ANTITUBERCULOTIC 4'-CYCLOHEXYLTHIOBENZANILIDES: COMBINATION OF FREE–WILSON METHOD IN QSAR WITH TOPLISS APPROACH*

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Dedicated to Professor Václav Horák, the founder of Czechoslovak school of chemistry of organic sulfur compounds, on the occasion of his 70th birthday.

On the basis of a preliminary study of antimycobacterial activity of thiobenzanilides against *Mycobacterium kansasii*, a group of 4'-cyclohexylthiobenzanilides have been prepared which exhibit a significant activity against the microorganism mentioned. The whole set of 35 thiobenzanilides was tested with *Mycobacterium tuberculosis*, and on the basis of the QSAR analysis conclusions have been made with regard to prognostics of structures suitable for further studies. The problem was solved by the method by Free and Wilson combined with the Topliss approach and by a Hansch type analysis.

In a previous communication[1] antituberculotic compounds were classified from the standpoint of dependences between lipophilicity and activity as follows: *a*) compounds exhibiting a parabolic dependence between overall lipophilicity and antituberculotic activity, *b*) compounds exhibiting a linear dependence for the same effects (the activity usually increased with increasing lipophilicity), *c*) compounds whose activity was only connected with physical parameters in partial sections of the molecule. On the basis of studies of relations between structure and antimycobacterial activity of thiobenzanilides against *Mycobacterium kansasii* the compound class studied can be included into the third group[2].

For the analysis of relations between antimycobacterial activity and structure of compounds of the third group it is usually convenient to adopt the Free–Wilson method, however, its application is limited. It is based on a separation of the resultant

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biologic activity into the effects of individual molecular sections varied (i.e. varying of substituents, molecular fragments etc.). In this way it is possible to reduce the number of necessary variations since the activity of a number of compounds can be calculated (predicted). However, when it is carried out in usual manner, it does not provide any prognosis outside the effects of the functional groups and/or fragments analyzed.

In our earlier communications we tried to extend the information potential of the Free–Wilson method, namely by additional correlations of separated contributions to activity (corresponding to effects of the molecular sections varied) with physical and physico-chemical properties of the respective molecular fragments[2 – 6]. Other possibilities are offered by the Topliss approach. In this case, however, a selection of substituents according to Topliss[7] must be carried out in the molecular section varied (i.e. with the following substituents of phenyl group in the anilide section of the molecule: H, 4′-CH_3, 4′-OCH_3, 4′-Cl and 3′,4′-Cl_2 and the same selection in benzoyl section where, however, 3-Br was used instead of 3,4-Cl_2). We supposed that a prognostics showing a reasonable selection of further substitution variations would be possible according to the recommendations used in the Topliss approach, after a separation carried out according to Free and Wilson.

In our previous study[2] we analyzed in this way the relations between structure and antimycobacterial activity against *Mycobacterium kansasii* within a group of thiobenzanilides where the substituents were varied according to the Topliss selection of substituents. The analysis was complemented by an equation of the Hansch type relating the antimycobacterial activity to the physico-chemical parameters of substituents in both molecular sections varied, see Eq. (*1*). The parameters corresponding to the substituents in the benzoyl section have the index 1, those for the anilide section index 2.

$$\log \mathrm{MIC} = 0.477\,\pi_1 - 1.452\,\sigma_1 - 0.692\,(\pi_2^-)^2 + 1.035\,\pi_2^- + 1.749\,, \qquad (1)$$

$$r = 0.747 \qquad s = 0.34 \qquad F = 7.90 \qquad n = 30\,.$$

We arrived at a conclusion that one of possible ways of increasing activity consists in increasing the lipophilicity of the anilide section of molecule.

The aim of the present work, therefore, is the preparation of 4-cyclohexylthiobenzanilides as representatives of model compounds suggested in this way. Moreover we wanted to study the activity against *Mycobacterium tuberculosis* in the whole set of thiobenzanilides synthetized in our laboratory, particularly from the standpoint of the structure–activity dependence. An approximate estimate had to be made again by the procedure by Topliss after a separation carried out according to Free and Wilson.

EXPERIMENTAL

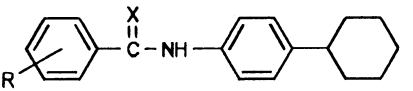
Chemicals. The preparation of thiosalicylanilides *XI – XXXV* was described in the previous communication[2], that of compounds *I – X* is given in this paper. The melting points of the compounds prepared were measured with a Kofler apparatus and were not corrected. The IR spectra were measured with a Perkin–Elmer 577 apparatus in KBr pellets. The samples for analysis were dried at 60 °C at 2.5 kPa 2 days.

