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RESEARCH ARTICLE

5-Nitrothiazolylthiosemicarbazones: Synthesis and antimycobacterial evaluation against tubercular and non-tubercular mycobacterial species

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Abstract
Nineteen 5-nitrothiazolylthiosemicarbazones were synthesized from 5-nitrothiazole by three-step synthesis and evaluated for *in vitro* activities against seven mycobacterial species. Among them, *N*-(5-nitro-1,3-thiazol-2-yl)-2-((*Z*)-4-[(phenylmethyl)oxy]phenylmethylidene)hydrazine-1-carbothioamide (**4m**) was found to be the most active compound with a minimum inhibitory concentration (MIC) of 0.23 μM against *Mycobacterium tuberculosis* H37 Rv, and was three times more potent than isoniazid and equally active as rifampicin. Compound **4m** also inhibited six non-tubercular mycobacteria with MICs ranging from 1.88 to 30.25 μM.

Keywords: *Tuberculosis; antimycobacterial activity; thiosemicarbazones; thiazole derivatives*

Introduction

Mycobacterium tuberculosis (MTB), a human pathogen causing tuberculosis (TB), is responsible for the deaths of millions of people every year, and continues to claim more lives than any other single infectious agent[1]. A chief reason that TB persists as a global killer and is on the rise in parts of the world is that existing antibiotics require up to 6 months of daily use, making it difficult for people to complete the treatment. Those who miss doses, in turn, fuel the emergence of drug-resistant strains of MTB (MDR-TB) that cause the illness. The treatment of MDR-TB is characterized by relatively less effective, poorly tolerated, and expensive drugs that may need to be administered for years[2]. A key element in these events is the explosive combination of TB and the human immune-deficiency virus (HIV) epidemic[3]. This convergence of HIV and TB poses difficult problems, not only because the viral infection increases mortality from TB, but also because optimization of antiviral and antimycobacterial drugs given together presents further difficulties: for example, rifampicin inhibits RNA polymerase but also induces CYP450 enzymes, which metabolize certain anti-HIV drugs. These problems illustrate the importance but also the difficulties inherent in finding new drugs to treat TB. Even in terms of drug-sensitive disease, the duration of therapy is still very long, and the drugs used have toxic effects. Unfortunately, the last truly novel drug, rifampicin, approved for the treatment of TB was discovered 45 years ago; this partially explains the inadequacy of the present armamentarium of antitubercular drugs. Earlier we reported the antitubercular activities of various oxazolyl and *N*-hydroxy-thiosemicarbazones which inhibited MTB with a minimum inhibitory concentration (MIC) of 0.05 μg/mL[4,5]. In continuation we present herein preliminary results concerning the synthesis and *in vitro* antimycobacterial activities of novel 5-nitrothiazolylthiosemicarbazones.

Materials and methods

Chemistry

Melting points were measured on an electrothermal melting point apparatus (Buchi BM530) in open capillary tubes and are uncorrected. ^{1}H-nuclear magnetic resonance (NMR)

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spectra were scanned on a Jeol Fx 400 MHz NMR spectrometer using dimethylsulfoxide (DMSO)-d_6 as solvent. Chemical shifts are expressed in δ (ppm) relative to tetramethylsilane. Elemental analyses (C, H, and N) were performed on a PerkinElmer model 240C analyzer and the data were within ±0.4% of the theoretical values.

Synthesis of N-(5-nitrothiazol-2-yl) hydrazinecarbothioamide (3)

To a solution of 2-amino-5-nitrothiazole (0.01 mol) in absolute ethanol (20 mL) were added potassium hydroxide (0.01 mol) and carbon disufhide (0.75 mL), and the mixture was stirred at 0–5°C for 1 h to form the potassium salt of dithiocarbamate (**2**). To the stirred mixture of dithiocarbamate salt was added hydrazine hydrate (0.01 mol) and the mixture was refluxed for 3 h with stirring. Completion of the reaction was monitored using thin layer chromatography (TLC). Ethanol was distilled off and finally crystals of thiosemicarbazide were filtered, with yield: 92%; M.P.: 150–152°C.

