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Short communication

Synthesis and preliminary evaluation of benzimidazole derivatives as antimicrobial agents

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Dedicated to Professor Dr Roland Mayer on the occasion of his 75th birthday

Abstract

A series of 2-alkylsulphanylbenzimidazoles was synthesised and the compounds were evaluated for their in vitro antimicrobial 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 non-tuberculous mycobacteria as well as antifungal activities against *Candida albicans*, *Candida tropicalis*, *Candida krusei*, *Candida glabrata*, *Trichosporon beigelii*, *Trichophyton mentagrophytes* and *Aspergillus fumigatus* were expressed as the corresponding MIC values. The substances exhibited appreciable antimycobacterial activity, in particular, against non-tuberculous mycobacteria. The activity of the most active compound in the set, 3,5-dinitro derivative **4t**, exceeded that of the standard isoniazide against *M. kansasii* and *M. avium*. The antifungal activities of the compounds were relatively low. A weak antifungal effect was observed against the dermatophyte *Trichophyton mentagrophytes*. None of the compounds showed significant inhibitory activity against yeasts.

Keywords: Antimycobacterial activity; Antifungal activity; 2-Alkylsulphanyl derivatives; Benzimidazole

1. Introduction

The deterioration of the epidemiological situation caused by infectious diseases is becoming a world-wide health problem. In the past few decades, tuberculosis is regaining its place among those infections, which more than often progress to a fatal end. Nearly one-third of the world's population is infected with *Mycobacterium tuberculosis*, with approximately three million patients deceasing every year [1].

The increasing incidence of tuberculosis in both developed and developing countries is one of the most alarming aspects of the disease. For example, India, South Africa, some of the states of the former Soviet Union (Latvia, Lithuania, and Estonia), and USA [2] belong to the areas most affected by the disease. The second aspect contributing to tuberculosis incidence is the rise in the occurrence of the multidrug resistant strains of *Mycobacterium tuberculosis*. In addition, a high number of infections with non-tuberculous mycobacteria, especially in immunocompromised patients such as those infected with the human immunodeficiency virus (HIV) [3], render the treatment even more difficult. Of this group of *Mycobacterium* species, the *Mycobacterium avium* complex (MAC), naturally resistant towards a number of antimycobacterial drugs [4], is considered to be the most dangerous strain. For the treatment of tuberculosis, isoniazid (INH), rifampicin, pyrazinamide, ethambutol, and streptomycin, generally administered as a four-drug combination, are the most widely used drugs. However, the protracted use of these agents is one of the principal factors, which led to the development of new resistant strains. Therefore, there is an urgent need for the design, synthesis, and assay of new potential drugs.

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In our previous work, we reported that the alkylsulphanyl group bound to an electron-deficient carbon atom in various heterocycles is one of the pharmacophores of antimycobacterial activity [5]. A significant antimycobacterial activity was observed in a large series of alkylsulphanyl derivatives of pyridine [6–9], with 4-benzylsulphanyl derivatives of pyridine-2-carbonitrile/2-carbothioamide being the most promising substances [10]. As regards their activity profile, the compounds exhibited antimycobacterial effect against both *M. tuberculosis* and nontuberculous mycobacteria. The values of MIC were within the range 4–125 μmol L^{-1}. Compared to a standard, the derivatives matched the activity of INH against *M. tuberculosis* (MIC 4 μmol L^{-1}) and were superior to the drug against nontuberculous mycobacteria (MIC 500 μmol L^{-1} for INH).

The above results prompted us to continue our ongoing search for new substances containing heterocyclic moieties other than pyridine. According to the literature, a large number of alkylsulphanyl derivatives of 1,2,4-triazole [11–14], tetrazole [15–17] benzothiazole [18,19] and benzimidazole [20–22] were evaluated against various strains of mycobacteria. Even though the majority of these compounds displayed a rather moderate activity, some of the derivatives of benzothiazole and benzimidazole exhibited appreciable antimycobacterial effect. Because of the interesting chemical properties of the benzimidazole ring, we decided to synthesise a set of alkylsulphanyl derivatives of benzimidazole.

In this paper, we describe the results of antimycobacterial activity evaluation of the derivatives of benzimidazole-2-thiol, bearing an alkyl chain on the sulphur atom in position 2. The alkyl group is either a linear or branched $C_{2-6,16}$ carbon chain, allyl, phenethyl and benzyl fragment. The benzyl moiety is further modified by groups with electron-accepting (NO_2, CN, CF_3) and electron-donating (Cl, F, Br, OCH_3) properties.

During our studies on new potential antimycobacterial agents, we noted that some of them also showed antifungal activity, and this fact prompted us to evaluate the newly prepared compounds for antifungal properties as well.

2. Chemistry

The synthetic pathways leading to 2-benzylsulphanyl derivatives of benzimidazole are depicted in Fig. 1. Benzimidazole-2-thiol (**2**), which serves as a convenient starting material in the syntheses, was prepared according to a literature procedure [23], by heating a mixture of *o*-phenylenediamine (**1**), potassium hydroxide, and carbon disulphide in 96% ethanol at reflux for 3 h. The reaction afforded the starting compound **2** in 70% yield.

