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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Novel antitubercular diallyl/dibenzylthiosemicarbazones endowed with high activity toward multi-drug-resistant tuberculosis

Dharmarajan Sriram · Debjani Banerjee · Tara Sasank Thripuraribhatla Venkata Naga Varuna · Manikandaprabu Sankar · Perumal Yogeeswari

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Abstract Novel diallyl and dibenzylthiosemicarbazones were prepared by three-step reactions. The compounds were tested for their in vitro activity against *Mycobacterium tuberculosis* H37Rv (MTB) and multi-drug-resistant *Mycobacterium tuberculosis* (MDR-TB). Most of the compounds showed excellent activity toward MDR-TB. Among the thirty compounds (**4**,**5a–o**) tested *N*,*N*-dibenzyl-2-((5-nitrofuran-2-yl)methylene)hydrazinecarbothioamide (**5g**) was found to be the most potent compound MICs of 0.55 and 0.12 μM against MTB and MDR-TB.

Keywords Diallyl/dibenzyl thiosemicarbazones · Multi-drug-resistant *Mycobacterium tuberculosis* · Tuberculosis

Introduction

Mycobacterium tuberculosis (MTB) is a notorious pathogen whose increasing resistance to antibiotics and heightened lethality in combination with AIDS makes it a major health concern worldwide (Maartens and Wilkinson, 2007). One-third of the world's population is thought to be infected with MTB; eight million people worldwide develop tuberculosis (TB) annually while nearly two million die (WHO report 2008). TB causes more deaths than any other infectious agent in the world. The World Health Organization estimates that 11.4 million people worldwide are infected with both MTB and HIV. Additionally, multi-drug-resistant TB (MDR-TB) (Zignol *et al*., 2006; Rattan *et al*., 1998) has been found in all regions of the world. Treatment for MDR-TB often requires the use of special drugs, all of which can produce serious side effects while only providing a 40–60% survival rate (Rivers and Mancera, 2008). Therefore, there is a pressing need to develop novel TB chemotherapeutics which is active against both drug-sensitive and drug-resistant MTB. Earlier, we have reported antitubercular activities of various oxazolyl and *N*-hydroxy thiosemicarbazones which inhibited MTB with MIC of 0.05 μg/ml (Sriram *et al*., 2006, 2007) (Fig. 1). As a continuation our earlier work, we present herein the preliminary results concerning the synthesis and the in vitro antitubercular activities of novel diallyl and dibenzyl thiosemicarbazones.

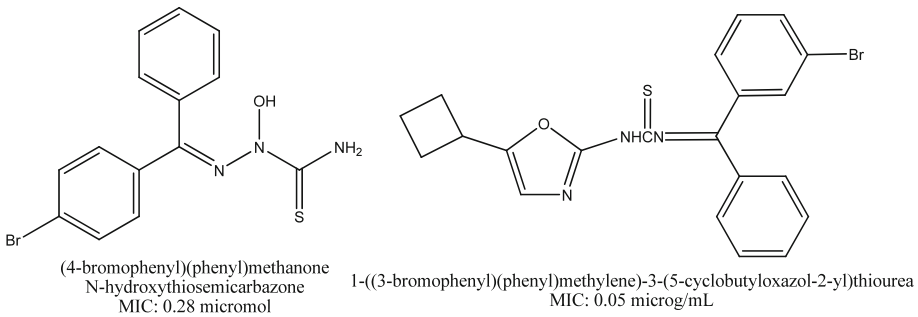
Experimental

Chemistry

Melting points were taken on an electrothermal melting point apparatus (Buchi BM530) in open capillary tubes and are uncorrected. ^{1}H-NMR spectra were scanned on a JEOL Fx 400 MHz NMR spectrometer using DMSO-d_6 as solvent. Chemical shifts are expressed in δ (ppm) relative to tertamethylsilane. Elemental analyses (C, H, and N) were performed on Perkin Elmer model 240C analyzer and the data were within ±0.4% of the theoretical values.

