Molecules **2001**, *6*, 603-613

molecules

ISSN 1420-3049
http://www.mdpi.org

Some Anilides of 2-Alkylthio- and 2-Chloro-6-Alkylthio-4-Pyridinecarboxylic Acids: Synthesis and Photosynthesis-Inhibiting Activity

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Received: 16 February 2001; in revised form 28 May 2001 / Accepted: 1 June 2001 / Published: 30 June 2001

Abstract: Many compounds containing a -CONH- group display photosynthesis inhibiting activity. Based on this structural feature, a group of anilides of 2-alkylthio- (**1b-4f**) or 2-chloro-6-alkylthio-4-pyridinecarboxylic acids (**5a-6c**) was synthesised. The prepared compounds were tested for their inhibition of the oxygen evolution rate (OER) in spinach chloroplasts. A quasi-parabolic dependence between photosynthesis-inhibiting activity and the lipophilicity of the compounds was determined for **1b-4f** as well as for **5a-6c**. The inhibitory activity of compounds **1b-4f** was higher than that of **5a-6c** for comparable lipophilicity values.

Keywords: 2-Alkylthio-4-pyridinecarboxylic acids; Anilides; Photosynthesis inhibition

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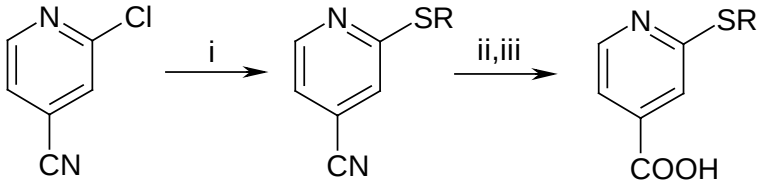
Introduction

Many herbicides acting as photosynthesis inhibitors possess in their molecules an >N-C(=X)- group (X=O or N, not S) and a hydrophobic residue in close vicinity to this group. Shipman concluded that the hydrophilic part of a herbicide binds electrostatically to the terminus of an α-helix at a highly charged amino acid, whereas the hydrophobic part of the inhibitors extends into the hydrophobic part of the membrane [1]. Recently, pronounced photosynthesis-inhibiting activity has been found for alkoxy substituted phenylcarbamates [2,3] as well as for the local anesthetic of the anilide type – trimecaine [4,5,6], i.e., for compounds with -CONH- groups in their molecules.

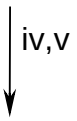
In order to bring together the structural features of the abovementioned compounds and our previous results with 2-alkyl-4-pyridinecaboxylic acids anilides [7], some anilides of 2-alkylthio-4-pyridinecarboxylic (**1b-4f**) and 2-chloro-6-alkylthio-4-pyridinecarboxylic acids (**5a-6c**) were synthesised.

Results and Discussion

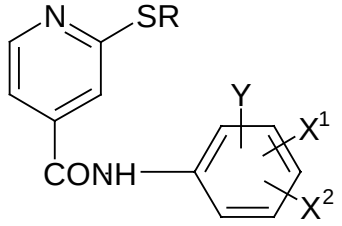
The synthesis of the anilides is shown in Schemes 1 and 2. The 2-alkylthio-4-cyanopyridines were synthesised as described previously [8]. Subsequent treatment with ethanolic sodium hydroxide solution afforded the corresponding acids **1-4**. 2-chloro-6-alkylthio-4-pyridinecarboxylic acids **5** and **6** were obtained by a similar procedure from 2-chloro-6-alkylthio-4-carbamoylpyridines [9]. The anilides were prepared from the acids by reaction of the corresponding acyl chlorides with substituted anilines and aminophenols (Scheme 3). The melting points, yields, and elemental analyses for compounds are given in Tables 2 and 4, and IR and ^{1}H-NMR spectroscopic data in Tables 3 and 5.



1-4

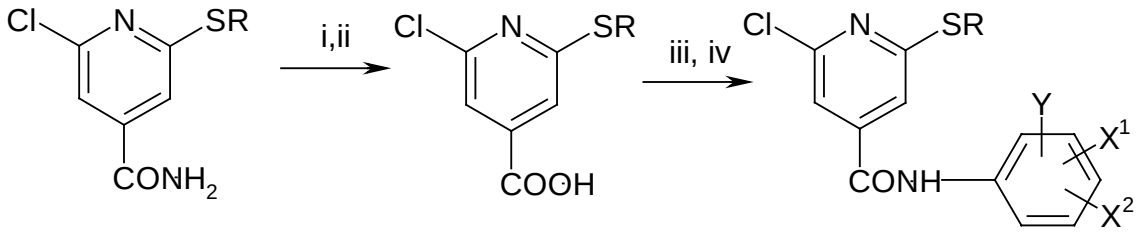


Reagents: i) $Na^+ {}^-SR$; ii) NaOH; iii) HCl;
iv) $SOCl_2$; v) substituted aniline



