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New benzimidazole derivatives as antimycobacterial agents

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Abstract

A set of 2-alkylsulfanyl derivatives of 5-methylbenzimidazole was synthesized and evaluated for antimycobacterial activity. The structures of the compounds were confirmed by ^{1}H NMR and IR data, and their purity by elemental analysis. Antimycobacterial activities against *Mycobacterium tuberculosis* and nontuberculous mycobacteria were expressed as the minimum inhibitory concentration. The substances exhibited significant antimycobacterial activity, in particular against both strains of *Mycobacterium kansasii*. The effect of the most active compound in the set, 3,5-dinitro derivative **3t**, exceeded that of the standard isoniazide against *M. kansasii* and *Mycobacterium avium*.

Keywords: Antimycobacterial activity; 2-Alkylsulfanyl derivatives; 5-Methylbenzimidazole-2-thiol; *Mycobacterium tuberculosis*; Nontuberculous mycobacteria

1. Introduction

Contrary to general expectations, the incidence of mycobacterial disease has significantly increased worldwide since 1990. Not only has there been a resurgence of tuberculosis, which nowadays still remains a major public health problem, but other mycobacteria, especially the *Mycobacterium avium* complex, have emerged as important pathogens due mainly to the AIDS epidemic [1]. The prevalence of HIV infection, and the emergence of drug-resistant and multi-drug-resistant strains of *Mycobacterium tuberculosis* are contributing to the worsening impact of the disease [2]. The World Health Organization (WHO) estimates that one-third of the population is infected with *M. tuberculosis*, the agent that causes pulmonary tuberculosis, and that 3 million people per year decease because of this illness.

There is therefore a pressing need to develop novel chemotherapeutic agents to hinder the emergence of resistance and, ideally, shorten the duration of therapy of this disease [3,4].

Extensive development of the structure–activity relationship (QSAR) at our department revealed an alkylsulfanyl group bound to an electron-deficient carbon atom in various heterocycles as a potential pharmacophore for antimycobacterial activity. In our previous work, we reported that a significant antimycobacterial activity was observed in a large series of alkylsulfanyl derivatives of pyridine [5–7]. The most promising substances were 4-benzylsulfanyl derivatives of pyridine-2-carbonitrile/2-carbothioamide [8]. The significant activity of the prepared compounds prompted us to search for new substances containing heterocyclic moieties other than pyridine but bearing an alkylsulfanyl moiety in the molecule. Using several published studies, we chose benzimidazole because of its broad spectrum of activities (antibacterial, antifungal [9], antihelminthic [10], antiparasitic [11], and especially a promising tuberculostatic activity [12–15]) and interesting chemical properties of the benzimidazole ring.

In order to enhance the activity of benzimidazole, we tried to substitute the hydrogen atoms at 5- and 2-positions. Thus, we synthesized derivatives of benzimidazole in which the CH_3 group on the benzimidazole ring at 5-position is maintained while the benzyl moiety on the sulfur atom at 2-position is further modified by groups with electron-accepting (NO_2, CN, CF_3) and electron-donating (Cl, F, Br, OCH_3) properties in order to optimize the lipophilic and steric characteristic.

In the present paper, we wish to report the synthesis and antimycobacterial activity of 2-alkylsulfanyl

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derivatives of 5-methylbenzimidazole and a comparison with the activity of a previously synthesized set of benzimidazoles.

2. Chemistry

2-Benzylsulfanyl derivatives of 5-methylbenzimidazole (**3**, **4**) were synthesized as outlined in Scheme 1. The commercially available 5-methyl benzimidazole-2-thiol (**1**) serves as a convenient starting material in the syntheses and it is alkylated with benzyl halides (**2**).

The reaction depicted in Scheme 1 was used for the preparation of benzylsulfanyl derivatives **3**. Starting thiol **1** was converted to the corresponding sodium salt by dissolving in a methanolic solution of sodium methanolate, and the resultant salt was subjected to a nucleophilic substitution upon an addition of alkyl halides **2**. The treatment of **1** with various benzyl chlorides or bromides **2** was carried out in *N*,*N*-dimethylformamide (DMF) at room temperature and yielded various monosubstituted and disubstituted 2-benzylsulfanyl derivatives **3**. Compounds bearing a C≡N (**3z**, **3aa**) group were further transformed into the corresponding thioamide (**4a**, **4b**) through reaction with hydrogen sulfide in the presence of triethylamine and pyridine.

