QUANTITATIVE RELATIONS BETWEEN CHEMICAL STRUCTURE AND HEPATOTOXICITY OF THIOBENZAMIDES*

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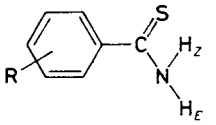
Dedicated to Dr Karel Bláha to commemorate his credit for introduction of the QSAR methods in Czechoslovakia.

^{1}H NMR chemical shifts of thioamide protons have been determined for a group of thiobenzamides, and the values obtained have been correlated with the Hammett constants. From the relations found the σ_m and σ_p values of thioamide group and some other σ constants describing the total effect of two substituents in the phenyl group have been calculated. The relation between the hepatotoxicity for rats (expressed as log ALT) and the Hammett constants is described by the equation of parabola.

Studies of quantitative relations between chemical structure and biological activity belong to standard methods of bioorganic chemistry[1]. Thiobenzamides were investigated in a number of laboratories as potential antituberculotics. Most reports are quoted in one of our previous communications[2]. The herbicidal activity of 2,6-dichlorothiobenzamide is generally known, the compound is used under the name Chlorthiamid. Much effort was also spent on studies of organ toxicity of thiobenzamides (see the survey[3]). The hepatotoxicity of thiobenzamides seems to be correlatable with the Hammett constants of the substituents present therein[4]. The aim of this present communication is to verify this relation in a group of the thiobenzamides *I*–*XX* having substituents in the aromatic ring. The group investigated



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also comprises thioterephthalamide (*IX*) and thioisophthalamide (*VIII*), hence it was necessary to determine the σ_m and σ_p values of the thioamide group. Similarly, it was also necessary to express the substituent effects in the disubstituted derivatives *IV*, *XII*, and *XIII*. These electronic parameters can be calculated from the correlation of chemical shifts of the protons of thioamide group (^{1}H NMR spectra) with the Hammett constants. The most suitable method for investigation of hepatotoxicity probably consists in the measurement of activity of the serum alaninaminotransferase (ALT), which has already been shown for thiobenzamides by Hanzlik et al.[5]. A damage of liver cells by the compounds investigated causes a transition of alaninaminotransferase into blood, hence the activity of this enzyme in the blood is increased.

EXPERIMENTAL

Materials. Preparation of compounds *I*, *III*, *V*, *VI*, *X*–*XII*, *XIV*–*XVII*, *IXX*, *XX* is described elsewhere[6–8]. For this present study the following compounds were prepared: 3-bromothiobenzamide[9] (*II*), 3,5-dibromothiobenzamide (*IV*), 3-methoxythiobenzamide (*XVIII*), 4-trifluoromethylthiobenzamide[10] (*VII*), thioisophthalamide[11] (*VIII*), thioterephthalamide[11] (*IX*), 3,5-dichlorothiobenzamide[12] (*XIII*). The preparations were accomplished by introduction of pre-dried hydrogen sulphide into solutions of the respective nitriles in a 1 : 1 pyridine–triethylamine mixture at room temperature for 6 hours to 2 days (TLC test). The reaction mixtures were poured onto ice and the products precipitated were purified by crystallization. All the thiobenzamides exhibited the melting points identical with those published in literature, the only exceptions being thioisophthalamide (*VIII*), m.p. 212°C (ref.[11] 200°C), and thioterephthalamide (*IX*), m.p. 265°C (ref.[11] 263°C). 4-Trifluoromethylthiobenzamide (*VII*) melted at 136°C, verification with ref.[10] was not possible. 3-Methoxythiobenzamide (*XVIII*), yield 65%, m.p. 100–101°C, for C_8H_9NOS (167·22) calculated: 57·46% C, 5·43% H, 8·38% N, 19·17% S; found: 57·13% C, 5·31% H, 8·03% N, 19·38% S. 3,5-Dibromothiobenzamide (*IV*), yield 89%, m.p. 106–107°C, for $C_7H_5Br_2NS$ (294·997) calculated: 28·50% C, 1·71% H, 54·17% Br, 4·75% N, 10·87% S; found: 28·63% C, 1·86% H, 54·28% Br, 4·66% N, 10·41% S.

^{1}H NMR Spectra. All the spectra were measured with a Tesla BS 497 (100 MHz) apparatus in dimethyl sulphoxide at 21°C. The δ(ppm) values obtained with tetramethylsilane as the internal standard are presented in Table I.

Evaluation of Hepatotoxicity. The experiments were carried out with rat males of the Wistar strain (from the breeding station of Pharmaceutical Faculty), weight 180–250 g. The animals were kept in cages (8 to 10 in each) and fed with the standard pelletized diet (product of Velaz n.p.) before and after the experiment. The water intake ad libitum. The substances were applied i.p. after dissolution in a 60 : 25 : 15 triethyleneglycol–water–ethanol mixture (the doses of thiobenzamides 0·72 mmol/kg, 4 ml/kg, those of thioisophthalamide and thioterephthalamide 0·36 mmol/kg, 4 ml/kg). Due to low solubility, thioterephthalamide (*IX*) was applied in suspension. The hepatotoxicity could not be determined at the conditions given with the compounds *XIV*, *XV*, and *XIX* due to perishing of the experimental animals. The animals were killed by decapitation 24 hours after application of the substance tested (or the solvent), and blood samples were taken from aorta carotis. The serum was obtained after 20–30 min standing by means of centrifugation, and the activity of alaninaminotransferase was determined therein (Bio-La test according to Reitmann and Frankel[13] in the modification by Ševela[14]). The activity of alaninaminotransferase was determined in μcat/l, and its logarithms (log ALT) are given in Table I.

