COMBINATION OF THE TOPLISS APPROACH WITH THE FREE–WILSON ANALYSIS IN THE STUDY OF ANTIMYCOBACTERIAL ACTIVITY OF 4-ALKYLTHIOBENZANILIDES*

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Received January 16, 1997
Accepted April 26, 1997

Dedicated to Professor Dr Vaclav Horak (Georgetown University) on the occasion of his 75th birthday.

On the basis of a preliminary study of the antimycobacterial activity of thiobenzanilides, a group of 4′-isopropyl- and 4′-butylthiobenzanilides have been synthesized and tested against *Mycobacterium tuberculosis*, *Mycobacterium kansasii*, *Mycobacterium avium* and *Mycobacterium fortuitum*. The effect of the substituents on minimum inhibitory concentrations was calculated by the Free–Wilson method. The separated values were analyzed by the Topliss approach. The substitution of the thiobenzanilide in position 4′ by the isopropyl group or butyl group increased the antimycobacterial activity less than the cyclohexyl group. The substitution in position 4 decreased the activity by the steric effect.

Key words: Thiobenzanilides; Tuberculostatics; *Mycobacterium* spp.; Structure–activity relationships; Free–Wilson analysis, Topliss approach.

Mycobacterial diseases produced by atypical mycobacterial strains have become a serious health problem at present. The diseases caused by *Mycobacterium avium* are particularly dangerous. Antituberculotic drugs were developed with an assumed effect against *Mycobacterium tuberculosis*, and the principal aim of research into antileprotic agents was development of compounds active against *Mycobacterium leprae*. Other mycobacterial strains escaped the attention of research laboratories as they were not considered to be pathogenic for man. Even nowadays they endanger the human popula-

* Part XII in the series Relationships Between the Chemical Structure of Substances and Their Antimycobacterial Activity to Atypical Strains; Part XI: see ref.[1].

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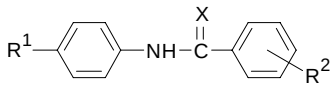
tion only infrequently but the result of their attack is, as a rule, fatal. The research team of the present authors is therefore systematically engaged in the study of the relationships between the structure and antimycobacterial activity of drugs with the aim of revealing general rule which would make possible development of chemotherapeutic agents against atypical mycobacterial strains.

A wide spectrum of antimycobacterial effect is found in a number of compounds containing a thioxo group, such as thiobenzamides[2], thiooxalamides[3], 2-(alkysulfanyl)-pyridine-4-carbothioamides[4], 4-(alkylsulfanyl)pyridine-2-carbothioamides[5], and thiobenzanilides[6,7]. The latter study has revealed that the antimycobacterial effect of thiobenzanilides is favorably affected by introducing cyclohexyl into position 4′. The present paper describes preparation, antimycobacterial evaluation and analysis of the relationships between the structure and antimycobacterial activity of two additional groups of compounds containing an alkyl in position 4′, namely 4′-isopropylthiobenzanilides and 4′-butylthiobenzanilides.



	R^2
1, 7, 12, 18	H
2, 8, 13, 19	4-CH_3
3, 9, 14, 20	4-OCH_3
4, 11, 15, 22	3-Br
5, 10, 16, 21	4-Cl
6, 17	3-NO_2

In formulae **1**-**11**: X = O; **12**-**22**: X = S;
1-**6**, **12**-**17**: $R^1 = CH(CH_3)_2$; **7**-**11**, **18**-**22**: $R^1 = (CH_2)_3CH_3$

All the anilides under study **1–11** (Table I) were prepared by the reaction of benzoyl chloride and substituted benzoyl chlorides with corresponding 4-isopropylaniline or 4-butylaniline in pyridine. Thiobenzanilides **12–22** (Table II) were prepared from the corresponding anilides by the reaction with phosphorus pentasulfide in pyridine. The selection of substitution modifications on the benzoyl moiety was carried out according to Topliss[8]. This approach brings an advantage – in most cases it is possible to determine the structure–activity relationship from the order of activity of substances. In contrast to the original Topliss approach, 3-bromo derivatives **15** and **22** were synthesized instead of 3,4-dichloro derivatives. This substitution is in accord with the original Topliss intention as in comparison with 4-chloroderivative lipophilicity and electron-acceptor properties are also increased. Nevertheless, 3-bromo derivatives can provide

