2*H*-1,3-Benzoxazine-2,4(3*H*)-diones Substituted in Position 6 as Antimycobacterial Agents

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Cyclization of salicylanilides substituted in position 5 gave the corresponding 3-(R-phenyl)-2*H*-1,3-benzoxazine-2,4(3*H*)-diones substituted in position 6. Their minimum inhibition concentrations against *Mycobacterium tuberculosis*, *M. kansasii*, and *M. avium* are reported. Most of the studied compounds possess activity against *M. kansasii* and *M. avium* greater than Isoniazid. The antimycobacterial profile of the compounds was evaluated according to the criteria based on vector algebra, such as cosine coefficient. Quantitative structure—activity relationships were analyzed by the Free—Wilson and Hansch method. The antimycobacterial activity increases with increasing electron-accepting ability and lipophilicity of the substituents on the phenyl ring, whereas the effect of substituents in the 6 position seems to be more complex.

For several years, research in our laboratories has been directed towards the search for new classes of compounds with antimycobacterial activity [1, 2]. In a further probe in this direction, pharmacophoric studies of thiobenzamides [3] and thiolactames [4, 5], compounds bearing alkylsulfanyl group bound to an electron-deficient carbon atom in heterocycle [6, 7], simplified analogues of clofazimine [8, 9], compounds containing disulfandiyl group bound in heterocycle [10, 11], and derivatives of 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-dione [12, 13] were undertaken.

In the case of 3-phenyl-2*H*-1,3-benzoxazine-2,4-(3*H*)-diones and their 6-bromo and 6-chloro derivatives, initial studies [14, 15] indicated that besides the influence of the halogen substituents it is only electron-acceptor properties of the substituent(s) on the phenyl ring that enhance the activity of the compounds against *Mycobacterium tuberculosis* and *M. kansasii*. On the contrary, both electronic and hydrophobic terms must be considered in 6,8-dihalo derivatives for an adequate description of physicochemical influences on inhibition of growth of *M. tuberculosis* and nontuberculous mycobacteria [13]. This discrepancy can be explained by the fact that the antimycobacterial data obtained for different mycobacterium strains (and in different laboratories) were compared. In order to examine this explanation, a series of 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones was subjected to antimycobacterial testing by the National Reference Laboratory for Mycobacterium Kansasii [12]. The results were congruent with the analysis of the data for 6,8-dihalo compounds [13], *i.e.* antimycobacterial activities increased with increasing electron-withdrawing properties and with increasing hydrophobicity of substituents on the phenyl ring. Isosteric 3-phenylquinazoline-2,4(3*H*)-diones appeared to be inactive [12]. The replacement of both oxo groups or at least of that in the 4 position by thioxo group yielded analogues with greater activity against *M. tuberculosis* and *M. kansasii* but with lesser activity against *M. avium* [4].

The purpose of the present study is to investigate the structure—antimycobacterial activity relationships in the group of 53 compounds, 3-(R^1-phenyl)-2*H*-1,3-benzoxazine-2,4(3*H*)-diones, in which fluorine, chlorine, bromine, or nitro group was introduced into the 6 position, with the hope to find out the influence of substitution in position 6 on antimycobacterial activity of compounds under study. The selection of substituents was similarly undertaken as in the previous study of antimycobacterial activity of salicylanilides [16].

THEORETICAL

Antimycobacterial Profiles

Activities were calculated as logarithms of reciprocal values of numerical values of minimum inhibitory concentrations (MICs). Numerical values of MICs expressed in mmol dm^{-1} were taken. (*Note*: Data in Table 2 are given in μmol dm^{-3}.)

Activities against *Mycobacterium tuberculosis* (A_1), *M. avium* (A_2), and *M. kansasii* (A_3) form the components of vector $\boldsymbol{A}$ representing the evaluated compound

$$\boldsymbol{A} = (A_1, A_2, A_3) \quad (1)$$

Complex criterion S_0 was calculated according to the formula

$$S_0 = \frac{1}{\sqrt{3}} \sum_{i=1}^{3} A_i \quad (2)$$

Complex criterion S_0 can be decomposed into two components

$$S_0 = k_0 \cdot g(\boldsymbol{A}) \quad (3)$$

where k_0 is the cosine coefficient and $g(\boldsymbol{A})$ the norm of vector $\boldsymbol{A}$,

$$g(\boldsymbol{A}) = \left(\sum_{i=1}^{3} A_i^2 \right)^{1/2} \quad (4)$$

so that division of the complex criterion S_0 by the norm $g(\boldsymbol{A})$ gives the cosine coefficient k_0 (Table 3).

Denoting $\boldsymbol{a}$ the vector of fragment contributions a_i to activities A_i, analogous relation holds true

$$s_0 = k_0 \cdot g(\boldsymbol{a}) \quad (5)$$

Results are given in Table 5.

Quantitative Structure—Activity Relationships

The values of Hammett constants σ and hydrophobic constants π were taken from Ref. [17]. *Free—Wilson* analyses [18] in the *Fujita—Ban* modification [19] and Hansch type equations were calculated with the use of the linear regression module of the ADSTAT program, version 2.00 (TriloByte, Pardubice, Czech Republic).

The logarithms of the partition coefficients (log P, octanol—water) were calculated using the ChemOffice package (fragmentations according to *Ghose* and *Crippen* [20], *Viswanadhan et al.* [21], and *Broto et al.* [22]), QSAR Properties, a ChemPlus extension for HyperChem [23], and the PALLAS program which uses fragmentation developed by *Rekker* [24].

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 (C, H, N) were performed on a CHNS-O CE elemental analyzer (Fisions EA 1110, Milan) and were within ± 0.4 % of the theoretical values. The IR spectra were measured in KBr pellets on a Nicolet Impact 400 apparatus; the wavenumbers are given in cm^{-1}. The stretching vibrations of C=O group [ν(C=O)] were found in the regions 1693—1716 cm^{-1} and 1764—1779 cm^{-1}, characteristic of 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones. TLC was performed on precoated silica gel plates with a fluorescent indicator Silufol UV 254 + 366, cyclohexane—acetone (3:1), to check the purity of the compounds. The ^{1}H NMR and ^{13}C NMR spectra of the new compounds were recorded in DMSO-d_6 solutions at ambient temperature on a Varian Mercury-Vx BB 300 spectrometer operating at 300 MHz. Chemical shifts were recorded as δ values and were indirectly referenced to tetramethylsilane *via* the solvent signal (2.49 for ^{1}H or 39.7 for ^{13}C) and they are given in Table 1.

6-Substituted 3-(R^1-Phenyl)-2*H*-1,3-benzoxazine-2,4(3*H*)-diones

The title compounds *I—IV* were synthesized from the respective salicylanilides with 55—65 % yield by procedure described previously [4]. Compounds *IIa—IIc, IIe—IIg,* and *Va—Vl* were described recently in Ref. [4] and Ref. [12], respectively. Other compounds described in literature: *Ia* [25], *Ib* [25], *Ic* [26], *Id* [25], *Ie* [27], *IIc* [27], *IVf* [28].

6-Bromo-3-(3,4-dichlorophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (If), m.p. = 250—251 °C. For $C_{14}H_6BrCl_2NO_3$ (M_r = 378.0) w_i(calc.): 43.45 % C, 1.56 % H, 3.62 % N; w_i(found): 43.08 % C, 1.60 % H, 3.47 % N.

6-Bromo-3-(3-chlorophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (Ig), m.p. = 249—251 °C. For $C_{14}H_7BrClNO_3$ (M_r = 352.6) w_i(calc.): 47.69 % C, 2.00 % H, 3.97 % N; w_i(found): 47.85 % C, 2.40 % H, 3.93 % N.

6-Bromo-3-(3-nitrophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (Ih), m.p. = 278—279 °C. For $C_{14}H_7BrN_2O_5$ (M_r = 363.1) w_i(calc.): 46.31 % C, 1.94 % H, 7.71 % N; w_i(found): 46.81 % C, 1.91 % H, 8.07 % N.

6-Bromo-3-(4-dimethylaminophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (Ij), m.p. = 320—322 °C. For $C_{16}H_{13}BrN_2O_3$ (M_r = 361.2) w_i(calc.): 53.21 % C, 3.63 % H, 7.76 % N; w_i(found): 53.14 % C, 3.76 % H, 7.47 % N.

6-Bromo-3-(4-fluorophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (Ik), m.p. = 223—225 °C. For $C_{14}H_7Br$-

FNO_3 (M_r = 336.1) w_i(calc.): 50.03 % C, 2.10 % H, 4.17 % N; w_i(found): 50.12 % C, 2.54 % H, 4.12 % N.

6-Bromo-3-(3-fluorophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (Il), m.p. = 210—212 °C. For $C_{14}H_7BrFNO_3$ (M_r = 336.1) w_i(calc.): 50.03 % C, 2.10 % H, 4.17 % N; w_i(found): 49.75 % C, 2.16 % H, 4.08 % N.

