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Heterocyclic Benzazole Derivatives with Antimycobacterial In Vitro Activity

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Abstract—The series of 2-benzylsulfanyl derivatives of benzoxazole and benzothiazole were synthesized, evaluated for their in vitro antimycobacterial activity against *Mycobacterium tuberculosis* and non-tuberculous mycobacteria, and the activity expressed as the minimum inhibitory concentration (MIC) in μmol/L. The substances bearing two nitro groups (**4e**, **4f**, **5e**, **5f**) or a thioamide group (**4i**, **4j**, **5i**, **5j**) exhibited appreciable activity particularly against non-tuberculous strains. The most active compounds were subjected to the toxicity assay and were evaluated as moderately cytotoxic.

Worldwide, tuberculosis (TB) still remains a major public health problem, and it is estimated that new infections of humans by *Mycobacterium tuberculosis* are greater than 8 million annually, and more than 3 million people will die from this disease each year.[1] Therefore, the development of specific and potent chemotherapeutics is an important goal in pharmaceutical research. An urgent need for novel antituberculous agents is justified by several important factors: (a) the incidence and prevalence of opportunistic infections due to nontuberculous mycobacteria, nowadays especially the *Mycobacterium avium* complex, seen particularly in persons with AIDS; (b) the emergence of multidrug resistant strains of *M. tuberculosis* to currently administered therapeutic agents; (c) immigration from countries where TB is common (Eastern Europe and Africa), social problems (war, famine, homelessness); (d) inadequate funding for TB control and other public health efforts.[2]

In our earlier paper, we have reported a large number of derivatives of pyridine,[3–5] which showed potential antimycobacterial activity and detailed QSAR findings which support the hypothesis that an alkylsulfanyl group bound to an electron deficient carbon atom in various heterocycles is responsible for antimycobacterial activity.[6] Significant antimycobacterial activity was observed in a series of 4-benzylsulfanyl derivatives of pyridine-2-carbonitrile/2-carbothioamide.[7] Continuing our search for new substances containing heterocyclic moieties other than pyridin, we designed the structure of benzimidazole. The set of benzimidazole with a different substituted benzylsulfanyl moiety in position 2 elicited remarkable activity on both tuberculous and especially nontuberculous strains of mycobacteria, where it exceeded the activity of the standard INH.[8] To investigate the substituent effect at positions 2 and 5, we introduced a methyl group into position 5 of the benzimidazole core. A newly prepared set of 2-benzylsulfanyl derivatives of 5-methylbenzimidazole exhibited comparable or even improved antituberculous potency in comparison with benzimidazole derivatives. A most significant effect in both sets was exhibited by mononitro-, dinitro- and thioamide groups in the benzyl moiety.[9] In our further efforts to investigate benzylsulfanyl derivatives, we focused on other heterocyclic compounds. We decided to simply replace the atom of nitrogen of the benzimidazole ring with the corresponding isosteric atom of

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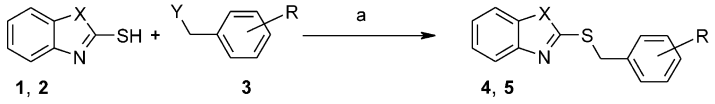
oxygen and sulfur, respectively. In the present communication, we wish to report the synthesis and preliminary antimycobacterial evaluation, including toxicity of the 2-benzylsulfanyl derivatives of benzoxazole/benzothiazole. The results mentioned above demonstrate that the most active derivatives are modified by nitro and thioamide groups.

The synthetic scheme employed for the preparation of the desired compounds (**4**, **5**) is depicted in Schemes 1 and 2. The synthesis utilized commercially available benzoxazole-2-thiol (**1**) and benzothiazole-2-thiol (**2**). Thiols (**1**, **2**) were converted to the corresponding sodium salts by dissolving in an ethanolic solution of sodium ethanolate, and the resulting salts were subjected to a nucleophilic substitution upon the addition of a benzyl halide (**3**). The reaction was carried out in *N*,*N*-dimethylformamide (DMF) at room temperature for 2–6 h and furnished products **4a**–**h** and **5a**–**h** (Scheme 1).[10] The benzylsulfanyl derivatives bearing a CN group (**4g**, **4h**, **5g**, **5h**) were further converted into the corresponding thioamide (**4i**, **4j**, **5i**, **5j**) through the reaction with hydrogen sulfide in the presence of pyridine/triethylamine solution (Scheme 2).[11]

