CORRELATION OF STRUCTURAL PARAMETERS WITH ANTITUBERCULOTIC ACTIVITY IN A GROUP OF 2-BENZAMIDOBENZOTHIAZOLES*

Karel WAISSER[a], Jiří KUNEŠ[a] and Želmíra ODLEROVÁ[b]

[a] *Department of Inorganic and Organic Chemistry, Pharmaceutical Faculty, Charles University, 501 65 Hradec Králové*

[b] *Research Institute of Preventive and Clinical Medicine, 833 01 Bratislava*

Received February 12, 1991
Accepted March 14, 1991

Dedicated to Professor Alois Vystrčil on the occasion of his 70th birthday.

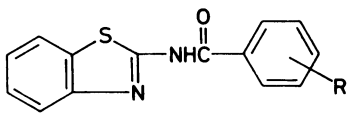
Fourteen 2-benzamidobenzothiazole derivatives substituted in the benzoyl group and 2-cinnamamidobenzothiazole have been synthetized. The prediction of active structures by the method by Topliss gives incorrect results but, as shown on the basis of the Hansch QSAR analysis, the 2-(4-dimethylaminobenzamido)benzothiazole synthetized on the basis of the QSAR procedure by Topliss behaves anomalously also in the procedure by Hansch. Regression equations of the Hansch type have been found in which the antituberculotic activity is interpreted by lipophilicity and electronic effects of substituents. When tested in vitro, the most active compounds are comparable with ethionamide.

The first antituberculotic derivatives of benzothiazole were prepared as early as in the forties[1,2], nevertheless, this group of compounds has been continuously investigated by some teams developing antituberculotics until now. Within the last 45 years more than 1 000 antituberculotic compounds were synthesized, some of which being protected by patents[3]. Among the most thoroughly investigated antimycobacterial derivatives of benzothiazole are 2-alkylthiobenzothiazoles. In contrast to communications of other workers we have focused our attention on 2-benzamidobenzothiazoles whose antituberculotic derivatives are dealt with in 1 report only[4].

The aim of the present communication was to prepare 2-benzamidobenzothiazoles substituted in the benzoyl group (*I*–*XIV*) and 2-cinnamamidobenzothiazole (*XV*) and to study their antimycobacterial activity. The selection of variable substituents followed the procedure by Topliss[5], i.e. in the first plan we wanted to prepare a group involving 2-benzamidobenzothiazole (*I*), 2-(4-methylbenzamido)benzothiazole (*II*), 2-(4-methoxybenzamido)benzothiazole (*III*), 2-(4-chlorobenzamido)-

* Part LIX in the series On Antituberculotics; Part LVIII: Collect. Czech. Chem. Commun *56*, 2395 (1991).

benzothiazole (*IV*), and 2-(3-bromobenzamido)benzothiazole (*V*). The last compound mentioned was used instead of the 3,4-dichlorobenzamido derivative recommended by Topliss. According to the ideas by Topliss, the mere activity order in such a group should provide an estimate of functional dependence between structure and biological (in our case antituberculotic) activity. The results should serve for a prediction of other active structures.



I, R = H
II, R = 4-CH_3
III, R = 4-OCH_3
IV, R = 4-Cl
V, R = 3-Br
VI, R = 4-$N(CH_3)_2$
VII, R = 4-OC_2H_5
VIII, R = 4-OC_3H_7
IX, R = 4-$OCH(CH_3)_2$
X, R = 4-$OCH_2CH{=}CH_2$
XI, R = 4-OC_4H_9
XII, R = 4-$OCH_2CH(CH_3)_2$
XIII, R = 4-OC_5H_{11}
XIV, R = 4-$OCH_2C_6H_5$

