RELATIONS BETWEEN STRUCTURE AND ANTITUBERCULOTIC ACTIVITY OF 4-ALKOXYBENZOIC ACIDS*

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Dedicated to Professor Václav Horák on the occasion of his 70th birthday.

Antimycobacterial activity of a series of alkoxybenzoic acids including 4-methoxybenzoic acid (*II*), 4-ethoxybenzoic acid (*III*), 4-propoxybenzoic acid (*IV*), 4-butoxybenzoic acid (*V*), 4-pentoxybenzoic acid (*VI*), 4-allyloxybenzoic acid (*IX*), 4-isopropoxybenzoic acid (*VII*), 4-isobutoxybenzoic acid (*VIII*), and 4-benzyloxybenzoic acid (*X*) has been determined and found to increase with the lipophilicity of the compounds expressed by the corresponding HPLC capacity factors. Also determined were the pK_a values of the compounds mentioned. The most active compound, 4-pentoxybenzoic acid (*VI*), is comparable with commercial antituberculotics when tested in vitro.

Our previous communication[1] dealt with the relations between structure and antituberculotic activity of 2-benzamidobenzothiazoles. The most active of them contained propoxy, isopropoxy, and allyloxy groups at the 4-position of the benzamido group. This fact made us presume that the mechanism of action can be connected with the alkoxybenzoic acids liberated after hydrolysis in a mycobacterial cell. Therefore we decided to examine whether alkoxybenzoic acids possess antituberculotic activity, and – in positive case – investigate the relations between their structure and antituberculotic activity. A correlation between structure and antimycobacterial activity in a group of aliphatic carboxylic acids was found by Hansch and Clayton[2]. It was a parabolic de-

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pendence between logarithm of the maximum inhibitory concentration (log MIC) and logarithm of distribution coefficient (octanol–water system), the optimum of activity being at the value $\log P_0 = 1.82$. No similar study for aromatic carboxylic acids has been published yet. The main aim of the present paper is a QSAR study focused on antituberculotic 4-alkoxybenzoic acids.

EXPERIMENTAL

Chemicals. All the alkoxybenzoic acids were prepared by alkylation of 4-hydroxybenzoic acid using the method by Jones[3], and their melting points agreed with the literature data[4]. All other chemicals inclusive of those for preparation of the buffers were of p.a. purity grade. The distilled water used as the solvent in the measurements of acid-base equilibria was rid of oxygen and carbon dioxide by bubbling nitrogen. Methanol for UV and redistilled water were used for the HPLC.

Ionization constants. The K_a ionization constants of benzoic acid (*I*) and alkoxybenzoic acids *II* – *X* were estimated spectrophotometrically by a method described in our earlier paper[5]. The pH values were measured by means of a Radiometer PHM-64 apparatus with a combined glass electrode GK-2401-B. The calibration was carried out with the help of standard buffers: phosphate (pH 6.87) and hydrogenphthalate (pH 4.01). The spectrophotometry was performed in 35 mm quartz cells of 100 ml volume. The adopted Pye–Unicam SP-1700 spectrophotometer was equipped with a thermostated cell holder (25 °C). The solutions of compounds *I* – *X* with the concentrations of $1.97 - 2.21 \cdot 10^{-5}$ mol l^{-1} were acidified to contain 0.01 mol l^{-1} HCl and titrated in the cell by adding small volumes of 1 M NaOH. Because of the limited water solubility of the compounds studied their stock solutions had to be prepared in ethanol, and the solutions titrated contained 1% (v/v) ethanol. The absorbance (*A*) of the titrated solutions was measured at selected wavelengths (see Table I), and their pH values were measured continuously. The values of mixed constants pK_a^M were calculated by the method of nonlinear regression from the experimental curves *A*–pH each of which involved a set of 20 – 25 points (in the pH region ca from 3.2 to 5.3) using the program[6]

TABLE I
Thermodynamic pK_a constants of benzoic acid (*I*) and 4-alkoxybenzoic acids *II* – *X* determined by spectrophotometry at 25 °C. Λ analytical wavelengths; *N* number of *A*–pH curves processed