The structures of the compounds were confirmed by ^{1}H NMR and IR spectral data, and their purity by elemental analysis. The singlet of the benzylic CH_2S group at 4.47–4.90 ppm and the singlet of CH_3 group at 2.24–2.38 ppm was observed in the ^{1}H NMR spectra of 5-methylbenzimidazole derivatives **3**, **4**. The spectra of all substances displayed multiplets in the aromatic region indicating the presence of the benzimidazole ring and double doublet at 6.93–6.95 ppm belonging to *H* at position 6 of the benzimidazole core. In the spectra of carbothioamides **4a** and **4b**, N–H resonances were apparent between 9.4 and 9.9 ppm.

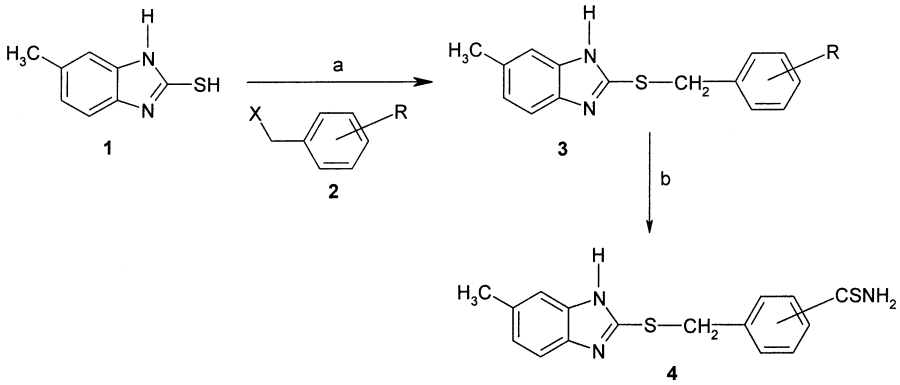
The IR spectra of compounds **3**, **4** were also in agreement with the structures. The N–H absorption band between 3500 and 3220 cm^{-1} was particularly characteristic. The CH_3 group exhibited four characteristic vibrations. The bands at 1380 and 1450 cm^{-1} were assigned to symmetric and asymmetric deformations and peaks at 2870 and 2960 cm^{-1} to stretching symmetric and asymmetric vibrations. The interpretation of nitro derivatives displayed bands at 1350 and 1530 cm^{-1}. Compounds having the C≡N group (**3z**, **3aa**) exhibited characteristic frequencies at 2229 and 2231 cm^{-1} and the diagnostic bands of the carbothioamide function (C=S stretching vibration) in derivatives **4a** and **4b** were at 1274 and 1273 cm^{-1}, respectively.

Chemical and physical data of compounds **3** and **4** are described in Tables 1 and 2.

3. Experimental

3.1. Chemistry

Melting points were determined on a Kofler block and are uncorrected. Analytical samples were dried over P_4O_{10} at 82 or 61 °C and 2.4–2.6 kPa for 8–10 h. Elemental analyses were performed on a CHNS-O CE instrument (FISONS EA 1110) and were within ±0.4% of the calculated values. IR spectra were obtained on a Nicolet Impact 400 spectrometer in KBr pellets. NMR spectra were recorded in DMSO-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). Coupling constants (*J*) are given in Hz. Reactions were monitored and the purity of products checked by TLC (Silufol UV 254 Kavalier, Votice, Czech Republic, and Merck TLC plates silica gel 60 F_{254}, aluminum back) in acetone–light petroleum. The plates were visualized using UV light, iodine fumes and/or dipping in a solution of $Ce(SO_4)_2{\cdot}4H_2O$, $H_3Mo_{12}O_{40}P{\cdot}xH_2O$, H_2SO_4, H_2O and subsequent heating. Preparative thin-layer chromatography was carried out on silica gel 60 F_{254} (0.015–0.040 mm, Merck).



Scheme 1. (a) Na, CH_3OH, DMF; (b) H_2S, TEA, pyridine.

