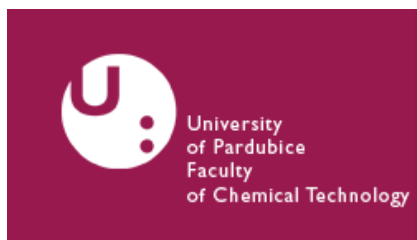




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Preliminary Study of Photosynthesis-inhibiting Activity of Substituted Phenylcarbamoylphenyl *N*-alkylcarbamates

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Abstract: In this study, a series of nine 4-chloro-2-(3,4-dichlorophenylcarbamoyl)phenyl *N*-alkylcarbamates was prepared. The procedures for synthesis of the compounds are presented. The compounds were analyzed using RP-HPLC to determine lipophilicity. They were tested for their activity related to inhibition of photosynthetic electron transport (PET) in spinach (*Spinacia oleracea* L.) chloroplasts. For the compounds, the relationships between the lipophilicity and the chemical structure of the studied compounds are discussed, as well as their structure-activity relationships (SAR).

Keywords: 4-Chloro-2-(3,4-dichlorophenylcarbamoyl)-phenyl *N*-alkylcarbamates; Lipophilicity; PET inhibition; Spinach chloroplasts; Structure-activity relationships.

INTRODUCTION

Salicylanilides are an important class of aromatic compounds with a wide range of pharmacological activities. A number of them showed antibacterial [1], antimycobacterial [2,3], antifungal [2,4]. In the recent past, a number of organic carbamates have been found to be potential antibacterial, antimycobacterial and antiviral agents [5,6]. The carbamate residue present in these new molecules contributes as a core component [7] or, incorporated into a known molecule, contributes to the improvement of its pharmacodynamic and pharmacokinetic properties [8]. In particular, carbamate was successfully used to protect phenolic drugs [9].

The presence of an amide or thioamide (-NHCO- or -NHCS-) group, is characteristic of a number of herbicides acting as photosynthesis inhibitors (acylanilides, thioacylanilides, phenylcarbamates, ureas, etc.), e.g. [10-13]. A wide spectrum of biological effects of salicylanilides includes in addition herbicidal activity, e.g. [14,15]. Moreover, salicylanilides were found to be uncouplers of photosynthetic phosphorylation [16].

The inhibitory activity of the R'-substituted salicylanilides related to the inhibition of oxygen evolution rate in spinach chloroplasts correlated with the hydrophobic parameter π and the Hammett constant σ of the R' substituents and the compounds were found to interact with the intermediate D[•] (Tyr_D) in the photosynthetic apparatus of spinach chloroplasts [14]. Intermediate D[•] is situated at the 161st position in D₂ protein occurring at the donor side of photosystem (PS) 2. In the presence of R'-substituted salicylanilides, the decreased intensity of the fluorescence emission band at 686 nm (belonging to the chlorophyll-protein complexes mainly in PS 2 [17]) suggested PS 2 as the site of action of the studied inhibitors. Upon addition of 1,5-diphenylcarbazide (DPC, an artificial electron donor of PS 2 with a known site of action in the intermediate Z[•]/D[•] on the donor side of PS 2 [18]) to chloroplasts treated with R' substituted salicylanilides, inhibition of the oxygen evolution rate was practically completely restored indicating that the own core of PS 2 (P 680) and a part of the electron transport chain - at least up to plastoquinone - remained intact. These results are in accordance with those obtained for 3-bromo- and 3,5-dibromosalicylanilides [19] and 3-methylsalicylanilides [15]. However, it could be stressed that 3,5-dibromosalicylanilides with R' = 3-F and R = 3-Cl interacted not only with the intermediate D[•] (Tyr_D) but also with Z[•] intermediate (Tyr_Z) which is situated in the 161st position in D1 protein occurring at the donor side of PS 2 [19].

