Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 501–506

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and Antimycobacterial Activity of New *S*-alkylisothiosemicarbazone Derivatives

Maria Teresa Cocco,[a,*] Cenzo Congiu,[a] Valentina Onnis,[a] Maria Luisa Pellerano[b] and Alessandro De Logu[b]

[a]*Dipartimento di Tossicologia, Università di Cagliari, Via Ospedale 72-09124 Cagliari, Italy*
[b]*Dipartimento di Scienze Chirurgiche e Trapianti d'Organo, Sezione di Microbiologia e Virologia, Università di Cagliari, Via Palabanda 14-09123 Cagliari, Italy*

Received 9 July 2001; accepted 9 August 2001

Abstract—A new series of *S*-alkylisothiosemicarbazones of 3- and 4-pyridincarboxaldehyde and 4-fluoro- and 4-trifluoromethylbenzaldehyde was synthesized and evaluated for biological activity against various *Mycobacterium* strains. Inhibitory activity against *Mycobacterium tuberculosis* H37Rv ATCC 27294 and INH-R ATCC 35822 was compared with activity against clinical isolated *Mycobacteria* as well as against MOTT. Some of newly prepared compounds showed best inhibitory values against clinical isolated *Mycobacteria*, besides to low citotoxicity values. © 2002 Elsevier Science Ltd. All rights reserved.

Introduction

Tuberculosis (TB) is a contagious disease with high mortality worldwide. The statistics indicate that 3 million people worldwide die annually from complication of tuberculosis[1,2] and there are estimated 8 million of new cases each year, 95% of which occur in developing countries.[3] Therefore the incidence of tuberculosis is rising at alarming rates coincident with the increase of AIDS cases. In addition, about a third of the world's population harbors a dormant *Mycobacterium tuberculosis* infection, representing a significant reservoir of disease for the future. The disease resurgence is attributed to emergence of drug-resistant strains, immigration from high-prevalence countries combined with poor access to healthcare in inner cities.

Furthermore, in the late 1950s, species of mycobacteria other than *M. tuberculosis* (MOTT) were being encountered with increasing frequency in medical practices.[4] MOTT are considered saprophytic mycobacteria, that can cause a wide range of infections in patients with predisposing conditions. Among the species potentially pathogenic in humans, *M. avium-intracellulare* (MAI complex or MAC), *M. Kansasii*, *M. fortuitum-chelonae* complex, *M. scrofulaceum*, *M. szulgai*, *M. marinum* and *M. xenopi* are particularly important for they clinical relevance. Pulmonary manifestations of MAC infections are similar to those of *M. tuberculosis*.[5] Usually, conditions predisposing humans to pulmonary infections with MAC include chronic obstructive pulmonary disease, recurrent pneumonia, inactive or active tuberculosis, bronchogenic carcinoma[6] and cystic fibrosis.[7] However, MAC was considered of low pathogenicity for humans until AIDS epidemic. Chronic pulmonary disease resembling classical tuberculosis is the most common manifestation following infection with *M. kansasii*.[8–10] However, occasional cases of extra pulmonary infections have been reported, including osteomyelitis,[11] lymphadenitis[12] and soft tissue infections.[13] Disseminated diseases due to *M. kansasii* have been observed in the presence of severe immunosuppression and in patients with AIDS.[14] *M. fortuitum* and *M. chelonae* have been associated with a variety of infections involving skin, lungs, CNS, bone and disseminated disease.[15,16] *M. fortuitum* may colonize the respiratory tract of immunocompromised patients or with chronic obstructive pulmonary disease. MOTT are frequently resistant to the commonly used antituberculosis drugs. Macrolides and quinolones show high MIC values against *M. kansasii*, *M. scrofulaceum*, *M. avium-intracellulare*, *M. chelonae*, and *M. fortuitum*.[17] In the case of M. *avium-intracellulare*, it seems that this resistance is due to the impermeability of the bacteria to the

*Corresponding author. Tel.: +39-0706-758645; fax: +39-0706-758612; e-mail: tcocco@unica.it

0968-0896/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0968-0896(01)00310-8

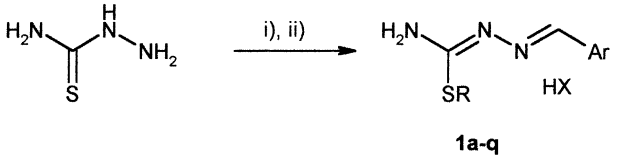
drugs,[18,19] but many patients treated with multiple-drugs regimens respond favorably to the therapy. Furthermore, many strains of MOTT rifampin-resistant,[20] beta-lactame-resistant[21] but also multidrug-resistant[22] have been recently isolated. Therefore, new molecules for the therapy of infection sustained by MOTT and multidrug resistant *M. tuberculosis* (MDRTB) are needed. In continuation of our efforts to obtain new antimycobacterial agents[23] we became interested to isothiosemicarbazones as analogoues of thiosemicarbazones. In fact thiosemicarbazone derivatives are known for their antibacterial,[24,25] antimalarial,[26,27] antineoplastic[28,29] and antiviral[30–34] properties. In a previous paper, we have described the evaluation of antimicrobial activity of isothiosemicarbazones derived from 5-nitrofuraldehyde and 4-nitrobenzaldehyde.[35] In this paper, we wish to report on the synthesis of a new series of isothiosemicarbazones derived from 4-fluorobenzaldehyde, 4-(trifluoromethyl)benzaldehyde, 3- and 4-pyridinecarboxaldehyde and on the results of biologic screening against *M. tuberculosis* H37Rv ATCC 27294 and *M. tuberculosis* resistant to isoniazid (INH-R) ATCC 35822 and clinical isolated strains of *Mycobacteria* as well as against MOTT.