6-Chloro-3-(4-methoxyphenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (IId), m.p. = 216—218 °C. For $C_{15}H_{10}ClNO_3$ (M_r = 303.7) w_i(calc.): 59.32 % C, 3.32 % H, 4.61 % N; w_i(found): 59.26 % C, 2.86 % H, 4.82 % N.

6-Chloro-3-(3-nitrophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (IIh), m.p. = 278—279 °C. For $C_{14}H_7ClN_2O_5$ (M_r = 318.7) w_i(calc.): 52.77 % C, 2.21 % H, 8.79 % N; w_i(found): 52.49 % C, 2.18 % H, 8.64 % N.

6-Chloro-3-(4-nitrophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (IIi), m.p. = 304—306 °C. For $C_{14}H_7ClN_2O_5$ (M_r = 318.7) w_i(calc.): 52.77 % C, 2.21 % H, 8.79 % N; w_i(found): 52.78 % C, 2.39 % H, 8.65 % N.

6-Chloro-3-(4-dimethylaminophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (IIj), m.p. = 325—327 °C. For $C_{16}H_{13}ClN_2O_3$ (M_r = 316.7) w_i(calc.): 60.67 % C, 4.14 % H, 8.85 % N; w_i(found): 60.60 % C, 4.12 % H, 8.92 % N.

6-Chloro-3-(4-fluorophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (IIk), m.p. = 233—235 °C. For $C_{14}H_7ClFNO_3$ (M_r = 291.7) w_i(calc.): 57.65 % C, 2.42 % H, 4.80 % N; w_i(found): 57.40 % C, 2.34 % H, 4.84 % N.

6-Chloro-3-(3-fluorophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (IIl), m.p. = 212—214 °C. For $C_{14}H_7ClFNO_3$ (M_r = 291.7) w_i(calc.): 57.65 % C, 2.42 % H, 4.80 % N; w_i(found): 57.62 % C, 2.36 % H, 4.83 % N.

6-Fluoro-3-phenyl-2H-1,3-benzoxazine-2,4(3H)-dione (IIIa), m.p. = 264—265 °C. For $C_{14}H_8FNO_3$ (M_r = 257.2) w_i(calc.): 65.37 % C, 3.13 % H, 5.45 % N; w_i(found): 65.28 % C, 3.20 % H, 5.95 % N.

6-Fluoro-3-(4-methylphenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (IIIb), m.p. = 231—233 °C. For $C_{15}H_{10}FNO_3$ (M_r = 271.3) w_i(calc.): 66.42 % C, 3.72 % H, 5.16 % N; w_i(found): 66.37 % C, 3.80 % H, 5.00 % N.

3-(4-Bromophenyl)-6-fluoro-2H-1,3-benzoxazine-2,4(3H)-dione (IIIc), m.p. = 242—244 °C. For $C_{14}H_7BrFNO_3$ (M_r = 336.1) w_i(calc.): 50.03 % C, 2.10 % H, 4.17 % N; w_i(found): 49.94 % C, 2.14 % H, 4.15 % N.

6-Fluoro-3-(4-methoxyphenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (IIId), m.p. = 204—206 °C. For $C_{15}H_{10}FNO_4$ (M_r = 287.3) w_i(calc.): 62.72 % C, 3.51 % H, 4.88 % N; w_i(found): 62.78 % C, 2.99 % H, 4.96 % N.

3-(4-Chlorophenyl)-6-fluoro-2H-1,3-benzoxazine-2,4(3H)-dione (IIIe), m.p. = 222—224 °C. For $C_{14}H_7ClFNO_3$ (M_r = 291.7) w_i(calc.): 57.65 % C, 2.42 % H, 4.80 % N; w_i(found): 57.34 % C, 2.52 % H, 4.32 % N.

3-(3,4-Dichlorophenyl)-6-fluoro-2H-1,3-benzoxazine-2,4(3H)-dione (IIIf), m.p.= 223—225 °C. For $C_{14}H_6Cl_2FNO_3$ (M_r = 326.1) w_i(calc.): 51.56 % C, 1.85 % H, 4.30 % N; w_i(found): 51.84 % C, 1.87 % H, 4.06 % N.

3-(3-Chlorophenyl)-6-fluoro-2H-1,3-benzoxazine-2,4(3H)-dione (IIIg), m.p. = 206—208 °C. For $C_{14}H_7ClFNO_3$ (M_r = 291.7) w_i(calc.): 57.65 % C, 2.42 % H, 4.80 % N; w_i(found): 57.50 % C, 2.41 % H, 4.72 % N.

6-Fluoro-3-(3-nitrophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (IIIh), m.p. = 221—223 °C. For $C_{14}H_7FN_2O_3$ (M_r = 302.2) w_i(calc.): 55.64 % C, 2.33 % H, 9.27 % N; w_i(found): 55.42 % C, 2.36 % H, 9.21 % N.

3-(4-Dimethylaminophenyl)-6-fluoro-2H-1,3-benzoxazine-2,4(3H)-dione (IIIj), m.p. = 285—287 °C. IR spectrum, $\tilde{\nu}/cm^{-1}$: 1706 and 1769. For $C_{16}H_{13}FN_2O_3$ (M_r = 300.3) w_i(calc.): 64.00 % C, 4.36 % H, 9.33 % N; w_i(found): 63.61 % C, 4.35 % H, 9.32 % N.

6-Fluoro-3-(4-fluorophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (IIIk), m.p. = 217—218 °C. For $C_{14}H_7F_2NO_3$ (M_r = 275.2) w_i(calc.): 61.10 % C, 2.56 % H, 5.09 % N; w_i(found): 60.82 % C, 2.73 % H, 5.05 % N.

6-Fluoro-3-(3-fluorophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (IIIl), m.p. = 210—211 °C. For $C_{14}H_7FNO_3$ (M_r = 275.2) w_i(calc.): 61.10 % C, 2.56 % H, 5.09 % N; w_i(found): 60.85 % C, 2.71 % H, 5.08 % N.

6-Nitro-3-phenyl-2H-1,3-benzoxazine-2,4(3H)-dione (IVa), m.p. = 225—227 °C. For $C_{14}H_8N_2O_5$ (M_r = 284.2) w_i(calc.): 59.16 % C, 2.84 % H, 9.86 % N; w_i(found): 58.94 % C, 2.91 % H, 9.82 % N.

3-(4-Methylphenyl)-6-nitro-2H-1,3-benzoxazine-2,4(3H)-dione (IVb), m.p. = 222—224 °C. For $C_{15}H_{10}N_2O_5$ (M_r = 298.3) w_i(calc.): 60.41 % C, 3.38 % H, 9.39 % N; w_i(found): 60.16 % C, 3.50 % H, 9.48 % N.

3-(4-Bromophenyl)-6-nitro-2H-1,3-benzoxazine-2,4(3H)-dione (IVc), m.p. = 223—225 °C. For $C_{14}H_7BrN_2O_5$ (M_r = 363.1) w_i(calc.): 46.31 % C, 1.94 % H, 7.71 % N; w_i(found): 46.05 % C, 2.02 % H, 7.61 % N.

3-(4-Methoxyphenyl)-6-nitro-2H-1,3-benzoxazine-2,4(3H)-dione (IVd), m.p. = 208—209 °C. For $C_{15}H_{10}N_2O_6$ (M_r = 314.3) w_i(calc.): 57.33 % C, 3.21 % H, 8.91 % N; w_i(found): 57.14 % C, 3.21 % H, 8.79 % N.

3-(4-Chlorophenyl)-6-nitro-2H-1,3-benzoxazine-2,4(3H)-dione (IVe), m.p. = 233—235 °C. For $C_{14}H_7ClN_2O_5$ (M_r = 318.7) w_i(calc.): 52.77 % C, 2.21 % H, 8.79 % N; w_i(found): 52.43 % C, 2.24 % H, 8.77 % N.

6-Nitro-3-(3-chlorophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (IVg), m.p. = 306—308 °C. For $C_{14}H_7ClN_2O_5$ (M_r = 318.7) w_i(calc.): 52.77 % C, 2.21 % H, 8.79 % N; w_i(found): 52.39 % C, 2.27 % H, 8.71 % N.

6-Nitro-3-(3-nitrophenyl)-2H-1,3-benzoxazine-2,4-(3H)-dione (IVh), m.p. = 302—304 °C. For $C_{14}H_7N_3O_7$ (M_r = 329.2) w_i(calc.): 51.08 % C, 2.14 % H, 12.76 % N; w_i(found): 50.70 % C, 2.22 % H, 12.89 % N.

