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New pyridine derivatives as potential antimicrobial agents

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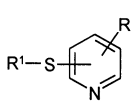
Abstract

A set of pyridine derivatives bearing a substituted alkylthio chain or a piperidyl ring in position 2 or 4 were synthesized, and their antimycobacterial and antifugal activities were evaluated. Chemical structures were confirmed by IR and NMR data, and by elemental analysis. Minimum inhibitory concentrations (MIC) were used for the evaluation of microbiological activity in vitro. The compounds were moderately active against both *Mycobacterium tuberculosis* and nontuberculous mycobacteria. The most active compound was 2-cyanomethylthiopyridine-4-carbonitrile (**7**) with MIC against *Mycobacterium kansasii* in the range of 8–4 μmol/l. The antifungal activities of the compounds were relatively low.

Keywords: Pyridine; Alkylthiopyridine; Piperidylpyridine; Antimycobacterial activity; Antifungal activity

1. Introduction

For several years, research in our laboratories has been directed towards the search for new compounds with antimycobacterial and antifungal activity. As a part of this programme, we have been investigating pyridine derivatives. Recently, we reported the synthesis and in vitro antimycobacterial and antifungal activities of various alkylthiopyridine derivatives bearing an alkylthio group in position 2 or 4 [1–4], as given by the general formula in Fig. 1. Pyridinecarbothioamides in particular showed significant activities. The most antimycobacterially active compounds were found in the



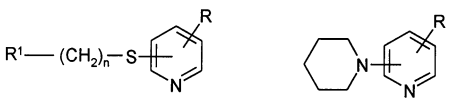
R = CN; $CSNH_2$

R^1 = alkyl C_1-C_{16}; cycloalkyl C_6; benzyl; subst. benzyl

Fig. 1.

series of 4-benzylthiopyridine-2-carbothioamides [3] with minimum inhibitory concentrations (MICs) of 4 μmol/l. As regards the spectrum of the antimycobacterial activity, they acted efficiently against both *Mycobacterium tuberculosis* and atypical mycobacterial strains. Significant antifungal activity against some yeasts and dermatophytes was observed only in the case of 2-alkylthiopyridine-4-carbothioamides [1], while the other derivatives displayed a weak or no activity at all.

In this work we decided to study the modulation of the alkylthio chain of pyridine carbothioamides and pyridine carbonitriles by substitution with the following groups: –OH, –CN, $–CSNH_2$, –COOR, $–CONHNH_2$. Another modification of the molecule was the replacement of the alkylthio chain both in pyridine carbonitriles and pyridine carbothioamides with the piperidine ring (Fig. 2).



R = CN; $CSNH_2$

R^1 = OH; CN; $CSNH_2$; $COOC_2H_5$; $CONHNH_2$. n = 1-3

Fig. 2.

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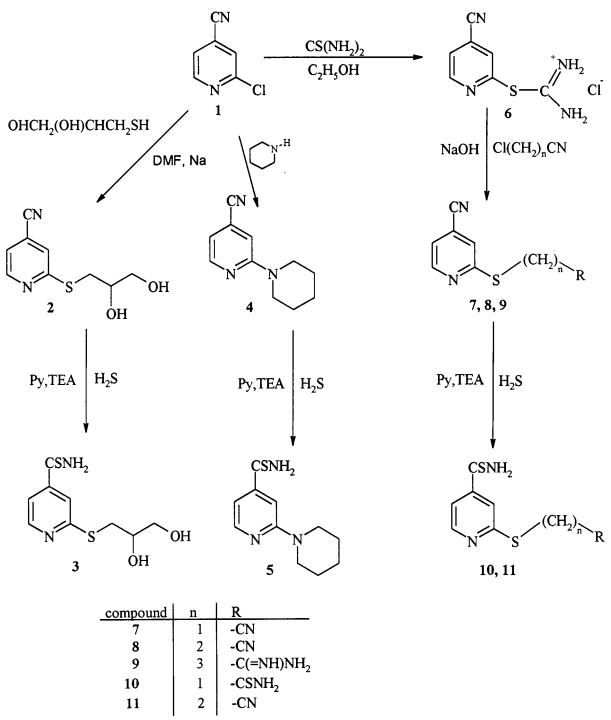
The target compounds were tested for their antimycobacterial and antifungal activity in vitro. MIC expressed in μmol/l was used for the evaluation of potential activity in vitro. Isoniazide and ketoconazole were employed as reference substances for antimycobacterial and antifungal activity, respectively.

