RELATIONSHIPS BETWEEN THE CHEMICAL STRUCTURE OF SUBSTANCES AND THEIR ANTIMYCOBACTERIAL ACTIVITY AGAINST ATYPICAL STRAINS. PART 18. 3-PHENYL-2*H*-1,3-BENZOXAZINE-2,4(3*H*)-DIONES AND ISOSTERIC 3-PHENYLQUINAZOLINE-2,4(1*H*,3*H*)-DIONES

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Dedicated to Professor Otto Exner on the occasion of his 75th birthday.

A series of 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones **2** and 3-phenylquinazoline-2,4(1*H*,3*H*)-diones **5** substituted on the phenyl rings were synthesized. The target compounds as well as the intermediates were tested against *Mycobacterium tuberculosis*, *M. kansasii*, and *M. avium*. The replacement of the oxygen atom by nitrogen resulted in a decrease or loss of antimycobacterial activity. 2-[(Ethoxycarbonyl)amino]benzanilides **4** appeared to be inactive. Salicylanilides **1** and 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones **2** exhibit significant activity against both *M. tuberculosis* and nontuberculous mycobacteria (the MICs within the range of 4–250 μmol/l for all compounds). The antimycobacterial activity of the compounds increases with increasing both electron-withdrawing properties and hydrophobicity of the substituent(s) on the phenyl moiety. The antimycobacterial profile of the compounds was analyzed according to the criteria based on vector algebra, such as cosine coefficients. Moreover, salicylanilides **1** exhibit activity against other microorganisms tested by the agar diffusion method.

Key words: Tuberculostatics; Antimicrobial activity; QSAR; 1,3-Benzoxazines; Quinazolines; Salicylanilides; Anthranilanilides; Cosine coefficient.

+ For Part 17, see ref.[1]

The search for substances active against *Mycobacterium avium* is presently one of the primary tasks of medicinal chemistry. Disseminated infection with the *M. avium* complex is the most common systematic bacterial infection complicating AIDS (ref.[2]). In the Czech Republic, an increase in the occurrence of *M. kansasii* has been observed in some areas[3].

We have reported on a series of 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones and their 6-mono- and 6,8-dihalogeno derivatives as a novel group of antimycobacterially active compounds[4]. Recently, initial studies of structure–activity relationships of 6-halogeno derivatives have been published[5]. Besides the influence of the halogen substituent, the studies proved that the activity of the compounds against *M. tuberculosis* and *M. kansasii* increases with increasing electron-acceptor properties of substituent(s) on the phenyl ring. On the contrary, for an adequate explanation of physicochemical influences on inhibition of *M. tuberculosis* and nontuberculous mycobacteria, both electronic and hydrophobic terms must be considered in 6,8-dihalogeno compounds[4b]. Other biological properties of 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones were also reviewed[6].

As an extension of our synthetic study of 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones, a group of isosteric 3-phenylquinazoline-2,4(1*H*,3*H*)-diones substituted on the 3-phenyl ring was also investigated. The present report describes their synthesis and the activity of these compounds, as well as of other synthesized intermediates, against *Mycobacterium tuberculosis, M. kansasii*, and *M. avium*. With new antimycobacterial data at hand, the quantitative structure–activity relationships were reinvestigated. Furthermore, the results of antimicrobial screening of some of these compounds are reported. In addition, the compounds were evaluated with regard to the considered therapeutical use, *i.e.*, as broad-spectrum antimycobacterial agents. For the sake of clarity, a very brief outline of this procedure is also provided.

No drug produces only a single effect: a drug is adequately described only by means of the full profile of effects. As a measure of *i*-th biological activity, the logarithm of the reciprocal value of molar concentration C_i of the evaluated compound required to produce *i*-th effect in the predetermined intensity is used:

$$A_i = \log 1/C_i = -\log C_i \ , \qquad i = 1, \ldots, n \ . \tag{1}$$

Each compound is then represented by an activity vector,

$$\boldsymbol{A} = (A_1, \ldots, A_n) \quad , \tag{2}$$

where n is the number of the activities studied.

The evaluation of the compounds with regard to the considered therapeutical use is based on the idea of the comparison with an "ideal" drug of predefined profile of activities making full use of the vector representation. Accordingly, the "ideal" drug is represented by vector $\boldsymbol{B}$,

$$\boldsymbol{B} = (B_1, \ldots, B_n) \quad , \tag{3}$$

or, more conveniently, by the unit vector $\boldsymbol{U}$ ($g(\boldsymbol{U}) = 1$),

$$\boldsymbol{U} = (U_1, \ldots, U_n) = \boldsymbol{B}/g(\boldsymbol{B}) \quad . \tag{4}$$

The Euclidean norm of vector, *e.g.* $\boldsymbol{A}$,

$$g(\boldsymbol{A}) = \left(\sum_{i=1}^{n} A_i^2 \right)^{1/2} \quad , \tag{5}$$

then characterizes the size of the vector.

For the purpose of an evaluation, a complex criterion S was proposed[7] as equal to scalar (dot) product of vectors representing both compounds:

$$S = (\boldsymbol{A}, \boldsymbol{U}) \quad . \tag{6}$$

The term "complex" is given by the fact that the criterion S can be decomposed into two components,

$$S = g(\boldsymbol{A})\ k(\boldsymbol{A}, \boldsymbol{U}) \quad , \tag{7}$$

of which the first concerns the evaluated compound itself and the other reflects its similarity to the "ideal" drug. The former is the above mentioned Euclidean norm of the activity vector, $g(\boldsymbol{A})$, as a measure of the overall po-

tency of the evaluated compound and the latter is the cosine coefficient $k(\boldsymbol{A}, \boldsymbol{U})$,

$$k(\boldsymbol{A}, \boldsymbol{U}) = S/g(\boldsymbol{A}) \ , \tag{8}$$

as a measure of the relative similarity of the evaluated compound to the "ideal" drug.

The following two situations are of great importance. In the case of broad-spectrum drugs, the "ideal" drug is represented by the unit vector $\boldsymbol{U}_0$; its components are given by

$$U_{0i} = 1/\sqrt{n} \ , \qquad i = 1, \ldots, n \tag{9}$$

so that the formula for calculating the complex criterion S_0 can be written as

$$S_0 = \frac{1}{\sqrt{n}} \sum_{i=1}^{n} A_i \ . \tag{10}$$

In the case of the selectivity of the *s*-th effect, the "ideal" drug is represented by the unit vector $\boldsymbol{U}_s$, the components of which are given by

$$U_{si} = \begin{cases} (n-1) / \sqrt{n(n-1)} & \text{for } i = s \ , \\ -1 / \sqrt{n(n-1)} & \text{for } i \neq s \ , \ i = 1, \ldots, n \ . \end{cases} \tag{11}$$

The negative sign attributed to all but the s-th effect reflects the fact that they are considered to be undesired. The formula for calculating the complex criteria S_s (complex selectivities) can be written as

$$S_s = \frac{1}{\sqrt{n(n-1)}} \left(nA_s - \sum_{i=1}^{n} A_i \right), \quad \text{where } s = 1, \ldots, n \ . \tag{12}$$

The respective cosine coefficients k_0 and k_s are then calculated according to Eq. (*8*).

The application of Hansch or Free–Wilson approach to the complex criteria *S* is evident[7].

EXPERIMENTAL

The melting points were determined on a Kofler apparatus and are uncorrected. 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 CHNS-O CE elemental analyser (Fisions EA 1110, Milano). The IR spectra were measured in KBr pellets on a Nicolet Impact 400 apparatus; the wavenumbers are given in cm^{-1}. The ^1H NMR spectra of new compounds were recorded in $CDCl_3$ solutions (with the exception of compounds **1j**, **2k**, **5c**–**5e**, and **5f** which were measured in $((CD_3)_2SO)$ 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 (for ^1H 7.26 in $CDCl_3$ and 2.49 in $((CD_3)_2SO)$. The purity of the compounds was checked by TLC (Silufol UV254, Kavalier, Votice, Czech Republic) in benzene–acetone (1 : 3) for compounds **1**, in cyclohexane–acetone (1 : 3) for compounds **2**, and in chloroform–acetone (1 : 3 and 1 : 9) for compounds **3**–**5**, using UV detection.