4′-Cyclohexylbenzanilides *I – V*

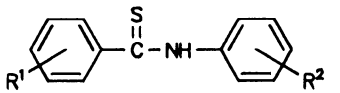
The corresponding chloride (23 mmol) was added dropwise into a mixture of 4 g (22.8 mmol) 4-cyclohexylaniline and 20 ml pyridine with stirring and cooling in an ice bath. The reaction mixture was left to stand at room temperature 3 days, whereafter it was poured into 100 ml saturated sodium carbonate solution. The separated product was collected by suction, washed with saturated sodium carbonate solution, with water, and recrystallized from ethanol. The results are given in Table I.

4′-Cyclohexylthiobenzanilides *VI – X*

A mixture of 14 mmol corresponding 4′-cyclohexylbenzanilide and 10 ml pyridine was refluxed with stirring and treated with 7.1 mmol phosphorus pentasufide added portionwise. The reaction mixture was refluxed for another 3 h. After cooling, it was poured into 100 ml water. The next day, the raw product was collected by suction (if it precipitated as a solid) or separated as a viscous oil from the aqueous phase (if it did not solidify) and recrystallized from ethanol. The results are given in Table I.



	R	X		R	X
I	H	O	*VI*	H	S
II	4-CH_3	O	*VII*	4-CH_3	S
III	4-OCH_3	O	*VIII*	4-OCH_3	S
IV	4-Cl	O	*IX*	4-Cl	S
V	3-Br	O	*X*	3-Br	S



XI – XL

For R^1, R^2 see Table II

TABLE I

4'-Cyclohexylbenzanilides and 4'-cycloheylthiobenzanilides, logarithm of their minimum inhibitory concentration (log MIC, μmol/l) against *Mycobacterium kansasii* and their most important absorption bands in infrared spectroscopy

Compound	R^1 X	Yield %	M. p. °C	Formula (M. w.)	Calculated/Found % C	% H	% N	% S	log MIC[a]	IR, cm^{-1}	
I	H	84	210	$C_{19}H_{21}NO$	81.68	7.58	5.01	–	–	2 880, 2 950	ν(C–H)
	O			(279.4)	81.59	7.84	5.11	–		1 660	ν(C=O)
II	4-CH_3	76	203 – 205	$C_{20}H_{23}NO$	81.87	7.90	4.77	–	–	2 850, 2 920, 3 030	ν(C–H)
	O			(293.4)	81.90	8.08	4.54	–		1 640	ν(C=O)
III	4-OCH_3	92	197 – 199	$C_{20}H_{23}NO_2$	77.64	7.49	4.53	–	–	2 900, 2 980	ν(C–H)
	O			(309.4)	77.76	7.60	4.40	–		1 675, 1 635	ν(C=O)
IV[b]	4-Cl	83	253 – 254	$C_{19}H_{20}ClNO$	72.72	6.42	4.46	–	–	2 850, 2 960, 3 025	ν(C–H)
	O			(313.8)	72.35	6.46	4.46	–		1 645	ν(C=O)
V[c]	3-Br	87	200 – 202	$C_{19}H_{20}BrNO$	63.70	5.63	3.91	–	–	2 860, 2 980	ν(C–H)
	O			(358.3)	63.62	5.71	4.09	–		1 670	ν(C=O)
VI	H	82	122	$C_{19}H_{21}NS$	77.24	7.16	4.74	10.85	0.477	2 860, 2 930, 3 000	ν(C–H)
	S			(295.4)	77.22	7.08	4.66	11.36		3 200	ν(N–H)
VII	4-CH_3	71	112 – 113	$C_{20}H_{23}NS$	77.62	7.49	4.53	10.36	1.792	2 860, 2 930	ν(C–H)
	S			(306.5)	77.64	7.43	4.48	10.86		3 230	ν(N–H)
VIII	4-OCH_3	52	144 – 145	$C_{20}H_{25}NOS$	73.81	7.12	4.30	9.85	1.176	2 850, 2 915	ν(C–H)
	S			(325.5)	73.62	7.09	4.34	9.96		3 260	ν(N–H)
IX	4-Cl[d]	72	175	$C_{19}H_{20}ClNS$	69.18	6.11	4.25	9.72	1.792	2 880, 2 960	ν(C–H)
	S			(329.9)	68.93	6.04	4.32	10.12		3 280	ν(N–H)
X	3-Br[e]	39	183 – 184	$C_{19}H_{20}BrNS$	60.96	5.38	3.75	8.56	1.176	2 860, 2 930	ν(C–H)
	S			(374.3)	60.98	5.27	3.89	9.05		3 200	ν(N–H)

[a] For comparison: log MIC isonicotinohydrazide 1.491. [b] Calculated: 11.30% Cl; found: 11.61% Cl. [c] Calculated: 22.30% Br; found 22.23% Br. [d] Calculated: 10.75% Cl; found: 10.62% Cl. [e] Calculated 21.34% Br; found: 21.64% Br.

