Synthesis and Antimycobacterial Activity of Salicylanilides Substituted in Position 5

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A set of 57 salicylanilides was synthesized, with the substitution being varied at positions 5, 4′, and 3′. The substances were evaluated for antimycobacterial activity against the strains of *Mycobacterium tuberculosis*, *Mycobacterium kansasii*, and *Mycobacterium avium*. Structure—activity relationships were determined using the Free—Wilson method, which was further combined with the approach of Hansch. Antimycobacterial activity becomes higher with increasing electron-accepting ability of the substituents on the phenyl ring, and with increasing their lipophilicity. The influence of the substituents in position 5 is more complex.

The biological (antimicrobial, neuroleptic, analgetic, antiinflammatory, molluscoidean) activity, allergenic properties, and toxicity of salicylanilides were reviewed in the previous paper [1]. The photosynthesis-inhibiting activity was found [2] as well. The mechanism of biological activity is not usually known. At present, mycobacterial infections caused by *Mycobacterium avium* constitute a serious problem. This type of infections endangers mainly birds, but it has been recently found that they are transferable to humans as well. These cases are somewhat rare, but they come, as a rule, to a fatal end. Following the results described in the review paper [1], we presume that it may be possible to modify the antimycobacterial effect of salicylanilides towards activity against *M. avium*. The results of studies on salicylanilides could also initiate further research of benzoxazine derivatives, as 2*H*-1,3-benzoxazin-2,4(3*H*)-diones are likely to have a similar pharmacophore [3, 4]. Thus, we decided to study the antimycobacterial activity of salicylanilides, in particular against the above strains which are potentially pathological. Antimycobacterial activity was assessed against the strains of *Mycobacterium tuberculosis*, *Mycobacterium kansasii*, and *Mycobacterium avium*. For the purposes of this study, we selected a series of salicylanilides substituted in position 5 (H, Br, Cl, F, NO_2). The compounds were further modified by structural changes on the phenyl ring in the anilide part of the molecule in order to achieve larger variations of electronic and hydrophobic parameters. The whole set consisted of 57 substances; nevertheless, a small number of them was not subjected to biological evaluation due to a limited solubility in water (no biological activity was detected within the range of their solubility).

The compounds are not commercially available, but a number of them have been described in the literature. We have reported the data on antimycobacterial activity of unsubstituted salicylanilides [5], which had been prepared before.

The results of biological assays were subjected to a QSAR analysis based upon a combination of the Free—Wilson and Hansch methods with modifications introduced in [6—8]. In our approach, the Free—Wilson analysis is carried out first. From the obtained values expressing the influence of structural fragments on the biological activity, a relationship to physical and chemical molecular parameters is determined (*e.g.* to the substituent constants). The analysis is subsequently completed by attempting to find a correlation between biological activity and physicochemical data. For the salicylanilides described in this paper, the Free—Wilson analysis was carried out with regard to all kinds of biological activity which were observed, the values of the logarithms of partition coefficients (in octanol—water) were calculated using five differ-

ent ways, and their correlations to hydrophobic constants were studied. Following a careful consideration of significant structural parameters, attempts to find relationships between the substituent constants and the biological activity of salicylanilides were made.

EXPERIMENTAL

The melting points were determined on a Kofler apparatus. The samples for analysis and antimycobacterial tests were dried over P_4O_{10} at 61 °C and 66 Pa for 24 h. Elemental analyses were performed on a CHNSO CE elemental analyzer (FISONS EA 1110, Milano). The IR spectra were measured in KBr pellets on a Nicolet Impact 400 apparatus; the absorption bands ν(C=O) of salicylanilides were found in the region of 1612—1655 cm^{-1}. The NMR spectra were recorded on a Varian Mercury-Vx BB 300 spectrometer operating at 300 MHz in deuteriochloroform. Chemical shifts were recorded as δ values and were indirectly referenced to tetramethylsilane *via* the solvent signal (7.26 for ^1H). The ^1H NMR spectra are summarized in Table 2.

All regression equations were calculated with the use of the Multireg H program (P. Klemera) for Microsoft Excel. The values of the Hammett constants σ and hydrophobic substituent constants π were taken from the literature [9] and are summarized in Table 4. The results of Free—Wilson calculations are given in Table 5. The logarithms of the partition coefficients were calculated using the CS ChemOffice program package, the PALLAS program, and the HyperChem package. The calculated partition coefficients are summarized in Table 6.

Salicylanilides *Ia—Vl*

All compounds were prepared by both methods.

Method *A*

A solution of phosphorus trichloride (10 mmol) in anhydrous pyridine (10 cm^3) was added dropwise to a substituted aniline (20 mmol) in anhydrous pyridine (10 cm^3). After 30 min of stirring, a solution of salicylic acid (20 mmol) in pyridine (10 cm^3) was added to the mixture. After 3 h of heating, the reaction mixture was poured into 10 % aqueous sodium carbonate (200 cm^3), and the pH of the solution was adjusted to 8. The resultant precipitate was filtered off, washed with water, and crystallized from ethanol—water (yields 50—80 %).