Table 1
Chemical and physical data of compounds **3** and **4**

Comp. no.	R	Formula	M.w.	Yield (%)	M.p. (°C)
3a	H	$C_{15}H_{14}N_2S$	254.4	66	142.5–147
3b	4-Cl	$C_{15}H_{13}ClN_2S$	288.8	66	178–181
3c	3-Cl	$C_{15}H_{13}ClN_2S$	288.8	63	142–145
3d	2-Cl	$C_{15}H_{13}ClN_2S$	288.8	71	127–131
3e	4-F	$C_{15}H_{13}FN_2S$	272.3	75	145.5–149.5
3f	3-F	$C_{15}H_{13}FN_2S$	272.3	78	142–147
3g	2-F	$C_{15}H_{13}FN_2S$	272.3	66	113–115
3h	4-Br	$C_{15}H_{13}BrN_2S$	333.3	58	194–197 [15]
3I	3-Br	$C_{15}H_{13}BrN_2S$	333.3	69	144.5–148
3j	4-CH_3	$C_{16}H_{16}N_2S$	268.4	66	149.5–157
3k	3-CH_3	$C_{16}H_{16}N_2S$	268.4	83	135.5-142
3l	4-OCH_3	$C_{16}H_{16}N_2OS$	284.4	67	144.5–151.5
3m	3-OCH_3	$C_{16}H_{16}N_2OS$	284.4	69	122–127
3n	4-NO_2	$C_{15}H_{13}N_3O_2S$	299.4	80	163–172
3o	3-NO_2	$C_{15}H_{13}N_3O_2S$	299.4	22	159.5–161.5
3p	2-NO_2	$C_{15}H_{13}N_3O_2S$	299.4	43	130–142
3q	2,6-Cl,F	$C_{15}H_{12}ClFN_2S$	306.8	73	139.5–144
3r	3,4-$(Cl)_2$	$C_{15}H_{12}Cl_2N_2S$	323.2	67	125–128 [15]
3s	3,4-$(F)_2$	$C_{15}H_{12}F_2N_2S$	290.3	68	133–141
3t	3,5-$(NO_2)_2$	$C_{15}H_{12}N_4O_4S$	344.3	37	132–138
3u	2,4-$(NO_2)_2$	$C_{15}H_{12}N_4O_4S$	344.3	56	179–188
3v	2,6-F,NO_2	$C_{15}H_{12}FN_3O_2S$	317.3	57	75–85
3w	4-CF_3	$C_{16}H_{13}F_3N_2S$	322.6	90	135–156
3x	3-CF_3	$C_{16}H_{13}F_3N_2S$	322.6	87	89–94
3y	3,5-$(CF_3)_2$	$C_{17}H_{12}F_6N_2S$	390.4	85	116–120
3z	4-CN	$C_{16}H_{13}N_3S$	279.4	83	197–201
3aa	3-CN	$C_{16}H_{13}N_3S$	279.4	80	136–142
4a	4-$CSNH_2$	$C_{16}H_{15}N_3S_2$	313.4	81	101–111
4b	3-$CSNH_2$	$C_{16}H_{15}N_3S_2$	313.4	94	75–85

Lit. [15] gives 189 °C (**3h**), 197 °C (**3r**).

*3.1.1. General procedure for the preparation of compounds **3a–z**, **aa***

5-Methylbenzimidazole-2-thiol **1** (6 mmol) in dry *N*,*N*-dimethylformamide (8 ml) was added to a solution of sodium (6 mmol) in dry methanol (2.5 ml). After 10 min of stirring at room temperature, benzyl halide **2** (6 mmol) was added in 2–3 portions, and the resultant suspension was stirred for 2–8 h. The reaction mixture was then poured into an ice bath and left overnight. The solid was filtered off, washed with cold water (2×30 ml) and air-dried. The crude product was dissolved in acetone, filtered, adhered to silica gel and purified by TLC preparative thin-layer chromatography using acetone–light petroleum (1:2, 1:3, 1:4, 1:5, 1:6) as the eluent to give white or brownish needles of pure compounds **3** in 22–90% yields (Tables 1 and 2).

*3.1.2. General procedure for the preparation of compounds **4a–4b***

Dry hydrogen sulfide was passed through a solution of a cyano compound (**3z**, **3aa**) (2 mmol) dissolved in a mixture of dry pyridine (7 ml) and triethylamine (0.7 ml). The reaction mixture was maintained at room temperature for 4–5 h and then heated at 45 °C for an additional hour. After cooling, the mixture was poured onto crushed ice with intensive stirring, the precipitated product was filtered off, washed with cold water, and air-dried. The residue was purified by preparative thin-layer chromatography using acetone–light petroleum (1:1, 1:2) as the eluent to give the yellow needles of pure product **4** in 81–94% yield (Tables 1 and 2).