3-(4-Dimethylaminophenyl)-6-nitro-2H-1,3-benzoxazine-2,4(3H)-dione (IVj), m.p. = 273—274 °C. For $C_{16}H_{13}N_3O_5$ (M_r = 327.3) w_i(calc.): 58.72 % C, 4.00 % H, 12.84 % N; w_i(found): 58.70 % C, 4.14 % H, 12.79 % N.

Table 1. NMR Spectra of the New Compounds

Compound	NMR spectra, δ
If	^{1}H NMR: 8.07 (d overlapping with next signal, 1H, J = 2.18 Hz, H-5), 8.04 (dd, 1H, J= 8.73 Hz, J = 2.18 Hz, H-7), 7.82 (d, 1H, J = 8.73 Hz, H-5′), 7.77 (d, 1H, J = 2.42 Hz, H-2′), 7.51 (d, 1H, J = 8.73 Hz, H-8), 7.46 (dd, 1H, J = 8.73 Hz, J = 2.43 Hz, H-6′); ^{13}C NMR: 159.9, 152.0, 147.3, 139.4, 133.1, 132.3, 131.7, 131.5, 131.0, 129.6, 129.6, 119.4, 117.4, 116.9.
Ig	^{1}H NMR: 8.06 (d, 1H, J = 2.17 Hz, H-5), 8.03 (dd, 1H, J = 8.76 Hz, J = 2.17 Hz, H-7), 7.54—7.58 (m, 3H, H-2′, H-5′, H-6′), 7.51 (d, 1H, J = 8.76 Hz, H-8), 7.37—7.44 (m, 1H, H-4′); ^{13}C NMR: 159.9, 152.0, 147.4, 139.3, 136.6, 133.4, 131.2, 129.6, 129.4, 128.9, 127.9, 119.4, 117.4, 117.0.
Ih	^{1}H NMR: 8.07 (d overlapping with next signal, 1H, J = 2.18 Hz, H-5), 8.04 (dd, 1H, J= 8.73 Hz, J = 2.18 Hz, H-7), 7.82 (d, 1H, J = 8.73 Hz, H-5′), 7.77 (d, 1H, J = 2.42 Hz, H-2′), 7.51 (d, 1H, J = 8.73 Hz, H-8), 7.46 (dd, 1H, J = 8.73 Hz, J = 2.43 Hz, H-6′); ^{13}C NMR: 159.9, 152.0, 147.3, 139.4, 133.1, 132.3, 131.7, 131.5, 131.0, 129.6, 129.6, 119.4, 117.4, 116.9.
Ik	^{1}H NMR: 8.05 (d, 1H, J = 2.41 Hz, H-5), 8.02 (dd, 1H, J = 8.68 Hz, J = 2.41 Hz, H-7), 7.49 (d overlapping with next signal, 1H, J = 8.68 Hz, H-8), 7.42—7.50 (m, 2H, H-2′, H-6′), 7.30—7.39 (m, 2H, H-3′, H-5′); ^{13}C NMR: 163.9 and 160.6 (J_{CF} = 245.17 Hz), 160.1, 152.0, 147.6, 139.2, 131.5 and 131.4 (J_{CF} = 3.09 Hz), 131.1 and 131.0 (J_{CF} = 8.90 Hz), 129.6, 119.3, 117.3, 117.1, 116.5 and 116.2 (J_{CF} = 22.86 Hz).
Il	^{1}H NMR: 8.06 (d, 1H, J = 2.54 Hz, H-5), 8.03 (dd, 1H, J = 8.62 Hz, J = 2.54 Hz, H-7), 7.51—7.61 (m, 1H, H-2′), 7.50 (d overlapping with previous signal, 1H, J = 8.62 Hz, H-8), 7.25—7.39 (m, 3H, H-4′, H-5′, H-6′); ^{13}C NMR: 163.9 and 160.7 (J_{CF} = 244.42 Hz), 159.9, 152.0, 147.4, 139.3, 136.7 and 136.6 (J_{CF} = 10.72 Hz), 131.2 and 131.0 (J_{CF} = 9.13 Hz), 129.6, 125.3 and 125.3 (J_{CF} = 3.17 Hz), 119.4, 117.4, 117.0, 116.5 and 116.3 (J_{CF} = 20.68 Hz), 116.4 and 116.1 (J_{CF} = 23.47 Hz).
IId	^{1}H NMR: 7.92 (d, 1H, J= 2.16 Hz, H-5), 7.89 (dd, 1H, J = 8.89 Hz, J = 2.16 Hz, H-7), 7.54 (d, 1H, J = 8.89 Hz, H-8), 7.26—7.34 (AA′ part of an AA′BB′ system, 2H, H-2′, H-6′), 7.00—7.08 (BB′ part of an AA′BB′ system, 2H, H-3′, H-5′), 3.79 (s, 3H, OCH_3); ^{13}C NMR: 160.3, 159.6, 151.6, 147.8, 136.3, 129.9, 129.6, 127.8, 126.7, 119.1, 116.8, 114.6, 55.7.
IIh	^{1}H NMR: 8.41 (t, 1H, J = 2.14 Hz, H-2′), 8.35 (ddd, 1H, J = 8.04 Hz, J = 2.14 Hz, J= 1.34 Hz, H-4′), 7.96 (d, 1H, J = 2.28 Hz, H-5), 7.93 (dd overlapping with next signal, 1H, J = 8.84 Hz, J = 2.28 Hz, H-7), 7.89—7.93 (m, 1H, H-6′), 7.84 (t, 1H, J = 8.04 Hz, H-5′), 7.59 (d, 1H, J = 8.84 Hz, H-8); ^{13}C NMR: 160.1, 151.6, 148.4, 147.5, 136.6, 136.3, 136.0, 131.1, 129.8, 126.7, 124.4, 124.3, 119.2, 116.6.
IIi	^{1}H NMR: 8.30—8.48 (AA′ part of an AA′BB′ system, 2H, H-3′, H-5′), 7.85—8.03 (m, 2H, H-5, H-7), 7.68—7.80 (BB′ part of an AA′BB′ system, 2H, H-2′, H-6′), 7.59 (d, 1H, J = 8.33 Hz, H-8); ^{13}C NMR: 160.0, 151.6, 147.9, 147.2, 141.0, 136.6, 130.6, 129.8, 126.7, 124.8, 119.2, 116.7.