1b,d; 2b,f; 3a,b,d-f; 4a,b,e,f

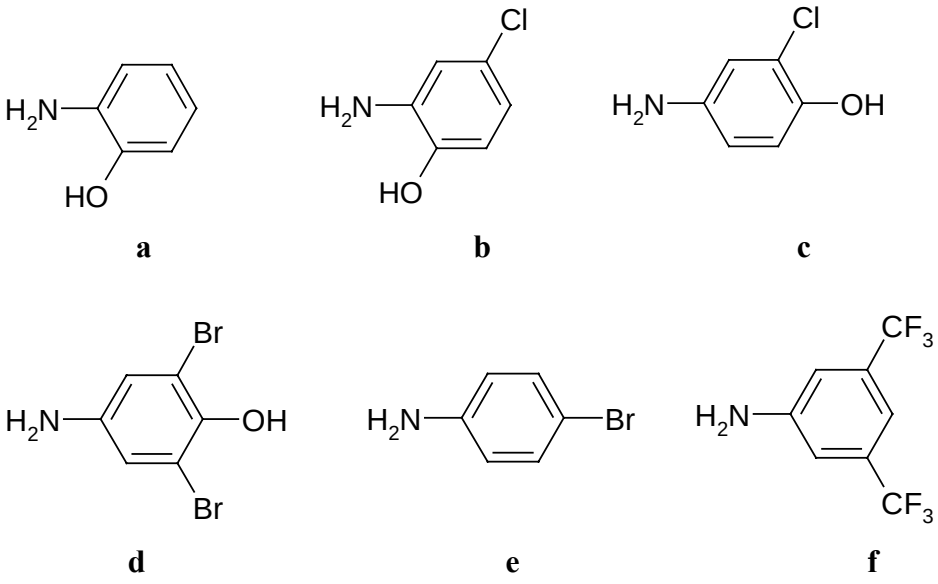
Scheme 1: Preparation of 2-alkylthio-4-pyridinecarboxylic acids and derived anilides.



5,6 **5a-c; 6a-c**

Reagents: i) NaOH; ii) HCl; iii) $SOCl_2$; iv) substituted aniline

Scheme 2: Preparation of 2-chloro 6-alkylthio-4-pyridinecarboxylic acids and derived anilides.



a **b** **c**

d **e** **f**

Scheme 3: Aminophenols and anilines used.

The biological activity of anilides of 2-alkylthio-4-pyridinecarboxylic acids (**1b-4f**) and 2-chloro-6-alkylthio-4-pyridinecarboxylic acids (**5a-6c**) with regards to inhibition of oxygen evolution rate in spinach chloroplasts was investigated. The inhibitory activity of the compounds has been expressed by IC_{50} values (Table 1). The IC_{50} values, i.e. molar concentrations of the compounds causing 50 % activity decrease with respect to the untreated control, varied in the range of 6.0 - 69.1 μmol.dm^{-3} for **1b-4f** and 14.2 - 32.5 μmol.dm^{-3}, respectively, for **5a-6c**.

A quasi-parabolic dependence of photosynthesis-inhibiting activity upon the lipophilicity (log P) of the compounds was determined for **1b-4f** as well as for **5a-6c** (Table 1). The comparison of the biological activity of compounds **1b-4f** and **5a-6c** having the same lipophilicity showed that the introduction of a halogen substituent in the 6 position led to a partial decrease of the biological activity. The previous study with anilides of 2-alkyl-4-pyridinecarboxylic acids showed that the site of their inhibitory action is the intermediate Z^+/D^+ corresponding to the tyrosine radicals Tyr_Z and Tyr_D

which are situated at 161th position in D_1 and D_2 proteins located on the donor side of photosystem (PS) 2 [10]. The same site of action in the photosynthetic apparatus of spinach chloroplasts can also be expected for the studied compounds **1b-4f** and **5a-6c.**

From the quasi-parabolic course of the dependence log ($1/IC_{50}$) *vs.* log P it can be assumed that the most active inhibitors are compounds with sufficiently high lipophilicity for securing their passage through the lipidic parts of the biological membranes, but enabling also their sufficiently high concentration in the aqueous phase. This is necessary for their interaction with the intermediates Z^+/D^+ situated at the lumenal side of photosynthetic membranes in D_1 and D_2 proteins [11].