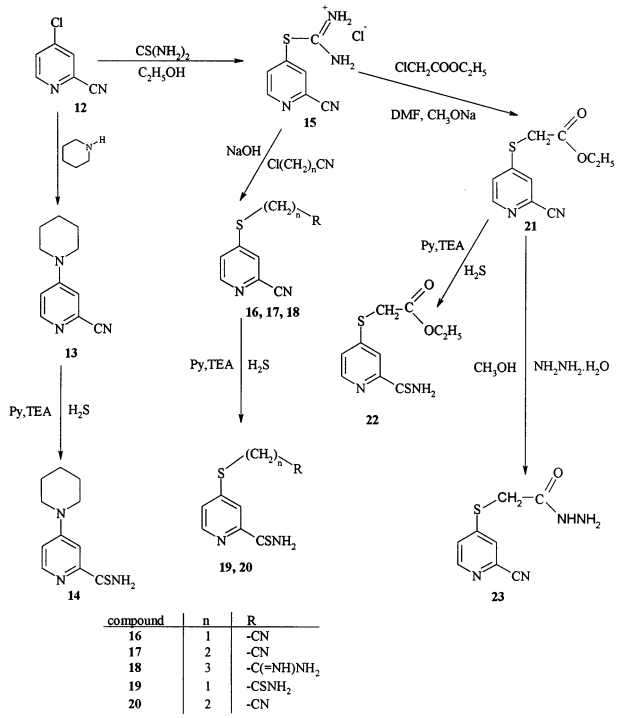
2. Chemistry

The synthetic pathways leading to the 2-alkylthio derivatives are depicted in Scheme 1. The chloro derivative **1**, that served as the starting material in the syntheses, was prepared according to a literature procedure [5]. Condensation of **1** with 3-mercaptopropan-1,2-diol in *N*,*N*-dimethylformamide (in the presence of sodium) afforded the hydroxyalkylthio derivative **2** which was further converted into the corresponding pyridinecarbothioamide **3** by the addition of hydrogen sulfide. The piperidyl derivative **4** was obtained by the treatment of **1** with piperidine, and then further converted into the corresponding pyridinecarbothioamide **5** by addition of hydrogen sulfide. The treatment of **1** with thiourea in ethanol afforded the thiuronium salt **6** in 80% yield, and the crude product was used in subsequent reactions. The reactions with chloroacetonitrile and 3-chloropropionitrile in NaOH solution gave the cyanoalkylthio derivatives **7** and **8**, respectively. The reaction of **6** with 4-chlorobutyronitrile under the same



compound	n	R
7	1	-CN
8	2	-CN
9	3	-C(=NH)NH$_2$
10	1	-CSNH$_2$
11	2	-CN

Scheme 1.



compound	n	R
16	1	-CN
17	2	-CN
18	3	-C(=NH)NH$_2$
19	1	-CSNH$_2$
20	2	-CN

Scheme 2.

conditions did not lead to the cyanoalkylthio derivative, but furnished the amidinoalkylthio derivative **9.** The structural assignment for this last compound is based on its ^{1}H NMR and ^{13}C NMR spectra. The spectra show a disubstituted linear chain –CH_2–CH_2–CH_2, two non-equivalent NH protons, a nitrile group, and a thioguanidin carbon. Compounds **7** and **8** were treated with hydrogen sulfide in pyridine, giving rise to the corresponding pyridinecarbothioamides **10** and **11**. The cyano group in the cyanomethylthio chain of **7** was transformed into the thiocarbamoyl group (**10**), whereas the cyanoethylthio chain of **8** remained intact (**11**).

Chloropyridine **12** prepared from 2-methylpyridine by an established method [6,7] was employed as the starting material for the synthesis of 4-alkylthio derivatives (Scheme 2). The piperidyl derivatives **13** and **14** were obtained via the same synthetic pathways as the 2-piperidyl derivatives **4** and **5**. The other 4-alkylthio derivatives were prepared from the thiuronium salt **15** obtained upon treatment of **12** with thiourea in ethanol. The reactions of **15** with chloroacetonitrile, 3-chloropropionitrile, and 4-chlorobutyronitrile in a NaOH solution gave analogous compounds as in the case of the 2-alkylthio derivatives described above. The cyanoalkylthio derivatives **16** and **17** were prepared by the reactions of **15** with chloroacetonitrile and 3-chloropropionitrile, respectively. They were subsequently subjected to treatment with hydrogen sulfide to afford the

pyridinecarbothioamides **19** and **20**. The reaction of **15** with 4-chlorobutyronitrile in NaOH solution led to the amidinoalkylthio derivative **18**. The condensation of **15** with ethyl chloroacetate in *N*,*N*-dimethylformamide in the presence of sodium methoxide produced ethoxycarbonylmethylthio derivative **21**, which was easily converted into the related pyridinecarbothioamide **22** by addition of hydrogen sulfide to the carbonitrile group of the pyridine ring. The ester function on the alkyl chain of **21** was converted into the hydrazide group by the reaction with hydrazine hydrate (**23**).

The structures of all compounds were confirmed by elemental analyses, IR and ^{1}H NMR spectra.

