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Synthesis of some anilides of substituted pyrazine-2-carboxylic acids and their photosynthesis-inhibiting activity

Martin Dolezal^{1a*}, Miroslav Miletin¹, Jiri Hartl¹, Katarina Kralova², Jiri Kunes³

¹Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy, Charles University, 500 05 Hradec Kralove, Czech Republic

^aE-mail: dolezalm@faf.cuni.cz, Tel.: +420 49 5067272, Fax: +420 49 5512423

²Institute of Chemistry, Faculty of Natural Sciences, Comenius University, 842 15 Bratislava, Slovak Republic

³Department of Inorganic and Organic Chemistry, Faculty of Pharmacy, Charles University, 500 05 Hradec Kralove, Czech Republic

* Author to whom correspondence should be addressed.

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Abstract: Condensation of chlorides of substituted pyrazine-2-carboxylic acids with ring substituted anilines yielded a series of anilides of 6-chloropyrazine-2-carboxylic, 5-(1,1-dimethylethyl)-pyrazine-2-carboxylic or 6-chloro-5-(1,1-dimethylethyl)pyrazine-2-carboxylic acids. Products were tested for their photosynthesis-inhibiting activity. The most active inhibitor of oxygen evolution rate in spinach chloroplasts was 3,5-bis(trifluoromethyl)anilide of 6-chloropyrazine-2-carboxylic acid ($IC_{50} = 0.026 \text{ mmol dm}^{-3}$), the most active antialgal compound was 3-methylanilide of 6-chloro-5-(1,1dimethylethyl)pyrazine-2-carboxylic acid ($IC_{50} = 0.063 \text{ mmol dm}^{-3}$). The elimination of phenolic group in the benzene moiety led to an decreased photosynthesis-inhibitory activity.

Keywords: Anilides of 6-chloro-5-alkylpyrazine-2-carboxylic acids; Photosynthesis inhibition; Spinach chloroplasts; *Chlorella vulgaris*.

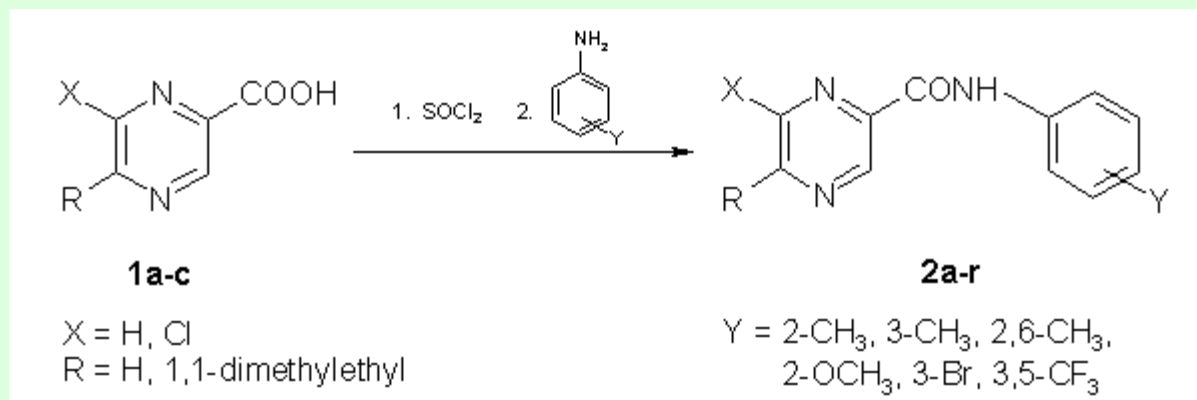
Introduction

Various compounds possessing -NHCO- group, *e. g.* acyl and thioacyl anilides, benzanilides, phenyl carbamates, etc., were found to inhibit photosynthetic electron transport [1--4]. Anilides of 2-alkylpyridine-4-carboxylic [5,6], 2-alkylsulfanyl-4-pyridinecarboxylic [6,7] acids inhibited oxygen evolution rate in *Chlorella vulgaris* and their inhibitory activity depended on the lipophilicity of the compounds. Several esters of alkoxy substituted phenylcarbamic acids (APA) showed the antialgal activity against *Chlorella vulgaris* [8--10]. The inhibitory efficiency of APA concerning chlorophyll production in *Chlorella vulgaris* depended on the lipophilicity of the alkoxy substituent and also on its position on the aromatic ring [8--10]. The antialgal activity of APA correlated with the antifungal activity of these compounds against *Candida albicans* [10]. We have recently reported the synthesis of a series of anilides prepared from some or 5-alkylpyrazine-2-carboxylic acids and some aminophenols [11].

