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[A0019]

Photosynthesis Inhibiting 2-(6-Acetamidobenzothiazolethio)acetic Acid Esters

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Abstract: The preparation, spectroscopic characterisation and biological activity 2-(6-acetamido-benzolethio)acetic acid esters are reported in this communication.

Keywords: Electron transfer inhibition, QSAR, lipophilicity, 2-(6-acetamidobenzothiazolethio)-acetic acid esters

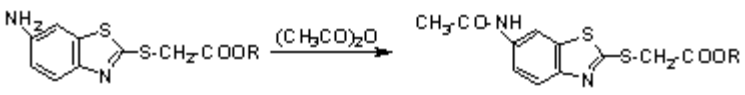
Introduction

2-Alkylthio-6-aminobenzothiazoles have shown good antimycobacterial 1, antiyeast 2-4, anticandidous 5, and antialgal 6 activity, as well as inhibition of photosynthetic electron transport in spinach chloroplasts 7. Their N-formyl 3, 4, 8 and N-acetyl 9, 10 derivatives have manifested antimycobacterial 8, 9, antifungal, anticandidous 10, antialgal and photosynthesis inhibiting 11 activity too.

Another antimicrobially active group of compounds, 2-(alkoxycarbonylmethyl-thio)-6-amino-benzothiazoles 12 have shown good antiyeast activity against *Saccharomyces cerevisae* and several derivatives exhibited good anticandidous activity against the clinical pathogen *Candida Cruzei*, the best of them being n-hexyl and benzyl derivatives 12, 13. The antialgal efficiency of these compounds has been low, but they have manifested interesting inhibition of photosynthetic electron transport in spinach chloroplasts - the inhibitory activity has been increasing with the increasing lipophilicity of the molecules 13, 14.

Results and Discussion

Based on the above experience with biologically active benzothiazole derivatives, thirteen new 2-(6-acetamidobenzothiazolethio)acetic acid esters (Table 1) have been synthesized by acetylation of 2-(alkoxycarbonylmethylthio)-6-aminobenzothiazoles 12 with acetic anhydride.



Compound R Compound R

1 -CH_3 **8** -$(CH_2)_4CH_3$

2 -C_2H_5 **9** -$(CH_2)_5CH_3$

3 -$(CH_2)_2CH_3$ **10** -$(CH_2)_6CH_3$

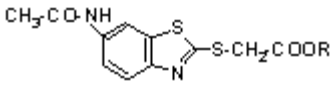
4 -CH_2-CH=CH_2 **11** -$(CH_2)_7CH_3$

5 -CH_2-CCH **12** -$(CH_2)_8CH_3$

6 -$(CH_2)_3CH_3$ **13** -CH_2-C_6H_5

7 -$CH(CH_3)C_2H_5$

Table 1. Characterisation of the prepared 2-(6-acetamidobenzothiazolethio)acetic acid esters.



	Formula			W_i(calc.) %			
Comp.	M_r			W_i(found) %		Yield %	M.p. (^oC)
		C	H	N	S		
1	$C_{12}H_{12}N_2O_3S_2$	48.63	4.08	9.45	21.64	94.5	154.5-155.5
	296.37	48.40	4.02	9.26	21.65		
2	$C_{13}H_{14}N_2O_3S_2$	50.30	4.55	9.03	20.66	99.8	131.0-132.5
	310.40	50.53	4.59	9.04	20.80		
3	$C_{14}H_{16}N_2O_3S_2$	51.83	4.97	8.63	19.77	92.5	111.5-112.5
	324.42	51.65	5.03	8.63	19.99		
4	$C_{14}H_{14}N_2O_3S_2$	52.16	4.38	8.69	19.89	86.8	119-120
	322.41	52.49	4.36	8.43	20.19		
5	$C_{14}H_{12}N_2O_3S_2$	52.48	3.78	8.74	20.02	90.5	123.0-124.5
	320.39	52.29	3.66	8.77	19.73		
6	$C_{15}H_{18}N_2O_3S_2$	53.23	5.36	8.28	18.95	74.0	113-114
	338.45	53.42	5.37	8.34	18.97		
7	$C_{15}H_{18}N_2O_3S_2$	53.23	5.36	8.28	18.95	88.6	117-118
	338.45	53.02	5.41	8.16	18.83		