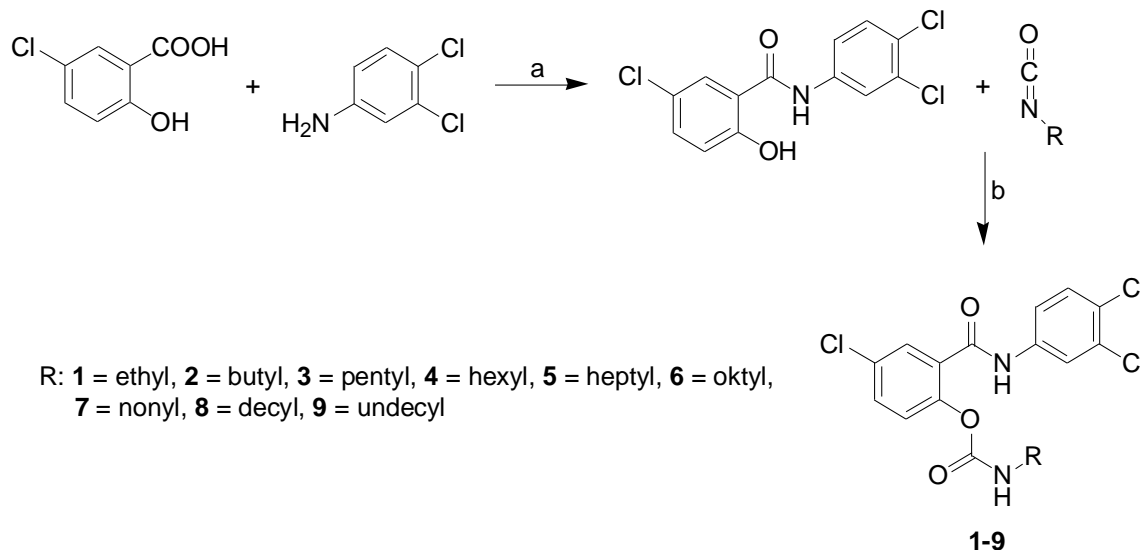
This is a follow-up paper to our previous articles [20–27] dealing with syntheses and especially herbicidal activity of various aromatic ring-substituted like-carboxamide derivatives. The compounds were tested for their photosynthesis-inhibiting activity (the inhibition of photosynthetic electron transport) in spinach chloroplasts (*Spinacia oleracea* L.). Lipophilicity (log *k*) of the compounds was determined using RP-HPLC. Relationships among the structure and *in vitro* inhibitory activity related to inhibition of photosynthetic electron transport (PET) in spinach chloroplasts of the carbamates are discussed.

RESULTS AND DISCUSSION

The preparation of the carbamates is outlined in Scheme 1. They were routinely prepared by the reaction of 5-chlorosalicylic acid with 3,4-dichlorophenylamine in chlorobenzene with PCl₃ [4] By using microwave irradiation, the reaction time was shortened from several hours to minutes. For the synthesis of the corresponding carbamates, a suspension of salicylanilide in acetonitrile (MeCN) was treated with one equivalent of triethylamine (TEA), adding then the corresponding isocyanate. This reaction was performed at room temperature due to thermal instability of the products. The prepared carbamates **1-9** are described in Table 1. The yields of the synthesized compounds varied in the interval of 35–80%. Prepared carbamates

were described as potential antituberculosic active compounds and their characterisation by means of infrared, NMR spectroscopy and elemental analyses [6].

Scheme 1. Synthesis of 4-Chloro-2-(3,4-dichlorophenylcarbamoyl)-phenyl *N*-alkylcarbamates **1-9**: (a) PCl_3 , chlorobenzene, microwave irradiation; (b) TEA, MeCN.



The hydrophobicity ($\log P/\text{Clog } P$ values) of the studied compounds **3**, **4**, **5a-5g**, and **6a-6g** were calculated using two commercially available programs (ChemDraw Ultra 10.0 and ACD/LogP). Their hydrophobicities were measured experimentally by RP-HPLC determination of their capacity factors (k) with subsequent calculation of $\log k$. This procedure measures the retention times of the compounds under isocratic conditions with various amounts of an organic modifier (methanol) in the mobile phase. Using an end-capped non-polar C_{18} stationary RP column, the capacity factor k can be calculated. $\log k$ is used as a lipophilicity index converted to the $\log P$ scale [28]. The results are shown in Table 1.