The alkylsulphanyl derivatives were prepared via three procedures. Method A was used for the preparation of benzylsulphanyl derivatives **4**. The treatment of **2** with various benzyl chlorides or bromides **3** was carried out in *N*,*N*-dimethylformamide (DMF) in the presence of sodium methanolate at room temperature. The process required 2–8 h depending on the alkylating

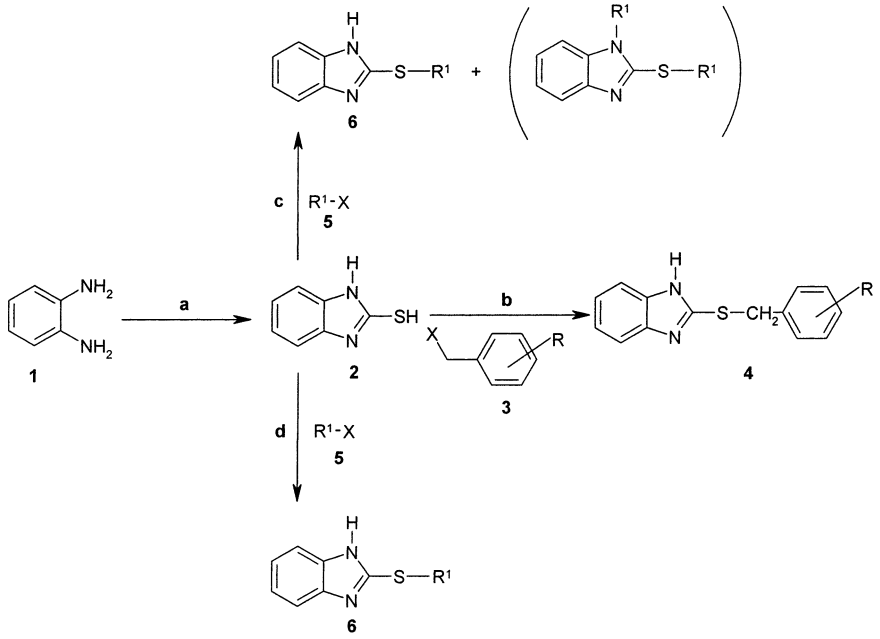

Fig. 1. Reagents: (a) CS_2, KOH, ethanol; (b) Method A: Na, CH_3OH, DMF; (c) Method B: Na, CH_3CH_2OH; (d) Method C: KOH, $(C_4H_9)_4N^+Br^-$, cyclohexane.

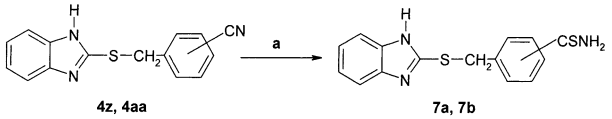

Fig. 2. Reagents: (a) H_2S, TEA, pyridine.

agent and furnished products **4** in 40–90% yields. The synthesis of alkylsulphanyl derivatives **6** was carried out in ethanol at reflux (method B). Thiol **2** was converted to the corresponding sodium salt by dissolving in an ethanolic solution of sodium ethanolate, and the resultant salt was subjected to a nucleophilic substitution upon the addition of an alkyl halide **5**. The yields varied between 19 and 47%. In most reactions in boiling ethanol, a second, more lipophilic product began to form after several hours. These undesired byproducts were identified as *S*,*N*-dialkylated compounds by IR (vanishing of the N–H bond vibration) and NMR spectroscopy. Thus, by terminating the reaction at an appropriate stage, moderate to good yields of the *S*-alkylated compounds could be obtained. Method C, which was used for the preparation of some of compounds **6** was based on the nucleophilic substitution under phase-transfer conditions. The method involved the alkylation of **2** with alkyl halides **5** in a two-phase cyclohexane–aqueous potassium hydroxide system with tetrabutylammonium bromide as a phase-transfer catalyst. The reactions with short-chain alkyl halides (ethyl bromide, propyl bromide) led to the formation of the *S*-alkylated products with acceptable selectivity.

The benzylsulphanyl derivatives bearing the CN group **4z**, **4aa** were further converted into the corresponding carbothioamides **7a** and **7b** by the addition of hydrogen sulphide in pyridine/triethylamine solution (Fig. 2).

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 4.5–4.6 ppm was typical in the ^{1}H-NMR spectra of the benzylsulphanyl derivatives, while the corresponding signal in the alkylsulphanyl derivatives was shifted upfield and split into a triplet. The spectra of all substances displayed multiplets in the aromatic region indicating the presence of the benzimidazole ring, and the NH resonance was apparent as a broad singlet between 12.0 and 13.0 ppm. In the spectra of substances with the carbothioamide group **7a** and **7b**, N–H resonances were apparent between 9.4 and 9.9 ppm (Table 5).

IR spectra of 2-alkylsulphanyl and 2-benzylsulphanyl derivatives of benzimidazole were also in agreement with the structures. The N–H absorption band between 3500 and 3220 cm^{-1} was particularly characteristic. The compounds having the CN group (**4z**, **4aa)** exhibited characteristic frequencies at 2227 and 2233 cm^{-1} and the diagnostic bands of the carbothioamide function (C=S stretching vibration) in the derived derivatives **7a** and **7b** were at 1437 and 1438 cm^{-1}, respectively. The spectra of all alkylsulphanyl derivatives showed the absorption signals of alkyl group vibrations between 3000 and 2840 cm^{-1} (Table 5).

3. Biology

In vitro antimycobacterial activity of the compounds was evaluated against *Mycocterium tuberculosis* CNCTC My 331/88, *Mycobacterium kansasii* CNCTC My 235/80, *Mycobacterium kansasii* 6509/96 and *Mycobacterium avium* CNCTC My 330/88 using the micromethod for the determination of the minimum inhibitory concentration. 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.

In vitro antifungal activity was assessed on a panel of one ATCC (*Candida albicans* ATCC 44859), and seven clinical isolates of yeasts (*Candida tropicalis* 156, *Candida krusei* E28, *Candida glabrata* 20/I) and filamentous fungi (*Trichosporon beigelii* 1188, *Trichophyton mentagrophytes* 445, *Aspergillus fumigatus* 231, *Absidia corymbifera* 272) using the microdilution format of the NCCLS M27-A guidelines.

