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Synthesis and Antimycobacterial Activity of Pyridylmethylsulfanyl and Naphthylmethylsulfanyl Derivatives of Benzazoles, 1,2,4-Triazole, and Pyridine-2-carbothioamide/-2-carbonitrile

A set of four types of benzazoles, 1,2,4-triazole, and pyridine-2-carbonitrile/-2-carbothioamide substituted with 1-naphthylmethylsulfanyl or pyridylmethylsulfanyl was prepared to modify the structure of benzylsulfanyl derivatives of the above-mentioned heterocycles. The compounds were evaluated for *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis, M. avium*, and two strains of *M. kansasii*. The activities were expressed as the minimum inhibitory concentration (MIC). The MIC values fall into a range of 2 to >1000 μmol/L. Introduction of a pyridyl moiety into the molecule mostly decreased the activity. A naphthyl moiety did not influence the activity in comparison with a phenyl. The most active substances were 4-(3-pyridylmethylsulfanyl)pyridine-2-carbothioamide (**7b**) (MIC = 2 − >62.5 μmol/L) and 4-(1-naphthylmethylsulfanyl)pyridine-2-carbothioamide (**7d**) (MIC = 2 − >32 μmol/L).

Keywords: 2-Arylmethylsulfanylbenzazoles; 3-Arylmethylsulfanyl-1,2,4-triazoles; 4-Arylmethylsulfanylpyridines; Antimycobacterial activity

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Introduction

Worldwide, tuberculosis (TB) still remains a major public health problem [1]. Although we have highly effective drugs and proven regimens, the treatment of TB is not satisfactory in some features. There are many voices calling for accelerating discovery and/or development of cost-effective new TB drugs that will shorten the duration of TB treatment or otherwise simplify its completion, provide more effective treatment for drug-resistant tuberculosis and/or improve the treatment of latent TB infection [2].

The research of potential antimycobacterial compounds revealed a lot of pharmacophores that are responsible for the mentioned activity. The alkylsulfanyl group can be counted as one of them [3, 4]. There are many different heterocycles bearing an alkylsufanyl group, prepared as potential antimycobacterial active compounds [5]. Recently, 6-mercaptopurine derivatives were reported as highly active agents [6, 7].

We have synthesized and tested derivatives of pyridine [8], benzimidazole [9], 5-methylbenzimidazole [10], benzoxazole, benzothiazole [11], and triazole [12]. The studies were dealing especially with their benzylsulfanyl derivatives with different simple mono- and di-substitutions on the benzyl moiety. In all series of heterocycles, we observed the same relationship between the activity and the type of the benzyl substituent; namely, the activity increases with electron-withdrawing substituents (CF_3, NO_2 groups). Lipophilicity of the molecule plays some role in the antimycobacterial activity, especially as regards the efficacy against non-tuberculous mycobacteria [13].

On the basis of these findings, we tried to enhance the activity of the compounds under study by replacing the benzyl moiety with a pyridylmethyl and with a naphthylmethyl core.

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Results and discussion

Chemistry

The types of compounds under study and their preparation are outlined in Table 1 and Scheme 1. The syntheses were based on nucleophilic substitution of the chlorine atom in the alkyl halide with a chosen thiolate. The following types of heterocycles served as thiolate sources: benzimidazole-2-thiol (**1**), 5-methylbenzimidazole-2-thiol (**2**), benzoxazole-2-thiol (**3**),

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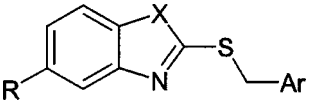
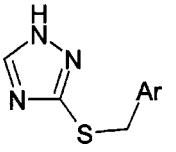
Table 1. Types of studied compounds.

