SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF NEW 4-(BENZYLSULFANYL)PYRIDINE DERIVATIVES

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Dedicated to the memory of Dr Miroslav Protiva, one of the greatest Czech medicinal chemists.

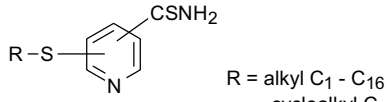
A series of 4-(benzylsulfanyl)pyridine-2-carbonitriles **2** and 4-(benzylsulfanyl)pyridine-2-carbothioamides **3** was synthesized and their antimycobacterial and antifungal activities were tested. The prepared compounds exhibit significant activity both against *M. tuberculosis* and nontuberculous mycobacteria. The most active compounds show the MICs of 4 μmol/l. The antifungal activities of **2** are very weak, compounds **3** are inactive against all the tested fungi.

Key words: Pyridinecarbonitriles; Pyridinecarbothioamides; Tuberculostatics; Antimycobacterial activity; Antifungal activity; QSAR.

The current search for new antimycobacterial agents is very urgent as tuberculosis has become the major emerging opportunistic infection. The developing resistance to conventional antimycobacterial agents is a stimulating factor in the research of new, more selective compounds[1]. In addition, also the infections caused by atypical mycobacteria (nontuberculosis mycobacteria) show a rising frequency, the greatest problem beeing the *Mycobacterium avium* complex. Disseminated infection with the *M. avium* complex is the most common systematic bacterial infection complicating AIDS (ref.[2]). In the Czech Republic an increase in the occurrence of *M. kansasii* has been observed in some areas[3].

During our studies on new potent antimycobacterial agents, we found that some compounds showed also antifungal activity. This fact prompted us to investigate systematically both of these biological activities.

In our previous work concerning the pyridine derivatives, we have reported synthesis and antimicrobial activity of compounds of general structure **1**.



1

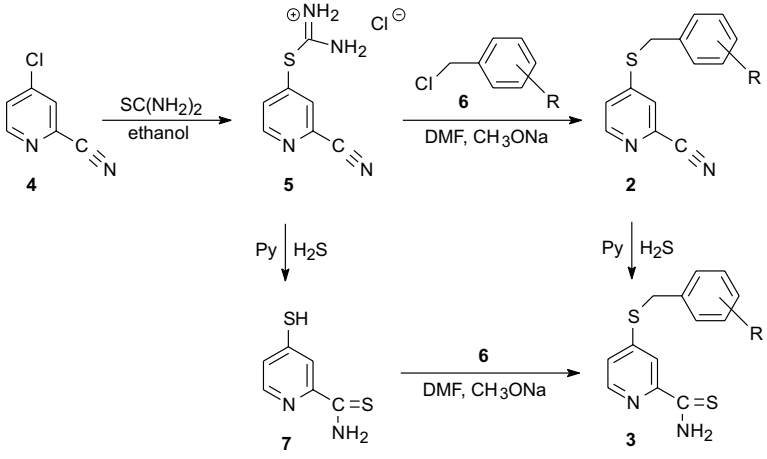
Especially 2-(alkylsulfanyl)pyridine-4-carbothioamides and 4-(alkylsulfanyl)pyridine-2-carbothioamides showed an interesting level of activity *in vitro*[4]. Regarding the spectrum of the antimycobacterial activity, they exhibited efficacy both against *M. tuberculosis* and against atypical mycobacterial strains. Moreover, *in vivo* results indicated that the activity of 2-(propylsulfanyl)pyridine-4-carbothioamide was comparable with the activity of isoniazide. A study of quantitative relationships between the structure and activity of 2-alkylsulfanyl derivatives brought us to an idea to modify the alkylsulfanyl group and a series of benzylsulfanyl derivatives bearing various substituents on the phenyl ring was prepared.

In the present paper, we report the results of antimycobacterial and antifungal activities of new 4-(benzylsulfanyl)pyridine-2-carbonitriles **2** and 4-(benzylsulfanyl)pyridine-2-carbothioamides **3**.

Nitrile **4** as the key starting compound was prepared from 2-methylpyridine by a method described in the literature[5,6]. Treatment of **4** with thiourea in ethanol afforded thiuronium salt **5** in 71% yield. Crude product **5** was used in further reactions. Condensation of **5** with the appropriate benzyl chlorides **6** in *N*,*N*-dimethylformamide in the presence of sodium methoxide produced corresponding benzyl derivatives **2**, which were further converted by addition of hydrogen sulfide in pyridine into the related pyridinecarbothioamides **3** in good yields. Only compounds **3s**, **3t**, and **3u**, having at least one nitro group in the benzyl ring, were obtained by reacting **7** with the corresponding nitrobenzyl chlorides. Compound **7** was obtained by a treatment of thiuronium salt **5** with hydrogen sulfide in pyridine (Scheme 1).

