A Note on the Antitubercular Activities of 1-Aryl-5-benzylsulfanyltetrazoles

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A set of 32 1-phenyl-5-benzylsulfanyltetrazoles substituted on the phenyl ring as well as on the benzyl moiety was synthesized. The compounds were evaluated for *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis*. The activity against *M. tuberculosis* becomes higher with increasing electron-accepting properties of the substituents on the phenyl ring. On the other hand, any substitution on the benzylic moiety decreases the activity.

Keywords: Benzylsulfanyltetrazoles; *Mycobacterium tuberculosis*; Tuberculostatics; Phase-transfer catalysis

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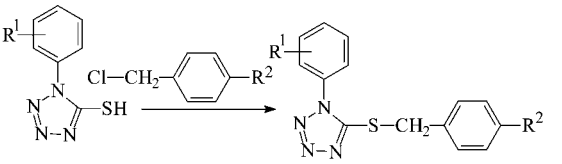
Introduction

The search for structurally novel tuberculostatics is the central theme of research in our group. In previous studies, we focused on antimycobacterial compounds containing the alkylsulfanyl group [1−4], which was identified as a pharmacophore of antimycobacterial activity [5]. Recently, we found that substitution with the benzylsulfanyl group increases the activity of 1-aryl-5-alkylsulfanyltetrazoles [6]. The goal of this study was to determine the structure-activity relationships in the group of the 1-aryl-5-benzylsulfanyltetrazoles substituted on both the phenyl and benzyl rings. In studying the relationships between the structure and antimycobacterial activity, we followed up on the conclusions made in our previous work [6], i.e. that the 5-benzylsulfanyl derivatives are more active than other 5-alkylsulfanyl derivatives.



	R^1		R^2
1	H	**a**	H
2	4-Br	**b**	4-Cl
3	4-Cl	**c**	4-CH_3
4	3,4-Cl_2	**d**	4-CF_3
5	4-CH_3	**e**	4-F
6	4-OCH_3	**f**	4-OCH_3

Scheme 1. Synthesis and structures of 1-aryl-5-benzylsulfanyltetrazoles.

Results

Chemistry

The starting 1-aryltetrazole-5-thiols were prepared from the corresponding substituted anilines following an established protocol [7, 8]. All 5-alkylsulfanyl-1-aryltetrazoles were prepared by the alkylation of 1-aryltetrazol-5-thiols with substituted benzyl chlorides (purchased from Aldrich Chemical Company, Prague, Czech Republic) in toluene/aqueous potassium hydroxide systems using tetrabutylammonium bromide as a phase transfer catalyst. Structures are summarized in Scheme 1. The chemical and physical data of the new 1-phenyl-5-benzylsulfanyltetrazoles are summarized in Table 1. Compounds described in previous papers [6] have not been included in the Table. NMR spectra are given in Table 2.

Microbiology

The tetrazoles were tested for their *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* CNCTC My 331/88, obtained from the Czech National Collection of Type Cultures (CNCTC). The antimycobacterial activities are summarized in Table 3. In several cases, the minimum inhibitory concentration could not be determined due to the limited solubility of the compounds.

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Table 1. Physical data of the new compounds and yields of the syntheses.

Compounds	Mp [°C]	Yield [%]	Compounds	Mp [°C]	Yield [%]
1d	92–93	84	**4a**	49–51	52
1e	91–93	78	**4b**	88–90.5	40
1f	oil	49	**4c**	87.5–89	71
2a	93.5–94	92	**4e**	76–77.5	80
2b	124–125	87	**5d**	98.5–100	90
2c	113–114	76	**5e**	79–80.8	85
2d	95–97	92	**5f**	36.5–38	56
2e	104–106	51	**6a**	42–44	64
3d	74–75	77	**6c**	60–62	52
3e	100–102	76	**6e**	79–83	97
3f	49–51	72	**6f**	60.5–62.5	65

Table 2. ^{1}H- and ^{13}C-NMR spectra of 1-aryl-5-benzylsulfanyltetrazoles.