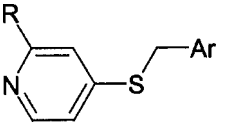
Compounds 1a–4d



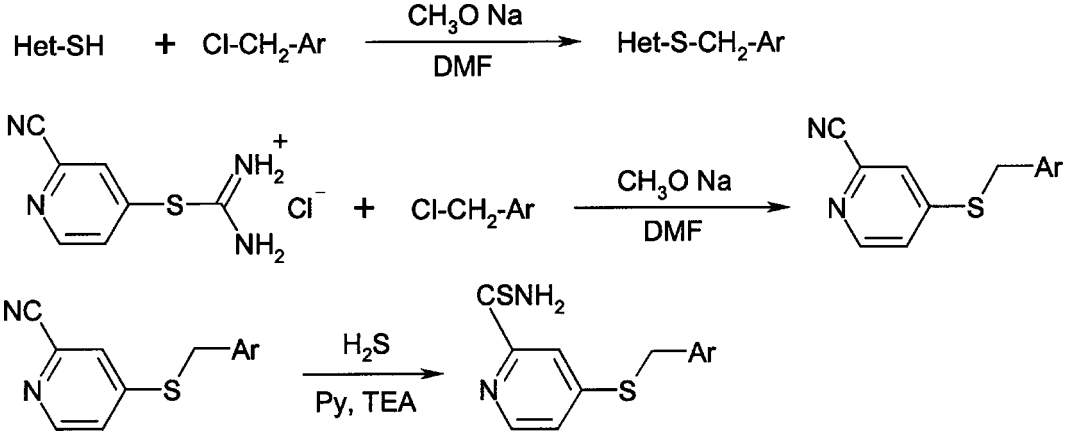
Compounds 5a–5d



Compounds 6a–7d



No.	X	R	Ar	No.	Ar	No.	R	Ar
1a	NH	H	2-pyridyl	**5a**	2-pyridyl	**6a**	CN	2-pyridyl
1b	NH	H	3-pyridyl	**5b**	3-pyridyl	**6b**	CN	3-pyridyl
1c	NH	H	4-pyridyl	**5c**	4-pyridyl	**6c**	CN	4-pyridyl
1d	NH	H	1-naphtyl	**5d**	1-naphtyl	**6d**	CN	1-naphtyl
2a	NH	CH_3	2-pyridyl			**7a**	$CSNH_2$	2-pyridyl
2b	NH	CH_3	2-pyridyl			**7b**	$CSNH_2$	3-pyridyl
2c	NH	CH_3	2-pyridyl			**7c**	$CSNH_2$	4-pyridyl
2d	NH	CH_3	2-pyridyl			**7d**	$CSNH_2$	1-naphtyl
3a	O	H	2-pyridyl					
3b	O	H	3-pyridyl					
3c	O	H	4-pyridyl					
3d	O	H	1-naphtyl					
4a	S	H	2-pyridyl					
4b	S	H	3-pyridyl					
4c	S	H	4-pyridyl					
4d	S	H	1-naphtyl					



Scheme 1. Preparation of compounds **1**–**7**.

benzothiazole-2-thiol (**4**), and 1,2,4-triazole-3-thiol (**5**) (all commercially available), and 2-cyanopyridine-4-thiuronium chloride, which was prepared from 2-methylpyridine by a five-step synthesis described in the literature [8, 14, 15]. Alkylating agents were presented by 2-, 3-, and 4-chloromethylpyridinium chlorides and by 1-naphthylmethyl chloride. The reactions were carried out according to the method previously applied for related compounds [9, 11, 12].

Starting compounds (**1**–**6**) were converted to the corresponding sodium salts by dissolving in a methanolic

solution of sodium methanolate. The resultant salts were substituted by addition of the appropriate alkylating agents. The reaction was carried out in DMF at room temperature for 1−23 h.

The nitrile group in molecule **6** provided further possibilities of modification. A thioamide group was obtained by addition of hydrogen sulfide in pyridine/triethylamine.

The structures of the compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and IR spectral data; their purities were checked by elemental analysis.

^{1}H NMR spectra confirmed the structures of all compounds. The singlets at 2.36−2.38 ppm in compounds **2** belong to the 5-methyl group of benzimidazole. The singlets of the benzylic SCH_2 groups are located at 4.33−5.15 ppm. The spectra of all substances display multiplets in the aromatic region. The singlet of hydrogen in position 5 on the triazole ring (**5**) falls into the region at 8.44−8.61 ppm and is overlapped in **5b**, **c**. Carbothioamide hydrogens (**7**) resonate as two broad singlets at 9.9 and 10.2 ppm.

IR spectra were also in agreement with the structures. The N-H absorption band between 3450 and 3200 cm^{-1} was particularly characteristic (compounds **1**, **2**, **5**, and **7**). Compounds **6** having a C≡N group exhibited characteristic frequencies at 2232−2238 cm^{-1}. H bridges in compounds **1**, **2**, and **5** fall into the region between 2850 and 2400 cm^{-1}.

Antimycobacterial activity

All the prepared compounds were evaluated for *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis, M. avium*, and two strains of *M. kansasii*. For MIC values, expressed in μmol/L, see Table 2. To compare the activities of new compounds, Table 2 includes the activities of the standard isoniazide (INH) and of formerly studied benzylsulfanyl derivatives as the basic structures that were modified in this work. In several cases (denoted >), MIC values could not be determined due to limited solubility of the compounds in the testing medium.

The antimycobacteriological assessment revealed that the compounds under study possess only moderate or slight activity, except for the pyridine-2-carbothioamides (**7b**−**d**). The MIC values are generally within a range of 2 − >1000 μmol/L, most often at 125 or 1000 μmol/L (according to the type of the heterocycle) against all evaluated strains. By comparing their MIC values with INH, they were less active against *M. tuberculosis* 331/88 and *M. kansasii* 6509/96 than INH. On the other hand, in some cases, the compounds exhibited comparable or better activities against *M. kansasii* 235/80 and *M. avium* 330/88 than INH.

