QSAR Study Concerning Photosynthesis Inhibition in Algae and Plant Chloroplasts by 2-Alkylthio-6-R-benzothiazoles
I. 2-Alkylthio-6-aminobenzothiazoles, 3-(2-Alkylthio-6-benzothiazolylaminomethyl)-2-benzothiazolinethiones, 3-(2-Alkylthio-6-benzothiazolylaminomethyl)-6-bromo-2-benzothiazolinones

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Activity of 2-alkylthio-6-aminobenzothiazoles and their 6-*N*-substituted derivatives (3-(2-alkylthio-6-benzothiazolylaminomethyl)-2-benzothiazolinethiones and 3-(2-alkylthio-6-benzothiazolylaminomethyl)-6-bromo-2-benzothiazolinones) concerning photosynthesis inhibition in algae *Chlorella vulgaris* and in spinach chloroplasts has been correlated with lipophilicity and dipole moments of the effectors using the Hansch method. Parameters of the Hansch equation were calculated by theoretical methods. The benzene and the thiazole rings of 2-alkylthio-6-R-benzothiazoles are the electron-donor part of the investigated benzothiazole effectors. Antialgal activity of the compounds of the series containing bromine showed relatively high efficiency for all investigated derivatives with negligible differences.

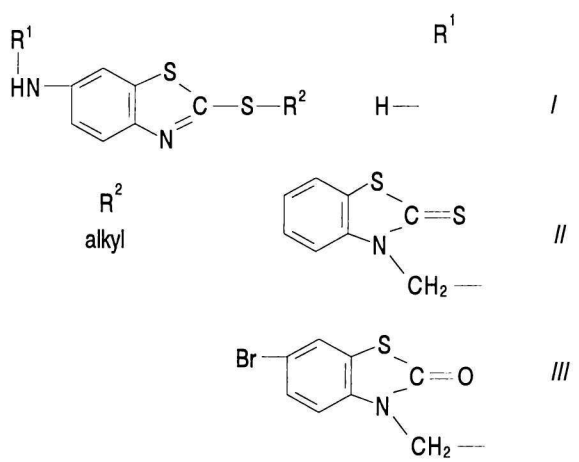
Benzothiazole derivatives belong to biologically active compounds showing a great variety of biological, mainly antimicrobial effects [1—3]. Antifungal [4], antiyeast [5], plant growth-regulating [6, 7], antiprotozoal [8], antiviral [9], antituberculotic [10, 11], and anthelminthic activities [12] of a great number of benzothiazole derivatives have been confirmed. 2-Alkylthio-6-R-benzothiazoles inhibit also chlorophyll synthesis in algae as well as oxygen evolution rate in plant chloroplasts at the Hill reaction [13] and these effects show good correlation with some of the above-mentioned biological activities of benzothiazole effectors [4, 13]. From the results of QSAR studies concerning 2-alkylthio-6-R-benzothiazoles it can be concluded that the biological activity of these effectors depends on lipophilicity and on certain steric parameters, *e.g.* on the branching of the 2-alkylthio substituent [14].

This paper is aimed to investigate the correlation between lipophilicity as well as dipole moments and inhibitory activity of 6-amino-2-alkylthiobenzothiazoles and their 6-*N*-substituted derivatives concerning photosynthetic processes in algae *Chlorella vulgaris* and in plant chloroplasts.

EXPERIMENTAL

Syntheses of the studied compounds 2-alkylthio-6-aminobenzothiazoles (*I*) (alkyl = C_2H_5—n-C_7H_{15}, n-C_9H_{19}, and $CH_2C_6H_5$), 3-(2-alkylthio-6-benzothiazolylaminomethyl)-2-benzothiazolinethiones (*II*) (alkyl = C_2H_5—n-C_9H_{19}, $CH_2C_6H_5$, $CH_2CH(CH_3)_2$, $CH(CH_3)C_2H_5$, $CH_2CH{=}CH_2$, CH_2CH_2OH), and 3-(2-alkylthio-6-benzothiazolylaminomethyl)-6-bromo-2-benzothiazolinones (*III*) (alkyl = n-C_3H_7—n-C_8H_{17}, $CH_2C_6H_5$, and $CH_2CH{=}CH_2$) have been described in the previous papers [10, 15, 16].

