Third International Electronic Conference on Synthetic Organic Chemistry (ECSOC-3), www.reprints.net/ecsoc-3.htm, September 1-30, 1999

[C0005]

Synthesis of some anilides of 2-alkylsulfanyl- and 2-chloro-6-alkylsulfanyl-4-pyridinecarboxylic acids and their photosynthesis-inhibiting activity

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Received: 16 August 1999 / Uploaded: 21 August 1999

Abstract: A group of anilides of 2-alkylsulfanyl- (*I*) or 2-chloro-6-alkylsulfanyl-4-pyridinecarboxylic acids (*II*) was synthesised. The prepared compounds were tested for inhibition of photosynthesis upon oxygen evolution rate in spinach chloroplasts. The IC_{50} values varied in the range of 6.0--69.1 μmol.dm^{-3} for *I* and 14.2--32.5 μmol.dm^{-3} for *II*. The inhibitory activity of *I* and *II* showed a quasi-parabolic dependence upon the lipophilicity (log P) of the compounds. For compounds with comparable lipophilicity the presence of chloro substituent in position 2 of the pyridine moiety led to the decrease of inhibitory activity.

Keywords: 2-Alkylsulfanyl-4-pyridinecarboxylic acids; Anilides; Photosynthesis inhibition.

Introduction

Studies of relationship between the chemical structure and biological activity have shown that number of herbicides acting as photosynthesis inhibitors possess in their molecules an >N-C(=X)- group, where X = O or N, not S, and a hydrophobic moiety in close vicinity to this group [1,2]. According to Shipman [3,4] the hydrophilic part of a herbicide binds electrostatically to the terminus of an alfa-helix at a highly charged amino acid, whereby the hydrophobic part of the inhibitors extends into the hydrophobic part of the membrane. Recently, pronounced photosynthesis-inhibiting activity has been reported for alkoxysubstituted phenylcarbamates [5,6], as well as for the local anesthetic of anilide type -- trimecaine [7,8,9], i. e., for compounds with -CONH- group in their molecules.

Continuing our previous work on anilides of 2-alkyl-4-pyridinecaboxylic acids [10], we report the results of photosynthesis-inhibiting activity of new anilides of 2-alkylsulfanyl-4-pyridinecarboxylic (*I*) acids and 2-chloro-6-

alkylsulfanyl-4-pyridinecarboxylic acids (*II*).

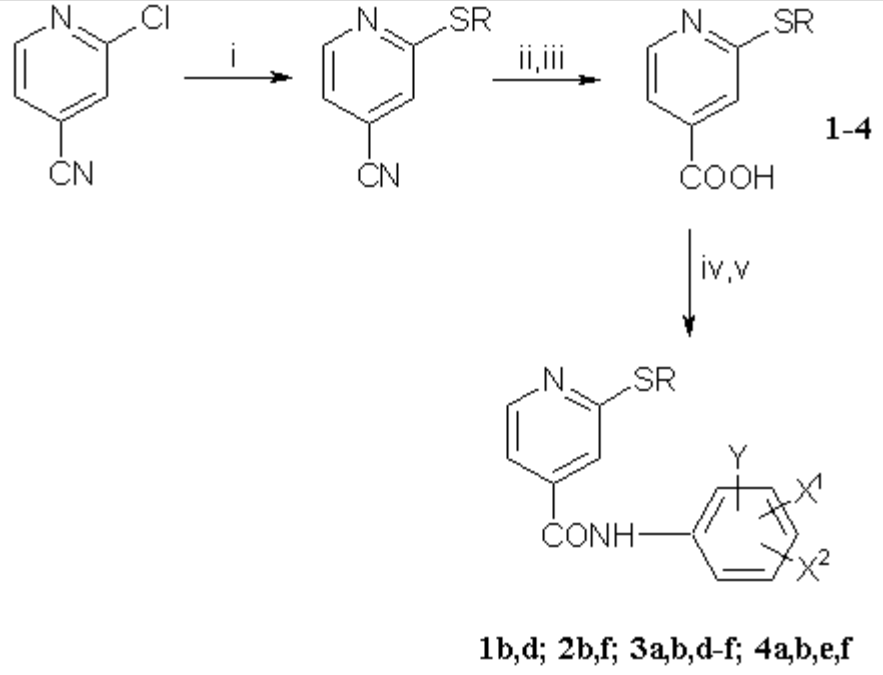
Results and Discussion

The synthesis of anilides is shown in Schemes 1 and 2.