4. Results and discussion

The results of the biological screening of the derivatives are summarised in Tables 1 and 2. In several cases (denoted $>$), the minimum inhibitory concentration (MIC) could not be determined due to the limited solubility of the compounds in the testing medium.

The values of antimycobacterial activity of the benzylsulphanyl derivatives **4** and **7** are shown in Table 1. For the sake of comparison, we also included the values of MICs of **2** and the standard isoniazide (INH). The results revealed that the compounds exhibited in vitro activity against all tested mycobacterial strains. The values of MICs are generally within the range 4–500 $\mu mol\ L^{-1}$, most often between 8 and 125 $\mu mol\ L^{-1}$. The compounds were less active than INH against *M. tuberculosis* 331/88 and *M. kansasii* 6509/96. On the other hand, in some cases, the compounds possessed a better activity against *M. kansasii* 235/80 and *M. avium* 330/88 than INH. A significant activity was observed for the compounds containing two nitro groups in positions 3 and 5 (**4t**) and 2 and 4 (**4u**) in the benzyl moiety. Compound **4t** displayed the highest activity of the set (MIC = 4–8 $\mu mol\ L^{-1}$). A promising effect was shown by the thioamide derivative **7a**, with MIC 8–62 $\mu mol\ L^{-1}$. It is worthy to note that the activity of the

Table 1
In vitro antimycobacterial activity of compounds **2**, **4**, **7** expressed as MIC (μmol L^{-1})

Compounds	Strains									
	Myobacterium tuberculosis My 331/88		*Myobacterium kansasii* My 235/80			*Myobacterium kansasii* My 6509/96			*Myobacterium* My 330/88	
	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d	14 d	21 d
2	500	1000	250	250	500	125	125	250	500	1000
4a	250	>250	16	>32	>125	32	>62	>125	125	>125
4b	>32	>125	>16	>32	>62	32	>62	>62	>16	>32
4c	>125	>250	>16	>32	>62	32	>>62	>62	62	>62
4d	>125	>125	125	>125	>125	32	>32	>62	62	>62
4e	62	125	32	62	125	32	62	62	62	62
4f	125	125	32	32	62	32	62	62	125	125
4g	>125	>125	125	125	125	32	62	62	125	>125
4h	>32	>125	>32	>62	>125	62	>62	>125	>62	>125
4i	>62	>250	>62	>62	>125	62	>125	>250	>62	>125
4j	>125	>125	32	>62	>125	32	62	>62	32	62
4k	>125	>125	32	125	>125	32	62	>62	62	62
4l	125	>125	62	62	62	32	32	62	32	32
4m	125	250	32	62	125	16	32	62	32	125
4n	62	>62	8	16	16	8	8	8	8	16
4o	>32	>125	>32	>62	>250	>32	>62	>250	>16	>62
4p	250	250	125	>125	>125	125	250	250	250	250
4q	62	>62	32	62	125	32	62	125	16	62
4r	>32	>62	>32	>32	>62	32	62	>62	16	>32
4s	62	62	32	62	62	16	32	32	32	32
4t	4	4	4	8	8	4	8	8	8	8
4u	4	8	8	16	32	8	8	16	16	32
4v	62	125	125	125	250	62	62	250	125	>250
4w	16	32	32	>32	>62	16	>32	>62	>32	>62
4x	62	62	32	62	>62	32	62	62	62	62
4y	62	>62	62	>62	>125	32	62	>62	>62	>125
4z	32	>62	>62	>250	>500	>32	>500	>500	32	62
4aa	>62	>62	62	>62	>62	62	>62	>250	>62	>62
7a	16	32	8	8	8	8	8	16	32	62
7b	62	62	62	125	125	62	125	125	125	125
INH	0.5	1	>250	>250	>250	2	4	4	>250	>250

Table 2
In vitro antimycobacterial activity of compounds **6** expressed as MIC (μmol L^{-1})

Compounds	Strains									
	Mycobacterium tuberculosis My 331/88		*Mycobacterium kansasii* My 235/80			*Mycobacterium kansasii* My 6509/96			*Myobacterium* My 330/88	
	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d	14 d	21 d
6a	>500	>500	500	>500	>500	500	>500	>500	500	>500
6b	>125	>250	125	250	>125	125	250	250	125	125
6c	>125	>250	>125	>125	>250	125	>125	>250	125	>125
6d	125	125	62	125	125	62	62	125	62	>62
6e	62	62	62	62	62	32	62	62	62	62
6f	>62	>62	>62	>125	>250	>62	>62	>250	>62	>125
6g	>125	>250	>250	>500	>1000	>250	>250	1000	>250	>500
6h	>1000	>1000	1000	>1000	>1000	1000	1000	1000	1000	1000
6i	500	1000	500	1000	1000	250	500	500	500	1000
6j	500	500	250	500	500	250	500	500	250	250
INH	0.5	1	>250	>250	>250	2	4	4	>250	>250

4-nitro derivative (**4n**) exceeded that of INH against nontuberculous mycobacteria (MIC 8–16 μmol L^{-1}), while the activity against *M. tuberculosis* was lower (MIC = 62 μmol L^{-1}).

The compounds with an aliphatic alkyl chain **6** are summarised in Table 2. The values of MICs are in the range 32–1000 μmol L^{-1} against all the tested mycobacterial strains. The results clearly show that the most active compounds in the series are pentyl (**6d**) and hexyl (**6e**) derivatives with activity between 62 and 125 μmol L^{-1}. The activities of the two derivatives exceed that of INH against *M. kansasii* 235/80 and *M. avium* 330/88. The lowest MICs were observed for **6h**, **6i** (MIC = 250–1000 μmol L^{-1}).