Structures of compounds **2** and **3** were confirmed by elemental analyses, IR and ^{1}H NMR spectral data. All compounds **2** showed in their IR spectra a strong band at 2 240 cm^{-1}, which is characteristic of the C≡N group, and compounds **3** showed characteristic bands of amides at 3 260–3 300 and 1 640 cm^{-1} (Tables I and II). In the ^{1}H NMR spectra the signals of the

pyridine moiety appeared separate from those of the substituted phenyl ring. In both the nitrile and the carbothioamide sets, the pyridine protons were observed as doublets of doublets coupled with one another; the larger coupling constant between H-5 and H-6 was in the range of 5.2–5.5 Hz while both these hydrogens had smaller couplings with H-3. Hydrogen atom H-6 was shifted most downfield in the nitrile series due to the presence of the pyridine nitrogen. On the other hand, in the carbothioamide series, H-3 lies even more downfiled since it is deshielded by the thiocarbamoyl group. Chemical shifts and multiplicities of protons in the phenyl moiety depend on the substitution (Tables III and IV).



2, 3, 6	R	2, 3, 6	R
a	H	**k**	4-CH_3
b	2-Cl	**l**	3-NO_2
c	3-Cl	**m**	4-NO_2
d	4-Cl	**n**	4-OCH_3
e	2-F	**o**	3,4-Cl_2
f	3-F	**p**	3,4-F_2
g	4-F	**r**	2-Cl-6-F
h	3-Br	**s**	2-F-6-NO_2
i	4-Br	**t**	2,4-$(NO_2)_2$
j	3-CH_3	**u**	3,5-$(NO_2)_2$

SCHEME 1

Antimycobacterial activity of the prepared compounds was tested *in vitro* against *Mycobacterium tuberculosis* CNCTC 331/88, *Mycobacterium kansasii* CNCTC 235/80, *Mycobacterium kansasii* 509/96 (clinical isolate), and *Mycobacterium avium* CNCTC 33/88 using the micromethod for the determina-

tion of the minimum inhibitory concentration (MIC). *In vitro* antigungal activity against *Trichophyton mentagrophytes* 445, *Candida albicans* ATCC 44859, *Candida tropicalis* 156, *Candida krusei* E28, *Candida glabrata* 20/I, *Trichosporon beigelii* 1188, *Aspergillus fumigatus* 231, and *Absidia corymbifera* 272 was determined using the microdilution broth test. All strains, except of *C. albicans*, were clinical isolates, identified by conventional morphological and biochemical methods.

EXPERIMENTAL

The melting points were determined on a Kofler apparatus and are uncorrected. Analytical samples were dried over P_4O_{10} at 60 °C and 30 Pa for 8–10 h. Elemental analyses were performed on CHNS-O CE instrument (FISONS EA 1110). IR spectra were obtained on a Nicolet Impact 400 spectrometer in KBr pellets. The NMR spectra were recorded in $CDCl_3$ solutions (with the exception of 4-sulfanylpyridine-2-carbothioamide which was measured in CD_3OD) at ambient temperature on a Varian Mercury-VX BB 300 spectrometer operating at 300 MHz. Chemical shifts were recorded as δ values in ppm and were indirectly referenced to tetramethylsilane (TMS). Coupling constants (*J*) are given in Hz. The signals were assigned to the corresponding protons only if an unequivocal assignment could be made (1D decoupling experiments were done when necessary). The reactions and purity of all the prepared compounds was checked by TLC (Silufol UV254, Kavalier, Votice, Czech Republic) in ethyl acetate–light petroleum (2 : 3) using UV detection.

2-Cyanopyridine-4-thiuronium Chloride (**5**)

4-Chloropyridine-2-carbonitrile (**4**; 3.5 g, 25 mmol) and thiourea (2.0 g, 26 mmol) were dissolved in ethanol (20 ml) and the solution was stirred at 100 °C for 15 min. After cooling, the resulting salt was filtered off, affording **5** as a grey-green product; yield 3.9 g (71%), m.p. 193–196 °C.

Preparation of 4-(Benzylsulfanyl)pyridine-2-carbonitriles **2**. General Procedure

To a stirred solution of **5** (1.5 g, 7 mmol) in dry DMF (8 ml) a solution of sodium (0.2 g, 8 mmol) in dry methanol (2.5 ml) was added at room temperature, followed by the appropriate benzyl chloride **6** (7 mmol). After stirring at room temperature for 2–4 h, the solvent was evaporated *in vacuo* and the residue was diluted with water (75 ml). The precipitated solid was filtered off, washed with cold water and crystallized from ethanol or ethanol–water (Table I).

4-Sulfanylpyridine-2-carbothioamide (**7**)

Dry hydrogen sulfide was passed through a solution of **5** (2.2 g, 10 mmol) in a mixture of dry pyridine (18 ml) and triethylamine (1.8 ml) at 70 °C for 3 h. After cooling, the mixture was diluted with water (75 ml), the solution was acidified with 1 M H_2SO_4 to pH 6, the formed precipitate was filtered off and crystallized from water to give **7** as yellow crystals;

