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Short Communication

On the relationship between the substitution pattern of thiobenzanilides and their antimycobacterial activity

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Abstract

The goal of this work was to shed more light on a preliminary finding about the relationship between the substitution in the thioacyl part of thiobenzanilides and their antituberculous effect. Thus, we prepared a set of 14 derivatives, out of which eight had not yet been reported, and the compounds were evaluated for antimycobacterial activity on a panel of four *Mycobacteria* species, including *Mycobacterium tuberculosis* CNCTC My 331/88, *Mycobacterium kansasii* CNCTC My 235/80, *Mycobacterium avium* CNCTC My 330/88 and *M. kansasii* 6509/96. While the contribution of the substituents with differing electronic and lipophilicity characteristics in position 3 to the antituberculous activity was negligible, we found that unsubstituted position 4 in the thioacyl part appears to be a prerequisite for a thiobenzanilide derivative to possess appreciable biological activity. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Thiobenzanilides; Antimycobacterial activity; Nontuberculous strains of mycobacteria

1. Introduction

In the process of optimizing the structure of thiobenzanilide, Waisser et al. found out that the antimycobacterial activity of its derivatives was favourably influenced by the introduction of a lipophilic substituent into position 4′ (in the anilide part of the molecule). Therefore, they prepared a group of compounds with the isopropyl, butyl [1] and cyclohexyl [2] moieties in this position and tested their antimycobacterial activity against *Mycobacterium tuberculosis*, *Mycobacterium kansasii*, *Mycobacterium avium* and *Mycobacterium fortuitum*. In order to quantitatively evaluate the influence of the alkyl substituents on the antimycobacterial activity of the derivatives, the experimental data were analyzed according to the Free–Wilson approach. The increase of biological activity was most significant in the case of the cyclohexyl derivative; this effect was probably linked to lipophilicity.

The substituents in the thioacyl part of the molecule were selected in accordance with the approach described by Topliss [3], which serves as a standard tool for determining structure–activity relationships (SARs) from the order of biological activities in a series of derivatives. Thus, the following functional groups were introduced into the thioacyl part of the molecule: H, 4-Cl, 4-CH_3, 4-CH_3O, 3-Br, 3-NO_2. Compared to the substitution recommended by Topliss (H, 4-Cl, 4-CH_3, 4-OCH_3 and 3,4-diCl), the 3-bromo compound was prepared in lieu of the 3,4-dichloro substitution, and the 3-nitro derivative was added to the set. 3-Bromo derivatives (derived from 3-bromobenzoic acid) are more easily accessible, and their biological evaluation can also supply information about the steric hindrance of position 4 in relationship to the biological activity. This change falls in line with the original Topliss's idea as well, because in comparison with the 4-chloro derivative, both lipophilicity and electron-accepting properties are enhanced by introducing the 3-bromo function, even though not so markedly as in the case of the 3,4-dichloro compound.

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The order of antimycobacterial activities in the subseries of 4′-isopropyl and 4′-butylthiobenzanilides bearing various functional groups in the thioacyl part indicated a steric influence of substitution in position 4, which led to a substantial decrease in the activity. Consequently, the most efficient substances against the tested strains of mycobacteria were those with unsubstituted *p*-position.

Following on from these results, we set out to synthesise a larger series of 3-substituted 4′-alkylthiobenzanilides in order to further investigate these findings.

2. Experimental

2.1. Chemistry

M.p.s were determined on a Kofler block and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded for $CDCl_3$ solutions at ambient temperature on a Varian Mercury-Vx BB 300 spectrometer operating at 300 MHz for ^{1}H. Chemical shifts were recorded as δ values in parts per million (ppm), and were indirectly referenced to tetramethylsilane (TMS) via the solvent signal (7.26 for ^{1}H, 77.0 for ^{13}C in $CDCl_3$). Infrared spectra were