The effect of the studied compounds (Formula 1) on chlorophyll production in stationarily cultured *Chlorella vulgaris* (7 d, 16 h light/8 h dark) was investi-



Formula 1

gated according to Ref. [17]. For low solubility of the studied compounds in water these were dissolved in *N*,*N*-dimethylformamide (DMF) and the DMF content in the control samples was the same as in samples containing benzothiazoles. The effect of studied compounds on the rate of oxygen evolution in spinach chloroplasts was investigated spectrophotometrically (Specord UV—VIS, Zeiss, Jena) according to Ref. [13]. The inhibition of chlorophyll synthesis in algae as well as the photosynthesis inhibition in plant chloroplasts were expressed by pI_{50} values corresponding to negative logarithms of concentrations (mol dm^{-3}) causing 50 % inhibition with respect to the control. The decrease of photosynthetic activity due to the presence of DMF was taken into account.

Physicochemical properties of benzothiazole derivatives have been studied by theoretical methods, whereas at lipophilicity calculations their additive properties were taken into account [18]. For determination of the electron structure of studied compounds the quantum-chemical calculations using the AM1 method with the standard parametrization [19] applied on some substituents were used. For determination of the structure of studied derivatives and for calculation of their dipole moments the method of molecular mechanics according to Allinger has been used [20].

The correlation between biological activity and physicochemical properties of studied benzothiazole derivatives has been studied by parabolic [21] and bilinear [22, 23] models.

RESULTS AND DISCUSSION

pI_{50} Values concerning the inhibitory activity of studied compounds with respect to chlorophyll synthesis in *Chlorella vulgaris* and oxygen evolution rate in spinach chloroplasts, as well as calculated values of the logarithm of lipophilicity (log *P*) and of the dipole moment (μ) are summarized in Table 1.

Recently it was found that the studied compounds inhibit the rate of electron transport through photosynthetic apparatus due to the interaction with the oxygen-evolving complex, especially with Z/D intermediate in the donor side of the photosynthetic centre PS 2 [24]. The most intense rise of the inhibition of photosynthetic activity of spinach chloroplasts with

Table 1. pI_{50} Values of Benzothiazole Derivatives according to Formula 1 Concerning Inhibition of Oxygen Evolution Rate in Spinach Chloroplasts and of Chlorophyll Production in *Chlorella vulgaris*, Values of Logarithms of Lipophilicity (log *P*), and Values of Dipole Moments (μ)

R^1	R^2	pI_{50}		log *P*	$\mu \cdot 10^{29}$ / C m
		Chloroplasts	*Chlorella vulgaris*		
I	C_2H_5	3.46	4.48	2.00	1.076
	C_3H_7	4.06	4.48	2.47	1.086
	C_4H_9	4.20	4.60	2.86	1.099
	C_5H_{11}	4.59	4.58	3.26	1.096
	C_6H_{13}	4.64	4.78	3.66	1.106
	C_7H_{15}	4.37	4.73	4.05	1.102
	C_9H_{19}	4.06	–	4.84	1.112
	$CH_2C_6H_5$	4.27	4.42	3.43	1.149
II	CH_3	–	4.20	3.99	2.068
	C_2H_5	3.20	4.25	4.33	2.068
	C_3H_7	3.56	4.30	4.80	2.048
	C_4H_9	3.87	4.60	5.19	2.038
	C_5H_{11}	3.95	4.77	5.59	2.005
	C_6H_{13}	4.22	4.78	5.99	2.018
	C_7H_{15}	4.11	4.96	6.38	1.998
	C_8H_{17}	3.91	4.96	6.78	1.998
	C_9H_{19}	3.87	4.85	7.17	1.998
	$CH_2C_6H_5$	3.95	4.95	5.76	2.091
	$CH_2CH(CH_3)_2$	4.11	4.82	5.20	2.038
	$CH(CH_3)C_2H_5$	3.90	4.48	5.21	2.061
	$CH_2CH{=}CH_2$	–	4.70	4.73	2.098
	CH_2CH_2OH	–	4.44	3.54	2.151
III	C_3H_7	3.60	5.00	4.85	1.072
	C_4H_9	3.56	5.06	5.25	1.066
	C_5H_{11}	3.63	5.06	5.65	1.029
	C_6H_{13}	3.90	5.06	6.04	0.996
	C_7H_{15}	3.87	4.87	6.44	0.952
	C_8H_{17}	–	4.97	6.84	0.929
	$CH_2C_6H_5$	3.27	5.00	5.82	1.145
	$CH_2CH{=}CH_2$	3.66	–	4.78	0.919

For R^1 see Formula 1.

the prolongation of the alkyl chain has been shown by derivatives of the homologous series *I*, by 6-*N* substitution (series *II* and *III*, respectively) the inhibitory activity has been lowered. Based on the above findings it can be assumed that the quasi-parabolic course of the dependence of pI_{50} *vs.* alkyl chain length is probably connected with the too high lipophilicity of the compounds with longer alkyl chain causing their limited penetrability through the hydrophilic regions of thylakoid membranes.