Salicylanilides **1a**–**1l**

The title compounds were synthesized from salicylic acid and the respective aniline as described previously[4b].

Salicylanilide (**1a**). M.p. l33–135 °C (ref.[8] m.p. 136–137 °C and ref.[9] m.p. 134–135 °C). IR: 1 612.

4′-Methylsalicylanilide (**1b**). M.p. 154–156 °C (ref.[8] m.p. 156–158 °C). IR: 1 603.

4′-Bromosalicylanilide (**1c**). M.p. 168–170 °C (ref.[8] m.p. 169–172 °C). IR: 1 614.

4′-Methoxysalicylanilide (**1d**). M.p. 158–160 °C (ref.[10] m.p. 159–160 °C). IR: 1 628.

4′-Chlorosalicylanilide (**1e**). M.p. 165–167 °C (ref.[8] m.p. 168–170 °C). IR: 1 615.

3′,4′-Dichlorosalicylanilide (**1f**). M.p. 213–215 °C (ref.[11] m.p. 213–216 °C). IR: 1 614.

3′-Chlorosalicylanilide (**1g**). M.p. 173–175 °C (ref.[8] m.p. 175–177 °C). IR: 1 616.

3′-Fluorosalicylanilide (**1h**). M.p. 151–153 °C. For $C_{13}H_{10}FNO_2$ (231.2) calculated: 67.53% C, 4.36% H, 6.06% N; found: 67.53% C, 4.60% H, 6.11% N. IR: 1 615. ^1H NMR ($CDCl_3$): 11.81 bs, 1 H (OH); 8.00 bs, 1 H (NH); 7.59–7.42 m, 3 H (H-4, H-6, H-2′); 7.39–7.29 m, 1 H (H-5′); 7.26–7.21 m, 1 H (H-6′); 7.07–7.01 m, 1 H (H-3); 6.97–6.86 m, 2 H (H-5, H-4′).

4′-Fluorosalicylanilide (**1i**). M.p. 158–160 °C (ref.[12] m.p. 160–161 °C). IR: 1 619.

3′-Nitrosalicylanilide (**1j**). M.p. 220–221 °C. For $C_{13}H_{10}N_2O_4$ (258.2) calculated: 60.47% C, 3.90% H, 10.85% N; found: 60.47% C, 4.00% H, 10.87% N. IR: 1 643. ^1H NMR ($(CD_3)_2SO$): 10.70 bs, 1 H (OH); 8.80–8.70 m, 1 H (H-2′); 8.10–8.01 m, 1 H (H-4′); 8.00–7.92 m, 1 H (H-6′); 7.92–7.86 m, 1 H (H-6); 7.62 t, 1 H, J = 8.0 (H-5′); 7.48–7.39 m, 1 H (H-4); 7.03–6.92 m, 2 H (H-3, H-5).

4′-Nitrosalicylanilide (**1k**). M.p. 233–235 °C (ref.[13] 230 °C). IR: 1 616.

4′-(Dimethylamino)salicylanilide (**1l**). M.p. 150–152 °C. For $C_{14}H_{16}N_2O_2$ (256.3) calculated: 70.29% C, 6.29% H, 10.93% N; found: 69.97% C, 6.46% H, 11.14% N. IR: 1 615. ^1H NMR ($CDCl_3$): 12.23 s, 1 H (OH); 7.83 bs, 1 H (NH); 7.53–7.47 m, 1 H (H-6); 7.47–7.41 m, 1 H (H-4); 7.41–7.34 m (AA′BB′), 2 H (H-2′, H-6′); 7.05–6.99 m, 1 H (H-3); 6.94–6.86 m, 1 H (H-5); 6.78–6.71 m (AA′BB′), 2 H (H-3′, H-5′); 2.95 s, 6 H (2 × (CH_3)).

3-Phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones **2a**–**2l**

The title compounds were synthesized from salicylanilides **1** as described previously[4b].

3-Phenyl-2H-1,3-benzoxazine-2,4(3H)-dione (**2a**). M.p. 246–247 °C (ref.[8] m.p. 247–248 °C). IR: 1 770, 1 693.

3-(4-Methylphenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (**2b**). M.p. 224–225 °C (ref.[8] m.p. 226–227 °C). IR: 1 761, 1 697.

3-(4-Bromophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (**2c**). M.p. 250–252 °C (ref.[8] m.p. 247–248 °C). IR: 1 772, 1 695.

3-(4-Methoxyphenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (**2d**). M.p. 206–207 °C (ref.[4a] m.p. 207–208 °C). IR: 1 763, 1 701.

3-(4-Chlorophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (**2e**). M.p. 242–244 °C (ref.[8] m.p. 243–244 °C). IR: 1 772, 1 708.

3-(3,4-Dichlorophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (**2f**). M.p. 212–214 °C (ref.[14] m.p. 192–195 °C). For $C_{14}H_7Cl_2NO_3$ (308.1) calculated: 54.57% C, 2.29% H, 4.55% N; found: 54.55% C, 2.36% H, 4.34% N. IR: 1 774, 1 712.

3-(3-Chlorophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (**2g**). M.p. 196–198 °C (ref.[8] m.p. 198–200 °C). IR: 1 756, 1 709.

3-(3-Fluorophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (**2h**). M.p. 192–193 °C. For $C_{14}H_8FNO_3$ (257.2) calculated: 65.37% C, 3.13% H, 5.45% N; found: 65.72% C, 3.27% H, 5.55% N. IR: 1 769, 1 697. ^{1}H NMR ($CDCl_3$): 8.16–8.08 m, 1 H (H-5); 7.82–7.72 m, 1 H (H-7); 7.56–7.32 m, 3 H (H-6, H-8, H-5′); 7.25–7.16 m, 1 H (H-4′); 7.16–7.02 m, 2 H (H-2′, H-6′).

3-(4-Fluorophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (**2i**). M.p. 237–238 °C. For $C_{14}H_8FNO_3$ (257.2) calculated: 65.37% C, 3.13% H, 5.45% N; found: 65.33% C, 3.34% H, 5.48% N. IR: 1 772, 1 709. ^{1}H NMR ($CDCl_3$): 8.17–8.09 m, 1 H (H-5); 7.82–7.73 m, 1 H (H-7); 7.46–7.34 m, 2 H (H-6, H-8); 7.34–7.17 m, 4 H (H-2′, H-3′, H-5′, H-6′).

3-(3-Nitrophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (**2j**). M.p. 204–206 °C. For $C_{14}H_8N_2O_5$ (284.2) calculated: 59.16% C, 2.84% H, 9.86% N; found: 58.72% C, 2.81% H, 9.76% N. IR: 1 768, 1 705. ^{1}H NMR ($CDCl_3$): 8.41–8.32 m, 1 H (H-4′); 8.28–8.21 m, 1 H (H-2′); 8.17–8.10 m, 1 H (H-5); 7.85–7.64 m, 3 H (H-7, H-5′, H-6′); 7.51–7.35 m, 2 H (H-6, H-8).

3-(4-Nitrophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (**2k**). M.p. 250–252 °C. For $C_{14}H_8N_2O_5$ (284.2) calculated: 59.16% C, 2.84% H, 9.86% N; found: 58.73% C, 3.09% H, 9.97% N. IR: 1 767, 1 707. ^{1}H NMR ($(CD_3)_2SO$): 8.43–8.33 m (AA′BB′), 2 H (H-3′, H-5′); 8.04–7.97 m, 1 H (H-5); 7.92–7.83 m, 1 H (H-7); 7.80–7.72 m (AA′BB′), 2 H (H-2′, H-6′); 7.55–7.44 m, 2 H (H-6, H-8).

3-(4-Dimethylaminophenyl)-2H-1,3-benzoxazine-2,4(3H)-dione (**2l**). M.p. 302–303 °C. For $C_{16}H_{14}N_2O_3$ (282.3) calculated: 68.08% C, 5.00% H, 9.92% N; found: 68.22% C, 5.08% H, 9.93% N. IR: 1 760, 1 706. ^{1}H NMR ($CDCl_3$): 8.17–8.11 m, 1 H (H-5); 7.78–7.69 m, 1 H (H-7); 7.44–7.31 m, 2 H (H-6, H-8); 7.18–7.10 m (AA′BB′), 2 H (H-2′, H-6′); 6.85–6.77 m (AA′BB′), 2 H (H-3′, H-5′); 3.01 s, 6 H (2 × CH_3).