D. Sriram (✉) · D. Banerjee · T. S. T. V. N. Varuna · M. Sankar · P. Yogeeswari
Medicinal Chemistry & Antimycobacterial Research Laboratory, Pharmacy Group, Birla Institute of Technology & Science—Pilani, Hyderabad Campus, Jawahar Nagar, Hyderabad 500078, Andhra Pradesh, India
e-mail: drdsriram@yahoo.com

Fig. 1 Earlier reported molecules



*General method for the synthesis of thiosemicarbazides (**3a–b**)*

To a solution of diallylamine (**1a**)/dibenzylamine (**1b**) (0.01 mol) in absolute ethanol (20 ml) was 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 corresponding potassium salt of dithiocarbamates (**2a–b**). To the stirred mixture of dithiocarbamate salts was added hydrazine hydrate (0.01 mol) and the stirring was continued at 80°C for 1 h and on adding crushed ice to obtain corresponding thiosemicarbazide which is converted to hydrochloride salts (**3a–b**). **3a**: Yield: 48%; mp: 280–282°C; **3b**: Yield: 47.2%; mp: >300°C.

*General method for the synthesis of thiosemicarbazones (**4,5a–o**)*

To a solution of **3a–b** hydrochloride (0.003 mol) in methanol, an equimolar quantity of sodium acetate in water and the appropriate aldehyde or ketone in methanol were added. The mixture was stirred under heating for 30 min and the resultant precipitate was filtered off and dried. The product was recrystallized from 95% ethanol.

*2-[(Z)-Phenylmethylidene]-N,N-diprop-2-enylhydrazine-1-carbothioamide (**4a**)* Yield: 60.8%; mp: 172–174°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.69 (d, 4H, C-1 methylene proton of allyl), 5.17 (dd, 2H, C-3 H *cis* of allyl), 5.2 (dd, 2H, C-3 H *trans* of allyl), 5.86–5.88 (m, 2H, C-2 H of allyl), 7.16–7.36 (m, 5H, aryl-H), 7.49 (s, 1H, benzylidine H), 11.9 (s, 1H, D_2O exchangeable NH); Chemical Formula: $C_{14}H_{17}N_3S$ Calculated for C, 64.83; H, 6.61; N, 16.20; Found: C, 64.78; H, 6.63; N, 16.22.

*2-[(Z)-(2-Nitrophenyl)methylidene]-N,N-bis(phenylmethyl) hydrazine-1-carbothioamide (**5b**)* Yield: 40.4%; mp: 106–108°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.76 (s, 4H, CH_2 of benzyl), 7.257.26 (m, 10H, aryl-H), 7.70–7.73 (m, 4H, Ar–H), 7.75 (s, 1H, benzylidine H), 10.4 (s, 1H, D_2O exchangeable NH); Chemical Formula: $C_{22}H_{20}N_4O_2S$ Calculated for C, 65.33; H, 4.98; N, 13.85; Found: C, 65.28; H, 4.96; N, 13.89.

*2-[(Z)-(4-Nitrophenyl)methylidene]-N,N-diprop-2-enylhydrazine-1-carbothioamide (**4c**)* Yield: 60.1%; mp: 161–163°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.69 (d, 4H, C-1 methylene proton of allyl), 5.17 (dd, 2H, C-3 H *cis* of allyl), 5.2 (dd, 2H, C-3 H *trans* of allyl), 5.84–5.88 (m, 2H, C-2 H of allyl), 7.72–7.73 (m, 4H, aryl-H), 7.49 (s, 1H, benzylidine H), 11.9 (s, 1H, D_2O exchangeable NH); Chemical Formula: $C_{14}H_{16}N_4O_2S$ Calculated for C, 55.25; H, 5.30; N, 18.41; Found: C, 55.21; H, 5.33; N, 18.42.

*2-[(Z)-(4-Chlorophenyl)methylidene]-N,N-bis(phenylmethyl) hydrazine-1-carbothioamide (**5d**)* Yield: 54.9%; mp: 144–146°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.76 (s, 4H, CH_2 of benzyl), 7.25–7.26 (m, 10H, aryl-H), 7.72–7.73 (m, 4H, Ar–H), 7.75 (s, 1H, benzylidine H), 10.4 (s, 1H, D_2O exchangeable NH); Chemical Formula: $C_{22}H_{20}ClN_3S$ Calculated for C, 67.08; H, 5.12; N, 10.67; Found: C, 67.12; H, 5.07; N, 10.64.