The activity of compounds depends on the type of the heterocycle and the type of aryl in the arylmethylsulfanyl part of the molecule. As regards the type of the heterocycle, the following dependences can be observed. Methylation of the benzimidazole scaffold (**2**) enhances the activity in comparison with non-methylated benzimidazole compounds (**1**). The same relationship has been observed in the benzylsulfanyl series of benzimidazole [9] and 5-methylbenzimidazole [10]. Benzoxazole (**3**) and benzothiazole (**4**) derivatives proved to be comparably active as 5-methylbenzimidazole (**2**). 1,2,4-Triazole derivatives (**5**) were the least active compounds in the set. Also, the recently prepared benzylsulfanyl derivatives of 1,2,4-triazole displayed low activity [12]. In the case of pyridine derivatives, thioamides (**7**) were more active than nitriles (**6**). The same relationship has been observed earlier [8, 13]; the activity is probably connected with the presence of a thioamide group.

As regards the arylmethylsulfanyl moiety, the antimycobacterial activity can be summarized as follows. Replacement of phenyl with 1-naphthyl enhanced the activity against *M. tuberculosis* (**1d**, **2d**, **5d**, and **7d**) and against non-tuberculous strains, the activity being similar to that of benzyl derivatives. In pyridylmethylsulfanyl derivatives, in **1**−**5**, the expected increase in the activity by replacement of phenyl with pyridyl did not occur. There are no differences between the activities of the isomers **a**, **b**, and **c**. The situation is different for **6** and especially **7** derivatives. The 2-pyridylmethylsulfanyl derivative **6a** displays better efficacy than **6b** or **6c**. On the other hand, lower MIC values were observed for **7b** and **c** than for **7a**. In these cases, we succeeded in increasing the activity and obtaining antimycobacterially active compounds. 4-(3-Pyridylmethylsulfanyl)pyridine-2-carbothioamide (**7b**) and 4-(1-naphthylmethylsulfanyl)pyridine-2-carbothioamide (**7d**) were the most active compounds in this set.

Acknowledgments

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Table 2. Antimycobacterial activities of prepared compounds expressed as MIC.

Compound	Strain									
	Mycobacterium tuberculosis My 331/88		*Mycobacterium avium* My 330/88		*Mycobacterium kansasii* My 235/80			*Mycobacterium kansasii* 6509/96		
	14 days	21 days	14 days	21 days	7 days	14 days	21 days	7 days	14 days	21 days
	(μmol/L)									
1a	250	500	500	1000	500	1000	>1000	500	1000	1000
1b	500	1000	1000	1000	500	1000	1000	500	500	1000
1c	250	500	500	1000	500	1000	1000	500	1000	1000
1d	32	62.5	62.5	125	62.5	62.5	125	62.5	62.5	62.5
1phenyl	250	>250	125	>125	16	>32	>125	32	>62.5	>125
2a	125	250	250	500	500	500	1000	250	250	500
2b	250	500	250	250	500	500	500	250	250	250
2c	125	250	125	250	250	500	500	250	250	250
2d	32	62.5	62.5	>62.5	62.5	62.5	62.5	62.5	62.5	62.5
2phenyl	62.5	62.5	62.5	>62.5	16	62.5	125	16	32	62.5
3a	32	62.5	125	250	250	500	1000	250	250	500
3b	125	250	125	250	125	250	250	125	250	250
3c	125	250	125	250	62.5	125	125	62.5	125	125
3d	>125	>125	125	250	62.5	125	>250	125	250	>500
3phenyl	250	500	62.5	125	125	250	250	32	125	250
4a	250	250	125	125	125	250	250	125	250	250
4b	125	125	125	125	62.5	125	125	62.5	62.5	125
4c	125	125	125	125	62.5	125	125	62.5	125	125
4d	500	>500	500	1000	125	500	1000	250	500	1000
4phenyl	250	500	32	62.5	62.5	500	>500	62.5	125	250
5a	1000	>1000	1000	1000	500	1000	>1000	500	1000	>1000
5b	1000	>1000	1000	>1000	1000	>1000	>1000	500	1000	>1000
5c	1000	>1000	1000	>1000	1000	>1000	>1000	500	1000	>1000
5d	250	250	250	250	125	250	250	125	250	250
5phenyl	500	1000	500	1000	1000	>1000	>1000	1000	>1000	>1000
6a	8	8	62.5	125	32	62.5	125	32	62.5	125
6b	1000	1000	1000	1000	500	1000	1000	500	1000	1000
6c	1000	1000	1000	1000	500	1000	1000	250	500	1000
6d	>125	>250	>125	>125	62.5	>62.5	>125	>62.5	>125	>250
6phenyl	125	125	125	125	–	32	62.5	–	62.5	62.5
7a	125	250	250	1000	250	500	1000	250	500	1000
7b	2	2	>32	>62.5	8	8	>32	16	16	>32
7c	8	16	32	62.5	8	16	32	16	32	62.5
7d	2	4	>32	>32	4	8	16	>16	>32	>32
7phenyl	16	32	16	32	–	8	32	–	16	32
INH	0.5	1	>250	>250	>250	>250	>250	4	4	8

(Department of Inorganic and Organic Chemistry, Faculty of Pharmacy, Charles University), and Mrs. B. Janíčková and Mrs. M. Malíková for antimycobacterial evaluation (National Reference Laboratory for *Mycobacterium kansasii*, Regional Institute of Hygiene).