Results and Discussion

Chemistry

The synthesis of the target isothiosemicarbazones **1a–q** is depicted in Scheme 1. Treatment of thiosemicarbazide with the appropriate alkyl halide in ethanol gave the alkyl isothiosemicarbazide salt intermediates. These latter were directly condensed with aldehydes without isolating them from reaction mixture (Table 1).

The IR and ^{1}H NMR spectra of **1** were consistent with the assigned structure. The ^{1}H NMR spectra of compounds **1a–q** show a singlet at 8.12–8.50 ppm relative to the proton on the C=N double bond, while the signal of NH_2 appears at 7.73–9.91 ppm as one or two broads singlets. In the IR spectra of **1a–q** the bands in the region 3450–3100 cm^{-1} are due to the stretching frequencies ν (NH_2). The typical bands of ν (C=N) and ν (C=C) appear in the frequency range 1670-1560 cm^{-1}.



Scheme 1. Synthesis of isothiosemicarbones **1a–q**. Reagents and conditions: (i) RX, EtOH; (ii) ArCHO, EtOH.

Biological evaluation

The effects on the growth of *Mycobacteria* of compounds **1a–q** was evaluated against *M. tuberculosis* H37Rv ATCC 27294, *M. tuberculosis* INH-R ATCC 35822 and *M. avium* ATCC 19421. The antimycobacterial activity of compounds **1a–q** was also evaluated against six strains of *M. tuberculosis*, six strains of *M. tuberculosis* INH-R, six strains of *M. avium* and *M. phlei*, *M. fortuitum*, *M. kansasii*, *M. szulgai*, *M. gordonae*, *M. chelonei*, *M. intracellulare*, *M. scrofulaceum*, *M. bovis*, all isolates from patients with active clinical infection.

Several of the tested compounds showed a specific activity against *M. tuberculosis* and *M. avium* (Tables 2 and 3). In particular, compounds **1e** and **1h** exhibited a MIC of 12.5 μg/mL against *M. tuberculosis* H37Rv ATCC 27294, and at the same concentration compound **1k**

Table 2. Cytotoxicity[a] and antibacterial activity against ATCC strains of *M. tuberculosis* of isothiosemicarbazone derivatives **1a–q**

Compound	MNTD (μg/mL)	MIC (μg/mL)	
		M. tuberculosis H37Rv ATCC27294	*M. tuberculosis INH-R* ATCC35822
1a	>1000	>100	>100
1b	>1000	>100	>100
1c	>1000	>100	>100
1d	250	>100	>100
1e	125	12.5	25
1f	62.5	50	25
1g	250	>100	100
1h	250	12.5	25
1i	125	100	12.5
1j	62.5	50	25
1k	500	25	12.5
1l	250	25	12.5
1m	62.5	50	100
1n	62.5	25	25
1o	250	>100	50
1p	>1000	50	25
1q	62.5	100	50
INH	>1000	0.09	100

[a]Expressed as maximum non toxic dose (MNTD).

Table 1. Isothiosemicarbazone derivatives **1a–q**

Compd	R	Ar	X	Compd	R	Ar	X
1a	CH_3	3-Pyridyl	I	**1j**	$CH_2CH{=}CH_2$	4-$CF_3C_6H_4$	Br
1b	CH_3	4-Pyridyl	I	**1k**	*n*-C_4H_9	3-Pyridyl	Br
1c	CH_3	4-FC_6H_4	I	**1l**	*n*-C_4H_9	4-Pyridyl	Br
1d	C_2H_5	3-Pyridyl	Br	**1m**	*n*-C_4H_9	4-FC_6H_4	Br
1e	C_2H_5	4-FC_6H_4	Br	**1n**	*n*-C_4H_9	4-$CF_3C_6H_4$	Br
1f	C_2H_5	4-$CF_3C_6H_4$	Br	**1o**	$CH_2C_6H_5$	3-Pyridyl	Br
1g	$CH_2CH{=}CH_2$	3-Pyridyl	Br	**1p**	$CH_2C_6H_5$	4-Pyridyl	Br
1h	$CH_2CH{=}CH_2$	4-Pyridyl	Br	**1q**	$CH_2C_6H_5$	4-FC_6H_4	Br
1i	$CH_2CH{=}CH_2$	4-FC_6H_4	Br				

inhibited the growth of *M. tuberculosis* INH-R ATCC 35822. Interestingly, several compounds, such as **1e**, **1k**, **1l**, **1o**, **1p** and **1q** were slightly more active against the strains of *M. tuberculosis*, *M. tuberculosis* INH-R and *M. avium* isolated from clinical specimens (Table 4).

