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Pyrazinecarboxamides, Their Synthesis and Evaluation as Potential Herbicides

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Abstract: The condensation of substituted pyrazinecarboxylic acid chlorides with ring substituted anilines yielded nine substituted pyrazinecarboxylic acid amides. The photo-synthesis inhibition activity of a series of pyrazine derivatives was investigated. The synthesis, analytical and biological data of the newly synthesized compounds are presented in this paper. The most active inhibitor of the oxygen evolution rate in spinach chloroplasts was 2-(5-methyl-pyrazine-2-carboxamido)benzoic acid (**9**, IC_{50} = 85.0 μmol·L^{-1}).

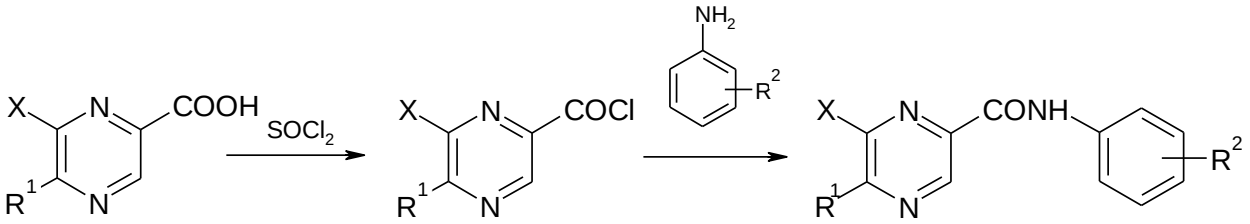
Keywords: synthesis of pyrazinecarboxamides, photosynthesis inhibiting activity, lipophilicity.

Introduction

The pyrazine nucleus is a part of many polycyclic compounds of biological and/or industrial significance. The widespread occurrence of pyrazine derivatives in nature, especially in the flavours of many food systems, their effectiveness at very low concentrations as well as the still increasing applications of synthetic pyrazines in the flavour and fragrance industry are responsible for the high interest in these compounds. Pyrazines occur naturally in a wide range of food items [1]. Pyrazine is a weaker base than pyridine, due to the induction effect of the second nitrogen. Some pyrazines, especially dihydropyrazines, are essential for all forms of life. Several pyrazine derivatives have been used as antioxidants. These compounds have shown important therapeutic applications, for example an antimycobacterial activity [1-6]. Furthermore, a simple pyrazine compound, 3-amino-6-chloro-pyrazine-6-carboxylic acid, has shown anti-auxin behaviour [7]. On the basis of the results of our previous studies [3-6], novel pyrazine derivatives were designed and prepared and their herbicidal activity was evaluated. The aim of this work is the synthesis of the title compounds and evaluation of their photosynthesis-inhibitory activity.

Results and Discussion

Alkylated and/or chlorinated pyrazine-2-carboxylic acid amides connected via -CONH- bridge with substituted anilines were synthesized using currently known synthetic pathways [3-6], see **Scheme 1**.



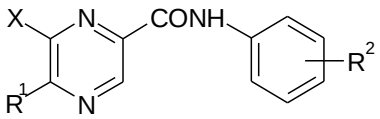
X = H, Cl
R^1 = CH_3, $C(CH_3)_3$
R^2 = 3-CCH, 3-CN, 2-CN-4-NO_2, 3-NO_2, 3-Cl, 3-Br, 3-I-4-CH_3

1 - 9

Scheme 1. Synthesis of the discussed substituted pyrazine-2-carboxamides **1-9**.

The majority of the compounds involved in this study inhibited the photosynthetic electron transport in spinach chloroplasts. From the obtained data [8] it can be concluded that the activity of investigated compounds related to inhibition of the oxygen evolution rate (OER) in spinach chloroplasts depends on their lipophilicity as well as on the electron accepting or withdrawing effects of the substituents. Therefore we designed in preference the compounds with the lipophilic and/or electron-withdrawing substituents in the benzene moiety (3-Br; 3-Cl; 4-CH_3-3-I; -C≡CH; -C≡N). Additionaly we prepared two compounds with the hydrophilic and/or electron-donating substituents in the benzene moiety (-NO_2, -COOH). Based on the obtained results it could be assumed that the biological activity of the studied pyrazinecarboxamides did not depend exclusively on the compound lipophilicity but it was also affected by electron accepting or withdrawing power of the substituents on the benzene ring. However, the values of their inhibitory activities were rather low, see **Table 1**.

Table 1. Inhibition of oxygen evolution rate (OER) in spinach chloroplasts (IC_{50}), and calculated lipophilicity (log *P*) of compounds **1-9** in comparison with the herbicide diurone (DCMU).



