Preparation and Photosynthesis-Inhibiting Activity of 1-[(2-Chlorophenyl)carbamoyl]naphthalen-2-yl Alkylcarbamates

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Abstract: In this study a series of eight 1-[(2-chlorophenyl)carbamoyl]naphthalen-2-yl alkylcarbamates was prepared and characterized. The discussed compounds were prepared by microwave-assisted and conventional synthesis. The compounds were tested for their activity related to inhibition of photosynthetic electron transport (PET) in spinach (*Spinacia oleracea* L.) chloroplasts. The PET-inhibiting activity of the compounds was moderate; the highest activity within the series of compounds was observed for 1-[(2-chlorophenyl)-carbamoyl]naphthalen-2-yl propylcarbamate, and the compounds were found to inhibit PET in photosystem II.

Keywords: Alkylcarbamates; Hydroxynaphthalene-carboxamides; PET inhibition; Spinach chloroplasts; Structure-activity relationships.

INTRODUCTION

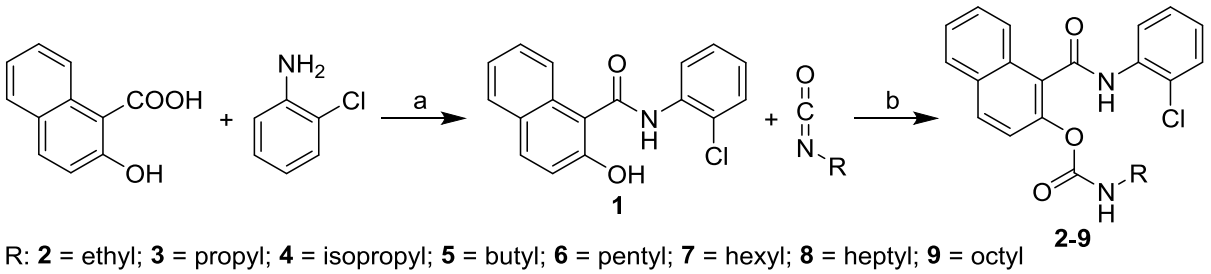
Although naphthalene can be considered as the simplest compound from the group of arenes, it is one of the most interesting arenes. Naphthalene-based drugs include not only clinically used anti-infective chemotherapeutics, e.g, naficillin, naftifine, terbinafine, tolnaftate, but also other compounds with significant antimicrobial effect, e.g., dye naftol [1–3]. The naphthalene scaffold can be found in many other bioactive compounds, e.g. [1,3–7]; therefore this scaffold can be considered as a privileged structure [8–11].

Our research group prepared and tested naphthalenecarboxamides and various positional isomers of hydroxynaphthalenecarboxamides as potential antimicrobial and antiprotozoal compounds [12–19]. The presence of an amide (–CONH–) group in the structure of compounds enables interactions with various enzymes or enzymatic systems [20–22 and refs. thereon] and is also characteristic for a number of herbicides acting as photosynthesis inhibitors, e.g., [23–31]. Although at present approximately 20 modes of action of herbicides are known [32], over 50% of commercially available herbicides act by reversible binding to photosystem II (PS II) [33], and due to this interaction, interruption of the photosynthetic electron transport occurs [34–36].
In the context of the previously-described compounds [12–18], new 1-[(2-chlorophenyl)-carbamoyl]naphthalen-2-yl alkylcarbamates were prepared and tested for their photosynthesis-inhibiting activity – the inhibition of photosynthetic electron transport (PET) in spinach chloroplasts (*Spinacia oleracea* L.). The structure-activity relationships are discussed.

RESULTS AND DISCUSSION

All the studied compounds were prepared according to Scheme 1. In the first step, *N*-(2-chlorophenyl)-2-hydroxynaphthalene-1-carboxamide (**1**) was synthesized by the microwave-assisted method [14]. In the second step, a modified method using triethylamine for activation of the phenolic group was used [22,29]. Addition of activated compound **1** to appropriate alkyl carbamates yielded a series of eight 1-[(2-chlorophenyl)carbamoyl]-naphthalen-2-yl alkylcarbamates (**2**–**9**).

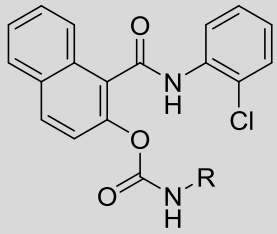
Scheme 1. Synthesis of 1-[(2-chlorophenyl)carbamoyl]naphthalen-2-yl alkylcarbamates **2**–**9**: (a) PCl_3, chlorobenzene, MW; (b) TEA, acetonitrile, ambient temperature.