The presented study is concerned in the synthesis of another series of anilides prepared from substituted pyrazine-2-carboxylic acids and alkylated, resp. alkoxyated (2-, 3-methyl; 2,6-dimethyl, 2-methoxy) or halogenated (3-bromo; 3,5-bis(trifluoromethyl)) anilines. The aim of this work is to search for the structure--activity relationships and to determine the importance of increased hydrophobic properties for photosynthesis-inhibiting evaluation of newly prepared pyrazine-2-carboxylic acid anilides.

Results and Discussion

The synthesis of anilides is shown in Scheme 1. Condensation of chlorides of 6-chloropyrazine-2-carboxylic (**1a**) [12], 5-(1,1-dimethylethyl)pyrazine-2-carboxylic (**1b**) [11] or 6-chloro-5-(1,1-dimethylethyl)pyrazine-2-carboxylic (**1c**) [11] acids with ring substituted anilines yielded a series of 18 anilides of mentioned substituted pyrazine-2-carboxylic acids **2a--r**.



Scheme 1: Preparation of anilides of substituted pyrazine-2-carboxylic acids **2a--r**.

The melting points, yields, and elemental analyses for the compounds prepared are given in Table 1, and the IR and ^1H NMR spectral data in Table 2. Calculated $\log P$ values of anilides **2a--r** are shown in Table 3.

Biological activity of prepared anilides **2a--r** concerning inhibition of oxygen evolution rate in spinach chloroplasts was investigated. The inhibitory activity of the compounds has been expressed as IC_{50} values (see Table 3).

Additionally some inhibition of chlorophyll production in green algae *Chlorella vulgaris* was studied at the anilides **2f**, **2l**, **2m**, **2n**, **2o** and **2p**. Results of their antialgal activity are given in Table 4.

The studied compounds inhibited photosynthetic electron transport in spinach chloroplasts what was reflected in the inhibition of oxygen evolution rate. The IC_{50} values varied in the range from 0.026 (**2m**) to 1.072 mmol dm^{-3} (**2a**). In general, the photosynthesis-inhibiting activity of the studied compounds depended on their lipophilicity showing a quasi-parabolic course. However, the studied compounds could be divided into two group. The compounds with 2- CH_3 substituent on the phenyl ring (**2a**, **2b**, **2c**, **2p**, **2q** and **2r**) had lower biological activity than the other investigated compounds with comparable $\log P$ values. Consequently, we assume that the methyl substituent in *ortho* position of the benzene ring is disadvantageous from the viewpoint of interactions with the photosynthetic apparatus. On the other hand, compound **2m** exhibited higher inhibitory activity than expected.

The antialgal activity of six studied compounds (**2f**, **2l**, **2m**, **2n**, **2o** and **2p**) showed a quasi-parabolic dependence upon $\log P$ with maximum activity for compounds having $\log P$ in the range from 3.18 to 5.16. With the further increasing of the lipophilicity a dramatic decrease of antialgal activity was observed.

Table 1. Analytical data of the anilides **2a--r**.

Compd.	X	R	Y	Formula M. w.	% Calculated % Found						M. p. / °C Yield / %
					C	H	N	F	Cl	Br	