All newly synthesized compounds were evaluated for their antimycobacterial activity against a set of four mycobacterial strains, *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).[12] The MIC values are summarized in Table 1.[13] In several cases (denoted >), the minimum inhibitory concentration could not be determined due to limited solubility of the compounds in the testing medium. For the sake of comparison, we also included the MIC values of the starting materials (**1**, **2**) and the standard isoniazide (INH). A detailed observation of the set of benzoxazole derivatives (**4**) showed that MIC values are generally within a range of 2–500 μmol/L, most often between 8 and 32 μmol/L. The compounds were less active than INH against *M. tuberculosis* 331/88 and against *M. kansasii* 6509/96. On the other hand, the compounds possessed a better activity against *M. kansasii* 235/80 and *M. avium* 330/88 than INH. The starting benzoxazole-2-thiol (**1**) was only slightly active (MIC = 250–1000 μmol/L). The introduction of the benzylsulfanyl moiety resulted in an increase in the activity. The unsubstituated benzylsulfanyl derivative (**4a**) displayed a range of activities from 32 to 500 μmol/L with a better activity against non-tuberculous strains. In contrast to initial expectations, mononitro derivatives (**4b**, **4c**, **4d**) displayed only low potency against all strains tested (MIC = 32–> 250 μmol/L). The incorporation of a second nitro group, leading to compounds **4e**, **4f**, generally produced an enhancement of the activity. Both compounds **4e** and **4f** exhibited significant activity against *M. kansasii* 235/80 (MIC_{4e} = 2–4 μmol/L, MIC_{4f} = 4 μmol/L) and exceeded the activity of INH (MIC = > 250 μmol/L). The activity against *M. kansasii* 6509/96 was 2-fold less than that of INH. The compounds showed only weak activity (MIC = 8 μmol/L) against *M. tuberculosis* 331/88 and the activity of INH was not reached (MIC_{INH} = 0.5–1 μmol/L). MICs against *M. avium* 330/88 could not be determined due to insolubility of compounds in the testing medium. With regard to cyano compounds (**4g**, **4h**), none of them showed significant activity. The conversion of the cyano group into a thioamide group afforded more potent compounds **4i**, **4j** against all strains tested than the corresponding cyano compounds **4h**, **4g** but they did not

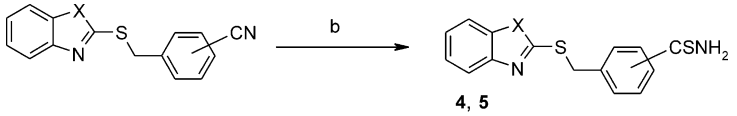


Number	X
1, 4	O
2, 5	S

Y = Cl, Br

4, 5	R
a	H
b	4-NO_2
c	3-NO_2
d	2-NO_2
e	3,5-NO_2
f	2,4-NO_2
g	4-CN
h	3-CN

Scheme 1. Reagents and conditions: (a) Na, ethanol, DMF.



4, 5	
i	4-$CSNH_2$
j	3-$CSNH_2$

Scheme 2. Reagents and conditions: (a) H_2S, TEA, pyridine.

Table 1. In vitro antimycobacterial activity of compounds **1**, **2**, **4a**–**j**, **5a**–**j** expressed as MIC (μmol/L)

Compd	Strains									
	Mycobacterium tuberculosis My 331/88		*Mycobacterium kansasii My 235/80*			*Mycobacterium kansasii 6 509/96*			*Mycobacterium avium My 330/88*	
	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d	14 d	21 d
1	500	500	500	1000	1000	250	500	1000	250	500
4a	250	500	125	250	250	32	125	250	62	125
4b	>62	>125	32	32	>62	32	32	>32	>32	>62
4c	>62	125	32	>32	>32	32	32	>32	>32	>62
4d	>250	>250	125	125	>125	62	>62	>62	>125	>125
4e	8	8	2	4	4	4	8	8	>16	>32
4f	8	8	4	4	4	4	8	8	>16	>32
4g	>62	>62	32	32	>32	16	32	>32	62	62
4h	125	125	32	62	125	32	62	62	62	62
4i	8	16	8	16	16	8	16	16	32	32
4j	8	16	8	32	32	8	32	32	62	62
2	62	62	62	125	>125	62	>62	>62	>62	>62
5a	250	500	62	500	>500	62	125	250	32	62
5b	>32	>125	32	>62	>250	32	>32	>125	32	>62
5c	>62	>125	62	>125	>125	32	62	>62	62	62
5d	125	500	62	125	500	32	32	125	125	>250
5e	2	2	16	16	>62	NE[a]	32	>62	>62	>62
5f	2	4	8	8	16	8	8	>32	>32	>32
5g	62	62	125	250	>500	16	32	32	32	>32
5h	62	250	125	250	500	32	62	125	32	62
5i	4	8	4	8	8	4	8	8	16	32
5j	8	16	8	16	16	8	16	16	32	62
INH	0.5	1	>250	>250	>250	2	2	4	>250	>250