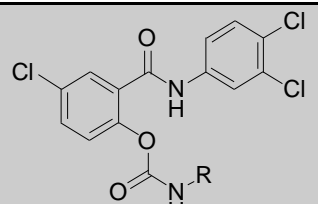
The results obtained with respect to all compounds show that the experimentally determined lipophilicities ($\log k$ values) are lower than those indicated by the calculated $\log P/\text{Clog } P$ as shown in Figure 1.

As expected, 4-chloro-2-(3,4-dichlorophenylcarbamoyl)phenyl ethylcarbamate (**1**) showed the lowest lipophilicity, whereas 4-chloro-2-(3,4-dichlorophenylcarbamoyl)phenyl undecylcarbamate (**9**) was the most lipophilic within each series. The calculated $\log P/\text{Clog } P$ data and the determined $\log k$ values correspond to the expected trend in lipophilicity, increasing within each series of evaluated compounds (ethyl \ll butyl $<$ pentyl $<$ hexyl $<$ heptyl $<$ oktyl $<$ nonyl $<$ decyl $<$ undecyl derivatives). This dependence is approximately linear. The $\log k$ data corresponds to the lipophilicity within the series of compounds discussed.

The evaluated carbamates showed a wide range of activities (from 56 to 505 $\mu\text{mol/L}$) related to inhibition of photosynthetic electron transport (PET) in spinach chloroplasts, see Table 1. 4-Chloro-2-(3,4-dichlorophenylcarbamoyl)phenyl butylcarbamate (**2**) expressed the highest PET-inhibiting activity (IC_{50} : 56.4 $\mu\text{mol/L}$). PET inhibition by compounds **8** and **9** could not be determined due to precipitation of the compounds during the experiments.

The PET-inhibiting activity was expressed by negative logarithm of IC_{50} value (compound concentration in mol/L causing 50% inhibition of PET). Despite the relatively low inhibitory activity of the studied compounds, correlations between $\log (1/\text{IC}_{50})$ and $\log k$ of compounds **1-7** were determined, see Figure 2.

Table 1. The calculated lipophilicities ($\log P/\text{Clog } P$), the determined $\log k$ values and IC_{50} [$\mu\text{mol/L}$] values related to PET inhibition in spinach chloroplasts of compounds **1-7** in comparison with standard 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU).

					
Comp.	R	$\log k$	$\log P/\text{Clog } P$ ChemOffice	$\log P$ ACD/LogP	PET inhibition IC_{50} [$\mu\text{mol/L}$]
1	C ₂ H ₅	1.0234	4.35 / 4.8194	4.88 ± 0.41	82.3
2	C ₄ H ₉	1.0457	5.25 / 5.8774	5.94 ± 0.41	56.4
3	C ₅ H ₁₁	1.0601	5.67 / 6.4064	6.47 ± 0.41	99.2
4	C ₆ H ₁₃	1.0611	6.08 / 6.9354	7.01 ± 0.41	143.5
5	C ₇ H ₁₅	1.0667	6.50 / 7.4644	7.54 ± 0.41	169.08
6	C ₈ H ₁₇	1.0845	6.92 / 7.9934	8.07 ± 0.41	431.6
7	C ₉ H ₁₉	1.0880	7.34 / 8.5224	8.60 ± 0.41	505.5
8	C ₁₀ H ₂₁	1.0894	7.77 / 9.0514	9.13 ± 0.41	^a
9	C ₁₁ H ₂₃	1.0903	8.17 / 9.58041	9.66 ± 0.41	^a
DCMU	—	—	—	—	1.9

^aprecipitation during the experiment.