3. Experimental

3.1. Chemistry

The melting points were determined on a Kofler apparatus and are uncorrected. Analytical samples were dried over P_2O_5 at 60°C and 30 Pa for 8–10 h. Elemental analyses were performed on CHNS–O CE instrument (FISONS EA 1110). The results were within $\pm$0.4% of the calculated values. IR spectra were obtained on a Nicolet Impact 400 spectrometer in KBr pellets. The ^{1}H NMR and ^{13}C NMR spectra were recorded for $CDCl_3$ or d_6-DMSO solutions at ambient temperature on a Varian Mercury-VX BB 300 spectrometer operating at 300 MHz. Chemical shifts were recorded as δ values in parts per million (ppm), and were indirectly referenced to tetramethylsilane via the solvent signal (7.26 for ^{1}H). Multiplicities are given together with the coupling constant(s) (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 UV_{254}, Kavalier, Votice, Czech Republic) in ethyl acetate–petroleum ether (2:3) using UV detection.

3.1.1. 2-(2,3-Dihydroxypropylthio)pyridine-4-carbonitrile (2)

Sodium (0.4 g, 18 mmol) was added at room temperature to a stirred solution of 3-mercaptopropan-1,2-diol (1.5 ml, 18 mmol) in dry DMF (8 ml), followed by **1** (2.5 g, 18 mmol). After heating for 3 h at 50°C, DMF was evaporated in vacuo and the residue was diluted with water. The solution was acidified with 1 M H_2SO_4 to pH 6, and extracted with ether. The organic layer was dried (Na_2SO_4) and the solvent was evaporated to dryness under reduced pressure. The crude product was recrystallized from ethanol (2.9 g, 76% yield), m.p. 70–73°C; IR (KBr, cm^{-1}) 2238 (C≡N), 3416 (O–H), 1070 and 1120 (C–O). ^{1}H NMR^{300} ($CDCl_3$, ppm) δ 8.52 (dd, $J(6,5) = 5.2$ Hz, $J(6,3) = 1.1$ Hz, 1H, H6), 7.50 (dd, $J(3,5) = 1.7$ Hz, $J(3,6) = 1.1$ Hz 1H, H3), 7.24 (dd, $J(5.6) = 5.2$ Hz, $J(5.3) = 1.7$ Hz 1H, H5), 4.10–3.85 (m, 1H, H2-alkyl), 3.69 (dd overlapped, $J(3a,3b) = 11.5$ Hz, $J(3a,2) = 4.2$ Hz, 1H, H3a-alkyl), 3.64 (dd overlapped, $J(3b,3a) = 11.5$ Hz, $J(3b,2) = 4.9$ Hz, 1H, H3b-alkyl), 3.41 (dd, $J(1a,1b) = 14.8$ Hz, $J(1a,2) = 5.1$ Hz, 1H, H1a-alkyl), 3.33 (dd, $J(1b,1a) = 14.8$ Hz, $J(1b,2) = 6.3$ Hz, 1H, H1b-alkyl), 2.87 (bs, $\sim$1H, OH). *Anal.* Calc. $C_9H_{10}N_2O_2S$ (C,H,N,S).

3.1.2. General procedure for the preparation of the pyridinecarbothioamides (3, 5, 10, 11, 14, 19, 20, 22)

Dry triethylamine (0.7 ml) was added to the solution of the appropriate pyridinecarbonitrile (**2, 4, 7, 8, 13, 16, 17, 21**) (1 mmol) in dry pyridine (7 ml), and dry hydrogen sulfide was passed through the mixture at 40–50°C for 2–6 h. After cooling, the mixture was diluted with water (75–100 ml), the precipitated solid was filtered off, and crystallized from ethanol.

3.1.2.1. 2-(2,3-Dihydroxypropylthio)pyridine-4-carbothioamide (3). Yield 82%, m.p. 102–105°C; IR (KBr, cm^{-1}) 3166 and 1652 (N–H), 1432 (C=S), 3417 (O–H), 1037 and 1168 (C–O). ^{1}H NMR^{300} (d_6-DMSO, ppm) δ 8.45 (bd, $J(6,5) = 5.2$ Hz, 1H, H6), 7.52 (bs, 1H, H3), 7.38 (dd, $J(5.6) = 5.2$ Hz, $J(5.3) = 1.7$ Hz 1H, H5), 3.75–3.60 (m, 1H, H2-alkyl), 3.45–3.30 (m, 3H, H3a, 3b, 1a-alkyl), 3.11 (dd, $J(1b,1a) = 13.5$ Hz, $J(1b,2) = 7.2$ Hz, 1H, H1b-alkyl). *Anal.* Calc. $C_9H_{12}N_2O_2S_2$ (C,H,N,S).