EXPERIMENTAL

Chemicals

All the substances studied were prepared by acylation of 2-aminobenzothiazole with the corresponding acyl chlorides: The equivalent amount of the liquid chloride (or pyridine solution of the equivalent amount of solid acyl chloride) was added portionwise to a solution of 0·02 mol 2-aminobenzothiazole in 5 ml pyridine with stirring and cooling. After 12 h standing the reaction mixture was treated with saturated solution of sodium carbonate, the separated product was collected by suction and several times recrystallized from hot ethanol. The samples for analysis and further tests were dried over phosphorus pentoxide at 1·5 kPa at 61°C for 3 days. 2-Benzamidobenzothiazole (*I*) 98%, m.p. 187—188°C (ref.[6] gives m.p. 187°C); 2-cinnamamidobenzothiazole (*XV*), 92%, m.p. 246°C (ref.[7] gives m.p. 245—246°C). The properties of the newly prepared compounds *II*—*XIV* are given in Table I.

Physical Measurements

The ^{1}H NMR spectra for verification of the structures were measured with a Tesla BS 497 (100 MHz) apparatus in deuteriochloroform. Dimethyl sulfoxide was used as the solvent for obtaining the values of chemical shift of amidic proton as the electronic parameter in the QSAR relations. Table II presents the δ(ppm) values related to tetramethylsilane. The IR spectra for verification of the structures were measured with a Perkin–Elmer 577 apparatus using the KBr disc procedure. All the substances exhibited the characteristic vibrations of amides, viz. ν(C=O) in the region of 1 650—1 682 cm^{-1}, ν(N—H) in the region of 3 370—3 470 cm^{-1}, and δ(N—H) in the region of 1 525—1 565 cm^{-1}.

Thin-Layer Partition Chromatography of Compounds *I*—*V*

The measurements were carried out on Silufol UV 254 (Kavalier, Votice; silica gel with starch binder and UV indicator) impregnated with silicone oil Lukoil M 100. The impregnation was carried out in the ascending manner using a 5% Lukoil solution in ether in a closed vessel over-

TABLE I

Yields, melting points, and elemental analyses of 2-benzamidobenzothiazoles *II*—*XIV*

Compound	Yield %	M.p. °C	Formula (M.w.)	Calculated/Found			
				H C	% H	% N	% S
II	70	189	$C_{15}H_{12}N_2OS$	67·14	4·51	10·44	11·95
			(268·3)	67·43	4·61	10·44	12·00
III	71	197	$C_{15}H_{12}N_2O_2S$	63·36	4·54	9·85	11·28
			(284·4)	62·85	4·06	9·69	11·36
IV[a]	78	225	$C_{14}H_9ClN_2OS$	58·23	3·14	9·70	11·10
			(288·8)	58·23	3·22	9·73	11·11
V[b]	81	174	$C_{14}H_9BrN_2OS$	50·47	2·72	8·41	9·62
			(333·2)	50·38	2·95	8·13	9·86
VI	71	211	$C_{15}H_{15}N_3OS$	64·62	5·10	14·13	10·78
			(297·4)	64·71	5·18	14·46	10·35
VII	90	159·5	$C_{16}H_{14}H_2O_2S$	64·41	4·73	9·39	10·75
			(298·4)	64·34	4·64	9·36	10·78
VIII	82	155·5	$C_{17}H_{16}N_2O_2S$	65·36	5·16	8·97	10·26
			(312·4)	65·42	5·11	8·96	10·25
IX	67	139·5	$C_{17}H_{16}N_2O_2S$	65·36	5·16	8·97	10·26
			(312·4)	65·24	5·30	8·77	10·20
X	82	166·5	$C_{17}H_{14}N_2O_2S$	65·79	4·55	9·03	10·33
			(310·4)	65·65	4·49	9·01	10·46
XI	81	147	$C_{18}H_{18}N_2O_2S$	66·23	5·56	8·58	9·82
			(326·4)	66·07	5·45	8·56	9·78
XII	71	133	$C_{18}H_{18}N_2O_2S$	66·23	5·56	8·58	9·82
			(326·4)	66·22	5·37	8·71	9·90
XIII	71	122	$C_{19}H_{20}H_2O_2S$	67·03	5·92	8·23	9·42
			(340·4)	67·16	5·80	8·17	9·30
XIV	86	150	$C_{21}H_{16}N_2O_2S$	69·98	4·48	7·77	8·90
			(360·4)	70·17	4·42	7·20	8·67