Experimental

Chemistry

Melting points were determined on a Kofler block and are uncorrected. Analytical samples were dried over P_4O_{10} at 76 °C or at room temperature and 2.4–2.6 kPa for 3–8 h.

Elemental analyses were performed on a CHNS-O CE instrument (FISONS EA 1110). All values of C, H, N, and S were within ±0.4% of the calculated data. IR spectra were obtained on a Nicolet impact 400 spectrometer in KBr pellets. NMR spectra were recorded in DMSO-d_6 or in acetone-d_6 solutions 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 referred to tetramethylsilane (TMS). Reactions were monitored and purity of products checked by TLC (Silufol VU 254 Kavalier, Votice, Czech Republic; and Merck TLC plates silica gel 60 F_{254}, aluminium back) in acetone/light petroleum or ethyl acetate/light petroleum. The plates were visualized using UV light, iodine fumes and/or dipping in a solution of $Ce(SO_4)$ $4H_2O$, $H_3Mo_{12}O_{40}P \times H_2O$, H_2SO_4 and H_2O, and subsequent heating. Column chromatography and preparative TLC were carried out using silica gel 60 F_{254} (0.015–0.040 mm; Merck).

The following compounds have been described in the literature: **1a** [16, 17], **1b** [18], **1c** [18–20]; **2a** [21]; **3a** [22], **3b** [22, 23], **3c** [18], **4a** [24], **4b** [25], **4c** [18, 20], **4d** [26], **5a** [24]. Only characteristics of new compounds are described below.

*General procedure for the preparation of compounds **1a–6d***

Arylthiol (**1**–**5**) or thiuronium chloride (**6**) (6 mmol) in dry *N,N*-dimethylformamide (DMF) (4 mL) was added to a solution of sodium (0.14 g, 6 mmol for 1-naphthylmethyl chloride; 0.28 g, 12 mmol for chloromethylpyridinium chlorides) in dry methanol (3 mL and 5 mL, respectively). After 10 min of stirring at room temperature, halide (**a**, **b**, **c**, or **d**) (6 mmol) was added. The resultant suspension was stirred with a $CaCl_2$ cap at room temperature for 2–5 h. The course of reactions was monitored by TLC. The reaction mixture was then treated to obtain the product in one of the following ways:

Method A – applied for **1d**, **2d**, **3d**, **4d**, **5d**, and **6d**.

The reaction mixture was poured into an ice bath (ca. 150 mL) and left overnight. The solid was filtered off, washed with cold water and air-dried. The crude product was purified by crystallization or by column chromatography (**6d**).

Method B – applied for **1a–c**, **2a–c**, **3a–c**, and **4a–c**.

The reaction mixture was poured into water (ca. 150 mL) and placed in a freezer overnight. After defrosting, the solid product was filtered off, air-dried and purified by crystallization or by column chromatography (**2a**, **3c**, **4a**).

Method C – applied for **5a–c**, **6b**.

The mixture was evaporated *in vacuo* and left to solidify for 2–4 days. The crude product was crystallized first from water (**5a–c**: 5–15 mL) or water/ethanol 5 : 1 (**6b**: 40 mL), and then from ethyl acetate.

Method D – applied for **6a**, **6c**.

The reaction mixture was evaporated *in vacuo*. The residue was suspended in water (20 mL) and extracted three times into 20 mL ethyl acetate. The organic layer was dried over Na_2SO_4, concentrated, and the crude product was purified by preparative TLC using light petroleum/ethyl acetate 4 : 6 as eluent.

*2-(1-Naphthylmethylsulfanyl)benzimidazole (**1d**)*

Yield: 81%. Mp = 166–168 °C (ethanol). Anal. $C_{18}H_{14}N_2S$. ^{1}H NMR (DMSO-d_6): δ = 5.07 (2H, s), 7.10–7.18 (2H, m), 7.31–7.71 (6H, m), 7.86 (1H, d, *J* = 8.3 Hz), 7.91–8.00 (1H, m), 8.16–8.27 (1H, m), 12.6 (1H, bs). ^{13}C NMR (DMSO-d_6): δ = 33.5, 121.7, 124.1, 125.7, 126.2, 126.7, 127.8, 128.5, 128.9, 131.2, 133.7, 149.9.