Compd.	X	R^1	R^2	IC_{50} [mmol dm^{-3}]	log ($1/IC_{50}$)	log *P*
1	H	CH_3	3-Br	0.648	3.1882	2.12
2	H	CH_3	3-C≡CH	0.668	3.1753	1.45
3	Cl	$C(CH_3)_3$	3-C≡CH	0.385	3.4146	3.78
4	Cl	$C(CH_3)_3$	3-C≡N	0.375	3.4256	3.65
5	H	CH_3	3-Cl	**0.174**	3.7588	**1.85**
6	H	CH_3	3-NO_2	0.402	3.3966	1.19
7	H	CH_3	2-C≡N-4-NO_2	0.550	3.2597	0.70
8	H	CH_3	3-I-4-CH_3	0.317	3.4985	3.14
9	H	CH_3	2-COOH	**0.075**	4.1266	**0.85**
DCMU	-	-	-	0.0019	-	2.76

The dependence of OER-inhibiting activity in the suspension of spinach chloroplasts (expressed as log ($1/IC_{50}$)) on the lipophilicity of the studied compounds is presented in Fig. 1. For compounds **3, 4, 5, 8** and **9** the OER-inhibiting activity showed a linear decrease with increasing values of lipophilicity parameter (log *P*). On the other hand, the biological activity of compounds **1, 2, 6** and **7** was significantly lower and decrease of OER-inhibiting activity with increasing log *P* values was less sharp (see the **Fig 1**). From these results it is evident that the biological activity of the studied compounds depended both on the compound lipophilicity as well as on the values of Hammett constants σ on the substituents R^2 [9].

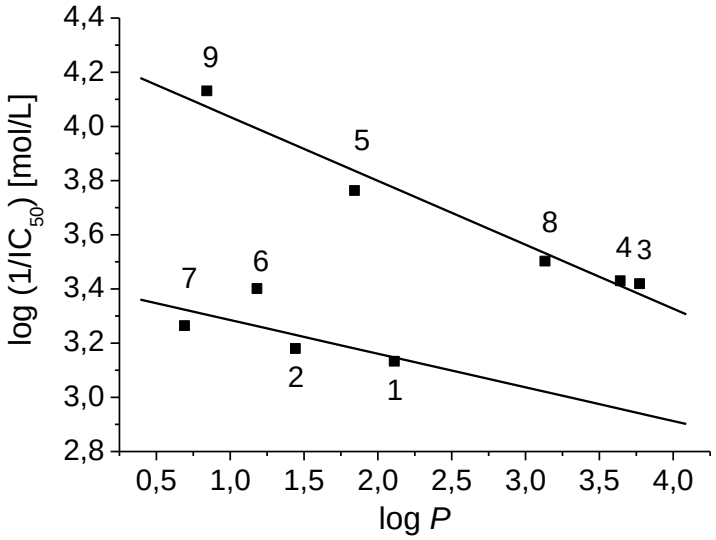


Fig. 1 Relationships between the OER inhibition log ($1/IC_{50}$) [$mmol.L^{-1}$] in spinach chloroplasts and lipophilicity (log *P*) of the studied compounds **1**-**9**.

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Experimental

General
All organic solvents used for the synthesis were of analytical grade. The solvents were dried and freshly distilled under argon atmosphere. The reactions were monitored and the purity of the products was checked by TLC (Silufol UV 254, Kavalier Votice, Czech Republic) using petroleum ether/EtOAc (9:1) as developing solvents. The plates were visualized using UV light (254 nm). Melting points (uncorrected) were determined on Boetius PHMK 05 (VEB Kombinat Nagema, Radebeul, Germany). Elemental analyses were performed on the automatic microanalyser CHNS-O CE instrument (FISONS EA 1110, Milano, Italy). Infrared spectra were recorded in Nicolet Impact 400 spectrometer in KBr pellets. Infrared spectra were recorded in Smart MIRacle™ ATR ZnSe, Nicolet™ 6700 FT-IR Spectrometer, Thermo Scientifis, U.S.A.

General procedure for pyrazinecarboxamide synthesis.
A mixture of acid, *i.e.* 5-methylpyrazine-2-carboxylic, or 5-*tert*-butyl-6-chloropyrazine-2-carboxylic [6] acids, respectively, (50.0 mmol) and thionyl chloride (5.5 mL, 75.0 mmol) in dry toluene (20 mL) was refluxed for about 1 h. Excess of thionyl chloride was removed by repeated evaporation with dry toluene *in vacuo*. The crude acyl chloride dissolved in dry acetone (50 mL) was added dropwise to a stirred solution of the corresponding substituted amine (50.0 mmol) and pyridine (50.0 mmol) in 50 mL of dry acetone at room temperature. After the addition was complete, stirring continued for another 30 min. The reaction mixture was then poured into 100 mL of cold water and the crude amide was collected and recrystalized from aqueous ethanol.