General method for the synthesis of thiosemicarbazones (4a–s)

To a solution of **3** (0.003 mol) in dimethylformamide (DMF), an appropriate aldehyde or ketone was added. The pH was maintained at 4–5 by adding glacial acetic acid (GAA). The mixture was refluxed with stirring for 10–15 h and completion of the reaction was monitored using TLC. The mixture was added to crushed ice to obtain final target products **4a–s**. Product was recrystallized from 95% ethanol.

*N-(5-Nitro-1,3-thiazol-2-yl)-2-[(Z)-phenylmethylidene]hydrazine-1-carbothioamide (**4a**)* Yield: 40.8%; M.P.: > 250°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.12–7.17 (m, 1H, Ar-H), 7.27–7.31 (m, 2H, Ar-H), 7.42–7.45 (m, 2H, Ar-H), 7.80 (s, 1H, benzylidine H), 7.99 (s, 1H, H of thiazole), 11.9 (s, 2H, D_2O exchangeable NH); Chemical Formula: $C_{11}H_9N_5O_2S_2$; Calculated for C, 42.99; H, 2.95; N, 22.79; Found: C, 43.04; H, 2.90; N, 22.76%.

*2-[(Z)-(2-Hydroxyphenyl)methylidene]-N-(5-nitro-1,3-thiazol-2-yl)hydrazine-1-carbothioamide (**4b**)* Yield: 50.3%; M.P.: 167°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 6.96–6.99 (m, 1H, Ar-H), 7.14–7.18 (m, 1H, Ar-H), 7.21–7.25 (m, 1H, Ar-H), 7.33 (s, 1H, benzylidine H), 7.36–7.38 (m, 1H, Ar-H), 7.99 (s, 1H, H of thiazole), 11.4 (s, 1H, OH); 11.9 (s, 2H, D_2O exchangeable NH); Chemical Formula: $C_{11}H_9N_5O_3S_2$; Calculated for C 40.86; H, 2.81; N, 21.66; Found: C, 40.88; H, 2.80; N, 21.70%.

*2-[(Z)-(2-Methylphenyl)methylidene]-N-(5-nitro-1,3-thiazol-2-yl)hydrazine-1-carbothioamide (**4c**)* Yield: 41.7%; M.P.: 184°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.78 (s, 3H, CH_3), 6.98–7.00 (m, 1H, Ar-H), 7.27–7.29 (m, 1H, Ar-H), 7.33–7.34 (m, 1H, Ar-H), 7.64 (s, 1H, benzylidine H), 7.99 (s, 1H, H of thiazole), 8.13–8.14 (m, 1H, Ar-H), 11.9 (s, 2H, D_2O exchangeable NH); Chemical Formula: $C_{12}H_{11}N_5O_2S_2$; Calculated for C, 44.85; H, 3.45; N, 21.79; Found: C, 44.88; H, 3.43; N, 21.76%.

*2-(Z)-[4-(Dimethylamino)phenyl]methylidene-N-(5-nitro-1,3-thiazol-2-yl)hydrazine-1-carbothioamide (**4d**)* Yield: 80.1%; M.P.: > 250°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.44 (s, 6H, CH_3), 6.44–6.46 (m, 2H, Ar-H), 7.64–7.66 (m, 2H, Ar-H), 7.80 (s, 1H, benzylidine H), 7.98 (s, 1H, H of thiazole), 11.9 (s, 2H, D_2O exchangeable NH); Chemical Formula: $C_{13}H_{14}N_6O_2S_2$; Calculated for C, 44.56; H, 4.03; N, 23.98; Found: C, 44.52; H, 3.98; N, 23.96%.