8	$C_{16}H_{20}N_2O_3S_2$	54.52	5.72	7.95	18.19	85.2	116.5-118.5
	352.48	54.55	5.74	7.92	18.09		
9	$C_{17}H_{22}N_2O_3S_2$	55.71	6.05	7.64	17.49	79.1	77-79
	366.50	56.03	6.21	7.64	17.44		
10	$C_{18}H_{24}N_2O_3S_2$	56.81	6.36	7.36	16.85	47.4	82.5-84.5
	380.53	57.04	6.44	7.31	16.88		
11	$C_{19}H_{26}N_2O_3S_2$	57.84	6.64	7.10	16.25	38.0	71.5-73.5
	394.56	58.06	6.74	7.07	16.25		
12	$C_{20}H_{28}N_2O_3S_2$	58.79	6.91	6.86	15.70	39.2	80-81
	408.59	58.50	6.92	6.76	15.63		
13	$C_{18}H_{16}N_2O_3S_2$	58.04	4.33	7.52	17.22	99.3	122.5-124.0
	372.47	58.09	4.42	7.48	17.43		

The structure of compounds **1**-**13** has been verified by ^{1}H NMR spectra. The chemical shifts of the hydrogen atoms of the benzothiazole skeleton are not influenced by the alkyl substituents.

The synthesized compounds were tested for photosynthesis inhibiting activity, they inhibited oxygen evolution rate (OER) in spinach chloroplasts. The photosynthesis - inhibiting activity was expressed by IC_{50} values, i.e. by molar concentrations causing 50% decrease of OER with respect to the untreated control sample (Table 2).

Table 2: Calculated values of log P and IC_{50} of the studied compounds concerning OER inhibition in spinach chloroplasts.

Compound	log P	IC_{50} (mol dm^{-3})
1	0.86	-
2	1.20	857
3	1.67	514
4	1.60	411
5	1.14	350
6	2.07	83
7	2.09	-
8	2.46	56
9	2.86	47
10	3.26	106
11	3.65	430
12	4.05	1879
13	2.64	622

OER = oxygen evolution rate

IC_{50} = molar concentration of the inhibitor causing 50 % decrease of activity against the control

The dependence of photosynthesis inhibiting activity on the lipophilicity of the derivatives with R = n-alkyl and allyl showed quasi-parabolic course with maximum activity at hexyl derivative (IC_{50} = 47 mol dm^{-3}). Whereas the biologically active compounds, in order to reach their site of action, must penetrate through several compartments of thylakoid membranes (e.g. series of lipid bilayers separated by aqueous layers), the highest biological effect is shown by molecules with suitable lipophilicity, which enables to cross both above mentioned compartments. The passage of the short-chain compounds through the thylakoid membranes is limited due to their too low partition coefficient and the number of inhibitors reaching the site of action in proteins situated on the inner side of thylakoid membranes is insufficient. On the other hand, the long-chain compounds due to intense interaction with membrane lipids, remain predominantly incorporated in the lipidic part of the membrane without reaching and damaging the corresponding membrane proteins. Similar results have been obtained also for the dependence of photosynthesis inhibiting activity on the lipophilicity of 2-alkylthio-6-aminobenzothiazoles [7], 6-acetamido-2-alkylthiobezothiazoles [11] and 2-(alkoxycarbonylmethylthio)-6-aminobenzothiazoles [13]. Based on the results obtained by EPR spectroscopy it was shown that the site of the above benzothiazole derivatives in the photosynthetic apparatus of spinach chloroplasts is the donor side of photosystem 2, upstream of the site of diphenylcarbazide action, i.e. in the oxygen evolving complex [14, 15].

Conclusion

The influence of quantum chemical parameters obtained by AM1 method 16 as well as of the calculated values of lipophilicity 17 upon inhibition of oxygen evolution in spinach chloroplasts caused by 2-(6-acetamidobenzothiazolethio) acetic acid esters was studied.

