NEW GROUPS OF POTENTIAL ANTITUBERCULOTICS: 3-ARYL-2*H*,4*H*-BENZ[*e*][1,3]OXAZINE-2,4-DIONES. COMPARISON OF THE TOPLISS APPROACH WITH REGRESSION ANALYSIS*

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Dedicated to the pioneer of QSAR, Professor R. Zahradník, on the occasion of his 65th birthday.

3-Phenyl-2*H*,4*H*-benz[*e*][1,3]oxazine-2,4-dione (*I*) and its derivatives *II* – *XI*, substituted on the phenyl ring, can be regarded as a new group of potential antituberculotics. Their activity increases with increasing electron-accepting properties of the substituents. Introduction of bromine into the position 6 also positively influences the activity. The compounds are active in vitro against *Mycobacterium tuberculosis* and *M. kansasii*. The activity of some of them (*VIII*, *IX*) exceeds that of commercial tuberculostatics used as standards.

The return of tuberculosis, caused by an overall immunity decrease in the human population, AIDS and appearance of resistant strains of mycobacteria, has brought back the development of new tuberculostatics into the laboratories of leading pharmaceutical companies[1 – 3]. This communication reports on a novel group of antituberculostatics, 3-phenyl-2*H*,4*H*-benz[*e*][1,3]oxazine-2,4-dione (*I*) and its substitution derivatives *II* – *XI*. The compounds were modified first on the phenyl ring attached in position 3, the basic structural modifications being performed according to the method recommended by Topliss[4]. The advantage of this method is that from the order of activities of the studied compounds one can make conclusion about the effect of the dominant physicochemical parameter on the activity. Since it appeared that the electron-accepting properties of

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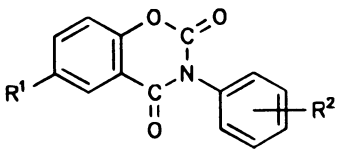
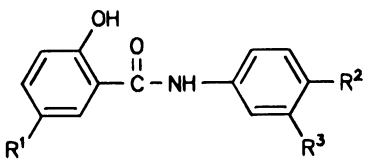
substituents have a positive influence, we enhanced this effect by introducing bromine atom into position 6 (compounds *VIII – XI*).



I – XI

For R^1, R^2 see Table I

The studied 3-aryl-2*H*,4*H*-benz[*e*][1,3]oxazine-2,3-diones (*I – XI*) were prepared by reaction of isopropyl chloroformate with the corresponding salicylanilides *XII – XXII*. The obtained 3-aryl-2*H*,4*H*-benz[*e*][1,3]oxazine-2,4-diones exhibit two characteristic ν(CO) absorption maxima in the infrared spectra.



	R^1	R^2	R^3
XII	H	H	H
XIII	H	Br	H
XIV	H	Cl	H
XV	H	Cl	Cl
XVI	H	CH_3	H
XVII	H	OCH_3	H

	R^1	R^2	R^3
XVIII	H	cyclohexyl	H
XIX	Br	H	H
XX	Br	Cl	H
XXI	Br	CH_3	H
XXII	Br	OCH_3	H

EXPERIMENTAL

The melting points were determined on a Kofler block and are uncorrected. IR spectra were recorded on a Perkin–Elmer 577 or Specord IR 75 (Carl Zeiss) instruments in KBr pellets. Samples for analysis, spectral measurements and antimycobacterial assays were dried at 61 °C and 1.5 kPa over phosphorus pentoxide for 3 days.

General Procedure for Preparation of Salicylanilides *XVIII*, *XXI* and *XXII*

A solution of phosphorus trichloride (2.2 ml, 25 mmol) in pyridine (20 ml) was added dropwise to an ice-cooled mixture of the substituted aniline (50 mmol) in anhydrous pyridine (20 ml). After stirring for 20 min, a solution of salicylic acid (6.9 g, 50 mmol) in pyridine (20 ml) was added. The mixture was stirred at 100 °C for 3 h and the pyridine was distilled off under diminished pressure. The residue was dissolved in 10% solution of sodium carbonate and the solution was adjusted to pH 8. Next day, the precipitate was filtered, washed with 10% sodium carbonate solution and water, and dried.

4'-Cyclohexylsalicylanilide (XVIII); yield 88%, m.p. 139 °C (ethanol). For $C_{19}H_{21}NO_2$ (295.4) calculated: 77.26% C, 7.17% H, 4.74% N; found: 77.44% C, 6.82% H, 4.67% N.