The starting benzimidazole-2-thiol (**2**) is characterised by activity within the range 125–1000 μmol L^{-1}. Comparison of the data for compounds **2** to those of the benzylsulphanyl derivatives indicates that the activity is linked to the substitution at position 2 of the benzimidazole ring, as the substitution of the hydrogen atom of the –SH group at this position with an alkyl/arylalkyl chain enhances the activity.

A more significant effect against the above mentioned strains was displayed by the benzylsulphanyl derivatives **4** and **7**. The activity was further improved upon modification of the benzyl moiety by electron-withdrawing groups. The most active derivative was the 3,5-dinitro compound **4t**. As regards the substitution with an aliphatic chain, the optimum chain length was found to be 5–6 carbon atoms.

These findings about the relationship between the structure of alkylsulphanyl derivatives and their antimycobacterial activity appear to be in agreement with our previous results on pyridine derivatives [6–8,10].

The complete series of benzylsulphanyl derivatives (**4** and **7**) was also evaluated for antifungal activity. The results are summarised in Table 3. We observed that only three of the eight fungi strains were susceptible to some of the derivatives tested. The most sensitive strains were *Trichophyton mentagrophytes* 445, *Aspergillus fumigatus* 231 and *Absidia corymbifera* 272. As far as the antifungal effect is concerned, some monosubstituted (**4e**, **4f**, **4l**, **4m**, **4p**) and disubstituted (**4q**, **4s**, **4t**, **4u**, **4v**) derivatives showed rather weak activities between 62 and 125 μmol L^{-1}. The values of MICs against all the remaining strains were higher than 125 μmol L^{-1}. It is evident from Table 3 that the activity of the standard ketoconazole (0.98–31.25 μmol L^{-1}) was not achieved.

Table 3
In vitro antifungal activity of some benzylsulphanyl derivatives **4** expressed as MIC (μmol L^{-1})

MIC	Strains		
	TM (72 h)	AC (24 h)	AF (24 h)
62	**4e**, **4f**, **4l**, **4m**, **4p**, **4q**, **4s**, **4t**, **4u**	**4f**	**4e**, **4s**
125	**4v**	**4e**	

TM: *Trichophyton mentagrophytes* 445; AC: *Absidia corymbifera* 272; AF: *Aspergillus fumigatus* 231. Standard Ketoconazole: TM (MIC 0.98), AC (MIC 31.25), AF (MIC 7.81).

The most active compound **4t** 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 11.4 and 9.7 μg mL^{-1}. The cytotoxic effect was evaluated on HeLa cells. The cytotoxic concentration expressed as CC_{50} (CC_5) was 15.3 (3.5) μg mL^{-1}. According to the values of GI and CC, compound **4t** can be considered as moderately toxic.

5. Experimental

5.1. Chemistry

The melting points were determined in 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 CHNS-O CE instrument (FISONS EA 1110) and were within ± 0.4% of the calculated values. IR spectra were obtained in a Nicolet Impact 400 spectrometer in KBr pellets and $CHCl_3$. NMR spectra were recorded in DMSO-d_6 solutions at ambient temperature in 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. The reactions were monitored and the purity of the products was checked by TLC (Silufol UV 254 Kavalier, Votice, Czech republic and E. Merck TLC plates silica gel 60 F_{254}, aluminium back) in acetone–light petroleum (for benzylsulphanyl derivatives), ethylacetate–light petroleum (for alkylsulphanyl derivatives). The plates were visualised 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 in silica gel 60 F_{254} (0.015–0.040 mm, Merck). Silica gel 60 (0.015–0.040 mm, Merck) was used for column chromatography.

The following compounds had been described in the literature: **4a**, R = H [24–26]; **4b**, R = 4-Cl [26,27]; **4d**, R = 2-Cl [28]; **4n**, R = 4-NO_2 [26]; **4u**, R = 2,4-$(NO_2)_2$ [24]; **6a**, R^1 = isopropyl [24]; **6b**, R^1 = butyl [27,29,30]; **6c**, R^1 = isobutyl [30]; **6e**, R^1 = hexyl [29]; **6h**, R^1 = ethyl [30,31]; **6i**, R^1 = allyl [30–32]; **6j**, R^1 = propyl [30,31].

5.1.1. General procedure for compounds *4a–z*, *aa*

Benzimidazol-2-thiol **2** (0.75 g, 5 mmol) in dry *N*,*N*-dimethylformamide (8 mL) was added to a solution of