*2-[(Z)-(2-Methylphenyl)methylidene]-N,N-diprop-2-enylhydrazine-1-carbothioamide (**4e**)* Yield: 44.0%; mp: 174–175°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.78 (s, 3H, 2-methyl), 3.69 (d, 4H, C-1 methylene proton of allyl), 5.17 (dd, 2H, C-3 H *cis* of allyl), 5.2 (dd, 2H, C-3 H *trans* of allyl), 5.86–5.88 (m, 2H, C-2 H of allyl), 7.71–7.73 (m, 4H, aryl-H), 7.49 (s, 1H, benzylidine H), 11.9 (s, 1H, D_2O exchangeable NH); Chemical Formula: $C_{15}H_{19}N_3S$ Calculated for C, 65.90; H, 7.00; N, 15.37; Found: C, 55.21; H, 5.33; N, 18.42.

*2-[(Z)-(2-Hydroxyphenyl)methylidene]-N,N-bis(phenylmethyl) hydrazine-1-carbothioamide (**5f**)* Yield: 43.0%; mp: 154–155°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.76 (s, 4H, CH_2 of benzyl), 7.25–7.26 (m, 10H, aryl-H), 7.72–7.73 (m, 4H, Ar–H), 7.75 (s, 1H, benzylidine H), 10.4 (s, 1H, D_2O exchangeable NH), 11.4 (s, 1H, OH); Chemical Formula: $C_{22}H_{21}N_3OS$ Calculated for C, 70.37; H, 5.64; N, 11.19; Found: C, 70.32; H, 5.67; N, 11.20.

*2-[(Z)-(5-Nitrofuran-2-yl)methylidene]-N,N-diprop-2-enylhydrazine-1-carbothioamide (**4g**)* Yield: 46.8%; mp: 142–144°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.69 (d, 4H, C-1 methylene proton of allyl), 5.17 (dd, 2H, C-3 H *cis* of allyl), 5.2 (dd, 2H, C-3 H *trans* of allyl), 5.86–5.88 (m, 2H, C-2 H of allyl), 7.54–7.55 (m, 2H, aryl-H), 7.49 (s, 1H, benzylidine H), 11.9 (s, 1H, D_2O exchangeable NH); Chemical Formula: $C_{12}H_{14}N_4O_3S$ Calculated for C, 48.97; H, 4.79; N, 19.04; Found: C, 48.96; H, 4.80; N, 19.10.

*2-[(Z)-1-Phenylethylidene]-N,N-bis(phenylmethyl)hydrazine-1-carbothioamide (**5h**)* Yield: 46.5%; mp: 128–129°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.13 (s, 3H, methyl), 4.76 (s, 4H, CH_2 of benzyl), 7.25–7.26 (m, 10H, aryl-H), 7.72–7.73 (m, 5H, Ar–H), 10.4 (s, 1H, D_2O exchangeable NH); Chemical Formula: $C_{23}H_{23}N_3S$ Calculated for C, 73.96; H, 6.21; N, 11.25; Found: C, 70.32; H, 5.67; N, 11.20.

*2-[(Z)-1-(2-Hydroxyphenyl)ethylidene]-N,N-diprop-2-enylhydrazine-1-carbothioamide (**4i**)* Yield: 65.2%; mp: 136–137°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.13 (s, 3H, methyl), 3.69 (d, 4H, C-1 methylene proton of allyl), 5.17 (dd, 2H, C-3 H *cis* of allyl), 5.2 (dd, 2H, C-3 H *trans* of allyl), 5.86–5.88 (m, 2H, C-2 H of allyl), 7.71–7.73 (m, 4H, aryl-H), 11.4 (s, 1H, OH), 11.9 (s, 1H, D_2O exchangeable NH), Chemical Formula: $C_{15}H_{19}N_3OS$ Calculated for C, 62.25; H, 6.62; N, 14.52; Found: C, 62.18; H, 6.63; N, 14.50.