The inhibitory activity concerning chlorophyll production in *Chlorella vulgaris* increased with the alkyl chain prolongation in the series *I* and *II*, with derivatives of the series *III* it remained practically unchanged. The antialgal effects of the studied series showed an opposite sequence with respect to that obtained by the study of the inhibition of oxygen evolution rate in spinach chloroplasts — the most active inhibitors were the compounds containing bromine. These differences in the inhibitory activities of the three investigated series with respect to both studied biological systems can be connected with different chemical composition of algal and chloroplast membranes.

From the quantum-chemical study it is evident that the benzene and thiazole rings of the 6-*N*-substituted 2-alkylthio-6-aminobenzothiazoles are the electron-donor part of the molecule of studied compounds. From Fig. 1 illustrating the distribution of the molecular electrostatic potential on the van der Waals surface for n-propyl derivatives of the investigated series it is evident that the negative charge and the lowest electrostatic potential is concentrated on this part of the molecules. Alkyl substituent does not affect significantly the electron structure of the electron-donor part of the molecule.

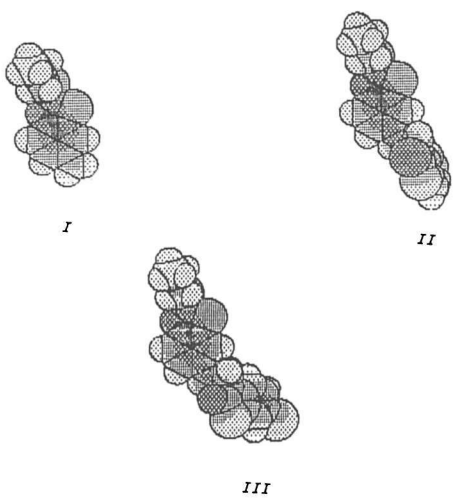


Fig. 1. Distribution of molecular electrostatic potential on the van der Waals surface for n-propyl derivatives of investigated benzothiazole derivatives (symbols according to Formula 1; the density of the electron charge is proportional to the intensity of crosshatching).

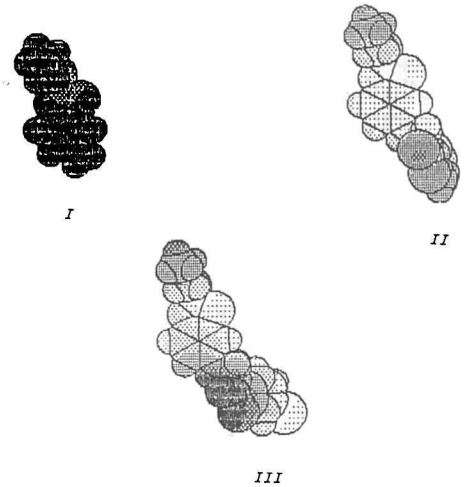


Fig. 2. Distribution of lipophilicity on the van der Waals surface for n-propyl derivatives of investigated benzothiazole derivatives (symbols according to Formula 1; the density of the electron charge is indirectly proportional to the intensity of crosshatching).

Fig. 2 demonstrates the distribution of lipophilicity on the van der Waals surface for the above-mentioned three propyl derivatives. It is evident that with n-propyl derivatives of all three series the highest values of lipophilicity take place at the site of alkyl chain substitution. With the prolongation of the alkyl chain the highest lipophilicity value in the molecule is shifted towards the alkyl chain. This is demonstrated in Fig. 3 for ethyl, n-butyl, n-hexyl, and n-nonyl derivatives of the series *I*. Figs. 1—3 were drawn using the program MGP [18].