Table 4
Yields and physicochemical properties of the compounds prepared

Compound number	R	R[1]	Formula (molecular weight)	Reaction time (h)	Yield (%)	M.p. (°C) (crystallisation solvent)
4a	H	–	$C_{14}H_{12}N_2S$ (240.3)	5	68	182–183 [a] (EtOH–H_2O)
4b	4-Cl	–	$C_{14}H_{11}ClN_2S$ (274.8)	4	51	180–181 [b] (EtOH–H_2O)
4c	3-Cl	–	$C_{14}H_{11}ClN_2S$ (274.8)	3.5	47	145–147 (EtOH–H_2O)
4d	2-Cl	–	$C_{14}H_{11}ClN_2S$ (274.8)	6	44	160–164 [c] (MeOH–H_2O)
4e	4-F	–	$C_{14}H_{11}FN_2S$ (258.3)	4.5	62	142–146 (EtOH–H_2O)
4f	3-F	–	$C_{14}H_{11}FN_2S$ (258.3)	4.5	43	153–155 (MeOH–H_2O)
4g	2-F	–	$C_{14}H_{11}FN_2S$ (258.3)	5.5	70	171–173 (EtOH–H_2O)
4h	4-Br	–	$C_{14}H_{11}BrN_2S$ (319.2)	4	63	198–201 (EtOH)
4i	3-Br	–	$C_{14}H_{11}BrN_2S$ (319.2)	7	72	161–163 (EtOH)
4j	4-CH_3	–	$C_{15}H_{14}N_2S$ (254.4)	7.5	71	157–161 (EtOH–H_2O)
4k	3-CH_3	–	$C_{15}H_{14}N_2S$ (254.4)	8	63	125–131 (EtOH–H_2O)
4l	4-OCH_3	–	$C_{15}H_{14}N_2OS$ (270.4)	3	37	124–129 (EtOH–H_2O)
4m	3-OCH_3	–	$C_{15}H_{14}N_2OS$ (270.4)	3.5	58	116–123 (EtOH–H_2O)
4n	4-NO_2	–	$C_{14}H_{11}N_3O_2S$ (285.3)	6	56	188–191 [d] (MeOH–H_2O)
4o	3-NO_2	–	$C_{14}H_{11}N_3O_2S$ (285.3)	5	70	111–113 (MeOH–H_2O)
4p	2-NO_2	–	$C_{14}H_{11}N_3O_2S$ (285.3)	3	84	133–135 (MeOH–H_2O)
4q	2-F-6-Cl	–	$C_{14}H_{10}FClN_2S$ (292.8)	3	62	174–178 (EtOH–H_2O)
4r	3.4-Cl_2	–	$C_{14}H_{10}Cl_2N_2S$ (309.2)	4	45	173–174 (EtOH–H_2O)
4s	3.4-F_2	–	$C_{14}H_{10}F_2N_2S$ (276.3)	6	51	127–130 (EtOH–H_2O)
4t	3.5-$(NO_2)_2$	–	$C_{14}H_{10}N_4O_4S$ (330.3)	5	46	155–158 (MeOH–H_2O)
4u	2.4-$(NO_2)_2$	–	$C_{14}H_{10}N_4O_4S$ (330.3)	4	25	178–182 [e] (EtOH–H_2O)
4v	2-F-6-NO_2	–	$C_{14}H_{10}FN_3O_2S$ (303.3)	3	62	130–134 (MeOH–H_2O)
4w	4-CF_3	–	$C_{15}H_{11}F_3N_2S$ (308.3)	3	85	200–204 (EtOH)
4x	3-CF_3	–	$C_{15}H_{11}F_3N_2S$ (308.3)	5	91	148–149.5 (EtOH)
4y	3.5-$(CF_3)_2$	–	$C_{16}H_{10}F_6N_2S$ (376.3)	4	96	139–144 (EtOH–H_2O)
4z	4-CN	–	$C_{15}H_{11}N_3S$ (265.3)	4	97	178–184 (EtOH–H_2O)
4aa	3-CN	–	$C_{15}H_{11}N_3S$ (265.3)	5.5	97	153–158 (EtOH–H_2O)
6a	–	$(CH_3)_2CH$	$C_{10}H_{12}N_2S$ (192.3)	12.5	45	181–184.5 [f] (EtOAc)
6b	–	C_4H_9	$C_{11}H_{14}N_2S$ (206.3)	8	47	133.5–135 [g] (EtOAc)
6c	–	$(CH_3)_2CH_2CH$	$C_{11}H_{14}N_2S$ (206.3)	9.5	19	165–167 [h] (EtOAc)
6d	–	C_5H_{11}	$C_{12}H_{16}N_2S$ (220.3)	12	44	103–106 (EtOAc)
6e	–	C_6H_{13}	$C_{13}H_{18}N_2S$ (234.4)	14.5	30	103–106 [i] (EtOAc)
6f	–	C_6H_5–CH_2–CH_2	$C_{15}H_{14}N_2S$ (254.4)	7	47	151–152 (EtOAc)
6g	–	$C_{16}H_{33}$	$C_{23}H_{38}N_2S$ (374.6)	18	35	89–90 (EtOAc)
6h	–	C_2H_5	$C_9H_{10}N_2S$ (178.3)	12	44	172–173.5 [j] (EtOAc)
6i	–	CH_2=CH–CH_2	$C_{10}H_{10}N_2S$ (190.3)	8.5	18	133.5–135 [k] (EtOAc)
6j	–	C_3H_7	$C_{10}H_{12}N_2S$ (192.3)	20	63	161–162 [l] (EtOAc)
7a	4-$CSNH_2$	–	$C_{15}H_{13}N_3S_2$ (299.4)	1.5	83	182–186 (EtOH–H_2O)
7b	3-$CSNH_2$	–	$C_{15}H_{13}N_3S_2$ (299.4)	2.5	83	148–155 (EtOH–H_2O)

[a] Refs. [24–26] give 184, 180–182, 187–188 °C.
[b] Refs. [26,27] give 185–186, 185 °C.
[c] Ref. [28] gives 141 °C.
[d] Ref. [26] gives 191–192 °C.
[e] Ref. [24] gives 181 °C.
[f] Ref. [24] gives 187 °C.
[g] Refs. [27,29,30] give 135, 134, 134–134.5 °C.
[h] Ref. [30] gives 167 °C.
[i] Ref. [29] gives 105 °C.
[j] Refs. [30,31] give 173, 173–174 °C.
[k] Refs. [30–32] give 139–140, 140–142 °C.
[l] Refs. [30,31] give 161, 161–162 °C.

sodium (0.12 g, 5 mmol) in dry methanol (2.5 mL). After 10 min of stirring at room temperature, a benzyl halide **3** (5 mmol) was added in 2–3 portions, and the resultant suspension was stirred for 3–8 h. The reaction mixture was then poured into an ice bath and left at overnight. The solid was filtered off, washed with cold water (2×30 mL) and air-dried. The crude products were purified by preparative TLC using acetone–light petroleum (1:2, 1:3, 1:4, 1:5 or 1:6), followed by crystallisation from ethanol, ethanol–water or methanol–water to afford the white or yellowish needles of the pure compounds in 25–97% yields (Table 4).