Table 1
Some characteristic of the benzanilides **1**–**14**

Comp.	Code	Yield (%)	M.p. (°C)	M.p. (°C) [Reference]	IR (CO) (cm^{-1})
4′-Isopropylbenzanilide	**1**	91	161–165	159–162 [1]	1650
3-Chloro-4′-isopropylbenzanilide	**2**	75	141–142		1651
3-Methyl-4′-isopropylbenzanilide	**3**	94	112–114		1655
3-Methoxy-4′-isopropylbenzanilide	**4**	82	108–111		1648
3-Bromo-4′-isopropylbenzanilide	**5**	81	146–148	144–147 [1]	1654
3-Nitro-4′-isopropylbenzanilide	**6**	79	151–152	143–146 [1]	1652
4′-Butylbenzanilide	**7**	77	123–124	112–115 [1]	1649
3-Chloro-4′-butylbenzanilide	**8**	81	125–130		1652
3-Methyl-4′-butylbenzanilide	**9**	77	85–87		1652
3-Methoxy-4′-butylbenzanilide	**10**	69	69–71		1647
3-Bromo-4′-butylbenzanilide	**11**	73	130–132	128–131 [1]	1652
3-Nitro-4′-butylbenzanilide	**12**	83	147–149		1652
4′-Cyklohexylbenzanilide	**13**	94	204–206	195–210 [2]	1647
3-Chloro-4′-cyklohexylbenzanilide	**14**	95	208–209		1649

Table 2
^{1}H NMR spectroscopic data of the benzanilides

Code	^{1}N NMR (300 MHz, $CDCl_3$)
2	δ 1.24 (d, 6H, J = 6.87 Hz, CH_3), 2.97–2.81 (m, 1H, CH), 7.23–7.17 (m AA′, BB′, 2H, H3′, H5′), 7.35 (t, 1H, J = 7.87 Hz, H5), 7.47 (ddd, 1H, J = 7.87, 1.76, 1.1 Hz, H4), 7.55–7.50 (m AA′, BB′, 2H, H2′, H6′), 7.69 (dt, 1H, J = 7.87, 1.76 Hz, H6), 7.81 (t, 1H, J = 1.76 Hz, H2), 8.07 (bs, 1H, NH)
3	δ 1.25 (d, 6H, J = 6.86 Hz, CH_3), 2.40 (s, 3H, CH_3), 2.99–2.82 (m, 1H, CH), 7.24–7.18 (m AA′, BB′, 2H, H3′, H5′), 7.35–7.31 (m, 2H, H4, H5), 7.59–7.53 (m AA′, BB′, 2H, H2′, H6′), 7.65–7.60 (m, 1H, H6), 7.69–7.65 (m, 1H, H2), 7.96 (bs, 1H, NH)
4	δ 1.25 (d, 6H, J = 7.14 Hz, CH_2), 2.98–2.82 (m, 1H, CH), 3.83 (s, 3H, OCH_3), 7.05 (ddd, 1H, J = 7.42, 2.47, 1.79 Hz, H4), 7.25–7.18 (m AA′, BB′, 2H, H3′, H5′), 7.44–7.33 (m, 3H, H2, H5, H6), 7.59–7.52 (m AA′, BB′, 2H, H2′, H6′), 7.96 (bs, 1H, NH)
8	δ 0.93 (t, 3H, J = 7.55 Hz, CH_3), 1.43–1.28 (m, 2H, CH_2), 1.65–1.52 (m, 2H, CH_2), 2.59 (t, 2H, J = 7.55 Hz, CH_2), 7.19–7.11 (m AA′, BB′, 2H, H3′, H5′), 7.35 (t, 1H, J = 7.83 Hz, H5), 7.46 (dd, 1H, J = 1.97, 0.97 Hz, H4), 7.55–7.47 (m AA′, BB′, 2H, H2′, H6′), 7.72–7.67 (m, 1H, H6), 7.81 (t, 1H, J = 1.97 Hz, H2), 8.02 (bs, 1H, NH)
9	δ 0.93 (t, 3H, J = 7.51 Hz, CH_3), 1.44–1.29 (m, 2H, CH_2), 1.66–1.53 (m, 2H, CH_2), 2.39 (s, 3H, CH_3), 2.59 (t, 2H, J = 7.51 Hz, CH_2), 7.21–7.12 (m, AA′, BB′, 2H, H3′, H5′), 7.36–7.30 (m, 2H, H4, H5), 7.59–7.50 (m AA′, BB′, 2H, H2′, H6′), 7.70–7.59 (m, 2H, H2, H6), 7.98 (bs, 1H, NH)
10	δ 0.93 (t, 3H, J = 7.51 Hz, CH_3), 1.43–1.28 (m, 2H, CH_2), 1.66–1.52 (m, 2H, CH_2), 2.59 (t, 2H, J = 7.51 Hz, CH_2), 3.81 (s, 3H, OCH_3), 7.04 (ddd, 1H, J = 7.97, 2.47, 1.37 Hz, H4), 7.19–7.12 (m AA′, BB′, 2H, H3′, H5′), 7.43–7.27 (m, 3H, H2, H5, H6), 7.58–7.50 (m AA′, BB′, 2H, H2′, H6), 8.04 (bs, 1H, NH)
12	δ 0.93 (t, 3H, J = 7.41 Hz, CH_3), 1.42–1.27 (m, 2H, CH_2), 1.65–1.51 (m, 2H, CH_2), 2.59 (t, 2H, J = 7.41 Hz, CH_2), 7.21–7.11 (m, AA′, BB′, 2H, H3′, H5′), 7.58–7.48 (m AA′, BB′, 2H, H2′, H6′), 7.65 (t, 1H, J = 7.97 Hz, H5), 8.28–8.18 (m, 2H, H6, NH), 8.40–8.32 (m, 1H, H4), 8.69–8.66 (m, 1H, H2)
14	δ 1.48–1.21 (m, 5H, CH_2), 1.97–1.69 (m, 5H, CH_2), 2.59–2.43 (m, 1H, CH), 7.25–7.19 (m AA′, BB′, 2H, H3′, H5′), 7.42 (t, 1H, J = 7.83 Hz, H5), 7.56–7.48 (m AA′, BB′, 2H, H2′, H6′), 7.75–7.70 (m, 2H, H4, H6), 7.84 (t, 1H, J = 1.79 Hz, H2)