Table 1. IC_{50} values for inhibition of oxygen evolution rate in spinach chloroplasts and calculated logP values of the compounds tested..

Compd.	$IC_{50} \cdot 10^6$ (mol.dm^{-3})	calculated logP	Compd.	$IC_{50} \cdot 10^6$ (mol.dm^{-3})	calculated logP
1b	7.3	4.55 ± 0.44	**4b**	6.0	5.13 ± 0.46
2b	8.0	4.90 ± 0.45	**4f**	35.2	7.01 ± 0.55
2f	13.9	6.79 ± 0.54	**5a**	32.5	4.03 ± 0.44
3a	12.7	4.24 ± 0.42	**5b**	14.2	5.41 ± 0.46
3b	4.8	5.62 ± 0.44	**5c**	16.2	4.64 ± 0.45
3d	11.3	6.66 ± 0.58	**6a**	19.7	4.57 ± 0.44
3f	69.1	7.50 ± 0.54	**6b**	18.3	5.94 ± 0.46
4a	10.3	3.75 ± 0.43	**6c**	14.2	5.17 ± 0.45

Acknowledgements

This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (Research Plan No. 111600001), by the Grant Agency of Charles University (Grant No. 26/1998-BCH), and by the Scientific Grant Agency VEGA of the Slovak Republic (No. 1/7262/20).

Experimental

General

Column chromatography was performed on silica gel (Silpearl, Kavalier Votice). Melting points were determined on a Kofler block, and are uncorrected. IR spectra were recorded on a Nicolet Impact 400 spectrometer in chloroform. ^{1}H-NMR spectra were determined for solutions in $CDCl_3$ or DMSO (substituted 4-pyridinecarboxylic acids) with a BS 587 (Tesla, Brno) 80 MHz apparatus or a Varian Mercury - Vx BB 300 spectrometer operating at 300 MHz. Chemical shifts were recorded as δ values in parts per million (ppm), and were indirectly referenced to tetramethylsilane via the solvent signal (7.26 for ^{1}H). Multiplicities are given together with the coupling constants (in Hz). Elemental analyses

were performed on a EA 1110 CHNS-O CE INSTRUMENTS elemental analyser. Lipophilicity of the compounds was computed using a program ACD/LogP version 1.0 (Advanced Chemistry Development Inc., Toronto).

Synthesis of 2-alkylthio-4-cyanopyridines and 2-phenylmethylthio-4-cyanopyridine.

2-Chloro-4-cyanopyridine (10 mmol) and the appropriate thiol (10 mmol) were dissolved in 10 mL of anhydrous N,N-dimethylformamide. To this solution sodium methoxide (10 mmol) in 5 mL methanol was added dropwise with stirring under a nitrogen atmosphere at 20 °C. The stirring was continued until TLC (6:1 hexane-ethyl acetate) indicated completion of the reaction. The reaction mixture was concentrated *in vacuo* and after evaporation of the solvents, the 2-alkylthio-4-cyanopyridines or 2-phenylmethylthio-4-cyanopyridine were distilled off from the oily product. The boiling points corresponded with the previously described [8].

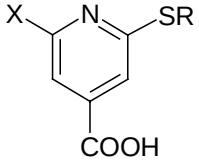
Synthesis of 2-chloro-6-alkylthio-4-carbamoylpyridines

2,6-Dichloro-4-carbamoylpyridine [13] (10 mmol) and the appropriate thiol (10 mmol) were dissolved in anhydrous *N,N*-dimethylformamide (10 mL). To the stirred solution sodium methoxide (10 mmol) in methanol (5 mL) was added dropwise. The reaction mixture was stirred at room temperature until TLC (2:1 hexane-ethyl acetate) indicated the reaction was complete. The mixture was then poured into cold water. The crude product was filtered off, purified by column chromatography (2:1 hexane-ethyl acetate), and recrystallised from aqueous ethanol. The boiling points and spectral data agreed with those previously described [9].

Synthesis of 2-alkylthio, 2-phenylmethylthio and 2-chloro-6-alkylthio-4-pyridinecarboxylic acids ***1-6***

The 2- or 2,6-substituted 4-cyano or carbamoylpyridine (10 mmol) in 10 mL of ethanol was mixed with 25% aqueous sodium hydroxide (30 mmol) and refluxed until the evolution of the ammonia ceased. The reaction mixture was then diluted with twice its volume of water and acidified with 10% hydrochloric acid to pH 4-5. The crude product was collected, washed with water, and recrystallised from aqueous ethanol. TLC for checking of the purity of final products was performed using hexane-ethyl acetate-acetic acid (50:45:5) as the mobile phase. The yields, melting points, and elemental analyses are given in Table 2, IR spectral data and ^{1}H-NMR chemical shifts in Table 3.