TABLE I
Yields and physicochemical properties of compounds **2a**–**2u**

Compound	R	Formula M.w.	Yield %	M.p. °C	Calculated/Found				IR cm^{-1}
					% C	% H	% N	% S	
2a	H	$C_{13}H_{10}N_2S$	68	90.5–92.5	69.01	4.46	12.39	14.17	2 234
		226.3			69.03	4.80	12.53	14.08	
2b[a]	2-Cl	$C_{13}H_9ClN_2S$	83	100–101	60.00	3.49	10.77	12.30	2 235
		260.7			60.13	3.43	10.82	12.42	
2c[b]	3-Cl	$C_{13}H_9ClN_2S$	61	76.5–78	60.00	3.49	10.77	12.30	2 237
		260.7			60.25	3.40	10.73	12.14	
2d[c]	4-Cl	$C_{13}H_9ClN_2S$	76	102.5–104	60.00	3.49	10.77	12.30	2 239
		260.7			59.90	3.64	10.70	12.34	
2e	2-F	$C_{13}H_9FN_2S$	46	103–105	63.92	3.71	11.47	13.12	2 236
		244.3			64.27	3.65	11.59	13.26	
2f	3-F	$C_{13}H_9FN_2S$	30	67–69	63.92	3.71	11.47	13.12	2 240
		244.3			63.76	3.78	11.57	13.19	
2g	4-F	$C_{13}H_9FN_2S$	65	58–60	63.92	3.71	11.47	13.12	2 235
		244.3			63.81	3.66	11.70	12.87	
2h[d]	3-Br	$C_{13}H_9BrN_2S$	82	70–73	51.16	2.97	9.18	10.50	2 235
		305.2			51.47	2.95	9.16	10.41	
2i[e]	4-Br	$C_{13}H_9BrN_2S$	80	112–113	51.16	2.97	9.18	10.50	2 236
		305.2			51.24	3.02	8.80	10.11	
2j	3-CH_3	$C_{14}H_{12}N_2S$	65	52–54	69.97	5.03	11.66	13.34	2 237
		240.3			69.96	4.88	11.92	13.35	
2k	4-CH_3	$C_{14}H_{12}N_2S$	63	78–81.5	69.97	5.03	11.66	13.34	2 238
		240.3			69.80	4.99	11.57	13.28	
2l	3-NO_2	$C_{13}H_9N_3O_2S$	83	142–144	57.56	3.34	15.49	11.82	2 240
		271.3			57.73	3.54	15.50	11.66	
2m	4-NO_2	$C_{13}H_9N_3O_2S$	85	140.5–143	57.56	3.34	15.49	11.82	2 240
		271.3			57.72	3.23	15.78	11.49	
2n	4-OCH_3	$C_{14}H_{12}N_2OS$	83	107–109.5	65.60	4.72	10.93	12.51	2 234
		256.3			65.44	4.65	11.29	12.51	
2o	3,4-Cl_2	$C_{13}H_8Cl_2N_2S$	81	85–87	52.90	2.73	9.49	10.86	2 240
		295.2			52.69	3.02	9.29	10.68	

TABLE I
(*Continued*)

Compound	R	Formula M.w.	Yield %	M.p. °C	Calculated/Found % C	% H	% N	% S	IR cm^{-1}
2p	3,4-F_2	$C_{13}H_8F_2N_2S$	84	73–75	59.53	3.07	10.68	12.22	2 243
		262.3			59.71	3.31	10.56	11.91	
2r[f]	2-Cl-6-F	$C_{13}H_8ClFN_2S$	87	121.5–125	56.02	2.89	10.05	11.50	2 234
		278.7			55.92	2.80	10.00	11.39	
2s	2-F-6-NO_2	$C_{13}H_8FN_3O_2S$	60	115.5–117.5	53.98	2.79	14.53	11.08	2 235
		289.3			53.91	2.76	14.23	11.07	
2t	2,4-$(NO_2)_2$	$C_{13}H_8N_4O_4S$	46	118.5–120	49.37	2.55	17.71	10.14	2 238
		316.3			49.55	2.54	17.50	10.11	
2u	3,5-$(NO_2)_2$	$C_{13}H_8N_4O_4S$	82	148–151	49.37	2.55	17.71	10.14	2 245
		316.3			49.32	2.80	17.72	9.79	

[a] % Cl calculated: 13.45, found: 13.18; [b] % Cl calculated: 13.45, found: 13.33; [c] % Cl calculated: 13.45, found: 13.58; [d] % Br calculated: 26.18, found: 25.97; [e] % Br calculated: 26.18, found: 26.24; [f] % Cl calculated: 12.72, found: 12.52.

yield 1.4 g (89%), m.p. 212–215 °C. For $C_6H_6N_2S_2$ (170.3) calculated: 42.33% C, 3.55% H, 16.45% N, 37.66% S; found: 42.24% C, 3.52% H, 16.38% N, 37.65% S. ^{1}H NMR (CD_3OD): 7.83 d, 1 H, *J*(3,5) = 1.9 (H-3); 7.46 dd, 1 H, *J*(5,6) = 6.3, *J*(5,3) = 1.9 (H-5); 7.68 d, 1 H, *J*(6,5) = 6.3 (H-6).

Preparation of 4-(Benzylsulfanyl)pyridine-2-carbothioamides **3a**–**3r**.
General Procedure

A 4-(benzylsulfanyl)pyridine-2-carbonitrile **2** (1 mmol) was dissolved in dry pyridine (6 ml), then dry triethylamine (0.6 ml) was added and dry hydrogen sulfide was passed through the mixture at 50 °C for 3–5 h. After cooling, the mixture was diluted with water (75 ml), the precipitated solid was filtered off and crystallized from ethanol (Table II).