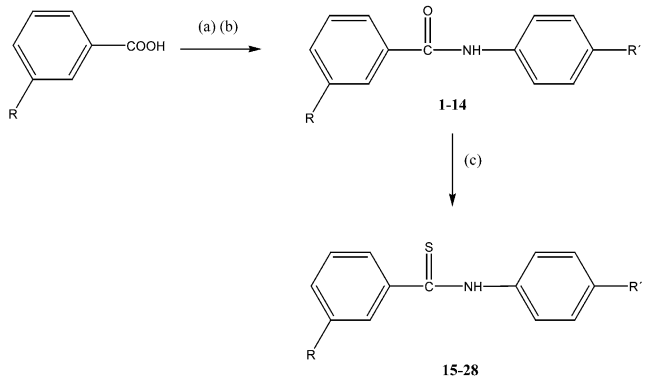
Table 3
^{13}C NMR spectroscopic data of the benzanilides

Code	^{13}C NMR (75 MHz, $CDCl_3$)
2	δ 24.0, 33.6, 120.6, 125.1, 126.9, 127.4, 129.9, 131.6, 134.8, 135.2, 136.8, 145.6, 164.5
3	δ 21.3, 24.0, 33.6, 120.4, 123.9, 126.9, 127.8, 128.1, 132.4, 135.0, 135.6, 138.5, 145.1, 165.9
4	δ 24.0, 33.6, 55.4, 112.4, 117.9, 118.7, 120.3, 126.9, 129.6, 135.5, 136.5, 145.3, 159.9, 165.5
8	δ 13.9, 22.2, 33.6, 35.0, 120.5, 125.1, 127.4, 128.9, 129.9, 131.6, 134.8, 135.1, 136.8, 139.6, 164.5
9	δ 13.9, 21.3, 22.2, 33.6, 35.0, 120.3, 123.9, 127.8, 128.5, 128.8, 132.4, 135.0, 135.5, 138.5, 139.1, 165.9
10	δ 13.9, 22.2, 33.6, 35.0, 55.3, 112.3, 117.9, 118.7, 120.3, 128.8, 129.6, 135.5, 136.5, 139.2, 159.8, 165.6
12	δ 13.9, 22.3, 33.6, 35.1, 120.7, 121.8, 126.2, 129.0, 130.0, 133.4, 134.8, 136.6, 140.1, 148.1, 163.4
14	δ 26.1, 26.8, 34.5, 44.0, 120.4, 125.0, 127.3, 127.4, 130.1, 131.8, 135.0, 135.2, 136.9, 145.0, 164.2