3.1.2.2. 2-(1-Piperidyl)pyridine-4-carbothioamide (5). Yield 86%, m.p. 161–163°C; IR (KBr, cm^{-1}) 3274 and 1603 (N–H), 1458 (C=S), 2944 and 2852 (CH), 1241 (C–N). ^{1}H NMR^{300} ($CDCl_3$, ppm) δ 8.19 (dd, $J(6,5) = 5.2$ Hz, $J(6,3) = 0.8$ Hz, 1H, H6), 7.67 (bs, $\sim$1H, $CSNH_2$), 7.09 (dd, $J(3,5) = 1.7$ Hz, $J(3,6) = 0.8$ Hz, 1H, H3), 6.72 (dd, $J(5,6) = 5.2$ Hz, $J(5,3) = 1.7$ Hz, 1H, H5), 3.65–3.50 (m, 4H, H2,6-piperidine), 1.75–1.50 (m, 6H, H3,4,5-piperidine). *Anal.* Calc. $C_{11}H_{15}N_3S$ (C,H,N,S).

3.1.2.3. 2-Thiocarbamoylmethylthiopyridine-4-carbothioamide (10). Yield 90%, m.p. 135–138°C; IR (KBr, cm^{-1}) 3143 and 1615 (N–H), 1433 (C=S). ^{1}H NMR^{300} (d_6-DMSO, ppm) δ 8.46 (dd, $J(6,5) = 5.2$ Hz, $J(6,3) = 0.7$ Hz, 1H, H6), 7.58 (dd, $J(3,5) = 1.7$ Hz, $J(3,6) = 0.7$ Hz, 1H, H3), 7.41 (dd, $J(5,6) = 5.2$ Hz, $J(5,3) = 1.7$ Hz, 1H, H5), 4.28 (s, 2H, SCH_2). *Anal.* Calc. $C_8H_9N_3S_3$ (C,H,N,S).

3.1.2.4. 2-(2-Cyanoethylthio)pyridine-4-carbothioamide (11). Yield 83%, m.p. 110–113°C; IR (KBr, cm^{-1}) 3297 and 1637 (N–H), 2258 (C=N), 1400 (C=S). ^{1}H

NMR^{300} ($CDCl_3$, ppm) δ 8.48 (dd, $J(6,5) = 5.2$ Hz, $J(6,3) = 1.0$ Hz, 1H, H6), 7.68 (bs, ~1H, $CSNH_2$), 7.52 (dd, $J(3,5) = 1.7$ Hz, $J(3,6) = 1.0$ Hz, 1H, H3), 7.36 (dd, $J(5,6) = 5.2$ Hz, $J(5,3) = 1.7$ Hz, 1H, H5), 3.45 (t, $J = 7.1$ Hz, 2H, SCH_2), 2.86 (t, $J = 1.7$ Hz, 2H, CH_2CN). *Anal.* Calc. $C_9H_9N_3S_2$ (C,H,N,S).

3.1.2.5. 4-(1-Piperidyl)pyridine-2-carbothioamide ***(14)***. Yield 71%, m.p. 160–162°C; IR (KBr, cm^{-1}) 3305 and 1596 (N–H), 1451 (C=S), 2940 and 2850 (CH), 1235 (C–N). ^{1}H NMR^{300} ($CDCl_3$, ppm) δ 9.62 (bs, 1H, $CSNH_2$), 8.14 (d, $J(3,5) = 2.7$ Hz, 1H, H3), 8.11 (d, $J(6,5) = 6.0$ Hz, 1H, H6), 7.33 (bs, ~1H, $CSNH_2$), 6.70 (dd, $J(5,6) = 6.0$ Hz, $J(5,3) = 2.7$ Hz, 1H, H5), 3.50–3.30 (m, 4H, H2,6-piperidine), 1.75–1.55 (m, 6H, H3,4,5-piperidine). *Anal.* Calc. $C_{11}H_{15}N_3S$ (C,H,N,S).

3.1.2.6. 4-Thiocarbamoylmethylthiopyridine-2-carbothioamide ***(19)***. Yield 93%, m.p. 176–179°C; IR (KBr, cm^{-1}) 3174 and 1613 (N–H), 1432 (C=S). ^{1}H NMR^{300} (d_6-DMSO, ppm) δ 8.40 (dd, $J(6,5) = 5.2$ Hz, $J(6,3) =$ 0.6 Hz, 1H, H6), 8.33 (bd, $J(3,5) = 1.9$ Hz, 1H, H3), 7.49 (dd, $J(5,6) = 5.2$ Hz, $J(5,3) = 1.9$ Hz, 1H, H5), 4.24 (s, 2H, SCH_2). *Anal.* Calc. $C_8H_9N_3S_3$ (C,H,N,S).