*2-[(Z)-1-(4-Hydroxyphenyl)ethylidene]-N,N-bis(phenylmethyl) hydrazine-1-carbothioamide (**5j**)* Yield: 39%; mp: 221–222°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.13 (s, 3H, methyl), 4.76 (s, 4H, CH_2 of benzyl), 7.25–7.26 (m, 10H, aryl-H), 7.72–7.73 (m, 4H, Ar–H), 10.4 (s, 1H, D_2O exchangeable NH), 11.2 (s, 1H, OH); Chemical Formula: $C_{23}H_{23}N_3OS$ Calculated for C, 70.92; H, 5.95; N, 10.79; Found: C, 70.32; H, 5.67; N, 11.20.

*2-[(Z)-1-(4-Aminophenyl)ethylidene]-N,N-diprop-2-enylhydrazine-1-carbothioamide (**4k**)* Yield: 40.2%; mp: >250°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.13 (s, 3H, methyl), 3.69 (d, 4H, C-1 methylene proton of allyl), 5.17 (dd, 2H, C-3 H *cis* of allyl), 5.2 (dd, 2H, C-3 H *trans* of allyl), 5.86–5.88 (m, 2H, C-2 H of allyl), 6.27 (s, 2H, amino), 7.71–7.73 (m, 4H, aryl-H), 11.4 (s, 1H, D_2O exchangeable NH); Chemical Formula: $C_{15}H_{20}N_4S$ Calculated for C, 62.47; H, 6.99; N, 19.43; Found: C, 62.46, H, 7.02, N, 19.40.

*2-[(Z)-1-(4-Nitrophenyl)ethylidene]-N,N-bis(phenylmethyl) hydrazine-1-carbothioamide (**5l**)* Yield: 64.6%; mp: 138–139°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.13 (s, 3H, methyl), 4.76 (s, 4H, CH_2 of benzyl), 7.25–7.26 (m, 10H, aryl-H), 7.72–7.73 (m, 4H, Ar–H), 10.4 (s, 1H, D_2O exchangeable NH), Chemical Formula: $C_{23}H_{22}N_4O_2S$ Calculated for C, 66.01; H, 5.30; N, 13.39; Found: C, 70.32; H, 5.67; N, 11.20.

*2-[(Z)-1-(4-Fluorophenyl)ethylidene]-N,N-diprop-2-enylhydrazine-1-carbothioamide (**4m**)* Yield: 34.0%; mp: 134–136°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.13 (s, 3H, methyl), 3.69 (d, 4H, C-1 methylene proton of allyl), 5.17 (dd, 2H, C-3 H *cis* of allyl), 5.2 (dd, 2H, C-3 H *trans* of allyl), 5.86–5.88 (m, 2H, C-2 H of allyl), 7.71–7.73 (m, 4H, aryl-H), 11.4 (s, 1H, D_2O exchangeable NH); Chemical Formula: $C_{15}H_{18}FN_3S$ Calculated for C, 61.83; H, 6.23; N, 14.42; Found: C, 61.84, H, 6.22, N, 14.40.

*2-(Diphenylmethylidene)-N,N-bis(phenylmethyl)hydrazine-1-carbothioamide (**5n**)* Yield: 49.6%; mp: 164–165°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.76 (s, 4H, CH_2 of benzyl), 7.25–7.26 (m, 10H, aryl-H), 7.33–7.39 (m, 10H, Ar–H), 10.4 (s, 1H, D_2O exchangeable NH), Chemical Formula: $C_{28}H_{25}N_3S$ Calculated for C, 77.21; H, 5.79; N, 9.65; Found: C, 77.22; H, 5.77; N, 9.70.