Method *B*

A suspension of substituted salicylic acid (0.02 mol) and a substituted aniline (0.02 mol) in chlorobenzene (100 cm^3) was heated under reflux in the presence of PCl_3 (0.01 mol) for 3 h. The reaction mixture was filtered while hot, and the product allowed to crystallize upon cooling, which yielded 80—95 % of the theoretical amount. The product was recrystallized from ethanol—water (Table 1).

Antimycobacterial Susceptibility Assay

For the evaluation of antimycobacterial activity of the substances *in vitro*, the following strains were used: *M. tuberculosis* CNCTC My 331/88, *M. kansasii* CNCTC My 235/80, *M. avium* CNCTC My 330/88, obtained from the Czech National Collection of Type Cultures (CNCTC), National Institute of Public Health, Prague.

Antimycobacterial activity of the compounds against these strains was determined by the microdilution broth method, in the Šula semisynthetic medium (SEVAC, Prague).

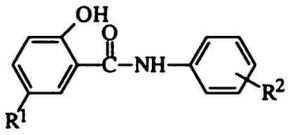
The compounds were added to the medium in a dimethyl sulfoxide solution. The following numerical values of concentrations ($c/(\mu mol\ dm^{-3})$) were used: 1000, 500, 250, 125, 62, 31, 16, 8, and 4. (The concentration of DMSO in medium 10 %, basic suspension of strains was carried out according to a standard having a concentration of 1 mg of bacterial mass per 1 cm^3.) The minimum inhibitory concentrations were determined after incubation at 37 °C for 14 d and 21 d by visual control. MIC was the lowest concentration of an antimycobacterially active substance (see the above concentrations), at which the inhibition of the growth of mycobacteria occurred. The DMSO has no inhibitory effect on this condition. The results are given in Table 3.

RESULTS AND DISCUSSION

All salicylanilides were prepared by the treatment of salicylic acid with the appropriate aniline derivatives in the presence of phosphorus trichloride in pyridine or chlorobenzene as a solvent. A strong absorption band in the region of $\tilde{\nu}$ = 1600—1655 cm^{-1} assignable to an aromatic amide vibration ν(C=O) was apparent in their infrared spectra. As the structure of the compounds is rather simple, the known ones were identified by the melting points, while the newly prepared substances were characterized by ^1H NMR spectra and their purity was checked by elemental analysis. The compounds are shown in Table 1 and their ^1H NMR spectral data are given in Table 2.

First physical and chemical parameters of the compounds were evaluated. Five approaches were used to calculate the logarithms of the partition coefficients (log P, octanol—water); three different fragmentations were employed in the ChemOffice package (fragmentations according to *Ghose* and *Crippen* [10], *Viswanadhan et al.* [11], and *Broto et al.* [12]). The

Table 1. Characteristics of the Compounds



Compound	R^1	R^2	Formula M_r	w_i(calc.)/% w_i(found)/%			M.p./°C	
				C	H	N	Found	Ref.
Ia	Br	H	$C_{13}H_{10}BrNO_2$ 292.1				225—227	225—227 [16]
Ib	Br	4-CH_3	$C_{14}H_{12}BrNO_2$ 306.2				253—255	251—253 [17]
Ic	Br	4-Br	$C_{13}H_9Br_2NO_2$ 371.0				240—241	240—241 [18]
Id	Br	4-OCH_3	$C_{14}H_{12}BrNO_3$ 326.6				236—237	236—237 [17]
Ie	Br	4-Cl	$C_{13}H_9BrClNO_2$ 326.6				239—241	238—243 [16]
If	Br	3,4-Cl_2	$C_{13}H_8BrCl_2NO_2$ 361.0				234—236	235—238 [16]
Ig	Br	3-Cl	$C_{13}H_9BrClNO_2$ 326.6	47.82 48.16	2.78 3.05	4.29 4.04	224—226	–
Ih	Br	3-NO_2	$C_{13}H_9BrN_2O_4$ 337.1	46.32 46.62	2.69 2.53	8.31 8.32	238—240	–
Ij	Br	4-$N(CH_3)_2$	$C_{15}H_{15}BrN_2O_2$ 335.2	53.75 53.78	4.51 4.11	8.36 8.48	249—251	–
Ik	Br	4-F	$C_{13}H_9BrFNO_2$ 310.1				231—233	234—235 [19]
Il	Br	3-F	$C_{13}H_9BrFNO_2$ 310.1	50.35 50.40	2.93 2.92	4.52 4.95	247—249	–
IIa	Cl	H	$C_{13}H_{10}ClNO_2$ 247.7				209—211	209—211 [16]
IIb	Cl	4-CH_3	$C_{14}H_{12}ClNO_2$ 261.7				225—227	215—217 [20]
IIc	Cl	4-Br	$C_{13}H_9BrClNO_2$ 326.6				224—226	224—226.5 [16]
IId	Cl	4-OCH_3	$C_{14}H_{12}ClNO_3$ 277.7				204—206	205 [21]
IIe	Cl	4-Cl	$C_{13}H_9Cl_2NO_2$ 316.6				229—231	231—232 [16]
IIf	Cl	3,4-Cl_2	$C_{13}H_8Cl_3NO_2$ 282.1				248—250	246—248 [16]
IIg	Cl	3-Cl	$C_{13}H_9Cl_2NO_2$ 282.1				216—218	216 [21]
IIh	Cl	3-NO_2	$C_{13}H_9ClN_2O_4$ 292.7	53.35 53.36	3.10 3.14	9.57 9.44	241—243	–
IIi	Cl	4-NO_2	$C_{13}H_9ClN_2O_4$ 292.7				260—262	260 [21]
IIj	Cl	4-$N(CH_3)_2$	$C_{15}H_{15}ClN_2O_2$ 290.8	61.97 61.94	5.20 4.98	9.63 9.69	232—234	–
IIk	Cl	4-F	$C_{13}H_9FClNO_2$ 265.7				234—236	237—238 [19]
IIl	Cl	3-F	$C_{13}H_9FClNO_2$ 265.7	58.77 58.63	3.41 3.31	5.27 5.29	234—236	–
IIIa	F	H	$C_{13}H_{10}FNO_2$ 231.2				204—205	199—200 [19]
IIIb	F	4-CH_3	$C_{14}H_{12}FNO_2$ 245.3	68.56 68.79	4.93 5.19	5.71 5.95	214—215	–
IIIc	F	4-Br	$C_{13}H_9BrFNO_2$ 310.1				217—218	213—214 [19]