recorded in $CDCl_3$ on a Nicolet Impact 400 spectrophotometer. Low resolution mass spectra were measured on a Magnum Finnigan Mat apparatus. Elemental analysis was carried out on a CHNS-OCE FISONS EA 1110 instrument. Analytical thin-layer chromatography (TLC) was conducted on E. Merck TLC plates (silica gel 60 F_{254}, aluminum back), and the plates were visualised under UV light and in iodine vapours. Silica gel 60 (230–400 mesh) for column chromatography was purchased from E. Merck.

2.2. Preparation of benzanilides

A *p*-substituted aniline (1–3 g) was dissolved in Py (5 ml), and an equivalent amount of an *m*-substituted benzoyl chloride was added to the solution upon cooling. The reaction mixture was allowed to stand for 12 h, and a saturated aq. solution of Na_2CO_3 was then added. The crude compound was filtered off and either recrystallised from EtOH or purified by chromatography (Tables 1–3).



(a) $SOCl_2$, (b) subst. aniline in Py, (c) P_2S_5 in Py

Compound	R	R′
1, 15	H	$-CH(CH_3)_2$
2, 16	-Cl	$-CH(CH_3)_2$
3, 17	$-CH_3$	$-CH(CH_3)_2$
4, 18	$-OCH_3$	$-CH(CH_3)_2$
5, 19	-Br	$-CH(CH_3)_2$
6, 20	$-NO_2$	$-CH(CH_3)_2$
7, 21	H	$-CH_2CH_2CH_2CH_3$
8, 22	-Cl	$-CH_2CH_2CH_2CH_3$
9, 23	$-CH_3$	$-CH_2CH_2CH_2CH_3$
10, 24	$-OCH_3$	$-CH_2CH_2CH_2CH_3$
11, 25	Br	$-CH_2CH_2CH_2CH_3$
12, 26	$-NO_2$	$-CH_2CH_2CH_2CH_3$
13, 27	H	$-C_6H_{12}$
14, 28	-Cl	$-C_6H_{12}$

Scheme 1.

2.3. Preparation of thiobenzanilides

A benzanilide (0.5–1 g) was mixed with a solution of an equivalent amount of P_2S_5 in Py, and the reaction mixture was heated at reflux for 10 h. The solution was poured into water (25–30 ml), and the resultant mixture was allowed to stand until the initially formed oily material turned into a solid. The crude product was purified by chromatography (Tables 4–6).