IIk	^{1}H NMR: 7.95 (d, 1H, J = 2.78 Hz, H-5), 7.91 (dd, 1H, J = 8.63 Hz, J = 2.78 Hz, H-7), 7.52—7.62 (m, 1H, H-2′), 7.57 (d overlapping with previous signal, 1H, J = 8.63 Hz, H-8), 7.26—7.39 (m, 3H, H-4′, H-5′, H-6′); ^{13}C NMR: 163.9 and 160.7 (J_{CF} = 244.50 Hz), 160.0, 151.6, 147.4, 136.7 and 136.6 (J_{CF} = 10.87 Hz), 136.5, 131.2 and 131.0 (J_{CF} = 8.98 Hz), 129.8, 126.7, 125.3 and 125.3 (J_{CF} = 3.02 Hz), 119.1, 116.7, 116.5 and 116.3 (J_{CF} = 20.68 Hz), 116.4 and 116.1 (J_{CF} = 24.00 Hz).
IIl	^{1}H NMR: 7.95 (d, 1H, J = 2.78 Hz, H-5), 7.91 (dd, 1H, J = 8.63 Hz, J = 2.78 Hz, H-7), 7.52—7.62 (m, 1H, H-2′), 7.57 (d overlapping with previous signal, 1H, J = 8.63 Hz, H-8), 7.26—7.39 (m, 3H, H-4′, H-5′, H-6′); ^{13}C NMR: 163.9 and 160.7 (J_{CF} = 244.50 Hz), 160.0, 151.6, 147.4, 136.7 and 136.6 (J_{CF} = 10.87 Hz), 136.5, 131.2 and 131.0 (J_{CF} = 8.98 Hz), 129.8, 126.7, 125.3 and 125.3 (J_{CF} = 3.02 Hz), 119.1, 116.7, 116.5 and 116.3 (J_{CF} = 20.68 Hz), 116.4 and 116.1 (J_{CF} = 24.00 Hz).
IIIa	^{1}H NMR: 7.69—7.78 (m, 2H, H-5, H-7), 7.53—7.61 (m, 1H, H-8), 7.45—7.53 (m, 3H, H-3′, H-4′, H-5′), 7.38—7.45 (m, 2H, H-2′, H-6′); ^{13}C NMR: 160.3 and 157.0 (J_{CF} = 242.76 Hz), 160.2 and 160.2 (J_{CF} = 2.87 Hz), 149.1 and 149.1 (J_{CF} = 1.74 Hz), 147.6, 135.2, 129.3, 129.1, 128.7, 124.1 and 123.8 (J_{CF} = 24.60 Hz), 119.2 and 119.1 (J_{CF} = 8.30 Hz), 116.4 and 116.3 (J_{CF} = 8.60 Hz), 113.1 and 112.8 (J_{CF} = 25.20 Hz).
IIIb	^{1}H NMR: 7.68—7.77 (m, 2H, H-5, H-7), 7.52—7.62 (m, 1H, H-8), 7.24—7.34 (m, 4H, H-2′, H-3′, H-5′, H-6′), 2.36 (s, 3H, CH_3); ^{13}C NMR: 160.2 and 157.0 (J_{CF} = 242.46 Hz), 160.2, 149.1 and 149.1 (J_{CF} = 1.96 Hz), 147.6, 138.5, 132.6, 129.8, 128.4, 124.0 and 123.7 (J_{CF} = 24.60 Hz), 119.1 and 119.0 (J_{CF} = 8.30 Hz), 116.4 and 116.3 (J_{CF} = 8.60 Hz), 113.1 and 112.8 (J_{CF} = 25.20 Hz).
IIIc	^{1}H NMR: 7.69—7.79 (m, 4H, H-5, H-7, H-2′, H-6′), 7.55—7.62 (m, 1H, H-8), 7.35—7.41 (BB′ part of an AA′BB′ system, 2H, H-3′, H-5′); ^{13}C NMR: 160.3 and 157.0 (J_{CF} = 242.84 Hz), 160.1 and 160.1 (J_{CF} = 2.57 Hz), 149.1 and 149.1 (J_{CF} = 1.96 Hz), 147.4, 134.6, 132.4, 131.0, 124.2 and 123.9 (J_{CF} = 24.68 Hz), 122.3, 119.2 and 119.1 (J_{CF} = 8.30 Hz), 116.3 and 116.2 (J_{CF} = 8.60 Hz), 113.2 and 112.8 (J_{CF} = 25.20 Hz).
IIId	^{1}H NMR: 7.68—7.78 (m, 2H, H-5, H-7), 7.51—7.61 (m, 1H, H-8), 7.27—7.36 (AA′ part of an AA′BB′ system, 2H, H-2′, H-6′), 6.99—7.08 (BB′ part of an AA′BB′ system, 2H, H-3′, H-5′), 3.79 (s, 3H, OCH_3); ^{13}C NMR: 160.4 and 157.2 (J_{CF} = 243.06 Hz), 159.6, 149.3 and 149.2 (J_{CF} = 1.74 Hz), 148.0, 129.9, 127.8, 124.2 and 123.8 (J_{CF} = 24.60 Hz), 119.3 and 119.2 (J_{CF} = 8.00 Hz), 116.5 and 116.4 (J_{CF} = 8.30 Hz), 114.6, 113.3 and 112.9 (J_{CF} = 25.20 Hz), 55.7.
IIIe	^{1}H NMR: 7.70—7.78 (m, 2H, H-5, H-7), 7.54—7.62 (m overlapping with AA′ part of an AA′BB′ system, 3H, H-8, H-2′, H-6′), 7.42—7.48 (BB′ part of an AA′BB′ system, 2H, H-3′, H-5′); ^{13}C NMR: 160.3 and 157.1 (J_{CF} = 243.06 Hz), 160.1 and 160.1 (J_{CF} = 2.87 Hz), 149.1 and 149.1 (J_{CF} = 1.96 Hz), 147.4, 134.1, 133.7, 130.7, 129.4, 124.2 and 123.9 (J_{CF} = 24.68 Hz), 119.2 and 119.1 (J_{CF} = 8.00 Hz), 116.3 and 116.2 (J_{CF} = 8.60 Hz), 113.2 and 112.8 (J_{CF} = 25.20 Hz).
IIIf	^{1}H NMR: 7.82 (d, J = 8.6 Hz, 1H, H-5′), 7.72—7.79 (m, 3H, H-5, H-7, H-2′), 7.57—7.64 (m, 1H, H-8), 7.47 (dd, 1H, J = 8.6 Hz, J = 2.3 Hz, H-6′); ^{13}C NMR: 160.3 and 157.1 (J_{CF} = 243.06 Hz), 160.0 and 160.0 (J_{CF} = 2.57 Hz), 149.0 and 149.0 (J_{CF} = 2.04 Hz), 147.2, 135.0, 132.1, 131.5, 131.4, 130.9, 129.4, 124.4 and 124.0 (J_{CF} = 24.60 Hz), 119.3 and 119.1 (J_{CF} = 8.30 Hz), 116.2 and 116.1 (J_{CF} = 8.60 Hz), 113.2 and 112.9 (J_{CF} = 25.20 Hz).