N-*(3-bromophenyl)-5-methylpyrazine-2-carboxamide* (**1**). Yield 78%; Anal. Calcd. for $C_{12}H_{10}BrN_3O$ (291.0): 49.34% C, 3.45% H, 14.38% N; Found: 49.61% C, 3.38% H, 14.44% N; Mp 124.5-125.5 °C; IR cm^{-1}: 3325 (N-H), 2918 (methyl), 1680 (C=O), 1613 (phenyl), 1540 (N-H), 1377, 1272, 1164, and 1025 (pyrazine).

N-*(3-ethynylphenyl)-5-methylpyrazine-2-carboxamide* (**2**). Yield 82%; Anal. Calcd. for $C_{14}H_{11}N_3O$ (237.1): 70.87% C, 4.67% H, 17.71% N; Found: 71.02% C, 4.62% H, 17.39% N. Mp 138.0-139.0 °C; IR cm^{-1}: 3321 (N-H), 2975, 2875 (methyl, ethynyl), 1685 (C=O), 1598 (phenyl), 1530 (N-H), 1370, 1300, 1167, and 1022 (pyrazine).

5-tert-*Butyl-6-chloro*-N-*(3-ethynylphenyl)pyrazine-2-carboxamide* (**3**). Yield 88%; Anal. Calcd. for $C_{17}H_{16}ClN_3O$ (313.1): 65.07% C, 5.14% H, 13.39% N; Found: 65.14% C, 5.11% H, 13.24% N; Mp 140.0-141.0 °C; IR cm^{-1}: 3325 (N-H), 2965, 2933, 2921, 2869 (*tert*-butyl, ethynyl), 1685 (C=O), 1590 (phenyl), 1519 (N-H), 1407, 1278, and 1143 (pyrazine).

5-tert-*Butyl-6-chloro*-N-*(3-cyanophenyl)pyrazine-2-carboxamide* (**4**). Yield 81%; Anal. Calcd. For $C_{16}H_{15}ClN_4O$ (314.1): 61.05% C, 4.80% H, 17.80% N; Found: 60.87% C, 4.74% H, 17.57% N; Mp 129.0-130.0 °C; IR cm^{-1}: 3325 (N-H), 2990, 2978, 2967 (*tert*butyl), 2229 (CN), 1699 (C=O), 1618 (phenyl), 1532 (N-H), 1269, 1149, and 1025 (pyrazine).

N-*(3-chlorophenyl)-5-methylpyrazine-2-carboxamide* (**5**). Yield 77%; Anal. Calcd. for C12H10ClN3O (247.1): 58.19% C, 4.07% H, 16.97% N; Found: 58.20% C, 4.14% H, 17.07% N. Mp 92.5-93.5 °C; IR cm^{-1}: 3327 (N-H), 2928 (methyl), 1679 (C=O), 1594 (phenyl), 1518 (N-H), 1276, 1125, and 1027 (pyrazine).

5-Methyl-N-*(3-nitrophenyl)pyrazine-2-carboxamide* (**6**). Yield 79%; Anal. Calcd. for C12H10N4O3 (258.1): 55.81% C, 3.90% H, 21.70% N; Found: 55.70% C, 3.90% H, 21.67% N. Mp 111.5-112.0 °C; IR cm^{-1}: 3338 (N-H), 2928 (methyl), 1683 (C=O), 1620 (phenyl), 1515 (N-H), 1340, 1278, 1149, and 1029 (pyrazine).

N-*(2-cyano-4-nitrophenyl)-5-methylpyrazine-2-carboxamide* (**7**). Yield 67%; Anal. Calcd. for C13H9N5O3 (283.1): 55.13% C, 3.20% H, 24.73% N; Found: 55.40% C, 3.44% H, 24.57% N. Mp 204.7-205.5 °C; IR cm^{-1}: 3328 (N-H), 2929 (methyl), 2229 (CN), 1685 (C=O), 1600 (phenyl), 1505 (N-H), 1320, 1267, 1123, and 1083 (pyrazine).

N-*(3-iodo-4-methylphenyl)-5-methylpyrazine-2-carboxamide* (**8**). Yield 85%; Anal. Calcd. for C13H12IN3O (353.0): 44.21% C, 3.42% H, 11.90% N; Found: 44.30% C, 3.34% H, 11.77% N.

Mp 146.0-147.0 °C; IR cm^{-1}: 3338 (N-H), 2924 (methyl), 2856 (methyl), 1695 (C=O), 1600 (phenyl), 1508 (N-H), 1372, 1286, 1174, and 1028 (pyrazine).