IIId	F	4-OCH_3	$C_{14}H_{12}FNO_3$	64.36	4.63	5.36	211—212	–
			261.3	64.48	4.60	5.57		
IIIe	F	4-Cl	$C_{13}H_9FClNO_2$				205—207	205—206 [19]
			265.7					
IIIf	F	3,4-Cl_2	$C_{13}H_8FCl_2NO_2$	53.03	2.69	4.67	254—256	–
			300.1	52.07	2.82	4.65		
IIIg	F	3-Cl	$C_{13}H_9FClNO_2$	58.77	3.41	5.27	235—237	–
			265.7	58.66	3.42	5.16		
IIIh	F	3-NO_2	$C_{13}H_9FN_2O_4$	56.53	3.25	10.14	236—237	–
			276.2	56.57	3.62	10.07		
IIIj	F	4-$N(CH_3)_2$	$C_{15}H_{15}FN_2O_2$	65.68	5.51	10.21	196—198	–
			274.3	65.51	5.74	10.23		
IIIk	F	4-F	$C_{13}H_9F_2NO_2$				200—202	202—203 [19]
			249.2					
IIIl	F	3-F	$C_{13}H_9F_2NO_2$	62.65	3.64	5.62	195—197	–
			249.2	62.53	3.57	5.67		
IVa	NO_2	H	$C_{13}H_{10}N_2O_4$				227—228	221—224 [21]
			258.2					
IVb	NO_2	4-CH_3	$C_{14}H_{12}N_2O_4$				232—234	232—233 [22]
			272.3					
IVc	NO_2	4-Br	$C_{13}H_9BrN_2O_4$				242—244	242—245 [23]
			337.1					
IVd	NO_2	4-OCH_3	$C_{14}H_{12}N_2O_5$				195—196	194—195 [22]
			288.3					
IVe	NO_2	4-Cl	$C_{13}H_9ClN_2O_4$				251—253	252—255 [23]
			292.7					
IVf	NO_2	3,4-Cl_2	$C_{13}H_8Cl_2N_2O_4$				280—282	280—282 [16]
			327.1					
IVg	NO_2	3-Cl	$C_{13}H_9ClN_2O_4$				228—230	230 [23]
			292.7					
IVh	NO_2	3-NO_2	$C_{13}H_9N_3O_6$				232—234	230—231 [22]
			303.2					
IVj	NO_2	4-$N(CH_3)_2$	$C_{15}H_{15}N_3O_4$	59.80	5.02	13.95	260—262	–
			301.3	60.01	5.23	13.96		
IVk	NO_2	4-F	$C_{13}H_9FN_2O_4$				238—240	240 [23]
			276.2					
IVl	NO_2	3-F	$C_{13}H_9N_2O_4F$	56.53	3.28	10.14	216—218	–
			276.2	56.13	3.30	10.13		
Va	H	H	$C_{13}H_{11}NO_2$				133—135	136—137 [18]
			213.2					
Vb	H	4-CH_3	$C_{14}H_{13}NO_2$				154—156	155—156 [24]
			227.3					
Vc	H	4-Br	$C_{13}H_{10}BrNO_2$				168—170	169—172 [18]
			292.1					
Vd	H	4-OCH_3	$C_{14}H_{13}NO_3$				158—160	159—160 [25]
			243.3					
Ve	H	4-Cl	$C_{13}H_{10}ClNO_2$				165—167	167—168 [22]
			247.7					
Vf	H	3,4-Cl_2	$C_{13}H_9Cl_2NO_2$				213—215	213—216 [16]
			282.1					
Vg	H	3-Cl	$C_{13}H_{10}ClNO_2$				173—175	175—177 [18]
			247.7					
Vh	H	3-NO_2	$C_{13}H_{10}N_2O_4$				220—221	220—221 [3]
			258.2					
Vi	H	4-NO_2	$C_{13}H_{10}N_2O_4$				232—234	230 [21]
			258.2					
Vj	H	4-$N(CH_3)_2$	$C_{15}H_{16}N_2O_2$				150—152	150—152 [3]
			256.3					
Vk	H	4-F	$C_{13}H_{10}FNO_2$				158—160	160—161 [19]
			231.2					
Vl	H	3-F	$C_{13}H_{10}FNO_2$				151—153	151—153 [3]
			231.2					