Table 1. (Continued)

Compound	NMR spectra, δ
IIIg	^{1}H NMR: 7.72—7.80 (m, 2H, H-5, H-7), 7.53—7.63 (m, 4H, H-8, H-2′, H-5′, H-6′), 7.39—7.46 (m, 1H, H-4′); ^{13}C NMR: 160.3 and 157.1 (J_{CF} = 242.76 Hz), 160.1 and 160.0 (J_{CF} = 2.57 Hz), 149.1 and 149.0 (J_{CF} = 1.96 Hz), 147.4, 136.5, 133.2, 131.0, 129.2, 128.8, 127.7, 124.2 and 123.9 (J_{CF} = 24.60 Hz), 119.2 and 119.1 (J_{CF} = 8.30 Hz), 116.3 and 116.2 (J_{CF} = 8.30 Hz), 113.2 and 112.8 (J_{CF} = 25.20 Hz).
IIIh	^{1}H NMR: 8.42 (t, 1H, J = 2.22 Hz, H-2′), 8.35 (ddd, 1H, J = 8.06 Hz, J = 2.22 Hz, J = 1.34 Hz, H-4′), 7.92 (dt, 1H, J = 8.06 Hz, J = 1.34 Hz, H-6′), 7.85 (d, 1H, J = 8.06 Hz, H-5′), 7.73—7.82 (m, 2H, H-5, H-7), 7.58—7.65 (m, 1H, H-8); ^{13}C NMR: 160.5 and 157.3 (J_{CF} = 243.36 Hz), 160.4, 160.3, 149.3 and 149.2 (J_{CF} = 2.04 Hz), 148.4, 147.6, 136.4, 136.0, 131.0, 124.5 and 124.2 (J_{CF} = 24.60 Hz), 124.3, 119.4 and 119.3 (J_{CF} = 8.98 Hz), 116.4 and 116.3 (J_{CF} = 8.60 Hz), 113.3 and 113.0 (J_{CF} = 25.51 Hz).
IIIk	^{1}H NMR: 7.72—7.80 (m, 2H, H-5, H-7), 7.56—7.64 (m, 1H, H-8), 7.44—7.52 (m, 2H, H-2′, H-6′), 7.31—7.40 (m, 2H, H-3′, H-5′); ^{13}C NMR: 163.7 and 160.5 (J_{CF} = 245.10 Hz), 160.4 and 157.1 (J_{CF} = 243.36 Hz), 160.3, 149.2 and 149.1 (J_{CF} = 2.04 Hz), 147.6, 131.5 and 131.4 (J_{CF} = 3.17 Hz), 131.0 and 130.9 (J_{CF} = 8.83 Hz), 124.1 and 123.8 (J_{CF} = 24.60 Hz), 119.2 and 119.1 (J_{CF} = 8.30 Hz), 116.4 and 116.3 (J_{CF} = 8.30 Hz), 116.4 and 116.1 (J_{CF} = 22.86 Hz), 113.2 and 112.8 (J_{CF} = 25.13 Hz).
IIIl	^{1}H NMR: 7.70—7.80 (m, 2H, H-5, H-7), 7.51—7.63 (m, 2H, H-8, H-2′), 7.26—7.39 (m, 3H, H-4′, H-5′, H-6′); ^{13}C NMR: 163.9 and 160.7 (J_{CF} = 244.19 Hz), 160.5 and 157.3 (J_{CF} = 243.36 Hz), 160.2, 149.3 and 149.2 (J_{CF} = 1.74 Hz), 147.5, 136.8 and 136.7 (J_{CF} = 10.56 Hz), 131.1 and 131.0 (J_{CF} = 8.90 Hz), 125.4 and 125.3 (J_{CF} = 3.17 Hz), 124.4 and 124.1 (J_{CF} = 24.60 Hz), 119.3 and 119.2 (J_{CF} = 8.30 Hz), 116.5 and 116.2 (J_{CF} = 20.60 Hz), 116.5 and 116.1 (J_{CF} = 23.77 Hz), 116.4 and 116.3 (J_{CF} = 8.60 Hz), 113.3 and 113.0 (J_{CF} = 25.20 Hz).
IVa	^{1}H NMR: 8.61—8.67 (m, 2H, H-5, H-7), 7.75 (d, 1H, J = 9.62 Hz, H-8), 7.47—7.58 (m, 3H, H-3′, H-4′, H-5′), 7.39—7.45 (m, 2H, H-2′, H-6′); ^{13}C NMR: 159.9, 156.5, 147.2, 144.5, 135.0, 131.2, 129.6, 129.4, 128.7, 123.2, 118.7, 116.1.
IVb	^{1}H NMR: 8.60—8.66 (m, 2H, H-5, H-7), 7.74 (d, 1H, J = 9.61 Hz, H-8), 7.25—7.36 (m, 4H, H-2′, H-3′, H-5′, H-6′), 2.37 (s, 3H, CH_3); ^{13}C NMR: 160.0, 156.5, 147.3, 144.5, 139.0, 132.4, 131.1, 130.0, 128.4, 123.3, 118.7, 116.1, 21.1.
IVc	^{1}H NMR: 8.62—8.67 (m, 2H, H-5, H-7), 7.72—7.79 (m overlapping with AA′ part of an AA′BB′ system, 3H, H-8, H-2′, H-6′), 7.35—7.42 (BB′ part of an AA′BB′ system, 2H, H-3′, H-5′); ^{13}C NMR: 160.2, 156.8, 147.4, 144.9, 134.7, 133.0, 131.6, 131.3, 123.6, 123.0, 119.1, 116.4.
IVd	^{1}H NMR: 8.59—8.65 (m, 2H, H-5, H-7), 7.73 (d, 1H, J = 9.64 Hz, H-8), 7.29—7.36 (AA′ part of an AA′BB′ system, 2H, H-2′, H-6′), 7.03—7.09 (BB′ part of an AA′BB′ system, 2H, H-3′, H-5′), 3.81 (s, 3H, OCH_3); ^{13}C NMR: 160.1, 159.8, 156.5, 147.4, 144.4, 131.1, 129.8, 127.5, 123.3, 118.7, 116.1, 114.7, 55.7.
IVe	^{1}H NMR: 8.65 (dd overlapping with next signal, 1H, J = 8.05 Hz, J = 2.68 Hz, H-7), 8.62—8.65 (m, 1H, H-5), 7.74—7.79 (m, 1H, H-8), 7.58—7.65 (AA′ part of an AA′BB′ system, 2H, H-2′, H-6′), 7.42—7.49 (BB′ part of an AA′BB′ system, 2H, H-3′, H-5′); ^{13}C NMR: 159.9, 156.5, 147.1, 144.5, 134.1, 133.9, 131.3, 130.7, 129.7, 123.3, 118.8, 116.0.
IVg	^{1}H NMR: 8.65 (dd overlapping with next signal, 1H, J = 9.63 Hz, J = 2.71 Hz, H-7), 8.63—8.65 (m, 1H, H-5), 7.75—7.80 (m, 1H, H-8), 7.54—7.61 (m, 3H, H-2′, H-5′, H-6′), 7.39—7.45 (m, 1H, H-4′); ^{13}C NMR: 159.8, 156.4, 147.0, 144.6, 136.3, 133.5, 131.4, 131.3, 129.6, 128.8, 127.8, 123.2, 118.8, 116.0.
IVh	^{1}H NMR: 8.63—8.72 (m, 2H, H-5, H-7), 8.33—8.44 (m, 2H, H-2′, H-4′), 7.77—7.96 (m, 3H, H-8, H-6′, H-5′); ^{13}C NMR: 159.9, 156.4, 148.5, 147.0, 144.7, 136.0, 135.8, 131.5, 131.2, 124.6, 124.2, 123.3, 118.8, 115.9.
IVk	^{1}H NMR: 8.60—8.68 (m, 2H, H-5, H-7), 7.75 (d, 1H, J = 9.62 Hz, H-8), 7.35—7.55 (m, 4H, H-2′, H-3′, H-5′, H-6′); ^{13}C NMR: 164.0 and 160.7 (J_{CF} = 45.86 Hz), 160.0, 156.5, 147.2, 144.5, 131.3 and 131.2 (J_{CF} = 3.32 Hz), 131.2, 131.0 and 130.9 (J_{CF} = 8.75 Hz), 123.2, 118.8, 116.7, 116.4 and 116.1 (J_{CF} = 21.51 Hz).
IVl	^{1}H NMR: 8.65 (dd overlapping with next signal, 1H, J = 8.86 Hz, J = 2.78 Hz, H-7), 8.63—8.65 (m, 1H, H-5), 7.75—7.80 (m, 1H, H-8), 7.55—7.64 (m, 1H, H-2′), 7.28—7.42 (m, 3H, H-4′, H-5′, H-6′); ^{13}C NMR: 163.9 and 160.7 (J_{CF} = 44.49 Hz), 159.8, 156.4, 147.0, 144.6, 136.4 and 136.3 (J_{CF} = 10.64 Hz), 131.3 and 131.2 (J_{CF} = 11.17 Hz), 125.2 and 125.2 (J_{CF} = 3.17 Hz), 123.8, 118.8, 116.7 and 116.5 (J_{CF} = 20.90 Hz), 116.3 and 116.0 (J_{CF} = 22.03 Hz).

3-(4-Fluorophenyl)-6-nitro-2H-1,3-benzoxazine-2,4(3H)-dione (IVk), m.p. = 243—244 °C. For $C_{14}H_7FN_2O_5$ (M_r = 302.2) w_i(calc.): 55.64 % C, 2.33 % H, 9.27 % N; w_i(found): 55.81 % C, 2.50 % H, 9.51 % N.

3-(3-Fluorophenyl)-6-nitro-2H-1,3-benzoxazine-2,4(3H)-dione (IVl), m.p. = 265—267 °C. For $C_{14}H_7FN_2O_5$ (M_r = 302.2) w_i(calc.): 55.64 % C, 2.33 % H, 9.27 % N; w_i(found): 55.46 % C, 2.27 % H, 9.23 % N.

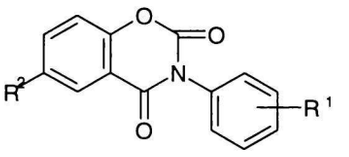
Antimycobacterial Susceptibility Assay

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, and *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 three strains was determined in the Šula semisynthetic medium (SEVAC, Prague). The compounds were added to the medium in a dimethyl sulfoxide solution. The following concentrations (c/(μmol dm^{-3})) were used: 1000, 500, 250, 125, 62, 31, 16, 8, and 4. The minimum inhibitory concentrations (MICs) were determined after incubation at 37 °C for 14 days and 21 days. In several cases, espe-

cially for dimethylamino derivatives (*Ij, IIj, IIIj, IVj, Vj*), the minimum inhibitory concentrations could not be determined due to the limited solubility of the compounds in water. MIC was the lowest concentration of a substance, at which the inhibition of the growth of mycobacteria occurred. Isoniazid was used as standard. The experimental conditions are described in our previous paper [16]. The results are given in Table 2.

RESULTS AND DISCUSSION

The synthesis of the title compounds (Formula 1) was straightforward. The starting 5-substituted salicylanilides were prepared by standard procedure from the known salicylic acids and the corresponding anilines in chlorobenzene as reported previously [16]. Cyclization of salicylanilides with ethyl chloroformate in pyridine [13] afforded the corresponding 6-substituted 3-(R^1-phenyl)-2*H*-1,3-benzoxazine-2,4(3*H*)-diones *I*—*IV*. The prepared compounds gave satisfactory elemental analyses. Their IR spectra showed characteristic absorption maxima of two C=O groups. ^{1}H and ^{13}C NMR spectra are presented for the new compounds in Experimental (Table 1).



I—*V*

	R^2		R^1		R^1
I	Br	*a*	H	*g*	3-Cl
II	Cl	*b*	4-CH_3	*h*	3-NO_2
III	F	*c*	4-Br	*i*	4-NO_2
IV	NO_2	*d*	4-OCH_3	*j*	4-$N(CH_3)_2$
V	H	*e*	4-Cl	*k*	4-F
		f	3,4-Cl_2	*l*	3-F

Formula 1. Structure of the studied compounds.