Compounds **1** were usually more active against *M. tuberculosis* than *M. avium*. However, it is interesting to point out that some compounds showed a specific activity against *M. avium*. Compound **1m**, which exhibited a MIC of 50 and 100 μg/mL against *M. tuberculosis* H37Rv ATCC 27294 and *M. tuberculosis* INH-R ATCC 35822, respectively, was significantly more active against *M. avium* ATCC 19421 (MIC 6.25 μg/mL) and the strains of *M. avium* isolated from clinical specimens (MIC 3.12–6.25 μg/mL). Compound **1i**, which was almost ineffective in inhibiting the growth of *M. tuberculosis* H37Rv ATCC 27294 (MIC 100 μg/mL), showed a MIC of 12.5 μg/mL against *M. avium* ATCC 19421. Compound **1f** exhibited the most interesting activity against *M. avium* ATCC 19421 (MIC 0.78 μg/mL). A good activity against *M. intracellulare*, which is frequently resistant to the commonly used antituberculosis drugs, was shown by compound **1n** (MIC 25 μg/mL). Furthermore several of compounds inhibited the growth of *M. kansasii* (MIC 12.5–50 μg/mL).

Table 3. Antibacterial activity against *M. avium* ATCC 19421 and clinical isolates of MOTT of isothiosemicarbazone derivatives **1a–q**[a]

Comp.	MIC (μg/mL)									
	M.a.[a]	M.p.	M.f.	M.k.	M.sz.	M.g.	M.c.	M.i.	M.sc.	M.b.
1a	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
1b	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
1c	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
1d	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
1e	>100	>100	>100	12.5	>100	>100	>100	>100	>100	12.5
1f	0.78	>100	>100	25	25	50	100	100	50	50
1g	>100	>100	>100	>100	>100	>100	>100	>100	>100	100
1h	>100	>100	>100	25	>100	>100	>100	>100	>100	12.5
1i	12.5	>100	>100	50	>100	>100	>100	>100	>100	>100
1j	50	100	>100	25	50	50	100	100	50	50
1k	12.5	>100	>100	25	>100	100	>100	>100	100	100
1l	12.5	>100	>100	25	>100	50	>100	>100	100	25
1m	6.25	>100	>100	50	100	50	>100	100	>100	25
1n	6.25	100	>100	>100	100	100	100	25	50	25
1o	50	>100	>100	50	>100	>100	>100	>100	>100	50
1p	25	>100	>100	25	>100	50	100	100	>100	50
1q	25	>100	>100	25	50	50	>100	>100	>100	50
INH	12.5	12.5	3.12	3.12	1.56	12.5	50	100	12.5	0.78

[a]M.a., *M. avium* ATCC19421; M.p., *M. phlei*; M.f., *M. fortuitum*; M.k., *M. kansasii*; M.sz., *M. szulgai*; M.g., *M. gordonae*; M.c., *M. chelonei*; M.i., *M. intracellulare*; M.sc., *M.scrofulaceum*; M.b., *M. bovis*.

Table 4. Range of activity against clinical isolates of *M. tuberculosis*, INH-R *M. tuberculosis* and *M. avium* of compounds **1a–q**

Compound	MIC (μg/mL)		
	M. tuberculosis[a]	*M. tuberculosis* INH-R[a]	*M. avium*[a]
1c	12.5–100	12.5–100	25–100
1e	3.12–6.25	3.12–6.25	6.25–100
1h	6.25–25	12.5–25	100–>100
1i	100–>100	100–>100	12.5–25
1k	6.25–12.5	1.56–12.5	12.5
1l	6.25–12.5	1.56–6.25	12.5
1m	12.5–50	12.5–50	3.12–6.25
1o	12.5–50	12.5–100	50
1p	6.25–12.5	3.12–25	12.5–25
1q	12.5–50	12.5–25	6.25–25
INH	0.045–0.18	50–200	6.25–>100

[a]Six strains assayed.

Cell cytotoxicity of compounds **1a–q** was tested in vitro on cultures of Vero cells. With the exception of compounds **1f**, **1j**, **1m**, **1n** and **1q** which showed to be toxic at concentrations higher than 62.5 μg/mL and **1e** and **1i** which showed to be toxic at concentration higher than 125 μg/mL, the tested compounds exhibited high values of maximum non toxic dose (MNTD) on Vero cells, ranging from 250 μg/mL, for **1d**, **1g**, **1h**, **1l** and **1o**, to 500–1000 μg/mL for the other members of the series. Results of cytotoxicity assays, expressed as MNTD, are reported in Table 2.