Evaluation of Antibacterial Activity

The antibacterial activity of compounds *VI – XL* against *Mycobacterium tuberculosis* $H_{37}R_v$ was evaluated on a semisynthetic substrate by Šula containing bovine blood serum. The substances tested were dissolved in dimethyl sulfoxide, the concentrations being 1 000, 500, 250, 125, 62, 31, 15, 7, and 3 μmol/l. The minimum inhibitory concentration was determined after 15 days incubation at 37 °C and its values are summarized in Table II.

The antimycobacterial evaluation of compounds *VI – X* (prepared in this work) against *Mycobacterium kansasii* was carried out on a Stauton synthetic substrate without peptides under the conditions described in the previous communication[2]. The results are summarized in Table I.

Calculations. All the calculations were carried out with the use of the Multireg H program and an IQ-152 computer (ZPA, Nový Bor). Table III presents a survey of calculated contributions of the varied structural section to the activity. The same table also gives the values of substituent constants used in other analyses which were predominantly taken from the book by Kuchař and Rejholec[8].

DISCUSSION

When monitoring the activity against *Mycobacterium kansasii*, one can conclude that the choice of new substituent was correct, and a repeated analysis of this group of compounds by the procedure by Free and Wilson shows that it increases the activity in the most distinct way among all the substitution analogues studied. The introduction of physico-chemical parameters (σ and π^-) of cyclohexyl into the regression equation describing the dependence of antimycobacterial activity against *Mycobacterium kansasii* upon structure (see Eq. (*1*)) has led to an only little improvement of the regression coefficients (see Eq. (*2*)).

$$\log \mathrm{MIC}_{\mathrm{kans}} = 0.640\,\pi_1 - 1.517\,\sigma_1 - 0.422\,(\pi_2^-)^2 + 0.751\,\pi_2^- + 1.682\,, \qquad (2)$$

$$r = 0.785 \qquad s = 0.36 \qquad F = 12.07 \qquad n = 35\,.$$

The symbols of Eq. (*2*) agree with those in the previous communication, i.e. the substituents have indexes 1 and 2 in the thioacyl and anilide sections of the molecule, respectively.

However, the present work for the first time studies the activity in vitro against *Mycobacterium tuberculosis*, viz. on the substrate with peptides by Šula. Nevertheless, the analysis by the Free–Wilson method[9] showed that, although 4′-cyclohexyl affects the resulting activity in a very favourable way, it does not reach the effect of 3′-Cl and 4′-Cl. The method by Topliss is based on the principle that the structure–activity dependence is deduced from the activity order of compounds containing the substituents recommended by him[7]. From the order of contributions of the substituents in thioacyl section to the resulting activity it is possible to conclude that the dependence on the Hammett constants is linear. More difficult is to estimate the structure–activity interrelations in the anilide section of the molecule. The order of increasing activity of substi-

TABLE II

Logarithm of the minimum inhibitory concentration (log MIC, μmol/l) of thiobenzanilides against *Mycobacterium tuberculosis* $H_{37}R_v$