*2-(Z)-[4-(Methyloxy)phenyl]methylidene-N-(5-nitro-1,3-thiazol-2-yl)hydrazine-1-carbothioamide (**4e**)* Yield: 41.7%; M.P.: 246°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.84 (s, 3H, -OCH_3), 6.73–6.75 (m, 2H, Ar-H), 7.31–7.34 (m, 2H, Ar-H), 7.80 (s, 1H, benzylidine H), 7.98 (s, 1H, H of thiazole), 11.9 (s, 2H, D_2O exchangeable NH); Chemical Formula: $C_{12}H_{11}N_5O_3S_2$; Calculated for C, 42.72; H, 3.29; N, 20.76; Found: C, 42.70; H, 3.33; N, 20.91%.

*2-[(Z)-(2-Nitrophenyl)methylidene]-N-(5-nitro-1,3-thiazol-2-yl)hydrazine-1-carbothioamide (**4f**)* Yield: 70.1%; M.P.: 167°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 6.69–6.70 (m, 2H, Ar-H), 7.72–7.73 (m, 1H, Ar-H), 7.78–7.79 (m, 1H, Ar-H), 7.9 (s, 1H, benzylidine H), 8.0 (s, 1H, H of thiazole), 11.9 (s, 2H, D_2O exchangeable NH); Chemical Formula: $C_{11}H_8N_6O_4S_2$; Calculated for C, 37.50; H, 2.29; N, 23.85; Found: C, 37.53; H, 2.31; N, 23.90%.

*2-[(Z)-(4-Nitrophenyl)methylidene]-N-(5-nitro-1,3-thiazol-2-yl)hydrazine-1-carbothioamide (**4g**)* Yield: 80.2%; M.P.: > 250°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.80 (s, 1H, benzylidine H), 7.96–7.97 (m, 2H, Ar-H), 8.0 (s, 1H, H of thiazole), 8.23–8.24 (m, 2H, Ar-H), 11.9 (s, 2H, D_2O exchangeable NH); Chemical Formula: $C_{11}H_8N_6O_4S_2$; Calculated for C, 37.50; H, 2.29; N, 23.85; Found: C, 37.51; H, 2.34; N, 23.86%.

*2-[(Z)-(3-Bromophenyl)methylidene]-N-(5-nitro-1,3-thiazol-2-yl)hydrazine-1-carbothioamide (**4h**)* Yield: 70.6%; M.P.: > 250°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.21–7.23 (m, 1H, Ar-H), 7.37–7.38 (m, 1H, Ar-H), 7.46–7.47 (m, 1H, Ar-H),7.75 (s, 1H, benzylidine H), 7.78–7.79 (m, 1H, Ar-H), 8.0 (s, 1H, H of thiazole), 11.9 (s, 2H, D_2O exchangeable NH); Chemical Formula: $C_{11}H_8BrN_5O_2S_2$; Calculated for C, 34.21; H, 2.09; N, 18.13; Found: C, 34.22; H, 2.13; N, 18.12%.

*2-[(Z)-(4-Fluorophenyl)methylidene]-N-(5-nitro-1,3-thiazol-2-yl)hydrazine-1-carbothioamide (**4i**)* Yield: 40.2%; M.P.: 184–185°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.30–7.32 (m, 2H, Ar-H), 7.54–7.58 (m, 2H, Ar-H), 7.80 (s, 1H, benzylidine H), 8.0 (s, 1H, H of thiazole), 11.9 (s, 2H, D_2O exchangeable NH); Chemical Formula: $C_{11}H_8FN_5O_2S_2$; Calculated for C, 40.61; H, 2.48; N, 21.53; Found: C, 40.64; H, 2.43; N, 21.50%.

*2-[(Z)-(4-Chlorophenyl)methylidene]-N-(5-nitro-1,3-thiazol-2-yl)hydrazine-1-carbothioamide (**4j**)* Yield: 52.8%; M.P.: 178–179°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.40–7.42 (m, 2H, Ar-H), 7.49–7.50 (m, 2H, Ar-H), 7.80 (s, 1H, benzylidine H), 8.0 (s, 1H, H of thiazole), 11.9 (s, 2H, D_2O exchangeable NH); Chemical Formula: $C_{11}H_8ClN_5O_2S_2$; Calculated for C, 38.65; H, 2.36; N, 20.49; Found: C, 38.64; H, 2.40; N, 20.53%.