The compounds were tested *in vitro* against *M. tuberculosis*, *M. kansasii*, and *M. avium*. Values of the logarithms of the partition coefficients (log *P*, octanol—water) calculated by different ways could be mutually correlated (r = 0.932—0.999, s = 0.02—0.04) with the exception of the correlation between *Rekker* style and *Hyperchem* approach (r = 0.884, s =

Table 2. Antimycobacterial Activity of Compounds *I*—*V*

Compound	MIC/(μmol dm^{-3}) Incubation 14 days/21 days			Compound	MIC/(μmol dm^{-3}) Incubation 14 days/21 days		
	M. tuber.	*M. kans.*	*M. avium*		*M. tuber.*	*M. kans.*	*M. avium*
Ia	31/31	31/62	62/62	*IIIg*	8/16	4/8	16/31
Ib	16/16	62/62	31/31	*IIIh*	16/31	16/31	16/31
Ic	4/4	8/8	16/16	*IIIk*	31/31	8/16	31/31
Ie	8/8	8/8	16/16	*IIIl*	16/16	16/31	31/62
If	4/4	4/4	16/16	*IVa*	62/62	31/31	500/500
Ig	4/8	8/8	16/16	*IVb*	62/62	31/62	250/250
Ih	8/8	16/16	31/31	*IVc*	31/31	8/16	125/125
Ik	16/16	16/16	31/31	*IVd*	125/250	62/62	1000/>1000
Il	8/16	8/8	31/31	*IVe*	16/31	16/31	125/125
IIa[a]	31/31	4/4	16/31	*IVf*	31/31	8/16	125/125
IIb[a]	16/16	4/8	8/16	*IVg*	16/31	16/16	−[b]/−[b]
IIc[a]	4/4	4/4	4/8	*IVh*	62/125	16/31	250/250
IId	62/62	4/4	16/31	*IVj*	−[b]/−[b]	125/−[b]	−[b]/−[b]
IIe[a]	4/4	4/4	8/8	*IVk*	31/31	31/62	500/500
IIf[a]	4/4	4/4	8/8	*IVl*	31/31	31/31	250/250
IIg[a]	8/16	4/4	8/8	*Va*[c]	125/125	125/250	62/125
IIh	8/8	4/8	16/16	*Vb*[c]	62/62	125/125	31/62
IIi	8/8	4/4	8/8	*Vc*[c]	31/31	16/31	31/31
IIj	31/−[b]	4/8	62/−[b]	*Vd*[c]	62/125	125/250	31/31
IIk	8/8	4/4	16/31	*Ve*[c]	16/31	16/16	62/62
IIl	16/16	4/8	16/16	*Vf*[c]	8/8	4/4	8/16
IIIa	31/62	8/16	31/31	*Vg*[c]	31/31	8/8	31/31
IIIb	16/31	8/8	16/31	*Vh*[c]	16/31	31/62	16/31
IIIc	4/8	8/8	8/16	*Vi*[c]	16/16	16/16	16/31
IIId	31/31	16/62	16/31	*Vk*[c]	125/125	62/62	62/125
IIIe	8/8	8/31	8/16	*Vl*[c]	31/62	31/62	62/125
IIIf	8/8	4/4	8/8	INH[d]	4/4	500/500	500/500

a) Data from Ref. [4]. *b*) Not determined. *c*) Data from Ref. [12]. *d*) Isoniazid.

Table 3. Evaluation of Compounds *I*—*V* as Broad-Spectrum Antimycobacterial Agents

Compound	Complex criterion S_0		Norm of vector $g(\boldsymbol{A})$		Cosine coefficient k_0	
	14 days	21 days	14 days	21 days	14 days	21 days
Ia	2.440	2.266	2.452	2.279	0.995	0.994
Ib	2.606	2.606	2.639	2.639	0.988	0.988
Ic	3.632	3.632	3.657	3.657	0.993	0.993
Ie	3.458	3.458	3.467	3.467	0.997	0.997
If	3.806	3.806	3.838	3.838	0.992	0.992
Ig	3.632	3.458	3.657	3.467	0.993	0.997
Ih	3.119	3.119	3.146	3.146	0.991	0.991
Ik	2.945	2.945	2.954	2.954	0.997	0.997
Il	3.293	3.119	3.327	3.146	0.990	0.991
IIa	3.293	3.127	3.355	3.210	0.982	0.974
IIb	3.632	3.285	3.657	3.294	0.993	0.997
IIc	4.153	3.980	4.153	3.987	1.000	0.998
IId	3.119	2.953	3.230	3.080	0.965	0.959
IIe	3.980	3.980	3.987	3.987	0.998	0.998
IIf	3.980	3.980	3.987	3.987	0.998	0.998
IIg	3.806	3.632	3.814	3.657	0.998	0.993
IIh	3.632	3.458	3.657	3.467	0.993	0.997
IIi	3.806	3.806	3.814	3.814	0.998	0.998
IIj	2.953	-[b]	3.080	-[b]	0.959	-[b]
IIk	3.632	3.466	3.657	3.525	0.993	0.983
IIl	3.458	3.285	3.493	3.294	0.990	0.997
IIIa	2.953	2.606	2.992	2.639	0.987	0.988
IIIb	3.285	2.953	3.294	2.992	0.997	0.987
IIIc	3.806	3.458	3.814	3.467	0.998	0.997
IIId	2.945	2.440	2.954	2.452	0.997	0.995
IIIe	3.632	3.119	3.632	3.146	1.000	0.991
IIIf	3.806	3.806	3.814	3.814	0.998	0.998
IIIg	3.632	3.119	3.657	3.146	0.993	0.991
IIIh	3.111	2.614	3.111	2.614	1.000	1.000
IIIk	2.953	2.779	2.992	2.789	0.987	0.996
IIIl	2.945	2.606	2.954	2.639	0.997	0.988
IVa	1.742	1.742	1.956	1.956	0.891	0.891
IVb	1.916	1.742	2.025	1.811	0.947	0.962
IVc	2.603	2.429	2.737	2.514	0.951	0.967
IVd	1.219	-[b]	1.508	-[b]	0.808	-[b]
IVe	2.595	2.264	2.696	2.317	0.963	0.977
IVf	2.603	2.429	2.737	2.514	0.951	0.967
IVh	2.082	1.740	2.247	1.859	0.927	0.936
IVk	1.916	1.742	2.155	1.956	0.889	0.891
IVl	2.090	2.090	2.217	2.217	0.943	0.943
Va	1.740	1.390	1.758	1.412	0.990	0.985
Vb	2.090	1.916	2.133	1.932	0.980	0.992
Vc	2.779	2.614	2.789	2.614	0.996	1.000
Vd	2.090	1.740	2.133	1.859	0.980	0.936
Ve	2.771	2.606	2.813	2.639	0.985	0.988
Vf	3.806	3.632	3.814	3.657	0.998	0.993
Vg	2.953	2.953	2.992	2.992	0.987	0.987
Vh	2.945	2.440	2.954	2.452	0.997	0.995
Vi	3.111	2.945	3.111	2.954	1.000	0.997
Vk	1.916	1.740	1.932	1.758	0.992	0.990
Vl	2.440	1.916	2.452	1.932	0.995	0.992
INH[a]	1.732	1.732	2.435	2.435	0.711	0.711

a) Isoniazid. *b*) Not determined.

0.30). Further correlations were found with constants π (of R^1), but not with constants π^-. In order to determine the influence of the substituents from the acyl part of the molecule (R^2), we carried out the separation of the calculated log *P* values according to the *Fujita* and *Ban* [19] 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 general, the MICs of *I*—*V* are within the range of 4—250 μmol dm^{-3} (Table 2). The same range of

Table 4. Results of Free—Wilson/Fujita—Ban Analyses

Fragment	Contribution[a] to activity against *M. tuberculosis*		*M. kansasii*		*M. avium*	
	14 days	21 days	14 days	21 days	14 days	21 days
μ^b	1.040	0.963	1.296	1.114	1.207	0.986
At position 6						
Br	0.559	0.600	0.347	0.454	0.098[c]	0.303
Cl	0.514	0.594	0.869	0.923	0.482	0.539
F	0.406	0.372	0.551	0.432	0.315	0.302
NO_2	−0.039[c]	−0.043[c]	0.167[c]	0.136[c]	−0.907	−0.643
H[d]	0	0	0	0	0	0
At phenyl						
H[d]	0	0	0	0	0	0
4-CH_3	0.233[c]	0.236[c]	−0.060[c]	0.000[c]	0.298	0.239
4-Br	0.715	0.715	0.414	0.477	0.536	0.534
4-OCH_3	−0.053[c]	−0.138[c]	−0.117[c]	−0.132[c]	0.096[c]	0.243
4-Cl	0.709	0.655	0.354	0.359[c]	0.416	0.474
3,4-Cl_2	0.772	0.832	0.655	0.775	0.594	0.652
3-Cl	0.652	0.474	0.474	0.594	0.369	0.456
3-NO_2	0.471	0.356	0.176[c]	0.121[c]	0.295	0.299
4-NO_2	0.650	0.687	0.366[c]	0.522	0.499	0.547
4-$N(CH_3)_2$	–	–	–	–	–	–
4-F	0.235[c]	0.295	0.118[c]	0.179[c]	0.060[c]	0.060[c]
3-F	0.414	0.354	0.179[c]	0.181[c]	0.120[c]	0.118[c]
r	0.921	0.926	0.888	0.870	0.964	0.964
s	0.194	0.197	0.240	0.289	0.171	0.148
F	14.39	15.49	9.58	7.99	32.60	31.96
n	51	51	51	51	50	49

a) The contributions are expressed in log{1/MIC} scale (MIC in mmol dm^{-3}). *b*) Predicted activity for *Va* (reference compound). *c*) Value is not significantly ($p < 0.05$) different from the contribution of the fragment on the reference compound. *d*) Fragment on the reference compound; zero values of contribution by definition.