*2-[(Z)-1-Methylpropylidene]-N,N-diprop-2-enylhydrazine-1-carbothioamide (**4o**)* Yield: 46.1%; mp: 154–155°C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.05 (t, 3H, CH_3 of ethyl), 2.0 (s, 3H, methyl), 2.27 (q, 2H, CH_2 of ethyl), 3.69 (d, 4H, C-1 methylene proton of allyl), 5.17 (dd, 2H, C-3 H *cis* of allyl), 5.2 (dd, 2H, C-3 H *trans* of allyl), 5.86–5.88 (m, 2H, C-2 H of allyl), 8.57 (s, 1H, D_2O exchangeable NH); Chemical Formula: $C_{11}H_{19}N_3S$ Calculated for C, 58.63; H, 8.50; N, 18.65; Found: C, 58.60, H, 8.53, N, 18.68.

In vitro antimycobacterial activity

All compounds were screened for their in vitro antimycobacterial activity against MTB, MDR-TB, and MC_2 in Middlebrook 7H11 agar medium supplemented with OADC by agar dilution method similar to that recommended by the National Committee for Clinical Laboratory Standards for the determination of MIC in triplicates. The MDR-TB clinical isolate was obtained from Tuberculosis Research Center, Chennai, India, and was resistant to isoniazid, rifampicin, ethambutol and ofloxacin. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound required to give complete inhibition of bacterial growth.

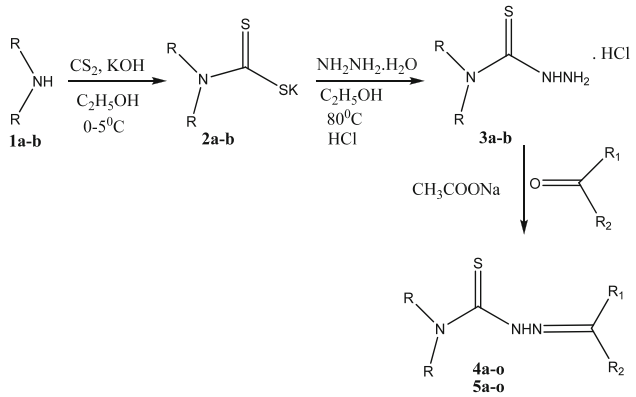
Cytotoxicity

Some compounds were further examined for toxicity (IC_{50}) in a mammalian Vero cell line at concentrations of 62.5 μg/ml. After 72 h of exposure, viability was assessed

on the basis of cellular conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay.

Results and discussion

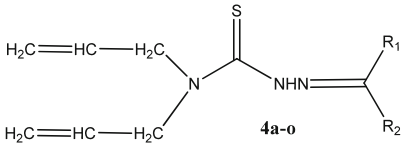
The synthesis of diallyl and dibenzyl thiosemicarbazides (**3a–b**) was carried out in two steps with 76 and 64% yield, respectively, as shown in Scheme 1. First, to a solution of diallylamine/dibenzylamine (0.01 mol) in ethanol (10 ml) were added potassium hydroxide (0.01 mol) and carbon disulfide (0.75 ml), and the mixture was stirred at 0–5°C for 1 h to form corresponding potassium salt of dithiocarbamates (**2a–b**). 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 compounds **3a–b**. Diallyl/dibenzylthiosemicarbazide hydrochloride on condensation with various carbonyl compounds in the presence of sodium acetate afforded various novel diallyl/dibenzylthiosemicarbazones (**4**,**5a–o**) (Table 1) in 39–63% 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 1H 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 10.4 and 11.9 ppm corresponding to NH proton of dibenzyl and diallyl thiosemicarbazones, respectively. Compounds **4**,**5a–g** showed singlet at δ 7.5 ppm corresponding to carbimino H of benzaldehyde, and compounds **4**,**5h–m** showed singlet at δ 2.13 ppm corresponding to carbimino CH_3 of acetophenone. Compounds **4a–o** showed doublet at δ 3.69–3.71 ppm, which corresponds to C-1 methylene proton of allyl, multiplet at δ 5.86–5.89 ppm, which corresponds to C-2 CH proton of allyl, double doublet at δ 5.17 ppm, which corresponds to C-3 H*cis* proton of allyl, and another double doublet at δ 5.20 ppm, which corresponds to C-3 H*trans* proton of allyl group. Compounds **5a–o** showed singlet at δ 4.76 ppm, which corresponds to CH_2 of benzyl protons and aromatic protons of benzyl group showed multiplet at δ 7.25–7.27 ppm. The elemental analysis results were within ±0.4% of the theoretical values.