Table 2. ^{1}H NMR Spectra of New Salicylanilides

	Chemical shift δ
Ig	10.49 (s, 1H, OH), 7.98 (d, 1H, J = 2.47 Hz, H-6), 7.87—7.91 (m, 1H, H-2′), 7.53—7.61 (m, 2H, H-4, H-6′), 7.38 (t, J = 8.1 Hz, H-5′), 7.15—7.21 (m, 1H, H-4′), 6.95 (d, 1H, J = 8.79 Hz, H-3)
Ih	10.72 (s, 1H, OH), 8.71—8.74 (m, 1H, H-2′), 8.01—8.10 (m, 1H, H-4′), 7.95—8.00 (m, 1H, H-6′), 7.98 (d overlapped, J = 2.48 Hz, 1H, H-6), 7.64 (t, J = 8.24 Hz, 1H, H-5′), 7.57 (dd, 1H, J = 8.79 Hz, J = 2.48 Hz, H-4), 6.96 (d, 1H, J = 8.79 Hz, H-3)
Ij	10.24 (s, 1H, OH), 8.13 (d, J = 2.47 Hz, 1H, H-6), 7.55 (dd, J = 8.79 Hz, J = 2.47 Hz, 1H, H-4), 7.43—7.51 (m, 2H (AA′, BB′), H-2′, H-6′), 6.92 (d, 1H, J = 8.79 Hz, H-3), 6.68—6.76 (m, 2H (AA′, BB′), H-3′, H-5′), 2.86 (s, 6H, CH_3)
Il	10.50 (s, 1H, OH), 7.98 (d, J = 2.48 Hz, 1H, H-6), 7.68 (dt, 1H, J = 11.54 Hz, J = 1.92 Hz, H-6′), 7.56 (dd, 1H, J = 8.79 Hz, J = 2.48 Hz, H-4), 7.33—7.48 (m, 2H, H-2′, H-5′), 6.90—7.00 (m, 1H, H-4′), 6.95 (d, 1H, J = 8.79 Hz, H-3)
IIh	10.76 (s, 1H, OH), 8.82 (t, 1H, J = 2.47 Hz, H-2′), 8.02 (ddd, 1H, J = 8.24 Hz, J = 2.47 Hz, J = 1.1 Hz, H-4′), 7.95 (ddd, 1H, J = 8.24 Hz, J = 2.47 Hz, J = 1.1 Hz, H-6′), 7.86 (d, J = 2.74 Hz, 1H, H-6), 7.62 (t, J = 8.24 Hz, 1H, H-5′), 7.44 (dd, 1H, J = 8.79 Hz, J = 2.74 Hz, H-4), 7.00 (d, 1H, J = 8.79 Hz, H-3)
IIj	10.23 (s, 1H, OH), 8.01 (d, J = 2.47 Hz, 1H, H-6), 7.39—7.53 (m, 3H, H-4, H-2′, H-6′), 6.97 (d, 1H, J = 8.79 Hz, H-3), 6.67—6.78 (m, 2H (AA′, BB′), H-3′, H-5′), 2.86 (s, 6H, CH_3)
IIl	10.50 (s, 1H, OH), 7.87 (d, 1H, J = 2.75 Hz, H-6), 7.68 (dt, 1H, J = 11.54 Hz, J = 1.92 Hz, H-6′), 7.33—7.48 (m, 3H, H-4, H-2′, H-5′), 7.00 (d, 1H, J = 8.79 Hz, H-3), 6.90—6.99 (m, 1H, H-4′)
IIIb	10.33 (s, 1H, OH), 7.77 (dd, 1H, J = 9.75 Hz, J = 3.16 Hz, H-6), 7.53—7.61 (m, 2H (AA′, BB′), H-2′, H-6′), 7.23—7.33 (m, 1H, H-4), 7.12—7.20 (m, 2H (AA′, BB′), H-3′, H-5′), 6.99 (dd, 1H, J = 9.06 Hz, J = 4.67 Hz, H-3), 2.26 (s, 3H, CH_3)