Various alkyl substituents do not substantially influence the distribution of the molecular electrostatic potential 17 and of charge density at the atoms, for this reason the studied biological activity of compounds **1**-**13** does not depend from the obtained quantum chemical parameters. The biological activity is substantially influenced by lipophilicity. Its parabolic dependence from the biological activity has got high statistical significance.

$$\log(1/IC_{50}) = (3.37000.4596)\log P - (0.71020.0868)\log^2 P - (0.59340.5506)$$

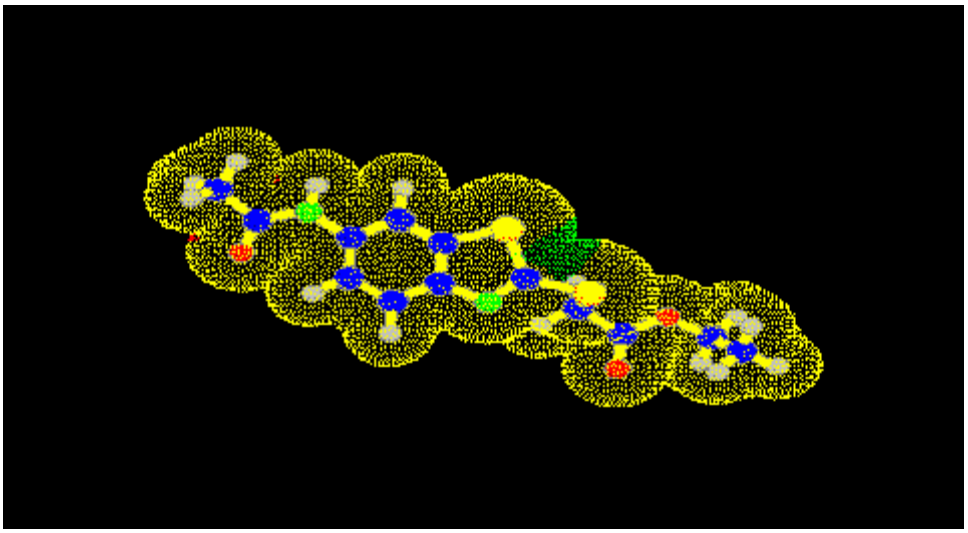
f = 0.958 s = 0.1874 F = 33.6 n = 9

Using the bilinear model 18, 19 gave better results, which were statistically more significant, and for this model the best value of the lipophilicity ($\log P_0$) was calculated as well.

$$\log(1/IC_{50}) = (1.37550.1251)\log P - (3.64720.3030)\log(+1) + (1.31080.2326)$$

f = 0.9799 s = 0.13040 F = 72.6 n = 9 $\log P_0$ = 2.71 = 1.1690 . 10^{-3}

The F-test value is statistically significant at the 99 % level of probability. From these facts it results, and it can be shown on the distribution of the lipophilicity on the Van der Waals surface of the molecules (Fig. 1) as well, that the biological efficiency of the molecule decreases when the value of logP is higher or lower than the value caculated for $\log P_0$. The compound **9** (R = n-hexyl) with the best biological activity has the largest green area of lipophilicity (Fig. 1).



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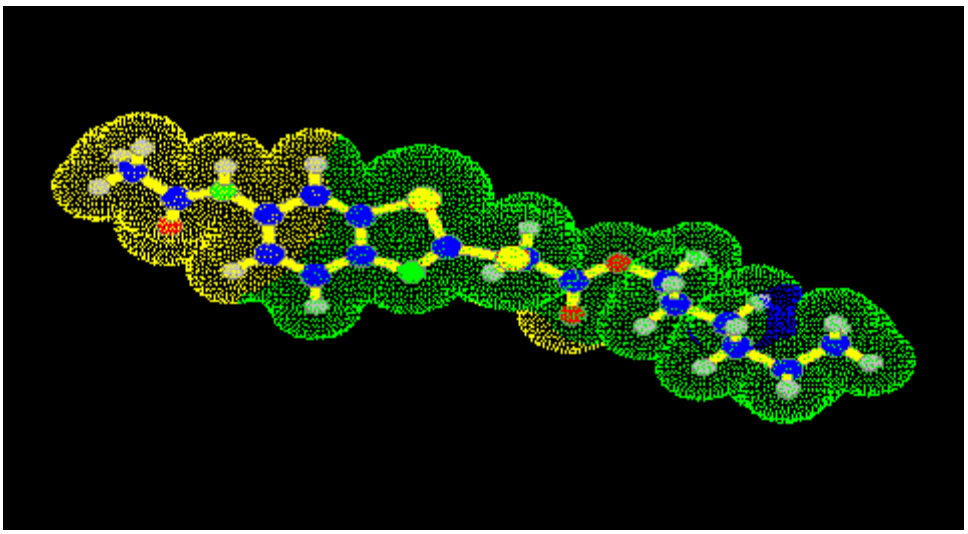