The dependence of the biological activity on lipophilic and electron parameters of the studied benzothiazole derivatives characterized by lipophilicity and

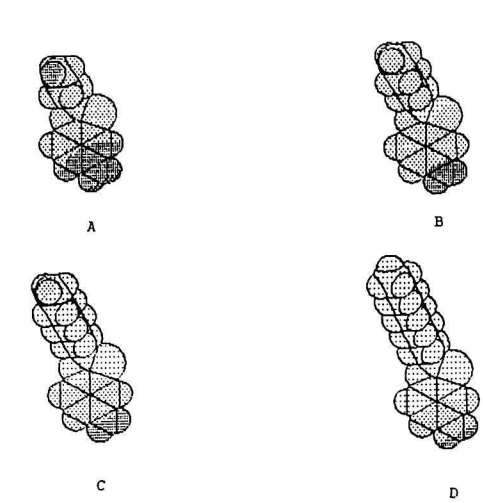


Fig. 3. Distribution of lipophilicity on the van der Waals surface for ethyl (A), n-butyl (B), n-hexyl (C), and n-nonyl (D) derivatives of 2-alkylthio-6-aminobenzothiazole (series *I*). The density of the electron charge is indirectly proportional to the intensity of crosshatching).

dipole moments has been expressed by the regression equations (*1*—*13*).

Relationships between the structure and inhibitory activity of the studied effectors with respect to the oxygen evolution rate in spinach chloroplasts using the parabolic model are reflected by the regression equations (*1*—*6*).

$$pI_{50}(I) = -0.283(\pm 0.729) + 2.594(\pm 0.442)\log P - 0.352(\pm 0.064)(\log P)^2 \quad (1)$$
$$r_i^2 = 0.891;\ s = 0.15;\ F = 20.39;\ N = 8$$

$$pI_{50}(I) = 4.842(\pm 2.569) + 3.037(\pm 0.408)\log P - 0.407(\pm 0.057)(\log P)^2 - 5.393(\pm 2.636) \times 10^{29}\,\mu \quad (2)$$
$$r_i^2 = 0.949;\ s = 0.11;\ F = 23.65;\ N = 8$$

$$pI_{50}(II) = -6.417(\pm 1.628) + 3.443(\pm 0.569)\log P - 0.281(\pm 0.049)(\log P)^2 \quad (3)$$
$$r_i^2 = 0.867;\ s = 0.12;\ F = 26.12;\ N = 11$$

$$pI_{50}(II) = -3.935(\pm 3.824) + 3.441(\pm 0.587)\log P - 0.284(\pm 0.051)(\log P)^2 - 1.176(\pm 1.627) \times 10^{29}\,\mu \quad (4)$$
$$r_i^2 = 0.876;\ s = 0.12;\ F = 16.55;\ N = 11$$

$$pI_{50}(III) = 14.064(\pm 8.410) - 3.912(\pm 3.051)\log P + 0.363(\pm 0.274)(\log P)^2 \quad (5)$$
$$r_i^2 = 0.386;\ s = 0.20;\ F = 1.26;\ N = 7$$

$$pI_{50}(III) = 5.254(\pm 8.240) + 0.085(\pm 3.265)\log P + 0.002(\pm 0.294)(\log P)^2 - 2.094(\pm 1.146) \times 10^{29}\,\mu \quad (6)$$
$$r_i^2 = 0.709;\ s = 0.16;\ F = 2.44;\ N = 7$$

where r_i is the correlation index, s the standard deviation, F the value of F-test and N is the number of molecules taken for calculation.

By inserting of the dipole moment in the correlation eqn (*1*) the correlation index and the value of F-test are increased (eqn (*2*)). That means that the activity of effectors of the series *I* depends not only on lipophilicity, but also on electron structure of the molecule. From the statistical parameters it follows that the activity of effectors of the series *II* depends probably only on the lipophilicity (eqns (*3*) and (*4*)). With effectors of the series *III* the dependence of the biological activity both on the lipophilicity and dipole moment cannot be excluded (eqns (*5*) and (*6*)), but the statistical parameters are not significant. The F-test values for eqns (*1*—*4*) are statistically significant at the 99 % level of probability.