R: **2** = ethyl; **3** = propyl; **4** = isopropyl; **5** = butyl; **6** = pentyl; **7** = hexyl; **8** = heptyl; **9** = octyl

All the predicted molecular descriptors (lipophilicity, molar volumes and Taft constants) were calculated using the ACD/Percepta ver. 2012 program (Advanced Chemistry Development, Toronto, ON, Canada), see Table 1. The lipophilicity of compounds **2**–**9**, expressed as log *P* values, ranged from 3.94 (compounds **2**, R = C_2H_5) to 7.19 (compound **9**, R = C_8H_{17}). Logically lipophilicity increases with lengthening of the alkyl tail. Isopropyl showed lower lipophilicity value than propyl. For individual substituents – alkyl tails of the discussed compounds, also electronic properties expressed as Taft polar constants σ* were predicted; they ranged from -0.25 to -0.11. Experience has shown that a parameter representing the bulk of substituents (i.e. tail length/branching) of each compound relative to other members of the same series may often be correlated with biological activity [21,22,29–31]; therefore molar volume MV [cm^{-3}] was calculated also for the hydrophobic *N*-alkyl tail.

Table 1. Structure of 1-[(2-chlorophenyl)carbamoyl]naphthalen-2-yl alkylcarbamates **2**–**9**, calculated values of log *P* of compounds, Taft polar constants σ*, molar volume MV [cm^{-3}] of R substituents (calculated using ACD/Percepta ver. 2012) and IC_{50} [mmol/L] values related to PET inhibition in spinach chloroplasts of tested compounds in comparison with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) standard.



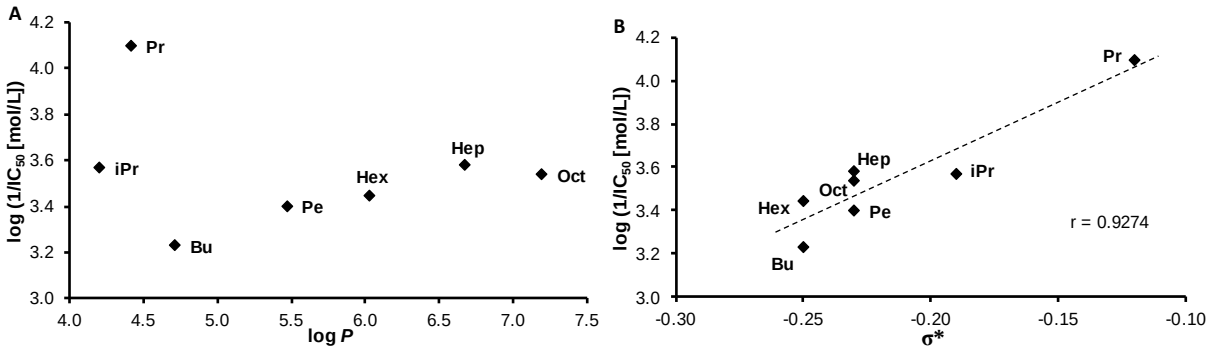
Comp.	R	log *P*	σ*	MV [cm^3]	PET inhibition IC_{50} [mmol/L]
2	C_2H_5	3.94	-0.11	47.29	-
3	C_3H_7	4.41	-0.12	63.80	0.080
4	$CH(CH_3)_2$	4.20	-0.19	64.18	0.271
5	C_4H_9	4.71	-0.25	80.31	0.589
6	C_5H_{11}	5.47	-0.23	96.81	0.396
7	C_6H_{13}	6.03	-0.25	113.32	0.358
8	C_7H_{15}	6.67	-0.23	129.83	0.263
9	C_8H_{17}	7.19	-0.23	146.33	0.290
DCMU	–	–	–		0.002

The PET-inhibiting activity was expressed by negative logarithm of IC_{50} value (compound concentration in mol/L causing 50% inhibition of PET). The evaluated 1-[(2-chlorophenyl)-carbamoyl]naphthalen-2-yl alkylcarbamates showed relatively low PET-inhibiting activity related to PET inhibition in spinach (*Spinacia oleracea* L.) chloroplasts with IC_{50} values ranging from 0.080 to 0.589 mmol/L, see Table 1. The least lipophilic compound **2** ($R = C_2H_5$) was inactive, and it was not possible to determine its IC_{50} value. 1-[(2-Chlorophenyl)carbamoyl]naphthalen-2-yl propylcarbamate (**3**) had the highest PET-inhibiting activity (IC_{50} = 0.080 mmol/L) within the whole investigated series. While ethyl derivative **2** was inactive due to low lipophilicity, propyl derivative **3** showing sufficient lipophilicity together with suitable aqueous solubility was the most active compound. With the elongation of the alkyl chain in the R substituent, the aqueous solubility of the evaluated derivatives decreased, and at higher concentrations they precipitated from the solution during the experiment. The lowest solubility was shown by butyl derivative **5**. The dependence of the PET-inhibiting activity log(1/IC_{50} [mol/L]) of compounds **3**–**9** in spinach chloroplasts on lipophilicity (expressed as log *P*) and electronic properties (expressed as Taft polar constants σ*) of the alkyl tail is shown in Figure 1. The dependence on Taft polar constants σ* is linear; the PET inhibiting activity linearly increases (correlation coefficient r = 0.9274, n = 7) with the increase of electron-withdrawing properties. A strong dependence of PET inhibition on the electron-withdrawing effect of substituents in individual series of many PET inhibitors was observed [13–17,31,37]