Figure 1. Comparison of the $\log P/\text{Clog } P$ values computed using two the programs with the calculated $\log k$ values. Compounds **1-9** are ordered according to the increase in $\log k$ values.

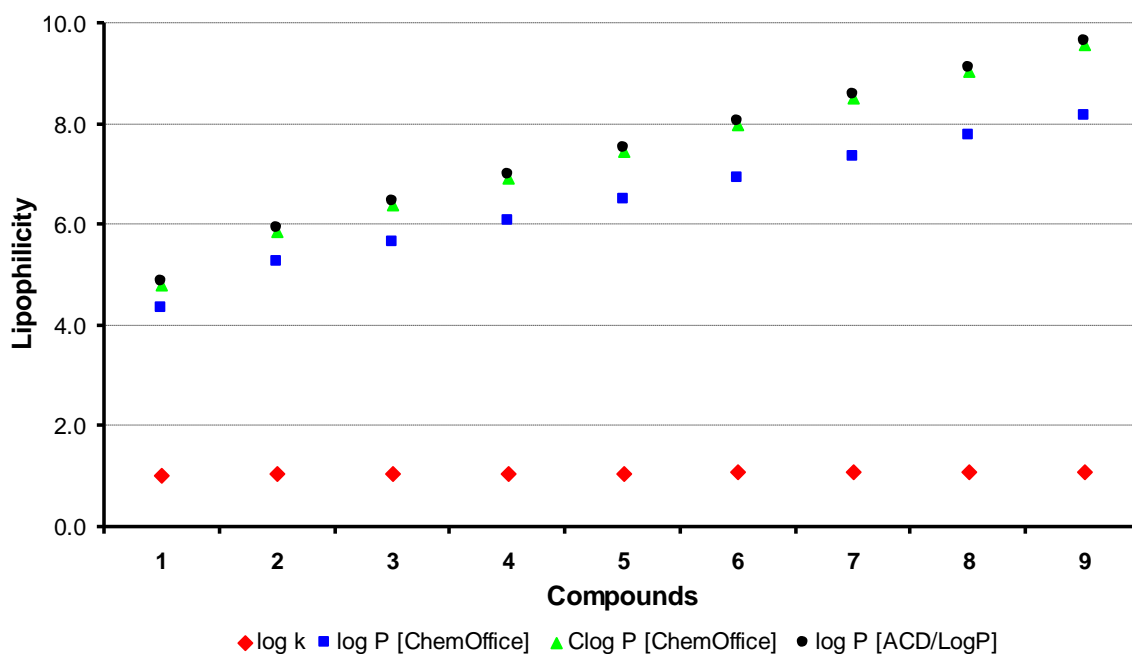
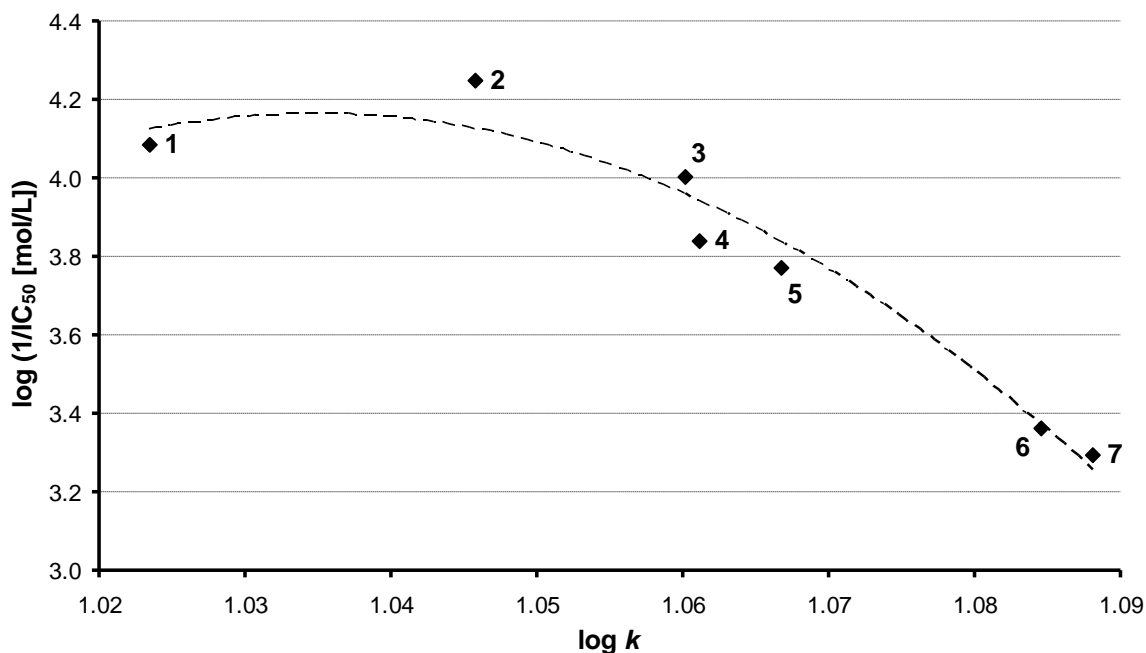