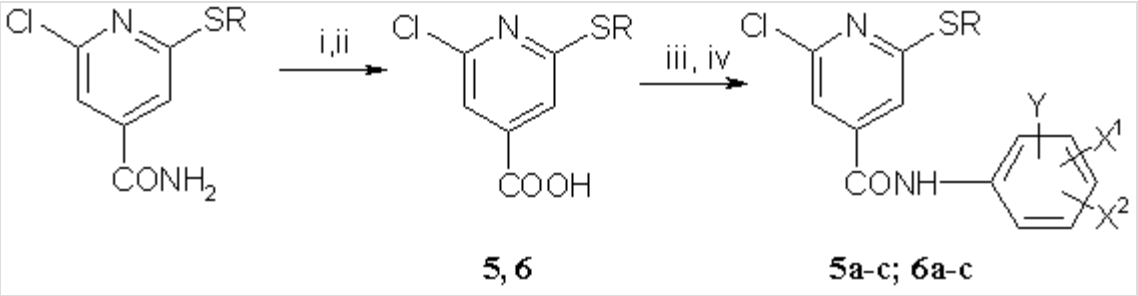
Starting from 2-chloro-4-cyanopyridine, 2-alkylsulfanyl-4-cyanopyridines were synthesised as described previously [11]. Subsequent treatment with ethanolic sodium hydroxide solution afforded corresponding acids **1--4**. The acids were converted directly to anilides via the corresponding acyl chlorides (Scheme 1) by reaction with substituted anilines or aminophenols (Fig. 1).

The reaction of 2,6-dichloro-4-amidopyridine with the appropriate thiolate gave the corresponding 2-chloro-6-alkylsulfanyl-4-amidopyridines [12], which were subsequently hydrolyzed to 2-chloro-6-alkylsulfanyl-4-pyridinecarboxylic acids **5** and **6**. The anilides were prepared from the acids in a manner analogous to that described above (Scheme 2).

The melting points, yields, and elemental analyses for the compounds prepared are given in Tables 1 and 2, and the IR and ^{1}H NMR spectral data in Tables 3 and 4.



Scheme 1: Preparation of 2-alkylsulfanyl-4-pyridinecarboxylic acids and related anilides (*I*).
i) Na^+ $^-$SR; ii) NaOH; iii) HCl; iv) $SOCl_2$; v) substituted aniline



Scheme 2: Preparation of 2-chloro-6-alkylsulfanyl-4-pyridinecarboxylic acids and related anilides (*II*).
i) NaOH; ii) HCl; iii) $SOCl_2$; iv) substituted aniline

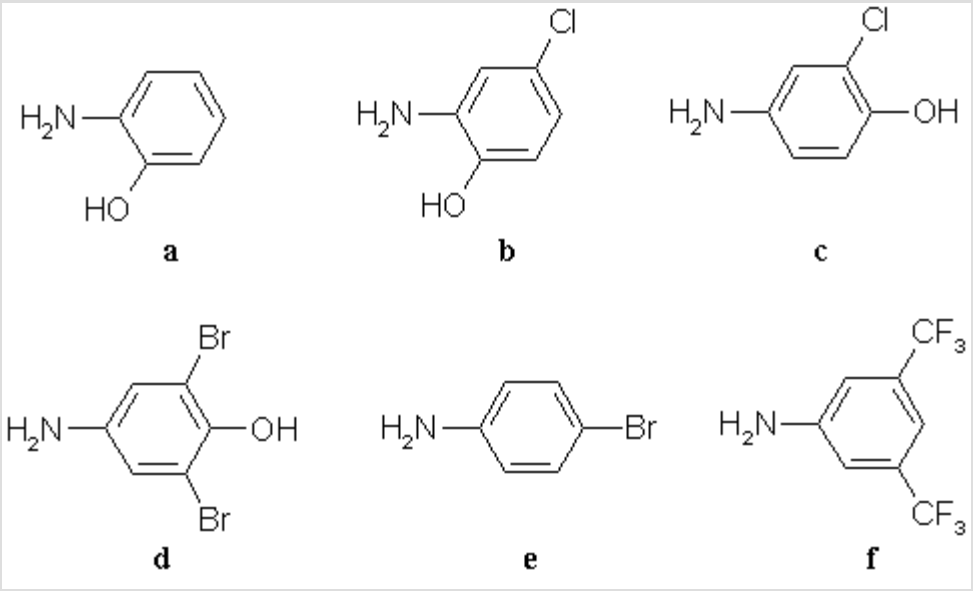
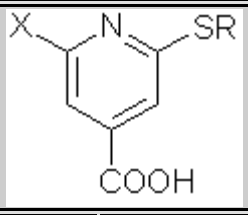


Fig. 1: Aminophenols and anilines used.