3.1.2.7. 4-(2-Cyanoethylthio)pyridine-2-carbothioamide ***(20)***. Yield 92%, m.p. 110–113°C; IR (KBr, cm^{-1}) 3243 and 1597 (N–H), 2248 (C≡N), 1429 (C=S). ^{1}H NMR^{300} ($CDCl_3$, ppm) δ 9.64 (bs, ~1H, $CSNH_2$), 8.55 (dd, $J(3,5) = 1.9$ Hz, $J(3,6) = 0.8$ Hz, 1H, H3), 8.36 (dd, $J(6,5) = 5.2$ Hz, $J(6,3) = 0.8$ Hz 1H, H6), 7.68 (bs, ~1H, $CSNH_2$), 7.30 (dd, $J(5,6) = 5.2$ Hz, $J(5,3) = 1.9$ Hz, 1H, H5), 3.36 (t, $J = 7.1$ Hz, 2H, SCH_2), 2.80 (t, $J = 7.1$ Hz, 2H, CH_2CN). *Anal.* Calc. $C_9H_9N_3S_2$ (C,H,N,S).

3.1.2.8. Ethyl-(2-thiocarbamoylpyridine-4-ylthio)acetate ***(22)***. Hydrogen sulfide was passed through at room temperature. 83% yield, m.p. 123–124°C; IR (KBr, cm^{-1}) 3282 and 1571 (N–H), 1428 (C=S), 1721 (C=O). ^{1}H NMR^{300} (d_6-DMSO, ppm) δ 8.39 (dd, $J(6,5) = 5.2$ Hz, $J(6,3) = 0.6$ Hz, 1H, H6), 8.30 (dd, $J(3,5) = 1.9$ Hz, $J(3,6) = 0.6$ Hz 1H, H3), 7.45 (dd, $J(5.6) = 5.2$ Hz, $J(5.3) = 1.9$ Hz 1H, H5), 4.12 (q overlapped, $J_1 = 7.1$ Hz, $J_2 = 14.0$ Hz, 2H, OCH_2), 4.11 (s overlapped, 2H, SCH_2), 1.17 (t, $J = 7.1$ Hz, 3H, CH_3). *Anal.* Calc. $C_{10}H_{12}N_2O_2S_2$ (C,H,N,S).

3.1.3. General procedure for the preparation of the piperidyl derivatives of the pyridinecarbonitriles ***(4, 13)***

A mixture of chloropyridinecarbonitrile (**1, 12**) (0.7 g, 5 mmol) and piperidine (1.25 ml, 15 mmol) was heated under reflux for 1 h. The resultant solid was then collected and recrystallized from ethanol.

3.1.3.1. 2-(1-Piperidyl)pyridine-4-carbonitrile ***(4)***. Yield 74%, m.p. 53.5–55°C; IR (KBr, cm^{-1}) 2234 (C≡N), 2943 and 2853(CH), 1254 (C–N). ^{1}H NMR^{300} ($CDCl_3$, ppm) δ 8.24 (dd, $J(6,5) = 5.0$ Hz, $J(6,3) = 1.0$ Hz, 1H, H6), 6.79 (bt, $J(3,6) = J(3,5) = 1.0$ Hz, 1H, H3), 6.67 (dd, $J(5,6) = 5.0$ Hz, $J(5,3) = 1.0$ Hz, 1H, H5), 3.60–3.50 (m, 4H, H2,6-piperidine), 1.75–1.55 (m, 6H, H3,4,5-piperidine). *Anal.* Calc. $C_{11}H_{13}N_3$ (C,H,N,S).

3.1.3.2. 4-(1-Piperidyl)pyridine-2-carbonitrile ***(13)***. Yield 63%, m.p. 84–85.5°C; IR (KBr, cm^{-1}) 2234 (C≡N), 2918 and 2854 (CH), 1267 (C–N). ^{1}H NMR^{300} ($CDCl_3$, ppm) δ 8.22 (bd, $J(6,5) = 6.0$ Hz, 1H, H6), 7.00 (bd, $J(3,5) = 2.8$ Hz, 1H, H3), 6.73(dd, $J(5,6) = 6.0$ Hz, $J(5,3) = 2.8$ Hz, 1H, H5), 3.40–3.30 (m, 4H, H2,6-piperidine), 1.75–1.55 (m, 6H, H3,4,5-piperidine). *Anal.* Calc. $C_{11}H_{13}N_3$ (C,H,N,S).

3.1.4. General procedure for the preparation of the thiuronium salts ***(6, 15)***

Chloropyridinecarbonitrile (**1, 12**) (3.5 g, 25 mmol) and thiourea (2.0 g, 26 mmol) were dissolved in ethanol (20 ml), and the solution was heated at 100°C for 15 min. After cooling, the resultant salt was filtered off.

3.1.4.1. 4-Cyanopyridine-2-thiuronium salt ***(6)***. Yield 75%, m.p. 190–203°C.

3.1.4.2. 2-Cyanopyridine-4-thiuronium salt ***(15)***. Yield 71%, m.p. 193–196°C.