The antimicrobial activity of compounds **1a–q** was also evaluated against six Gram-positive species (*Staphilococcus aureus*, *S. epidermidis*, *Streptococcus agalactiae*, *S. faecalis*, *Bacillus licheniformis* and *B. subtilis*), five Gram-negative species (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Proteus mirabilis*, *Klebsiella pneumoniae*) isolated from clinical specimens and against *Candida albicans* ATCC E10931. Compounds **1a–q** were ineffective on inhibiting the growth of the strains of the Gram-negative species tested. A weak activity against *S. aureus* was shown by compounds **1p** and **1q** (MIC 100 μg/mL) and by compound **1p** against *S. epidermidis* (MIC 100 μg/mL). Compounds **1i**, **1m**, **1p** and **1q** showed a poor activity against *C. albicans* (MIC 100 μg/mL).

Experimental Protocols

Chemistry

Melting points were determined on a Kofler hot-stage and are uncorrected. Proton NMR spectra were recorded on a Varian Unity 300 spectrometer. The coupling constants were recorded in hertz (Hz) and the chemical shift are reported in part per million (δ, ppm) downfield from tetramethylsilane (TMS), which was used as internal standard. Infrared spectra were obtained with a Perkin-Elmer 398 spectrophotometer. Elemental analyses were carried out with a Carlo Erba model 1106 Elemental Analyzer and the values found were within ±0.4% of theoretical values.

General procedure for the synthesis of isothiosemicarbazones 1a–q

A suspension of thiosemicarbazide 0.45 g (5 mmol) and the appropriate alkyl halide (5.3 mmol) in ethanol (50 mL) was refluxed until the solid was completely dissolved. Then the appropriate aldheyde (5 mmol) was added and the heating was continued for further 30 min. After cooling to room temperature, the isothiosemicarbazone

hydrohalide was filtered off, then washed with diethyl ether. Isothiosemicarbazones **1b**, **1i**, **1j**, **1m** and **1n** were previously described.[36,37]

3-Pyridincarboxyaldehyde *S*-methylisothiosemicarbazone hydroiodide (1a). Yield 72%. Mp 168–170 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 2.64 (s, 3H, CH_3), 7.52, 8.62, 9.03 (m, 4H, pyridyl), 8.35 (s, 1H, CH), 9.38 (br s, 2H, NH_2). IR (Nujol) 3400, 3270, 2740, 1660, 1615, 1600 cm^{-1}. Anal. calcd for $C_8H_{10}N_4S$·HI: C, 29.83; H, 3.44; N, 17.39. Found: C, 29.87; H, 3.43; N, 17.42.

4-Pyridincarboxyaldehyde *S*-methylisothiosemicarbazone hydroiodide (1b). Yield 75%. Mp 197–198 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 2.57 (s, 3H, CH_3), 7.90, 8.86 (br s, 2H, NH_2), 8.09, 8.73 (m, 4H, pyridyl), 8.29 (s, 1H, CH). IR (Nujol) 3380, 3190, 1630, 1590 cm^{-1}. Anal. calcd for $C_8H_{10}N_4S$·HI: C, 29.83; H, 3.44; N, 17.39. Found: C, 29.80; H, 3.42; N, 17.36.

4-Fluorobenzaldehyde *S*-methylisothiosemicarbazone hydroiodide (1c). Yield 90%. Mp 185–187 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 2.68 (s, 3H, CH_3), 7.30, 7.99 (m, 4H, Ar), 8.28 (s, 1H, CH), 9.54 (br s, 2H, NH_2). IR (Nujol) 3240, 3160, 3080, 1625, 1595 cm^{-1}. Anal. calcd for $C_9H_{10}FN_3S$·HI: C, 31.87; H, 3.27; N, 12.39. Found: C, 31.90; H, 3.25; N, 12.42.

3-Pyridincarboxyaldehyde *S*-ethylisothiosemicarbazone hydrobromide (1d). Yield 82%. Mp 185–187 °C (2-PrOH). ^{1}H NMR (DMSO-d_6) δ 1.28 (t, 3H, J = 7.0 Hz, CH_3), 3.33 (q, 2H, J = 7.0 Hz, CH_2), 7.89, 8.84, 9.34 (m, 4H, pyridyl), 8.50 (s, 1H, CH), 9.91 (br s, 2H, NH_2). IR (Nujol) 3345, 1630, 1560, 1530 cm^{-1}. Anal. calcd for $C_9H_{12}N_4S$·HBr. C, 37.38; H, 4.53; N, 19.37. Found: C, 37.43; H, 4.55; N, 19.33.

4-Fluorobenzaldehyde *S*-ethylisothiosemicarbazone hydrobromide (1e). Yield 64%. Mp 101–103 °C. ^{1}H NMR (DMSO-d_6) δ 1.26 (t, 3H, J = 7.1 Hz, CH_3), 3.27 (q, 2H, J = 7.1 Hz, CH_2), 7.28, 7.95 (m, 4H, Ar), 8.29 (s, 1H, CH), 9.49 (br s, 2H, NH_2). IR (Nujol) 3340, 3250, 3100, 1600 cm^{-1}. Anal. calcd for $C_{10}H_{12}FN_3S$·HBr: C, 39.23; H, 4.28; N, 13.72. Found: C, 39.19; H, 4.30; N, 13.68.