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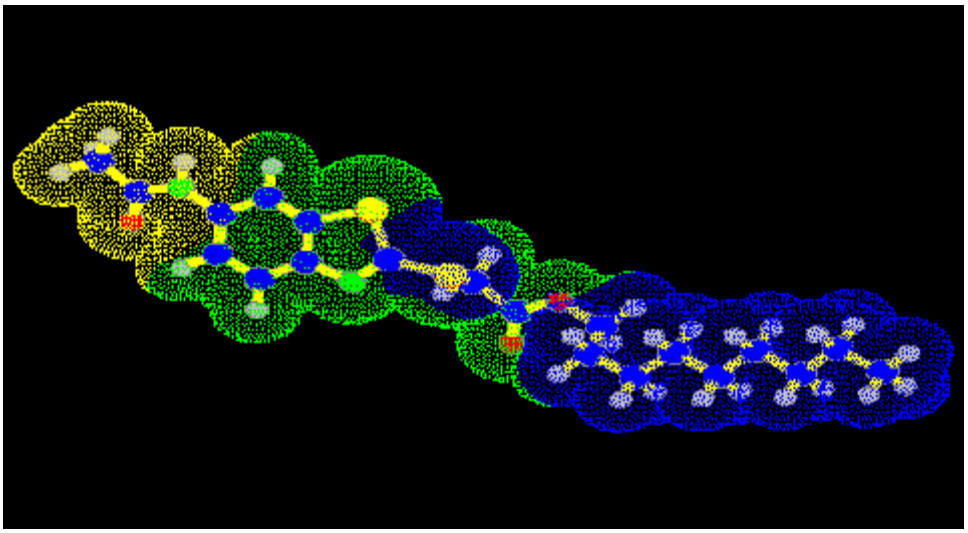

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Figure 1. Distribution of lipophilicity on the Van der Waals surface (the highest value of the lipophilicity is in the blue colour area) for compounds **2**, **9** and **12**.

Experimental Part

General

The starting 2-(alkoxycarbonylmethylthio)-6-aminobezothiazoles were prepared and purified according to [12]. Melting points were determined on a Kofler hotstage apparatus and are uncorrected. ^{1}H NMR spectra were obtained on TESLA BS 587 spectrometer (80 MHz) in deuterated dimethyl sulfoxide (DMSO) solution. Tetramethylsilane was used as internal standard.

The oxygen evolution rate in spinach chloroplasts was determined spectrophotometrcally (Specord UV VIS, Carl Zeiss, Jena, Germany) by Hill reaction at constant chlorophyll concentration (30 g cm^{-1}) using 2,6-dichloro-phenol-indophenol as electron acceptor [11]. The compounds were dissolved in DMSO because of their too low water solubility. The applied DMSO concentration (up to 5%) did not affect OER.

Results of ^{1}H NMR analysis (80 MHz, deuterated DMSO)

6-Acetamidobenzothiazole skeleton

10.15 (NH, s, 1H); f. 39 (H-4, d, J=1.6 Hz, 1H); 7.75 (H-7, d, J=8.8 Hz, 1H); 7.50 (H-6, dd, J=8.8 and 1.6 Hz, 1H); 2.09 ($COCH_3$, s, 3H).

-S-CH_2COOR substituents

1 : 4.31 (SCH_2, s, 2H); 3.71 (OCH_3, s, 3H).

2 : 4.29 (SCH_2, s, 2H); 4.16 (OCH_2, q, 2H); 1.20 (CH_3, t, 3H).

3 : 4.29 (SCH_2, s, 2H); 4.08 (OCH_2, t, 2H); 1.60 (CH_2, sx, 2H); 0.86 (CH_3, t, 3H)

4 : 4.34 (SCH_2, s, 2H); 4.65 (OCH_2, d, J=5.1 Hz, 2H); 5.9 (=CH, m, 1H); 5.2 (=CH_2, m, 2H)

5 : 4.35 (SCH_2, s, 2H); 4.81 (OCH_2, d, J=2.4 Hz, 2H); 3.58 (CH, t, J'2.4 Hz, 1H)

6 : 4.29 (SCH_2, s, 2H); 4.12 (OCH_2, t, 2H); 1.4 (CH_2CH_2, m, 4H); 0.82 (CH_3, t, 3H)

7 : 4.25 (SCH_2, s, 2H); 4.81 (CH, sx, 1H); 1.54 (CH_2, qi, 2H); 1.17 (CH_3, d, 3H); 0.83 (CH_3, t, 3H).