IIId	10.30 (s, 1H, OH), 7.79 (dd, 1H, J = 9.61 Hz, J = 2.75 Hz, H-6), 7.54—7.65 (m, 2H (AA′, BB′), H-2′, H-6′), 7.22—7.32 (m, 1H, H-4), 6.87—7.03 (m, 3H, H-3, H-3′, H-5′), 3.75 (s, 3H, OCH_3)
IIIf	10.55 (s, 1H, OH), 8.08 (d, 1H, J = 2.20 Hz, H-2′), 7.56—7.67 (m, 3H, H-6, H-5′, H-6′), 7.25—7.33 (m, 1H, H-4), 6.99 (dd, 1H, J = 9.07 Hz, J = 4.67 Hz, H-3)
IIIg	10.49 (s, 1H, OH), 7.90 (t, 1H, J = 1.92 Hz, H-2′), 7.67 (dd, 1H, J = 9.61 Hz, J = 3.30 Hz, H-6), 7.58 (ddd, 1H, J = 8.24 Hz, J = 1.92 Hz, J = 1.10 Hz, H-6′), 7.37 (t, 1H, J = 8.24 Hz, H-5′), 7.25—7.34 (m, 1H, H-4), 7.17 (ddd, 1H, J = 8.24 Hz, J = 1.92 Hz, J = 1.10 Hz, H-4′), 7.00 (dd, 1H, J = 9.06 Hz, J = 4.67 Hz, H-3)
IIIh	10.66 (s, 1H, OH), 8.69 (t, 1H, J = 1.92 Hz, H-2′), 7.99 (ddd, 1H, J = 8.24 Hz, J = 1.92 Hz, J = 1.10 Hz, H-4′), 7.91 (ddd, 1H, J = 8.24 Hz, J = 1.92 Hz, J = 1.10 Hz, H-6′), 7.65 (dd, 1H, J = 9.48 Hz, J = 3.16 Hz, H-6), 7.58 (t, 1H, J = 8.24 Hz, H-5′), 7.22—7.31 (m, 1H, H-4), 6.97 (dd, 1H, J = 9.07 Hz, J = 4.67 Hz, H-3)
IIIj	10.21 (s, 1H, OH), 7.82 (dd, 1H, J = 9.89 Hz, J = 3.30 Hz, H-6), 7.44—7.54 (m, 2H (AA′, BB′), H-2′, H-6′), 7.22—7.32 (m, 1H, H-4), 6.97 (dd, 1H, J = 9.06 Hz, J = 4.67 Hz, H-3), 6.67—6.75 (m, 2H (AA′, BB′), H-3′, H-5′), 2.85 (s, 6H, CH_3)
IIIl	10.42 (s, 1H, OH), 7.67—7.75 (m, 3H, H-6, H-2′, H-6′), 7.25—7.33 (m, 1H, H-4), 7.15—7.24 (m, 2H, H-4′, H-5′), 6.99 (dd, 1H, J = 9.06 Hz, J = 4.67 Hz, H-3)
IVj	11.06 (s, 1H, OH), 8.83 (d, J = 3.48 Hz, 1H, H-6), 8.15—8.23 (m, 1H, H-4), 7.46—7.54 (m, 2H (AA′, BB′), H-2′, H-6′), 6.98 (d, 1H, J = 9.20 Hz, H-3), 6.70—6.78 (m, 2H (AA′, BB′), H-3′, H-5′), 2.87 (s, 6H, CH_3)
IVl	10.66 (s, 1H, OH), 8.67 (d, 1H, J = 2.89 Hz, H-6), 8.26 (dd, 1H, J = 9.20 Hz, J = 2.89 Hz, H-4), 7.68 (dt, 1H, J = 11.54 Hz, J = 1.92 Hz, H-6′), 7.33—7.49 (m, 2H, H-2′, H-5′), 7.15 (d, 1H, J = 9.20 Hz, H-3), 6.91—7.01 (m, 1H, H-4′)