Figure 2. The relationships between the PET-inhibiting activity $\log (1/IC_{50})$ [mol/L] in spinach chloroplasts and the determined lipophilicity $\log k$ of the studied compounds **1-7**.



Thus, it could be concluded that lipophilicity (significantly affected by the length of the substituted alkyl chain/substituent bulkiness) was decisive for photosynthesis-inhibiting activity. The parabolic dependence of PET-inhibiting activity on lipophilicity ($\log k$) of the studied compounds can be observed in Figure 2. Within the series of carbamates **1-7**, activity increases from ethyl to butyl derivative and then the PET-inhibition decreases with lipophilicity increase.

Question if propyl, isopropyl, isobutyl or *tert*-butyl express higher PET-inhibiting activity than compound **2** (*n*-butyl derivative) can not be answered based on the results discussed herein and now it is under investigation.

EXPERIMENTAL

Synthesis

All the discussed compounds were synthesized as described previously [6].

Lipophilicity HPLC determination (capacity factor k / calculated $\log k$)

A Waters Alliance 2695 XE HPLC separation module and a Waters Photodiode Array Detector 2996 (Waters Corp., Milford, MA, USA) were used. A Symmetry[®] C₁₈ 5 μ m, 4.6 \times 250 mm, Part No. WAT054275 (Waters Corp., Milford, MA, USA) chromatographic column was used. The HPLC separation process was monitored by Millennium32[®] Chromatography Manager Software, Waters 2004 (Waters Corp., Milford, MA, USA). A mixture of MeOH p.a. (70.0%) and H₂O-HPLC – Mili-Q Grade (30.0%) was used as a mobile phase. The total flow of the column was 1.0 mL/min, injection 30 μ L, column temperature 45 $^{\circ}$ C and sample temperature 10 $^{\circ}$ C. The detection wavelength of 210 nm was chosen. The KI methanolic solution was used for the dead time (t_D) determination. Retention times (t_R) were measured in minutes.

The capacity factors k were calculated using the Millennium32[®] Chromatography Manager Software according to formula $k = (t_R - t_D)/t_D$, where t_R is the retention time of the solute,

whereas t_D denotes the dead time obtained via an unretained analyte. Log k , calculated from the capacity factor k , is used as the lipophilicity index converted to log P scale [28]. The log k values of the individual compounds are shown in Table 1.

Lipophilicity calculations

Log P , *i.e.* the logarithm of the partition coefficient for *n*-octanol/water, was calculated using the programs CS ChemOffice Ultra ver. 10.0 (CambridgeSoft, Cambridge, MA, USA) and ACD/LogP ver. 1.0 (Advanced Chemistry Development Inc., Toronto, Canada). Clog P values (the logarithm of *n*-octanol/water partition coefficient based on established chemical interactions) were generated by means of CS ChemOffice Ultra ver. 10.0 (CambridgeSoft, Cambridge, MA, USA) software. The results are shown in Table 1.