4-Trifluoromethylbenzaldehyde *S*-ethylisothiosemicarbazone hydrobromide (1f). Yield 63%. Mp 164–165 °C. ^{1}H NMR (DMSO-d_6) δ 1.28 (t, 3H, J = 7.0 Hz, CH_3), 3.28 (q, 2H, J = 7.0 Hz, CH_2), 7.80, 8.13 (m, 4H, Ar), 8.39 (s, 1H, CH), 9.71 (br s, 2H, NH_2). IR (Nujol) 3450, 3250, 1640, 1610, 1590 cm^{-1}. Anal. calcd for $C_{11}H_{12}F_3N_3S$·HBr: C, 37.09; H, 3.68; N, 11.80. Found: C, 37.14; H, 3.70; N, 11.75.

3-Pyridincarboxyaldehyde *S*-allylisothiosemicarbazone hydrobromide (1g). Yield 81%. Mp 227–228 °C. ^{1}H NMR (DMSO-d_6) δ 3.47 (m, 2H, CH_2), 5.03, 5.20 (m, 2H, CH_2), 5.80 (m, 1H, CH), 8.12 (s, 1H, CH), 7.92, 8.56, 8.80, 9.01(m, 4H, pyridyl and NH_2). IR (Nujol) 3410, 3040, 1670, 1610, 1590 cm^{-1}. Anal. calcd for $C_{10}H_{12}N_4S$·HBr: C, 39.88; H, 4.35; N, 18.60. Found: C, 39.93; H, 4.37; N, 18.54.

4-Pyridincarboxyaldehyde *S*-allylisothiosemicarbazone hydrobromide (1h). Yield 77%. Mp 180–182 °C. ^{1}H NMR (DMSO-d_6) δ 3.36 (m, 2H, CH_2), 5.13, 5.30 (m, 2H, CH_2), 5.85 (m, 1H, CH), 8.04 (br s, 2H, NH_2), 8.23, 8.75 (m, 4H, pyridyl), 8.34 (s, 1H, CH). IR (Nujol) 3360, 2650, 2600, 1630, 1600, 1570 cm^{-1}. Anal. calcd for $C_{10}H_{12}N_4S$·HBr: C, 39.88; H, 4.35; N, 18.60. Found: C, 39.83; H, 4.34; N, 18.63.

4-Fluorobenzaldehyde *S*-allylisothiosemicarbazone hydrobromide (1i). Yield 73%. Mp 161–163 °C. ^{1}H NMR (DMSO-d_6) δ 4.00 (m, 2H, CH_2), 5.20, 5.34 (m, 2H, CH_2), 5.86 (m, 1H, CH), 7.30, 7.98 (m, 4H, Ar), 7.73, 9.67 (br s, 2H, NH_2), 8.35 (s, 1H, CH). IR (Nujol) 3260, 3090, 1630, 1600 cm^{-1}. Anal. calcd for $C_{11}H_{12}FN_3S$·HBr: C, 41.52; H, 4.12; N, 13.21. Found: C, 41.58; H, 4.10; N, 13.24.

4-Trifluoromethylbenzaldehyde *S*-allylisothiosemicarbazone hydrobromide (1j). Yield 56%. Mp 163–164 °C. ^{1}H NMR (DMSO-d_6) δ 4.02 (m, 2H, CH_2), 5.20, 5.35 (m, 2H, CH_2), 5.89 (m, 1H, CH), 7.81, 8.13 (m, 4H, Ar), 8.43 (s, 1H, CH), 9.77 (s, 2H, NH_2). IR (Nujol) 3040, 1630, 1610, 1570 cm^{-1}. Anal. calcd for $C_{12}H_{12}F_3N_3S$·HBr: C, 39.14; H, 3.56; N, 11.41. Found: C, 39.20; H, 3.54; N, 11.44.

3-Pyridincarboxyaldehyde *S*-butylisothiosemicarbazone hydrobromide (1k). Yield 75%. Mp 173–174 °C (2-PrOH). ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H, CH_3), 1.35 (m, 2H, CH_2), 1.55 (m, 2H, CH_2), 3.27 (m, 2H, CH_2), 7.55, 8.42, 8.63, 9.08 (m, 4H, pyridyl), 8.38 (s, 1H, CH), 9.57 (br s, 2H, NH_2). IR (Nujol) 3260, 3100, 1625 cm^{-1}. Anal. calcd for $C_{11}H_{16}N_4S$·HBr: C, 41.65; H, 5.40; N, 17.66. Found: C, 41.70; H, 5.37; N, 17.64.

