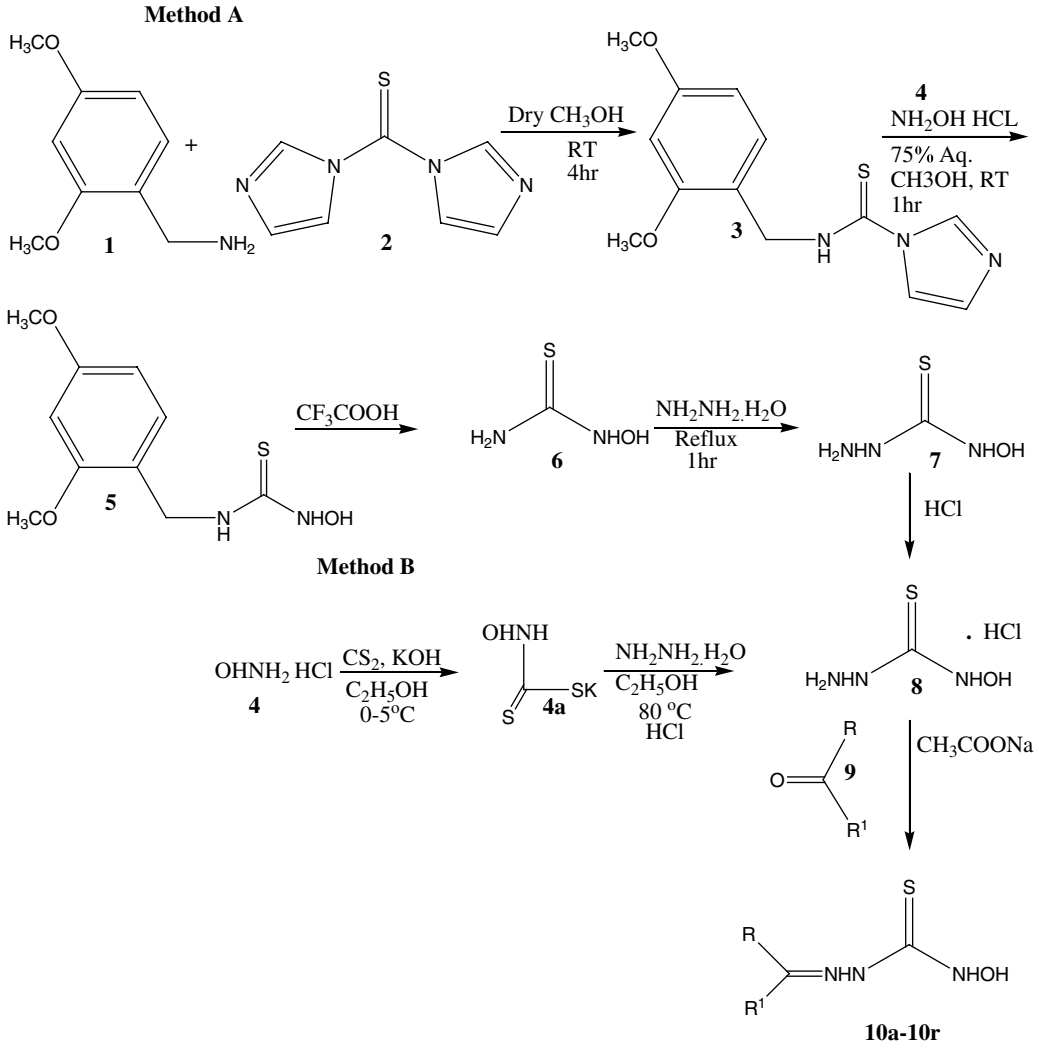
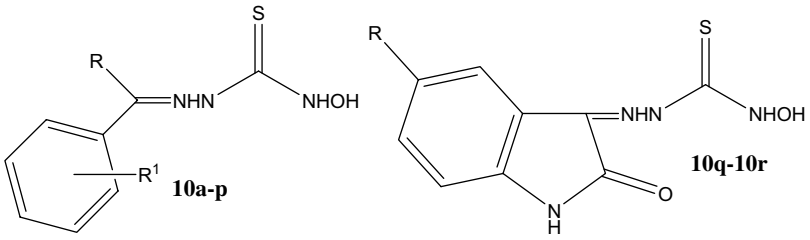


Scheme 1. Synthetic protocol of titled compounds.

gave the 1-(2,4-dimethoxybenzyl)-3-hydroxythiourea (**5**). The dimethoxy benzyl group was eliminated from **5** with trifluoroacetic acid giving hydroxyl thiourea (**6**), which in turn was refluxed with hydrazine hydrate to yield *N*-hydroxythiosemicarbazide (**7**) and when treated with hydrochloric acid furnishes *N*-hydroxythiosemicarbazide hydrochloride salt (**8**) with 62% overall yield. In the second method, the synthesis of compound **8** was carried out in two steps with 74% yield as shown in Scheme 1. First, to a solution of hydroxylamine hydrochloride (0.01 mol) in ethanol (10 ml) were added potassium hydroxide (0.01 mol) and carbon disulfide (0.75 ml), and the mixture was stirred at 0–5 °C for 1 h to form a potassium salt of dithiocarbamate (**4a**). To the stirred mixture was added hydrazine hydrate (0.01 mol) and the stirring was continued for 1 h at 80 °C followed by treatment with hydrochloric acid to give compound **8**. *N*-Hydroxythiosemicarbazide hydrochloride on condensation with various carbonyl compounds (**9**) in the presence of sodium acetate afforded various *N*-hydroxythiosemicarbazones (**10a**–**r**) (Table 1) in 43–83% yields. The purity of the compounds was checked by TLC and elemental analyses; and the compounds of this study were identified by spectral data. In the ^{1}H NMR spectra, the signals of the respective protons of the prepared derivatives were verified on the basis of their chemical shifts, multiplicities, and coupling constants. The spectra of all the compounds showed a D_2O exchangeable singlet at δ 8.5 ppm corresponding to NH proton of hydrazide, broad peak at δ 9.5 ppm corresponding to hydroxyl NH proton, and doublet at δ 10.4 ppm corresponding to OH proton. Compounds **10a**–**j** showed singlet at δ 8.1 ppm corresponding to carbimino H of benzaldehyde, and compounds **10k**–**o** showed singlet at δ 2.14 ppm corresponding to carbimino CH_3 of acetophenone. The elemental analysis results were within ±0.4% of the theoretical values.[11]