Preparation of 4-(Benzylsulfanyl)pyridine-2-carbothioamides **3s**–**3u**.
General Procedure

To a stirred solution of **7** (1.5 g, 9 mmol) in dry DMF (8 ml) a solution of sodium (0.2 g, 9 mmol) in dry methanol (3 ml) was added, followed by the corresponding benzyl chloride **6** (9 mmol). After stirring at room temperature for 1–2.5 h, the solvent was evaporated *in*

TABLE II
Yields and physicochemical properties of compounds **3a**–**3u**

Compound	R	Formula M.w.	Yield %	M.p. °C	Calculated/Found % C	% H	% N	% S	IR cm^{-1}
3a	H	$C_{13}H_{12}N_2S_2$	87	125–127	59.97	4.65	10.76	24.63	3 232
		260.4			60.05	4.71	10.71	24.72	1 598
3b[a]	2-Cl	$C_{13}H_{11}ClN_2S_2$	90	169.5–172	52.96	3.76	9.50	21.75	3 231
		294.8			52.73	3.74	9.64	21.72	1 597
3c[b]	3-Cl	$C_{13}H_{11}ClN_2S_2$	92	143–145.5	52.96	3.76	9.50	21.75	3 284
		294.8			52.91	3.75	9.69	21.73	1 616
3d[c]	4-Cl	$C_{13}H_{11}ClN_2S_2$	95	163–166	52.96	3.76	9.50	21.75	3 247
		294.8			52.95	3.73	9.69	21.81	1 602
3e	2-F	$C_{13}H_{11}FN_2S_2$	85	152–153.5	56.09	3.98	10.06	23.03	3 242
		278.4			56.14	4.19	10.02	23.56	1 595
3f	3-F	$C_{13}H_{11}FN_2S_2$	80	121–123	56.09	3.98	10.06	23.03	3 316
		278.4			55.91	3.90	9.95	23.18	1 615
3g	4-F	$C_{13}H_{11}FN_2S_2$	87	154–155.5	56.09	3.98	10.06	23.03	3 297
		278.4			55.87	3.97	9.89	23.50	1 600
3h[d]	3-Br	$C_{13}H_{11}BrN_2S_2$	92	152–155.5	46.16	3.28	8.29	18.92	3 259
		338.0			46.01	3.21	8.26	19.00	1 617
3i[e]	4-Br	$C_{13}H_{11}BrN_2S_2$	90	157–161	46.16	3.28	8.29	18.92	3 299
		338.0			45.94	3.55	8.56	18.95	1 637
3j	3-CH_3	$C_{14}H_{14}N_2S_2$	92	132.5–134.5	61.28	5.14	10.21	23.37	3 299
		274.4			61.57	5.42	10.07	23.42	1 640
3k	4-CH_3	$C_{14}H_{14}N_2S_2$	95	137–138	61.28	5.14	10.21	23.37	3 309
		274.4			61.14	5.10	9.97	23.46	1 624
3l	3-NO_2	$C_{13}H_{11}N_3O_2S_2$	77	167–169	51.13	3.63	13.76	21.00	3 286
		305.4			51.44	3.50	14.05	21.17	1 637
3m	4-NO_2	$C_{13}H_{11}N_3O_2S_2$	68	139.5–142	51.13	3.63	13.76	21.00	3 321
		305.4			50.83	3.55	13.65	21.27	1 619
3n	4-OCH_3	$C_{14}H_{14}N_2OS_2$	92	131–133	57.90	4.86	9.65	22.08	3 324
		290.4			57.95	4.73	9.73	22.23	1 610
3o	3,4-Cl_2	$C_{13}H_{10}Cl_2N_2S_2$	89	135–137	47.57	3.07	8.54	19.50	3 258
		328.0			47.54	2.71	8.34	19.41	1 609

TABLE II
(*Continued*)

Compound	R	Formula M.w.	Yield %	M.p. °C	Calculated/Found % C	% H	% N	% S	IR cm^{-1}
3p	3,4-F_2	$C_{13}H_{10}F_2N_2S_2$	90	131–134	52.69	3.40	9.45	21.61	3 258
		296.0			52.82	3.17	9.25	21.68	1 610
3r[f]	2-Cl-6-F	$C_{13}H_{10}ClFN_2S_2$	88	146–148.5	49.92	3.22	8.96	20.50	3 237
		312.8			49.79	3.11	8.92	20.68	1 605
3s	2-F-6-NO_2	$C_{13}H_{10}FN_3O_2S_2$	67	147–149.5	48.29	3.12	12.99	19.83	3 274
		323.4			48.18	3.13	12.76	19.78	1 621
3t	2,4-$(NO_2)_2$	$C_{13}H_{10}N_4O_4S_2$	80	147.5–150	44.57	2.88	15.99	18.30	3 312
		350.4			44.34	2.78	15.90	18.36	1 604
3u	3,5-$(NO_2)_2$	$C_{13}H_{10}N_4O_4S_2$	34	160–163	44.57	2.88	16.00	18.27	3 255
		350.4			44.20	3.11	15.80	18.05	1 594

[a] % Cl calculated: 12.03, found: 11.90; [b] % Cl calculated: 12.03, found: 11.78; [c] % Cl calculated: 12.03, found: 12.16; [d] % Br calculated: 23.35, found: 23.50; [e] % Br calculated: 23.35, found: 23.75; [f] % Cl calculated: 11.33, found: 11.35.

vacuo and the residue was diluted with water (100 ml). The precipitated dolid was filtered off and crystallized from ethanol (Table II).