[a]NE, not evaluated; d, days.

reach the activity of dinitro derivatives (**4e**, **4f**). It is worth noting that the 4-substituted derivative (**4i**) had generally greater activity than its 3-substituted analogue (**4j**). The compound **4i** showed better activity, particularly against *M. avium* 330/88, and was the most active compound against this strain in the set (MIC = 32 μmol/L). In the case of benzothiazole derivatives (**5**), in vitro evaluation revealed similar trends as in the benzoxazole set (**4**). Thus, the starting benzothiazole-2-thiol (**2**) and its 2-benzylsulfanyl derivative (**5a**) were found to be only weakly active. Identically, as in the case of the benzoxazole set, mononitro derivatives (**5b**, **5c**, **5d**) exhibited no pronounced activity against all tested strains. The derivatives bearing two nitro groups **5e** and **5f** exhibited the highest activity of both sets against *M. tuberculosis* 331/88 ($MIC_{\mathbf{5e}}$ = 2 μmol/L and $MIC_{\mathbf{5f}}$ = 2–4 μmol/L) and were only 2-fold less active than INH (MIC = 0.5–1 μmol/L). The activity against non-tuberculous strains was weak and did not reach the activity of dinitro derivatives of benzoxazole. A similar effect as in the set of benzoxazole was revealed for cyano and thioamide derivatives. The thioamide derivatives (**5i**, **5j**) exhibited promising activity, particularly **5i** showed an improvement in the activity against *M. avium* 330/88 (MIC = 16–32 μmol/L) and both strains of *M. kansasii* (MIC = 4–8 μmol/L).

Table 2. Cytotoxic (CC_{50}) evaluation of compounds **4e**, **4f**, **4i**, **4j**, **5e**, **5f**, **5i**, **5j** expressed in μmol/L

Compd	HeLa CC_{50}	S.I.[a]
4e	>150.9	>18.9
4f	136.1	17.0
4i	78.6	9.8
4j	71.9	9.0
5e	>143.9	>72.0
5f	>143.9	>72.0
5i	>158.0	>39.5
5j	36.7	4.6

[a]S.I. = $CC_{50}/MIC_{M.\ tuberculosis\ My\ 331/88,\ 14d}$.

The most active derivatives of each set were also subjected to cytotoxic effect assay against the HeLa cells. As reported in Table 2, the values expressed as CC_{50} are ranging from 36.7 to >158.0 μmol/L. The values of CC_{50} were further compared with the microbial data and expressed as Safety Indexes (S.I.). The data revealed that compounds can considered as moderately toxic.[14]

In conclusion, the results revealed that incorporation of the benzylsulfanyl moiety into position 2 is important to the antimycobacterial activity. The substitution of the phenyl ring enhances the activity (**4e**, **4f**, **4i**, **4j**, **5e**, **5f**, **5i**, **5j**). The further modification of the most active compounds and also mechanism of action are in progress.