Compound	Substituent	pK_a	Λ, nm	*N*
I	H	4.209 ± 0.010[a]	232	4
II	OCH_3	4.477 ± 0.015[a]	227/258	6
III	OC_2H_5	4.476 ± 0.013	230/260	8
IV	$O(CH_2)_2CH_3$	4.488 ± 0.010	227/260	6
V	$O(CH_2)_3CH_3$	4.484 ± 0.022	227/260	8
VI	$O(CH_2)_4CH_3$	4.490 ± 0.019	245	4
VII	$OCH(CH_3)_2$	4.459 ± 0.018	225/265	8
VIII	$OCH_2CH(CH_3)_2$	4.478 ± 0.013	227/265	8
IX	$OCH_2CH{=}CH_2$	4.452 ± 0.023	226/258	6
X	$OCH_2C_6H_5$	4.440 ± 0.018	270	4

[a] pK_a from ref.[8]: *I* 4.204, *II* 4.496.

CHEMSTAT for PC-AT. The pK_a^M constants were transformed into the thermodynamic constants pK_a (neglecting the effect of 1% ethanol) by introducing the correction for real ionic strength according to ref.[7]. At least four *A*–pH curves were measured for each compound, and the final pK_a values were obtained as arithmetic mean of the individual results (see Table I).

High performance liquid chromatography. The measurements were carried out on a liquid chromatograph consisting of a high pressure pump Varian 8500, a programable UV-VIS detector Hewlett–Packard HP 1050, and an integrator Hewlett–Packard HP 3394. The analysis was carried out at the wavelength of 254 nm. The measurement was performed on a chromatographical column of 250 mm length and 4 mm inner diameter packed with Silasorb SPH C_{18}, 7.5 μm (Lachema, Brno). Methanolic solutions of the compounds were injected into the column by means of six-way valve through a 3 μl loop. The mobile phase was formed by a methanol–water mixture (60 : 40 v/v) acidified with several drops of 10% perchloric acid to pH 2.7, flow rate 0.8 ml min^{-1}. The HPLC retention characteristics were measured at 20 °C. Methanolic solution of potassium iodide was used for estimation of the retention dead time. The capacity factor k' was calculated from the retention time of solute t_R and the retention dead time t_0 using Eq. (*1*):

$$k' = (t_R - t_0)/t_0 . \qquad (1)$$

The k' values found are summarized in Table II.

Microbiological evaluation. The antimycobacterial activity was tested with *Mycobacterium tuberculosis* $H_{37}Rv$ and *Mycobacterium kansasii* PKG 8, on a liquid synthetical Sauton substrate without added peptides. The compounds were added to the substrate after dissolution in dimethyl sulfoxide, the resulting concentrations were 1 000, 500, 250, 125, 62, 31, 15, and 7 μmol l^{-1}. For compound *VIII* showing a partial inhibition at 125 μmol l^{-1}, the concentration of 188 μmol l^{-1} was evaluated too. Simultaneously tested were the commercial antituberculotics ethionamide (ETH) and isoniazide (INH). The minimum inhibitory concentration (MIC) was determined after 14 days incubation at 37 °C. The results are given in Table II.

TABLE II
Antimycobacterial activities in the minimum inhibitory concentration (MIC) of 4-alkoxybenzoic acids *II* – *X*, ethionamide (ETH), and isoniazide (INH), capacity factors (k'), and hydrophobic substituent constants (π)

Compound	MIC (μmol l^{-1})		k'	π
	M. tuberculosis	*M. kansasii*		
II	–	–	2.14	–0.03[a]
III	500	1 000	3.90	0.47[a]
IV	61	125	8.16	0.97[a]
V	7	62	16.93	1.55
VI	7	7	34.86	2.07
VII	31	250	5.47	0.85[a]
VIII	188	250	15.95	1.50
IX	500	500	4.83	0.62
X	15	15	14.08	1.37[a]
ETH	15	15	–	–
INH	<7	<7	–	–

[a] Ref.[9].

QSAR calculations. All the calculations of regression equations were performed with the program[10] W-6 for a Sharp PC 1211 microcomputer.