Antimycobacterial Activity

For the evaluation of antimycobacterial activity of the substances *in vitro*, the following strains were used: *Mycobacterium tuberculosis* CNCTC My 331/88, *Mycobacterium kansasii* CNCTC My 235/80, *Mycobacterium avium* CNCTC My 330/88, obtained from the Czech National Collection of Type Cultures (CNCTC), National Institute of Public Health, Prague, and a clinical isolate *Mycobacterium kansasii* 6 509/96. Antimycobacterial activity of the compounds against these strains was determined in Šula's semisynthetic medium (SEVAC, Prague). The compounds were added to the medium as dimethyl sulfoxide solutions. The following concentrations were used: 1 000, 500, 250, 125, 62, 31, 16, 8, and 4 μmol/l. The minimum inhibitory concentrations were determined after incubation at 37 °C for 14 and 21 days. MIC was the lowest concentration of a substance, at which the inhibition of the growth of mycobacteria occurred.

TABLE III
^{1}H NMR data of compounds **2a–2u**

Compound	Pyridine[a]			Benzene	$-CH_2-$ (s)	Other
	H-3 (dd)	H-5 (dd)	H-6 (dd)			
2a	7.48	7.28	8.43	7.30–7.45 m, 5 H	4.25	
2b	7.50	7.29	8.46	7.40–7.50 m, 2 H (H-3,H-4); 7.20–7.35 m, 2 H (H-5,H-6)	4.35	
2c	7.46	7.26	8.45	7.38–7.42 m, 1 H (H-2); 7.25–7.35 m, 3 H (H-4,H-5,H-6)	4.21	
2d	7.46	7.25	8.44	7.33 s, 4 H (H-2,H-3,H-5,H-6)	4.14	
2e	7.51	7.31	8.46	7.05–7.20 m, 2 H (H-3,H-5); 7.25–7.35 m, 1 H (H-4); 7.35–7.45 m, 1 H (H-6)	4.27	
2f	7.46	7.26	8.44	7.12 dt, 1 H, *J*(2,F) = 9.4, *J* = 1.9 (H-2); 6.95–7.05 m, 1 H (H-4); 7.30–7.40 m, 1 H (H-5); 7.15–7.20 m, 1 H (H-6)	4.23	
2g	7.47	7.27	8.44	7.37 m, 12 H (H-2,H-6); 7.05 m, 2 H (H-3,H-5)	4.22	
2h	7.46	7.26	8.45	7.5 t, 2 H, *J* = 1.7 (H-2); 7.40–7.50 m, 1 H (H-4); 7.30–7.35 m, 1 H (H-5); 7.22 d, 1 H, *J*(5,6) = 7.7 (H-6)	4.20	
2i	7.46	7.25	8.45	7.27 m (AA′BB′), 2 H (H-2,H-6); 7.49 m (AA′BB′), 2 H (H-3,H-5)	4.19	
2j	7.40–7.50 m	7.10–7.30 m	8.42	7.10–7.30 m, 4 H (H-2,H-4,H-6)	4.20	2.35 s[b]
2k	7.47	7.28	8.42	7.28 m (AA′BB′), 2 H (H-2,H-6); 7.16 m (AA′BB′), 2 H (H-3,H-5)	4.21	2.35 s[b]
2l	7.48	7.28	8.48	8.30 t, 1 H, *J* = 1.8 (H-2); 8.20 dm, 1 H (H-4); 7.57 t, 1 H, *J* = 7.9 (H-5); 7.75 bd, 1 H, *J* = 7.9 (H-6)	4.35	

TABLE III
(*Continued*)

Compound	Pyridine[a]			Benzene	$-CH_2-$ (s)	Other
	H-3 (dd)	H-5 (dd)	H-6 (dd)			
2m	7.47	7.27	8.47	7.59 m (AA′BB′), 2 H (H-2,H-6); 8.23 m (AA′BB′), 2 H (H-3,H-5)	4.33	
2n	7.47	7.27	8.43	7.30 m (AA′BB′), 2 H (H-2,H-6); 6.88 m (AA′BB′), 2 H (H-3,H-5)	4.20	3.81 s[c]
2o	7.46	7.25	8.47	7.51 d, 1 H, *J*(2,6) = 2.2 (H-2); 7.44 d, 1 H, *J*(5,6) = 8.3 (H-5); 7.24 dd, 1 H, *J*(6,5) = 8.3, *J*(6,2) = 2.2 (H-6)	4.19	
2p	7.46	7.25	8.46	7.10–7.25 m, 3 H (H-2,H-5,H-6)	4.20	
2r	7.59	7.41	8.50	7.00–7.10 m, 1 H (H-5); 7.20–7.30 m, 2 H (H-3,H-4)	4.38, 4.39	
2s	7.56	7.38	8.50	7.90 dt, 1 H, *J*(5,4) = 8.0, *J*(5,3) = *J*(5,F) = 1.4 (H-5); 7.40–7.55 m, 2 H (H-3,H-4)	4.62, 4.63	
2t	7.52	7.30	8.52	8.93 d, 1 H, *J*(3,5) = 2.3 (H-3); 8.46 dd, 1 H, *J*(5,6) = 8.5, *J*(5,3) = 2.3 (H-5); 7.83 d, 1 H, *J*(6,5) = 8.5 (H-6)	4.71	
2u	7.51	7.30	8.53	8.63 d, 2 H, *J*(2,5;4) = 1.9 (H-2,H-5); 9.01 t, 1 H, *J*(4,2) = *J*(4,5) = 1.9 (H-4)	4.45	