*N-(5-Nitro-1,3-thiazol-2-yl)-2-(Z)-[2-(trifluoromethyl) phenyl]methylidenehydrazine-1-carbothioamide (**4k**)* Yield: 84.6%; M.P.: 166–167°C; ^{1}H-NMR (DMSO-d_6)

δ (ppm): 7.39 (s, 1H, benzylidine H), 7.59–7.60 (m, 1H, Ar-H), 7.66–7.67 (m, 1H, Ar-H), 8.0 (s, 1H, H of thiazole), 8.03–8.06 (m, 1H, Ar-H), 8.22–8.24 (m, 1H, Ar-H), 11.9 (s, 2H, D_2O exchangeable NH); Chemical Formula: $C_{12}H_8F_3N_5O_2S_2$; Calculated for C, 38.40; H, 2.15; N, 18.66; Found: C, 38.43; H, 2.11; N, 18. 70%.

*2-[(Z)-(2,6-Dichlorophenyl)methylidene]-N-(5-nitro-1,3-thiazol-2-yl)hydrazine-1-carbothioamide (**4l**)* Yield: 84.6%; M.P.: 166–167°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.08–7.10 (m, 1H, Ar-H), 7.34–7.36 (m, 2H, Ar-H), 7.98 (s, 1H, benzylidine H), 8.5 (s, 1H, H of thiazole), 11.9 (s, 2H, D_2O exchangeable NH); Chemical Formula: $C_{11}H_7Cl_2N_5O_2S_2$; Calculated for C, 35.12; H, 1.88; N, 18.61; Found: C, 35.13; H, 1.91; N, 18.64%.

*N-(5-Nitro-1,3-thiazol-2-yl)-2-((Z)-4-[(phenylmethyl) oxy]phenylmethylidene)hydrazine-1-carbothioamide (**4m**)* Yield: 40.4%; M.P.: 188–190°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.98 (s, 2H, CH_2 of benzyl), 6.68–6.70 (m, 2H, Ar-H), 7.04–7.06 (m, 1H, Ar-H), 7.11–7.12 (m, 2H, Ar-H), 7.13–7.14 (m, 2H, Ar-H), 7.27–7.28 (m, 2H, Ar-H), 7. 80 s, 1H, benzylidine H), 8.0 (s, 1H, H of thiazole), 11.9 (s, 2H, D_2O exchangeable NH); Chemical Formula: $C_{18}H_{15}N_5O_3S_2$; Calculated for C, 52.29; H, 3.66; N, 16.94; Found: C, 52.26; H, 3.70; N, 16.98%.

*N-(5-Nitro-1,3-thiazol-2-yl)-2-[(Z)-1-phenylethylidene] hydrazine-1-carbothioamide (**4n**)* Yield: 54.4%; M.P.: 188–189°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.32 (s, 3H, CH_3), 7.25–7.27 (m, 3H, Ar-H), 7.39–7.42 (m, 2H, Ar-H), 7.95 (s, 1H, H of thiazole), 9.77 (s, 2H, D_2O exchangeable NH); Chemical Formula: $C_{12}H_{11}N_5O_2S_2$; Calculated for C, 44.85; H, 3.45; N, 21.79; Found: C, 44.90; H, 3.49; N, 21.76%.

*2-[(Z)-1-(2-Hydroxyphenyl)ethylidene]-N-(5-nitro-1,3-thiazol-2-yl)hydrazine-1-carbothioamide (**4o**)* Yield: 50.1%; M.P.: 146–147°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.21 (s, 3H, CH_3), 6.95–6.97 (m, 1H, Ar-H), 7.14–7.16 (m, 1H, Ar-H), 7.18–7.19 (m, 1H, Ar-H), 7.34–7.35 (m, 1H, Ar-H), 7.95 (s, 1H, H of thiazole), 10.40 (s, 2H, D_2O exchangeable NH); Chemical Formula: $C_{12}H_{11}N_5O_3S_2$; Calculated for C, 42.72; H, 3.29; N, 20.76; Found: C, 42.76; H, 3.26; N, 20.79%.