3.2. Antimycobacterial activity

In vitro antimycobacterial activity of the compounds was evaluated against *M. tuberculosis* CNCTC My 331/88, *Mycobacterium kansasii* CNCTC My 235/80, *M. kansasii* 6509/96, and *M. avium* CNCTC My 330/88 using the micromethod for the determination of the minimum inhibitory concentration (MIC). 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. Antimycobacterial activities of the compounds were determined in a Šula semisynthetic medium (SEVAC, Prague). The compounds were added to the medium in dimethylsulfoxide solutions. The following concentrations were used: 500, 250, 125, 62, 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

Table 2
^{1}H NMR and IR spectroscopic data of the compounds **3** and **4**

Comp. no.	NMR δ (ppm)	IR ν, δ (cm^{-1})
3a	2.37 (3H, s), 4.53 (2H, s), 6.93 (1H, dd, $J_1 = 1.4$ Hz, $J_2 = 8.2$ Hz), 7.05–7.55 (7H, m), 12.43 (1H, bs)	3432 (N–H), 3063–2645 (H-bridges), 2972, 2858, 1442, 1395 (CH_3)
3b	2.37 (3H, s), 4.52 (2H, s), 6.93 (1H, dd, $J_1 = 1.3$ Hz, $J_2 = 8.2$ Hz), 7.10–7.50 (2H, m overlapped), 7.30–7.40 (2H, m AA′BB′), 7.40–7.50 (2H, m AA′BB′), 12.44 (1H, bs)	3444 (N–H), 3062–2684 (H-bridges), 2981, 2851, 1441, 1396 (CH_3)
3c	2.37 (3H, s), 4.53 (2H, s), 6.93 (1H, dd, $J_1 = 1.7$ Hz, $J_2 = 8.0$ Hz), 7.15–7.45 (5H, m), 7.50–7.55 (1H, m), 12.45 (1H, bs)	3432 (N–H), 3066–2737 (H-bridges), 2996, 2860, 1437, 1392 (CH_3)
3d	2.37 (3H, s), 4.61 (2H, s), 6.94 (1H, dd, $J_1 = 1.4$ Hz, $J_2 = 8.3$ Hz), 7.05–7.40 (4H, m), 7.48 (1H, dd, $J_1 = 1.8$ Hz, $J_2 = 7.5$ Hz), 7.56 (1H, dd, $J_1 = 2.1$ Hz, $J_2 = 7.0$ Hz), 12.46 (1H, bs)	3432 (N–H), 3058–2722 (H-bridges), 2996, 2860, 1444, 1395 (CH_3)
3e	2.37 (3H, s), 4.52 (2H, s), 6.93 (1H, d, $J = 8.2$ Hz), 7.05–7.20 (3H, m), 7.40–7.60 (3H, m), 12.43 (1H, bs)	3447 (N–H), 3043–2684 (H-bridges), 2989, 2858, 1441, 1399 (CH_3)
3f	2.37 (3H, s), 4.54 (2H, s), 6.93 (1H, dd, $J_1 = 1.0$ Hz, $J_2 = 8.3$ Hz), 7.00–7.10 (1H, m), 7.20–7.60 (5H, m), 12.45 (1H, bs)	3427 (N–H), 3053–2686 (H-bridges), 2976, 2852, 1446, 1396 (CH_3)
3g	2.37 (3H, s), 4.55 (2H, s), 6.94 (1H, d, $J = 8.3$ Hz), 7.00–7.55 (6H, m), 12.46 (1H, bs)	3432 (N–H), 3038–2618 (H-bridges), 2991, 2864, 1441, 1397 (CH_3)