4-Pyridincarboxyaldehyde *S*-butylisothiosemicarbazone hydrobromide (1l). Yield 73%. Mp 181–182 °C (2-PrOH). ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H, CH_3), 1.35 (m, 2H, CH_2), 1.57 (m, 2H, CH_2), 3.42 (m, 2H, CH_2), 8.12, 8.74 (m, 4H, pyridyl), 8.32 (s, 1H, CH), 8.87 (br s, 2H, NH_2). IR (Nujol) 3370, 1630, 1590 cm^{-1}. Anal. calcd for $C_{11}H_{16}N_4S$·HBr: C, 41.65; H, 5.40; N, 17.66. Found: C, 41.59; H, 5.38; N, 17.70.

4-Fluorobenzaldehyde *S*-butylisothiosemicarbazone hydrobromide (1m). Yield 79%. Mp 177–178 °C (2-PrOH). ^{1}H NMR (DMSO-d_6) δ 0.84 (t, 3H, J = 7.4 Hz, CH_3), 1.35 (m, 2H, CH_2), 1.55 (m, 2H, CH_2), 3.25 (m, 2H, CH_2), 7.28, 7.96 (m, 4H, Ar), 8.30 (s, 1H, CH), 9.56 (br s, 2H, NH_2). IR (Nujol) 3260, 3130, 1630, 1600, 1585 cm^{-1}. Anal. calcd for $C_{12}H_{16}FN_3S$·HBr: C, 43.12; H, 5.13; N, 12.57. Found: C, 43.07; H, 5.15; N, 12.61.

4-Trifluoromethylbenzaldehyde *S*-butylisothiosemicarbazone hydrobromide (1n). Yield 57%. Mp 194–196 °C. ^{1}H NMR (DMSO-d_6) δ 0.86 (t, 3H, CH_3), 1.36 (m, 2H, CH_2), 1.59 (m, 2H, CH_2), 3.26 (m, 2H, CH_2), 7.80, 8.13 (m, 4H, Ar), 8.39 (s, 1H, CH), 9.74 (br s, 2H, NH_2). IR (Nujol) 3255, 3080, 2770, 1640, 1630, 1610, 1570 cm^{-1}. Anal. calcd for $C_{13}H_{16}F_3N_3S$·HBr: C, 40.63; H, 4.46; N, 10.94. Found: C, 40.70; H, 4.44; N, 10.99.

3-Pyridincarboxyaldehyde *S*-benzylisothiosemicarbazone hydrobromide (1o). Yield 72%. Mp 136–138 °C (2-PrOH). ^{1}H NMR (DMSO-d_6) δ 4.54 (s, 2H, CH_2), 7.32 (m, 5H, Ar), 7.55, 8.38, 8.64, 9.05 (m, 4H, pyridyl), 8.35 (s, 1H, CH), 9.40 (br s, 2H, NH_2). IR (Nujol) 3190, 2700, 1590, 1560 cm^{-1}. Anal. calcd for $C_{14}H_{14}N_4S$·HBr: C, 47.87; H, 4.30; N, 15.95. Found: C, 47.92; H, 4.27; N, 15.99.

4-Pyridincarboxyaldehyde *S*-benzylisothiosemicarbazone hydrobromide (1p). Yield 86%. Mp 189–190 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 4.42 (s, 2H, CH_2), 7.30 (m, 5H, Ar), 8.17, 8.74 (m, 4H, pyridyl), 8.34 (s, 1H, CH), 8.60 (br s, 2H, NH_2). IR (Nujol) 3350, 3040, 2650, 1630, 1600, 1570 cm^{-1}. Anal. calcd for $C_{14}H_{14}N_4S$·HBr: C, 47.87; H, 4.30; N, 15.95. Found: C, 47.82; H, 4.32; N, 15.90.

4-Fluorobenzaldehyde *S*-benzylisothiosemicarbazone hydrobromide (1q). Yield 64%. Mp 209–210 °C (2-PrOH). ^{1}H NMR (DMSO-d_6) δ 4.62 (s, 2H, CH_2), 7.33, 7.96 (m, 9H, Ar and pyridyl), 8.33 (s, 1H, CH), 9.76 (br s, 2H, NH_2). IR (Nujol) 3240, 3060, 1615, 1590, 1570 cm^{-1}. Anal. calcd for $C_{15}H_{14}FN_3S$·HBr: C, 48.92; H, 4.11; N, 11.41. Found: C, 48.87; H, 4.09; N, 11.45.

Biological assays

For antimicrobial and cytotoxicity studies, compounds **1a**–**q** were dissolved in dimethylsulfoxide at 10 mg/mL concentration and kept at −20 °C. The working solutions were prepared in the same medium used for tests. To avoid interference by the solvent,[38] the highest DMSO concentration was 1%.