3.1.5. General procedure for the preparation of the cyanoalkylthio pyridinecarbonitriles ***(7, 8, 16, 17)***

Sodium hydroxide (0.75 g, 18 mmol) followed by chloroacetonitrile (0.5 ml, 9 mmol) or 3-chloropropionitrile (0.7 ml, 9 mmol) were added to a suspension of the appropriate thiuronium salt (**1**, **15**) (2 g, 9 mmol) in hot water (50 ml). The solution was heated for 5 min (**7** and **16**) or 0.5 h (**8** and **17**), the resultant solid was collected, and recrystallized from ethanol.

3.1.5.1. 2-Cyanomethylthiopyridine-4-carbonitrile ***(7)***. Yield 80%, m.p. 94–96°C; IR (KBr, cm^{-1}) 2241 and 2248(C≡N). ^{1}H NMR^{300} ($CDCl_3$, ppm) δ 8.68 (dd, $J(6,5) = 5.2$ Hz, $J(6,3) = 1.1$ Hz, 1H, H6), 7.47 (t, $J(3,6) = J(3,5) = 1.1$ Hz, 1H, H3), 7.31 (dd, $J(5,6) = 5.2$ Hz, $J(5,3) = 1.1$ Hz, 1H, H5), 4.02 (s, 2H, SCH_2). *Anal.* Calc. $C_8H_5N_3S$ (C,H,N,S).

3.1.5.2. 2-(2-Cyanoethylthio)pyridine-4-carbonitrile ***(8)***. Yield 51%, m.p. 98–101°C; IR (KBr, cm^{-1}) 2239 and 2253(C≡N). ^{1}H NMR^{300} ($CDCl_3$, ppm) δ 8.58 (dd, $J(6,5) = 5.0$ Hz, $J(6,3) = 1.2$ Hz, 1H, H6), 7.42 (t, $J(3,6) = J(3,5) = 1.2$ Hz, 1H, H3), 7.23 (dd, $J(5,6) = 5.0$ Hz, $J(5,3) = 1.2$ Hz, 1H, H5), 3.45 (t, $J = 7.0$ Hz, 2H, SCH_2), 2.85 (t, $J = 7.0$ Hz, 2H, CH_2CN). *Anal.* Calc. $C_9H_7N_3S$ (C,H,N,S).

*3.1.5.3. 4-Cyanomethylthiopyridine-2-carbonitrile (**16**).* Yield 86%, m.p. 130–132°C; IR (KBr, cm^{-1}) 2245 (C≡N). ^1H NMR[300] ($CDCl_3$, ppm) δ 8.64 (dd, $J(6,5) = 5.2$ Hz, $J(6,3) = 0.7$ Hz, 1H, H6), 7.60 (dd, $J(3,5) = 1.9$ Hz, $J(3,6) = 0.7$ Hz, 1H, H3), 7.43 (dd, $J(5,6) = 5.2$ Hz, $J(5,3) = 1.9$ Hz, 1H, H5), 3.81 (s, 2H, SCH_2). *Anal.* Calc. $C_8H_5N_3S$ (C,H,N,S).

*3.1.5.4. 4-(2-Cyanoethylthio)pyridine-2-carbonitrile (**17**).* Yield 62%, m.p. 95–98°C; IR (KBr, cm^{-1}) 2233 and 2243(C≡N). ^1H NMR[300] ($CDCl_3$, ppm) δ 8.54 (dd, $J(6,5) = 5.4$ Hz, $J(6,3) = 0.6$ Hz, 1H, H6), 7.51 (t, $J(3,5) = 1.9$ Hz, $J(3,6) = 0.6$ Hz, 1H, H3), 7.33 (dd, $J(5,6) = 5.4$ Hz, $J(5,3) = 1.9$ Hz, 1H, H5), 3.33 (t, $J = 7.1$ Hz, 2H, SCH_2), 2.79 (t, $J = 7.1$ Hz, 2H, CH_2CN). *Anal.* Calc. $C_9H_7N_3S$ (C,H,N,S).

*3.1.6. General procedure for the preparation of the butanamidine derivatives (**9, 18**)*

Sodium hydroxide (0.5 g, 12 mmol) followed by 4-chlorobutyronitrile (0.5 ml, 6 mmol) were added to a suspension of the appropriate thiuronium salt (**1**, **15**) (1.3 g, 6 mmol) in hot water (70 ml), and the solution was heated at reflux for 3 h. After cooling, the precipitate was collected, and recrystallized from ethanol.