remaining two approaches were based on the PALLAS program which uses fragmentation developed by *Rekker* [13] and HyperChem [14]. Values calculated by different ways could be mutually correlated (r = 0.970—0.998, s = 0.03—0.12), and further correlations were found with constants π, but not with constants π^-. In order to determine the influence of the substituents from the acyl part of the molecule, we carried out the separation of the calculated log P values according to the *Fujita* and *Ban* [15] modification of the Free—Wilson approach. Within the series of our compounds, both groups of separated data correlated to both π_m values and π_p values (the correlation to π_p was somewhat more significant).

In the group of salicylanilides, there are two functional groups, which can be considered as the probable active centres with regard to the mechanism of action, *i.e.* the amide function and the hydroxy group. Substituents R^2 (in the anilide part of the molecule) can strongly modify the electronic structure of the anilide moiety, but their influence over the hydroxy group is weaker. On the other hand, substituents R^1 (in the acyl portion of the molecule) can effect electron density in both groups; the influence on the amide and

Table 3. Logarithms of Minimum Inhibitory Concentrations (log{MIC}) of Salicylanilides

Compound	log{MIC/(μmol dm^{-3})} Incubation 14 d/21 d		
	M. tuberculosis	*M. kansasii*	*M. avium*
Ia	1.204/1.491	1.491/1.491	1.491/1.792
Ib	1.204/1.204	1.204/*a*	*a*/*a*
Ic	0.602/0.602	0.903/0.903	1.204/1.204
Id	*a*/*a*	*a*/*a*	*a*/*a*
Ie	0.602/0.602	0.903/1.204	1.204/1.204
If	0.602/0.602	0.903/0.903	0.903/0.903
Ig	0.602/0.903	1.204/1.204	1.491/1.491
Ih	0.903/0.903	1.204/1.204	1.491/1.491
Ij	*a*/*a*	*a*/*a*	*a*/*a*
Ik	0.903/0.903	0.903/0.903	1.491/1.491
Il	0.903/0.903	0.903/1.204	1.491/1.491
IIa	1.491/1.491	0.602/0.602	1.204/1.491
IIb	1.204/1.491	0.602/0.602	1.204/1.204
IIc	0.903/1.204	0.602/0.602	0.903/0.903
IId	*a*/*a*	0.602/0.903	1.491/1.491
IIe	0.602/0.602	0.903/0.903	0.903/0.903
IIf	0.602/0.903	0.602/0.602	1.204/1.204
IIg	0.602/0.903	0.602/0.903	0.903/1.204
IIh	0.903/0.903	0.903/0.903	1.204/1.204
IIi	0.602/0.903	0.602/0.602	0.903/0.903
IIj	*a*/*a*	0.903/0.903	1.491/1.792
IIk	1.204/1.204	0.602/0.602	1.204/1.204
IIl	0.903/0.903	0.903/0.903	1.204/1.204
IIIa	1.491/1.792	1.204/1.491	1.491/1.792
IIIb	1.491/1.792	1.204/1.204	1.204/1.491
IIIc	0.602/0.903	0.602/0.903	0.903/1.204
IIId	1.792/1.792	1.792/1.792	1.204/1.204
IIIe	0.903/0.903	0.903/0.903	1.204/1.204
IIIf	0.602/0.602	0.602/0.602	0.903/0.903
IIIg	0.903/1.204	*a*/*a*	1.204/1.204
IIIh	0.903/1.204	1.204/1.491	1.204/1.491
IIIj	1.491/*a*	*a*/*a*	*a*/*a*
IIIk	1.204/1.204	0.903/0.903	1.204/1.204
IIIl	1.491/1.491	1.204/1.204	1.491/1.491
IVa	1.204/1.491	0.903/1.204	2.398/2.398
IVb	1.204/1.204	0.903/1.204	2.097/2.097
IVc	1.204/1.204	0.903/0.903	1.491/1.792
IVd	1.491/1.491	0.903/1.204	2.398/2.398
IVe	1.204/1.204	0.903/0.903	1.792/1.792
IVf	0.903/0.903	0.903/0.903	1.491/1.792
IVg	1.204/1.204	0.903/1.204	1.792/1.792
IVh	1.491/1.491	0.903/1.204	2.097/2.097
IVj	1.491/1.491	0.903/1.204	2.699/3.000
IVk	1.204/1.491	0.903/0.903	2.097/2.097
IVl	1.491/1.491	1.204/1.204	1.792/2.097
Va	1.792/1.792	2.097/2.398	1.792/2.097
Vb	1.792/1.792	1.792/2.097	1.491/1.792
Vc	1.204/1.491	1.204/1.491	1.491/1.491
Vd	1.792/1.792	2.398/2.398	1.792/2.097
Ve	1.491/1.491	1.491/1.491	1.491/1.491
Vf	0.903/0.903	0.602/0.903	1.204/1.491
Vg	1.204/1.204	0.903/0.903	1.491/1.491
Vh	1.204/1.204	1.792/*a*	1.491/*a*
Vi	0.903/1.204	1.204/1.204	1.491/1.491
Vj	2.398/2.398	2.398/*a*	2.097/2.398
Vk	1.792/1.792	1.792/2.097	1.491/1.792
Vl	1.491/1.491	1.792/1.792	1.792/1.792

a) The minimum of inhibitory activity is impossible to estimate for the small solubility of the compound.

Table 4. Substituent Constants σ, π, and π^-

Substituent	σ	π	π^-
H	0	0	0
p-CH_3	−0.17	0.60	0.48
m-Br	0.39	0.96	1.17
p-Br	0.23	1.19	1.13
p-OCH_3	−0.27	−0.03	−0.12
p-Cl	0.23	0.73	0.93
m-Cl	0.37	0.73	1.04
m,*p*-Cl_2	0.6	1.5	2.97
m-NO_2	0.71	−0.05	0.54
p-NO_2	0.78	0.02	0.45
p-$N(CH_3)_2$	−0.83	−0.08	0.69
m-F	0.34	0.22	0.47
p-F	0.06	0.15	0.31