Table 2. Analytical data of the prepared 2-alkylthio- and 2-chloro-6-alkylthio-4-pyridinecarboxylic acids.



Compd.	Formula M. w.	R X	M. p. °C Yield %	Calculated / Found % C	% H	% N	%S	%Cl
1	$C_9H_{11}NO_2S$	C_3H_7	141-143	54.80	5.62	7.10	16.25	-
	197.3		80	54.55	5.79	6.95	16.08	
2	$C_{10}H_{13}NO_2S$	iC_4H_9	137-139	56.85	6.20	6.63	15.17	-
	211.3		78	56.61	6.41	6.46	14.93	
3	$C_{11}H_{15}NO_2S$	C_5H_{11}	135-137	58.64	6.71	6.22	14.23	-
	225.3		82	58.48	6.89	6.05	14.02	
4	$C_{13}H_{11}NO_2S$	$CH_2C_6H_6$	196-197 [a]	-	-	-	-	-
	245.3		76					
5	$C_9H_{10}ClNO_2S$	C_3H_7	119-120	46.66	4.35	6.05	13.84	15.30
	231.7	Cl	77	46.45	4.21	6.19	13.65	15.55
6	$C_{10}H_{12}ClNO_2S$	C_4H_9	93-95	48.88	4.92	5.70	13.05	14.43
	245.7	Cl	75	48.67	4.85	5.82	12.87	14.65

a) ref. [12] M. p. 195-196°C

Table 3. IR and ^{1}H-NMR spectroscopic data of the 2-alkylthio and 2-chloro-6-alkylthio-4-pyridinecarboxylic acids (DMSO).

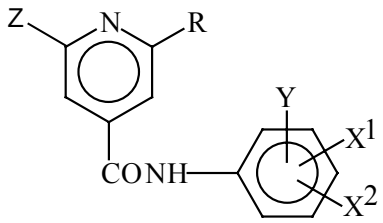
Compd.	IR (cm^{-1})	δ ^{1}H-NMR (ppm)
1	2975, 2935, 2890 (CH-aliph.) 2480 (COOH) 1725 (CO)	($CDCl_3$): 1.06 t, J=7.3 Hz, 3H, CH_3; 1.76 m, 2H, CH_2; 3.18 t, J= 7.3 Hz, 2H, SCH_2; 7.56 m, 1H, H-5; 7.78 m, 1H, H-3; 8.59 m, 1H, H-6 [a]
2	2960, 2930, 2870 (CH-aliph.) 2470 (COOH) 1725 (CO)	($CDCl_3$): 1.05 d, J=6.6 Hz, 6H, 2xCH_3; 1.96 m, 1H, CH<; 3.11 d, J= 6.9 Hz, 2H, SCH_2; 7,53 d, J=4.95 Hz, 1H, H-5; 7.78 m, 1H, H-3; 8.59 d, J=4.95 Hz, 1H, H-6 [a]
3	2970, 2925, 2860 (CH-aliph.) 2450 (COOH) 1730 (CO)	($CDCl_3$): 0.91 t, J=7 Hz, 3H, CH_3; 1.3 - 1.5 m, 4H, 2xCH_2; 1.73 m, 2H, CH_2; 3.19 t, J=7.3 Hz, 2H, SCH_2; 7.56 d, J=4.95 Hz, 1H, H-5; 7.79 m, 1H, H-3; 8.62 d, J=4.95 Hz, 1H, H-6 [a]
5	2970, 2934, 2874 (CH-aliph.) 2658 (COOH) 1705 (CO)	(DMSO-d_6): 1.05 t, J=7.2 Hz, 3H, CH_3; 1.47-2.10 m, 2H, CH_2; 3.18 t, J=7.1 Hz, 2H, SCH_2; 7.54 d, J=0.7 Hz, 1H, H-5; 7.67 d, J=0.7 Hz, 1H, H-3; 11.65 s, 1H, COOH
6	2962, 2933, 2873 (CH-aliph.) 2541 (COOH) 1707 (CO)	(DMSO-d_6): 0.96 t, J=6.2 Hz, 3H, CH_3; 1.21-1.98 m, 4H, CH_2; 3.20 t, J=7.1 Hz, 2H, SCH_2; 7.53 d, J=1.1 Hz, 1H, H-5; 7.66 d, J=1.1 Hz, 1H, H-3; 11.62 s, 1H, COOH

a) A COOH signal was not observed in the ^{1}H-NMR spectrum.