[a] Calculated 12·28% Cl, found 12·48% Cl; [b] calculated 23·98% Br, found 24·29% Br.

night. After 12 h drying in the air the impregnation was repeated from the opposite side. The samples for chromatography were dissolved in chloroform. The elution was carried out with aqueous methanol (45, 50, 55, 60, 65, and 70%). Each substance was measured six times at each of the methanol concentrations mentioned. The results are presented in Table III along with the values extrapolated to 0% methanol in the mobile phase.

TABLE II

Parametrization of physico-chemical properties: the proton chemical shift (δ, ppm) of amide group, the Hammett substituent constants (σ), the hydrophobic substituent constants (π^-)

Compound	Substituent	δ	σ	π^-
I	H	12·90	0	0
II	4-CH_3	12·81	−0·17	0·48
III	4-OCH_3	12·73	−0·27	−0·12
IV	4-Cl	12·99	0·23	0·93
V	3-Br	13·03	0·39	1·17
VI	4-$N(CH_3)_2$	12·44	−0·83	−0·69
VII	4-OC_2H_5	12·70	−0·24	0·35
VIII	4-OC_3H_7	12·69	−0·25	0·85
IX	4-$OCH(CH_3)_2$	12·70	−0·45	0·73
X	4-$OCH_2CH{=}CH_2$	12·70	—	0·30
XI	4-OC_4H_9	12·68	−0·32	1·39
XII	4-$OCH_2CH(CH_3)_2$	12·70	−0·36	1·26
XIII	4-OC_5H_{11}	12·68	−0·34	1·98
XIV	4-$OCH_2C_6H_5$	12·74	−0·42	1·56

TABLE III

R_M values of partition TLC in various concentrations of methanol in water (vol. %), the R_M values extrapolated to 0% methanol

Compound	$R_M(70)$	$R_M(65)$	$R_M(60)$	$R_M(55)$	$R_M(50)$	$R_M(45)$	$R_M(0)$
I	0·066	0·207	0·286	0·514	0·642	1·027	2·540
II	0·052	0·253	0·418	0·643	0·678	1·196	2·912
III	−0·052	0·160	0·344	0·525	0·596	1·042	2·722
IV	0·061	0·300	0·478	0·702	0·813	1·169	2·987
V	0·095	0·333	0·543	0·806	0·839	1·483	3·549

Microbiological Evaluation

The antimycobacterial activity of the substances was determined on a liquid semisynthetical substrate by Šula (ÚSOL, Prague). The substances tested were added to the substrate after dissolution in dimethyl sulfoxide, the resulting concentrations being (in $\mu mol\ l^{-1}$): 1 000, 333, 111, 37, 12·3, 3·1, 1 (and exceptionally also 25). The minimum inhibition concentrations (MIC) were determined after 15 days incubation at 37°C. The antimycobacterial activity was determined with application of *Mycobacterium tuberculosis* $H_{37}Rv$ and *Mycobacterium kansasii* PKG8, the results are presented in Table IV.

Calculations

The values of substituent constants for the calculations were taken mainly from the book[8]. The π constant data were complemented in some cases on the basis of the values used in our laboratory for expressing the lipophilicity of alkyl groups[9]. The monoparameter regression equations were calculated on a microcomputer Sharp PC 1 211 using the W-6 program[10]. The multiparameter equations were calculated with an IQ-151 computer using the Multireg-H program.