Compound	R^1	R^2	log MIC[a]
VI	H	4′-c-C_6H_{11}	1.492
VII	4-CH_3	4′-c-C_6H_{11}	1.492
VIII	4-OCH_3	4′-c-C_6H_{11}	1.492
IX	4-Cl	4′-c-C_6H_{11}	1.792
X	3-Br	4′-c-C_6H_{11}	1.176
XI	H	H	2.097
XII	H	4′-CH_3	2.574
XIII	H	4′-OCH_3	2.097
XIV	H	4′-Cl	1.792
XV	H	3′,4′-Cl_2	1.176
XVI	H	4′-Br	1.176
XVII	4-CH_3	H	2.097
XVIII	4-CH_3	4′-CH_3	2.398
XIX	4-CH_3	4′-OCH_3	2.398
XX	4-CH_3	4′-Cl	2.273
XXI	4-CH_3	3′,4′-Cl_2	1.176
XXII	4-CH_3	4′-Br	2.097
XXIII	4-OCH_3	H	1.792
XXIV	4-OCH_3	4′-CH_3	2.398
XXV	4-OCH_3	4′-OCH_3	1.792
XXVI	4-OCH_3	4′-Cl	3.301
XXVII	4-OCH_3	3′,4′-Cl_2	1.492
XXVIII	4-OCH_3	4′-Br	3.301
XXIX	4-Cl	H	2.097
XXX	4-Cl	4′-CH_3	1.792
XXXI	4-Cl	4′-OCH_3	1.792
XXXII	4-Cl	4′-Cl	2.097
XXXIII	4-Cl	3′,4′-Cl_2	1.176
XXXIV	4-Cl	4′-Br	2.398
XXXV	3-Br	H	1.792
XXXVI	3-Br	4′-CH_3	1.792
XXXVII	3-Br	4′-OCH_3	1.792
XXXVIII	3-Br	4′-Cl	1.491
XXXIX	3-Br	3′,4′-Cl_2	1.491
XL	3-Br	4′-Br	1.491

[a] For comparison: log MIC isonicotinohydrazide 0.613 μmol/l.

tuent variations recommended by Topliss reads as follows: 4′-CH_3, 4′-Cl < H, 4′-OCH_3 < 3′,4′-Cl_2. The study[7] by Topliss gives no recommendation for this order. However, with regard to the above-mentioned effect of substituents upon the activity we supposed that the relation could be a three-parameter relation expressing a quadratic dependence of activity on lipophilicity together with a linear dependence on electron-acceptor properties of substituents.

The separation according to Free and Wilson, however, showed that the substitution with 4′-Br lead to contradictory results and the substances containing this substituent showed the largest deviations of the calculated values from the experimental ones. The exclusion of this subgroup of compounds from the set increased the statistical significance of the Free–Wilson analysis. The structure–activity relationship can then be expressed by Eq. (*3*).

$$\log \mathrm{MIC}_{tbc} = -0.714\,\sigma_1 - 0.405\,(\pi_2^-)^2 + 0.770\,\pi_2^- - 0.860\,\sigma_2 + 1.916\,, \qquad (3)$$

$$r = 0.790 \qquad s = 0.32 \qquad F = 10.36 \qquad n = 30$$

TABLE III

The Hammett substituent constants (σ), the hydrophobic substituents constants (π, π^-) and the activity contributions of Free–Wilson analysis (Δ log MIC)

Substituent	σ	π	π^-	Δ log MIC		
				a	*b*	*c*
4-H	0	0	–	−0.397	−0.116	0.018
4-CH_3	−0.17	0.60	–	0.264	0.101	0.118
4-OCH_3	−0.27	−0.03	–	0.290	0.337	0.191
4-Cl	0.23	0.73	–	0.095	−0.010	−0.062
3-Br	0.39	0.96	–	−0.253	−0.312	−0.264
4′-H	0	–	0	−0.078	0.088	0.122
4′-CH_3	−0.17	–	0.48	0.260	0.303	0.338
4′-OCH_3	−0.27	–	−0.12	−0.081	0.085	0.119
4′-Cl	0.23	–	0.93	0.403	0.303	0.338
3′,4′-Cl_2	0.60	–	1.97	−0.259	−0.586	−0.551
4′-c-C_6H_{11}	−0.13	–	2.46	−0.589	−0.399	−0.365
4′-Br	0.23	–	1.19	0.345	0.205	–
μ_0	–	–	–	1.871	1.887	1.853

[a] *M. kansasii*, $r = 0.774$, $s = 0.428$, $F = 3.59$, $n = 35$; [b] *M. tuberculosis*, $r = 0.745$, $s = 0.422$, $F = 3.01$, $n = 35$; [c] *M. tuberculosis*, $r = 0.798$, $s = 0.347$, $F = 3.89$, $n = 30$.

Thiobenzanilides belong among the class of compounds with antituberculotic activity in which the resulting activity is a function of local parameters. In our laboratory they are investigated with the aim of their utilization as drugs for treatment of mycobacterial skin diseases. The application of salicylanilides (e.g. Arilide) is reduced because of their allergen activity[10].

In conclusion it can be stated that a new class of potential antituberculotics has been found. The activity increase – as compared with the thiobenzamides studied earlier[11] – caused by introduction of aryl group on nitrogen atom of the thioamide group can be compared to a similar effect among thiourea derivatives. However, it is necessary to look for further structure modifications to increase the activity.

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