[a] 1 H, *J*(3,5) = 1.9–2.0, *J*(3,6) = 0.6–0.8 (H-3); 1 H, *J*(5,6) = 5.2–5.5, *J*(5,3) = 1.9–2.0 (H-5); 1 H, *J*(6,5) = 5.2–5.5, *J*(6,3) = 0.6–0.8 (H-6); [b] $-CH_3$; [c] $-OCH_3$.

TABLE IV
^{1}H NMR data of compounds **3a–3u**

Compound	Pyridine[a]			Benzene	$-NH_2$ (bs)	$-CH_2-$ (s)	Other
	H-3 (dd)	H-5 (dd)	H-6 (dd)				
3a	8.61	7.22	8.61	7.50–7.40 m, 2 H (H-2,H-6); 7.40–7.25 m, 3 H (H-3,H-4,H-5)	9.46, 7.70	4.29	
3b	8.64	7.20–7.30 m	8.28	7.45–7.55 m, 1 H (H-3); 7.35–7.45 m, 1 H (H-4); 7.20–7.30 m, 2 H (H-5,H-6)	9.47, 7.65	4.40	
3c	8.58	7.21	8.26	7.44 t, 1 H, J = 0.6 (H-2); 7.40–7.25 m, 3 H (H-4,H-5,H-6)	9.46, 7.21	4.25	
3d	8.58	7.20	8.26	7.30 d, 2 H, J = 8.5 (H-2,H-6); 7.38 d, 2 H, J = 8.5 (H-3,H-5)	9.46, 7.69	4.25	
3e	8.62	7.24	8.27	7.00–7.15 m, 2 H (H-3,H-5); 7.20–7.35 m, 1 H (H-4); 7.45 td, $J(1)$ = 8.0, $J(2)$ = 1.9 (H-6)	9.47, 7.71	4.32	
3f	8.59	7.21	8.26	7.35–7.15 m, 3 H (H-2,H-5,H-6); 7.00–6.90 m, 1 H (H-4)	9.45, 7.68	4.27	
3g	8.59	7.21	8.26	7.41 m, 2 H (H-2,H-6); 7.02 m, 2 H (H-3,H-5)	9.46, 7.67	4.26	
3h	8.59	7.21	8.27	7.61 t, 1 H, J = 1.6 (H-2); 7.25–7.15 m, 1 H (H-6); 7.45–7.35 m, 2 H (H-4,H-5)	9.45, 7.63	4.25	
3i	8.47	7.09	8.15	7.35 m (AA′BB′), 2 H (H-3,H-5); 7.21 m (AA′BB′), 2 H (H-2,H-6)	9.34, 7.54	4.13	
3j	8.61	7.22	8.23	7.25–7.30 m, 1 H (H-2); 7.20–7.25 m, 2 H (H-4,H-5); 7.05–7.15 m, 1 H (H-6)	9.46, 7.65	4.25	2.35 s[b]

TABLE IV
(*Continued*)