Study of inhibition photosynthetic electron transport (PET) in spinach chloroplasts

Chloroplasts were prepared from spinach (*Spinacia oleracea* L.) according to Masarovicova and Kralova [29]. The inhibition of photosynthetic electron transport (PET) in spinach chloroplasts was determined spectrophotometrically (Genesys 6, Thermo Scientific, USA), using an artificial electron acceptor 2,6-dichlorophenol-indophenol (DCIPP) according to Kralova *et al.* [30], and the rate of photosynthetic electron transport was monitored as a photoreduction of DCPIP. The measurements were carried out in phosphate buffer (0.02 mol/L, pH 7.2) containing sucrose (0.4 mol/L), MgCl₂ (0.005 mol/L) and NaCl (0.015 mol/L). The chlorophyll content was 30 mg/L in these experiments and the samples were irradiated (~ 100 W/m²) from 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. The inhibitory efficiency of the studied compounds was expressed by IC₅₀ values, *i.e.* by molar concentration of the compounds causing 50% decrease in the oxygen evolution rate relative to the untreated control. The comparable IC₅₀ value for a selective herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea, DCMU (Diurone[®]) was about 1.9 μmol/L [31]. The results are summarized in Table 1.

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REFERENCES

1. Vinsova, J.; Imramovsky, A. *Ces. Slov. Farm.* **2004**, *53*, 294–299.
2. Vinsova, J.; Imramovsky, A.; Buchta, V.; Ceckova, M.; Dolezal, M.; Staud, F.; Jampilek, J.; Kaustova, J. *Molecules* **2007**, *12*, 1–12.
3. Imramovsky, A.; Vinsova, J.; Monreal-Ferriz, J.; Dolezal, R.; Jampilek, J.; Kaustova, J.; Kunc, F. *Bioorg. Med. Chem.* **2009**, *17*, 3572–3579.
4. Imramovsky, A.; Vinsova, J.; Monreal-Ferriz, J.; Buchta, V.; Jampilek, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 348–351.
5. Ray, S.; Pathak, S.R.; Chaturvedi, D. *Drugs Future* **2005**, *30*, 161–180.
6. Monreal-Ferriz, J.; Vavrova, K.; Kunc, F.; Imramovsky, A.; Stolarikova, J.; Vavrikova, E.; Vinsova, J. *Bioorg. Med. Chem.* **2010**, *18*, 1054–1061.
7. Agouridas, C.; Denis, A.; Auger, J.M.; Benedetti, Y.; Bonnefoy, A.; Bretin, F.; Chantot, J.F.; Dussarat, A.; Fromentin, C.; D'Ambrieres, S.G.; Lachaud, S.; Laurin, P.; Le Martret, O.; Loyau, V.; Tessot, N. *J. Med. Chem.* **1998**, *41*, 4080–4100.