Preparation of Anthranilanilides **3a**–**3h**. General Procedure

A suspension of 2-(carboxyamino)benzoic anhydride (7.2 g, 44.2 mmol) in glacial acetic acid (300 ml) was added gradually to a stirred solution of the corresponding aniline (44.2 mmol) in the same solvent (100 ml). The stirred mixture was slowly heated to its reflux temperature and then poured into water (2 l). After 12 h, the product was filtered off and crystallized from aqueous ethanol (yield 70–85%).

Anthranilanilide (**3a**). M.p. 123–125 °C (ref.[15] m.p. 125–126.5 °C). IR: 1 644.

4′-Methylanthranilanilide (**3b**). M.p. 149–150 °C (ref.[15] m.p. 150–151 °C). IR: 1 635.

4′-Bromoanthranilanilide (**3c**). M.p. 161–163 °C (ref.[15] m.p. 148–149 °C). For $C_{13}H_{11}BrN_2O$ (267.1) calculated: 53.63% C, 3.81% H, 9.62% N; found: 53.15% C, 3.97% H, 9.42% N. IR: 1 637. ^{1}H NMR ($CDCl_3$): 7.79 bs, 1 H (NH); 7.50–7.40 m, 5 H (H-6, H-2′, H-3′, H-5′, H-6′); 7.30–7.22 m, 1 H (H-4); 6.75–6.67 m, 2 H (H-3, H-5); 5.49 bs, 2 H (NH_2).

4′-Methoxyanthranilanilide (**3d**). M.p. 123–125 °C (ref.[15] m.p. 125–126 °C). IR: 1 635.

3′,4′-Dichloroanthranilanilide (**3e**). M.p. 146–147 °C (ref.[16] m.p. 142–143 °C). For $C_{13}H_{10}Cl_2N_2O$ (281.1) calculated: 55.54% C, 3.59% H, 9.96% N; found: 55.87% C, 3.20% H, 9.43 % N. IR: 1 638.

4′-Fluoroanthranilanilide (**3f**). M.p. 131–132 °C. For $C_{13}H_{11}FN_2O$ (216.2) calculated: 67.82% C, 4.82% H, 12.17% N; found: 67.39% C, 4.97% H, 11.97% N. IR: 1 630. ^{1}H NMR ($CDCl_3$): 7.78 bs, 1 H (NH); 7.55–7.48 m, 2 H (H-2′, H-6′), 7.48–7.43 m, 1 H (H-6); 7.30–7.22 m, 1 H (H-4); 7.11–7.01 m, 2 H (H-3′, H-5′); 6.75–6.65 m, 2 H (H-3, H-5); 5.49 bs, 2 H (NH_2).

3′-Nitroanthranilanilide (**3g**). M.p. 189–190 °C. For $C_{13}H_{11}N_3O_3$ (257.2) calculated: 4.31% C, 60.70% H, 16.33% N; found: 60.25% C, 4.49% H, 16.22% N. IR: 1 633.

3′,4′-Dimethylanthranilanilide (**3h**). M.p. 114–116 °C. For $C_{15}H_{16}N_2O$ (240.3) calculated: 74.97% C, 6.71% H, 11.66% N; found: 75.1% C, 6.58% H, 11.86% N. IR: 1 636. ^{1}H NMR ($CDCl_3$): 7.66 s, 1 H (NH); 7.49–7.42 m, 1 H (H-6); 7.40–7.33 m, 1 H (H-2′); 7.31–7.20 m, 2 H (H-4, H-6′); 7.15–7.08 m, 1 H (H-5′); 6.75–6.65 m, 2 H (H-3, H-5); 5.50 bs, 2 H (NH_2); 2.27 s, 3 H (CH_3); 2.25 s, 3 H (CH_3).

Preparation of 2-[(Ethoxycarbonyl)amino]benzanilides **4a**–**4h**. General Procedure

Ethyl chloroformate (1 g, 9.24 mmol) was added dropwise to a stirred solution of an appropriate anilide **3** (7.7 mmol) in dry pyridine (15 ml) at 0 °C. The mixture was heated on a water bath for 2 h and then poured into water (100 ml). After 12 h, the product was filtered off and crystallized from aqueous ethanol (yield 75–85%).

2-[(Ethoxycarbonyl)amino]benzanilide (**4a**). M.p. 155–157 °C (ref.[17] m.p. 153–154 °C). IR: 1 738, 1 636. ^{1}H NMR ($CDCl_3$): 9.99 s, 1 H (NH); 8.18 bs, 1 H (NH); 8.32–8.25 m, 1 H (H-6); 7.66–7.59 m, 2 H (H-2′, H-6′); 7.56–7.50 m, 1 H (H-3); 7.47–7.36 m, 3 H (H-5, H-3′, H-5′); 7.24–7.15 m, 1 H (H-4′); 7.07–6.98 m, 1 H (H-4); 4.21 q, 2 H, J = 14.16, J = 7.14 (CH_2); 1.31 t, 3 H, J = 7.14 (CH_3).

4′-Methyl-2-[(ethoxycarbonyl)amino]benzanilide (**4b**). M.p. 162–164 °C. For $C_{17}H_{18}N_2O_3$ (298.3) calculated: 68.44% C, 6.08% H, 9.39% N; found: 68.65% C, 5.59% H, 9.81% N. IR: 1 706, 1 659. ^{1}H NMR ($CDCl_3$): 10.00 s, 1 H (NH); 8.30 bs, 1 H (NH); 8.30–8.15 m, 1 H (H-6); 7.55–7.45 m, 3 H (H-3, H-2′, H-6′); 7.44–7.32 m, 1 H (H-5); 7.24–7.15 m (AA′BB′), 2 H (H-3′, H-5′); 7.05–6.93 m, 1 H (H-4); 4.20 q, 2 H, J = 14.28, J = 7.15 (CH_2); 2.35 s, 3 H (CH_3); 1.30 t, 3 H, J = 7.15 (CH_3).

4′-Bromo-2-[(ethoxycarbonyl)amino]benzanilide (**4c**). M.p. 156–158 °C. For $C_{16}H_{14}BrN_2O_3$ (363.2) calculated: 52.91% C, 4.16% H, 7.71% N; found: 53.14% C, 4.45% H, 7.41% N. IR: 1 702, 1 655. ^{1}H NMR ($CDCl_3$): 9.86 s, 1 H (NH); 8.40 bs, 1 H (NH); 8.22–8.14 m, 1 H (H-6); 7.65–7.43 m, 5 H (H-3, H-2′, H-3′, H-5′, H-6′); 7.42–7.31 m, 1 H (H-5); 7.05–6.93 m, 1 H (H-4); 4.23 q, 2 H, J = 14.29, J = 7.15 (CH_2); 1.33 t, 3 H, J = 7.15 (CH_3).

4′-Methoxy-2-[(ethoxycarbonyl)amino]benzanilide (**4d**). M.p. 164–165 °C. For $C_{17}H_{18}N_2O_4$ (314.3) calculated: 64.96% C, 5.77% H, 8.91% N; found: 64.91% C, 5.77% H, 8.84% N. IR: 1 739, 1 635. ^{1}H NMR ($CDCl_3$): 10.08 s, 1 H (NH); 8.03 bs, 1 H (NH); 8.35–8.25 m, 1 H (H-6); 7.60–7.47 m, 1 H (H-3); 7.53–7.48 m (AA′BB′), 2 H (H-2′, H-6′); 7.47–7.37 m, 1 H

(H-5); 7.10–6.98 m, 1 H (H-4); 6.95–6.90 m (AA′BB′), 2 H (H-3′, H-5′); 4.20 q, 2 H, *J* = 14.10, *J* = 7.0 (CH_2); 3.82 s, 3 H (OCH_3); 1.29 t, 3 H, *J* = 7.0 (CH_3).