The synthesized compounds **10a**–**r** were tested for their antimycobacterial activity in vitro against MTB and INHR-MTB by agar dilution method in Middlebrook 7H11 supplemented with OADC media (Hi-Media) using double dilution technique (drug concentrations of 12.5, 6.25, 3.125, etc.) similar to that recommended by the National Committee for Clinical Laboratory

Table 1. Physical constants and antimycobacterial activity of the titled compounds



Compound	R	R^1	Yield (%)	Mp (°C)	MIC[a] in μM	IC_{50}[b] in μM
10a	H	H	48	85	64.02	NT
10b	H	2-OH	83	190	29.58	NT
10c	H	4-OH	42	245	29.58	NT
10d	H	4-$N(CH_3)_2$	59	240	52.45	NT
10e	H	4-OCH_3	73	165	14.95	NT
10f	H	4-CH_3	75	159	12.97	NT
10g	H	3-OCH_3, 4-OH	53	244	27.21	NT
10h	H	4-Cl	48	210	13.02	NT
10i	H	4-NO_2	47	>260	6.49	>260.15
10j	H	2-NO_2	55	213	6.49	>260.15
10k	CH_3	H	80	119	14.95	NT
10l	CH_3	2-OH	51	195	13.89	NT
10m	CH_3	4-CH_3	43	120	6.98	>279.90
10n	CH_3	4-Cl	48	140	3.20	>256.45
10o	CH_3	4-NO_2	47	202	0.78	>245.81
10p	C_6H_5	4-Br	70	70–75	0.28	>178.45
10q	H	—	45	259	64.02	NT
10r	F	—	42	238	24.58	NT
Ison	—	—	—	—	0.72	>455.77
Rifa	—	—	—	—	0.24	>75.94
Etha	—	—	—	—	15.31	>305.90

[a] Minimum inhibitory concentration required to inhibit growth of *M. tuberculosis* H37Rv.
[b] Cytotoxic concentration of drugs in VERO cells; NT, not tested.

Standards[12] for the determination of minimum inhibitory concentration (MIC). The MIC was defined as the minimum concentration of a compound required to inhibit the complete bacterial growth and MICs of the compounds was reported in Table 1. Rapid glance at the obtained results revealed that all the compounds exhibited very good antimycobacterial activity with MIC ranging from 0.28 to 64.02 μM. Among the synthesized compounds, (4-bromophenyl)(phenyl)methanone *N*-hydroxythiosemicarbazone (**10p**) was found to be the most potent compound (MIC: 0.28 μM) and was 2.5 times more active than standard isoniazid and almost equally active as rifampicin. When compared to ethambutol (MIC: 15.31 μM), eleven compounds (**10e–f**, **10h–p**) were more potent with MIC of 0.28–14.95 μM. Among the benzaldehyde and actophenone derived thiosemicarbazones (**10a–o**), substituents with strongly deactivating electron withdrawing groups like nitro and trifluoromethyl groups in the phenyl ring showed excellent antimycobacterial activity (**10i**, **10j**, **10o**) and this is followed by weakly deactivating groups like halogen derivatives (**10h**, **10p**). Electron donating groups like methyl and methoxy reduce the activity. With respect to the carbimino terminal, the order of activity was found to be (4-bromo)dipheylbenzophenone > (sub)acetophenone > (sub)benzaldehyde > isatin. The intermediate *N*-hydroxythiosemicarbazide did not show any inhibition up to 12.5 μg/mL (116.18 μM).

Some of the compounds (**10i–j**, **10m–p**) which showed good in vitro activity were further examined for toxicity (IC_{50}) in a mammalian VERO cell line at a concentration of 62.5 μg/mL. After 72 h exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay.[13] The compounds were non-toxic as represented in Table 1 and the selectivity index (IC_{50}/MIC) for the most active compound **10p** was more than 625.

In conclusion, it has been shown that the potency, selectivity, and low cytotoxicity of these compounds make them valid leads for synthesizing new compounds that possess better activity. Further structure–activity and mechanistic studies should prove fruitful.

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11. Physical and spectral data for compound **10p**: Yield: 60%; mp 123° C; IR (KBr): 3600, 3550, 3200, 1640 for cm; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.5–8.1 (m, 9H, Ar–H), 8.5 (s, 1H, NH of hydrazide), 9.5 (br s, 1H, NH attached with hydroxyl), 10.4 (d, 1H, OH); calculated for $C_{14}H_{12}BrN_3OS$: C, 48.01; H, 3.45; N, 12.00. Found: C, 47.91; H, 3.49; N, 11.99.
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