Cytotoxicity assay

Cell cytotoxicity of compounds **1a**–**q** was tested in vitro by two methods. In the first method, RPMI 1640 medium (Gibco) alone or medium containing compounds at concentrations ranging from 1000 to 31.5 μg/mL were inoculated onto cultures of Vero cells is six-well tissue culture plates. The cells were observed daily for 6 days for any signs of cell cytotoxicity compared with the controls. In the second method, a cell viability assay previously reported[39,40] was used. Monolayers of Vero cells in 96-multiwell plates were incubated with the testing compounds at concentrations of 1000–62.5 μg/mL in RPMI 1640 for 48 h and the medium replaced with 50 μL of 1 mg/mL solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) in RPMI without phenol red (Sigma). Cells were incubated at 37 °C for 3 h, the untransformed MTT removed and 50 μL of isopropanolic HCl 0.04 N was added to each well. After few min at room temperature to ensure that all crystals were dissolved, the plates were read with a 560 nm test wavelength and a 690 nm reference wavelength.

Determination of MICs

The determination of MIC against *Mycobacteria* was carried out by the two fold agar dilution method[41] using 7H11 agar (Difco Laboratories) containing compounds **1a**–**q** at concentration that range between 100 and 0.19 μg/mL on which 100 μL of the tested bacterial suspension were spotted. Suspension to be used for drug susceptibility testing were prepared from 7H9 broth cultures, washed, suspended in saline to a turbidity of no. 1 Mcfarland and then diluted to obtain inocula of 3×10^5 cells per well. The MICs of the compounds were determined after 7 days (rapid growing) or 21 days (slow growing) of cultivation at 37 °C (28–30 °C for *M. chelonei*) in a CO_2 (5% CO_2–95% humidified air) incubator. Isoniazid (INH) served as reference compound.

The MICs of various compounds against Gram-positive, Gram-negative and *C. albicans* were determined by a standard broth macro-dilution method.[42,43] Tests with Gram-positive and Gram-negative bacteria were carried out in Mueller Hinton broth DIFCO. Antifungal activity against *C. albicans* ATCC E10231 was evaluated in yeast extract peptone dextrose medium (Difco).[44] The compounds were diluted in the test medium to obtain final concentrations ranging from 100 to 0.19 μg/mL. Tubes were inoculated with 1×10^5 cells/mL and were incubated at 37 °C for 18 or 24 h.

References and Notes

1. Snider, D. E.; Raviglione, M.; Kochi, A. In *Global Burden of Tuberculosis, Tuberculosis: Pathogenesis, Protection and Control*; Bloom, B., Ed.; ASM: Washington, DC, 1994; p 3.
2. Shinnick, T. M.; King, C. H.; Quinn, F. D. *Am. J. Med. Sci*. **1995**, *309*, 92.
3. Snider, D. E., Jr.; La Montagne, J. R. *J. Infect. Dis*. **1994**, *169*, 1189.
4. Woods, G. L.; Washington, J. A. *Rev. Infect. Dis*. **1987**, *9*, 275.
5. Tsukamura, M.; Shimoide, H.; Kita, N.; Kawakumi, K.; Ito, T.; Nakajima, N.; Kondo, N.; Yamamoto, Y. *Rev. Infect. Dis*. **1981**, *3*, 997.
6. Etzkorn, E. T.; Aldarondo, S.; McAllister, C. K.; Matthews, J.; Ognibene, A. *J. Am. Rev. Respir. Dis*. **1986**, *134*, 442.
7. Kilbi, J. M.; Gilligan, P. H.; Yankaskas, J. R.; Highsmith, W. E., Jr.; Edwards, L. J.; Knowles, M. R. *Chest* **1992**, *102*, 70.
8. Hornick, D. B.; Dayton, C. S.; Bedell, G. N.; Fick, R. B., Jr. *Chest* **1988**, *93*, 550.
9. Contreras, M. A.; Cheung, O. T.; Sanders, D. E.; Goldstein, R. S. *Am. Rev. Respir. Dis*. **1988**, *137*, 149.
10. Al Jarad, N.; Demertzis, P.; Jones, D. J.; Barnes, N. C.; Rudd, R. M.; Gaya, H.; Wedzicha, J. A.; Hughes, D. T.; Empey, D. W. *Thorax* **1996**, *51*, 137.
11. Minkin, B. I.; Mills, C. L.; Bullock, D. W.; Burke, F. D. *J. Hand. Surg*. **1987**, *12*, 1992.
12. Yamauchi, T.; Ferrieri, P.; Anthony, B. F. *J. Med. Microbiol*. **1980**, *13*, 37.
13. Sutcker, W. L.; Lankford, L. L.; Tompsett, R. *Rev. Infect. Dis*. **1979**, *1*, 729.
14. Sherer, R.; Sable, R.; Sonnenberg, M.; Cooper, S.; Spencer, P.; Schwimmer, S.; Kocka, F.; Muthuswamy, P.; Kallick, C. *Ann. Intern. Med*. **1986**, *105*, 710.
15. Azadian, B. S.; Beck, A.; Curtis, J. R.; Cherrington, L. E.; Gower, P. E.; Phillips, M.; Eastwood, J. B.; Nicholls, J. *Tubercle* **1981**, *62*, 281.
16. Silcox, V. A.; Good, R. A.; Floyd, M. M. *J. Clin. Microbiol*. **1981**, *14*, 686.