TABLE IV

Antimycobacterial activity in minimum inhibitory concentration of 2-benzamidobenzothiazoles *I*—*XIV*, 2-cinnamamidobenzothiazole (*XV*), isonicotinohydrazide (INH), 2-ethylisonicotine thioamide (ETH)

Compound	MIC, μmol/l	
	M. tuberculosis	*M. kansasii*
I	1 000	1 000
II	111	111
III	25	111
IV	>1 000	>1 000
V	>1 000	>1 000
VI	>1 000	>1 000
VII	37	333
VIII	12·3	111
IX	12·3	111
X	12·3	333
XI	111	111
XII	37	111
XIII	1 000	1 000
XIV	111	333
XV	>1 000	>1 000
INH	4	111
ETA	12·3	37

DISCUSSION

From the comparison of the activity order of the first group of 5 synthetized compounds (i.e. *I*–*V*) with the table of characteristical order of physical properties of substituted phenyl groups in the study[5] by Topliss it follows that the activity could be a function of the Hammett constants and could increase with their decreasing values. Surprisingly, the dimethylamino derivative *VI*, which was synthesized on the basis of the prognosis by the Topliss approach, was completely inefficient.

After this failure we returned to variations of the most efficient compound *III* (i.e. the methoxy derivative) and prepared further 8 alkoxy derivatives (*VII*–*XIV*). The MIC values obtained with *Mycobacterium tuberculosis* led to an idea that the activity could be connected with both the electronic parameter and the lipophilicity of the molecules.

The lipophilicity of the first group of compounds (*I*–*V*) was investigated by means of partition chromatography on a thin layer, and the experimental R_M values were correlated with published π^- substituent constants. The correlation of the R_M data from the system containing 65% acetone with the substituent constants mentioned was excellent (see Eq. (*1*)). However, the same cannot be stated for the correlation of the R_M values extrapolated to zero methanol concentration whose application has spread especially under the influence of the papers by Biagi[11] (see Eq. (*2*)).

$$(R_M)_{65} = 0{\cdot}1217\pi^- + 0{\cdot}1907 \qquad (1)$$

$$r = 0{\cdot}986 \quad s = 0{\cdot}01 \quad F = 101{\cdot}58 \quad n = 5$$

$$(R_M)_0 = 0{\cdot}6050\pi^- + 2{\cdot}6444 \qquad (2)$$

$$r = 0{\cdot}892 \quad s = 0{\cdot}20 \quad F = 11{\cdot}63 \quad n = 5$$

We considered Eq. (*1*) a verification of the relation between lipophilicity and hydrophobic substituent constants, and therefore we stopped further experimental studies monitoring the lipophilicity and took the substituent constants π^- as sufficient for the rest of our study. The published σ constants were taken as the electronic parameter, but we could not find the Hammett constant of allyloxy group in available literature. Excluding the allyl derivative we found the regression equation (*3*) for the set of the active compounds investigated.

$$\log \text{MIC} = 0{\cdot}7654(\pi^-)^2 - 0{\cdot}6150\pi^- + 4{\cdot}2368\sigma + 2{\cdot}7212 \qquad (3)$$

$$r = 0{\cdot}916 \quad s = 0{\cdot}34 \quad F = 10{\cdot}40 \quad n = 10$$

The activity was followed up to the limit concentration of 1 000 μmol l^{-1}. Three compounds (*IV*, *V*, *VI*) were inactive even at this concentration. Sometimes they

can be included in the calculation if they are ascribed the activity corresponding to the next higher concentration step in the dilution scale used, i.e. 3 000 μmol l^{-1} in the cases given. The inclusion of compounds *IV* and *V* (fulfilling the condition mentioned) into the set does not substantially change the regression coefficients (Eq. (*4*)).

$$\log \text{MIC} = 0{\cdot}8390(\pi^-)^2 - 0{\cdot}9027\pi^- + 3{\cdot}0960\sigma + 2{\cdot}5538 \qquad (4)$$
$$r = 0{\cdot}936 \quad s = 0{\cdot}36 \quad F = 18{\cdot}90 \quad n = 12$$