3′,4′-Dichloro-2-[(ethoxycarbonyl)amino]benzanilide (**4e**). M.p. 174–176 °C. For $C_{16}H_{14}Cl_2N_2O_3$ (353.2) calculated: 54.41% C, 4.00% H, 7.93% N; found: 54.53% C, 3.80% H, 7.94% N. IR: 1 727, 1 673. ^{1}H NMR ($CDCl_3$): 9.80 s, 1 H (NH); 8.45 bs, 1 H (NH); 8.20–8.13 m, 1 H (H-6); 7.98–7.93 m, 1 H (H-2′); 7.50–7.41 m, 3 H (H-3, H-5′, H-6′); 7.42–7.32 m, 1 H (H-5); 7.05–6.95 m, 1 H (H-4); 4.25 q, 2 H, *J* = 14.28, *J* = 7.15 (CH_2); 1.34 t, 3 H, *J* = 7.15 (CH_3).

4′-Fluoro-2-[(ethoxycarbonyl)amino]benzanilide (**4f**). M.p. 163–165 °C. For $C_{16}H_{14}FN_2O_3$ (302.3) calculated: 63.57% C, 5.00% H, 9.27% N; found: 63.30% C, 4.98% H, 9.24% N. IR: 1 707, 1 656. ^{1}H NMR ($CDCl_3$): 9.95 s, 1 H (NH); 8.22 bs, 1 H (NH); 8.28–8.23 m, 1 H (H-6); 7.65–7.55 m, 2 H (H-2′, H-6′); 7.55–7.47 m, 1 H (H-3); 7.45–7.35 m, 1 H (H-5); 7.15–6.97 m, 3 H (H-4, H-3′, H-5′); 4.22 q, 2 H, *J* = 14.29, *J* = 7.14 (CH_2); 1.31 t, 3 H, *J* = 7.14 (CH_3).

3′-Nitro-2-[(ethoxycarbonyl)amino]benzanilide (**4g**). M.p. 183–185 °C. For $C_{16}H_{15}N_3O_5$ (329.3) calculated: 58.36% C, 4.59% H, 8.97% N; found: 57.86% C, 4.39% H, 12.78% N. IR: 1 725, 1 659.

3′,4′-Dimethyl-2-[(ethoxycarbonyl)amino]benzanilide (**4h**). M.p. 133–135 °C. For $C_{18}H_{20}N_2O_3$ (312.4) calculated: 69.21% C, 6.45% H, 8.97% N; found: 69.56% C, 6.22% H, 8.63% N. IR: 1 717, 1 663. ^{1}H NMR ($CDCl_3$): 10.06 s, 1 H (NH); 8.02 bs, 1 H (NH); 8.36–8.26 m, 1 H (H-6); 7.56–7.48 m, 1 H (H-3); 7.48–7.35 m, 2 H (H-5, H-2′); 7.35–7.27 m, 1 H (H-6′); 7.18–7.10 m, 1 H (H-5′); 7.07–6.96 m, 1 H (H-4); 4.20 q, 2 H, *J* = 14.28, *J* = 7.0 (CH_2); 2.28 s, 3 H (CH_3); 2.26 s, 3 H (CH_3); 1.30 t, 3 H, *J* = 7.0 (CH_3).

Preparation of 3-Phenylquinazoline-2,4(1*H*,3*H*)-diones **5a**–**5h**. General Procedure

An appropriate benzanilide **4** (0.5 g) was heated at 250–270 °C for 10–20 min. The product was crystallized from aqueous acetone (yield 75–85%).

3-Phenylquinazoline-2,4(1H,3H)-dione (**5a**). M.p. 280–281 °C (ref.[18] m.p. 295–296 °C and ref.[19] m.p. 272 °C). For $C_{14}H_{10}N_2O_2$ (238.2) calculated: 70.58% C, 4.23% H, 11.75% N; found: 70.18% C, 4.18% H, 11.85% N. IR: 1 724, 1 672. ^{1}H NMR ($CDCl_3$): 8.17–8.13 m, 1 H (H-5), 7.60–7.46 m, 4 H (H-7, H-3′, H-4′, H-5′); 7.35–7.30 m, 2 H (H-2′, H-6′); 7.28–7.20 m, 1 H (H-6); 6.94–6.89 m, 1 H (H-8).

3-(4-Methylphenyl)quinazoline-2,4(1H,3H)-dione (**5b**). M.p. 275–277 °C (ref.[20] m.p. 263–265 °C). For $C_{14}H_{12}N_2O_2$ (252.3) calculated: 71.42% C, 4.79% H, 11.10% N; found: 71.34% C, 4.85% H, 11.17% N. IR: 1 720, 1 672. ^{1}H NMR ($CDCl_3$): 8.17–8.08 m, 1 H (H-5); 7.53–7.43 m, 1 H (H-7); 7.38–7.31 m (AA′BB′), 2 H (H-2′, H-6′); 7.24–7.16 m, 3 H (H-6, H-3′, H-5′); 6.85–6.78 m, 1 H (H-8); 10.19 s, 1 H (NH); 2.45 s, 3 H (CH_3).

3-(4-Bromophenyl)quinazoline-2,4(1H,3H)-dione (**5c**). M.p. 329–330 °C (ref.[21] m.p. 297 °C). For $C_{14}H_9BrN_2O_2$ (317.1) calculated: 53.02% C, 2.85% H, 8.83% N; found: 52.85% C, 2.83% H, 8.83% N. IR: 1 723, 1 672. ^{1}H NMR ($(CD_3)_2SO$): 7.94–7.87 m, 1 H (H-5); 7.71–7.62 m, 3 H (H-7, H-3′, H-5′); 7.32–7.25 m, 2 H (H-2′, H-6′); 7.24–7.15 m, 2 H (H-6, H-8).

3-(4-Methoxyphenyl)quinazoline-2,4(1H,3H)-dione (**5d**). M.p. 311–312 °C (ref.[20] m.p. 306–308 °C). For $C_{15}H_{12}N_2O_3$ (268.3) calculated: 67.16% C, 4.51% H, 10.44% N; found: 66.99% C, 4.44% H, 10.43% N. IR: 1 720, 1 669. ^{1}H NMR ($(CD_3)_2SO$): 7.95–7.88 m, 1 H (H-5); 7.72–7.63 m, 1 H (H-7); 7.25–7.16 m, 4 H (H-6, H-8, H-2′, H-6′); 7.04–6.96 m, 2 H (H-3′, H-5′).

3-(3,4-Dichlorophenyl)quinazoline-2,4(1H,3H)-dione (**5e**). M.p. 328–329 °C (ref.[21] m.p. 317 °C). For $C_{14}H_8Cl_2N_2O_2$ (307.1) calculated: 54.57% C, 2.94% H, 9.09% N; found: 54.76% C, 2.69% H, 8.94% N. IR: 1 725, 1 674. ^{1}H NMR ($(CD_3)_2SO$): 7.95–7.88 m, 1 H (H-5); 7.78–7.66 m, 3 H (H-7, H-2′, H-5′); 7.48–7.36 m, 1 H (H-6′); 7.26–7.18 m, 2 H (H-6, H-8).

3-(4-Fluorophenyl)quinazoline-2,4(1H,3H)-dione (**5f**). M.p. 280–281 °C (ref.[21] m.p. 272–274 °C). For $C_{14}H_9FN_2O_2$ (256.2) calculated: 65.62% C, 3.54% H, 10.93% N; found: 65.22% C, 3.49% H, 10.95% N. IR: 1 724, 1 672. ^{1}H NMR ($(CD_3)_2SO$): 7.92 dd, 1 H, J = 8.1, J = 1.5 (H-5); 7.73–7.64 m, 1 H (H-7); 7.41–7.17 m, 6 H (H-6, H-8 + C_6H_4F).

3-(3-Nitrophenyl)quinazoline-2,4(1H,3H)-dione (**5g**). M.p. 352–353 °C (ref.[21] m.p. >350 °C). For $C_{14}H_9N_3O_4$ (283.2) calculated: 59.37% C, 3.20% H, 14.84% N; found: 59.02% C, 3.25% H, 14.64% N. IR: 1 724, 1 676.