17. Yew, W. W.; Piddock, L. J.; Li, M. S.; Lyon, D.; Chan, C. Y.; Cheng, A. F. *J. Antimicrob. Chemother.* **1994**, *34*, 343.
18. Rastogi, N.; Frehel, C.; Ryter, A.; Ohayou, H.; Lesourd, M.; David, H. L. *Antimicrob. Agents Chemother.* **1981**, *20*, 666.
19. David, H. *Rev. Infect. Dis.* **1981**, *3*, 878.
20. Wallace, R. J.; Dunbar, D.; Brown, B. A.; Onyi, G.; Dunlap, R.; Ahn, C. H.; Murphy, D. T. *Clin. Infect. Dis.* **1994**, *18*, 736.
21. Fattorini, L.; Orefici, G.; Jin, S. H.; Scardaci, G.; Amicosante, G.; Franceschini, N.; Chopra, I. *Antimicrob. Agents Chemother.* **1992**, *36*, 1068.
22. Yamada, H.; Kohyama, T.; Terashi, K.; Mori, H.; Yamaguchi, A.; Arima, N.; Tanaka, H. *Intern. Med.* **1997**, *36*, 298.
23. Cocco, M. T.; Congiu, C.; Onnis, V.; Pusceddu, M. C.; Schivo, M. L.; De Logu, A. *Eur. J. Med. Chem.* **1999**, *34*, 1071.
24. Dobek, A. S.; Klayman, D. L.; Dickson, E. T., Jr.; Scovill, J. P.; Tramont, E. C. *Antimicrob. Agents Chemother.* **1980**, *18*, 27.
25. Dobek, A. S.; Klayman, D. L.; Dickson, E. T., Jr.; Scovill, J. P.; Oster, C. N. *Arzneim.-Forsch. (Drug Res.)* **1983**, *33*, 1583.
26. Klayman, D. L.; Bartosevich, J. F.; Griffin, T. S.; Mason, C. J.; Scovill, J. P. *J. Med. Chem.* **1979**, *22*, 855.
27. Klayman, D. L.; Scovill, J. P.; Bartosevich, J. F.; Mason, C. J. *J. Med. Chem.* **1979**, *22*, 1367.
28. Foye, W. O.; Almassian, B.; Eisenberg, M. S.; Maher, T. J. *J. Pharm. Sci.* **1990**, *79*, 527.
29. Campana, M.; Laborie, C.; Barbier, G.; Assan, R.; Milcent, R. *Eur. J. Med. Chem.* **1991**, *26*, 273.
30. Shipman, C., Jr.; Smith, S. H.; Drach, J. C.; Klayman, D. L. *Antiviral Res.* **1986**, *6*, 197.
31. Turk, S. R.; Shipman, C., Jr.; Drach, J. C. *Biochem. Pharmacol.* **1986**, *35*, 1539.
32. Easmon, J.; Heinisch, G.; Holzer, W.; Rosenwirth, B. *Arzneim.-Forsch. (Drug Res.)* **1989**, *39*, 1196.
33. Sidwell, R. W.; Huffman, J. H.; Schafer, T. W.; Shipman, C. *Chemotherapy* **1990**, *36*, 58.
34. Easmon, J.; Heinisch, G.; Holzer, W.; Rosenwirth, B. *J. Med. Chem.* **1992**, *35*, 3288.
35. Cocco, M. T.; Plumitallo, A.; Schivo, M. L.; De Logu, A. *Farmaco* **1990**, *45*, 1101.
36. Foye, W. O.; Almassian, B.; Marc, S.; Maher, T. J. *J. Pharm. Sci.* **1990**, *79*, 527.
37. Cardia, M. C.; Begala, M.; De Logu, A.; Maccioni, E.; Plumitallo, A. *Farmaco* **2000**, *55*, 93.
38. Jagannath, C.; Reddy, V. M.; Gangadharam, P. R. *J. Antimicrob. Chemother.* **1995**, *35*, 381.
39. Denizot, F.; Lang, R. *J. Immunol. Meth.* **1986**, *89*, 271.
40. Mosmann, T. *J. Immunol. Meth.* **1983**, *65*, 55.
41. Hopewell, P.; Cynamon, M.; Starke, J.; Iseman, M.; O'Brein, R. *Clin. Infect. Dis. 15, S282.* **1992**.
42. Trhupp, L. D. In *Antibiotics in Laboratory Medicine*; Lorian, V., Ed.; William & Wilkins: Baltimore, MD, 1986; Chapter 4.
43. Washington, J. A., II; Sutter, V. L. In *Manual of Clinical Microbiology*; Lennette, E. H., Spaulding, E. H., Truant, J. I., Eds., American Society of Microbiology: Washington, DC, 1980; Chapter 42.
44. Pfaller, M. A.; Rinaldi, M. G.; Galgiani, J. N.; Bartlett, M. S.; Body, B. A.; Espinel-Ingroff, A.; Fromtling, R. A.; Hall, G. S.; Hughes, C. E.; Odds, F. C.; Sugar, A. M. *Antimicrob. Agents Chemother.* **1990**, *34*, 1648.