

## Synthesis of 2-(6-Acetamidobenzothiazolethio)acetic Acid Esters as Photosynthesis Inhibitors

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**Abstract:** The synthesis and photosynthesis-inhibiting activity of 13 new 2-(6-acetamidobenzothiazolethio)acetic acid esters are reported. The new compounds were prepared by acetylation of 2-(alkoxycarbonylmethylthio)-6-aminobenzothiazoles with acetic anhydride. The structure of the compounds was verified by <sup>1</sup>H NMR spectra. The compounds inhibit photosynthetic electron transfer in spinach chloroplasts. The structure - activity relation was studied. Lipophilicity was found to influence substantially photosynthetic electron transfer.

**Keywords:** Electron transfer inhibition, QSAR, lipophilicity.

### Introduction

2-Alkylthio-6-aminobenzothiazoles have shown good antimycobacterial [1], antiyeast [2-4], anticandidous [5], and antialgal [6] activities, 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] activities too.

Another antimicrobially active group of compounds, 2-(alkoxycarbonylmethylthio)-6-aminobenzothiazoles [12] have shown good antiyeast activity against *Saccharomyces cerevisiae* and several derivatives exhibited good anticandidous activity against the clinical pathogen *Candida Cruzei*, the best of them being the n-hexyl and benzyl derivatives [12, 13]. The antialgal efficiency of

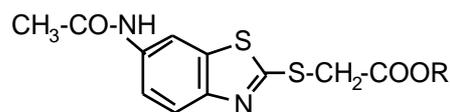
these compounds is low, but they have manifested interesting inhibition of photosynthetic electron transport in spinach chloroplasts - the inhibitory activity increases with the increasing lipophilicity of the molecules [13, 14].

### Results and Discussion

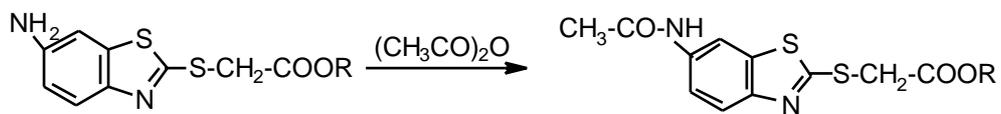
Based on the above experience with biologically active benzothiazole derivatives, thirteen new 2-(6-acetamidobenzothiazolethio)acetic acid esters (Table 1) have been synthesized by acetylation of 2-(alkoxycarbonylmethylthio)-6-amino-benzothiazoles [12] with acetic anhydride (Scheme 1).

The structures of compounds **1-13** were verified by <sup>1</sup>H NMR spectra. The chemical shifts of the hydrogen atoms of the benzothiazole skeleton are not influenced by the alkyl substituents.

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

Comp.	R	Formula M <sub>r</sub>	C	W <sub>i</sub> (calc.) % / W <sub>i</sub> (found) %			Yield %	M.p. (°C)
				H	N	S		
1	-CH <sub>3</sub>	C <sub>12</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub> S <sub>2</sub> 296.37	48.63	4.08	9.45	21.64	94.5	154.5-155.5
			48.40	4.02	9.26	21.65		
2	-C <sub>2</sub> H <sub>5</sub>	C <sub>13</sub> H <sub>14</sub> N <sub>2</sub> O <sub>3</sub> S <sub>2</sub> 310.40	50.30	4.55	9.03	20.66	99.8	131.0-132.5
			50.53	4.59	9.04	20.80		
3	-(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>	C <sub>14</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub> S <sub>2</sub> 324.42	51.83	4.97	8.63	19.77	92.5	111.5-112.5
			51.65	5.03	8.63	19.99		
4	-CH <sub>2</sub> CH=CH <sub>2</sub>	C <sub>14</sub> H <sub>14</sub> N <sub>2</sub> O <sub>3</sub> S <sub>2</sub> 322.41	52.16	4.38	8.69	19.89	86.8	119-120
			52.49	4.36	8.43	20.19		
5	-CH <sub>2</sub> C(CH <sub>3</sub> )	C <sub>14</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub> S <sub>2</sub> 320.39	52.48	3.78	8.74	20.02	90.5	123.0-124.5
			52.29	3.66	8.77	19.73		
6	-(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>	C <sub>15</sub> H <sub>18</sub> N <sub>2</sub> O <sub>3</sub> S <sub>2</sub> 338.45	53.23	5.36	8.28	18.95	74.0	113-114
			53.42	5.37	8.34	18.97		
7	-CH(CH <sub>3</sub> )C <sub>2</sub> H <sub>5</sub>	C <sub>15</sub> H <sub>18</sub> N <sub>2</sub> O <sub>3</sub> S <sub>2</sub> 338.45	53.23	5.36	8.28	18.95	88.6	117-118
			53.02	5.41	8.16	18.83		
8	-(CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub>	C <sub>16</sub> H <sub>20</sub> N <sub>2</sub> O <sub>3</sub> S <sub>2</sub> 352.48	54.52	5.72	7.95	18.19	85.2	116.5-118.5
			54.55	5.74	7.92	18.09		
9	-(CH <sub>2</sub> ) <sub>5</sub> CH <sub>3</sub>	C <sub>17</sub> H <sub>22</sub> N <sub>2</sub> O <sub>3</sub> S <sub>2</sub> 366.50	55.71	6.05	7.64	17.49	79.1	77-79
			56.03	6.21	7.64	17.44		
10	-(CH <sub>2</sub> ) <sub>6</sub> CH <sub>3</sub>	C <sub>18</sub> H <sub>24</sub> N <sub>2</sub> O <sub>3</sub> S <sub>2</sub> 380.53	56.81	6.36	7.36	16.85	47.4	82.5-84.5
			57.04	6.44	7.31	16.88		
11	-(CH <sub>2</sub> ) <sub>7</sub> CH <sub>3</sub>	C <sub>19</sub> H <sub>26</sub> N <sub>2</sub> O <sub>3</sub> S <sub>2</sub> 394.56	57.84	6.64	7.10	16.25	38.0	71.5-73.5
			58.06	6.74	7.07	16.25		
12	-(CH <sub>2</sub> ) <sub>8</sub> CH <sub>3</sub>	C <sub>20</sub> H <sub>28</sub> N <sub>2</sub> O <sub>3</sub> S <sub>2</sub> 408.59	58.79	6.91	6.86	15.70	39.2	80-81
			58.50	6.92	6.76	15.63		
13	-CH <sub>2</sub> -C <sub>6</sub> H <sub>5</sub>	C <sub>18</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub> S <sub>2</sub> 372.47	58.04	4.33	7.52	17.22	99.3	122.5-124.0
			58.09	4.42	7.48	17.43		