Table 5. Results of the Free—Wilson Analysis

Fragment	Δ log{MIC}		
	M. tuberculosis	*M. kansasii*	*M. avium*
5-Br	−0.6144	−0.5071	−0.1756
5-Cl	−0.5043	−0.9197	−0.4413
5-F	−0.3717	−0.5666	−0.3576
5-NO_2	−0.2609	−0.7153	0.4043
H	0	0	0
H	0	0	0
4′-CH_3	−0.0574	−0.1184	−0.1916
4′-Br	−0.5334	−0.4166	−0.4768
4′-OCH_3	0.1159	0.1730	0.0307
4′-Cl	−0.4760	−0.2388	−0.3564
3′,4′-Cl_2	−0.7140	−0.5370	−0.5342
3′-Cl	−0.5334	−0.3626	−0.2990
3′-NO_2	−0.3556	−0.0582	−0.1778
4′-NO_2	−0.7820	−0.4383	−0.3716
4′-$N(CH_3)_2$	0.2175	0.1452	0.3188
4′-F	−0.1750	−0.2388	−0.1778
3′-F	−0.1806	−0.0582	−0.1212
	μ_o = 1.7867	μ_o = 1.8011	μ_o = 1.7893
	r= 0.914	r = 0.854	r = 0.948
	s = 0.196	s = 0.281	s = 0.154
	F = 12.56	F = 6.62	F = 22.03
	n = 53	n = 53	n = 53

hydroxy functions will be proportional to the substituent constants σ_m and σ_p, respectively. This assumption was used as the starting point for further work. It can be concluded that substituent constants π (π_p are probably more suitable for the acyl part) and substituent constants σ (both σ_m and σ_p for the acyl part) can be used for the correlation of physical data with the biological activity of the compounds.

Antimycobacterial activity of the compounds was monitored after 14 d and 21 d of incubation. The logarithms of minimum inhibitory concentrations (log MIC) for both incubation periods are summarized in Table 3. As there is a correlation between the results of evaluation after 14 d and 21 d ($r > 0.93$, $s < 0.16$), only values obtained after a 14-day period were further processed, which is more common. Given the described correlation, it can be presumed that the processing of values obtained after longer periods of time would yield similar results.

The correlations according to the general Hansch equation (correlation log{MIC} with log P, log(P^2) and Hammett constants, see Table 4) were not significant. We carried out the Fujita and Ban [15] modification of the Free—Wilson analysis (Table 5).

It is a disadvantage of the Free—Wilson analysis that predictions can be made only within the mosaic of the structural fragments which were used. In the second part of this work, we attempted to generalize our conclusions using our own method based on correlating the contributions of individual substructures with the substituent constants corresponding to physicochemical properties of the compounds under investigation (Table 6). Thus, following the above considerations, correlations with the substituent constants were set up. Eqns (*1*—*3*) characterize the relationships between structure and antimycobacterial activity against *M. tuberculosis*, *M. kansasii*, and *M. avium*, respectively. In all the equations, index 1 denotes substituent constants from the acyl portion of the molecule, index 2 marks substituent constants from the anilide part. The remaining symbols are obvious and do not require explanation.

$$\log\{MIC_{tbc}\} = -0.298(\pm 0.070)(\pi_p)_1 - \\ -1.004(\pm 0.303)(\sigma_m)_1 + 0.621(\pm 0.251)(\sigma_p)_1 - \\ -0.264(\pm 0.057)(\pi)_2 - 0.496(\pm 0.078)(\sigma)_2 + \\ +1.688(\pm 0.063) \qquad (1)$$

$$r = 0.879 \quad s = 0.204 \quad F = 32.07 \quad n = 53$$

$$\log\{MIC_{kans}\} = -0.137(\pm 0.114)(\pi_p)_1 - \\ -1.754(\pm 0.512)(\sigma_m)_1 + 0.710(\pm 0.429)(\sigma_p)_1 - \\ -0.255(\pm 0.090)(\pi)_2 - 0.205(\pm 122)(\sigma)_2 + \\ +1.731(\pm 0.100) \qquad (2)$$

$$r = 0.733 \quad s = 0.325 \quad F = 10.92 \quad n = 53$$

$$\log\{MIC_{av}\} = -0.117(\pm 0.070)(\pi_p)_1 - \\ -1.359(\pm 0.303)(\sigma_m)_1 + 1.774(\pm 0.253)(\sigma_p)_1 - \\ -0.260(\pm 0.056)(\pi)_2 - 0.256(\pm 0.076)(\sigma)_2 + \\ +1.722(\pm 0.061) \qquad (3)$$

$$r = 0.887 \quad s = 0.199 \quad F = 34.93 \quad n = 53$$

All the three equations show a similar dependence of antimycobacterial activity on the structural parameters for all strains of mycobacteria which were evaluated. The compounds become more efficient antimycobacterial agents with increasing electron-accepting influence of the substituents on the amide function,