Table 5
^{1}H-NMR and IR spectroscopic data of the compounds prepared

Compound number	^{1}H-NMR δ (ppm)	IR ν, δ (cm^{-1})
4a	4.57 (2H, s), 7.05–7.15 (2H, m), 7.20–7.35 (3H, m), 7.40–7.55 (4H, m)	3441 (NH), 3071–2344 (H-bridges)
4b	4.53 (2H, s), 7.05–7.15 (2H, m), 7.30–7.40 (2H, m AA′BB′), 7.40–7.50 (4H, m)	3432 (NH), 3064–2054 (H-bridges)
4c	4.54 (2H, s), 7.05–7.15 (2H, m), 7.20–7.35 (2H, m), 7.35–7.50 (3H, m), 7.51 (1H, bs)	3443 (NH), 3071–2631 (H-bridges)
4d	4.65 (2H, s), 7.10–7.20 (2H, m), 7.20–7.65 (4H, m), 12.62 (1H, bs)	3451 (NH), 3070–2630 (H-bridges)
4e	4.53 (2H, s), 7.05–7.15 (4H, m), 7.35–7.50 (4H, m)	3440 (NH), 3070–2346 (H-bridges)
4f	4.55 (2H, s), 6.95–7.15 (3H, m), 7.20–7.35 (3H, m), 7.40–7.50 (2H, m)	3432 (NH), 3060–2614 (H-bridges)
4g	4.57 (2H, s), 7.05–7.25 (4H, m), 7.25–7.35 (1H, m), 7.40–7.50 (2H, m), 7.51 (1H, td overlapped, $J_1 = 1.6$ Hz, $J_2 = 7.7$ Hz)	3448 (NH), 3134–2638 (H-bridges)
4h	4.53 (2H, s), 7.05–7.15 (2H, m), 7.30–7.60 (2H, m overlapped), 7.35–7.45 (2H, m AA′BB′ overlapped), 7.45–7.55 (2H, m AA′BB′ overlapped)	3432 (NH), 3068–2611 (H-bridges)
4i	4.55 (2H, s), 7.05–7.15 (2H, m), 7.24 (1H, t, $J = 7.8$ Hz), 7.30–7.55 (4H, m), 7.67 (1H, t, $J = 1.7$ Hz)	3432 (NH), 3069–2629 (H-bridges)
4j	2.24 (3H, s), 4.51 (2H, s), 7.05–7.15 (4H, m), 7.30–7.35 (2H, m AA′BB′), 7.40–7.50 (2H, m)	3447 (NH), 3063–2630 (H-bridges), 2860 (CH_3), 1445 (CH_3), 1363 (CH_3)
4k	2.25 (3H, s), 4.52 (2H, s), 7.00–7.30 (6H, m), 7.30–7.45 (1H, m), 7.50–7.60 (1H, m)	3445 (NH), 3042–2450 (H-bridges), 2858 (CH_3), 1439 (CH_3), 1364 (CH_3)
4l	.69 (3H, s), 4.50 (2H, s), 6.80–6.90 (2H, m AA′BB′), 7.05–7.15 (2H, m), 7.30–7.40 (2H, m AA′BB′ overlapped), 7.30–7.40 (1H, m overlapped), 7.50–7.55 (1H, m)	3443 (NH), 3066–2635 (H-bridges)
4m	3.68 (3H, s), 4.52 (2H, s), 6.80 (1H, ddd, $J_1 = 1.0$ Hz, $J_2 = 2.5$ Hz, $J_3 = 8.2$ Hz), 6.95–7.05 (2H, m), 7.05–7.15 (2H, m), 7.21 (1H, t, $J = 7.7$ Hz), 7.30–7.60 (2H, m)	3438 (NH), 3063–2488 (H-bridges)
4n	4.68 (2H, s), 7.05–7.15 (2H, m), 7.35–7.55 (2H, m), 7.70–7.80 (2H, m AA′BB′), 8.10–8.20 (2H, m AA′BB′)	3440 (NH), 3077–2614 (H-bridges), 1518 (NO_2), 1348 (NO_2)
4o	4.69 (2H, s), 7.05–7.15 (2H, m), 7.35–7.55 (2H, m), 7.58 (1H, t, $J = 8.0$ Hz), 7.91 (1H, dm, $J = 8.0$ Hz), 8.07 (1H, ddd, $J_1 = 0.9$ Hz, $J_2 = 2.1$ Hz, $J_3 = 8.0$ Hz), 8.38 (1H, t, $J = 2.1$ Hz)	3443(NH), 3074–2448 (H-bridges), 1526 (NO_2), 1350 (NO_2)
4p	4.84 (2H, s), 7.05–7.15 (2H, m), 7.25–7.60 (2H, m overlapped), 7.51 (1H, td overlapped, $J_1 = 1.5$ Hz, $J_2 = 7.7$ Hz), 7.65 (1H, td, $J_1 = 1.5$ Hz, $J_2 = 7.7$ Hz), 7.78 (1H, dd, $J_1 = 1.5$ Hz, $J_2 = 7.7$ Hz), 8.02 (1H, dd, $J_1 = 1.5$ Hz, $J_2 = 7.7$ Hz)	3420 (NH), 3072–2633 (H-bridges), 1535(NO_2), 1351 (NO_2);
4q	4.68 (2H, d, $J = 1.7$ Hz), 7.10–7.20 (2H, m), 7.20–7.30 (1H, m), 7.35–7.50 (3H, m), 7.50–7.60 (1H, m)	3443 (NH), 3064–2476 (H-bridges)
4r	4.54 (2H, s), 7.05–7.15 (2H, m), 7.35–7.50 (2H, m overlapped), 7.43 (1H, dd overlapped, $J_1 = 1.9$ Hz, $J_2 = 8.2$ Hz), 7.54 (1H, d, $J = 8.2$ Hz), 7.73 (1H, d, $J = 1.9$ Hz)	3443 (NH), 3065–2636 (H-bridges)
4s	4.54 (2H, s), 7.05–7.15 (2H, m), 7.25–7.40 (2H, m), 7.40–7.50 (2H, m), 7.53 (1H, ddd, $J_1 = 2.2$ Hz, $J_2 = 8.0$ Hz, $J_3 = 11.8$ Hz)	3447 (NH), 3043–2502 (H-bridges)
4t	4.79 (2H, s), 7.05–7.15 (2H, m), 7.30–7.55 (2H, m), 8.66 (1H, t, $J = 2.2$ Hz), 8.83 (2H, d, $J = 2.2$ Hz)	3424 (NH), 2969–2811 (H-bridges), 1541(NO_2), 1345(NO_2)
4u	4.92 (2H, s), 7.05–7.15 (2H, m), 7.35–7.50 (2H, m), 8.08 (1H, d, $J = 8.5$ Hz), 8.47 (1H, dd, $J_1 = 2.5$ Hz, $J_2 = 8.5$ Hz), 8.69 (1H, d, $J = 2.5$ Hz)	3427 (NH), 2955–2602 (H-bridges), 1531(NO_2), 1345 (NO_2)
4v	4.82 (2H, d, $J = 1.4$ Hz), 7.05–7.15 (2H, m), 7.35–7.50 (2H, m), 7.50–7.70 (2H, m), 7.83 (1H, dt, $J_1 = 1.3$ Hz, $J_2 = 7.7$ Hz)	3423 (NH), 3096–2807 (H-bridges) 1530 (NO_2), 1352 (NO_2)
4w	4.64 (2H, s), 7.05–7.15 (2H, m), 7.40–7.50 (2H, m), 7.66 (4H, bs)	3432 (NH), 3074–2618 (H-bridges)
4x	4.64 (2H, s), 7.05–7.15 (2H, m), 7.40–7.50 (2H, m), 7.50–7.65 (2H, m), 7.75 (1H, bd, $J = 7.4$ Hz), 7.85 (1H, bs)	3446 (NH), 3079–2460 (H-bridges)