*2-[(Z)-1-(4-Methylphenyl)ethylidene]-N-(5-nitro-1,3-thiazol-2-yl)hydrazine-1-carbothioamide (**4p**)* Yield: 43.1%; M.P.: 149–150°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.32 (s, 3H, CH_3), 2.34 (s, 3H, 4-CH_3), 7.20–7.22 (m, 2H, Ar-H), 7.88–7.90 (m, 2H, Ar-H), 7.95 (s, 1H, H of thiazole), 9.77 (s, 2H, D_2O exchangeable NH); Chemical Formula: $C_{13}H_{13}N_5O_2S_2$; Calculated for C, 46.55; H, 3.91; N, 20.88; Found: C, 46.60; H, 3.94; N, 20.89%.

*2-[(Z)-1-(3-Aminophenyl)ethylidene]-N-(5-nitro-1,3-thiazol-2-yl)hydrazine-1-carbothioamide (**4q**)* Yield: 43.1%; M.P.: 149–150°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.32 (s, 3H, CH_3), 6.61–6.63 (m, 1H, Ar-H), 7.02 (s, 2H, NH_2 D_2O exchangeable), 7.14–7.16 (m, 1H, Ar-H), 7.18–7.19 (m, 1H, Ar-H), 7.26–7.28 (m, 1H, Ar-H), 7.95 (s, 1H, H of thiazole), 9.77 (s, 2H, D_2O exchangeable NH); Chemical Formula: $C_{12}H_{12}N_6O_2S_2$; Calculated for C, 42.85; H, 3.60; N, 24.98; Found: C, 42.87; H, 3.64; N, 24.90%.

*2-[(Z)-1-(4-Bromophenyl)ethylidene]-N-(5-nitro-1,3-thiazol-2-yl)hydrazine-1-carbothioamide (**4r**)* Yield: 38.4%; M.P.: 156–158°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.32 (s, 3H, CH_3), 7.51–7.53 (m, 2H, Ar-H), 7.91–7.93 (m, 2H, Ar-H), 7.95 (s, 1H, H of thiazole), 9.77 (s, 2H, D_2O exchangeable NH); Chemical Formula: $C_{12}H_{10}BrN_5O_2S_2$; Calculated for C, 36.01; H, 2.52; N, 17.50; Found: C, 36.10; H, 2.54; N, 17.49%.

*2-[(Z)-1-(4-Nitrophenyl)ethylidene]-N-(5-nitro-1,3-thiazol-2-yl)hydrazine-1-carbothioamide (**4s**)*
Yield: 40.1%; M.P.: 188–189°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.32 (s, 3H, CH_3), 7.51–7.90–7.91 (m, 2H, Ar-H), 7.95 (s, 1H, H of thiazole), 8.18–8.20 (m, 2H, Ar-H), 9.77 (s, 2H, D_2O exchangeable NH); Chemical Formula: $C_{12}H_{10}N_6O_4S_2$; Calculated for C, 39.34; H, 2.75; N, 22.94; Found: C, 39.36; H, 2.69; N, 23.1%.

Antimycobacterial activity in log-phase cultures

All compounds were screened in triplicate for their *in vitro* antimycobacterial activity against log-phase cultures of MTB, and non-tubercular mycobacterial (NTM) species such as *M. smegmatis* ATCC 14468, *M. microti* MTCC 1727, *M. vaccae* MTCC 997, *M. phlei* MTCC 1724, *M. fortuitum* MTCC 951, and *M. kansasii* MTCC 3058 in Middlebrook 7H11agar medium supplemented with OADC (albumin–dextrose–sodium chloride) by the agar dilution method, similar to that recommended by the National Committee for Clinical Laboratory Standards, for the determination of MIC[6]. The MDR-TB clinical isolate was obtained from the Tuberculosis Research Center, Chennai, India, and was resistant to isoniazid, rifampicin, and ciprofloxacin. The MIC is defined as the minimum concentration of compound required to give complete inhibition of bacterial growth.