Calculations. All the calculations were carried out with the SHARP PC 1211 microcomputer. The W-6 program[15] was used for the calculation of regression coefficients. The values of the Hammett constants were taken from the book by Hansch and Leo[16].

RESULTS AND DISCUSSION

The H_E and H_Z proton signals were assigned according to the study by Walter et al.[17]. The correlation equations (*1*)–(*2*) were found for a group of 15 mono-substituted thiobenzamides.

$$\sigma = 1{\cdot}457\delta_Z - 14{\cdot}187 \quad r = 0{\cdot}980 \quad n = 15 \quad F = 318{\cdot}94$$
$$s = 0{\cdot}09 \qquad (1)$$

TABLE I

The Hammett substituent constants (σ), the proton chemical shifts (δ_E and δ_Z) of thioamide group, and logarithm of the activity of serum alaninaminotransferase (log ALT) after application of the thiobenzamides

Compound	Substituent	σ	δ_E	δ_Z	log ALT
I	H	0	9·46	8·82	0·55
II	3-Br	0·39	9·59	9·98	−0·14
III	4-Br	0·23	9·54	9·91	−0·33
IV	3,5-Br_2	0·53[a]	9·69	10·11	0·77
V	3-CH_3	−0·07	9·40	9·76	1·20
VI	4-CH_3	−0·17	9·38	9·71	1·19
VII	4-CF_3	0·54	9·70	10·09	0·40
VIII	3-$CSNH_2$	0·25[a]	9·53	9·93	−0·17
IX	4-$CSNH_2$	0·30[a]	9·57	9·95	−0·01
X	3-Cl	0·37	9·60	9·98	−0·15
XI	4-Cl	0·23	9·53	9·90	−0·12
XII	3,4-Cl_2	0·45[a]	9·65	10·06	−0·06
XIII	3,5-Cl_2	0·54[a]	9·69	10·13	0·78
XIV	4-NH_2	−0·66	8·93	9·14	—
XV	4-$N(CH_3)_2$	−0·83	9·02	9·20	—
XVI	3-NO_2	0·71	9·84	10·17	0·40
XVII	4-NO_2	0·78	9·80	10·19	1·03
XVIII	3-OCH_3	0·12	9·45	9·82	0·48
XIX	4-OCH_3	−0·27	9·29	9·58	—
XX	4-SCH_3	0	9·40	9·72	0·45

[a] This communication.

$$\sigma = 1{\cdot}786\delta_E - 16{\cdot}811 \quad r = 0{\cdot}982 \quad n = 15 \quad F = 353{\cdot}97$$
$$s = 0{\cdot}09 \qquad (2)$$

Using Eqs (*1*) and (*2*) and the chemical shifts of the thioamide protons in thioisophthalamide (*VIII*) and thioterephthalamide (*IX*) we calculated the values of the Hammett constants for the thioamide group. Table I presents average values from both the determinations. A similar procedure was adopted to evaluate the effects of substituents in the aromatic ring on the thioamide group. It was somewhat surprising to find that for the Hammett constants of thioamide group σ_m has a lower value, which fact was confirmed by repeated measurements.

The compounds *VIII* and *IX* with two thioamide groups were applied to the experimental animals at lower concentrations (one half as compared with that of the other thiobenzamides, see Experimental). We expected to obtain, in this way, an equivalent effect at molecular level, because we supposed that the compounds mentioned can react with both their thioamide groups. The dependence of hepatotoxicity (expressed by the logarithm of the activity of serum alaninaminotransferase) on the Hammett constants is given by Eq. (*3*). A similar correlation also applies to the values of the chemical shifts of the protons of thioamide groups (Eq. (*4*) for the shifts in Hz).

$$\log \text{ALT} = 5{\cdot}377\sigma^2 - 3{\cdot}487\sigma + 5{\cdot}558 \qquad (3)$$
$$r = 0{\cdot}816 \quad s = 0{\cdot}30 \quad F = 13{\cdot}97 \quad n = 17$$

$$\log \text{ALT} = 19{\cdot}79\delta_Z^2 - 398{\cdot}17\delta_Z + 1962{\cdot}27 \qquad (4)$$
$$r = 0{\cdot}825 \quad s = 0{\cdot}30 \quad F = 14{\cdot}87 \quad n = 17$$

The statistical evaluation is acceptable with respect to the fact that the works with experimental animals always are loaded with a larger scattering of experimental values and that the method of equiconcentration activity values was used. Hence, it can be stated that for the compounds tested the dependence between hepatotoxicity and the Hammett constants can be expressed by a parabola in the interval examined. (The dependence on other electronic parameters, e.g. the chemical shifts values, will be similar, too, see Eq. (*4*)). Parabolic dependences between biological activities and electronic parameters are rare, nevertheless, some of them have already been described and were summarized by Franke[18]. Thus the study has shown that neither very low nor very high values of the Hammet constants are favourable, and any suggestions of structures of potential therapeutics or agrochemicals should be focused on the compounds lying near the minimum of the parabole (σ 0·325) or in the interval $0{\cdot}2 < \sigma < 0{\cdot}4$ where it is possible to expect the compounds of lowest hepatotoxicity.

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