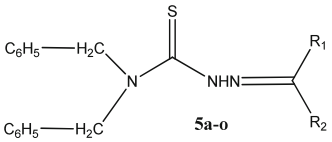
Scheme 1 Synthetic protocol of compounds

All compounds were screened for their in vitro antimycobacterial activity against MTB and MDR-TB by agar dilution method similar to that recommended by the National Committee for Clinical Laboratory Standards (1995) for the determination of MIC in duplicate. The minimum inhibitory concentration (MIC) of the synthesized compounds along with the standard drugs for comparison were reported (Table 1). In the first phase of screening against MTB, all the diallyl/dibenzyl thiosemicarbazones showed in vitro activity against MTB with MIC ranging from 0.5 to 110.93 μM. Two compounds (**5c** and **5g**) inhibited MTB with MIC of less than 1 μM and compound **5g** (MIC: 0.50 μM) was more potent than standard isoniazid (MIC: 0.66 μM). When compared to ofloxacin (MIC: 2.13 μM), three compounds (**5c**, **5d**, and **5g**) were found to be more active against MTB. Eight compounds (**5b**, **4c**, **5c**, **5d**, **4g**, **5g**, **5l**, and **5n**) were more potent than ethambutol (MIC: 4.71 μM). All the compounds were less active than rifampicin (MIC: 0.23 μM), against MTB. Compound *N*,*N*-dibenzyl-2-((5-nitrofuran-2-yl)methylene)hydrazinecarbothioamide (**5g**) was found to be the most active compound in vitro with a low MIC of 0.50 μM against MTB and was 1.3, 4.2, and 9.4 times more potent than isoniazid, ofloxacin, and ethambutol, respectively. Subsequently, some of the compounds were evaluated against MDR-TB, and among the 25 compounds screened, all the compounds inhibited MDR-TB with MIC ranging from 0.12 to 11.43 μM and were found to be more active than isoniazid (MIC: 45.57 μM), ofloxacin (MIC: 34.59 μM), and ethambutol (MIC: 37.68 μM). Eleven compounds (**4**,**5b–d**, **4**,**5g**, **5l**, and **4**,**5n**) inhibited MDR-TB with MIC of less than 3 μM and were more potent than rifampicin (MIC: 3.79 μM). Most of the compounds showed high activity (2–4 folds) toward MDR-TB rather than MTB. Compound **5g** was found to be the most active compound in vitro with MIC of 0.12 μM against MDR-TB and was 379, 31, 288, and 314 times more potent than isoniazid, rifampicin, ofloxacin, and ethambutol, respectively. With respect to structure–MTB activity relationship, the results demonstrated that dibenzylthiosemicarbazones (**5a–o**) were found to be more potent than diallylthiosemicarbazones (**4a–o**). In the carbimino terminal, benzaldehyde-derived thiosemicarbazones (**4**,**5a–f**) were more potent than acetophenone-derived thiosemicabazones (**4**,**5h–m**). The

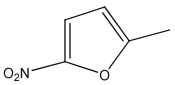
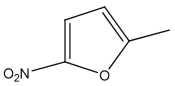
Table 1 Physical constants and antitubercular activities of thiosemicarbazones