3h	2.36 (3H, s), 4.50 (2H, s), 6.93 (1H, dd, $J_1 = 1.1$ Hz, $J_2 = 8.2$ Hz), 7.10–7.45 (2H, m overlapped), 7.30–7.40 (2H, m overlapped AA′BB′), 7.40–7.50 (2H, m AA′BB′), 12.43 (1H, bs)	3428 (N–H), 3061–2637 (H-bridges), 2981, 2858, 1439, 1396 (CH_3)
3I	2.37 (3H, s), 4.52 (2H, s), 6.93 (1H, dd, $J_1 = 1.0$ Hz, $J_2 = 8.1$ Hz), 7.20–7.40 (3H, m), 7.40–7.45 (2H, m), 7.66 (1H, t, $J = 1.8$ Hz), 12.45 (1H, bs)	3428 (N–H), 3065–2638 (H-bridges), 2989, 2861, 1440, 1394 (CH_3)
3j	2.24 (3H, s), 2.37 (3H, s), 4.48 (2H, s), 6.93 (1H, dd, $J_1 = 1.4$ Hz, $J_2 = 8.2$ Hz), 7.15–7.45 (2H, m overlapped), 7.05–7.15 (2H, m AA′BB′), 7.25–7.35 (2H, m overlapped AA′BB′), 12.40 (1H, bs)	3425 (N–H), 3023–2731 (H-bridges), 2980, 2861, 1438, 1389 (CH_3)
3k	2.25 (3H, s), 2.37 (3H, s), 4.49 (2H, s), 6.93 (1H, bd, $J = 8.2$ Hz), 7.00–7.10 (1H, m), 7.10–7.30 (4H, m), 7.30–7.50 (1H, m), 12.42 (1H, bs)	3432 (N–H), 3039–2641 (H-bridges), 2980, 2861, 1440, 1393 (CH_3)
3l	2.37 (3H, s), 3.69 (3H, s), 4.47 (2H, s), 6.80–6.90 (2H, m AA′BB′) 6.93 (1H, dd, $J_1 = 1.7$ Hz, $J_2 = 8.5$ Hz), 7.25–7.40 (2H, m overlapped AA′BB′), 7.10–7.50 (2H, m overlapped), 12.40 (1H, bs)	3424 (N–H), 3062–2637 (H-bridges), 2996, 2859, 1441, 1397 (CH_3), 1177, 1033 (C–O Ar–O–CH_3)
3m	2.37 (3H, s), 3.68 (3H, s), 4.49 (2H, s), 6.80 (1H, ddd, $J_1 = 1.1$ Hz, $J_2 = 2.5$ Hz, $J_3 = 8.2$ Hz), 6.90–7.05 (3H, m), 7.15–7.25 (1H, m overlapped), 7.20 (1H, t overlapped, $J = 7.8$ Hz), 7.32 (1H, bd, $J = 8.2$ Hz), 12.42 (1H, bs)	3432 (N–H), 3061–2610 (H-bridges), 2998, 2858, 1466, 1403 (CH_3), 1171, 1042 (C–O Ar–O–CH_3)
3n	2.36 (3H, s), 4.65 (2H, s), 6.93 (1H, dd, $J_1 = 1.1$ Hz, $J_2 = 8.2$ Hz), 7.10–7.40 (2H, m), 7.60–7.70 (2H, m AA′BB′), 8.10–8.20 (2H, m AA′BB′) 12.47 (1H, bs)	3427 (N–H), 3074–2797 (H-bridges), 2978, 2860, 1441, 1397 (CH_3), 1345, 1519 (NO_2)
3o	2.36 (3H, s), 4.66 (2H, s), 6.93 (1H, dd, $J_1 = 1.4$ Hz, $J_2 = 8.2$ Hz), 7.10–7.45 (2H, m), 7.57 (1H, t, $J = 7.8$ Hz), 7.89 (1H, dm, $J = 7.8$ Hz), 8.08 (1H, ddd, $J_1 = 1.1$ Hz, $J_2 = 2.5$ Hz, $J_3 = 8.2$ Hz), 8.36 (1H, t, $J = 2.0$ Hz), 12.46 (1H, bs)	3424 (N–H), 3043–2799 (H-bridges), 2984, 2865, 1440, 1397 (CH_3), 1352, 1528 (NO_2)