3-(3,4-Dimethylphenyl)quinazoline-2,4(1H,3H)-dione (**5h**). M.p. 262 °C. For $C_{16}H_{14}N_2O_2$ (266.3) calculated: 72.17% C, 2.86% H, 10.52% N; found: 72.15% C, 2.85% H, 10.56% N. IR: 1 727, 1 674. ^{1}H NMR ($CDCl_3$): 8.17–8.10 m, 1 H (H-5); 7.52–7.43 m, 1 H (H-7); 7.33–7.27 m, 1 H (H-5′); 7.25–7.15 m, 1 H (H-6); 7.11–7.02 m, 2 H (H-2′, H-6′); 6.88–6.80 m, 1 H (H-8); 10.05 s, 1 H (NH); 2.33 s, 3 H (CH_3); 2.29 s, 3 H (CH_3).

Microbiological Assays

Antimycobacterial Activity

For the evaluation of the antimycobacterial activity of the substances *in vitro*, the following strains were used: *Mycobacterium 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, and a clinic isolate of *M. kansasii* 6509/96. Antimycobacterial activity of the compounds against these strains was determined in the Šula semisynthetic medium (SEVAC, Prague). Each strain was simultaneously inoculated into a Petri dish containing the Löwenstein–Jensen medium for the control of the sterility of the inoculum and its growth. The compounds were added to the medium in dimethyl sulfoxide solutions. The following concentrations were used: 1 000, 500, 250, 125, 62, 31, 16, 8, and 4 μmol/l. The minimum inhibitory concentrations (MICs) were determined after incubation at 37 °C for 14 and 21 days. MIC was the lowest concentration of a substance, at which the inhibition of the growth occurred. Isoniazid was used as standard.

General Antimicrobial Activity

Antimicrobial activity was determined by the agar diffusion test[22]. The Gram-positive and Gram-negative bacteria, as well as the fungi used were from the culture collections (*Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 1031, and *Pseudomonas aeruginosa* ATCC 27922) or from the stock of the Hans Knöll Institute of Natural Products Research (*Mycobacterium smegmatis* SG 987, *Staphylococcus aureus* SG 511, *P. aeruginosa* SG 137, *Serratia marcescens* SG 621, and *Sporobolomyces salmonicolor* 549). Penetration mutant DC 2 and its parent strain *E. coli* DC 0 were described by Richmond *et al.*[23], *Pseudomonas aeruginosa* K799/WT and its penetration mutant K799/61 by Zimmermann[24]. *Stenotrophomas meltophilia* GN 12873 was kindly provided by Prof. S. Mitsuhashi, Eisome Institute, Gunma, Japan. Azlocilline was used as standard.

Calculations

Log P Calculations

The calculations of log *P* values were carried out with the HyperChem Suite for Windows (release 5.1), module ChemPlus 1.6 using atomic parameters derived by Ghose *et al.*[25] and later extended[26].

Quantitative Structure–Activity Relationship Study (QSARs)

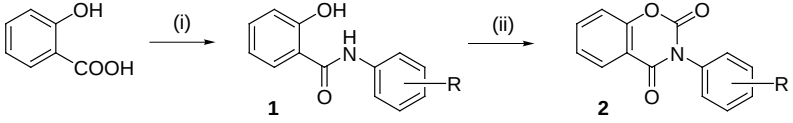
All regression equations were calculated with the use of the Multireg H program (Prof. P. Klemera) for Microsoft Excel. The values of Hammett constants σ and hydrophobic substituent constants π were taken from the literature[27].

Antimycobacterial Profile Evaluation

For this purpose, logarithms of reciprocal values of minimum inhibitory concentrations were calculated as if expressed in mmol/l to obtain only positive values. Activities against *Mycobacterium tuberculosis* CNCTC My 331/88, *M. kansasii* CNCTC My 235/80, and *M. avium* CNCTC My 330/88 form the components of vector ***A*** (in the given order) representing the evaluated compound. Subscripts 1, 2, and 3 are used to further indicate the respective strain. Complex criteria S_0 (Eq. (*10*)), S_1 to S_3 (Eq. (*12*)) and the norm $g(\boldsymbol{A})$ (Eq. (*5*)) were used for the calculation of the respective cosine coefficients k_0 to k_3 (Eq. (*8*)).

RESULTS AND DISCUSSION

The starting salicylanilides **1** were prepared from salicylic acid and an appropriate aniline in chlorobenzene. Treatment of **1** with ethyl chloroformate yielded the corresponding 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones **2** (Scheme 1). All salicylanilides **1** showed IR bands at 1 603–1 643 cm^{-1}, which are characteristic of the amide C=O group, and 1,3-benzoxazine-2,4(3*H*)-diones **2** showed characteristic absorption maxima of two C=O groups at 1 770–1 774 cm^{-1} and 1 693–1 712 cm^{-1}. The starting



1,2	R
a	H
b	4-CH_3
c	4-Br
d	4-OCH_3

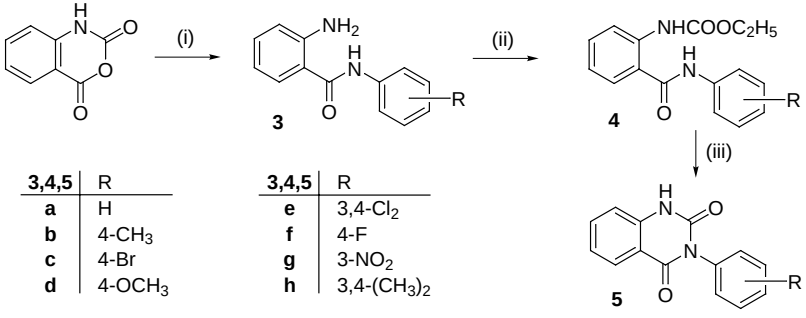
1,2	R
e	4-Cl
f	3,4-Cl_2
g	3-Cl
h	3-F

1,2	R
i	4-F
j	3-NO_2
k	4-NO_2
l	4-$N(CH_3)_2$

(i) substituted aniline, PCl_3/chlorobenzene; (ii) ethyl chloroformate/pyridine

SCHEME 1

anthranilanilides **3** were prepared from 2-(carboxyamino)benzoic anhydride and appropriate anilines in glacial acetic acid. Treatment of **3** with ethyl chloroformate yielded 2-[(ethoxycarbonyl)amino]benzanilides **4**, which were thermally cyclized to 3-phenylquinazoline-2,4(1*H*,3*H*)-diones **5** (Scheme 2). This method gives products of higher purity and in higher yields as compared with other methods. Anthranilanilides **3** showed characteristic bands of C=O groups at 1 630–1 644 cm^{-1}, 2-[(ethoxycarbonyl)-amino]benzanilides **4** at 1 635–1 673 cm^{-1} and 1 702–1 738 cm^{-1}, and 3-phenylquinazoline-2,4(1*H*,3*H*)-diones **5** at 1 669–1 676 cm^{-1} and 1 720–1 727 cm^{-1}. Most of the products have already been reported in the literature[8–21]. The spectroscopic data of the new compounds (**1h**, **1j**, **1l**, **2h**–**2l**, **3f**–**3h**, **4b**–**4h**, and **5h**) as well as of compounds with melting points different from the literature ones, are in agreement with the assigned structures. ^{13}C NMR spectra of 4′-substituted compounds **2** will be published separately[28].



3,4,5	R
a	H
b	4-CH_3
c	4-Br
d	4-OCH_3

3,4,5	R
e	3,4-Cl_2
f	4-F
g	3-NO_2
h	3,4-$(CH_3)_2$

(i) substituted aniline/glacial acetic acid; (ii) ethyl chloroformate/pyridine; (iii) heating

SCHEME 2

All the compounds prepared (with the exception of **2h**) were tested *in vitro* against mycobacterial strains *Mycobacterium tuberculosis*, *M. kansasii*, and *M. avium* obtained from the Czech National Collection of Type Cultures and a clinic isolate of *M. kansasii* 6509/96 (Tables I–IV). A comparison of the minimum inhibitory concentrations for anthranilanilides **3** and 3-phenylquinazoline-2,4(1*H*,3*H*)-diones **5** (Tables III and IV) with their hydroxy/oxa analogues (Tables I and II) indicates that replacement of the oxygen atom by nitrogen resulted in a decrease or loss of the antimycobacterial activity. 2-[(Ethoxycarbonyl)amino]benzanilides **4** appeared to be completely inactive. It is worth noting that 3′,4′-dichloroanthranilanilide **3e** displays the lowest MICs of the groups of compounds **3**, **4**, and **5**.