TABLE I
Characteristic data of 4′-isopropylbenzanilides **1–6** and 4′-butylbenzanilides **7–11**

Compound	M.p., °C Yield, %	Formula M.w.	Calculated/Found		
			% C	% H	% N
1	152–162[a]	$C_{16}H_{17}NO$	80.30	7.16	5.85
	82	239.3	80.13	7.15	5.79
2	155–157	$C_{17}H_{19}NO$	80.60	7.56	5.53
	93	253.3	80.43	7.71	5.71
3	165–167	$C_{17}H_{19}NO_2$	75.81	7.11	5.20
	65	269.3	75.63	7.38	5.22
4	144–147	$C_{16}H_{16}BrNO$	60.39	5.07	4.40
	83	318.2	60.07	4.60	4.47
5	239–241	$C_{16}H_{16}ClNO$	70.20	5.89	5.12
	98	273.8	70.12	5.88	5.15
6	143–146	$C_{16}H_{16}N_2O_3$	67.59	5.67	9.85
	85	284.3	67.66	5.32	9.85
7	112–115[b]	$C_{17}H_{19}NO$	80.60	7.56	5.53
	86	253.3	80.27	7.35	5.80
8	118–121	$C_{18}H_{21}NO$	80.86	7.92	5.24
	86	267.37	80.93	7.77	5.43
9	144–147[c]	$C_{18}H_{21}NO_2$	76.30	7.47	4.94
	82	283.8	76.40	7.47	4.67
10	193–196	$C_{17}H_{18}ClNO$	70.95	6.30	4.87
	90	287.8	71.26	6.43	4.58
11	128–131	$C_{17}H_{18}BrNO$	61.46	5.46	4.22
	87	332.2	61.62	4.99	4.72

[a] Ref.[10] gives m.p. 161.4–162 °C. [b] Ref.[11] gives m.p. 126 °C. [c] Ref.[12] gives m.p. 145.5–146 °C.

TABLE II
Characteristic data of 4′-isopropylthiobenzanilides **12–17** and 4′-butylthiobenzanilides **18–22**

Compound	M.p., °C Yield, %	Formula M.w.	Calculated/Found			
			% C	% H	% N	% S
12	66–69	$C_{16}H_{17}NS$	75.25	6.71	5.48	12.55
	58	255.4	74.84	6.49	5.57	12.26
13	105–108	$C_{17}H_{19}NS$	75.79	7.11	5.20	11.90
	43	269.4	75.70	7.22	4.81	11.95
14	99–101	$C_{17}H_{19}NOS$	71.54	6.71	4.91	11.23
	41	285.4	71.79	6.58	5.11	5.21
15	109–111	$C_{16}H_{16}BrNS$	57.49	4.82	4.19	11.06
	54	334.3	57.48	4.68	4.23	10.85
16	195–197	$C_{16}H_{16}ClNS$	66.31	5.56	4.83	9.59
	65	289.8	66.30	5.50	4.82	9.38
17	96–99	$C_{16}H_{16}N_2O_2S$	63.98	5.37	9.33	10.67
	39	300.4	63.84	5.36	9.14	9.24
18	69–72	$C_{17}H_{19}NS$	75.79	7.11	5.20	11.90
	57	269.4	75.48	7.09	5.14	11.82
19	71–74	$C_{18}H_{21}NS$	76.28	7.47	4.94	11.31
	63	283.4	76.29	7.58	4.80	11.09
20	105–108	$C_{18}H_{21}NOS$	72.20	7.07	4.68	10.71
	48	299.4	71.92	7.18	4.59	10.75
21	121–124	$C_{17}H_{18}ClNS$	67.20	5.97	4.61	10.55
	61	303.9	67.61	5.94	4.77	10.66
22	75–78	$C_{17}H_{18}BrNS$	58.62	5.21	4.02	9.20
	44	348.3	58.51	5.18	4.21	8.84

information about the steric hindrance of position 4 if it occurs (in connection with biological activity). The structures of the new products prepared in the present study were supported by their infrared spectra.