3p	2.36 (3H, s), 4.81 (2H, s), 6.93 (1H, dd, $J_1 = 1.4$ Hz, $J_2 = 8.2$ Hz), 7.10–7.25 (1H, m), 7.25–7.40 (1H, m), 7.51 (1H, td, $J_1 = 1.4$ Hz, $J_2 = 7.7$ Hz), 7.64 (1H, td, $J_1 = 1.4$ Hz, $J_2 = 7.6$ Hz), 7.75 (1H, dd, $J_1 = 1.7$ Hz, $J_2 = 7.7$ Hz), 8.02 (1H, dd, $J_1 = 1.4$ Hz, $J_2 = 8.2$ Hz), 12.41 (1H, bs)	3428 (N–H), 3068–2858 (H-bridges), 2923, 2858, 1442, 1395 (CH_3), 1342, 1524 (NO_2)
3q	2.38 (3H, s), 4.64 (2H, d, $J = 1.7$ Hz), 6.95 (1H, dd, $J_1 = 1.4$ Hz, $J_2 = 8.2$ Hz), 7.05–7.30 (2H, m), 7.30–7.55 (3H, m), 12.54 (1H, bs)	3432 (N–H), 3041–2718 (H-bridges), 2922, 2862, 1453, 1395(CH_3)
3r	2.36 (3H, s), 4.52 (2H, s), 6.93 (1H, dd, $J_1 = 1.9$ Hz, $J_2 = 8.2$ Hz), 7.15–7.40 (2H, m), 7.41 (1H, dd, $J_1 = 8.3$ Hz, $J_2 = 2.0$ Hz), 7.54 (1H, d, $J = 8.2$ Hz), 7.72 (1H, d, $J = 2.2$ Hz), 12.45 (1H, bs)	3432 (N–H), 3061–2629 (H-bridges), 2973, 2861, 1440, 1396 (CH_3)
3s	2.37 (3H, s), 4.51 (2H, s), 6.93 (1H, dd, $J_1 = 1.1$ Hz, $J_2 = 8.2$ Hz), 7.05–7.45 (4H, m), 7.51 (1H, ddd, $J_1 = 2.2$ Hz, $J_2 = 8.0$ Hz, $J_3 = 11.8$ Hz), 12.44 (1H, bs)	3428 (N–H), 3035–2615 (H-bridges), 2996, 2856, 1437, 1400 (CH_3)
3t	2.36 (3H, s), 4.76 (2H, s), 6.93 (1H, dd, $J_1 = 1.1$ Hz, $J_2 = 8.2$ Hz), 7.05–7.45 (2H, m), 8.66 (1H, t, $J = 2.2$ Hz), 8.81 (2H, d, $J = 2.2$ Hz), 12.47 (1H, bs)	3427 (N–H), 3100–2872 (H-bridges), 2996, 2872, 1439, 1388 (CH_3), 1344, 1541 (NO_2)
3u	2.35 (3H, s), 4.90 (2H, s), 6.92 (1H, dd, $J_1 = 1.7$ Hz, $J_2 = 8.3$ Hz), 7.00–7.55 (2H, m), 8.05 (1H, d, $J = 8.6$ Hz), 8.46 (1H, dd, $J_1 = 2.5$ Hz, $J_2 = 8.6$ Hz), 8.69 (1H, d, $J = 2.5$ Hz), 12.45 (1H, bs)	3427 (N–H), 3114–2869 (H-bridges), 2950, 2869, 1440, 1385 (CH_3), 1344, 1533 (NO_2)
3v	2.36 (3H, s), 4.79 (2H, s), 6.93 (1H, dd, $J_1 = 1.7$ Hz, $J_2 = 8.0$ Hz), 7.10–7.45 (2H, m), 7.50–7.75 (2H, m), 7.83 (1H, dt, $J_1 = 1.4$ Hz, $J_2 = 7.9$ Hz), 12.44 (1H, bs)	3432 (N–H), 3099–3058 (H-bridges), 2923, 2865, 1464, 1385 (CH_3), 1351, 1533 (NO_2)
3w	2.36 (3H, s), 4.61 (2H, s), 6.93 (1H, dd, $J_1 = 1.1$ Hz, $J_2 = 8.3$ Hz), 7.05–7.55 (2H, m), 7.65 (4H, bs)	3432 (N–H), 3069–2807 (H-bridges), 2999, 2864, 1440, 1395 (CH_3), 1122, 1160, 1276 (CF_3)
3x	2.36 (3H, s), 4.61 (2H, s), 6.93 (1H, dd, $J_1 = 1.5$ Hz, $J_2 = 8.1$ Hz), 7.15–7.40 (2H, m), 7.45–7.65 (2H, m), 7.73 (1H, bd, $J = 7.8$ Hz), 7.83 (1H, bs)	3432 (N–H), 3069–2807 (H-bridges), 2927, 2865, 1450, 1395 (CH_3), 1125, 1167, 1276 (CF_3)