Cytotoxicity

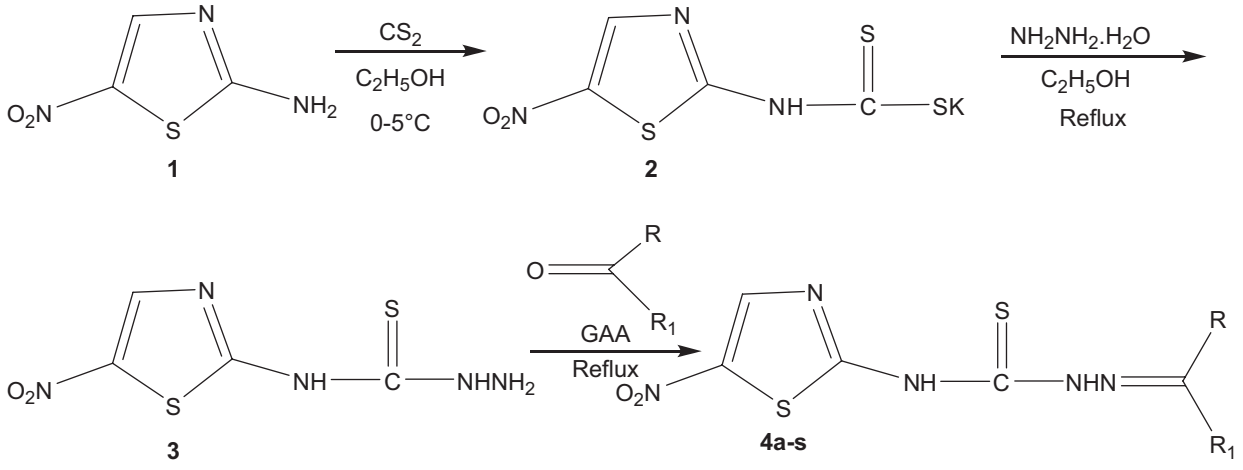
All the compounds were further examined for toxicity (IC_{50}) in a mammalian Vero cell line at a concentration of 62.5 μg/mL by the serial dilution method[7]. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into a formazan product using the Promega CellTiter 96 non-radioactive cell proliferation assay.

Results and discussion

Synthesis

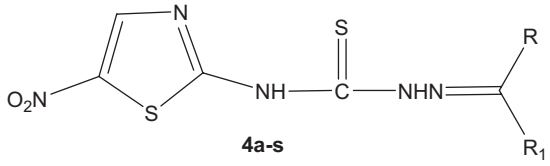
The synthesis of *N*-(5-nitrothiazol-2-yl)hydrazinecarbothioamide **(3)** was carried out in two steps with 92% yield, as shown in Scheme 1. First, to a solution of 2-amino-5-nitrothiazole (**1**) in ethanol were added potassium hydroxide and carbon disulfide, and the mixture was stirred to form the corresponding potassium salt of dithiocarbamate (**2**). To the stirred mixture was added hydrazine hydrate, and stirring was continued with reflux for 3 h followed by distillation to give compound **3**. Thiosemicarbazide on condensation with various carbonyl compounds in the presence of glacial acetic acid afforded various novel

5-nitrothiazolyl-2-thiosemicarbazones (**4a–s**) (Table 1). 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 11.9 and 9.77 ppm, corresponding to NH protons of benzaldehyde-derived thiosemicarbazones (**4a–m**) and acetophenone-derived thiosemicarbazones (**4n–s**), respectively. Compounds **4a–m** showed a singlet at δ ranging 7.4–7.8 ppm, corresponding to the carbimino H of benzaldehyde, and compounds **4n–s** showed a singlet at δ 2.32 ppm, corresponding to the carbimino CH_3 of acetophenone. All the



Scheme 1. Synthesis of compounds.

Table 1. Antimycobacterial activities of 5-nitrothiazolylthiosemicarbazones.