Table 4
Some characteristic of the thiobenzanilides **1**–**14**

Comp.	Code	Yield (%)	M.p. (°C)	M.p. (°C) [Reference]	LRMS
4′-Isopropylthiobenzanilide	**15**	10	67–70	66–69 [1]	255 [$M^{\cdot +}$]
3-Chloro-4′-isopropylthiobenzanilide	**16**	26	103–105		289 [$M^{+} - H$]
3-Methyl-4′-isopropylthiobenzanilide	**17**	5	79–85		269 [$M^{\cdot +}$]
3-Methoxy-4′-isopropylthiobenzanilide	**18**	42	81–84		285 [$M^{\cdot +}$]
3-Bromo-4′-isopropylthiobenzanilide	**19**	6	109–112	109–111 [1]	334 [$M^{\cdot +}$]
3-Nitro-4′-isopropylthiobenzanilide	**20**	45	100–102	96–99 [1]	299 [$M^{\cdot +}$]
4′-Butylthiobenzanilide	**21**	6	77–80	69–72 [1]	268 [$M^{\cdot +}$]
3-Chloro-4′-butylthiobenzanilide	**22**	7	93–96		302 [$M^{\cdot +} - H$]
3-Methyl-4′-butylthiobenzanilide	**23**	30	29–32		283 [$M^{\cdot +}$]
3-Methoxy-4′-butylthiobenzanilide	**24**	41	59–61		299 [$M^{\cdot +}$]
3-Bromo-4′-butylthiobenzanilide	**25**	11	79–82	75–78 [1]	348 [$M^{\cdot +}$]
3-Nitro-4′-butylthiobenzanilide	**26**	15	74–78		313 [$M^{\cdot +} - H$]
4′-Cyklohexylthiobenzanilide	**27**	9	115–119	122 [2]	294 [$M^{\cdot +} - H$]
3-Chloro-4′-cyklohexylthiobenzanilide	**28**	35	175–178		329 [$M^{\cdot +} - H$]