*5-Methyl-2-(3-pyridylmethylsulfanyl)benzimidazole (**2b**)*

Yield: 63%. Mp = 139–142.5 °C (toluene). Anal. $C_{14}H_{13}N_3S$. ^{1}H NMR (DMSO-d_6): δ = 2.37 (3H, s), 4.54 (2H, s), 6.93 (1H, dd, J_1 = 1.1 Hz, J_2 = 8.2 Hz), 7.08–7.51 (3H, m), 7.83 (1H, dm, *J* = 7.8 Hz), 8.42 (1H, dd, J_1 = 1.7 Hz, J_2 = 4.7 Hz), 8.63 (1H, d, *J* = 2.2 Hz), 12.46 (1H, s). ^{13}C NMR (DMSO-d_6): δ = 21.4, 32.5, 123.7, 134.3, 136.6, 148.6, 150.0.

*5-Methyl-2-(2-pyridylmethylsulfanyl)benzimidazole (**2c**)*

Yield: 54%. Mp = 181–187 °C (toluene). Anal. $C_{14}H_{13}N_3S$. ^{1}H NMR (DMSO-d_6): δ = 2.36 (3H, s), 4.53 (2H, s), 6.93 (1H, dd, J_1 = 1.4 Hz, J_2 = 8.2 Hz), 7.10–7.38 (2H, m), 7.39–7.38 (2H, m), 8.43–8.50 (2H, m), 12.48 (1H, bs). ^{13}C NMR (DMSO-d_6): δ = 21.4, 33.9, 123.0, 124.0, 128.4, 129.1, 130.9, 147.4, 148.6, 149.9.

*5-Methyl-2-(1-naphthylmethylsulfanyl)benzimidazole (**2d**)*

Yield: 48%. Mp = 161–168 °C (ethanol). Anal. $C_{19}H_{16}N_2S$. ^{1}H NMR (DMSO-d_6): δ = 2.38 (3H, s), 5.04 (2H, s), 6.91–7.00 (1H, m), 7.16–7.45 (3H, m), 7.50–7.72 (3H, m), 7.85 (1H, d, *J* = 8.2 Hz), 7.91–8.00 (1H, m), 8.16–8.25 (1H, m), 12.45 (1H, bs). ^{13}C NMR (DMSO-d_6): δ = 21.4, 33.6, 123.0, 124.1, 125.7, 126.2, 126.6, 127.8, 128.5, 128.9, 130.8, 131.2, 133.1, 133.7, 149.3.

*2-(1-Naphthylmethylsulfanyl)benzoxazole (**3d**)*

Yield: 41%. Mp = 90–93 °C (ethanol). Anal. $C_{18}H_{13}NOS$. ^{1}H NMR (DMSO-d_6): δ = 5.12 (2H, s), 7.27–7.39 (2H, m), 7.40–7.49 (1H, m), 7.51–7.78 (5H, m), 7.89 (1H, d, *J* = 8.5 Hz), 7.93–8.00 (1H, m). ^{13}C NMR (DMSO-d_6): δ = 33.9, 110.5, 118.6, 123.9, 124.6, 124.9, 125.7, 126.3, 126.9, 128.2, 129.0, 129.0, 131.1, 131.8, 133.7, 141.5, 151.5, 164.0.

*3-(3-Pyridylmethylsulfanyl)-1,2,4-triazole (**5b**)*

Yield: 90%. Mp = 126–128 °C (ethyl acetate). Anal. $C_8H_8N_4S$. ^{1}H NMR (DMSO-d_6): δ = 4.35 (2H, s, CH_2), 7.28–7.34 (1H, m, H5′), 7.74–7.80 (1H, m, H4′), 8.39–8.44 (1H, m, H6′), 8.53–8.57 (2H, m, H5, H2′). ^{13}C NMR (DMSO-d_6): δ = 32.6, 123.7, 134.5, 136.5, 145.1, 148.5, 150.0.

*3-(4-Pyridylmethylsulfanyl)-1,2,4-triazole (**5c**)*

Yield: 76%. Mp = 90–92.5 °C (ethyl acetate). Anal. $C_8H_8N_4S$. ^{1}H NMR (DMSO-d_6): δ = 4.33 (2H, s, CH_2), 7.34–7.38 (2H, m, H3′, H5′), 8.50–8.44 (3H, m, H5, H2′, H6′). ^{13}C NMR (DMSO-d_6): δ = 34.2, 124.0, 147.6, 149.8.