2-(5-methylpyrazine-2-carboxamido)benzoic acid (**9**). Yield 71%; Anal. Calcd. for C13H11N3O (257.1): 60.70% C, 4.31% H, 16.33% N; Found: 59.78% C, 4.24% H, 16.27% N. Mp 81.0-82.5 °C; IR cm^{-1}: 3335 (N-H), 2967 (methyl), 1709 (C=O), 1685 (C=O) 1618 (phenyl), 1518 (N-H), 1326, 1160, 1122, and 1025 (pyrazine).

Lipophilicity calculations
Log *P*, *i. e.* the logarithm of the partition coefficient *P* for *n*-octanol/water was calculated using the program ChemBioDraw Ultra ver. 11.0 (CambridgeSoft, Cambridge, MA, U.S.A.) The results are shown in Table 1.

Study of the inhibition of oxygen evolution rate in spinach chloroplasts.

Chloroplasts were prepared by the procedure of Walker from spinach (*Spinacia oleracea* L.) [10]. The inhibition of photosynthetic electron transport (PET) in spinach chloroplasts was determined spectrophotometrically (Genesys 6 UV VIS Spectrophotometer, USA) using an artificial electron acceptor 2,6-dichlorophenol-indophenol (DCIPP) according to Kráľová *et al*. [11] and the rate of photosynthetic electron transport was monitored as a photo-reduction of DCPIP. The measurements were carried out in a phosphate buffer (0.02 $mol \cdot L^{-1}$, pH 7.2) containing sucrose (0.4 $mol \cdot L^{-1}$), $MgCl_2$ (0.005 $mol \cdot L^{-1}$) and NaCl (0.015 $mol \cdot L^{-1}$). The chlorophyll content was 30 mg/L in these experiments and the samples were irradiated (~100 W/m^2) from a 10 cm distance with a halogen lamp (250 W) using a 4 cm water filter to prevent warming of the samples (suspension temperature 22 °C). The studied compounds were dissolved in DMSO due to their limited water solubility. The applied DMSO concentration (up to 4%) did not affect the photochemical activity in spinach chloroplasts (PET). The inhibitory efficiency of the studied compounds was expressed as the IC_{50} values, *i.e.* molar concentration of the compounds causing 50% decrease in the oxygen evolution relative to the untreated control. The comparable IC50 value for a selective herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea, (diurone, DCMU) was about 1.9 μmol L^{-1}. [12]. The results are summarised in **Table 1**.

References

1. Doležal, M. Biological active pyrazines of natural and synthetic origin. *Chem. Listy* **2006**, *100*, 959-966.
2. Brown, D.J. *The Pyrazines*. J. Wiley Sons, Inc., New York, **2002**.
3. Doležal, M.; Palek, L.; Vinšová, J.; Buchta, V.; Jampílek, J.; Kráľová, K. Substituted Pyrazinecarboxamides; Synthesis and Their Biological Evaluation. *Molecules* **2006**, *11*, 242-256.
4. Doležal, M.; Čmedlová, P.; Palek, L.; Vinšová, J.; Kuneš, J.; Buchta, V.; Jampílek, J.; Kráľová, K. Synthesis and antimycobacterial evaluation of substituted pyrazinecarboxamides. *Eur. J. Med. Chem.* **2008**, *43*, 1105-1113.
5. Doležal, M.; Miletín, M.; Kuneš, J.; Kráľová, K. Substituted amides of pyrazine-2-carboxylic acids, their synthesis and biological activity. *Molecules* **2002**, *7*, 363-373.
6. Doležal, M.; Hartl, J.; Miletín, M.; Macháček, M.; Kráľová, K. *Chem. Pap.* **1999**, *53*, 126-128.
7. Camper. N. D.; McDonald, S. K. Tissue and cell cultures as model system in herbicide research. *Rev. Weed Sci.* **1989**, *4*, 169-190.

8. Doležal, M.; Kráľová, K.; Šeršeň, F.; Miletín, M. The site of action of some anilides of pyrazine-2-carboxylic acids in the photosynthetic apparatus. *Folia Pharm. Univ. Carol.* **2001**, *26*, 13-20.
9. Hansch, C.; Leo, A.J. *Substituent Constants for Correlation Analysis in Chemistry and Biology*. John Wiley: New York, **1979**.
10. Walker, D. A. In *Methods in Enzymology*. Part C. Colowick, S.P.; Kaplan, N.O. Ed.; Academic Press: New York, **1980**; Vol. 69, pp. 94-104.
11. Kráľová, K.; Šeršeň, F.; Sidóová, E. Photosynthesis inhibition produced by 2-alkylthio-6-benzothiazoles. *Chem. Pap.* **1992**, *46*, 348-350.
12. Fedke, C. *Biochemistry and Physiology of Herbicide Action*. Springer Verlag: Berlin-Heidelberg-New York, **1982**.