8. Meng, Q.; Luo, H.; Liu, Y.; Li, W.; Zhang, W.; Yao, Q. *Bioorg. Med. Chem.* **2009**, *19*, 2808–2810.
9. Thorberg, S.; Berg, S.; Lundstrom, L.; Pettersson, B.; Wijkstrom, A.; Sanchez, D.; Lindberg, P.; Nilsson, J.G. *J. Med. Chem.* **1987**, *30*, 2008–2012.
10. Good, N.E. Inhibitors of the Hill reaction. *Plant Physiol.* **1961**, *36*, 788–803.
11. Kralova, K.; Sersen, F.; Cizmarik, J. *Gen. Physiol. Biophys.* **1992**, *11*, 261–267.
12. Kralova, K.; Sersen, F.; Kubicova, L.; Waisser, K. *Chem. Pap.* **1999**, *53*, 328–331.
13. Kralova, K.; Sersen, F.; Kubicova, L.; Waisser, K. *J. Trace Microprobe Techn.* **2000**, *18*, 251–256.
14. Kubicova, L.; Kralova, K.; Sersen, F.; Gregor, J.; Waisser, K. *Folia Pharm. Univ. Carol.* **2000**, *25*, 89–96.
15. Pravda, M.; Hrciarova, D.; Kralova, K. *Chem. Listy* **2003**, *97*, 1122–1123.
16. Black, C.C. *Biochim. Biophys. Acta* **1968**, *162*, 294–296.
17. Govindjee, S. *Aust. J. Plant Physiol.* **1995**, *22*, 131–160.
18. Jegerschoold, C.; Styring, S. *FEBS Lett.* **1991**, *280*, 87–90.
19. Kralova, K.; Kubicova, L.; Sersen, F.; Waisser, K. Book of Abstracts: *51st Congress of Chemical Societies*, Nitra, Slovakia, September 6-9, **1999**, H-PO66.
20. Jampilek, J.; Dolezal, M.; Kunes, J.; Buchta, V.; Kralova, K. *Med. Chem.* **2005**, *1*, 591–599.
21. Dolezal, M.; Palek, L.; Vinsova, J.; Buchta, V.; Jampilek, J.; Kralova, K. *Molecules* **2006**, *11*, 242–256.
22. Musiol, R.; Jampilek, J.; Kralova, K.; Richardson, D.R.; Kalinowski, D.; Podeszwa, B.; Finster, J.; Niedbala, H.; Palka, A.; Polanski, J. *Bioorg. Med. Chem.* **2007**, *15*, 1280–1288.
23. Musiol, R.; Tabak, D.; Niedbala, H.; Podeszwa, B.; Jampilek, J.; Kralova, K.; Dohnal, J.; Finster, J.; Mencil, A.; Polanski, J. *Bioorg. Med. Chem.* **2008**, *16*, 4490–4499.
24. Dolezal, M.; Cmedlova, P.; Palek, L.; Vinsova, J.; Kunes, J.; Buchta, V.; Jampilek, J.; Kralova, K. *Eur. J. Med. Chem.* **2008**, *43*, 1105–1113.
25. Jampilek, J.; Musiol, R.; Pesko, M.; Kralova, K.; Vejsova, M.; Carroll, J.; Coffey, A.; Finster, J.; Tabak, D.; Niedbala, H.; Kozik, V.; Polanski, J.; Csollei, J.; Dohnal, J. *Molecules* **2009**, *14*, 1145–1159.
26. Jampilek, J.; Musiol, R.; Finster, J.; Pesko, M.; Carroll, J.; Kralova, K.; Vejsova, M.; O'Mahony, J.; Coffey, A.; Dohnal, J.; Polanski, J. *Molecules* **2009**, *14*, 4246–4265.
27. Musiol, R.; Jampilek, J.; Nycz, J.E.; Pesko, M.; Carroll, J.; Kralova, K.; Vejsova, M.; O'Mahony, J.; Coffey, A.; Mrozek, A.; Polanski, J. *Molecules* **2010**, *15*, 288–304.
28. Pliska, V. In: *Lipophilicity in drug action and toxicology* (Methods and Principles in Medicinal Chemistry, Vol. 4). Pliska, V., Testa, B., van der Waterbeemd, H. (Eds.). 1st Ed. Wiley-VCH, Weinheim 1996, pp. 1–6.
29. Masarovicova, E.; Kralova, K. *Approaches to measuring plant photosynthesis activity*. In *Handbook of Photosynthesis* 2nd Ed.; Pessaraki M. Eds.; Taylor & Francis Group: Boca Raton, London-New York-Singapore, 2005; pp. 617–656.
30. Kralova, K.; Sersen, F.; Sidoova, E. *Chem. Pap.* **1992**, *46*, 348–350.
31. Fedke, C. *Biochemistry and Physiology of Herbicide Action*. Springer Verlag: Berlin-Heidelberg-New York, **1982**.