In the spectra of anilides **1–11** (Table III), absorptions of the amide carbonyl were clearly observed in the region between 1 645 and 1 655 cm^{-1} but they disappeared after replacement of the oxygen atom by the sulfur atom. The anilides absorb in the region of 3 280 and 3 300 cm^{-1}, whereas thiobenzanilides **12–22** in the region of 3 158–3 282 cm^{-1}. In all spectra it was possible to observe aromatic C–H vibration between 3 024 and 3 064 cm^{-1} and several absorption bands of alkyls, *e.g.* in the regions of 2 952–2 967 cm^{-1},

TABLE III
Infrared spectra of compounds **1–22**

Compound	ν(N–H)	$\nu(C–H)_{arom}$	$\nu(C–H)_{alif}$	ν(C=O)	δ(C–H)
1	3 300	3 050	2 978, 2 958, 2 927, 2 894	1 649	831
2	3 338	3 048	2 961, 2 922, 2 887, 2 870	1 655	827
3	3 325	3 050	2 967, 2 929, 2 907, 2 869	1 652	827
4	3 340	3 050	2 964, 2 927, 2 894, 2 872	1 651	828
5	3 351	3 036	2 958, 2 922, 2 891, 2 866	1 652	827
6	3 315	3 050	2 963, 2 928, 2 900, 2 871	1 652	827
7	3 325	3 050	2 961, 2 926, 2 874, 2 853	1 651	825
8	3 336	3 044	2 959, 2 925, 2 873, 2 858	1 654	822
9	3 331	3 050	2 959, 2 929, 2 872, 2 858	1 652	823
10	3 336	3 044	2 952, 2 929, 2 874, 2 858	1 651	824
11	3 324	3 044	2 961, 2 925, 2 874, 2 853	1 654	828
12	3 166	3 044	2 963, 2 927, 2 889, 2 871	–	831
13	3 186	3 064	2 987, 2 959, 2 932, 2 868	–	825
14	3 158	3 034	2 962, 2 934, 2 871, 2 839	–	837
15	3 187	3 051	2 963, 2 927, 2 893, 2 872	–	826
16	3 189	3 050	2 960, 2 931, 2 895, 2 869	–	840
17	3 282	3 050	2 963, 2 930, 2 896, 2 871	–	832
18	3 201	3 061	2 961, 2 927, 2 871, 2 856	–	826
19	3 244	3 050	2 958, 2 926, 2 867, 2 856	–	831
20	3 158	3 024	2 960, 2 931, 2 872, 2 858	–	833
21	3 187	3 055	2 960, 2 927, 2 871, 2 859	–	829
22	3 187	3 056	2 958, 2 930, 2 870, 2 857	–	826

2 922–2 929 cm^{-1}, and 2 853–2 894 cm^{-1}. The absorption maximum in the region of 822–840 cm^{-1}, which occurs in the spectra of all compounds, was assigned to the out-of-plane vibration of aromatic hydrogen atoms in the anilide moiety. The structure of the newly prepared compounds was then further confirmed by elemental analysis.

Antimycobacterial activity of 4′-isopropylthiobenzanilides **12–17** and 4′-butylthiobenzanilides **18–22** against *Mycobacterium tuberculosis*, *Mycobacterium kansasii*, *Mycobacterium avium*, and *Mycobacterium fortuitum* was evaluated. The antimycobacterial activity against *M. fortuitum* was not significant and it was not therefore further analyzed. The survey of antimicrobial activities of the compounds under study is given in Table IV. The experimentally found data were then further separated by the Free–Wilson method[9] to determine the effects of modifying substituents. The calculation also included the data of the activities of 4′-cyclohexylthiobenzanilides against *M. tuberculosis* and *M. kansasii* which were taken from the previous study[7]. The results are summarized in Table V. In the evaluation of the relationships between the structure and activity against *M. avium*, only the data from the present study could be used as this activity was not assayed in recent years.

TABLE IV
Minimum inhibitory concentration (MIC, μmol/l) of 4′-isopropylthiobenzamides **12–17** and 4′-butylthiobenzanilides **18–22**

Compound	MIC, μmol/l			
	M. tuberculosis	*M. kansasii*	*M. avium*	*M. fortuitum*
12	60	60	125	500 (250)[a]
13	125	250	1 000	>1 000
14	60	60	250	>1 000
15	30	60	125	>1 000
16	500	>1 000	>1 000	>1 000
17	30	250	1 000 (500)[a]	>1 000
18	30	60	250	250
19	250	500	500	500
20	60	60	1 000	1 000
21	125	250	>1 000	>1 000
22	125	125	250	500

[a] The values in brackets represent concentrations of partial inhibition.