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10. General procedure for the preparation of compounds **4a**–**h**, **5a**–**h**. Benzazole-2-thiol (**1**, **2**) (5 mmol) in dry DMF (8 mL) was added to a solution of sodium (5 mmol) in dry ethanol (2.5 mL). After 10 min of stirring at ambient temperature, benzyl halide (**3**) (5mmol) was added in 2–3 portions, and the resultant suspension was stirred for 2–6 h. The reaction mixture was then poured into an ice bath and left at it overnight. The solid was filtered off, washed with cold water (2×30 mL) and air-dried. The crude product was purified by preparative TLC using acetone–light petroleum (1:2, 1:3, 1:4, 1:5, 1:6), followed by crystallization from ethanol (95%) to afford the crystals of the pure product **4a**–**h**, **5a**–**h** in 35–97% yield.
11. General procedure for the preparation of compounds **4i**, **4j**, **5i**, **5j**. Dry hydrogen sulfide was passed through a solution of cyano compounds **4g**, **4h**, **5g**, **5h** (2 mmol) dissolved in a mixture of dry pyridine (7 mL) and triethylamine (0.7 mL). The reaction mixture was maintained at ambient temperature for 3 h and then heated at 45 °C for additional h. After cooling, the mixture was poured onto crushed ice with intensive stirring, the precipitated product was filtered off, washed with cold water (2×35 mL) and air-dried. Performed preparative TLC chromatography using acetone–light petroleum (1:1, 1:2) and crystallization from ethanol (95%) yielded products **4i**, **4j**, **5i**, **5j** as yellow crystals in 35–66% yield.
12. 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 in the 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). MICs were determined after incubation at 37 °C for 7, 14, and 21 days. MIC was the lowest concentration of the substance, at which the inhibition of the growth of mycobacteria occurred.
13. All prepared compounds were fully characterized by spectral methods, and their purity checked by elemental analyses. Representative data for the most potent derivatives: compound **4e**: yellow crystals, yield 62.31%, $M_r = 331.30$, mp = 96–106 °C, IR (KBr) ν_{max} 3107, 3086 $(C–H)_{Ar}$, 1627, 1596, 1454 $(C=C)_{Ar}$, 1541, 1345 (NO_2), 1505 (C=N), 1470 (CH_2), 1236 (Ar–H), 863, 731 (Ar–H 1H) cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6) δ 4.85 (2H, s), 7.26–7.37 (2H, m), 7.58–7.66 (2H, m), 8.69 (1H, t, $J = 2.2$ Hz), 8.87 (2H, d, $J = 2.2$ Hz), ^{13}C NMR (75 MHz, DMSO-d_6) 34.0, 110.5, 118.0, 118.5, 124.7, 125.0, 129.9, 141.2, 142.2, 148.1, 151.6, 163.5. Anal. calculated for $C_{14}H_9N_3O_5S$: C 50.76, H 2.74, N 12.68, O 24.15, S 9.68. Found: C 50.65, H 2.77, N 12.53, S 9.70. **4g**: white crystals, yield 79.67%, $M_r = 266.32$, mp = 102–106 °C, IR (KBr) ν_{max} 3048 $(C–H)_{Ar}$, 2227 (C≡N), 1604, 1509, 1453 $(C=C)_{Ar}$, 1470 (CH_2), 1242 (Ar–H), 831 (Ar–H 2H) cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6) δ 4.67 (2H, s), 7.26–7.36 (2H, m), 7.59–7.67 (2H, m), 7.68–7.73 (2H, m AA′BB′), 7.77–7.82 (2H, m AA′BB′), ^{13}C NMR (75 MHz, DMSO-d_6) 35.1, 110.5, 110.6, 118.6, 118.9, 124.7, 124.9, 130.2, 132.7, 141.3, 151.6, 163.7. Anal. calculated for $C_{15}H_{10}N_2OS$: C 67.65, H 3.78, N 10.52, O 6.01, S 12.04. Found: C 67.80, H 3.85, N 10.92, S 11.72. **5i**: yellow crystals, yield 66.37%, $M_r = 316.45$, mp = 143–160 °C, IR (KBr) ν_{max} 3425 (NH), 3061 $(C–H)_{Ar}$, 2924, 1426 (CH_2), 1629, 1455 $(C=C)_{Ar}$, 1273 (Ar–H), 1126 (C=S –NH—C=S), 850 (Ar–H 2H) cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6) δ 4.67 (2H, s), 7.32–7.40 (1H, m), 7.42–7.58 (1H, m overlapped), 7.50–7.58 (2H, m AA′BB′ overlapped), 7.77–7.92 (1H, m overlapped), 7.77–7.85 (2H, m AA′BB′ overlapped), 7.96–8.06 (1H, m), 9.47 (1H, s), 9.86 (1H, s), ^{13}C NMR (75 MHz, DMSO–d_6) 36.3, 121.4, 122.1, 124.8, 126.6, 127.7, 128.8, 134.9, 138.9, 140.2, 152.8, 166.0, 199.8. Anal. calculated for $C_{15}H_{12}N_2S_3$: C 56.93, H 3.82, N 8.85, S 30.39. Found: C 56.76, H 3.95, N 8.65, S 30.27.
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