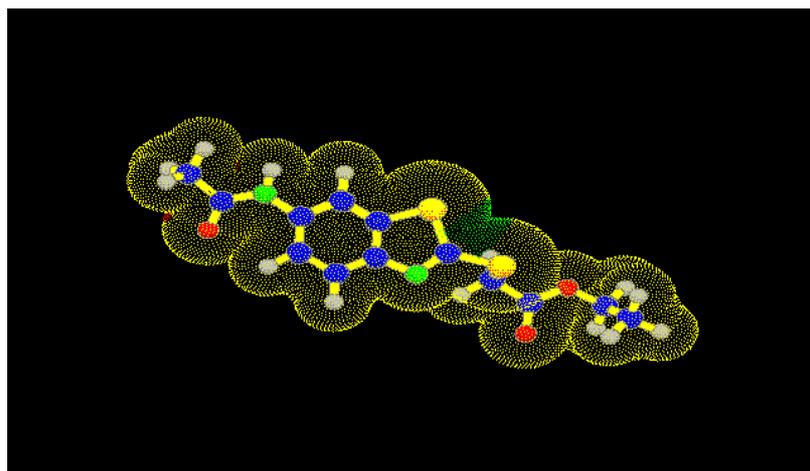
Scheme 1.

**Table 2.** Experimental values of  $IC_{50}$  of the studied compounds concerning OER inhibition in spinach chloroplasts and calculated  $\log P$ .

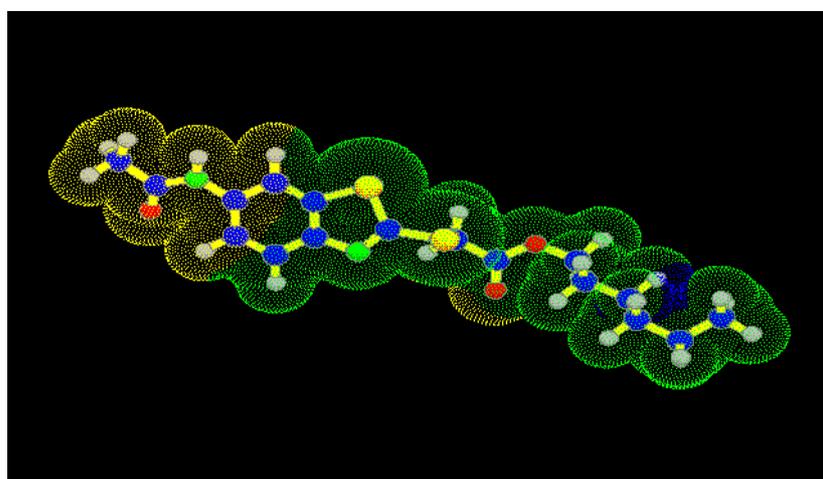
Compound	$\log P$	$IC_{50}$ ( $\mu\text{mol dm}^{-3}$ )
1	0.86	-
2	1.20	857
3	1.67	514
4	1.60	411
5	1.14	350
6	2.07	83
7	2.09	-
8	2.46	56
9	2.86	47
10	3.26	106
11	3.65	430
12	4.05	1879
13	2.64	622

OER = oxygen evolution rate.