Compound	R	R_1	IC_{50}[a] (μM)	Minimum inhibitory concentration (μM)						
				MTB[b]	MS[c]	MM[d]	MV[e]	MP[f]	MF[g]	MK[h]
4a	H	Phenyl	NT[i]	40.67	2.54	81.34	5.08	2.54	5.08	2.54
4b	H	2-OH-Phenyl	NT	77.31	9.68	38.66	77.32	4.82	38.66	77.32
4c	H	2-CH_3-Phenyl	NT	19.44	2.43	77.79	4.85	4.85	4.85	4.85
4d	H	4-$N(CH_3)_2$-Phenyl	NT	17.83	2.23	142.68	2.23	71.34	4.45	71.34
4e	H	4-OCH_3-Phenyl	NT	37.05	2.31	148.20	74.1	18.52	4.62	2.31
4f	H	2-NO_2-Phenyl	<177.38	2.21	2.21	70.95	141.9	35.47	8.88	4.43
4g	H	4-NO_2-Phenyl	<177.38	1.10	2.21	70.95	2.21	17.74	2.21	2.21
4h	H	3-Br-Phenyl	161.81	1.00	2.01	129.45	32.36	8.10	2.01	8.10
4i	H	4-F-Phenyl	>192.10	4.79	4.79	76.84	38.42	2.39	76.84	4.79
4j	H	4-Cl-Phenyl	182.85	4.56	2.28	36.57	36.57	2.28	73.14	36.57
4k	H	2-CF_3-Phenyl	>166.51	0.52	2.08	13.32	13.32	26.64	26.64	13.32
4l	H	2,5-$(Cl)_2$-Phenyl	NT	16.61	16.61	132.89	33.22	8.32	4.15	2.07
4m	H	4-Benzyloxy phenyl	>151.15	0.23	1.88	30.25	30.25	3.78	15.13	3.78
4n	CH_3	Phenyl	NT	19.44	2.42	77.79	77.79	9.74	77.79	77.79
4o	CH_3	2-OH-Phenyl	NT	18.52	2.31	37.05	74.10	4.62	148.20	9.27
4p	CH_3	4-CH_3-Phenyl	NT	9.33	4.65	74.53	74.53	4.65	37.27	2.33
4q	CH_3	3-NH_2-Phenyl	NT	9.30	2.32	74.32	148.63	4.63	297.27	4.63
4r	CH_3	4-Br-Phenyl	156.1	3.89	15.61	62.45	124.91	1.95	124.91	31.22
4s	CH_3	4-NO_2-Phenyl	<170.58	2.12	2.12	68.23	68.23	2.12	68.23	34.11
Ciprofloxacin			>188.59	4.71	2.35	2.35	4.71	4.71	4.71	9.45
Rifampicin			>75.94	0.23	1.89	30.38	3.80	30.38	1.89	7.59
Isoniazid			>455.73	0.66	45.57	22.82	182.3	91.15	22.82	182.3

[a]Cytotoxicity in mammalian Vero cell lines.
[b]*M. tuberculosis*; [c]*M. smegmatis*; [d]*M. microti*; [e]*M. vaccae*; [f]*M. phlei*; [g]*M. fortuitum*; [h]*M. kansasii*.
[i]Not tested.

compounds showed a singlet in the range of δ 7.8–8.0 ppm, which corresponds to the C-4 hydrogen of the thiazole ring. The elemental analysis results were within ±0.4% of the theoretical values.

Biological investigation

In the first phase, the compounds were screened for their *in vitro* antimycobacterial activity against log-phase cultures of MTB, and NTM species such as *M. smegmatis* ATCC 14468, *M. microti* MTCC 1727, *M. vaccae* MTCC 997, *M. phlei* MTCC 1724, *M. fortuitum* MTCC 951, and *M. kansasii* MTCC 3058 by the agar dilution method for determination of the MIC. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound required to give complete inhibition of bacterial growth. MICs of the synthesized compounds along with those of the standard drugs for comparison are reported (Table 1).