*General method for the synthesis of anilides of 2-alkylthio, 2-phenylmethylthio and 2-chloro-6-alkylthio-4-pyridinecarboxylic acids (**1b,d; 2b,f; 3a,b,d-f; 4a,b,e,f; 5a-c; 6a-c**).*

A mixture of the 2- or 2,6 substituted-4-pyridinecarboxylic acid (10 mmol) and thionyl chloride (15 mmol) in 10 mL of dry benzene was refluxed for about 1 h. The excess of thionyl chloride was removed by repeated evaporation of dry benzene solutions *in vacuo*. The resulting crude acyl chloride dissolved in 10 mL of dry acetone was added dropwise to a stirred solution of substituted aniline or aminophenol (10 mmol) in 10 mL of dry pyridine keeping the temperature at 10 °C. After addition of the aniline or aminophenol was complete, stirring at 10 °C was continued for another 30 min. The low temperature was essential in the case of aminophenols in order to avoid the partial esterification of acyl chloride. The reaction mixture was poured into 40 mL of cold water. Crude anilide was collected and recrystallised from aqueous ethanol. TLC was performed using hexane-ethyl acetate (50:50) as the mobile phase. The yields, melting points and elemental analyses of the anilides are given in Table 4, IR spectral data and ^{1}H-NMR chemical shifts in Table 5.

Table 4. Analytical data of the prepared anilides.



Compd.	Formula M. w.	R Y, Z	X^1 X^2	M. p. °C Yield %	Calculated / Found % C	% H	% N	% S	% Cl(Br,F)
1b	$C_{15}H_{15}ClN_2O_2S$	SC_3H_7	5′-Cl	161-163	55.81	4.68	8.68	9.93	10.98
	322.8	2′-OH, H	H	57	55.96	4.52	8.49	10.06	10.76
1d	$C_{15}H_{14}Br_2N_2O_2S$	SC_3H_7	3′-Br	152-153	40.38	3.16	6.28	7.19	35.82
	446.2	4′-OH, H	5′-Br	65	40.41	3.23	6.22	7.12	35.71
2b	$C_{16}H_{17}ClN_2O_2S$	S-*i*C_4H_9	5′-Cl	162-164	57.05	5.09	8.32	9.52	10.53
	336.8	2′-OH, H	H	58	57.11	5.07	8.27	9.56	10.45
2f	$C_{18}H_{16}F_6N_2OS$	S-*i*C_4H_9	3′-CF_3	164-165	51.18	3.82	6.63	7.59	26.99
	422.4	H, H	5′-CF_3	54	51.31	3.72	6.48	7.75	26.78
3a	$C_{17}H_{20}N_2O_2S$	SC_5H_{11}	H	123-125	64.53	6.37	8.85	10.13	-
	316.4	2′-OH, H	H	60	64.48	6.45	8.71	10.28	
3b	$C_{17}H_{19}ClN_2O_2S$	SC_5H_{11}	5′-Cl	153-155	58.19	5.46	7.98	9.14	10.10
	350.9	2′-OH, H	H	58	58.23	5.39	7.88	9.19	10.01
3d	$C_{17}H_{18}Br_2N_2O_2S$	SC_5H_{11}	3′-Br	120-122	43.06	3.83	5.91	6.76	33.70
	474.2	4′-OH, H	5′-Br	67	43.15	3.81	5.77	6.67	33.50
3e	$C_{17}H_{19}BrN_2OS$	SC_5H_{11}	4′-Br	94-95	53.83	5.05	7.39	8.45	21.07
	379.3	H, H	H	52	53.97	4.93	7.23	8.31	20.88