*3-(1-Naphthylmethylsulfanyl)-1,2,4-triazole (**5d**)*

Yield: 88%. Mp = 151–153 °C (ethanol). Anal. $C_{13}H_{11}N_3S$. ^{1}H NMR (DMSO-d_6): δ = 4.84 (2H, s, H5), 7.36–7.43 (1H, m, Ar), 7.50–7.61 (3H, m, Ar), 7.81–7.87 (1H, m, Ar), 7.91–7.96 (1H, m, Ar), 8.13–8.18 (1H, m, Ar), 8.61 (1H, bs, H5). ^{13}C NMR (DMSO-d_6): δ = 33.6, 124.1, 125.6, 126.2, 126.6, 127.6, 128.4, 128.9, 131.1, 133.4, 133.7, 145.1.

*4-(2-Pyridylmethylsulfanyl)pyridine-2-carbonitrile (**6a**)*

Yield: 6%. Mp = 81–84 °C (light petroleum/ethyl acetate 1: 1). Anal. $C_{12}H_9N_3S$. ^{1}H NMR (acetone-d_6): δ = 4.56 (2H, s,

CH_2), 7.28 (1H, ddd, J_1 = 7.6 Hz, J_2 = 4.8 Hz, J_3 = 1.1 Hz, H5′), 7.54–7.59 (1H, m, H3′), 7.66 (1H, dd, J_1 = 5.5 Hz, J_2 = 1.9 Hz, H5), 7.78 (1H, td, J_1 = 7.7 Hz, J_2 = 1.9 Hz, H4′), 7.94–7.98 (1H, m, H3), 8.46 (1H, dd, J_1 = 5.5 Hz, J_2 = 0.8 Hz, H6), 8.53 (1H, ddd, J_1 = 4.8 Hz, J_2 = 2.8 Hz, J_3 = 0.8 Hz, H6′). ^{13}C NMR (acetone-d_6): δ = 37.4, 118.0, 123.5, 124.0, 124.5, 126.1, 134.2, 137.9, 150.1, 151.0, 152.5, 157.3.

*4-(3-Pyridylmethylsulfanyl)pyridine-2-carbonitrile (**6b**)*

Yield: 38%. Mp = 102–104°C (ethyl acetate). Anal. $C_{12}H_9N_3S$. ^{1}H NMR (acetone-d_6): δ = 4.54 (2H, s, CH_2), 7.31–7.41 (1H, m, H5′), 7.60 (1H, dd, J_1 = 5.4 Hz, J_2 = 2.1 Hz, H5), 7.84–7.96 (2H, m, H3, H4′), 8.44–8.56 (2H, m, H6, H6′), 8.69–8.75 (1H, m, H2′). ^{13}C NMR (acetone-d_6): δ = 32.7, 117.9, 124.4, 124.6, 126.1, 132.5 134.4, 137.1, 149.8, 150.9, 151.3, 151.7.

*4-(4-Pyridylmethylsulfanyl)pyridine-2-carbonitrile (**6c**)*

Yield: 37%. Mp = 78–81°C (light petroleum/ethyl acetate 2:3). Anal. $C_{12}H_9N_3S$. ^{1}H NMR (acetone-d_6): δ = 4.53 (2H, s, CH_2), 7.46–7.51 (2H, m, H3′, H5′), 7.57 (1H, dd, J_1 = 5.4 Hz, J_2 = 2.1 Hz, H5), 7.84–7.87 (1H, m, H3), 8.48 (1H, dd, J_1 = 5.5 Hz, J_2 = 0.6 Hz, H6), 8.52–8.56 (2H, m, H2′, H6′). ^{13}C NMR (acetone-d_6): δ = 34.2, 117.8, 124.5, 124.6, 126.1, 134.4, 145.7, 151.0, 151.3, 151.5.

*4-(1-Naphthylmethylsulfanyl)pyridine-2-carbonitrile (**6d**)*

Yield: 31%. Mp = 125–129°C (light petroleum/ethyl acetate 1 : 1). Anal. $C_{17}H_{12}N_2S$. ^{1}H NMR (acetone-d_6): δ = 4.94 (2H, s), 7.46 (1H, dd, J_1 = 8.2 Hz, J_2 = 7.1 Hz), 7.50–7.63 (2H, m), 7.65 (1H, dm, J = 7.1 Hz), 7.69 (1H, dd, J_1 = 5.4 Hz, J_2 = 2.0 Hz), 7.89 (1H, d, J = 8.2 Hz), 7.96 (1H, dm, J = 7.60 Hz), 8.10 (1H, dd, J_1 = 2.0 Hz, J_2 = 0.6 Hz), 8.20 (1H, dm, J = 8.1 Hz), 8.50 (1H, dd, J_1 = 5.4 Hz, J_2 = 0.6 Hz). ^{13}C NMR (acetone-d_6): δ = 32.5, 117.6, 124.2, 124.3, 125.78, 125.82, 126.4, 126.7, 128.1, 128.8, 128.9, 131.1, 131.3, 132.8, 133.7, 150.5, 151.7.