Compound	Pyridine[a]			Benzene	$-NH_2$ (bs)	$-CH_2-$ (s)	Other
	H-3 (dd)	H-5 (dd)	H-6 (dd)				
3k	8.61	7.22	8.25	7.32 m (AA′BB′), 2 H (H-2,H-6); 7.14 m (AA′BB′), 2 H (H-3,H-5)	9.46, 7.65	4.26	2.33 s[b]
3l	8.57	7.23	8.28	8.33 t, 1 H, *J*(2,4) = *J*(2,6) = 2.0 (H-2); 8.14 ddd, 1 H, *J*(4,5) = 8.0, *J*(4,2) = 2.0, *J*(4,6) = 0.7 (H-4); 7.53 t, 1 H, *J*(5,4) = *J*(5,6) = 8.0 (H-5); 7.81 ddd, 1 H, *J*(6,5) = 8.0, *J*(6,2) = 2.0, *J*(6,4) = 0.7 (H-6)	9.43, 7.65	4.38	
3m	8.57	7.21	8.27	8.20 m (AA′BB′), 2 H (H-2,H-6); 7.64 m (AA′BB′), 2 H (H-3,H-5)	9.44, 7.67	4.36	
3n	8.60	7.22	8.25	7.35 m (AA′BB′), 2 H (H-2,H-6); 6.87 m (AA′BB′), 2 H (H-3,H-5)	9.47, 7.69	4.25	3.79 s[c]
3o	8.45	7.08	8.16	7.44 d, 1 H, *J*(2,6) = 2.2 (H-2); 7.29 d, 1 H, *J*(5,6) = 8.2 (H-5); 7.18 dd, 1 H, *J*(6,5) = 8.2, *J*(6,2) = 2.2 (H-6)	9.34, 7.64	4.11	
3p	8.56	7.21	8.27	7.05–7.35 m, 3 H (H-2,H-5,H-6)	9.46, 7.72	4.24	
3r	8.66	7.35	8.32	7.10–6.95 m, 1 H (H-5); 7.25–7.20 m, 2 H (H-3,H-4)	9.49, 7.69	4.45, 4.44	
3s	8.61	7.32	8.32	7.86 dt, 1 H, *J*(3,4) = 8.0, *J*(3,5) = *J*(3,F) = 1.4 (H-3); 7.50–7.35 m, 2 H (H-4,H-5)	9.46, 7.68	4.67, 4.66	
3t	8.58	7.22	8.30	8.89 d, 1 H, *J*(3,5) = 2.4 (H-3); 8.42 dd, *J*(5,6) = 8.5, *J*(5,3) = 2.4 (H-5); 7.93 d, 1 H, *J*(6,5) = 8.5 (H-6)	9.43, 7.72	4.75	
3u	8.54	7.26	8.32	8.96 t, 1 H, *J*(4,2) = *J*(4,6) = 1.9 (H-4); 8.69 d, 2 H, *J*(2,6;4) = 1.9 (H-2,H-6)	9.40, 7.65	4.48	

[a] 1 H, *J*(3,5) = 1.9, *J*(3,6) = 0.6–0.7 (H-3); 1 H, *J*(5,6) = 5.2, *J*(5,3) = 1.9–2.0 (H-5); 1 H, *J*(6,5) = 5.2–5.5, *J*(6,3) = 0.6–0.7 (H-6); [b] $-CH_3$; [c] $-OCH_3$.

Antifungal Activity

The microdilution broth test was used for the assessment of the antifungal effect. All the tested substances were dissolved in dimethyl sulfoxide. A two-fold dilution range of the compounds was used so that the first concentration was 1 mmol/l provided a given compound was soluble in dimethyl sulfoxide and stable in the culture tissue medium RPMI 1640 (Sevac, Prague). The test medium was buffered to pH 7.0 with 0.165 M morpholine–4-propanesulfonic acid. Drug-free controls were included. The yeast inocula were prepared from 24–72 h colonies grown on Sabouraud agar at 37 °C. Conidia from 5–10 day colonies were used to obtain suspension in filamentous fungi. The cell density in sterile 0.85% saline was adjusted by means of a Bürker's chamber. Antifungal activity of the compounds *in vitro* was expressed as the minimum inhibitory concentration (MIC) which was read after 24 and 48 h of static incubation at 35 °C. In the case of *Trichophyton mentagrophytes*, the MICs were recorded after 72 and 120 h incubation.

Calculation

All calculations were carried out using the Multireg H program (Klemera) for Microsoft Excel. The values of the substituent constants (σ and π) were taken from the literature[7].

RESULTS AND DISCUSSION

The results of antimycobacterial activity are collected in Tables V and VI. In several cases, the minimum inhibitory concentration could not be determined due to the limited solubility of the compounds in the tested medium.

The prepared compounds, in particular pyridinecarbothioamides **3**, exhibit significant *in vitro* activity against all tested mycobacterial strains. The values of MICs are within the range of 4–125 μmol/l for **2** and 4–62 μmol/l for **3**. By comparing their MIC values with isoniazide (INH), the studied compounds reach the activity of INH against *M. tuberculosis* (MIC 4 μmol/l) and their activities against nontuberculosis mycobacteria exceed that of INH. Whereas nontuberculosis mycobacteria are moderately susceptible towards INH, the newly prepared compounds display practically the same activity against all the tested strains. Pyridinecarbonitriles **2** exhibit a better efficacy against both strains of *M. kansasii* than agaisnt *M. tuberculosis* and *M. avium*. The highest activity was observed with 3,5-dinitrobenzyl substituted derivative **2u** (MIC 4–8 μmol/l), which is comparable with the activity of the corresponding pyridinecarbothioamide **3u**. The lowest MICs were observed for pyridinecarbothioamides **3** towards all tested strains. Compounds **3u**, **3m**, **3d**, and **3h** are the most active compounds of the series.

The results of antifungal activity, summarized in Table VII, indicate that only five of the eight fungi strains are slightly susceptible to some of the

tested pyridinecarbonitriles **2**. The remaining compounds **2** and **3** were essentially inactive against all groups of the fungi tested.