In the initial screening against MTB, the newer compounds showed activity with MICs ranging from 0.23 to 77.31 μM. Two compounds (**4k** and **4m**) showed excellent activity, with MIC < 1 μM. When compared to isoniazid (INH) (MIC: 0.66 μM), both compounds (**4k** and **4m**) were found to be more active, with MICs of 0.52 and 0.23 μM, respectively. Compound **4m** was also found to be equally active as rifampicin (RIF) (MIC: 0.23 μM). Eight compounds (**4f–h**, **4j**, **4k**, **4m**, **4r**, **4s**) were more potent than ciprofloxacin (CIP) (MIC: 4.71 μM). Compound *N*-(5-nitro-1,3-thiazol-2-yl)-2-((*Z*)-4-[(phenylmethyl)oxy] phenylmethylidene)hydrazine-1-carbothioamide (**4m**) was found to be the most active compound *in vitro* with an MIC of 0.23 μM against MTB, and was 2 and 19 times more potent than INH and CIP, respectively. With respect to the structure–MTB activity relationship, in the carbimino terminal, benzaldehyde-derived thiosemicarbazones (**4a–m**) were more potent than acetophenone-derived thiosemicabazones (**4n–s**). The antimycobacterial activity was enhanced by the introduction of strongly deactivating electron-withdrawing groups such as nitro and trifluoromethyl groups in the phenyl moiety, whereas the introduction of electron-donating groups such as hydroxyl, methyl, and dimethylamino groups decreased the activity. The introduction of the bulky benzyloxy group in the phenyl ring enhanced the activity.

All the compounds were also screened for atypical mycobacteria (AM) infection[8], an illness caused by a type of mycobacterium other than TB which causes a wide variety of infections such as abscesses, septic arthritis, and osteomyelitis. It can also infect the lungs, lymph nodes, gastrointestinal tract, skin, and soft tissues. The incidence of AM infections is rare, but it is increasing as the AIDS population grows. Populations at risk include individuals who have lung disease and those with weakened immune systems. The synthesized compounds inhibited *M. smegmatis* (MS) with MICs ranging from 1.88 to 16.61 μM, and all the compounds were more potent than INH (MIC: 45.57 μM); MS infects the lungs[9]. With regard to activity against *M. microti* (MM; which causes sepsis tuberculosa acutissima in immunocompetent persons[10]), the compounds showed activity with MICs ranging from 13.32 to 148.20 μM, and only one compound (**4k**) was more potent than INH (MIC: 22.82 μM). *M. vaccae*, which causes cutaneous and pulmonary infections[11], was inhibited by the synthesized compounds with MICs ranging from 2.21 to 148.63 μM, and all the compounds were more potent than INH (MIC: 182.3 μM). All the compounds also inhibited *M. phlei* (MP), which causes abscesses[12], with MICs ranging from 1.95 to 71.34 μM, and were more potent than INH (MIC: 91.15 μM). Against *M. fortuitum* (which causes infection in immunosuppressed persons[13]), the compounds showed excellent activity, with MICs ranging from 2.01 to 297.27 μM, and nine compounds were more potent than INH (MIC: 22.82 μM). The compounds were also screened against *M. kansasii*, which causes central nervous system infection and cutaneous lymphadenitis[14], and was inhibited with MICs ranging from 2.21 to 77.79 μM; all compounds were more potent than INH (MIC: 182.3 μM). Compound **4m** inhibited all seven mycobacterium species with MICs ranging from 0.23 to 30.25 μM, and was more potent than INH except against MM.

Some compounds were further examined for toxicity (IC_{50}) in a mammalian Vero cell line at a concentration of 62.5 μg/mL (Table 1). After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96 non-radioactive cell proliferation assay. The compounds with nitro group substitution were found to be toxic (**4f**, **4g**, and **4s**). Other compounds were not found to be toxic; these results are important, as these compounds with their decreased cytoliability are much more attractive in the development of a compound for the treatment of TB. This is primarily due to the fact that the eradication of TB requires a lengthy course of treatment, and the need for an agent with a high margin of safety becomes a primary concern. Compound **4m** showed a selectivity index (IC_{50}/MIC) of more than > 657 against log-phase MTB.

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