*3.1.6.1. 4-(4-cyanopyridine-2-ylthio)butanamidine (**9**).* Yield 23%, m.p. 103–106°C; IR (KBr, cm^{-1}) 2250 (C≡N), 3378 and 1657 (N–H), 1697 (C=N). ^1H NMR[300] (d_6-DMSO, ppm) δ 8.55 (dd, $J(6,5) = 5.2$ Hz, $J(6,3) = 0.5$ Hz, 1H, H6), 8.20 (bs, ~1H, NH), 7.71 (bs, ~1H, NH), 7.65 (dd, $J(3,5) = 1.5$ Hz, $J(3,6) = 0.5$ Hz, 1H, H3), 7.48 (dd, $J(5,6) = 5.2$ Hz, $J(5,3) = 1.5$ Hz, 1H, H5), 3.24 (t, $J = 7.1$ Hz, 2H, SCH_2), 2.62 (t, $J = 7.1$ Hz, 2H, CH_2), 1.96 (p, $J = 7.1$ Hz, 2H, CH_2CN). ^{13}C NMR (d_6-DMSO, ppm) δ 165.97, 158.67, 150.22, 142.06, 120.22, 119.74, 117.83, 28.28, 25.10, 15.66. *Anal.* Calc. $C_{10}H_{12}N_4S$ (C,H,N,S).

*3.1.6.2. 4-(2-cyanopyridine-4-ylthio)butanamidine (**18**).* Yield 30%, m.p. 99–102°C; IR (KBr, cm^{-1}) 2245 (C≡N), 3328 (N–H), 1694 (C=N). ^1H NMR[300] ($CDCl_3$, ppm) δ 8.37 (dd, $J(6,5) = 5.3$ Hz, $J(6,3) = 0.6$ Hz, 1H, H6), 8.03 (dd, $J(3,5) = 2.1$ Hz, $J(3,6) = 0.6$ Hz, 1H, H3), 7.85 (bs, ~1H, NH), 7.27 (dd, $J(5,6) = 5.3$ Hz, $J(5,3) = 2.1$ Hz, 1H, H5), 6.14 (bs, ~1H, NH), 3.20 (t, $J = 7.1$ Hz, 2H, SCH_2), 2.57 (t, $J = 7.1$ Hz, 2H, CH_2), 2.10 (p, $J = 7.1$ Hz, 2H, CH_2CN). ^{13}C NMR ($CDCl_3$, ppm) δ 166.40, 149.71, 149.47, 147.93, 123.35, 118.94, 118.49, 29.08, 24.20, 16.09. *Anal.* Calc. $C_{10}H_{12}N_4S$ (C,H,N,S).

*3.1.7. Ethyl-(2-cyanopyridine-4-ylthio)acetate (**21**)*

A solution of sodium (0.3 g, 15 mmol) in dry methanol (5 ml) followed by ethyl chloroacetate (1.2 ml, 12 mmol) were added at room temperature to a stirred solution of **15** (2.5 g, 12 mmol) in dry DMF (8 ml). After stirring at room temperature for 2 h, the solvent was evaporated in vacuo, and the residue was diluted with water (50 ml). The precipitated solid was filtered off, washed with cold water, and crystallized from ethanol (1.1 g, 42% yield), m.p. 45–47°C; IR (KBr, cm^{-1}) 2240 (C≡N), 1727 (C=O). ^1H NMR[300] ($CDCl_3$, ppm) δ 8.49 (dd, $J(6,5) = 5.4$ Hz, $J(6,3) = 0.8$ Hz, 1H, H6), 7.55 (dd, $J(3,5) = 1.9$ Hz, $J(3,6) = 0.8$ Hz, 1H, H3), 7.37 (dd, $J(5,6) = 5.4$ Hz, $J(5,3) = 1.9$ Hz, 1H, H5), 4.24 (q, $J_1 = 7.1$ Hz, $J_2 = 14.3$ Hz, 2H, OCH_2), 3.76 (s, 2H, SCH_2), 1.28 (t, $J = 7.1$ Hz, 3H, CH_3). *Anal.* Calc. $C_{10}H_{10}N_2O_2S$ (C,H,N,S).

*3.1.8. (2-Cyanopyridine-4-ylthio)acethydrazide (**23**)*

Some 30% hydrazine hydrate (7.5 ml) was added to a solution of **21** (1 g, 4.5 mmol) in dry ethanol, and the reaction mixture was maintained at room temperature for 3 h. The solution was evaporated to dryness, and the resultant solid was recrystallized from ethanol (0.65 g, 68% yield), m.p. 154–157°C; IR (KBr, cm^{-1}) 2240 (C≡N), 1651 (C=O). ^1H NMR[300] (d_6-DMSO, ppm) δ 8.49 (dd, $J(6,5) = 5.5$ Hz, $J(6,3) = 0.6$ Hz, 1H, H6), 7.99 (dd, $J(3,5) = 1.9$ Hz, $J(3,6) = 0.6$ Hz, 1H, H3), 7.61 (dd, $J(5,6) = 5.5$ Hz, $J(5,3) = 1.9$ Hz, 1H, H5), 3.81 (s, 2H, SCH_2). *Anal.* Calc. $C_8H_9N_4OS$ (C,H,N,S).