In contrast thereto, compound *VI* should be strongly active according to both Eqs (*3*) and (*4*), and its inclusion into the set substantially changes the regression coefficients and lowers the statistical significance of the regression. Hence it can be concluded that the dimethylamino derivative *VI* behaves anomalously with regard to the rest of the set. In conclusion we used the values of the δ chemical shift of hydrogen atom of amide group as the electronic parameter in the regression equations, which enabled the inclusion of allyl derivative *X*, too. Equations (*5*) and (*6*) apply to the sets of eleven and thirteen (*IV* and *V* added) substances, respectively.

$$\log \text{MIC} = 0{\cdot}8047(\pi^-)^2 - 0{\cdot}7340\pi^- + 7{\cdot}6951\delta - 96{\cdot}2660 \qquad (5)$$
$$r = 0{\cdot}957 \quad s = 0{\cdot}24 \quad F = 25{\cdot}33 \quad n = 11$$

$$\log \text{MIC} = 0{\cdot}8452(\pi^-)^2 - 0{\cdot}8781\pi^- + 6{\cdot}6796\delta - 83{\cdot}4091 \qquad (6)$$
$$r = 0{\cdot}966 \quad s = 0{\cdot}26 \quad F = 42{\cdot}22 \quad n = 13$$

These equations, too, indicate the anomalous behaviour of the dimethylamino derivative *VI*. The inclusion of experimental electronic parameter increased the statistical significance of the regression equations. The respective values of chemical shifts correlate with the Hammett constants according to Eq. (*7*).

$$\delta = 0{\cdot}4789\sigma + 12{\cdot}8647 \qquad (7)$$
$$r = 0{\cdot}966 \quad s = 0{\cdot}04 \quad F = 151{\cdot}91 \quad n = 13$$

The preparation of the cinnamamido derivative *XVI* was motivated by the fact that cinnamic acid itself possesses an antituberculotic effect. However, compound *XVI* turned out ot be inefficient as an antituberculotic.

In conclusion it can be stated that: (i) A set of compounds with significant antimycobacterial activity has been found. Compounds *VIII*, *IX*, and *X* (when tested in vitro against *Mycobacterium tuberculosis*) are comparable with ethionamide, which is a practically adopted antituberculotic. (ii) The activity was proved to depend on lipophilicity and electronic parameters. The dependence can be expressed by the equations of the Hansch type. (iii) The dimethylamino derivative *VI* deviates

from the dependence. The deviation could be ascribed to basic properties of this group which can behave as a second binding centre during interactions with proteins. (iv) The application of δ values instead of σ values to the regression equations leads to increase in statistical significance of the correlation. It is supposed that the Hammett constants for some alkoxy groups will have to be revised.

REFERENCES

1. Freedlander B. L., French F. A.: Proc. Soc. Exp. Biol. Med. *66*, 362 (1948); Chem. Abstr. *42*, 3037 (1948).
2. Binswanger L. A., Erlenmeyer H., Sorkin E., Suter U.: Helv. Chim. Acta *31*, 1975 (1948).
3. Waisser K., Kuneš J.: Cesk. Farm., in press.
4. Choungh Y. S., Kim K. W.: Yakkak Hoeji *15*, 83 (1971); Chem. Abstr. *80*, 59 890 (1974).
5. Topliss J. G.: J. Med. Chem. *20*, 463 (1977).
6. Kamala K., Rao P., Reddy K.: Indian J. Chem., B *22*, 1194 (1983).
7. Schultz H. W., Wiese G. A.: J. Am. Pharm. Assoc. *48*, 750 (1959).
8. Kuchař M., Rejholec V.: *Kvantitativní vztahy mezi strukturou a biologickou aktivitou.* Academia, Prague 1980.
9. Waisser K.: Folia Pharm. Univ. Carol. *13*, 17 (1989).
10. Waisser K., Exner O., Kuchař M., Tichý M.: *QSAR v příkladech.* Charles University, Prague 1985.
11. Biagi G. L., Barbaro A. M., Gamba M. F., Guerra M. C.: J. Chromatogr. *44*, 195 (1969).

Translated by J. Panchartek.