It follows from the found data that the effect of the butyl and isopropyl groups in position 4′ of thiobenzanilides on the increase of the activity of compounds under study is smaller than the effect of the cyclohexyl group. The effect could be ascribed to a decrease in the lipophilicity of the substituent. The order of activity of thioanilides substituted in the thiobenzoyl moiety indicates steric effect of substituents in position 4, which is manifested by a decrease in the activity. In order to demonstrate this effect, 3-nitro-4′-isopropylthiobenzanilide **17** was synthesized. Its activity against *M. tuberculosis* was one of the highest in the group of isopropylthiobenzanilides. However, its activity against the other mycobacterial strains under study was not significant. The obtained data contribute to further development of antimycobacterial thiobenzanilides.

EXPERIMENTAL AND CALCULATIONS

The melting points were determined with a Kofler apparatus and are uncorrect. The samples for analyses and antimycobacterial tests were dried over phosphorus pentoxide at 61 °C and 66 Pa for 24 h. Elemental analyses were performed on a C,H,N analyzer (Laboratorni pristroje, Prague). The IR spectra were measured in KBr on a Nicolet Impact 400 apparatus, wavelengths are given in cm^{-1} (Table III).

4′-Isopropylbenzanilides **1–6** and 4′-Butylbenzanilides **7–11**

A benzoyl chloride (23 mmol) was added dropwise into a stirred mixture of substituted aniline (22.8 mmol) and pyridine (20 ml) under cooling in an ice bath. The reaction mixture was allowed to stand at room

TABLE V
Results of the Free–Wilson structure–antimycobacterial activity analysis, contributions of fragments to log MIC values (Δ log MIC)

Fragment	Δ log MIC		
	M. tuberculosis[a]	*M. kansasii*[b]	*M. avium*[c]
H	–0.22	–0.30	–0.53
4-CH_3	0.18	0.30	0.22
4-OCH_3	–0.12	–0.30	–0.23
4-Cl	0.38	0.50	0.52
3-Br	–0.22	–0.20	0.02
4′-i-C_3H_7	0.16	0.06	0.04
4′-C_4H_9	0.16	0.12	–0.04
4′-C_6H_{11}	–0.32	–0.18	–
Common skeleton	1.82	2.00	2.78

Statistical evidence: [a] $r = 0.853$, $s = 0.28$, $F = 4.80$, $t = 2.31$, $n = 16$; [b] $r = 0.846$, $s = 0.31$, $F = 4.53$, $t = 2.31$, $n = 16$; [c] $r = 0.966$, $s = 0.15$, $F = 11.25$, $n = 10$.

temperature for 1 day, whereafter it was poured into a saturated sodium carbonate solution (100 ml). The separated product was filtered off, washed consecutively with a saturated sodium carbonate solution and water, and recrystallized from ethanol. The results are given in Table I.

4′-Isopropylthiobenzanilides **12–17** and 4′-Butylthiobenzanilides **18–22**

To a refluxing mixture of a 4′-alkylbenzanilide (14 mmol) and pyridine (10 ml), phosphorus pentasulfide (7.1 mmol) was added portionwise and the reaction mixture was refluxed for 3–8 h. After cooling, it was poured into water (100 ml). On the following day, the raw product was filtered off and recrystallized from ethanol. The results are given in Table II.

Microbiological Evaluation

The microbiological tests were carried out on a semisynthetic liquid medium with proteins by Sula (SEVAC, Prague). The following mycobacterial strains were used: *Mycobacterium tuberculosis* $H_{37}Rv$, *Mycobacterium kansasii* PKG 8, *Mycobacterium avium* No. 80/72 and *Mycobacterium fortuitum* No. 1 023. The substances were added after dissolution in dimethyl sulfoxide. The resulting concentrations of the compounds in the substrate were: 7, 15, 30, 60, 125, 250, 500 and 1 000 μmol/l. The minimum inhibition concentrations (MIC) were read after 15 days of incubation at 37 °C. The results are summarized in Table IV.

Calculations

All calculations were carried out using the Multireg program for Microsoft Excel. Table V presents a survey of the calculated contributions of the varied structural fragments to the activity.

The authors wish to thank Mrs D. Karlickova from the Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy, Charles University, for CHN elemental analysis and Mrs J. Zizkova from the Department of Inorganic and Organic Chemistry for elemental analysis of sulfur and IR spectra measurements. The work was supported by grant No. 35/95 of the Grant Agency of the Charles University and by grant No. 203/96/0262 of the Grant Agency of the Czech Republic.

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