Table 5
^{1}H NMR spectroscopic data of the thiobenzanilides **15**–**28**

Code	^{1}H NMR (300 MHz, $CDCl_3$)
15	δ 1.27 (d, 6H, J = 6.87 Hz, CH_3), 3.03–2.87 (m, 1H, CH), 7.34–7.26 (m, 2H, H3′, H5′), 7.55–7.38 (m, 3H, H3, H4, H5), 7.72–7.64 (m, 2H, H2′, H6′), 7.88–7.79 (m, 2H, H2, H6), 9.01 (bs, 1H, NH)
16	δ 1.27 (d, 6H, J = 6.86 Hz, CH_3), 3.03–2.85 (m, 1H, CH), 7.33–7.26 (m, AA′, BB′, 2H, H3′, H5′), 7.26 (d, 1H, J = 7.96 Hz, H4), 7.48–7.42 (m, 1H, H5), 7.70–7.62 (m, AA′, BB′overlaped, 3H, H6, H2′, H6′), 7.80 (t, 1H, J = 1.65 Hz, H2), 9.01 (bs, 1H, NH)
17	δ 1.27 (d, 6H, J = 7.14 Hz, CH_3), 2.42 (s, 3H, CH_3), 3.04–2.86 (m, 1H, CH), 7.36 (m, 4H, H4, H5, H3′, H5′), 7.73–7.56 (m, 4H, H2, H6, H2′, H6′), 8.97 (bs, 1H, NH)
18	δ 1.27 (d, 6H, J = 6.87 Hz, CH_3), 3.03–2.85 (m, 1H, CH), 3.86 (s, 3H, OCH_3), 7.07–6.98 (m, 1H, H4), 7.38–7.26 (m, 4H, H5, H6, H3′, H5′), 7.45–7.41 (m, 1H, H2), 7.73–7.63 (m, AA′, BB′, 2H, H2′, H6′), 9.02 (bs, 1H, NH)
19	δ 1.27 (d, 6H, J = 6.86 Hz, CH_3), 3.03–2.82 (m, 1H, CH), 7.35–7.26 (m, 3H, H4, H3′, H5′), 7.70–7.57 (m, AA′, BB′ overlaped, 3H, H5, H2′, H6′), 7.74 (d, 1H, J = 7.70 Hz, H6), 7.96 (t, 1H, J = 1.65 Hz, H2), 8.97 (bs, 1H, NH)
20	δ 1.27 (d, 6H, J = 6.86 Hz, CH_3), 3.05–2.83 (m, 1H, CH), 7.35–7.27 (m AA′, BB′2H, H3′, H5′), 7.61 (t, 1H, J = 7.97 Hz, H5), 7.72–7.64 (m, AA′, BB′, 2H, H2′, H6′), 8.20 (d, 1H, J = 7.97 Hz, H6), 8.31 (dd, J = 7.97 Hz, J = 1.38 Hz, H4), 8.59 (t, 1H, J = 1.38 Hz, H2), 9.18 (bs, 1H, NH)
21	0.94 (t, 3H, J = 7.41 Hz, CH_3), 1.45–1.29 (m, 2H, CH_2), 1.69–1.53 (m, 2H, CH_2), 2.64 (t, 2H, J = 7.41 Hz, CH_2), 7.30–7.21 (m, AA′, BB′, 2H, H3′, H5′), 7.55–7.38 (M 3H, H3, H4, H5), 7.71–7.62 (m, 2H, H2′, H6′), 7.89–7.79 (m, 2H, H2, H6), 9.01 (bs, 1H, NH)
22	δ 0.94 (t, 3H, J = 7.41 Hz, CH_3), 1.46–1.24 (m, 2H, CH_2), 1.69–1.51 (m, 2H, CH_2), 2.63 (t, 2H, J = 7.41 Hz, CH_2), 7.29–7.21 (m, 2H, H3′, H5′), 7.41–7.31 (m, 1H, H5), 7.53–7.42 (m, 1H, H4), 7.72–7.59 (m, 3H, H6, H2′, H6′), 7.84–7.78 (m, 1H, H2), 9.02 (bs, 1H, NH)
23	δ 0.94 (t, 3H, J = 7.41 Hz, CH_3), 1.46–1.28 (m, 2H, CH_2), 1.68–1.55 (m, 2H, CH_2), 2.64 (t, 2H, J = 7.41 Hz, CH_2), 7.28–7.21 (m, 2H, H3′, H5′), 7.34–7.28 (m, 2H, H4, H5), 7.71–7.56 (m, 4H, H2, H6, H2′, H6′), 8.99 (bs, 1H, NH)
24	δ 0.94 (t, 3H, J = 7.41 Hz, CH_3), 1.45–1.29 (m, 2H, CH_2), 1.69–1.55 (m, 2H, CH_2), 2.63 (t, 2H, J = 7.41 Hz, CH_2), 3.86 (s, 3H, OCH_3), 7.07–6.99 (m, 1H, H4), 7.28–7.21 (m, 2H, H3′, H5′), 7.37–7.30 (m, 2H, H5, H6), 7.45–7.40 (m, 1H, H2), 7.70–7.62 (m, 2H, H2′, H6′), 9.01 (bs, 1H, NH)
25	δ 0.94 (t, 3H, J = 7.41 Hz, CH_3), 1.45–1.30 (m, 2H, CH_2), 1.69–1.54 (m, 2H, CH_2), 2.63 (t, 2H, J = 7.41 Hz, CH_2), 7.33–7.22 (m, 3H, H4, H3′, H5′), 7.67–7.58 (m, 3H, H5, H2′, H6′), 7.74 (d, 1H, J = 7.97 Hz, H6), 7.96 (t, 1H, J = 1.81 Hz, H2), 8.97 (bs, 1H, NH)
26	δ 0.94 (t, 3H, J = 7.42 Hz, CH_3), 1.46–1.29 (m, 2H, CH_2), 1.69–1.55 (m, 2H, CH_2), 2.64 (t, 2H, J = 7.42 Hz, CH_2), 7.29–7.22 (m, AA′, BB′, 2H, H3′, H5′), 7.69–7.57 (m, 3H, H5, H2′, H6′), 8.20 (d, 1H, J = 7.96 Hz, H6), 8.31 (dd, 1H, J = 7.96, 1.37 Hz, H4), 8.59 (t, 1H, J = 1.37 Hz, H2), 9.17 (bs, 1H, NH)
27	δ 1.51–1.16 (m, 5H, CH_2), 1.97–1.68 (m, 5H, CH_2), 2.61–2.46 (m, 1H, CH), 7.32–7.24 (m, AA′, BB′, 2H, H3′, H5′), 7.54–7.38 (m, 3H, H3, H4, H5), 7.73–7.63 (m, AA′, BB′, 2H, H2′, H6′), 7.88–7.79 (m, 2H, H2, H6), 9.00 (bs, 1H, NH)
28	δ 1.53–1.16 (m, 5H, CH_2), 1.98–1.68 (m, 5H, CH_2), 2.62–2.44 (m, 1H, CH), 7.31–7.24 (m, 2H, H3′, H5′), 7.40–7.32 (m, 1H, H5), 7.50–7.42 (m, 1H, H4), 7.72–7.62 (m, 3H, H6, H2′, H6′), 7.84–7.79 (m, 1H, H2), 8.96 (bs, 1H, NH)