Table 2 (*Continued*)

Comp. no.	NMR δ (ppm)	IR ν, δ (cm^{-1})
3y	2.36 (3H, s), 4.68 (2H, s), 6.93 (1H, dd, $J_1 = 1.5$ Hz, $J_2 = 8.0$ Hz), 7.21 (1H, bs), 7.31 (1H, bd, $J = 8.3$ Hz), 7.95 (1H, bs), 8.20 (2H, bs)	3440 (N–H), 3054–2649 (H-bridges), 2988, 2865, 1442, 1399 (CH_3), 1134, 1178, 1279 (CF_3)
3z	2.36 (3H, s), 4.60 (2H, s), 6.93 (1H, dd, $J_1 = 1.4$ Hz, $J_2 = 8.2$ Hz), 7.00–7.55 (2H, m), 7.55–7.65 (2H, m AA′BB′), 7.70–7.80 (2H, m AA′BB′) 12.46 (1H, bs)	3428 (N–H), 3053–2626 (H-bridges), 2925, 2862, 1441, 1398 (CH_3), 2229 (C≡N)
3aa	2.37 (3H, s), 4.57 (2H, s), 6.93 (1H, dd, $J_1 = 1.6$ Hz, $J_2 = 8.2$ Hz), 7.15–7.40 (2H, m), 7.49 (1H, t, $J = 7.7$ Hz), 7.70 (1H, dt, $J_1 = 1.4$ Hz, $J_2 = 7.7$ Hz), 7.77 (1H, dt, $J_1 = 1.4$ Hz, $J_2 = 7.7$ Hz), 7.90 (1H, bt, $J = 1.1$ Hz), 12.45 (1H, bs)	3428 (N–H), 3061–2614 (H-bridges), 2923, 2862, 1438, 1397 (CH_3), 2231 (C≡N)
4a	2.37 (3H, s), 4.55 (2H, s), 6.93 (1H, dd, $J_1 = 1.7$ Hz, $J_2 = 8.2$ Hz), 7.10–7.40 (2H, m), 7.40–7.50 (2H, m AA′BB′), 7.75–7.85 (2H, m AA′BB′), 9.44 (1H, bs), 9.83 (1H, bs), 12.45 (1H, bs)	3408 (N–H), 3288–3191 (H-bridges), 2923, 2857, 1436, 1385 (CH_3), 1274 (C=S –NH–C=S)
4b	2.37 (3H, s), 4.57 (2H, s), 6.93 (1H, dd, $J_1 = 1.7$ Hz, $J_2 = 8.0$ Hz), 7.15–7.40 (2H, m overlapped), 7.32 (1H, t overlapped, $J = 7.8$ Hz), 7.55 (1H, dt, $J_1 = 1.5$ Hz, $J_2 = 7.8$ Hz), 7.69 (1H, dt, $J_1 = 1.5$ Hz, $J_2 = 7.8$ Hz), 7.99 (1H, t, $J = 1.5$ Hz), 9.49 (1H, bs), 9.87 (1H, bs), 12.41 (1H, bs)	3424 (N–H), 3297–3184 (H-bridges), 2923, 2857, 1433, 1387 (CH_3), 1273 (C=S –NH–C=S)

concentration of a substance at which the inhibition of the growth of mycobacteria occurred.

4. Results and discussion

The results of biological evaluation of newly prepared benzylsulfanyl derivatives **3** and **4** are summarized in Table 3. The values of antimycobacterial activity are expressed as the MIC. In several cases (denoted $>$), the MIC could not be determined due to limited solubility of the compounds in the testing medium. For the sake of comparison, the MIC values of **1** and the standard isoniazide (INH) are also included. All of these compounds (**1**, **3**, **4**) displayed in vitro activity against all mycobacterial strains tested. The MIC values are within a range of 4–500 μmol/l, most often between 4 and 62 μmol/l. By comparing their MIC values with INH, the compounds under study were less active against *M. tuberculosis* 331/88 than INH, and all but one (**3t**) against *M. kansasii* 6509/96. In contrast to INH, the derivatives were comparably active against both strains of *M. kansasii*, with better activity against *M. kansasii* 235/80. The compounds also possessed a more pronounced effect than INH against *M. avium* 330/88.