Regression equations (*7*—*13*) calculated using the parabolic model express the relationship between antialgal activity of the studied effectors and their structure.

$$pI_{50}(I) = 4.815(\pm 0.984) - 0.306(\pm 0.673)\log P + 0.071(\pm 0.111)(\log P)^2 \quad (7)$$
$$r_i^2 = 0.472;\ s = 0.12;\ F = 1.79;\ N = 7$$

$$pI_{50}(I) = 8.819(\pm 1.908) + 0.294(\pm 0.544)\log P - 0.014(\pm 0.087)(\log P)^2 - 4.541(\pm 2.017) \times 10^{29}\,\mu \quad (8)$$
$$r_i^2 = 0.804;\ s = 0.08;\ F = 4.10;\ N = 7$$

$$pI_{50}(II) = 2.913(\pm 1.154) + 0.447(\pm 0.437)\log P - 0.022(\pm 0.040)(\log P)^2 \quad (9)$$
$$r_i^2 = 0.659;\ s = 0.17;\ F = 10.62;\ N = 14$$

$$pI_{50}(II) = -4.956(\pm 4.048) + 0.767(\pm 0.418)\log P - 0.041(\pm 0.037)(\log P)^2 + 3.282(\pm 1.634) \times 10^{29}\,\mu \quad (10)$$
$$r_i^2 = 0.757;\ s = 0.15;\ F = 10.38;\ N = 14$$

$$pI_{50}(II) = -2.762(\pm 3.574) + 0.308(\pm 0.069)\log P + 2.814(\pm 1.596) \times 10^{29}\,\mu \quad (11)$$
$$r_i^2 = 0.727;\ s = 0.15;\ F = 14.63;\ N = 14$$

$$pI_{50}(III) = 3.578(\pm 2.167) + 0.546(\pm 0.747)\log P - 0.051(\pm 0.064)(\log P)^2 \quad (12)$$
$$r_i^2 = 0.361;\ s = 0.07;\ F = 1.13;\ N = 7$$

$$pI_{50}(III) = 3.524(\pm 2.514) + 0.613(\pm 0.956)\log P - 0.058(\pm 0.084)(\log P)^2 - 1.123(\pm 6.967) \times 10^{30}\,\mu \quad (13)$$
$$r_i^2 = 0.367;\ s = 0.08;\ F = 0.58;\ N = 7$$

Similarly to the results obtained with photosynthesis inhibition in plant chloroplasts, the activity of effectors of the series *I* depends on lipophilicity and dipole moment of the molecules, but the statistical parameters r_i and F are not significant (eqns (*7*) and (*8*)). The same conclusion can be made for the activity of effectors of the series *II*, whereby the contribution of the quadratic member of the regression equation is not significant as determined by t-test (eqns (*9*) and (*10*)). This is confirmed also by the statistical parameters of eqn (*11*) in which the quadratic regression coefficient $(\log P)^2$ was not used. All investigated derivatives of the series *III* showed relatively high antialgal activities, differing each from other only negligibly, which was confirmed also by statistical results (no correlation between the biological activity and studied physicochemical parameters). Besides the Hansch parabolic model for the relationship between biological activity and log P also the bilinear model has been used [22, 23]. The comparison of statistical parameters obtained by both the models is shown in Table 2. For comparison of both the models the partial F-test has been used, whereby the number of degrees of freedom was taken into account. It can be concluded that all the statistical parameters were better for the bilinear model, but the partial F-test [23] indicates that the results obtained by the bilinear model are not statistically more significant (the values of partial F-test were the most 1.44). The more pronounced deviation between both the models was obtained for log P_o values (value of log P corresponding to the highest activity) for eqn (*9*). With respect to the relatively high value of the

Table 2. Comparison of Some Statistical Parameters of Regression Equations and log P_o Values Calculated by Parabolic (P) and Bilinear Model (B)

Eqn	Model	log P_o	r_i	F-test	Partial F-test
(*1*)	P	3.689	0.944	20.39	0.05
	B	3.520	0.944	20.66	
(*3*)	P	6.121	0.931	26.12	0.43
	B	5.933	0.935	27.99	
(*5*)	P	5.394	0.621	1.26	0.04
	B	5.412	0.627	1.30	
(*7*)	P	2.151	0.687	1.79	0.05
	B	2.344	0.694	1.85	
(*9*)	P	10.124	0.812	10.62	1.44
	B	6.733	0.838	12.95	
(*12*)	P	5.341	0.601	1.13	0.49
	B	5.328	0.672	1.65	

partial F-test for this system (1.44) and to the extraordinarily high value of log P_o determined by the parabolic model (10.124) it seems that for this system the bilinear model is more suitable.

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