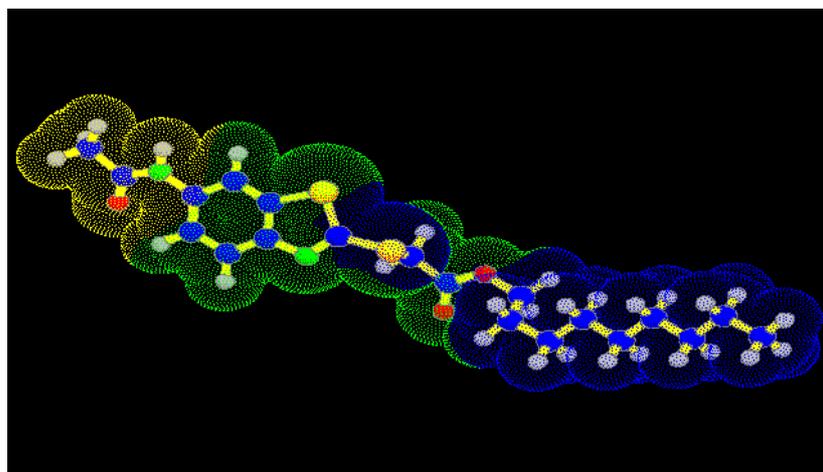
$IC_{50}$  = molar concentration of the inhibitor causing 50 % decrease of activity against the control.



2



9



12

**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**.

The synthesised compounds were tested for photosynthesis inhibiting activity, they inhibited the oxygen evolution rate (OER) in spinach chloroplasts. The photosynthesis - inhibiting activity was expressed by  $IC_{50}$  values, i.e. by molar concentrations causing a 50% decrease of OER with respect to the untreated control sample (Table 2). The dependence of photosynthesis inhibiting activity on the lipophilicity of the derivatives with R = n-alkyl and allyl showed a quasi-parabolic course with a maximum activity with the hexyl derivative ( $IC_{50} = 47 \mu\text{mol dm}^{-3}$ ). Whereas the biologically active compounds, in order to reach their site of action, must penetrate through several compartments of thylakoid membranes (e.g. a series of lipid bilayers separated by aqueous layers), the highest biological effect is shown by molecules with a suitable lipophilicity, which enables them to cross both the above mentioned compartments. The passage of the short-chain compounds through the thylakoid membranes is limited due to their too low partition coefficient, resulting in an insufficient number of inhibitors reaching the site of action in proteins situated on the inner side of thylakoid membranes. On the other hand, the long-chain compounds due to their strong interaction with membrane lipids, remain predominantly incorporated in the lipid part of the membrane without reaching and damaging the corresponding membrane proteins. Similar results were also obtained for the dependence of photosynthesis inhibiting activity on the lipophilicity of 2-alkylthio-6-aminobenzothiazoles [7], 6-acetamido-2-alkylthiobenzothiazoles [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 the photosystem 2, upstream of the site of diphenylcarbazine action, i.e. in the oxygen evolving complex [14, 15].

The influence of quantum chemical parameters obtained by the 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.

The various alkyl substituents do not substantially influence the distribution of the molecular electrostatic potential [17] or charge density at the atoms. For this reason the studied biological activity of compounds **1-13** does not depend upon the obtained quantum chemical parameters. The biological activity is substantially influenced by lipophilicity. Its parabolic dependence on the biological activity has high statistical significance.

$$\log(1/IC_{50}) = (3.3700 \pm 0.4596) \log P - (0.7102 \pm 0.0868) \log^2 P - (0.5934 \pm 0.5506)$$

$$f = 0.958 \quad s = 0.1874 \quad F = 33.6 \quad n = 9$$

Using the bilinear model [18, 19] gave better results, which were statistically more significant. For this model, the best value of the lipophilicity ( $\log P_0$ ) was also calculated.