2a	Cl	H	2-CH ₃	C ₁₂ H ₁₀ ClN ₃ O 247.7	58.19 58.02	4.07 4.14	16.97 16.86	--	14.31 14.19	--	97--99 75
2b	H	(CH ₃) ₃ C	2-CH ₃	C ₁₆ H ₁₉ N ₃ O 269,3	71.35 71.48	7.11 7.08	15.60 15.67	--	--	--	80--81 84
2c	Cl	(CH ₃) ₃ C	2-CH ₃	C ₁₆ H ₁₈ ClN ₃ O 303.8	63.26 63.15	5.97 5.82	13.83 13.96	--	11.67 11.86	--	114--115 78
2d	Cl	H	3-CH ₃	C ₁₂ H ₁₀ ClN ₃ O 247.7	58.19 58.08	4.07 4.11	16.97 16.80	--	14.31 14.48	--	83--84 79
2e	H	(CH ₃) ₃ C	3-CH ₃	C ₁₆ H ₁₉ N ₃ O 269,3	71.35 71.41	7.11 7.22	15.60 15.77	--	--	--	94--95 85
2f	Cl	(CH ₃) ₃ C	3-CH ₃	C ₁₆ H ₁₈ ClN ₃ O 303.8	63.26 63.40	5.97 6.08	13.83 14.01	--	11.67 11.74	--	98--99 84
2g	Cl	H	2-OCH ₃	C ₁₂ H ₁₀ ClN ₃ O ₂ 263.7	54.66 54.57	3.82 3.93	15.94 16.01	--	13.45 13.35	--	71--72 85
2h	H	(CH ₃) ₃ C	2-OCH ₃	C ₁₆ H ₁₉ N ₃ O ₂ 285.3	67.35 67.16	6.71 6.68	14.73 14.62	--	--	--	77--78 88
2i	Cl	(CH ₃) ₃ C	2-OCH ₃	C ₁₆ H ₁₈ ClN ₃ O ₂ 319.8	60.09 60.16	5.67 5.59	13.14 13.23	--	11.09 11.07	--	118--119 82
2j	Cl	H	3-Br	C ₁₁ H ₇ BrClN ₃ O 312.5	42.27 42.37	2.26 2.25	13.44 13.41	--	11.34 11.48	25.56 25.60	99--100 83
2k	H	(CH ₃) ₃ C	3-Br	C ₁₅ H ₁₆ BrN ₃ O 334.2	53.91 54.03	4.83 4.97	12.57 12.61	--	--	23.91 23.77	113--114 75
2l	Cl	(CH ₃) ₃ C	3-Br	C ₁₅ H ₁₅ BrClN ₃ O 368.7	48.87 48.79	4.10 4.22	11.40 11.28	--	9.62 9.77	21.67 21.78	104--105 62
2m	Cl	H	3,5-CF ₃	C ₁₃ H ₆ ClF ₆ N ₃ O 369.7	42.24 42.21	1.64 1.66	11.37 11.33	30.84 30.77	9.59 9.46	--	132--133 88
2n	H	(CH ₃) ₃ C	3,5-CF ₃	C ₁₇ H ₁₅ F ₆ N ₃ O 391.3	52.18 52.02	3.86 3.84	10.74 10.72	29.13 29.17	--	--	135--137 89
2o	Cl	(CH ₃) ₃ C	3,5-CF ₃	C ₁₇ H ₂₁ ClF ₆ N ₃ O 425.8	47.96 48.01	3.31 3.41	9.87 9.63	26.77 26.56	8.33 8.51	--	98--99 88
2p	Cl	H	2,6-CH ₃	C ₁₃ H ₁₂ ClN ₃ O 361.7	59.66 59.70	4.62 4.70	16.06 16.09	--	13.55 13.67	--	121--122 75
2q	H	(CH ₃) ₃ C	2,6-CH ₃	C ₁₈ H ₂₂ ClN ₃ O 331.8	72.06 72.09	7.47 7.45	14.83 14.84	--	--	--	84--85 78
2r	Cl	(CH ₃) ₃ C	2,6-CH ₃	C ₁₈ H ₂₂ ClN ₃ O 331.8	64.25 64.19	6.34 6.40	13.22 13.18	--	11.16 11.17	--	145--146 68

Table 2. IR and ¹H NMR spectral data of the anilides **2a-r**.

Compd.	IR (cm ⁻¹) (C=O)	¹ H-NMR (delta, ppm; J in Hz)											
		NH	H3	H5	H6	H2'	H3'	H4'	H5'	H6'	C(CH ₃) ₃	OCH ₃	CH ₃
							7.33--	7.33--	7.17--	8.15--			