4a-o



5a-o

No	R_1	R_2	Yield (%)	mp (°C)	CC_{50} (μM)	MIC (μM)	
						MTB	MDR-TB
4a	H	C_6H_5	60.8	172–174	NT	48.19	NT
5a	H	C_6H_5	56.2	110–111	174.02	17.40	8.71
4b	H	2-NO_2–C_6H_4	55.6	150–152	205.34	5.12	2.56
5b	H	2-NO_2–C_6H_4	40.4	106–108	<154.51	3.85	0.98
4c	H	4-NO_2–C_6H_4	60.1	161–163	<205.34	2.56	1.31
5c	H	4-NO_2–C_6H_4	62.4	113–115	<154.65	0.98	0.98
4d	H	4-Cl–C_6H_4	58.8	230–231	212.72	5.30	2.65
5d	H	4-Cl–C_6H_4	54.9	144–146	158.65	1.98	1.98
4e	H	2-CH_3–C_6H_4	44.0	174–175	>228.60	22.86	5.70
5e	H	2-CH_3–C_6H_4	54.3	136–137	167.33	8.38	4.71
4f	H	2-OH–C_6H_4	56.3	188–190	NT	45.39	NT
5f	H	2-OH–C_6H_4	43.0	154–155	166.44	8.33	4.16
4g	H		46.8	142–144	<212.34	2.65	0.67
5g	H		51.6	123–124	158.45	0.50	0.12
4h	CH_3	C_6H_5	42.0	140–141	228.60	22.86	11.43
5h	CH_3	C_6H_5	46.5	128–129	167.33	16.73	4.17
4i	CH_3	2-OH–C_6H_4	65.2	136–137	215.96	21.59	10.81
5i	CH_3	2-OH–C_6H_4	48.63	173–174	160.45	8.03	8.03
4j	CH_3	4-OH–C_6H_4	39.8	138–140	215.96	21.59	10.81
5j	CH_3	4-OH–C_6H_4	39.0	221–222	160.45	16.06	8.03
4k	CH_3	4-NH_2–C_6H_4	40.2	>250	NT	43.34	NT
5k	CH_3	4-NH_2–C_6H_4	56.1	231–133	160.86	16.08	8.04
4l	CH_3	4-NO_2–C_6H_4	43.2	84–85	<196.30	9.83	9.83
5l	CH_3	4-NO_2–C_6H_4	64.6	138–139	<149.33	3.72	1.86
4m	CH_3	4-F–C_6H_4	34.0	134–136	214.48	10.74	5.35
5m	CH_3	4-F–C_6H_4	41.6	136–138	159.64	7.99	3.98
4n	C_6H_5	C_6H_5	36.8	128–129	186.30	9.33	2.32
5n	C_6H_5	C_6H_5	49.6	164–165	143.48	3.58	1.79
4o	CH_3	C_2H_5	46.1	154–155	NT	110.93	NT
5o	CH_3	C_2H_5	74.8	148–149	NT	76.82	NT
Isoniazid					>455.8	0.66	45.57
Rifampin					>75.9	0.23	3.79
Ethambutol					>188.5	4.71	37.68
Ofloxacin					>155.3	2.13	34.59

NT not tested, *MIC* Minimum inhibitory concentration, *CC_{50}* cytotoxicity

antimycobacterial activity was enhanced by the introduction of electron withdrawing groups in the phenyl moiety, whereas introduction of electron donating groups decreased the activity. Replacement of phenyl ring with heteroaryl ring like 5-nitrofuranyl derived thiosemicarbazones (**4**,**5g**) enhances the activity many more times. Similarly, introduction of second aryl ring (**4**,**5n**) not changes the activity much, but replacement of aryl ring with alkyl chain (**4**,**5o**) reduces the activity drastically. When compared to our earlier reported *N*-hydroxy thiosemicarbazones these compounds are slightly less active.

Some compounds which showed good in vitro activities were further examined for toxicity (CC_{50}) in a mammalian Vero cell line at 62.5 μg/ml concentration (Gundersen *et al*., 2002). After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product and the results are reported in Table 1. Twenty-five compounds when tested showed IC_{50} values ranging from 143.48 to 228.60 μM. In general, nitro group-substituted compounds demonstrated more cytotoxicity. These results are important as the most active nitro-substituted compounds with their increased cytoliability are much less 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. The IC_{50} of compound **5g** was found to be 158.45 μM and showed selectivity index (CC_{50}/MIC) of more than 316 for MTB and 1320 for MDR-TB.

Conclusions

Screening of the antimycobacterial activity of these series, identified diallyl/dibenzylthiosemicarbazones as a better lead endowed with high activity toward MDR-TB, exhibiting MIC values between 0.12 and 11.43 μM. 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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