Table 6. Calculated Logarithms of Partition Coefficients

Compound	Substituent		Method of calculation				
	R^1	R^2	[10]	[11]	[12]	[14]	[13]
Ia	5-Br	H	3.28	3.32	3.07	3.79	4.00
Ib	5-Br	4-CH_3	3.77	3.78	3.49	4.26	4.44
Ic	5-Br	4-Br	4.11	4.11	3.96	4.58	4.89
Id	5-Br	4-OCH_3	3.15	3.06	3.20	3.54	3.96
Ie	5-Br	4-Cl	3.84	3.83	3.69	4.31	4.73
If	5-Br	3,4-Cl_2	4.40	4.35	4.31	4.82	5.43
Ig	5-Br	3-Cl	3.84	3.83	3.69	4.31	4.79
Ih	5-Br	3-NO_2	3.31	3.27	3.03	3.74	3.98
Ij	5-Br	4-$N(CH_3)_2$	3.56	3.58	3.90	4.05	3.97
Ik	5-Br	4-F	3.44	3.46	3.21	3.93	4.16
Il	5-Br	3-F	3.44	3.46	3.21	3.93	4.26
IIa	5-Cl	H	3.01	3.04	2.80	3.51	3.84
IIb	5-Cl	4-CH_3	3.50	3.51	3.22	4.26	4.28
IIc	5-Cl	4-Br	3.84	3.83	3.69	4.31	4.73
IId	5-Cl	4-OCH_3	2.88	2.79	2.93	3.26	3.80
IIe	5-Cl	4-Cl	3.57	3.56	3.42	4.03	4.57
IIf	5-Cl	3,4-Cl_2	4.12	4.08	4.04	4.55	5.27
IIg	5-Cl	3-Cl	3.57	3.56	3.42	4.03	4.63
IIh	5-Cl	3-NO_2	3.04	3.00	2.76	3.47	3.82
IIi	5-Cl	4-NO_2	3.04	3.00	2.76	3.47	3.76
IIj	5-Cl	4-$N(CH_3)_2$	3.29	3.31	3.64	3.78	3.81
IIk	5-Cl	4-F	3.17	3.18	2.94	3.65	3.99
IIl	5-Cl	3-F	3.17	3.18	2.94	3.65	4.10
IIIa	5-F	H	2.61	2.66	2.32	3.14	3.35
IIIb	5-F	4-CH_3	3.10	3.13	2.73	3.60	3.78
IIIc	5-F	4-Br	3.44	3.46	3.21	3.93	4.23
IIId	5-F	4-OCH_3	2.48	2.41	2.45	2.88	3.30
IIIe	5-F	4-Cl	3.17	3.18	2.94	3.65	4.07
IIIf	5-F	3,4-Cl_2	3.72	3.70	3.56	4.17	4.77
IIIg	5-F	3-Cl	3.17	3.18	2.94	3.65	4.13
IIIh	5-F	3-NO_2	2.64	2.62	2.28	3.09	3.33
IIIj	5-F	4-$N(CH_3)_2$	2.89	2.93	3.15	3.40	3.32
IIIk	5-F	4-F	2.77	2.8	2.45	3.28	3.50
IIIl	5-F	3-F	2.77	2.8	2.45	3.28	3.60
IVa	5-NO_2	H	2.48	2.48	2.14	2.95	3.04
IVb	5-NO_2	4-CH_3	2.97	2.94	2.56	3.42	3.47
IVc	5-NO_2	4-Br	3.31	3.27	3.03	3.74	3.92
IVd	5-NO_2	4-OCH_3	2.36	2.22	2.27	2.70	2.99
IVe	5-NO_2	4-Cl	3.04	3.00	2.76	3.47	3.76
IVf	5-NO_2	3,4-Cl_2	3.60	3.51	3.38	3.99	4.47
IVg	5-NO_2	3-Cl	3.04	3.00	2.76	3.47	3.82
IVh	5-NO_2	3-NO_2	2.52	2.43	2.10	2.90	3.02
IVj	5-NO_2	4-$N(CH_3)_2$	2.77	2.74	2.98	3.21	3.01
IVk	5-NO_2	4-F	2.64	2.62	2.28	3.09	3.19
IVl	5-NO_2	3-F	2.64	2.62	2.28	3.09	3.29
Va	H	H	2.45	2.52	2.18	3.00	3.05
Vb	H	4-CH_3	2.94	2.99	2.60	3.46	3.49
Vc	H	4-Br	3.28	3.32	3.07	3.79	3.94
Vd	H	4-OCH_3	2.32	2.27	2.31	2.74	3.01
Ve	H	4-Cl	3.01	3.04	2.80	3.51	3.78
Vf	H	3,4-Cl_2	3.57	3.56	3.42	4.03	4.48
Vg	H	3-Cl	3.01	3.04	2.80	3.51	3.84
Vh	H	3-NO_2	2.48	2.48	2.14	2.95	3.03
Vi	H	4-NO_2	2.48	2.48	2.1	2.95	2.97
Vj	H	4-$N(CH_3)_2$	2.73	2.79	3.02	3.26	3.02
Vk	H	4-F	2.61	2.66	2.32	3.14	3.20
Vl	H	3-F	2.61	2.66	2.32	3.14	3.31

and with the increase of their hydrophobicity. Another positive factor contributing to the activity is the electron-donating influence of the substituents on the hydroxy group. This finding can be utilized in the design of further salicylanilides with antimycobacterial activity. It also demonstrates the importance of the

union between physical organic chemistry and bioorganic chemistry.

In conclusion, salicylanilides described in this paper are important broad-spectrum antimycobacterial compounds, and the results can be utilized for the design of further compounds with significant activity. Conceptually, this work is an introduction to the study of similar derivatives of benzoxazine.

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