The data in Tables I and II show that the values of MICs are within the range of 4–250 μmol/l both for salicylanilides **1** and 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones **2**. By comparing their MICs with that of isoniazid (INH), the most active 3,4-dichloro analogues **2f** and **1f** have activity against *M. tuberculosis* comparable to that of INH (MIC = 4 μmol/l). However, all the tested compounds **1** and **2** possess activity against atypical mycobacteria greater than INH (MIC = 500 μmol/l). While nontuberculous mycobacteria are moderately susceptible towards INH, the newly prepared compounds display virtually the same activity against all the tested strains. A similar profile of antimycobacterial activity has recently been described for 4-(benzylsulfanyl)pyridine derivatives[29].

TABLE I
In vitro antimycobacterial activity of salicylanilides **1** expressed as MIC (μmol/l)

Compound	*M. tuberculosis* My 331/88		*M. kansasii* My 235/80		*M. kansasii* 6509/96		*M. avium* My 330/88	
	14 days	21 days	14 days	21 days	14 days	21 days	14 days	21 days
1a	62	62	125	250	62	125	62	125
1b	62	62	62	125	62	125	31	62
1c	16	31	16	31	16	16	31	31
1d	62	62	250	250	125	125	62	125
1e	31	31	31	31	16	31	31	31
1f	8	8	4	8	4	4	16	31
1g	16	16	8	8	8	8	31	31
1h	31	31	62	62	62	62	62	62
1i	62	62	62	125	62	62	31	62
1j	16	16	62	>62	16	31	31	>31
1k	8	16	16	16	16	16	31	31
1l	250	250	250	>250	[a]	[a]	125	250
INH[b]	4	4	500	500	[a]	[a]	500	500

[a] Not determined. [b] Isoniazid.

For the detailed evaluation of the differences in the antimycobacterial profiles of individual compounds (Tables V and VI), a complex criterion S_0 characterizing equipotency of the three compared activities, complex selectivities S_1–S_3 towards the individual mycobacterial strains and their decomposition into respective cosine coefficients k_0–k_3, and the norm of activity vector representing given compound, $g(\boldsymbol{A})$, were used. This treatment is somewhat more complex than that in previous papers[7] where the criterion S_0 and its decomposition were sufficient. MICs for *Mycobacterium tuberculosis*, *M. kansasii*, and *M. avium* obtained from CNCTC were used for the calculation. For better orientation, the clinic isolate of *M. kansasii* was omitted, as it exhibits the same or even better susceptibility than *M. kansasii* from the CNCTC.

TABLE II
In vitro antimycobacterial activity of 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones **2** expressed as MIC (μmol/l)

Compound	*M. tuberculosis* My 331/88		*M. kansasii* My 235/80		*M. kansasii* 6509/96		*M. avium* My 330/88	
	14 days	21 days	14 days	21 days	14 days	21 days	14 days	21 days
2a	125	125	125	250	62	125	62	125
2b	62	62	125	125	62	125	31	62
2c	31	31	16	31	16	31	31	31
2d	62	125	125	250	125	125	31	31
2e	16	31	16	16	16	16	62	62
2f	8	8	4	4	4	4	8	16
2g	31	31	8	8	4	8	31	31
2h	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
2i	125	125	62	62	62	62	62	125
2j	16	31	31	62	16	31	16	31
2k	16	16	16	16	16	16	16	31
2l	>125	>125	>125	>125	[a]	[a]	>125	>125

[a] Not determined.

TABLE III
In vitro antimycobacterial activity of anthranilanilides **3** expressed as MIC (μmol/l)

Compound	*M. tuberculosis* My 331/88		*M. kansasii* My 235/80		*M. kansasii* 6509/96		*M. avium* My 330/88	
	14 days	21 days	14 days	21 days	14 days	21 days	14 days	21 days
3a	>1 000	>1 000	>1 000	>1 000	>1 000	>1 000	500	500
3b	>250	250	>250	>250	>250	>250	250	>250
3c	125	125	125	125	62	125	>125	>125
3d	500	500	>500	>500	500	500	>250	>250
3e	62	62	31	62	31	31	>62	>62
3f	>250	>500	>500	>500	>250	>500	>125	>250
3g	500	500	>1 000	>1 000	>1 000	>1 000	>125	>250
3h	>125	>125	>125	>125	>125	>125	125	125

TABLE IV
In vitro antimycobacterial activity of 3-phenylquinazoline-2,4(1*H*,3*H*)-diones **5** expressed as MIC (μmol/l)

Compound	*M. tuberculosis* My 331/88		*M. kansasii* My 235/80		*M. avium* My 330/88	
	14 days	21 days	14 days	21 days	14 days	21 days
5a	500	>500	250	>250	>250	>500
5b	500	>500	250	500	500	>500
5c	250	>500	>250	>500	>125	>250
5d	>125	>500	>125	>250	>125	>500
5e	>62	>250	>125	>250	>125	>125
5f	>125	>250	>125	>250	>125	>125
5g	250	200	>125	>250	>62	>125
5h	250	500	>250	500	250	250

The highest values of the norm $g(\boldsymbol{A})$ correspond to the most potent 3,4-dichloro derivatives, **2f** and **1f**. The same is true for the values of complex criterion S_0, however, the non-zero values of the corresponding com-

TABLE V
Complex criteria S, norm $g(\boldsymbol{A})$, cosine coefficients k (see the text), and log P for salicylanilides **1**

Compound	S_0	S_1	S_2	S_3	$g(A)$	k_0	k_1	k_2	k_3	log P
	14 days / 21 days of incubation									
1a	1.916	0.125	–0.249	0.125	1.932	0.992	0.064	–0.129	0.064	2.997
	1.566	0.372	–0.370	–0.002	1.624	0.965	0.229	–0.228	–0.001	
1b	2.266	–0.123	–0.123	0.246	2.279	0.994	–0.054	–0.054	0.108	3.464
	1.916	0.125	–0.249	0.125	1.932	0.992	0.064	–0.129	0.064	
1c	2.945	0.117	0.117	–0.234	2.954	0.997	0.040	0.040	–0.079	3.788
	2.614	0.000	0.000	0.000	2.614	1.000	0.000	0.000	0.000	
1d	1.742	0.247	–0.495	0.247	1.811	0.962	0.137	–0.273	0.137	2.744
	1.566	0.372	–0.370	–0.002	1.624	0.965	0.229	–0.228	–0.001	
1e[a]	2.614	0.000	0.000	0.000	2.614	1.000	0.000	0.000	0.000	3.515
1f	3.632	0.000	0.369	–0.369	3.657	0.993	0.000	0.101	–0.101	4.033
	3.293	0.240	0.240	–0.480	3.327	0.990	0.072	0.072	–0.144	
1g[a]	3.119	–0.006	0.363	–0.357	3.146	0.991	–0.002	0.115	–0.114	3.515
1h[a]	2.266	0.246	–0.123	–0.123	2.279	0.994	0.108	–0.054	–0.054	3.136
1i	2.266	–0.123	–0.123	0.246	2.279	0.994	–0.054	–0.054	0.108	3.136
	1.916	0.125	–0.249	0.125	1.932	0.992	0.064	–0.129	0.064	
1j[b]	2.606	0.357	–0.363	0.006	2.639	0.988	0.135	–0.138	0.002	2.950
1k	3.119	0.363	–0.006	–0.357	3.146	0.991	0.115	–0.002	–0.114	2.950
	2.945	0.117	0.117	–0.234	2.954	0.997	0.040	0.040	–0.079	
1l[b]	1.216	–0.123	–0.123	0.246	1.241	0.980	–0.099	–0.099	0.198	3.261
INH[a,c]	1.732	1.712	–0.856	–0.856	2.435	0.711	0.703	–0.352	–0.352	–

[a] No difference between the values after 14 and 21 days of incubation. [b] After 14 days of incubation. [c] Isoniazid.