Compounds	NMR, δ
1d	^{1}H NMR (300 MHz, $CDCl_3$): δ 7.58–7.56 (bs, 4H, H2′, H3′, H5′, H6′), 7.55–7.49 (m, 5H, H2, H3, H4, H5, H6), 4.64 (s, 2H, SCH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.3, 139.6, 133.4, 130.2, 129.9 (q, J = 32.6 Hz), 129.8, 129.6, 126.5 (q, J = 272.6 Hz), 125.7 (q, J = 3.7 Hz), 123.7, 36.7
1e	^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56–7.47 (m, 5H, H2, H3, H4, H5, H6), 7.45–7.37 (m, 2H, H2′, H6′), 7.04–6.95 (m, 2H, H3′, H5′), 4.59 (s, 2H, SCH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.4 (d, J = 247.4 Hz), 153.6, 133.4, 131.1 (d, J = 3.4 Hz), 131.0 (d, J = 8.3 Hz), 130.1, 129.7, 123.7, 115.7 (d, J = 21.7 Hz), 36.7
1f	^{1}H NMR (300 MHz, $CDCl_3$): δ 7.55–7.49 (m, 5H, H2, H3, H4, H5, H6), 7.38–7.32 (m, AA′, BB′, 2H, H2′, H6′), 6.87–6.81 (m, AA′, BB′, 2H, H3′, H5′), 4.59 (s, 2H, SCH_2), 3.78 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.4, 154.0, 133.6, 130.5, 130.0, 129.7, 127.0, 123.7, 114.2, 55.3, 37.2
2a	^{1}H NMR (300 MHz, DMSO): δ 7.87–7.79 (m, AA′, BB′, 2H, H2, H6), 7.58–7.52 (m, AA′, BB′, 2H, H3, H5), 7.44–7.37 (m, 2H, H2′, H6′), 7.35–7.23 (m, 3H, H3′, H4′, H5′), 4.58 (s, 2H, SCH_2). ^{13}C NMR (75 MHz, DMSO): δ 154.2, 136.3, 133.2, 132.4, 129.3, 128.8, 128.0, 126.8, 124.0, 37.0
2b	^{1}H NMR (300 MHz, $CDCl_3$): δ 7.70–7.64 (m, AA′, BB′, 2H, H2, H6), 7.44–7.34 (m, 4H, H3, H5, H2′, H6′), 7.32–7.26 (m, AA′, BB′, 2H, H3′, H5′), 4.58 (s, 2H, SCH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.5, 134.2, 133.8, 133.0, 132.4, 130.6, 129.0, 125.2, 124.3, 36.9
2c	^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68–7.62 (m, AA′, BB′, 2H, H2, H6), 7.44–7.38 (m, AA′, BB′, 2H, H3, H5), 7.33–7.27 (m, AA′, BB′, 2H, H2′, H6′), 7.16–7.09 (m, AA′, BB′, 2H, H3′, H5′), 4.60 (s, 2H, SCH_2), 2.33 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.9, 138.2, 132.9, 132.5, 131.9, 129.5, 129.1, 125.2, 124.1, 37.6, 21.1
2d	^{1}H NMR (300 MHz, $CDCl_3$): δ 7.70–7.64 (m, AA′, BB′, 2H, H2, H6), 7.57 (bs, 4H, H2′, H3′, H5′, H6′), 7.43–7.37 (m, AA′, BB′, 2H, H3, H5), 4.64 (s, 2H, SCH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.3, 139.4, 133.0, 132.3, 130.3 (q, J = 33.0 Hz), 129.6, 125.7 (q, J = 3.8 Hz), 125.4 (q, J=274.4 Hz), 125.1, 124.3, 36.8
2e	^{1}H NMR (300 MHz, $CDCl_3$): δ 7.70–7.63 (m, AA′, BB′, 2H, H2, H6), 7.45–7.36 (m, 4H, H3, H5, H2′, H6′), 7.04–6.95 (m, 2H, H3′, H5′), 4.59 (s, 2H, SCH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.4 (d, J = 247.7 Hz), 153.6, 133.0, 132.4, 131.0, 131.0, (d, J = 8.3 Hz), 125.1, 124.1, 115.7 (d, J = 21.7 Hz), 36.8
3d	^{1}H NMR (300 MHz, $CDCl_3$): δ 7.57 (bs, 4H, H2′, H3′, H5′, H6′), 7.55–7.49 (m, AA′, BB′, 2H, H2′, H6′), 7.49–7.44 (m, AA′, BB′, 2H, H3′, H5′), 4.65 (s, 2H, SCH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.4, 139.4, 136.3, 131.8, 130.1, 129.9 (q, J = 33.0 Hz), 129.6, 125.9 (q, J = 275.2 Hz), 125.8 (q, J = 3.7 Hz), 125.0, 36.8
3e	^{1}H NMR (300 MHz, $CDCl_3$): δ 7.53–7.36 (m, 6H, H2, H3, H5, H6, H2′, H6′), 7.04–6.94 (m, 2H, H3′, H5′), 4.60 (s, 2H, SCH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.4 (d, J = 247.7 Hz), 153.6, 136.2, 131.9, 131.0, 131.0 (d, J = 8.3 Hz), 130.0, 124.9, 115.7 (d, J = 21.7 Hz), 36.8
3f	^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55–7.44 (m, 4H, H2, H3, H5, H6), 7.38–7.30 (m, AA′, BB′, 2H, H2′, H6′), 6.89–6.81 (m, AA′, BB′, 2H, H3′, H5′), 4.59 (s, 2H, CH_2), 3.97 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.5, 154.0, 136.1, 132.0, 130.5, 130.0, 126.9, 125.0, 114.2, 55.3, 37.4.