$$\log(1/IC_{50}) = (1.3755 \pm 0.1251) \log P - (3.6472 \pm 0.3030) \log( +1) + (1.3108 \pm 0.2326)$$

$$f = 0.9799, \quad s = 0.13040, \quad F = 72.6,$$

$$n = 9, \quad \log P_0 = 2.71, \quad = 1.1690 \cdot 10^{-3}$$

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

## Experimental

### General

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

The oxygen evolution rate (OER) in spinach chloroplasts was determined spectrophotometrically (Specord UV VIS, Carl Zeiss, Jena, Germany) by the Hill reaction. The measurements were carried out in phosphate buffer (20 mmol, pH=7.2) containing sucrose ( $0.4 \text{ mol dm}^{-3}$ ),  $\text{MgCl}_2$  ( $5 \text{ mmol dm}^{-3}$ ) and NaCl ( $15 \text{ mmol dm}^{-3}$ ) using 2, 6-dichlorophenolindophenol as electron acceptor. All samples contained the same amount of chlorophyll ( $30 \mu\text{g cm}^{-3}$ ) and they were irradiated ( $\sim 100 \text{ W m}^{-2}$ ) from 10cm distance with a halogen lamp (250 W) using a water filter to exclude warming of the samples (suspension temperature  $22^\circ\text{C}$ ) [20]. The compounds were dissolved in DMSO because of their too low water solubility. The applied DMSO concentration (up to 5%) did not affect the OER.

### Results of $^1\text{H}$ 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=8.8Hz, 1H); 7.50 (H-6, dd, J=8.8 and 1.6 Hz, 1H); 2.09 ( $\text{COCH}_3$ , s, 3H).

-S- $\text{CH}_2\text{COOR}$  substituents

**1** : 4.31 (SCH<sub>2</sub>, s, 2H); 3.71 (OCH<sub>3</sub>, s, 3H).  
**2** : 4.29 (SCH<sub>2</sub>, s, 2H); 4.16 (OCH<sub>2</sub>, q, 2H); 1.20 (CH<sub>3</sub>, t, 3H).  
**3** : 4.29 (SCH<sub>2</sub>, s, 2H); 4.08 (OCH<sub>2</sub>, t, 2H); 1.60 (CH<sub>2</sub>, sx, 2H); 0.86 (CH<sub>3</sub>, t, 3H)  
**4** : 4.34 (SCH<sub>2</sub>, s, 2H); 4.65 (OCH<sub>2</sub>, d, J=5.1 Hz, 2H); 5.9 (=CH, m, 1H); 5.2 (=CH<sub>2</sub>, m, 2H)  
**5** : 4.35 (SCH<sub>2</sub>, s, 2H); 4.81 (OCH<sub>2</sub>, d, J=2.4 Hz, 2H); 3.58 (CH, t, J'2.4 Hz, 1H)  
**6** : 4.29 (SCH<sub>2</sub>, s, 2H); 4.12 (OCH<sub>2</sub>, t, 2H); 1.4 (CH<sub>2</sub>CH<sub>2</sub>, m, 4H); 0.82 (CH<sub>3</sub>, t, 3H)  
**7** : 4.25 (SCH<sub>2</sub>, s, 2H); 4.81 (CH, sx, 1H); 1.54 (CH<sub>2</sub>, qi, 2H); 1.17 (CH<sub>3</sub>, d, 3H); 0.83 (CH<sub>3</sub>, t, 3H).  
**8** : 4.28 (SCH<sub>2</sub>, s, 2H); 4.10 (OCH<sub>2</sub>, t, 2H); 1.5 (CH<sub>2</sub>, m, 2H); 1.3 (CH<sub>2</sub>, m, 4H); 1.19 (CH<sub>3</sub>, t, 3H).  
**9** : 4.27 (SCH<sub>2</sub>, s, 2H); 4.10 (OCH<sub>2</sub>, t, 2H); 1.5 (CH<sub>2</sub>, m, 2H); 1.2 (CH<sub>2</sub>, m, 6H); 0.80 (CH<sub>3</sub>, t, 3H).  
**10** : 4.27 (SCH<sub>2</sub>, s, 2H); 4.10 (OCH<sub>2</sub>, t, 2H); 1.5 (CH<sub>2</sub>, m, 2H); 1.2 (CH<sub>2</sub>, m, 8H); 0.82 (CH<sub>3</sub>, t, 3H).  
**11** : 4.27 (SCH<sub>2</sub>, s, 2H); 4.10 (OCH<sub>2</sub>, t, 2H); 1.5 (CH<sub>2</sub>, m, 2H); 1.2 (CH<sub>2</sub>, m, 10H); 0.83 (CH<sub>3</sub>, t, 3H).  
**12** : 4.27 (SCH<sub>2</sub>, s, 2H); 4.09 (OCH<sub>2</sub>, t, 2H); 1.5 (CH<sub>2</sub>, m, 2H); 1.1 (CH<sub>3</sub>, t, 12H); 0.84 (CH<sub>3</sub>, t, 3H).  
**13** : 4.36 (SCH<sub>2</sub>, s, 2H); 5.20 (OCH<sub>2</sub>Ph, 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] (5 mmol). After solidifying, the reaction mixture was boiled for 10 min with water, filtered off and washed with hot water.

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

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**Samples Availability:** Samples are available from the authors and MDPI.