Table 4 (cont.)									
3f	$C_{19}H_{18}F_6N_2OS$	SC_5H_{11}	3′-CF_3	122-124	52.29	4.16	6.42	7.35	26.12
	436.4	H, H	5′-CF_3	56	52.16	4.21	6.36	7.21	25.95
4a	$C_{19}H_{16}N_2O_2S$	$SCH_2C_6H_5$	H	149-150	67.84	4.79	8.33	9.53	-
	336.4	2′-OH, H	H	60	67.57	4.97	8.09	9.75	
4b	$C_{19}H_{15}ClN_2O_2S$	$SCH_2C_6H_5$	5′-Cl	194-196	61.54	4.08	7.55	8.66	9.56
	370.9	2′-OH, H	H	78	61.65	3.86	7.41	8.72	9.38
4e	$C_{19}H_{15}BrN_2OS$	$SCH_2C_6H_5$	4′-Br	108-109	57.15	3.79	7.02	8.03	20.01
	339.3	H, H	H	55	57.31	3.71	6.87	8.14	19.85
4f	$C_{21}H_{14}F_6N_2OS$	$SCH_2C_6H_5$	3′-CF_3	141-143	55.26	3.09	6.14	7.02	24.98
	456.4	H, H	5′-CF_3	61	55.38	3.01	6.03	7.18	24.75
5a	$C_{15}H_{15}ClN_2O_2S$	SC_3H_7	H	138-139	55.81	4.68	8.68	9.93	10.98
	322.81	2′-OH, Cl	H	44	55.68	4.51	8.79	9.72	11.21
5b	$C_{15}H_{14}Cl_2N_2O_2S$	SC_3H_7	5′-Cl	152-153	50.43	3.95	7.84	8.97	19.85
	357.25	2′-OH, Cl	H	53	50.33	3.91	7.72	8.85	20.07
5c	$C_{15}H_{14}Cl_2N_2O_2S$	SC_3H_7	3′-Cl	144-146	50.43	3.95	7.84	8.97	19.85
	357.25	4′-OH, Cl	H	34	50.29	3.86	7.95	8.81	20.03
6a	$C_{16}H_{17}ClN_2O_2S$	SC_4H_9	H	123-125	57.05	5.09	8.32	9.52	10.53
	336.84	2′-OH, Cl	H	56	56.91	5.02	8.48	9.39	10.79
6b	$C_{16}H_{16}Cl_2N_2O_2S$	SC_4H_9	5′-Cl	158-160	51.76	4.34	7.55	8.63	19.10
	371.28	2′-OH, Cl	H	59	51.58	4.31	7.68	8.41	19.31
6c	$C_{16}H_{16}Cl_2N_2O_2S$	SC_4H_9	3′-Cl	115-117	51.76	4.34	7.55	8.63	19.10
	371.28	4′-OH, Cl	H	53	51.63	4.23	7.71	8.38	19.33

Table 5. IR and ^{1}H-NMR spectroscopic data of the prepared anilides.

Compd.	**IR (cm^{-1})**	**δ ^{1}H-NMR (ppm)**
1b	2965, 2932, 2873 (CH aliph.), 1651 (CO)	(DMSO-d_6): 1.00 t, J=7, 3H, CH_3; 1.66 m, 2H, CH_2; 3.18 t, J=7, 2H, SCH_2; 6.93 d, J=8.5, 1H, H-3′; 7.12 dd, J=8.5, J=2.4, 1H, H-4′; 7.53 dd, J=5.1, J=1.5, 1H, H-5; 7.73 qs, 2H, H-3 and H-6′; 8.60 d, J=5.1, 1H, H-6; 9.82 s, 1H, OH or NH; 10.08 s, 1H, NH or OH
1d	2975, 2945, 2890 (CH aliph.), 1650 (CO)	(DMSO-d_6): 1.00 t, J=7, 3H, CH_3; 1.75 m, 2H, CH_2; 3.18 t, J=7, 2H, SCH_2; 7.52 d, J=5.1, 1H, H-5; 7.71 s, 1H, H-3; 8,00 s, 2H, H-2′and H-6′; 8.62 d, J=5.1, 1H, H-6; 10.45 s, 1H, OH or NH; 10.51 s, 1H,NH or OH
2b	2975, 2940, 2890 (CH aliph.), 1655 (CO)	(DMSO-d_6): 1.01 d, J=7, 6H, 2xCH_3; 1.92 m, 1H, -CH<; 3.13 t, J=7.5, 2H, SCH_2; 6.95 d, J=8.5, 1H, H-3′; 7.13 dd, J=8.5, J=2.5, 1H, H-4′; 7.52 dd, J=5, J=1, 1H, H-5; 7.73 dd, J=1, J<1, 1H, H-3; 7.74 d, J=2.5, 1H, H-6′; 8.59 dd, J=5, J<1, 1H, H-6
2f	2962, 2929, 2869 (CH aliph.), 1662 (CO)	($CDCl_3$) 1.05 d, J=6.4, 6H, 2xCH_3; 1.94 m, 1H, CH; 3.14 d, J=6.7, 2H, SCH_2; 7.31 dd, J=5.2, J=1.5, 1H, H-5; 7.55 d, J=1.5, 1H H-3; 7.68 s, 1H, H-4′; 8.17 s, 3H, H-2′, H-6′ and NH; 8.57 d, J=5.2, 1H, H-6

Table 5 (cont.)