For quantitative evaluation of the structure–antimycobacterial activity relationships, a three-parameter regression equation was employed. Similarly to the previous work[8], the Hammett constant σ of the substituents on the phenyl ring served as one parameter, the second parameter being the

TABLE V
In vitro antimycobacterial activity of compounds **2a**–**2u** expressed as MIC (μmol/l)

Compound	*M. tuberculosis* 331/81		*M. kansasii* 235/80		*M. kansasii* 509/96		*M. avium* 330/88	
	14 d	21 d	14 d	21 d	14 d	21 d	14 d	21 d
2a	125	125	31	62	62	62	125	125
2b	–	–	16	31	–	–	62	–
2c	62	62	8	16	31	31	31	62
2d	31	31	16	31	16	16	16	31
2e	125	125	16	31	62	62	125	125
2f	62	125	31	62	62	62	62	125
2g	62	125	16	31	31	62	31	62
2h	62	62	16	16	16	31	16	31
2i	31	31	–	–	16	16	–	–
2j	62	125	16	16	31	62	31	31
2k	62	62	16	62	31	62	31	62
2l	–	–	–	–	–	–	250	–
2m	–	–	31	–	31	62	–	–
2n	–	–	31	–	–	–	–	–
2o	62	–	8	16	16	16	31	–
2p	125	250	31	62	16	62	16	31
2r	–	–	16	–	–	–	–	–
2s	–	–	62	62	62	125	125	125
2t	62	62	16	31	16	16	31	31
2u	16	31	8	8	4	8	31	62
INH	4	4	500	500	500	500	500	500

substituent hydrophobic constants π. The third parameter was the indicator variable *I*, which acquired only two values, 1 in the case of pyridine-2-carbonitriles **2**, and 0 in the case of pyridine-2-carbothioamides **3** (see Eqs (*1*) and (*2*)). Equations (*1*) and (*2*) show the correlation for the measured values of antimycobacterial activity against *Mycobacterium tuberculosis* after 14 and 21 days of incubation, respectively.

TABLE VI
In vitro antimycobacterial activity of compounds **3a**–**3u** expressed as MIC (μmol/l)

Compound	*M. tuberculosis* 331/81		*M. kansasii* 235/80		*M. kansasii* 509/96		*M. avium* 330/88	
	14 d	21 d	14 d	21 d	14 d	21 d	14 d	21 d
3a	16	31	8	31	16	31	16	31
3c	8	8	4	8	8	8	8	31
3d	8	8	8	8	8	8	8	8
3e	4	16	16	31	62	–	16	–
3f	8	16	8	16	16	31	16	62
3g	8	8	16	16	8	16	16	31
3h	8	8	8	8	8	8	8	–
3i	31	31	31	–	16	16	8	–
3j	8	31	8	8	8	8	8	31
3k	16	31	16	31	16	16	16	31
3l	4	4	4	16	16	16	8	16
3m	4	4	8	–	4	4	4	8
3n	–	–	16	31	16	31	16	–
3o	8	16	8	8	8	8	4	16
3p	8	8	16	31	16	31	8	31
3r	8	31	16	–	16	–	–	–
3t	8	31	–	–	16	–	16	31
3u	4	8	4	8	4	4	8	31
INH	4	4	500	500	500	500	500	500

TABLE VII
In vitro antifungal activity of some compounds **2** expressed as MIC (μmol/l)

Compound	*Trichophyton mentagrophytes* 445		*Candida albicans* 44859		*Candida tropicalis* 156		*Aspergillus fumigatus* 231		*Absidia corymbifera* 272	
	72 h	120 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
2a	250	250	250	500	>500	>500	250	250	500	500
2c	62.5	62.5	500	>500	>500	>500	62.5	125	125	125
2f	125	125	250	>500	>500	>500	250	250	>500	>500
2g	125	125	125	500	250	>500	250	250	500	500
2h	62.5	62.5	125	>500	>500	>500	62.5	>500	62.5	>500
2j	125	125	125	>500	500	>500	500	500	500	500
2k	62.5	250	62.5	500	>500	>500	>500	>500	>500	>500
2s	250	500	250	>500	>500	>500	250	>500	250	500
2t	>500	>500	500	>500	62.5	62.5	>500	>500	>500	>500
Ketoconazole	0.98	1.95	<0.24	<0.24	1.95	3.91	7.81	7.81	31.25	31.25

$$\log \mathrm{MIC}_{M.t.14d} = -0.308(\pm 0.094)\sigma + 0.012(\pm 0.006)\pi + 0.801(\pm 0.080)I + 0.998(\pm 0.062)$$

$$R = 0.922 \; s = 0.20 \; F = 45.28 \; n = 28 \qquad (1)$$

$$\log \mathrm{MIC}_{M.t.21d} = -0.323(\pm 0.121)\sigma + 0.017(\pm 0.007)\pi + 0.734(\pm 0.104)I + 1.160(\pm 0.079)$$

$$R = 0.878 \; s = 0.26 \; F = 25.74 \; n = 27 \qquad (2)$$

From the calculated equations it can be concluded that the activity of the compounds under study increases with increasing electron-acceptor properties and decreasing lipophilicity of the substituents on the benzyl group. Influence of the electronic parameters is predominant. Thioamides **3** are usually more active than the corresponding nitriles **2**. Similar equations were calculated for other strains of *Mycobacterium* but the statistical significance was worse (but still on the level of significance 0.05).

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