5-Bromo-4'-methylsalicylanilide (XXI); yield 84%, m.p. 251 – 253 °C. For $C_{14}H_{12}BrNO_2$ (306.1) calculated: 54.92% C, 3.95% H, 4.58% N; found: 54.75% C, 3.87% H, 4.53% N.

5-Bromo-4'-methoxysalicylanilide (XXII); yield 83%, m.p. 236 – 237 °C. For $C_{14}H_{12}BrNO_3$ (322.1) calculated: 52.19% C, 3.75% H, 4.35% N; found: 51.86% C, 3.64% H, 4.39% N.

The other salicylanilides were prepared according to the literature; *XII* – *XIV*, *XVI* and *XX* (ref.[5]), *XV* (ref.[6]), *XVII* (ref.[7]) and *XIX* (ref.[8]). Their melting points were in accord with the reported values[5 – 8].

General Procedure for Preparation of Compounds *VI* – *VIII*, *X* and *XI*

Isopropyl chloroformate (48 mmol) was added dropwise to a stirred solution of the salicylanilide (40 mmol) in dry pyridine (20 ml) under ice-cooling. The mixture was heated on a steam bath for 1 h and then poured into 5% hydrochloric acid (140 ml). Next day, the product was filtered, suspended in 5% potassium hydroxide solution, again filtered and crystallized from ethanol.

3-(4-Methoxyphenyl)-2H,4H-benz[e][1,3]oxazine-2,4-dione (VI); yield 42%, m.p. 207 – 208 °C. For $C_{15}H_{11}NO_4$ (269.2) calculated: 66.91% C, 4.12% H, 5.20% N; found: 66.57% C, 3.68% H, 5.13% N. IR spectrum: 1 710, 1 755 (C=O).

3-(4-Cyclohexylphenyl)-2H,4H-benz[e][1,3]oxazine-2,4-dione (VII); yield 74%, m.p. 231 °C. For $C_{20}H_{19}NO_3$ (321.4) calculated: 74.75% C, 5.96% H, 4.36% N; found: 74.93% C, 5.68% H, 4.17% N. IR spectrum: 1 690, 1 750 (C=O).

6-Bromo-3-phenyl-2H,4H-benz[e][1,3]oxazine-2,4-dione (VIII); yield 81%, m.p. 277 – 278 °C. For $C_{14}H_8BrNO_3$ (318.1) calculated: 52.86% C, 2.53% H, 4.40% N; found: 53.29% C, 2.78% H, 4.27% N. IR spectrum: 1 690, 1 760 (C=O).

6-Bromo-3-(4-methylphenyl)-2H,4H-benz[e][1,3]oxazine-2,4-dione (X); yield 93%, m.p. 252 – 253 °C. For $C_{15}H_{10}BrNO_3$ (332.1) calculated: 54.24% C, 3.03% H, 4.22% N; found: 54.19% C, 2.86% H, 4.15% N. IR spectrum: 1 702, 1 768 (C=O).

6-Bromo-3-(4-methoxyphenyl)-2H,3H-benz[e][1,3]oxazine-2,4-dione (XI); yield 53%, m.p. 219 – 221 °C. For $C_{15}H_{10}BrNO_4$ (348.1) calculated: 51.75% C, 2.90% H, 4.02% N; found: 51.70% C, 2.71% H, 4.03% N. IR spectrum: 1 700, 1 770 (C=O).

The other 3-aryl-2*H*,3*H*-benz[*e*][1,3]oxazine-2,4-diones have already been described and the substances obtained by us had melting points identical with those reported: *I* – *III*, *V* and *IX* (ref.[5]) and *IV* (ref.[9]).

Microbiological Assays

The antimycobacterial activity of the compounds against *Mycobacterium tuberculosis* $H_{37}Rv$ and *M. kansasii* PKG 8 was determined on Šula semisynthetic medium (manufactured by ÚSOL, Prague). The studied compounds were added to the medium in dimethyl sulfoxide solution; final

concentrations were 1 000, 333, 111, 37, 12.3 and 4 μmol l^{-1}. The minimum inhibitory concentrations were determined after incubation at 37 °C for 15 days and their values are given in Table I.

Calculations

The regression equations were calculated[10] using the W-6 program on a Sharp PC 1211 microcomputer. The values of Hammett constants were taken from a review of Hansch and coworkers[11].