plex are indicative of different MICs for the tested mycobacterial strains. To avoid this discrepancy, cosine coefficients k_0 and k_s obtained as the ratios $S_0/g(\boldsymbol{A})$ and $S_s/g(\boldsymbol{A})$ should be taken into consideration. Generally, the more the value of the cosine coefficient is close to 1, the more the profile of the evaluated compound relatively agrees with that of the "ideal" drug. As follows from Tables V and VI, the values of the cosine coefficient k_0

TABLE VI
Complex criteria *S*, norm $g(\boldsymbol{A})$, cosine coefficients *k* (see the text), and log *P* for 3-phenyl-2*H*-1,3-benz- oxazine-2,4(3*H*)-diones **2**

Compound	S_0	S_1	S_2	S_3	$g(A)$	k_0	k_1	k_2	k_3	log *P*
	14 days / 21 days of incubation									
2a	1.740	–0.125	–0.125	0.249	1.758	0.990	–0.071	–0.071	0.142	3.988
	1.390	0.123	–0.246	0.123	1.412	0.985	0.087	–0.174	0.087	
2b	2.090	0.002	–0.372	0.370	2.133	0.980	0.001	–0.174	0.174	4.455
	1.916	0.125	–0.249	0.125	1.932	0.992	0.064	–0.129	0.064	
2c	2.779	–0.117	0.234	–0.117	2.789	0.996	–0.042	0.084	–0.042	4.779
	2.614	0.000	0.000	0.000	2.614	1.000	0.000	0.000	0.000	
2d	2.090	0.002	–0.372	0.370	2.133	0.980	0.001	–0.174	0.174	3.735
	1.740	–0.125	–0.493	0.618	1.859	0.936	–0.067	–0.265	0.332	
2e	2.771	0.240	0.240	–0.480	2.813	0.985	0.085	0.085	–0.171	4.506
	2.606	0.006	0.357	–0.363	2.639	0.988	0.002	0.135	–0.138	
2f	3.806	–0.123	0.246	–0.123	3.814	0.998	–0.032	0.064	–0.032	5.024
	3.632	0.000	0.369	–0.369	3.657	0.993	0.000	0.101	–0.101	
2g[a]	2.953	–0.240	0.480	–0.240	2.992	0.987	–0.080	0.160	–0.080	4.506
2i	1.916	–0.249	0.125	0.125	1.932	0.992	–0.129	0.064	0.064	4.127
	1.740	–0.125	0.249	–0.125	1.758	0.990	–0.071	0.142	–0.071	
2j	2.945	0.117	–0.234	0.117	2.954	0.997	0.040	–0.079	0.040	3.941
	2.440	0.123	–0.246	0.123	2.452	0.995	0.050	–0.100	0.050	
2k	3.111	0.000	0.000	0.000	3.111	1.000	0.000	0.000	0.000	3.941
	2.945	0.117	0.117	–0.234	2.954	0.997	0.040	0.040	–0.079	

[a] No difference between the values after 14 and 21 days of incubation.

TABLE VII
Antimicrobial activity of salicylanilides **1**

Microorganism	Inhibition zone, mm												
	1a	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**1h**	**1i**	**1j**	**1k**	**1l**	**AZL**[a]
B. subtilis ATCC 663	A	15*p*	13/ 16*p*	0	13/ 15*p*	11	12.5	12*P*	12*P*	A13	17	0	31
S. aureus SG 511	16*P*	A	23	0	20	17.5	23	17	13*P*	15	21	0	33
P. aeruginosa K799/61	0	19	10	0	10	0	12	0	0	12	11	0	36
P. aeruginosa K799/WT	0	14*p*	10	0	10	0	0	18*p*	18*p*	13*P*	10	0	32
P. aeruginosa SG 137	0	15*p*	11	0	10.5	0	11.5	0	0	10.5	11	0	19
S. marcescens SG 621	10*P*	11*P*	11.5	0	11.5	10*P*	11.5	11.5	10.5*P*	11.5*p*	12	0	18*p*
P. aeruginosa ATCC 27853	0	16*p*	10.5	0	10.5	0	10.5	0	0	0	10.5	0	24
K. pneumoniae ATCC 10031	0	12*p*	14	0	14	10.5*P*	13.5	11*P*	0	12*P*	14	0	16*p*
E. coli ATCC 25922	0	13.5	10	0	10.5	0	10	A	0	10	11	0	12
S. meltophilia GN 12873	0	0	11	11	A	0	11	10.5	0	0	0	11.5	10*p*
E. coli DC 0	0	12*P*	10.5	0	10	0	10.5	A	0	11*P*	11	0	15
E. coli DC 2	A	19	14	0	14/ 21*P*	A	13.5	13*P*	A	13*P*	14/ 25*P*	0	29
M. smegmatis SG 987	0	0	20	0	20	17	18	16*P*	A	16*P*	22	0	0
S. salmonicolor 549	14*p*	13*P*	15.5	0	17	A	17	16	13	13*P*	17	0	0

[a] Azlocilline. *A*, Indication of inhibition; *p*, colonies in the inhibition zone; *P*, a number of colonies in the inhibition zone.

(0.936–1.000) justify the conclusion that the compounds represent broad-spectrum antimycobacterial agents. As can be seen for compounds **1c**, **1e**, and **2c** (results after 21 days of incubation), compounds with identical susceptibility to all considered strains have $k_0 = 1$ and, simultaneously, $k_1 = k_2 = k_3 = 0$. Ranking of the values of the cosine coefficients k_1–k_3 gives the order of susceptibilities to individual mycobacterial strains, where the maximum value indicates the most susceptible strain. For example, values of k_2 after 21 days of incubation indicate that compounds **1g**, **2e**, **2f**, **2g**, and **2i** exhibit a better efficacy against *M. kansasii* than against *M. tuberculosis* and *M. avium*. If two strains are equally susceptible, the respective cosine coefficients have the same value, which is positive if the other strain is less susceptible, or negative if the other strain is more susceptible. It is worth noting that the sum of all but one cosine coefficient k_s taken with the opposite sign is equal to the remaining cosine coeficient k_s.

Salicylanilides **1** and 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones **2** were also assessed for their ability to inhibit the growth of other microoganisms using the agar diffusion method, specified in DAB 9, at 100 μg/ml (ref.[22]) (Tables VII and VIII). Information about the influence of the penetrability of the compounds into the bacterial cell on their antibacterial activity is obtained by a comparison of the activity of "wild" type strain *E. coli* DC 0 and *P. aeruginosa* K799/WT with their penetration mutants DC 2 and K799/61 in antimicrobial assays. Stronger inhibition of the penetration mutants means hindered penetrability. A small difference between the inhibition of the penetration mutant and the "wild" type indicates good penetrability.

TABLE VIII
Antimicrobial activity of 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones **2**

Microorganism	Inhibition zone, mm											
	2a	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**	**2h**	**2i**	**2j**	**2k**	**2l**
S. aureus SG 511	0	0	A	0	14*P*	18.5	22	16	0	14	17	0
M. smegmatis SG 987	0	0	17*P*	0	16*P*	16	16*p*	18*P*	0	18*P*	17	0

A, Indication of inhibition; *p*, colonies in the inhibition zone; *P*, a number of colonies in the inhibition zone.