Table 5 (*Continued*)

Compound number	^{1}H-NMR δ (ppm)	IR ν, δ (cm^{-1})
4y	4.70 (2H, s), 7.05–7.15 (2H, m), 7.30–7.40 (1H, m), 7.45–7.55 (1H, m), 7.95 (1H, bs), 8.21 (2H, bs)	3443 (NH), 2961–2618 (H-bridges)
4z	4.63 (2H, s), 7.05–7.15 (2H, m), 7.40–7.50 (2H, m), 7.60–7.65 (2H, m AA′BB′), 7.75–7.80 (2H, m AA′BB′)	3428 (NH), 3088–2610 (H-bridges), 2227 (CN)
4aa	4.60 (2H, s), 7.05–7.15 (2H, m), 7.40–7.50 (2H, m overlapped), 7.50 (1H, t overlapped, $J = 7.7$ Hz), 7.70 (1H, dt, $J_1 = 1.5$ Hz, $J_2 = 7.7$ Hz), 7.79 (1H, dt, $J_1 = 1.5$ Hz, $J_2 = 7.7$ Hz), 7.92 (1H, bt, $J = 1.5$ Hz)	3432 (NH), 3065–2614 (H-bridges), 2233 (CN)
6a	1.38 (6H, d, $J = 6.6$ Hz), 3.85–4.05 (1H, m), 7.05–7.15 (2H, m), 7.35–7.50 (2H, m)	3439 (NH), 2965, 1403, 1351
6b	0.88 (3H, t, $J = 7.3$ Hz), 1.30–1.50 (2H, m), 1.60–1.75 (2H, m), 3.26 (2H, t, $J = 7.1$ Hz), 7.05–7.15 (2H, m), 7.35–7.50 (2H, m), 12.49 (1H, bs)	3443 (NH), 2956, 1433, 1394
6c	0.99 (6H, d, $J = 6.9$ Hz), 1.85–2.05 (1H, m), 3.18 (2H, d, $J = 6.9$ Hz), 7.05–7.15 (2H, m), 7.35–7.50 (2H, m), 12.5 (1H, bs)	3432 (NH), 2957, 1441, 1398
6d	0.85 (3H, t, $J = 7.2$ Hz), 1.20–1.45 (4H, m), 1.60–1.75 (2H, m), 3.25 (2H, t, $J = 7.3$ Hz), 7.05–7.15 (2H, m), 7.35–7.50 (2H, m), 12.5 (1H, bs)	3447 (NH), 2957, 1440, 1401
6e	0.84 (3H, t, $J = 7.1$ Hz), 1.15–1.30 (4H, m), 1.30–1.50 (2H, m), 1.60–1.75 (2H, m), 3.25 (2H, t, $J = 7.3$ Hz), 7.05–7.15 (2H, m), 7.35–7.45 (2H, m)	3447 (NH), 2957, 1440, 1401
6f	2.95–3.10 (2H, m), 3.45–3.60 (2H, m), 7.05–7.15 (2H, m), 7.20–7.25 (1H, m), 7.25–7.35 (4H, m), 7.40–7.50 (2H, m)	3432 (NH), 2959, 1439, 1402
6g	0.88 (3H, t, $J = 6.6$ Hz), 1.10–1.35 (24H, m), 1.35–1.50 (2H, m), 1.65–1.85 (2H, m), 3.33 (2H, t, $J = 7.4$ Hz), 7.15–7.25 (2H, m), 7.40–7.60 (2H, m)	3444 (NH), 2919, 2850, 1442, 1402
6h	1.35 (3H, t, $J = 7.4$ Hz), 3.25 (2H, q, $J = 7.4$ Hz), 7.05–7.15 (2H, m), 7.35–7.50 (2H, m)	3432 (NH), 2970, 1441, 1403
6i	3.94 (2H, dm, $J = 6.9$ Hz), 5.05–5.15 (1H, m), 5.25–5.35 (1H, m), 5.90–6.10 (1H, m), 7.00–7.20 (2H, m), 7.30–7.55 (2H, m)	3447 (NH), 2965, 1441, 1406
6j	0.98 (3H, t, $J = 7.3$ Hz), 1.65–1.80 (2H, m), 3.23 (2H, t, $J = 7.1$ Hz), 7.05–7.15 (2H, m), 7.50–7.30 (2H, m), 12.5 (1H, bs)	3432 (NH), 2967, 1433, 1397
7a	4.58 (2H, s), 7.05–7.15 (2H, m), 7.30–7.60 (4H, m), 7.75–7.85 (2H, m AA′BB′), 9.44 (1H, s), 9.83 (1H, s)	3446 (NH), 2965–2641 (H-bridges), 1434 (C = S)
7b	4.60 (2H, s), 7.05–7.15 (2H, m), 7.30–7.40 (1H, m overlapped), 7.33 (1H, t overlapped, $J = 7.8$ Hz), 7.45–7.55 (1H, m), 7.57 (1H, bd, $J = 7.8$ Hz), 7.70 (1H, bd, $J = 7.8$ Hz), 8.01 (1H, bt, $J = 1.8$ Hz), 9.50 (1H, s), 9.88 (1H, s)	3424 (NH), 1438 (C = S), 3076–2796 (H-bridges)