*Procedure for preparation of compounds **7a–d***

Dry hydrogen sulfide was passed through a solution of a cyano compound (**6a–d**) (2 mmol) dissolved in a mixture of dry pyridine (5 mL) and triethylamine (0.5 mL). The reaction mixture was maintained at room temperature for 1.5 h. The mixture was then poured into water (40 mL); the precipitated product was filtered off, washed with cold water and air-dried. The crude product was purified by crystallization from ethanol.

*4-(2-Pyridylmethylsulfanyl)pyridine-2-carbothioamide (**7a**)*

Yield: 86%. Mp = 225–228°C (ethanol). Anal. $C_{12}H_{11}N_3S_2$. ^{1}H NMR (acetone-d_6): δ = 4.51 (2H, s, CH_2), 7.26 (1H, ddd, J_1 = 7.4 Hz, J_2 = 4.9 Hz, J_3 = 1.1 Hz, H5′), 7.54–7.60 (2H, m, H5, H3′), 7.75 (1H, td, J_1 = 7.7 Hz, J_2 = 1.9 Hz, H4′), 8.34 (1H, dd, J_1 = 5.4 Hz, J_2 = 0.7 Hz, H6), 8.53 (1H, ddd, J_1 = 4.9 Hz, J_2 = 1.7 Hz, J_3 = 0.9 Hz, H6′), 8.58–8.61 (1H, m, H3), 9.23 (1H, bs, NH_2), 9.71 (1H, bs, NH_2). ^{13}C NMR (acetone-d_6): δ = 37.7, 122.3, 123.29, 123.33, 123.9, 137.7, 148.0, 150.2, 151.3, 151.8, 157.5, 196.0.

*4-(3-Pyridylmethylsulfanyl)pyridine-2-carbothioamide (**7b**)*

Yield: 56%. Mp = 190–192°C (ethanol). Anal. $C_{12}H_{11}N_3S_2$. ^{1}H NMR (DMSO-d_6): δ = 4.47 (2H, s), 7.35 (1H, ddd, J_1 = 7.8 Hz, J_2 = 4.7 Hz, J_3 = 0.7 Hz), 7.52 (1H, dd, J_1 = 5.2 Hz, J_2 = 1.9 Hz), 7.87 (1H, dm, J = 7.8 Hz), 8.36 (1H, dd, J_1 = 1.9 Hz, J_2 = 0.6 Hz), 8.38 (1H, dd, J_1 = 5.2 Hz, J_2 = 0.6 Hz), 8.45 (1H, dd, J_1 = 4.7 Hz, J_2 = 1.6 Hz), 8.68 (1H, d, J = 1.6 Hz), 9.90 (1H, bs), 10.21 (1H, bs). ^{13}C NMR (DMSO-d_6): δ = 31.5, 121.4, 122.8, 123.9, 132.5, 136.7, 147.6, 148.8, 149.2, 150.1, 151.8, 194.3.

*4-(4-Pyridylmethylsulfanyl)pyridine-2-carbothioamide (**7c**)*

Yield: 79%. Mp = 157–159°C (ethanol). Anal. $C_{12}H_{11}N_3S_2$. ^{1}H NMR (DMSO-d_6): δ = 4.47 (2H, s), 7.41–7.56 (3H, m), 8.33 (1H, bd, J = 1.9 Hz), 8.37 (1H, dd, J_1 = 5.2 Hz, J_2 = 0.6 Hz), 8.47–8.55 (2H, m AA′BB′), 9.89 (1H, bs), 10.20 (1H, bs). ^{13}C NMR (DMSO-d_6): δ = 33.0, 121.4, 122.8, 124.1, 145.8, 147.6, 149.1, 150.0, 151.8, 194.3.

*4-(1-Naphthylmethylsulfanyl)pyridine-2-carbothioamide (**7d**)*

Yield: 85%. Mp = 169–172°C (ethanol). Anal. $C_{17}H_{14}N_2S_2$. ^{1}H NMR (DMSO-d_6): δ = 4.90 (2H, s), 7.44 (1H, dd, J_1 = 8.2 Hz, J_2 = 7.1 Hz), 7.50–7.69 (3H, m), 7.68 (1H, bdd, J_1 = 7.1 Hz, J_2 = 0.8 Hz), 7.87 (1H, bd, J = 8.2 Hz), 7.95 (1H, dm, J = 7.5 Hz), 8.20 (1H, dm, J = 8.2 Hz), 8.41 (1H, bd, J = 5.2 Hz), 8.43 (1H, bd, J = 1.7 Hz), 9.93 (1H, bs), 10.22 (1H, bs). ^{13}C NMR (DMSO-d_6): δ = 32.7, 121.5, 122.9, 124.3, 125.7, 126.3, 126.6, 128.0, 128.7, 128.9, 131.3, 131.4, 133.7, 147.3, 150.2, 151.7, 194.5.