Table 6
^{13}C NMR spectroscopic data of the thiobenzanilides **15**–**28**

Code	^{13}C NMR (75 MHz, $CDCl_3$)
15	δ 23.9, 33.8, 123.6, 126.6, 127.0, 128.6, 131.2, 136.6, 143.1, 147.8, 198.0
16	δ 23.9, 33.8, 123.5, 124.7, 127.0, 129.8, 131.0, 134.6, 136.4, 144.6, 148.0, 196.1
17	δ 21.4, 23.9, 33.8, 123.4, 123.6, 127.0, 127.7, 128.5, 132.0, 136.7, 138.6, 143.3, 147.8, 198.3
18	δ 23.9, 33.8, 55.5, 112.6, 117.3, 118.1, 123.6, 127.0, 129.6, 136.6, 144.6, 147.8, 159.6, 197.7
19	δ 23.9, 33.8, 122.7, 123.5, 125.2, 127.0, 129.7, 130.1, 133.9, 136.4, 144.8, 148.1, 196.0
20	δ 23.8, 33.8, 121.1, 123.5, 125.4, 127.1, 129.7, 133.1, 136.2, 144.2, 147.9, 148.3, 194.6
21	δ 13.9, 22.3, 33.4, 35.2, 123.6, 126.6, 126.9, 128.6, 128.7, 128.9, 131.2, 136.6, 142.0, 143.2, 198.1
22	δ 13.9, 22.3, 33.4, 35.3, 123.5, 124.7, 127.0, 129.0, 129.8, 131.0, 134.6, 136.3, 142.2, 144.6, 196.1
23	δ 13.9, 21.4, 22.3, 33.4, 35.2, 123.4, 123.6, 127.6, 128.5, 128.9, 132.0, 136.6, 138.5, 141.9, 143.2, 198.3
24	δ 13.9, 22.3, 33.4, 35.2, 55.5, 112.6, 117.3, 118.1, 123.5, 128.9, 129.6, 136.5, 141.9, 144.6, 159.6, 197.7
25	13.9, 22.3, 33.4, 35.3, 122.7, 123.5, 125.3, 129.0, 129.7, 130.1, 133.9, 136.3, 142.2, 144.8, 196.0
26	δ 13.9, 22.3, 33.4, 35.3, 121.1, 123.5, 125.4, 129.0, 129.7, 133.2, 136.1, 142.5, 144.2, 147.9, 194.6
27	δ 26.0, 26.8, 34.3, 44.2, 123.5, 126.6, 127.3, 128.6, 131.2, 136.6, 143.2, 147.0, 197.9
28	δ 26.0, 26.8, 34.3, 44.2, 123.4, 124.7, 127.0, 127.4, 129.9, 131.0, 134.6, 136.4, 144.7, 147.3, 196.0

2.4. Biology

Three CNCTC (Czech National Collection of type cultures) strains (*M. tuberculosis* CNCTC My 331/88, *M. kansasii* CNCTC My 235/80 and *M. avium* CNCTC My 330/88), and a clinical isolate (*M. kansasii* 6509/96) were used for the evaluation of the biological activity of the target compounds. The tests were carried out in the semisynthetic Šula medium (SEVAC, Prague). Twofold serial dilutions of the compounds in Me_2SO (250–8

Table 7
Antimycobacterial activity of thiobenzanilides (MIC, μmol l^{-1})