Table 5. Free—Wilson/Fujita—Ban Analyses of Complex Criterion S_0, Complex Criterion s_0, Norm of Vector $g(\boldsymbol{a})$, and Cosine Coefficient k_0 for Fragments

Fragment	Contribution to S_0^a		Complex criterion s_0^b		Norm of vector $g(\boldsymbol{a})^b$		Cosine coefficient k_0^b	
	14 days	21 days	14 days	21 days	14 days	21 days	14 days	21 days
μ	2.032[c]	1.767[c]	2.046	1.768	2.054	1.772	0.996	0.998
At position 6								
Br	0.591	0.785	0.580	0.783	0.665	0.811	0.871	0.966
Cl	1.077	1.187	1.077	1.187	1.119	1.223	0.962	0.971
F	0.735	0.639	0.734	0.639	0.753	0.645	0.975	0.990
NO_2	−0.394	−0.315	−0.450	−0.318	0.923	0.659	−0.487	−0.482
H[d]	0	0	0	0	0	0	0	0
At phenyl								
H[d]	0	0	0	0	0	0	0	0
4-CH_3	0.272[e]	0.274[e]	0.272	0.274	0.383	0.336	0.710	0.816
4-Br	0.961	0.996	0.961	0.997	0.985	1.012	0.976	0.985
4-OCH_3	0.082[e]	0.002[e]	−0.043	−0.016	0.160	0.309	−0.266	−0.050
4-Cl	0.854	0.859	0.854	0.859	0.895	0.885	0.954	0.971
3,4-Cl_2	1.166	1.304	1.167	1.304	1.174	1.311	0.994	0.995
3-Cl	0.873	0.871	0.863	0.880	0.887	0.886	0.974	0.993
3-NO_2	0.544	0.448	0.544	0.448	0.583	0.480	0.933	0.933
4-NO_2	0.888	1.015	0.875	1.014	0.897	1.022	0.975	0.992
4-F	0.239[e]	0.308	0.238	0.308	0.270	0.350	0.884	0.880
3-F	0.412	0.377	0.412	0.377	0.467	0.415	0.882	0.909
r	0.960	0.962						
s	0.225	0.233						
F	28.59	29.98						
n	49	49						

a) Calculated for data from Table 3. *b*) Calculated for data from Table 4 (see the text). *c*) Complex criterion S_0 calculated for *Va* (reference compound). *d*) Fragment on the reference compound; zero values of contribution by definition. *e*) Value is not significantly ($p < 0.05$) different from the contribution of the fragment on the reference compound.

Table 6. QSARs Based on Eqn $\log\{1/\mathrm{MIC}\} = a(\sigma)_1 + b(\pi)_1 + cI_{\mathrm{Br}} + dI_{\mathrm{Cl}} + eI_{\mathrm{F}} + fI_{\mathrm{NO_2}} + g$

Mycobacterium	Days	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*r*	*s*	*F*	*n*	Eqn
M. tuberculosis	14	0.484	0.327	0.554	0.533	0.395	−0.050[a]	1.197	0.885	0.208	26.99	52	(*6*)
		± 0.089	± 0.058	± 0.094	± 0.087	± 0.091	± 0.091	± 0.070					
	21	0.484	0.369	0.591	0.594	0.353	−0.062[a]	1.071	0.897	0.209	30.10	51	(*7*)
		± 0.098	± 0.059	± 0.094	± 0.089	± 0.091	± 0.091	± 0.071					
M. kansasii	14	0.361	0.291	0.340	0.915	0.544	0.138[a]	1.300	0.870	0.242	23.76	53	(*8*)
		± 0.097	± 0.068	± 0.109	± 0.101	± 0.106	± 0.104	± 0.080					
	21	0.364	0.359	0.435	0.954	0.411	0.114[a]	1.132	0.840	0.285	18.04	52	(*9*)
		± 0.122	± 0.080	± 0.129	± 0.120	± 0.125	± 0.125	± 0.096					
M. avium	14	0.303	0.285	0.068[a]	0.458	0.298	−0.920	1.294	0.948	0.183	64.46	51	(*10*)
		± 0.078	± 0.051	± 0.082	± 0.077	± 0.080	± 0.083	± 0.062					
	21	0.269	0.293	0.257	0.539	0.281	−0.695	1.109	0.939	0.172	52.38	49	(*11*)
		± 0.083	± 0.049	± 0.078	± 0.074	± 0.076	± 0.080	± 0.059					
S_0[b]	14	0.639	0.518	0.556	1.098	0.713	−0.482	2.196	0.949	0.238	66.69	51	(*12*)
		± 0.102	± 0.067	± 0.107	± 0.100	± 0.104	± 0.107	± 0.080					
	21	0.678	0.587	0.741	1.187	0.605	−0.370	1.904	0.947	0.245	61.23	49	(*13*)
		± 0.118	± 0.069	± 0.110	± 0.105	± 0.107	± 0.114	± 0.084					

MIC expressed in mmol $\mathrm{dm^{-3}}$; $(\sigma)_1$ Hammett constant and $(\pi)_1$ hydrophobic constant of substituent on the phenyl ring; I_{X} dummy variable, $I_{\mathrm{X}} = 1$ if substituent X is present in the 6 position, otherwise $I_{\mathrm{X}} = 0$. *a*) Value is not significantly ($p < 0.05$) different from zero. *b*) Calculated for data from Table 3.

Table 7. QSARs Based on Eqn $\log\{1/\mathrm{MIC}\} = a(\sigma)_1 + b(\pi)_1 + c(\sigma_m)_2 + d(\sigma_p)_2 + e(\pi_p)_2 + f$

Mycobacterium	Days	*a*	*b*	*c*	*d*	*e*	*f*	*r*	*s*	*F*	*n*	Eqn
M. tuberculosis	14	0.474	0.320	1.253	−1.213	0.339	1.215	0.875	0.213	30.17	52	(*14*)
		±0.091	±0.060	±0.331	±0.274	±0.073	±0.071					
	21	0.492	0.360	1.083	−1.074	0.413	1.087	0.882	0.220	31.53	51	(*15*)
		±0.103	±0.062	±0.341	±0.283	±0.076	±0.075					
M. kansasii	14	0.329	0.259	1.917	−1.583	0.180[a]	1.371	0.711	0.340	9.63	53	(*16a*)
		±0.136	±0.095	±0.528	±0.435	±0.116	±0.112					
	14	0.558	0.364	1.913	−1.525	−0.053[a]	1.214	0.879	0.218	23.82	41	(*16b*)
		±0.107	±0.069	±0.337	±0.278	±0.080	±0.075					
	21	0.315[a]	0.326	1.367	−1.106	0.351	1.209	0.691	0.376	8.42	52	(*17a*)
		±0.161	±0.105	±0.584	±0.484	±0.129	±0.125					
	21	0.590	0.433	1.375	−1.097	0.120[a]	1.038	0.827	0.275	14.70	40	(*17b*)
		±0.150	±0.088	±0.427	±0.353	±0.101	±0.096					
M. avium	14	0.267	0.261	1.262	−2.331	0.260	1.351	0.886	0.263	32.83	51	(*18*)
		±0.112	±0.074	±0.407	±0.340	±0.090	±0.087					
	21	0.282	0.274	1.076	−1.867	0.364	1.146	0.875	0.240	28.15	49	(*19*)
		±0.115	±0.068	±0.372	±0.314	±0.083	±0.082					
S_0[b]	14	0.683	0.479	2.560	−2.887	0.413	2.253	0.865	0.359	25.54	49	(*20*)
		±0.172	±0.101	±0.557	±0.469	±0.125	±0.123					
	21	0.700	0.555	2.046	−2.330	0.634	1.965	0.872	0.370	27.37	49	(*21*)
		±0.178	±0.104	±0.574	±0.483	±0.128	±0.126					

MIC expressed in mmol $\mathrm{dm^{-3}}$; σ, σ_{m}, σ_{p} Hammett constants and π, π_{p} hydrophobic constants of substituent on the phenyl ring (subscript 1) or at the 6 position (subscript 2). The chloro derivatives *II* were omitted in calculation of eqns (*16b*) and (*17b*). *a*) Value is not significantly ($p < 0.05$) different from zero. *b*) Calculated for data from Table 3.

MICs was found for 5-salicylanilides [16]. By comparing the MICs of compounds *I*—*V* with that of Isoniazid (INH), the most active derivatives *If*, *IIc*, *IIe*, and *IIf* have *in vitro* activity against *M. tuberculosis* comparable to that of INH (MIC = 4 μmol $\mathrm{dm^{-3}}$). However, most of the tested compounds *I*—*V* possess activity against *M. kansasii* and *M. avium* greater than INH (MIC = 500 μmol $\mathrm{dm^{-3}}$). Only *M. avium* was less susceptible to compounds *IVa*, *IVd*, and *IVk* (MIC = 500 μmol $\mathrm{dm^{-3}}$ or more). While nontuberculous mycobacteria are moderately susceptible towards INH, the prepared compounds display virtually the same activity against all the tested strains.