Table 2. *(continued)*.

Compounds	NMR, δ
4a	^{1}H NMR (300 MHz, $CDCl_3$): δ 7.66 (d, 1H, J = 2.5 Hz, H2), 7.61 (d, 1H, J = 8.5 Hz, H5), 7.44−7.29 (m, 6H, H6, H2′, H3′, H4′, H5′, H6′), 4.63 (s, 2H, SCH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.9, 134.9, 134.6, 134.0, 132.6, 131.4, 129.2, 128.9, 128.3, 125.6, 122.7, 37.9
4b	^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67 (d, 1H, J = 2.5 Hz, H2), 7.62 (d, 1H, J = 8.5 Hz, H5), 7.41 (dd, overlaped, 1H, J = 8.5 Hz, J = 2.5 Hz, H6), 7.40−7.34 (m, AA′, BB′, 2H, H2′, H6′), 7.32−7.26 (m, AA′, BB′, 2H, H3′, H5′), 4.52 (s, 2H, SCH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.6, 134.7, 136.3, 134.1, 133.6, 132.5, 131.5, 130.6, 129.0, 125.5, 122.7, 37.0
4c	^{1}H NMR (300 MHz, $CDCl_3$): δ 7.65 (d, 1H, J = 2.5 Hz, H2), 7.60 (d, 1H, J = 8.5 Hz, H5), 7.41 (dd, 1H, J = 8.5 Hz, J = 2.5 Hz, H6), 7.33−7.26 (m, AA′, BB′, 2H, H2′, H6′), 7.18−7.09 (m, AA′, BB′, 2H, H3′, H5′), 4.60 (s, 2H, SCH_2), 2.33 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 154.0, 138.3, 134.5, 134.0, 132.6, 131.8, 131.4, 129.5, 129.1, 125.6, 122.7 37.7, 21.1
4e	^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67 (d, 1H, J = 2.5 Hz, H2), 7.62 (d, 1H, J = 8.5 Hz, H5), 7.44−7.37 (3H, m, H6, H2′, H6′), 7.05−6.96 (m, 2H, H3′, H5′), 4.60 (s, 2H, SCH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.5 (d, J = 247.9 Hz), 153.7, 134.7, 134.1, 132.5, 131.5, 131.0 (d, J = 8.3 Hz), 130.8 (d, J = 3.4 Hz), 125.5, 122.7, 115.8 (d, J = 21.7 Hz), 37.0
5d	^{1}H NMR (300 MHz, $CDCl_3$): δ 7.57 (bs, 4H, H2′, H3′, H5′, H6′), 7.41−7.30 (m, 4H, H2, H3, H5, H6), 4.63 (s, 2H, SCH_2), 2.43 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.3, 140.7, 139.7, 130.9, 130.3, 130.3 (q, J = 29.5 Hz), 129.6, 125.8 (q, J = 272.3 Hz), 125.7 (q, J = 4.0 Hz), 123.6, 36.6, 21.2
5e	^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44−7.29 (m, 6H, H2, H3, H5, H6, H2′, H6′), 7.04−6.95 (m, 2H, H3′, H5′), 4.58 (s, 2H, SCH_2), 2.43 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.4 (d, J = 247.4 Hz), 153.6, 140.6, 131.2 (d, J = 3.4 Hz), 131.0, 131.0 (d, J = 8.3 Hz), 130.3, 123.6, 115.7, (d, J = 21.7 Hz), 36.7, 21.2
5f	^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42−7.28 (m, 6H, H2, H3, H5, H6, H2′, H6′), 6.87−6.80 (m, 2H, H3′, H5′), 4.57 (s, 2H, SCH_2), 3.78 (s, 3H, OCH_3), 2.42 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.4, 153.9, 140.4, 131.0, 130.5, 130.2, 127.1, 123.6, 114.1, 55.2, 37.2, 21.2
6a	^{1}H NMR (300 MHz, $CDCl_3$): δ 7.45−7.25 (m, 7H, H2, H6, H2′, H3′, H4′, H5′, H6′), 7.04−6.96 (m, 2H, H3, H5), 4.60 (s, 2H, SCH_2), 3.86 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.7, 154.0, 135.3, 129.2, 128.8, 128.1, 126.2, 125.5, 114.8, 55.6, 37.5
6c	^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43−7.36 (m, AA′, BB′, 2H, H2, H6), 7.33−7.27 (m, AA′, BB′, 2H, H2′, H6′), 7.15−7.09 (m, AA′, BB′, 2H, H3′, H5′), 7.03−6.97 (m, AA′, BB′, 2H, H3, H5), 4.57 (s, 2H, SCH_2), 3.85 (s, 3H, OCH_3), 2.32 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.6, 154.1, 138.0, 132.1, 129.4, 129.1, 126.2, 125.4, 114.7, 55.6, 37.3, 21.1
6e	^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43−7.35 (m, 4H, H2, H6, H2′, H6′), 7.03−6.94 (m, 4H, H3, H5, H3′, H5′), 4.55 (s, 2H, SCH_2), 3.85 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.4 (d, J = 247.4 Hz), 160.7, 153.8, 131.2 (d, J = 3.5 Hz), 130.9 (d, J = 8.3 Hz), 126.1, 125.4, 115.6 (d, J = 21.4 Hz), 114.8, 55.6, 36.6
6f	^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43−7.37 (m, AA′, BB′, 2H, H2, H6), 7.36−7.30 (m, AA′, BB′, 2H, H2′, H6′), 7.03−6.97 (m, AA′, BB′, 2H, H3, H5), 6.87−6.80 (m, AA′, BB′, 2H, H3′, H5′), 4.56 (s, 2H, SCH_2), 3.85 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.6, 159.4, 154.1, 130.5, 127.1, 126.2, 125.5, 114.7, 114.1, 55.6, 55.2, 37.1