Biological activity of anilides of 2-alkylsufanyl-4-pyridinecarboxylic acids (*I*) and 2-chloro-6- alkylsulfanyl-4-pyridinecarboxylic acids (*II*) concerning inhibition of oxygen evolution rate in spinach chloroplasts was investigated. The inhibitory activity of the compounds has been expressed as IC_{50} values (Table 5). The IC_{50} values varied in the range of 6.0--69.1 μmol.dm^{-3} for the compounds *I* and 14.2--32.5 μmol.dm^{-3} for the compounds *II*.

The inhibitory activity of *I* and *II* showed a quasi-parabolic dependence upon the lipophilicity (log P) of the compounds. The comparison of the biological activity of compounds *I* and *II* having the same lipophilicity showed that the introduction of halogeno substituent in the position 6 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 [13]. The same site of action in the photosynthetic apparatus of spinach chloroplasts can be expected also for the studied compounds *I* and *II*. 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.

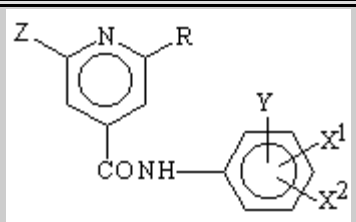
Table 1. Analytical data of the prepared 2-alkylsulfanyl- and 2-chloro-6-alkylsulfanyl-4-pyridinecarboxylic acids.



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

a) Ref. [14] m. p. 195--196°C)

Table 2. 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	$C_{15}H_{15}ClN_2O_2S$ 322.8	SC_3H_7 2'-OH, H	5'-Cl H	161--163 57	55.81 55.96	4.68 4.52	8.68 8.49	9.93 10.06	10.98 10.76
1d	$C_{15}H_{14}Br_2N_2O_2S$ 446.2	SC_3H_7 4'-OH, H	3'-Br 5'-Br	152--153 65	40.38 40.41	3.16 3.23	6.28 6.22	7.19 7.12	35.82 35.71
	$C_{16}H_{17}ClN_2O_2S$	$S\text{-}iC_4H_9$	5'-Cl	162--164	57.05	5.09	8.32	9.52	10.53