3a	2958, 2931, 2859 (CH aliph.), 1652 (CO)	(DMSO-d_6): 0.88 dist.t, CH_3; 1.38 m, 4H, 2xCH_2; 1.66 m, 2H, CH_2; 3.19 t, 2H, CH_2; 6.96 m, 3H, arom.; 7.55 m, 2H, H-5 and 1H arom.; 7.74 s, 1H, H-3; 8.59 d, J=5.1, 1H, H-6; 9.66 s, 1H, OH; 9.77 bs, 1H, NH
3b	2975, 2940, 2870 (CH aliph.), 1640 (CO)	(DMSO-d_6): 0.88 dist. t, 3H, CH_3; 1.36 m, 4H, $(CH_2)_2$; 1.63 m, 2H, CH_2; 3.20 t, J=7, 2H, SCH_2; 7.13 dd, J=8.5, J=2.5, 1H, H-4'; 7.53 dd, J=5, J=1.5, 1H, H-5; 7.72 dd, J=1.5, J=1, 1H, H-3; 7.75 d, J=2.5, 1H, H-6′; 8.61 dd, J=5, J=1, 1H, H-6
3d	2980, 2945, 2875 (CH aliph.), 1655 (CO)	(DMSO-d_6): 0.88 dist. t, 3H, CH_3; 1.37 m, 4H, $(CH_2)_2$; 1.66 m, 2H, CH_2; 3.19 t, J=7, 2H, SCH_2; 7.52 d, J=5.1, 1H, H-5; 7.71 s, 1H, H-3; 8,00 s, 2H, H-2′and H-6′; 8.61 d, J=5.1, 1H, H-6; 9.84 s, 1H, OH or NH; 10.44 s, 1H, NH or OH
3e	2952, 2926, 2852 (CH aliph.), 1658 (CO)	($CDCl_3$) 0.91 dist. t, 3H, CH_3; 1.38 m, 4H 2xCH_2; 1.71 m, 2H, CH_2; 3.19 t, J=7, 2H, SCH_2; 7.27 dd, J=5, J=1.5, 1H, H-5; 7.49 qs, 5H, H-3 and C_6H_4; 8.01 bs, 1 H, NH; 8.52 d, J= 5, 1H, H-6
3f	2958, 2930, 2858 (CH aliph.), 1669 (CO)	($CDCl_3$) 0.91 dist. t, 3H, CH_3; 1.38 m, 4H, 2xCH_2; 1.71 m, 2H, CH_2; 3.22 t, J=7, 2H, SCH_2; 7.32 dd, J=5.2, J=1.5, 1H, H-5; 7.55 d, J=1.5, 1H, H-3; 7.68 s, 1H, H-4′; 8.17 qs, 3H, H-2′, H-6′and NH; 8.58 d, J=5.2, 1H, H-6
4a	1660 (CO)	($CDCl_3$) 4.49 s, 2H, SCH_2; 6.95 m, 1H, H-4′ or H-5′; 7.03 m, 1H, H-3′ or H-6′; 7.16 m, 1H, H-5′ or H-4′; ca.7.25 m overlapped with the signal of solvent, H-6′ or H-3′; 7.27-7.45 m, 5H, C_6H_5; 7.39 overlapping dd, J=5.2 Hz, J=1.6 Hz, 1H, H-5; 7.58 m, 1H, H-3; 7.75 bs, 1H, NH; 8.14 bs, 1H, OH; 8.63 d, J=5.2 Hz, 1H, H-6
4b	1655 (CO)	($CDCl_3$) 4.49 s, 2H, SCH_2; 6.95 d, J=8.7 Hz, 1H, H-3′; 7.11 dd, J=8.7 Hz, J=2.5, 1H, H-4′; 7.27-7.45 m, 5H, C_6H_5; 7.37 overlapping dd, J=5.2 Hz, J=1.6 Hz, 1H, H-5; 7.55 m, 1H, H-6′; 7.56 m, 1H, H-3; 8.09 bs, 1H, NH; 8.64 dd, J=5.2 Hz, J=0.8 Hz,1H, H-6
4e	1653 (CO)	($CDCl_3$) 4.45 s, 2H, SCH_2; 7.31 m, 6H, H-5 and C_6H_5; 7.45 qs, 5H, H-3 and C_6H_4; 7.98 bs, 1 H, NH; 8.53 d, J= 5.2, 1H, H-6
4f	1668 (CO)	($CDCl_3$) 4.46 s, 2H, SCH_2; 7.36 m, 7H, H-3, H-5 and C_6H_5; 7.67 s, 1H, H-4′; 8.10 s, 2H, H-2′and H-6′; 8.24 bs, 1 H, NH; 8.57 d, J=5.2, 1H, H-6
5b	2965, 2931, 2872 (CH aliph.), 1655 (CO)	(DMSO-d_6): 0.86-1.33 m, 3H, CH_3; 1.50-2,02 m, 2H, CH_2; 3.20 t, J=7.2 Hz, 2H, CH_2; 6.90 d, J=8.5 Hz, 1H, H-3′; 7.00-7.23 m, 1H, NH; 7.10 dd, J_1=2.2 Hz, J_2=8.5 Hz, 1H, H-4′; 7.34 d, J=1.2 Hz, 1H, H-5; 7.46 d, J=1.2 Hz, 1H, H-3; 7.69 d, J=2.2 Hz, 1H, H-6′; 8.18 s, 1H, -OH
5c	2965, 2931, 2872 (CH aliph.), 1654 (CO)	(DMSO-d_6): 0.90-1.18 m, 3H, CH_3; 1.46-2.04 m, 2H, CH_2; 3.18 t, J=7.1 Hz, CH_2; 5.57 s, 1H, NH; 6.98 d, J=8.8 Hz, 1H, H-5′; 7.28 dd, J_1=2.4 Hz, J_2=8.8 Hz, 1H, H-6′; 7.29 d, J=1.2 Hz, 1H, H-5; 7.41 d, J=1.2 Hz, 1H, H-3; 7.73 d, J=2.4 Hz, 1H, H-2′; 7.83 s, 1H, OH