Discussion

In studying the relationship between the structure and antimycobacterial activity, we followed up on the conclusions made in our previous work [6], namely that 5-benzylsulfanyl derivatives are more active than other 5-alkylsulfanyl derivatives. Compounds **1a**, **3a**, and **4a** were the most active ones (approximately eight times less active than isoniazid). The activity against *M. tuberculosis* becomes higher with increasing electron-accepting properties of the substituents on the phenyl ring. On the other hand, any substitution on the benzylic moiety decreases the activity. The key factor that imposes limitations on potential use of the compounds was their low solubility in water. All 1-(4-bromophenyl)-5-benzylsulfanyltetrazoles and 1-(4-chlorophenyl) derivatives and 1-(3,4-dichlorophenyl) derivatives with lipophilic substituents on the benzyl ring were insoluble. Further research has to be focused on electron-accepting substituents possessing a low lipophilicity in the phenyl ring.

Table 3. Minimum inhibitory concentrations of 1-aryl-5-benzylsulfanyltetrazoles.

Compound	R^1	R^2	MIC [μmol/L]	Compound	R^1	R^2	MIC [μmol/L]
1a	H	H	16†	**3f**	4-Cl	4-OCH_3	32
1b	H	4-Cl	†,‡	**4a**	3,4-Cl_2	H	16
1c	H	4-CH_3	62.5†	**4b**	3,4-Cl_2	4-Cl	‡
1d	H	4-CF_3	‡	**4c**	3,4-Cl_2	4-CH_3	‡
1e	H	4-F	‡	**4e**	3,4-Cl_2	4-F	62.5
1f	H	4-OCH_3	§	**5a**†	4-CH_3	H	32†
2a	4-Br	H	‡	**5b**†	4-CH_3	4-Cl	‡
2b	4-Br	4-Cl	‡	**5c**$^{\delta}$	4-CH_3	4-CH_3	62.5†
2c	4-Br	4-CH_3	‡	**5d**	4-CH_3	4-CF_3	‡
2d	4-Br	4-CF_3	‡	**5e**	4-CH_3	4-F	‡
2e	4-Br	4-F	‡	**5f**	4-CH_3	4-OCH_3	32
3a	4-Cl	H	16†	**6a**	4-OCH_3	H	32
3b	4-Cl	4-Cl	†,‡	**6b**$^{\delta}$	4-OCH_3	4-Cl	62.5
3c	4-Cl	4-CH_3	†,‡	**6c**	4-OCH_3	4-CH_3	62.5
3d	4-Cl	4-CF_3	‡	**6e**	4-OCH_3	4-F	125
3e	4-Cl	4-F	‡	**6f**	4-OCH_3	4-OCH_3	32

† data from the ref. [6], ‡ not tested because of low solubility, § not tested because of being an oil.

Acknowledgments

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Experimental

General

The melting points were determined on a Kofler apparatus (C. Reichert, Vienna, Austria). The samples for analysis and antimycobacterial tests were dried over P_4O_{10} at 61 °C and 530 Pa for 24 h. Elemental analysis were performed on a CHNS-O CE elemental analyzer (Fisons EA 1110, Milano, Italy) and were within ± 0.4% of the theoretical values. The IR spectra were measured in $CHCl_3$ on a Nicolet Impact 400 apparatus (Nicolet Instruments Corporation, Madison, WI, USA) or in KBr pellets. TLC was performed on silica gel plates precoated with a fluorescent indicator Silufol UV 254 + 366 (Kavalier, Votice, Czech Republic), with petrolether-ethyl acetate (φ_r = 9:1), or petrolether-ether (φ_r = 9:1) as the mobile phase. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in DMSO-d_6 solution, or $CDCl_3$-*d* solution at ambient temperature on a Varian Mercury-Vx BB 300 spectrometer operating at 300 MHz (Varian, Palo Alto, CA, USA). Chemical shifts were recorded as δ (ppm) and were indirectly referenced to tetramethylsilane *via* the solvent signal (DMSO-d_6 2.49 for ^{1}H or 39.7 ^{13}C; $CDCl_3$-*d* 7.26 for ^{1}H or 77.0 for ^{13}C).

General Procedure for Preparation of 1-Aryl-5-benzylsulfanyl-1,2,3,4-tetrazoles

Tetrabutylammonium bromide (200 mg, 0.6 mmol) was added to a stirred suspension of a 1-aryltetrazol-5-thiol (5 mmol) and an alkyl chloride (5 mmol) in toluene (20 mL), and 1.1 M KOH solution (15 mL). The mixture was stirred and heated to reflux for 4 to 12.5 h until TLC showed the disappearance of the starting thiol (TLC in toluene-aceton (φ_r = 10 : 1). The toluene layer was washed with water, dried over Na_2SO_4, filtered, and the solvent evaporated under reduced pressure. The solid residue was dissolved in methanol, passed through charcoal and recrystallized from ethanol-water or purified by column chromatography (silica gel, mobile phase petrolether-ethyl acetate (φ_r = 9:1, or petrolether-ether 9:1).

Microbiological assay

Antimycobacterial susceptibility testing

The strains of *M. tuberculosis* CNCTC My 331/8, obtained from the Czech National Collection of Type Cultures (CNCTC), National Institute of Public Health, Prague, were used for the evaluation of *in vitro* antimycobacterial activity. The antimycobacterial activity of the compounds was determined in the Šula semisynthetic medium (SEVAC, Prague, Czech Republic). This medium (with bovine serum) is routinely used in the Czech Republic. Each strain was simultaneously inoculated into a Petri dish containing the Löwenstein-Jensen medium (from our lab) for the control of sterility of the inoculum and its growth. The compounds were added to the medium in DMSO solutions. The final concentrations were 1000, 500, 250, 125, 62.5, 31, 16, 8, 4, 2 μmol/L. The MICs were determined after incubation at 37 °C for 14 days (see Table 3). MIC was the lowest concentration of an antimycobacterially effective substance (on the above concentration scale), at which inhibition of the growth of the mycobacteria occurred. The evaluation was repeated three times and the values of the minimum inhibitory concentration were the same. The minimum inhibitory concentration of isoniazid is 2 μmol/L.

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