*5.1.2. General procedure for compounds **6a–g***

Benzimidazole-2-thiol (**2**) (0.6 g, 4 mmol) was added to a stirred solution of sodium (0.1 g, 4 mmol) in 96% ethanol (20 mL). After 10 min, an equivalent amount of an alkyl halide **5** in 96% ethanol (10 mL,) was added dropwise and the mixture was heated under reflux for 6–18 h. The reaction mixture was then cooled to room temperature, and concentrated to dryness in vacuo. The residue was redissolved in ethylacetate, and the solution washed with brine (3 ×). The organic layer was dried over Na_2SO_4 and the solvent removed. Purification of the crude products by column chromatography (light petroleum–ethylacetate, 85:15; light petroleum–ethylacetate, 95:5; light petroleum–chloroform, 1:1), followed by crystallisation from ethylacetate afforded pure compounds as white needles in 19–47% yield (Table 4).

*5.1.3. General procedure for compounds **6h–j***

Alkyl halide **5** (7 mmol) was added to a solution of benzimidazole-2-thiol (**2**) (1.0 g, 7 mmol) and tetrabutylammonium bromide (0.1 g, 0.35 mmol) in cyclohexane (20 mL). A 2 M aqueous solution of KOH (10.6 mL) was further added and the resulting two-phase system was vigorously stirred under reflux for 2–5 days depending on the reactivity of halide **5**. The reaction mixture was cooled to room temperature, the phases were separated, and the organic phase was washed with H_2O (3 ×) and dried over Na_2SO_4. The solvent was removed in vacuo, and subsequent purification of the

crude residue by column chromatography (light petroleum–ethylacetate, 85:15; light petroleum–ethylacetate, 95:5; light petroleum–chloroform, 1:1), followed by crystallisation from ethylacetate gave pure products as white solids in 18–63% yields (Table 4).

*5.1.4. General procedure for compounds **7a** and **7b***

Dry hydrogen sulphide was passed through the solution of a cyano compound (**4z**, **4aa)** (0.5 g, 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 3–4 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. Preparative TLC chromatography in acetone–light petroleum (1:1 or 1:2) and crystallisation from ethanol–water gave the products as yellow needles in 83% yields (Table 4).

5.2. Microbiological methods

5.2.1. Antimycobacterial activity

The antimycobacterial activities of the compounds were determined in the Šula semisynthetic medium (SEVAC, Prague). The compounds were added to the medium in dimethylsulphoxide solutions. The following concentration were used: 1000, 500, 250, 125, 62, 32, 16, 8, and 4 μmol L^{-1}. MICs were determined after incubation at 37 °C for 7, 14, and 21 days. MIC was the lowest concentration of a substance, at which the inhibition of the growth of mycobacteria occurred.

5.2.2. Antifungal activity

The microdilution broth test was used for the assessment of the antifungal effect. All substances were dissolved in dimethylsulphoxide. Two-fold serial dilutions of the compounds were used (1000–15 μmol L^{-1}) provided that a given compound was soluble in dimethylsulphoxide 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-propanesulphonic acid. Drug-free controls were included. The yeast inocula were prepared from 24 to 72 h colonies grown on the Sabouraud agar at 37 °C. Conidia from 5 to 10-day-old colonies were used to obtain suspensions of filamentous fungi. The cell density in a 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 h of static incubation at 35 °C. In the case of *Trichophyton mentagrophytes*, the MICs were recorded after 72 and 120 h of incubation.

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