2a	1692	9.42bs	9.40s	8.81s	--	--	7.22m	7.22m	7.10m	8.11m	--	--	2.40s
2b	1685	9.71bs	9.41dd J=1.37	8.65dd J=1.37	--	--	7.33-- 7.20m	7.33-- 7.20m	7.10td J=7.70	8.26d J=7.70	1.45s	--	2.40s
2c	1695	9.42s	9.28s	--	--	--	7.32-- 7.21m	7.32-- 7.21m	7.12td J=7.41 J=1.37	8.18-- 8.13m	1.56s	--	2.40s
2d	1692	9.35bs	9.39bs	8.80s	--	7.61-- 7.52m	--	7.61-- 7.52m	7.28t J=7.96	7.03-- 6.98m	--	--	2.39s
2e	1684	9.61s	9.40d J=1.51	--	8.62d J=1.51	7.65-- 7.61m	--	7.56-- 7.50m	7.27t J=7.69	6.98d J=7.69	1.45s	--	2.38s
2f	1694	9.32bs	9.27s	--	--	7.63-- 7.59m	--	7.57-- 7.51m	7.28t J=7.69	7.02-- 6.97m	1.55s	--	2.39s
2g	1690	10.04s	9.38s	8.78s	--	--	6.94dd J=7.96 J=1.64	7.13td J=7.69 J=1.51	7.03td J=7.69 J=1.51	8.52dd J=7.96 J=1.64	--	3.97s	3.97s
2h	1691	10.27bs	9.39d J=1.37	--	8.68d J=1.37	--	6.94dd J=7.96 J=1.64	7.11td J=7.69 J=1.53	7.02td J=7.69 J=1.53	8.59dd J=7.96 J=1.64	1.45s	3.96s	--
2i	1695	10.01bs	9.26s	--	--	--	6.94dd J=7.97 J=1.51	7.12td J=7.97 J=1.51	7.02td J=7.97 J=1.51	8.53dd J=7.97 J=1.51	1.55s	3.97s	--
2j	1701	9.38bs	9.38bs	8.82s	--	8.01t J=1.92	--	7.67ddd J=7.96 J=1.92 J=1.37	7.35-- 7.22m	7.35-- 7.22m	--	--	--
2k	1692	9.66bs	9.38d J=1.65	--	8.62d J=1.65	8.03t J=1.92	--	7.66dt J=7.65 J=1.92	7.32-- 7.21m	7.32-- 7.21m	1.45s	--	--
2l	1697	9.36bs	9.26s	--	--	8.02t J=1.92	--	7.66dt J=7.69 J=1.92	7.34-- 7.22m	7.34-- 7.22m	1.55s	--	--
2m	1681	9.66bs	9.41s	8.87s	--	8.87bs	--	7.70bs	--	8.87bs	--	--	--
2n	1699	9.94bs	9.41d J=1.51	--	8.64d J=1.51	8.28bs	--	7.66bs	--	8.28bs	1.46s	--	--
2o	1686	9.63bs	9.29s	--	--	8.29bs	--	7.68bs	--	8.29bs	1.56s	--	--
2p	1691	8.94bs	9.39s	8.83s	--	--	7.21-- 7.10m	7.21-- 7.10m	7.21-- 7.10m	--	--	--	2.28s
2q	1667	9.16bs	9.40d J=1.37	--	8.65d J=1.37	--	7.19-- 7.09m	7.19-- 7.09m	7.19-- 7.09m	--	1.46s	--	2.29s
2r	1710	8.91bs	9.27s	--	--	--	7.21-- 7.07m	7.21-- 7.07m	7.21-- 7.07m	--	1.57s	--	2.28s

Table 3. IC₅₀ values concerning inhibition of oxygen evolution rate in spinach chloroplasts by the tested anilides **2a--r** and calculated log *P* values of the compounds in comparison with standard (atrazine).

Compd.	IC ₅₀ [mmol dm ⁻³]	log <i>P</i>

2a	1.072	2.72 +- 0.41
2b	0.440	3.28 +- 0.40
2c	0.244	4.41 +- 0.42
2d	0.486	2.72 +- 0.41
2e	0.148	3.28 +- 0.40
2f	0.118	4.41 +- 0.42
2g	-- ^a	2.15 +- 0.42
2h	0.286	2.72 +- 0.41
2i	0.097	3.84 +- 0.43
2j	0.313	3.46 +- 0.48
2k	0.081	4.03 +- 0.48
2l	0.107	5.15 +- 0.50
2m	0.026	5.16 +- 0.54
2n	0.114	5.73 +- 0.53
2o	0.241	6.85 +- 0.55
2p	0.649	3.18 +- 0.41
2q	0.229	3.75 +- 0.40
2r	0.242	4.87 +- 0.42
Atrazine	0.001	1.03 +- 0.62
^a not measured (insoluble compound)		

Table 4. IC₅₀ values concerning inhibition of chlorophyll production in green algae *Chlorella vulgaris* by the tested anilides **2f**, **2l**, **2m**, **2n**, **2o** and **2p** and calculated log *P* values of the compounds.