Microbiology

In vitro antimycobacterial activity of the compounds was evaluated against *Mycobacterium tuberculosis* CNCTC My 331/88, *M. avium* CNCTC My 330/88, *M. kansasii* CNCTC My 235/80, and *M. kansasii* 6509/96. All strains were obtained from the Czech National Collection of Type Cultures (CNCTC), with the exception of *M. kansasii* 6509/96, which was a clinical isolate. The antimycobacterial activities of the compounds were determined by the micromethod for the determination of the minimum inhibitory concentration (MIC) in a Šula semi-synthetic medium (SEVAC, Prague). The compounds were added to the medium in dimethylsulfoxide solutions. The following concentrations were used: 1000, 500, 250, 125, 62.5, 32, 16, 8, 4, and 2 μmol/L. MIC values were determined after incubation at 37°C for 7, 14, and 21 days. MIC was the lowest concentration of a substance at which inhibition of growth of mycobacteria occurred.

References

[1] M. C. Raviglione, *Tuberculosis* **2003**, *83*, 4–14.

[2] http://www.tballiance.org/, Scientific Blueprint **2000**.

[3] K. Waisser, V. Klimešová, Ž. Odlerová, *Folia Pharm. Univ. Carol.* **1995**, *18*, 31–34.

[4] K. Waisser, J. Hladůvková, A. Hrabálek, V. Klimešová, L. Kubicová, J. Kuneš, K. Palát, M. Macháček, J. Vinšová, V. Buchta, P. Jílek, Ž. Odlerová, *Folia Pharm. Univ. Carol.* **1998**, *21–22*, 69–81.

[5] V. Klimešová, J. Kočí, L. Zahajská, *Ces. Slov. Farm.* **2002**, *51*, 26–36.

[6] A. Scozzafava, A. Mastrolorenzo, C. T. Supuran, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1675–1678.

[7] A. K. Pathak,V. Pathak, L. E. Seitz, W. J. Suling, R. C. Reynolds, *J. Med. Chem.* **2004**, *47*, 273–276.

[8] V. Klimešová, M. Svoboda, K. Waisser, M. Pour, J. Kaustová, *Collect. Czech. Chem. Commun.* **1999**, *64*, 417–434.

[9] V. Klimešová, J. Kočí, M. Pour, J. Stachel, K. Waisser, J. Kaustová, *Eur. J. Med. Chem.* **2002**, *37*, 409–418.

[10] V. Klimešová, J. Kočí, K. Waisser, J. Kaustová, *Farmaco* **2002**, *57*, 259–265.

[11] J. Kočí, V. Klimešová, K. Waisser, J. Kaustová, H. M. Dahse, U. Möllmann, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3275–3278.

[12] V. Klimešová, L. Zahajská, K. Waisser, J. Kaustová, U. Möllmann, *Farmaco* **2004**, *59*, 279–288.

[13] V. Klimešová, K. Palát, K. Waisser, J. Klimeš, *Int. J. Pharm.* **2000**, *207*, 1–6.

[14] T. Kato, H. Hayashi, *Yakugaku Zasshi* **1963**, *83*, 352–355 [Chem. Abstr. **1963**, *59*, 441569].

[15] M. Čeladník, Z. Koštálová, S. Jíška, K. Waisser, E. Kubala, K. Palát, *Ces. Slov. Farm.* **1976**, *25*, 181–185.

[16] D. Graber, R. A. Morge, J. C. Sih, *J. Org. Chem.* **1987**, *52*, 4620–4622.

[17] G. Quelever, G. Sturtz, *Bull. Soc. Chim. Fr.* **1997**, *134*, 817–822.

[18] G. T. Egyesuelt, DE 1804450 **1968** [Chem. Abstr. **1970**, *73*, P25481c].

[19] H. O. Hankovszky, K. Hideg, *Acta Chim. Acad. Sci. Hung.* **1969**, *61*, 69–77.

[20] K. Hideg, O. Hideg, F. Ordogh, L. Vaczy, G. Mehes, GB 1234058 **1971** [Chem. Abstr. **1971**, 75, 98570g].

[21] G. T. Egyesuelt, HU 156129 **1967** [Chem. Abstr. **1969**, *71*, 91479t].

[22] H. Schickaneder, H. Engler, I. Szelenyi, *J. Med. Chem.* **1987**, *30*, 547–551.

[23] J. C. Sih, D. R. Graber, *J. Org. Chem.* **1983**, *48*, 3842–3845.

[24] S. Evangelista, M. Ghelardoni, A. Meli, V. Pestellini, G. Viti, *Farmaco* **1988**, *43*, 901–908.

[25] H. Takagi, M. Sutani, M. Yotsutsuji, M. Hirose, S. Minami, Y. Watanabe, *Jpn. Kohaki Tokkyo Koho* **1997**, 26pp. [Chem. Abstr. **1997**, *127*, 34061].

[26] A. Gaplovsky, R. Hercek, P. Kuran, *Chem. Pap.* **2001**, *55*, 45–48.