2b	336.8	2'-OH, H	H	58	57.11	5.07	8.27	9.56	10.45
2f	$C_{18}H_{16}F_6N_2OS$ 422.4	S-*i*C_4H_9 H, H	3'-CF_3 5'-CF_3	164--165 54	51.18 51.31	3.82 3.72	6.63 6.48	7.59 7.75	26.99 26.78
3a	$C_{17}H_{20}N_2O_2S$ 316.4	SC_5H_{11} 2'-OH, H	H H	123--125 60	64.53 64.48	6.37 6.45	8.85 8.71	10.13 10.28	--
3b	$C_{17}H_{19}ClN_2O_2S$ 350.9	SC_5H_{11} 2'-OH, H	5'-Cl H	153--155 58	58.19 58.23	5.46 5.39	7.98 7.88	9.14 9.19	10.10 10.01
3d	$C_{17}H_{18}Br_2N_2O_2S$ 474.2	SC_5H_{11} 4'-OH, H	3'-Br 5'-Br	120--122 67	43.06 43.15	3.83 3.81	5.91 5.77	6.76 6.67	33.70 33.50
3e	$C_{17}H_{19}BrN_2OS$ 379.3	SC_5H_{11} H, H	4'-Br H	94--95 52	53.83 53.97	5.05 4.93	7.39 7.23	8.45 8.31	21.07 20.88
3f	$C_{19}H_{18}F_6N_2OS$ 436.4	SC_5H_{11} H, H	3'-CF_3 5'-CF_3	122--124 56	52.29 52.16	4.16 4.21	6.42 6.36	7.35 7.21	26.12 25.95
4a	$C_{19}H_{16}N_2O_2S$ 336.4	S$CH_2C_6H_5$ 2'-OH, H	H H	149--150 60	67.84 67.57	4.79 4.97	8.33 8.09	9.53 9.75	--
4b	$C_{19}H_{15}ClN_2O_2S$ 370.9	S$CH_2C_6H_5$ 2'-OH, H	5'-Cl H	194--196 78	61.54 61.65	4.08 3.86	7.55 7.41	8.66 8.72	9.56 9.38
4e	$C_{19}H_{15}BrN_2OS$ 339.3	S$CH_2C_6H_5$ H, H	4'-Br H	108--109 55	57.15 57.31	3.79 3.71	7.02 6.87	8.03 8.14	20.01 19.85
4f	$C_{21}H_{14}F_6N_2OS$ 456.4	S$CH_2C_6H_5$ H, H	3'-CF_3 5'-CF_3	141--143 61	55.26 55.38	3.09 3.01	6.14 6.03	7.02 7.18	24.98 24.75
5a	$C_{15}H_{15}ClN_2O_2S$ 322.81	SC_3H_7 2'-OH, Cl	H H	138--139 44	55.81 55.68	4.68 4.51	8.68 8.79	9.93 9.72	10.98 11.21
5b	$C_{15}H_{14}Cl_2N_2O_2S$ 357.25	SC_3H_7 2'-OH, Cl	5'-Cl H	152--153 53	50.43 50.33	3.95 3.91	7.84 7.72	8.97 8.85	19.85 20.07
5c	$C_{15}H_{14}Cl_2N_2O_2S$ 357.25	SC_3H_7 4'-OH, Cl	3'-Cl H	144--146 34	50.43 50.29	3.95 3.86	7.84 7.95	8.97 8.81	19.85 20.03
6a	$C_{16}H_{17}ClN_2O_2S$ 336.84	SC_4H_9 2'-OH, Cl	H H	123--125 56	57.05 56.91	5.09 5.02	8.32 8.48	9.52 9.39	10.53 10.79
6b	$C_{16}H_{16}Cl_2N_2O_2S$ 371.28	SC_4H_9 2'-OH, Cl	5'-Cl H	158--160 59	51.76 51.58	4.34 4.31	7.55 7.68	8.63 8.41	19.10 19.31
6c	$C_{16}H_{16}Cl_2N_2O_2S$ 371.28	SC_4H_9 4'-OH, Cl	3'-Cl H	115--117 53	51.76 51.63	4.34 4.23	7.55 7.71	8.63 8.38	19.10 19.33

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

Compd.	IR (cm^{-1})	delta ^{1}H NMR (ppm)

1	2975, 2935, 2890 (CH-aliph.) 2480 (COOH) 1725 (CO)	a)
2	2960, 2930, 2870 (CH-aliph.) 2470 (COOH) 1725 (CO)	a)
3	2970, 2925, 2860 (CH-aliph.) 2450 (COOH) 1730 (CO)	a)
5	2970, 2934, 2874 (CH-aliph.) 2658 (COOH) 1705 (CO)	11.65 s, 1H, COOH; 7.67 d, J=0.7 Hz, 1H, H-3; 7.54 d, J=0.7 Hz, 1H, H-5; 3.18 t, J=7.1 Hz, 2H, CH_2S; 1.47-2.10 m, 2H, CH_2; 1.05 t, J=7.2 Hz, 3H, CH_3
6	2962, 2933, 2873 (CH-aliph.) 2541 (COOH) 1707 (CO)	11.62 s, 1H, COOH; 7.66 d, J=1.1 Hz, 1H, H-3; 7.53 d, J=1.1 Hz, 1H, H-5; 3.20 t, J=7.1 Hz, 2H, CH_2S; 1.21-1.98 m, 4H, CH_2; 0.96 t, J=6.2 Hz, 3H, CH_3

a) ^{1}H NMR spectra of 2-alkylsulfanyl-4-pyridinecarboxylic acids were not measured.

Table 4. IR and ^{1}H NMR spectral data of the prepared anilides.