Table 5 (cont.)		
6a	2958, 2931, 2872 (CH aliph.), 1646 (CO)	(DMSO-d_6): 0.78-1.10 m, 3H, CH_3; 1.5-1.95 m, 4H, CH_2CH_2; 3.18 t, J=7.0 Hz, 2H, CH_2; 6.70-7.26 m, 3H, H-4´, H-5´, H-6´; 7.32 d, J=1.0 Hz, 1H, H-5; 7.43 d, J=1,2 Hz, 1H, H-3; 7.58 d, J=7.8 Hz, 1H, H-3´; 7.72 s, 1H, NH; 8.47 s, 1H, OH
6b	2959, 2931, 2872 (CH aliph.), 1656 (CO)	(DMSO-d_6): 0.73-1.32 m, 3H, CH_3; 1.50-2.02 m, 4H, CH_2CH_2; 3.22 t, J=7.2 Hz, 2H, CH_2; 6.90 d, J=8.3 Hz, 1H, H-3´; 7.00-7.23 m, 1H, NH; 7.10 dd overlapped by another signal, J_1=2.2 Hz, J_2=8.5 Hz, 1H, H-4´; 7.35 d, J=1.2 Hz, 1H, H-5; 7.45 d, J=1.2 Hz, 1H, H-3; 7.69 d, J=2.2 Hz, 1H, H-6´; 8.14 s, 1H, OH
6c	2959, 2930, 2872 (CH aliph.), 1649 (CO)	(DMSO-d_6): 0.96 t, J=6.4 Hz, 3H, CH_3; 1.14-1.93 m, 4H, CH_2CH_2; 3.19 t, J=7.1 Hz, CH_2; 5.62 s, H, NH; 7.27 dd ovelapped by d, J_1=2.4 Hz, J_2=8.8 Hz, 2x1 H, H-5´, H-6´; 7.29 d, J=1.2 Hz, 1H, H-5; 7.40 d, J=1.2 Hz, 1H, H-3; 7.73 d, J=2.4 Hz, 1H, H-2´; 7.91 s, 1H, OH

Biological assays

Chloroplasts were prepared from locally purchased spinach according to the procedure described by Walker [14]. The oxygen evolution rate (OER) in spinach chloroplasts was determined spectrophotometrically (Specord UV VIS Zeiss Jena, Germany) by the Hill reaction. The measurements were carried out in phosphate buffer (20 mmol, pH = 7.2) containing sucrose (0.4 $mol.dm^{-3}$), $MgCl_2$ (5 $mmol.dm^{-3}$) and NaCl (15 $mmol.dm^{-3}$) using 2,6-dichlorophenol-indophenol as electron acceptor.

Chlorophyll content in the samples was 30 $mg.dm^{-3}$ and the samples were irradiated (~ 100 $W.m^{-2}$) from 10cm distance with a halogen lamp (250 W) using a water filter to prevent warming of the samples (suspension temperature 22 ^oC). The compounds were dissolved in dimethyl sulfoxide (DMSO) because of their limited water solubility. The applied DMSO concentration (up to 5 %) did not affect OER.

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Sample availability: Supporting samples of the following compounds are available at MDPI: **5**, **1b**, **2f**, **3b**, **3e**, **3f**, **5a-c**, **6a-c**.