The starting 5-methylbenzimidazole-2-thiol (**1**) is characterized by the activity within a range of >250–500 μmol/l. Comparisons of the MIC values of **1** to those of the benzylsulfanyl derivative (**3a**) indicate that the antimycobacterial activity is connected with the presence of the benzyl moiety at 2-position of the 5-methylbenzimidazole ring. It was expected that a further modification of the benzyl moiety by electron-withdrawing groups might improve the activity. Thus, the 2-(3,5-dinitrobenzylsulfanyl)-5-methylbenzimidazol (**3t**) was the most active compound in the set, having activities ranging from 4 to 32 μmol/l. The activity exceeded that of INH against both strains of *M. kansasii*, against *M. tuberculosis* it was 4-fold less than that of INH. The compound was only moderately active (MIC = 32 μmol/l) against *M. avium*, but exceeded the potency of INH. The presence of the nitro group in positions 2 and 4 (**3u**) resulted in a slight decrease in activity (MIC = 8–62 μmol/l). Displacement of the nitro group in position 2 led to compound **3n**, which exhibited a considerable decrease in activity against *M. tuberculosis* (MIC = 32 μmol/l), on the other hand the activity against *M. avium* increased (MIC = 8 μmol/l). A promising effect was revealed for thioamide derivatives (**4**). The 4-substituated derivative (**4a**) had a greater activity (MIC = 8–16 μmol/l) than its 3-substituated analog (**4b**) with MIC = 62–125 μmol/l.

In a previous communication [16], we reported the synthesis and biological evaluation of a series of benzimidazole derivatives. The study revealed that the newly prepared set of 5-methylbenzimidazole derivatives displayed a better activity than that of the benzimidazole analog. The MIC values for the set of 5-methylbenzimidazoles and benzimidazoles varied between 4–62 and 8–125 μmol/l, respectively. The mono-, dinitro- and thioamide derivatives were the most active compounds in both sets.

On the basis of the pharmacophore analysis and knowledge about the relationship between the structure and antimycobacterial activity of pyridine [7,8,17,18] and benzimidazole derivatives [16], we postulated that alkylsulfanyl moiety is responsible for the observed antituberculotic activity. This finding is in agreement with the presented results. In addition, the activity of compound **4** is linked to the presence of a thioamide group, which is supposed to also be the pharmacophore of antimycobacterial activity.

Table 3
Antimycobacterial activity of compounds **1**, **3**, **4** expressed as MIC (μmol/l)

Comp. No.	Strains									
	M. tuberculosis My 332/88		*M. kansasii* My 235/80			*M. kansasii* My 6509/96			*M. avium* My 330/88	
	14 days	21 days	7 days	14 days	21 days	7 days	14 days	21 days	14 days	21 days
1	500	>250	500	500	500	500	500	500	500	>500
3a	62	62	16	62	125	16	32	62	62	>62
3b	32	32	16	32	62	4	16	16	32	32
3c	62	62	16	32	32	4	16	16	32	32
3d	32	32	8	32	32	4	16	16	32	62
3e	32	32	16	62	62	8	32	32	32	62
3f	32	62	32	32	62	8	16	32	62	62
3g	62	62	8	32	32	4	16	32	62	125
3h	32	>32	16	>32	>32	8	16	32	>32	>62
3i	32	62	8	16	32	4	16	16	32	62
3j	>32	>32	8	32	>32	4	16	32	32	>32
3k	32	62	16	32	32	4	16	32	62	62
3l	32	>32	16	32	>62	16	32	32	62	>62
3m	62	62	16	62	125	16	32	32	62	>32
3n	32	32	8	16	32	4	8	16	8	16
3o	62	>62	16	32	>62	8	16	32	32	>62
3p	62	62	125	125	>125	62	125	125	62	125
3q	16	32	16	32	62	8	16	16	8	16
3r	32	>32	8	16	32	4	8	16	32	>32
3s	32	>32	16	32	32	4	16	16	32	>62
3t	4	4	4	4	4	2	4	4	32	32
3u	8	8	8	8	32	8	8	8	62	>125
3v	62	125	>62	125	>125	>32	62	125	125	>125
3w	32	62	32	32	32	32	32	32	32	62
3x	32	32	62	62	62	32	32	62	32	62
3y	32	62	62	62	125	32	62	62	62	125
3z	32	62	>32	>32	>62	>32	>32	>32	32	32
3aa	62	>62.5	125	125	125	125	125	125	62	125
4a	8	8	8	8	8	8	8	8	16	16
4b	32	62	16	32	62	16	32	62	62	125
INH	1	1	500	500	500	4	8	8	250	500

The most active compound **3t** was also subjected to an antiproliferative effect assay against the K-562 and L-929 cell lines. The values expressed as GI_{50} (growth inhibition) are 7.1 and 12.0 μg/ml. The cytotoxic effect was evaluated on HeLa cells. The cytotoxic concentration expressed as CC_{50} (CC_5) was 14.0 (3.9) μg/ml. According to the values of GI and CC, compound **3t** can be considered as moderately toxic.

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