Evaluation of broad-spectrum antimycobacterial profiles of individual compounds was performed by the procedure described previously [29]. The results of calculations are summarized in Table 3. The norm of activity vector $g(\boldsymbol{A})$ is a measure of the overall antimycobacterial potency of the compound. Complex criterion S_0 is proposed for a comparison of the evaluated compound with the ideal broad-spectrum drug, *i.e.* compound possessing equal MICs against

all strains taken into consideration. Cosine coefficient k_0 is a measure of relative similarity to the ideal drug as the influence of the overall potency is eliminated. This aspect, of course, is of greater importance in the search for selectively acting compounds. The more the cosine coefficient is close to the value of 1, the more the profile of evaluated compound relatively agrees with that of the ideal drug. As follows from Table 3, the values of cosine coefficient k_0 (0.959—1.000) for 6-halogeno-3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones *I*—*III* justify the conclusion that the compounds represent broad-spectrum antimycobacterial agents. The values of cosine coefficient k_0 and other criteria for 6-nitro derivatives *IV* are less favourable due to lesser susceptibility of *M. kansasii* to these derivatives.

For quantitative analysis of the structure—antimycobacterial activity relationships by the Free—Wilson method (in the Fujita—Ban modification) the unsubstituted compound *Va* was chosen as the reference compound for all the analyses. Since limited data were available for dimethylamino derivatives *(Ij—Vj)*, contribution of the dimethylamino group could be calculated only for data after 14-day incubation of *M. kansasii*. As compound *IVd* showed to be inactive against *M. avium* after 21 days the respective value was not included in calculation. The results of Free—Wilson/Fujita—Ban analyses confirm that each considered fragment has a constant, independent, and additive contribution to the activity of the whole molecule against all tested strains (Table 4) and to the complex criterion S_0 (Table 5).

For substituents on the phenyl ring no contribution of any fragment was negative; they all were positive

Table 8. Substituent Constants σ and π [17]

Substituent	σ		π	
	meta	*para*	*meta*	*para*
H	0	0	[a]	0
CH_3	[a]	−0.17	[a]	0.60
Br	0.39	0.23	[a]	1.19
OCH_3	[a]	−0.27	[a]	−0.03
Cl[b]	0.37	0.23	0.77	0.73
NO_2	0.71	0.78	−0.05	0.02
$N(CH_3)_2$	[a]	−0.83	[a]	−0.08
F	0.34	0.06	0.22	0.15

a) Value not used in calculations. *b*) For 3,4-Cl_2 values of σ = 0.60 and π = 1.50 were used.

Table 9. Calculated Logarithms of Partition Coefficients (log *P*)

Compound	Method of calculation					Compound	Method of calculation				
	[24]	[25]	[26]	[27]	[28]		[24]	[25]	[26]	[27]	[28]
Ia	3.83	3.86	2.80	4.78	3.07	*IIIg*	3.72	3.72	2.67	4.64	3.20
Ib	4.32	4.33	3.22	5.25	3.51	*IIIh*	3.19	3.16	2.01	4.08	2.39
Ic	4.66	4.65	3.69	5.57	3.96	*IIIj*	3.45	3.47	2.88	4.39	2.38
Id	3.71	3.61	2.93	4.53	3.03	*IIIk*	3.32	3.35	2.18	4.27	2.57
Ie	4.39	4.38	3.42	5.30	3.80	*IIIl*	3.32	3.35	2.18	4.27	2.67
If	4.95	4.89	4.04	5.82	4.50	*IVa*	3.04	3.02	1.87	3.94	2.11
Ig	4.39	4.38	3.42	5.30	3.85	*IVb*	3.52	3.49	2.29	4.41	2.54
Ih	3.87	3.81	2.76	4.73	3.05	*IVc*	3.87	3.81	2.76	4.73	2.99
Ij	4.12	4.12	3.63	5.04	3.04	*IVd*	2.91	2.77	2.00	3.69	2.06
Ik	3.99	4.00	2.94	4.92	3.22	*IVe*	3.59	3.54	2.49	4.46	2.83
Il	3.99	4.00	2.94	4.92	3.33	*IVf*	4.15	4.06	3.11	4.98	3.53
IIa	3.56	3.58	2.53	4.51	2.91	*IVg*	3.59	3.54	2.49	4.46	2.89
IIb	4.05	4.05	2.95	4.97	3.35	*IVh*	3.07	2.97	1.83	3.89	2.08
IIc	4.39	4.38	3.42	5.30	3.80	*IVj*	3.32	3.28	2.71	4.21	2.07
IId	3.43	3.33	2.66	4.25	2.87	*IVk*	3.19	3.16	2.49	4.08	2.26
IIe	4.12	4.10	3.15	5.02	3.64	*IVl*	3.19	3.16	2.49	4.08	2.36
IIf	4.68	4.62	3.77	5.54	4.34	*Va*	3.00	3.07	1.91	3.99	2.12
IIg	4.12	4.10	3.15	5.02	3.69	*Vb*	3.49	3.53	2.33	4.45	2.55
IIh	3.59	3.54	2.49	4.46	2.89	*Vc*	3.83	3.86	2.80	4.78	3.01
IIi	3.59	3.54	2.49	4.46	2.83	*Vd*	2.88	2.81	2.04	3.73	2.08
IIj	3.85	3.85	3.37	4.77	2.88	*Ve*	3.56	3.58	2.53	4.51	2.85
IIk	3.72	3.72	2.67	4.64	3.06	*Vf*	4.12	4.10	3.15	5.02	3.55
IIl	3.72	3.72	2.67	4.64	3.17	*Vg*	3.56	3.58	2.53	4.51	2.90
IIIa	3.16	3.21	2.05	4.13	2.41	*Vh*	3.04	3.02	1.87	3.94	2.10
IIIb	3.65	3.67	2.46	4.59	2.85	*Vi*	3.04	3.02	1.87	3.94	2.04
IIIc	3.99	4.00	2.94	4.92	3.30	*Vj*	3.29	3.33	2.75	4.25	2.09
IIId	3.03	2.95	2.18	3.87	2.37	*Vk*	3.16	3.21	2.05	4.13	2.27
IIIe	3.72	3.72	2.67	4.64	3.14	*Vl*	3.16	3.21	2.05	4.13	2.38
IIIf	4.28	4.24	3.29	5.16	3.84						

or at least not significantly different from zero. The greatest values of contributions are found for the 3,4-dichloro substitution in all three strains. However, no other similarity is evident in the order of other fragment contributions. In this connection, it is noteworthy that *M. kansasii* showed maximum susceptibility (MIC = 4 μmol dm^{-3}) to all of compounds in the chloro series *II* regardless of substituents present at the phenyl ring (see below). For substituents at the 6 position, the contributions of halogen were in all but one case positive, the greatest values are found for bromine followed by chlorine in case of *M. tuberculosis*, while chlorine is the best choice in case of *M. avium* and *M. kansasii*. The contribution of nitro group to the activity against *M. tuberculosis* and *M. kansasii* is not significantly different from zero; but it is negative in *M. avium* resulting in negative value of contribution to the complex criterion S_0.

We also calculated complex criteria s_0 characterizing broad-spectrum profile of fragments from the contributions obtained by the Free—Wilson method. Additional information can be obtained by their decomposition into the norms of vector $g(\boldsymbol{a})$ and the cosine coefficients k_0 (Table 5).

As a first step in deriving Hansch type equations, the Hammett constant, σ, and Hansch hydrophobic substituent constant, π, were utilized instead of dummy variables describing the substituents on the phenyl ring in eqns (*6*—*13*) (Table 6). These equations provide the same explanation of the physicochemical influences of 3-phenyl substitution on the inhibition of *Mycobacterium* growth for all 6-substituted derivatives *I*—*IV* as that reported previously for 6-unsubstituted compounds *V* [12] and 6,8-dihalo analogues [13]. The compounds become more efficient antimycobacterial agents with increasing electron-accepting properties and with increasing hydrophobicity of the phenyl substituents. For substituents in the 6 position, the same pair of constants, σ_p and π_p, yielded equations which are not significant. No significant correlation could be found for the activity of the compounds using $(\log P)^2$, $\log P$, σ_1, and σ_2 terms. Acceptable correlations are given in eqns (*14*—*21*) (Tables 7—9). In these equations, addition of another electronic term, Hammett σ_m, gives a significant improvement. Eqns (*16b*) and (*17b*) obtained for the activity against *M. kansasii*, are the correlations with the chloro derivatives *II* deleted. Similar 5-parameter relationships provide a quantitative description of structural requirements for antimycobacterial activity of 5-substituted salicylanilides [16].

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