Compd.	IC ₅₀ [mmol dm ⁻³]	log <i>P</i>
2f	0.063	4.41 +- 0.42
2l	0.067	5.15 +- 0.50
2m	0.125	5.16 +- 0.54
2n	0.208	5.73 +- 0.53
2o	0.356	6.85 +- 0.55
2p	0.079	3.18 +- 0.41

Experimental

General

Melting points were determined on a Kofler apparatus and are uncorrected. Elemental analyses were obtained using an EA 1110 CE instrument (Fisons Instruments S. p. A., Milan). The IR spectra were recorded on a Nicolet Impact 400 spectrometer in KBr pellets. The ¹H NMR spectra were measured for solutions in CDCl₃ with a Varian Mercury - Vx BB 300 spectrometer operating at 300 MHz. Chemical shifts were recorded as delta values in parts per million (ppm), and were indirectly referenced to tetramethylsilane via the solvent signal (7.26 for ¹H). Multiplicities are given

together with the coupling constants (in Hz). Log *P* values were computed using a program ACD/Log P ver. 1.0 (Advanced Chemistry Development Inc., Toronto).

Synthesis of anilides 2a-r

A mixture of acid (*i. e.* 6-chloropyrazine-2-carboxylic [8], 5-(1,1-dimethylethyl)pyrazine-2-carboxylic [3] or 6-chloro-5-(1,1-dimethylethyl)-pyrazine-2-carboxylic [3] acids, 0.05 mol) and thionyl chloride (5.5 cm³, 75 mmol) in 20 cm³ of dry benzene was refluxed for about 1 h. Excess of thionyl chloride was removed by repeated evaporation with dry benzene in vacuo. The crude acyl chloride dissolved in 50 cm³ of dry acetone was added dropwise to a stirred solution of the corresponding substituted aniline (50 mmol) in 50 cm³ of dry pyridine keeping at the room temperature. After the addition was complete, stirring continued for another 30 min. The reaction mixture was then poured into 200 cm³ of cold water and the crude anilide was collected and recrystallized from aqueous ethanol.

Study of inhibition of oxygen evolution rate in spinach chloroplasts

The oxygen evolution rate in spinach chloroplasts was investigated spectrophotometrically (Specord UV VIS, Zeiss, Jena) in the presence of an electron acceptor 2,6-dichlorophenol--indophenol, by method described in Ref. [13]. The compounds were dissolved in dimethyl sulfoxide (DMSO) because of their low water solubility. The used DMSO volume fractions (up to 5 vol. %) did not affect the oxygen evolution. The inhibitory efficiency of the studied compounds has been expressed by IC₅₀ values, *i. e.* by molar concentration of the compounds causing 50 % decrease in the oxygen evolution relative to the untreated control. Comparable IC₅₀ value for a selective herbicide atrazine [14] is about 1.0 mmol dm⁻³.

Study of inhibition of chlorophyll production in green algae Chlorella vulgaris

The algae *Chlorella vulgaris* were cultivated statically at room temperature according to Sidoova *et al.* [15] (photoperiod 16 h light / 8 h dark; illumination 4000 lx; pH = 7.2). The effect of compounds **2f**, **2l**, **2m**, **2n**, **2o** and **2p** on algal chlorophyll (Chl) content was determined after 4-day cultivation in the presence of the tested compounds, expressing the response as percentage of the corresponding values obtained for control. The Chl content in the algal suspension was determined spectrophotometrically (Specord UV VIS, Zeiss Jena, Germany) after extraction into *N,N*-dimethylformamide according to Inskeep and Bloom [16]. The Chl content in the suspensions at the beginning of cultivation was 0.5 mg dm⁻³. Because of their low water solubility, the tested compounds were dissolved in DMSO. DMSO concentration in the algal suspensions did not exceed 0.25 v/v % and the control samples contained the same DMSO amount as the suspensions treated with the tested compounds.

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