Comp.	*M. tuberculosis* My 331/88	*M. avium* My 330/88	*M. kansasii* My 235/80	*M. kansasii* 6509/96
15	32	62.5	32	32
	62.5	62.5	62.5	62.5
			62.5	62.5
16	32	32	32	32
	32	62.5	62.5	62.5
			62.5	62.5
17	32	62.5	32	32
	62.5	62.5	62.5	62.5
			62.5	62.5
18	32	32	32	32
	62.5	62.5	62.5	
			62.5	
19	32	62.5	32	32
	32	32	62.5	32
			62.5	62.5
20	16	62.5	32	16
	32	62.5	62.5	32
			62.5	32
21	32	62.5	32	32
	62.5	62.5	62.5	62.5
			62.5	62.5
22	32	62.5	32	32
	62.5	62.5	62.5	62.5
			62.5	62.5
23	32	32	16	16
	32	32	32	32
			32	32
24	32	32	16	16
	32	32	32	32
			32	32
25	32	32	16	16
	32	32	32	62.5
			32	62.5
26	32	16	8	16
	32	32	16	16
			32	16
27	32	16	16	
	32	32	32	16
			32	32
28	16	32	16	16
	32	32	62.5	32
			62.5	62.5
Isoniazide	0.5	> 250	> 250	2.0
	1.0	250	> 250	4.0
			> 250	4.4
Rifampicine	0.25	16	0.125	0.125
			25	0.125
			25	0.25
Clofazimine	0.125	0.25	0.06	0.06
	0.125		0.06	0.06
	0.25	0.25	0.06	0.06

μmol l^{-1}) were used. Minimum inhibitory concentrations (MICs) were read after 14 and 21 days of incubation at 37 °C for *M. tuberculosis* and *M. avium*, , and after 7, 14 and 21 days for *M. kansasii*. The results are shown in Table 7.

3. Results and discussion

The compounds were prepared via a three-step process (Scheme 1). Commercially available substituted benzoic acids were treated with thionyl chloride to

afford the corresponding acyl chlorides, which were further converted into benzanilides by the reaction with various substituted anilines in pyridine. Finally, the oxo group was replaced with the thioxo moiety by heating the benzanilides with phosphorus pentasulphide in pyridine.

The structures of the compounds were confirmed by ^{1}H and ^{13}C NMR as well as by IR spectroscopy. The signal of the carbonyl group at 164–166 ppm was apparent in the ^{13}C NMR spectra of benzanilide derivatives, while the carbons of the thiocarbonyl function in the corresponding thiobenzanilides resonated at substantially lower field, in the range 194–199 ppm. In the IR spectra of benzanilides, the typical vibration bands of the C=O group were observed between 1647 and 1655 cm^{-1}. On the other hand, the vibrations of the C=S group in the thiobenzanilides appeared at lower frequencies, in the range 1198–1221 cm^{-1}. The structures of the target compounds were also corroborated by low resolution mass spectra; the spectrum of each compound contained a peak at *m*/*z* corresponding to the molecular ion. Finally, the purity of the compounds was confirmed by elemental analysis.

To test the assumption about the steric properties of the substituents in position 4 having been the cause of reduced activity of the 4-substituted thiobenzanilides [1], the target compounds were evaluated for antimycobacterial activity. The results, displayed in Table 7, clearly show that the activities of the compounds against nontuberculous strains of mycobacteria (*M. avium* My 330/88 and *M. kansasii* My 235/80) are superior to that of the standard antituberculotic isoniazid (INH), but lower than those of rifampicine and clofazimine. Hence, it is important for the antimycobacterial effect of thiobenzanilides that position 4 in the thioacyl part be unsubstituted. Interestingly, substituents with various electronic parameters and lipophilicity attached to C(3) do not significantly influence the activity. As regards substitution in the anilide moiety, derivatives with the isopropyl group in position 4′ are more active than those bearing the butyl chain. Further increase in the lipophilicity of the substituents in the anilide part does not lead to derivatives possessing higher activity (4′-cyclohexyl derivatives).

In summary, it appears that the presence of unsubstituted position 4 in the thioacyl part of the thiobenzanilide molecule seems to have a positive influence on the antimycobacterial activity, with the compounds possessing higher activity than that of the standard INH against some of the nontuberculous strains of mycobacteria. The compounds, which do not fit this requirement, usually display substantially lower activities [1].

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