RESULTS AND DISCUSSION

Quantitative differences in biological activity of compounds are usually ascribed to the effects of chemical and structural changes[9]. The pK_a constants can represent a suitable electronic parameter, and as the pK_a values[11] of the acids studied had not been obtained at identical conditions, we determined the necessary values experimentally ourselves. However, it turned out that the differences in pK_a values of the compounds studied were very small, being in the limits from 4.440 to 4.490. Hence the differences in mycobacterial activity of 4-alkoxybenzoic acids cannot be ascribed to them. Therefore we focused our attention to a study of hydrophobical properties.

The literature search carried out by Hansch and Lee[12] gives no values of distribution coefficients for the compounds studied. The book by Kuchař and Rejholec[9] gives five values of substituent hydrophobic constants π for *p*-alkoxy groups. We used these values for the correlation with chromatographical experimental values of logarithm of capacity factors (k') in Eq. (*2*). For evaluation of hydrophobic properties we used HPLC on an octadecylsilanized silica gel column, which is a frequently adopted method at present[13].

In the compound series studied the values of capacity factors correlate with the hydrophobic substituent constants π according to Eq. (*2*).

$$\pi = 1.687 \log k' - 0.529 , \qquad (2)$$

$$r = 0.990 \quad s = 0.09 \quad F = 141.46 \quad n = 5 .$$

Using Eq. (*2*) we calculated the values of hydrophobic substituent constants of the remaining substituents (see Table II).

The antimycobacterial activities of the compounds against *Mycobacterium tuberculosis* and *M. kansasii* mutually correlate according to Eq. (*3*).

$$\log \mathrm{MIC}_{\mathrm{kans}} = 0.9098 \log \mathrm{MIC}_{\mathrm{tbc}} + 0.5163 , \qquad (3)$$

$$r = 0.887 \quad s = 0.38 \quad F = 22.06 \quad n = 8 .$$

From Eq. (*3*) it can be deduced that the relations between structure and antimycobacterial activity will be similar in both groups of the activities investigated.

The relations between logarithm of capacity factors and logarithm of the minimum inhibitory concentrations against *Mycobacterium tuberculosis* and *Mycobacterium kansasii* are expressed in Eqs (*4*) and (*5*).

$$\log \mathrm{MIC}_{\mathrm{tbc}} = -2.212 \log k' + 3.950 , \qquad (4)$$

$$r = 0.947 \quad s = 0.265 \quad F = 52.46 \quad n = 8 ;$$

$$\log \mathrm{MIC}_{\mathrm{kans}} = -2.076 \log k' + 4.173 , \qquad (5)$$

$$r = 0.866 \quad s = 0.424 \quad F = 18.01 \quad n = 8 .$$

Similar dependences are also described by Eqs (*6*) and (*7*) using the hydrophobic substituent constants π instead of logarithm of capacity factors.

$$\log \mathrm{MIC}_{\mathrm{tbc}} = -1.334\pi + 3.249 , \qquad (6)$$

$$r = 0.940 \quad s = 0.283 \quad F = 45.29 \quad n = 8 ;$$

$$\log \mathrm{MIC}_{\mathrm{kans}} = -1.254\pi + 3.560 , \qquad (7)$$

$$r = 0.860 \quad s = 0.432 \quad F = 17.14 \quad n = 8 .$$

The study unambiguously proves that the antimycobacterial activity increases with lipophilicity in the series investigated. The statistical significance of the relations correlating the antimycobacterial activity with hydrophobic and structural parameters is lower for *M. kansasii* than for *M. tuberculosis*. Obviously another so far unknown factor makes itself felt in this case. Nevertheless the lipophilicity plays a dominant role in this case too.

The activity of 4-pentoxybenzoic acid (*VI*) is very high and comparable with that of commercial antituberculotics when tested in vitro on the Sauton substrate without peptides. The calculated value of logarithm of distribution coefficient of this compound (log *P* of benzoic acid + π of pentoxy group) is close to 4. However, this is a too high value for an activity in vivo to be expected[14]. Nevertheless, the study indicates that new antimycobacterial disinfectants could possibly be developed on a similar basis. The above-mentioned restriction is connected with transport factors.

When the results of the present study are compared with the analysis carried out earlier by Hansch and Clayton[2] in a series of aliphatic carboxylic acids, it can be seen that, although both the series exhibit a correlation between antimycobacterial activity and lipophilicity, the activity optima lie in different regions of values of logarithm of distribution coefficient.

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