Compd.	IR (cm^{-1})	delta ^{1}H NMR (ppm)
1b	2965, 2932, 2873 (CH aliph.) 1651 (CO)	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)	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)	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
3a	2958, 2931, 2859 (CH aliph.) 1652 (CO)	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)	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
	2980, 2945, 2875 (CH aliph.)	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,

3d	1655 (CO)	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)	a)
4b	1655 (CO)	a)
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
5a	2964, 2931, 2873 (CH aliph.) 1647 (CO)	1.06 t, J=7.2 Hz, 3H, CH_3; 1.49-2.01 m, 2H, CH_2; 3.19 t, J=7.1 Hz, 2H, CH_2S; 6.72-7.24 m, 3H, H-4', H-5', H-6'; 7.35 d, J=1.2 Hz, 1H, H-5; 7.37-7.64 m, 2H, -NH-, H-3'; 7.64 d, J=1.2 Hz, 1H, H-3; 8,23 s, 1H, OH
5b	2965, 2931, 2872 (CH aliph.) 1655 (CO)	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)	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
6a	2958, 2931, 2872 (CH aliph.) 1646 (CO)	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)	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; overlapping with 7.10 dd, 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)	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, 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

a) ^{1}H NMR spectra of the compounds were not measured

Table 5. IC_{50} values concerning inhibition of oxygen evolution rate in spinach chloroplasts by the tested compounds and calculated logP values of the compounds.

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

Experimental

General

The melting points were determined on a Kofler apparatus and are uncorrected. The IR spectra were recorded on a Nicolet Impact 400 spectrometer in chloroform; the wavenumbers are given in cm^{-1}. The ^1H NMR spectra were determined in $CDCl_3$ or DMSO solutions with TMS as the internal standard with a BS 587 (Tesla, Brno) 80 MHz apparatus. Column chromatography was performed on silica gel (Silpearl, Kavalier Votice). Elemental analyses were performed on an 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-alkylsulfanyl-4-cyanopyridines and 2-phenylmethylsulfanyl-4-cyanopyridine

2-Chloro-4-cyanopyridine (10 mmol) and appropriate thiol (10 mmol) were dissolved in 10 mL of anhydrous N,N-dimethylformamide. To the stirred solution, sodium methoxide (10 mmol) in of 5 mL methanol was added dropwise at 20 °C under a nitrogen atmosphere. The stirring continued until TLC (hexane : ethyl acetate, 6:1) indicated a complete reaction. The solvents were evaporated under reduced pressure and the 2-alkylsulfanyl-4-cyanopyridines or 2-phenylmethylsulfanyl-4-cyanopyridine were distilled off from the oily residue. The boiling points of the products were identical to those described previously [11].

Synthesis of 2-chloro-6-alkylsulfanyl-4-amidopyridines

2,6-Dichloro-4-amidopyridine [15] (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 (hexane : ethyl acetate 2:1) indicated a complete reaction. The mixture was then poured into cold water. The crude product was filtered off, purified by column chromatography (hexane : ethyl acetate, 2:1), and crystallised from aqueous ethanol. The boiling points and spectral data of the products were identical to those described previously [12].

*Synthesis of 2-alkylsulfanyl-, 2-phenylmethylsulfanyl- and 2-chloro-6-alkylsulfanyl-4-pyridinecarboxylic acids **1--6***

2-alkylsulfanyl-4-cyanopyridine or 2-chloro-6-alkylsulfanyl-4-amidopyridine (10 mmol) in 10 mL of ethanol was mixed with 25% aqueous solution of sodium hydroxide (30 mmol) and refluxed until the evolution of the ammonia ceased. Reaction mixture was then diluted with twice as high volume of water and acidified with 10% hydrochloric acid to pH 4--5. The crude product was collected, washed with water, and crystallised 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 1, IR spectral data and ^{1}H NMR chemical shifts in Table 3.

*Synthesis of anilides of 2-alkylsulfanyl, 2-phenylmethylsulfanyl and 2-chloro-6-alkylsulfanyl-4-pyridinecarboxylic acids **1b,d; 2b,f; 3a,b,d-f; 4a,b,e,f; 5a-c; 6a-c***

A mixture of 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. Excess of thionyl chloride was removed by repeated evaporation of dry benzene in vacuo. 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 aniline or aminophenol was complete, stirring at 10 °C continued for another 30 min. Using aminophenol as a reagent, the low temperature was essential 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 crystallised from aqueous ethanol. TLC was performed in hexane -- ethyl acetate (50:50) as the mobile phase. The yields, melting points and elemental analyses of anilides are given in Table 2, IR spectral data and ^{1}H NMR chemical shifts in Table 4.

Biological assays

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 °C). 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.

Acknowledgements. This study was supported by the Grant Agency of Charles University (Grant No. 26/1998) and by Scientific Grant Agency of Ministry of Education, Slovak Republic (grant No. 1/4013/97). Authors thank to Tomas Vojtisek for transformation of the poster into html format.

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