DISCUSSION

The most active compounds of the studied series (*VIII* and *IX*) were found relatively soon on the basis of the Topliss approach. In conclusion, it seemed interesting, how the results could be analyzed using the regression analysis. As a measure of the effect of electron-accepting properties we took the Hammett constants. The regression equation (*1*), analyzing the electron-acceptor effects on the activity against *M. tuberculosis*, shows in the first group of compounds (*I* – *VI*) a surprisingly high values of statistical criteria, unusual in biological correlations.

$$\log \mathrm{MIC_{tbc}} = -1.146\,\sigma + 1.186 \qquad (1)$$

$$r = 0.991,\ s = 0.056,\ F = 213.2,\ n = 6$$

TABLE I
Antimycobacterial activity in minimum inhibitory concentration (MIC) of 3-aryl-2*H*,4*H*-benz[*e*][1,3]-oxazine-2,4-diones *I* – *XI*

Compounds	R^1	R^2	log MIC[a], μmol l^{-1}	
			M. tuberculosis	*M. kansasii*
I	H	H	74	111
II	H	4-Br	37	37
III	H	4-Cl	37	111
IV	H	3,4-Cl_2	12.3	37
V	H	4-CH_3	111	333
VI	H	4-OCH_3	111	333
VII	H	4-cyclohexyl	>1 000	>1 000
VIII	Br	H	12.3	37
IX	Br	4-Cl	12.3	12.3
X	Br	4-CH_3	37	37
XI	Br	4-OCH_3	>1 000	>1 000

[a] For comparison: 4-aminobenzoic acid (PAS) MIC_{tbc} 111 μmol l^{-1}, MIC_{kans} 1 000 μmol l^{-1}; 2-ethylisonicotinthioamide (ETA) MIC_{tbc} 37 μmol l^{-1}, MIC_{kans} 37 μmol l^{-1}.

The completely inactive compound *VII* also belongs to the compounds without bromine in position 6; however, it contains cyclohexyl which is markedly different due to its lipophilicity and therefore derivatives with this substituent may differ in their behaviour.

The same relationship was observed for the regression equation (*2*) describing an analogous relationship for *M. kansasii*.

$$\log \mathrm{MIC}_{\mathrm{kans}} = -1.420\,\sigma + 2.125 \qquad (2)$$

$$r = 0.895,\ s = 0.213,\ F = 16.08,\ n = 6$$

Somewhat more difficulties we encounter when trying to present a quantitative description that would include also the effect of bromine in position 6. When we took this effect as proportional to the Hammett σ_m constant for bromine (i.e. 0.39) and added this value to the constants for the substituents on the phenyl ring, we obtained for the active compounds significant relationships (*3*) and (*4*).

$$\log \mathrm{MIC}_{\mathrm{tbc}} = -1.221\,\sigma + 1.799 \qquad (3)$$

$$r = 0.968,\ s = 0.105,\ F = 104.85,\ n = 9$$

$$\log \mathrm{MIC}_{\mathrm{kans}} = -1.420\,\sigma + 2.125 \qquad (4)$$

$$r = 0.912,\ s = 0.212,\ F = 34.55,\ n = 9$$

Application of a similar procedure using substituent constant σ_p (0.23) for the bromine in position 6 leads to a somewhat lower statistical significance of both relationships. The similarity of the structure–antimycobacterial activity relationship for the whole group of active compounds is obvious because also the logarithms of minimum inhibitory concentrations that have been used as a measure of tuberculostatic activity correlate with each other (see Eq. (*5*)).

$$\log \mathrm{MIC}_{\mathrm{kans}} = 1.083 \log \mathrm{MIC}_{\mathrm{tbc}} + 0.156 \qquad (5)$$

$$r = 0.877,\ s = 0.248,\ F = 23.32,\ n = 9$$

Also the two inactive compounds *VII* and *XI* exhibited no activity against both the bacterial strains.

Similarly to the Topliss approach, the regression analysis shows the importance of the electron-acceptor properties of substituents. Its only drawback consists in the inability of explaining why the compound *XI* is inactive. However, since also the regression equations show that this compound should not be interesting (the methoxy group in position 4 of the phenyl is an electron donor), a closer analysis would have no value for our further research.

The most significant of the compounds studied by us is compound *IX* whose activity against both mycobacteria strains exceeds those of the commercial tuberculostatics used as standards.

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