TABLE IX

QSAR for salicylanilides **1**. MICs after 14 days (1) or 21 days (2) of incubation;

$$\log 1/\mathrm{MIC}(\textit{Mycobacterium}) = a\,\sigma + b\,\pi + c \quad \text{(a)},$$

$$\log 1/\mathrm{MIC}(\textit{Mycobacterium}) = a\,\sigma + b \log P + c \quad \text{(b)}$$

Mycobacterium	Relationship	*a*	*b*	*c*	*r*	*s*	*F*	*n*	Eq.
M. tuberculosis My 331/88	1a	0.840 ±0.082	0.212 ±0.070	–1.732 ±0.045	0.970	0.119	73.37	12	(*13*)
	1b	0.886 ±0.072	0.245 ±0.099	–2.459 ±0.328	0.962	0.134	56.10	12	(*14*)
	2a	0.757 ±0.074	0.198 ±0.063	–1.762 ±0.04	0.971	0.107	73.14	12	(*15*)
	2b	0.800 ±0.081	0.230 ±0.096	–2.445 ±0.318	0.962	0.121	56.38	12	(*16*)
M. kansasii My 235/80	1a	0.655 ±0.149	0.670 ±0.127	–2.017 ±0.081	0.939	0.215	33.46	12	(*17*)
	1b	0.783 ±0.132	0.927 ±0.158	–4.809 ±0.519	0.949	0.197	40.62	12	(*18*)
	2a	1.226 ±0.244	0.457 ±0.149	–2.181 ±0.106	0.937	0.226	25.04	10	(*19*)
	2b	1.230 ±0.263	0.573 ±0.213	–3.853 ±0.697	0.927	0.243	21.26	10	(*20*)
M. kansasii 6509/96	1a	0.907 ±0.157	0.507 ±0.101	–1.891 ±0.077	0.946	0.169	34.23	11	(*21*)
	1b	0.886 ±0.170	0.666 ±0.146	–3.842 ±0.479	0.937	0.182	28.96	11	(*22*)
	2a	0.965 ±0.184	0.562 ±0.118	–2.041 ±0.090	0.938	0.198	29.23	11	(*23*)
	2b	0.949 ±0.218	0.703 ±0.187	–4.091 ±0.614	0.913	0.233	19.92	11	(*24*)

TABLE IX
(*Continued*)

Mycobacterium	Relationship	*a*	*b*	*c*	*r*	*s*	*F*	*n*	Eq.
M. avium My 330/88	1a	0.300 ±0.084	0.224 ±0.071	–1.745 ±0.045	0.885	0.12	16.19	12	(*25*)
	1b	0.346 ±0.087	0.280 ±0.104	–2.580 ±0.341	0.865	0.129	13.40	12	(*26*)
	2a	0.492 ±0.100	0.225 ±0.082	–1.936 ±0.051	0.935	0.124	27.79	11	(*27*)
	2b	0.549 ±0.097	0.278 ±0.112	–2.762 ±0.369	0.929	0.13	25.10	11	(*28*)
Complex criteria S_0[a]	1a	1.033 ±0.116	0.633 ±0.099	2.034 ±0.063	0.975	0.167	84.89	12	(*29*)
	1b	1.160 ±0.125	0.830 ±0.149	–0.455 ±0.492	0.968	0.187	67.00	12	(*30*)
	2a	1.386 ±0.225	0.517 ±0.138	1.815 ±0.098	0.956	0.209	37.47	10	(*31*)
	2b	1.390 ±0.251	0.649 ±0.203	–0.078 ±0.664	0.946	0.231	29.96	10	(*32*)

The values following ± sign are standard errors. [a] See Table V.

In contrast to salicylanilides **1**, 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones **2** were essentially inactive in the same antimicrobial assays. Only two out of fourteen strains, namely *Staphylococcus aureus* SG 511 and, as expected, *M. smegmatis* SG 987, were susceptible to compounds **2c**, **2e**–**2h**, **2j**, and **2k** (Table VIII). Moreover, compounds **2g** and **2k** inhibited the growth of *B. subtilis* ATCC 6633 (inhibition zone 12 mm in diameter with a number of colonies) and the mutant strain of *E. coli* DC 2 gave an indication of inhibition by the same compounds (inhibition zone of 13.5 mm). As iron depletion is one of the defense mechanisms found in the infected host, growth inhibition screening has also been performed against *Mycobacterium smegmatis* SG 987 cultured in the assay medium supplemented with 2,2′-bis(2-hydroxyphenyl)-2,2′-(ethylenediimino)diacetic acid (EDDHA, 50 μmol/l) as an iron chelator. Interestingly, some compounds, *e.g.* **1c**, **1e**, and **1k**, as well as **2g**, **2h**, and **2k**, showed increased activity under these conditions.

TABLE X

QSAR for 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones **2**. MICs after 14 days (1) or 21 days (2) of incubation;

$$\log 1/\mathrm{MIC}(\textit{Mycobacterium}) = a\,\sigma + b\,\pi + c \quad \text{(a)},$$

$$\log 1/\mathrm{MIC}(\textit{Mycobacterium}) = a\,\sigma + b \log P + c \quad \text{(b)}$$

Mycobacterium	Relationship	*a*	*b*	*c*	*r*	*s*	*F*	*n*	Eq.
M. tuberculosis My 331/88	1a	0.826 ±0.219	0.273 ±0.142	–1.873 ±0.110	0.865	0.234	10.38	10	(*33*)
	1b	0.826 ±0.235	0.313 ±0.203	–3.084 ±0.869	0.844	0.25	8.64	10	(*34*)
	2a	0.829 ±0.131	0.360 ±0.085	–2.006 ±0.066	0.952	0.141	33.80	10	(*35*)
	2b	0.820 ±0.152	0.451 ±0.132	–3.768 ±0.562	0.936	0.162	24.67	10	(*36*)
M. kansasii My 235/80	1a	0.973 ±0.186	0.567 ±0.120	–1.994 ±0.094	0.945	0.199	29.34	10	(*37*)
	1b	0.958 ±0.219	0.715 ±0.190	–4.787 ±0.811	0.924	0.233	20.38	10	(*38*)
	2a	1.041 ±0.271	0.651 ±0.176	–2.172 ±0.137	0.910	0.290	16.89	10	(*39*)
	2b	1.018 ±0.286	0.850 ±0.247	–5.505 ±1.057	0.901	0.304	15.01	10	(*40*)
M. kansasii 6509/96	1a	0.962 ±0.201	0.500 ±0.13	–1.839 ±0.101	0.929	0.216	22.17	10	(*41*)
	1b	0.943 ±0.211	0.658 ±0.183	–4.418 ±0.781	0.923	0.225	20.11	10	(*42*)
	2a	0.998 ±0.224	0.489 ±0.146	–1.992 ±0.113	0.916	0.240	18.31	10	(*43*)
	2b	0.984 ±0.245	0.619 ±0.212	–4.411 ±0.907	0.900	0.261	14.96	10	(*44*)
Complex criteria S_0[a]	1a	1.322 ±0.229	0.553 ±0.148	2.014 ±0.115	0.942	0.245	27.74	10	(*45*)
	1b	1.318 ±0.276	0.655 ±0.240	–0.532 ±1.025	0.915	0.295	18.08	10	(*46*)
	2a	1.295 ±0.242	0.702 ±0.157	1.726 ±0.122	0.944	0.259	28.75	10	(*47*)
	2b	1.284 ±0.297	0.860 ±0.258	–1.627 ±1.101	0.916	0.316	18.15	10	(*48*)

The values following ± sign are standard errors. [a] See Table V.

For the quantitative evaluation of the structure–antimycobacterial activity relationships, two-parameter regression equations were employed. The Hammett constant σ of the substituent on the phenyl ring served as one parameter, while the second parameter was the hydrophobic constant π of the same substituent or the calculated log *P* value. Only those equations for salicylanilides **1** and 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones **2** that are statistically significant at the 95% level or better are listed in Tables IX and X, respectively. No significant correlation could be found for activity of 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones **2** against *M. avium*. Regardless of that, highly significant correlation equations were obtained between the same parameters and the complex criteria S_0 which are linear combinations of three antimycobacterial activities not only for the salicylanilides **1** (Eqs (*29*)–(*32*)) but also for 3-phenyl-2*H*-1,3-benz- oxazine-2,4(3*H*)-diones **2** (Eqs (*45*)–(*48*)). The antimycobacterial activities of both groups of compounds, **1** and **2**, increase with increasing electron-withdrawing properties and with increasing hydrophobicity of the substituents on the phenyl ring (relationships (a)) or calculated log *P* (relationships (b)). Since the relationships (a) have higher correlation coefficients, they are considered to be more significant than the relationships (b). The structure–antimycobacterial activity relationships are similar not only in both groups of compounds, **1** and **2**, but the Eqs (*33*) and (*37*) are also consistent[30] with the relationships for the 6,8-dihalogeno-3-phenyl- 2*H*-1,3-benzoxazine-2,4(3*H*)-diones[4b]. The discrepancy arising from the comparison of the previous results[4b,5] can be explained by the fact that the MICs obtained for different mycobacterial strains (and in different laboratories) were compared.

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30. Instead of log MIC such as in refs[4b,5], log 1/MIC values are analyzed in this paper, which affects the sign of regression coefficients.