3.2. Microbiology

3.2.1. Antimycobacterial activity

For the evaluation of the antimycobacterial activity of the substances in vitro, the following strains were used: *M. tuberculosis* CNCTC My 331/88, *M. 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 of *M. kansasii* 6 509/96. The antimycobacterial activities of the compounds against these strains were determined in the Šula's semisynthetic medium (SEVAC, Prague). The compounds were added to the medium in dimethylsulfoxide solutions. The following concentrations were used: 1000, 500, 250, 125, 62, 31, 16, 8, and 4 μmol/l. MICs 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.

3.2.2. Antifungal activity

In vitro antifungal activities 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 were determined using the microdilution broth test. All strains,

except of *C. albicans*, were clinical isolates, identified by conventional morphological and biochemical methods. All substances were dissolved in dimethylsulfoxide. A two-fold dilution range of the solutions was used with the first concentration being 1 mmol/l provided that a given compound was soluble in dimethylsulfoxide 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- to 10-day colonies were used to obtain suspension in filamentous fungi. The cell density in sterile 0.85% saline was adjusted by means of the Bürker's chamber. Antifungal activity of the compounds in vitro was expressed as MIC which was determined 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.

4. Results and discussion

The antimycobacterial activities of the compounds compared to isoniazide (INH) are shown in Table 1. MIC values fall within the range of 1000–4 μmol/l. As compared to isoniazide, the compounds did not match the activity of INH against *M. tuberculosis*, but some of them displayed remarkable activity against nontuberculous mycobacteria. The most active derivative was 2-cyanomethylthiopyridine-4-carbonitrile (**7**) being highly efficient against *M. kansasii* (MIC in the range of 8–4 μmol/l). By comparing of MIC values, **7** turned out to be six to seven times more potent than the standard. It appears that the antimycobacterial activities of these compounds are related to the presence of the 2-cyanomethylthio moiety. This is in agreement with our previous hypothesis [8] that the alkylthio group bound to an electron-deficient carbon is the pharmacophore of antimycobacterial activity. Prolonging of the alkyl chain (**8**) caused a substantial decrease of activity. The 4-cyanoalkylthio isomers (**16, 17**) did not turn out to be particularly active either. Moderately active compounds (**10, 11, 19, 20**) with MIC values in the range of 31–250 μmol/l were obtained upon replacing the nitrile group on the pyridine ring with the thiocarbamoyl group, but all of them turned out to be more potent against nontuberculous mycobacteria than isoniazide. The other variations of the alkylthio chain as well as its replacement with the piperidine ring led to weakly active or inactive compounds.

As far as the antifungal activity is concerned, some of the compounds were found to be slightly active against the tested strains. Only *T. mentagrophytes*, *C. albicans*, *A. fumigatus* and *A. corymbifera* are generally susceptible to the new pyridine derivatives, which exhibited activity within the range of 125–1000 μmol/l. The rest of the fungi strains were not susceptible up to the concentration of 1000 μmol/l against all the compounds. Most of the moderately active compounds possess the thioamide group which we regard as a

Table 1
Antimycobacterial in vitro activity expressed as MIC (μmol/l)

Comp.	*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
2	1000	100	1000	1000	250	500	1000	250
3	250	500	500	500	250	500	–	250
4	500	500	500	500	250	250	500	500
5	500	500	500	500	250	500	500	500
7	31	31	4	4	8	8	62	125
8	250	250	500	500	500	500	500	1000
9	>1000	>1000	500	500	500	500	>1000	>1000
10	125	125	250	250	62	125	62	125
11	31	31	62	125	125	125	125	125
13	1000	1000	500	1000	500	500	1000	1000
14	500	500	125	250	250	500	125	250
16	500	500	500	500	500	500	500	1000
17	125	250	500	500	250	250	250	500
18	500	1000	500	1000	500	500	1000	>1000
19	62	62	31	31	31	62	62	62
20	250	250	62	125	125	125	250	250
21	500	1000	500	1000	500	500	1000	1000
22	500	1000	125	500	250	500	250	500
23	500	1000	500	500	500	500	250	500
INH	4	4	500	500	500	500	500	500

Table 2
Antifungal in vitro activity expressed as MIC (μmol/l)

Comp.	*Trichophyton mentagrophytes* 445		*Candida albicans* 44859		*Aspergillus fumigatus* 231		*Absidia corymbifera* 272	
	72 h	120 h	24 h	48 h	24 h	48 h	24 h	48 h
10	250	250	1000	1000	>1000	>1000	>1000	>1000
14	125	125	500	>1000	250	250	500	500
19	250	250	500	1000	>1000	>1000	>1000	>1000
Ket [a]	0.98	1.95	<0.24	<0.24	1.95	3.91	7.81	7.81

[a] Ketoconazole.

fundamental condition for the antifungal activity in compounds studied by us [1,4]. The most susceptible strain was *T. mentagrophytes*, and the most antifungally active compound turned out to be 4-(1-piperidyl)pyridine-2-carbothioamide (**14**). The results of antifungal activity of selected compounds are summarized in Table 2.

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