8 : 4.28 (SCH_2, s, 2H); 4.10 (OCH_2, t, 2H); 1.5 (CH_2, m, 2H); 1.3 (CH_2, m, 4H); 1.19 (CH_3, t, 3H).

9 : 4.27 (SCH_2, s, 2H); 4.10 (OCH_2, t, 2H); 1.5 (CH_2, m, 2H); 1.2 (CH_2, m, 6H); 0.80 (CH_3, t, 3H).

10 : 4.27 (SCH_2, s, 2H); 4.10 (OCH_2, t, 2H); 1.5 (CH_2, m, 2H); 1.2 (CH_2, m, 8H); 0.82 (CH_3, t, 3H).

11 : 4.27 (SCH_2, s, 2H); 4.10 (OCH_2, t, 2H); 1.5 (CH_2, m, 2H); 1.2 (CH_2, m, 10H); 0.83 (CH_3, t, 3H).

12 : 4.27 (SCH_2, s, 2H); 4.09 (OCH_2, t, 2H); 1.5 (CH_2, m, 2H); 1.1 (CH_3, t, 12H)); 0.84 (CH_3, t, 3H).

13 : 4.36 (SCH_2, s, 2H); 5.20 (OCH_2Ph, s, 2H); 7.33 (Ph, bs, 5H).

2-(6-Acetamidobenzothiazolethio)acetic Acid Esters 1 - 13

Acetic anhydride (0.01 mol, 1.0 g) was added to 2-(alkoxycarbonylmethylthio)-6-aminobenzothiazoles 12 (0.005 mol). After solidifying the reaction mixture was boiled for 10 min. with water, sucked off and washed

with hot water.

Samples for analysis and testing were crystallized from acetone-water (4:1-5:1) and from methanol (derivatives **7**-**12)** using charcoal.

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References and Notes

1. Sidoova, E.; Odlerova, Z.; Volna, F.; Blockinger, G. *Chem. Zvesti* **1979**, *33*, 830.

2. Sidoova, E.; Kuchta, T.; Zemanova, M.; Strakova, H. *CS Pat. 261699*, **1988**.

3. Kuchta, T.; Strakova, H.; Sidoova, E. *Cs. Farmacie* **1989**, *38*, 139.

4. Kuchta, T.; Bujdakova, H.; Sidoova, E. *Folia Microbiologica* **1989***, 34*, 504.

5. Kuchta, T.; Bujdakova, H.; Sidoova, E.; Neslusanova, H. *Acta Fac. Rerum. Natur. Univ. Comen. Microbiologia* **1990***, 17 - 18*, 49.

6. Bujdakova, H.; Kuchta, T.; Sidoova, E. *Acta Fac. Rerum. Natur. Univ. Comen. Microbiologia* **1991***, 19 - 20*, 37.

7. Kralova, K.; Loos, D.; Sersen, F.; Sidoova, E. *Chem. Papers* **1994***, 48,* 198.

8. Sidoova, E.; Odlerova, Z. *Chem. Papers* **1990***, 44*, 375.

9. Sidoova, E.; Odlerova, Z. *Chem. Papers* **1985***, 39*, 553.

10. Kuchta, T.; Sidoova, E. *Cs. Farmacie* **1989**, *38*, 310.

11. Kralova, K.; Sersen, F.; Sidoova, E. *Chem. Papers* **1992***, 46,* 348.

12. Sidoova, E.; Perjéssy, A.; Loos, D.; Kuchta, T. *Chem. Papers* **1992***, 46,* 420.

13 Kralova, K.; Bujdakova, H.; Kuchta, T.; Loos, D. *Pharmazie* **1994***, 49*, 460.

14. Sersen, F.; Kralova, K.; Sidoova, E. *Photosynthetica* **1993***, 29*, 147.

15. Kralova, K.;, Sersen, F.; Sidoova, E. *Gen. Physiol. Biophys.* **1993**, *12*, 421.

16 Dewar, M. J. S.; Zoebisch, E.; Healy, E.; Stewart, J. P. P. *J. Am. Chem. Soc.* **1985***, 107*, 3902.

17. Ertl, P. *Chem. Listy* **1992**, *86*, 465.

18. Kubyni, H. *Arzneim.-Forsch. (Drug Res.)* **1976**, *26*, 1991.

19. Kubyni, H.; Kehrhahn, O. H. *Arzneim.-Forsch. (Drug Res.)* **1978**, *28*, 598.

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