Besides physicochemical parameters, such as, for example, lipophilicity or electronic properties of the substituents, also appropriate concentration of the compound at the site of action in the photosynthetic apparatus is important for PET-inhibiting activity. A compound having very low aqueous solubility cannot pass through the hydrophilic regions of the thylakoid membrane to reach the site of action, which results in a significant decrease of inhibitory activity. The solubility

of butyl derivative **5** and derivatives with longer alkyl chains was similar and significantly lower than that of propyl (**3**) and isopropyl (**4**) derivatives, which resulted in a notable activity decrease; a slight increase of PET-inhibiting activity with further prolongation of the alkyl tail can be connected with the fact that a longer alkyl chain can be incorporated in the thylakoid membrane to a greater extent and subsequently cause membrane perturbation also at lower concentration. This effect is connected with the surface activity of these compounds (they can be considered as non-ionic surfactants) and with the alkyl tail length (molar volume), which is again reflected by lipophilicity. From the aspect of PET-inhibiting activity, the lipophilicity optimum for C_4–C_8 alkyl chains can be found for C_7, see Fig. 1A. With the further elongation of the alkyl chain (hydrophobic part) to octyl, so called 'cut-off' effect, i.e. the loss of biological activity usually observed for amphiphilic compounds was manifested [22,23,38–40].

Figure 1. Dependence of PET-inhibiting activity log(1/IC_{50} [mol/L]) of compounds **3**–**9** in spinach chloroplasts on lipophilicity expressed as log *P* (**A**) and electronic properties expressed as Taft polar constants σ* of alkyl tail (**B**).



The application of 2,5-diphenylcarbazide (DPC, artificial electron donor) that supplies electrons in the site of $Z^{\bullet}/D^{\bullet}$ intermediate, i.e. tyrosine radicals Tyr_Z and Tyr_D (or their surroundings) that are situated in D_1 and D_2 proteins on the donor side of PS II [36] to chloroplasts, the activity of which was inhibited by the most active compound **3** (up to 30% of the control) caused practically complete PET restoration. Therefore it can be concluded that the site of action of the studied 1-[(2-chlorophenyl)carbamoyl]naphthalen-2-yl alkylcarbamates is situated mainly on the donor side of PS II. The site of action situated on the donor side of PS II was found also for 2-alkylthio-6-R-benzothiazoles (R = 6-formamido-, 6-acetamido-, and 6-benzoylamino-) [41], anilides of 2-alkylpyridine-4-carboxylic acids [42], cationic surfactants [43,44] acting in the intermediates $Z^{\bullet}/D^{\bullet}$ and 2-alkylsulphanyl-4-pyridinecarbothioamides acting in the $D^{\bullet}$ intermediate [45].

EXPERIMENTAL

General

All reagents were purchased from Aldrich (Sigma-Aldrich, St. Louis, MO, USA), and Alfa (Alfa-Aesar, Ward Hill, Massachusetts, USA). TLC experiments were performed on alumina-backed silica gel 60 F254 plates (Merck, Darmstadt, Germany). The plates were illuminated under UV (254 nm) and evaluated in iodine vapour. The melting points were determined on Kofler hot-plate apparatus HMK (Franz Kustner Nacht KG, Dresden, Germany) and are uncorrected. Infrared (IR) spectra were recorded on a Smart MIRacle™ ATR ZnSe for Nicolet™ Impact 410 FT-IR spectrometer (Thermo Scientific, West Palm Beach, FL, USA). The spectra were obtained by accumulation of 256 scans with 2 cm^{-1} resolution in the region of 4000–600 cm^{-1}. All ^{1}H- and ^{13}C-NMR spectra were recorded on a

Varian Mercury 300 MHz NMR spectrometer (300 MHz for ^{1}H and 75.5 MHz for ^{13}C, Varian, Palo Alto, CA, USA) in DMSO-d_6. ^{1}H and ^{13}C chemical shifts (δ) are reported in ppm. High-resolution mass spectra were measured using a high-performance liquid chromatograph Dionex UltiMate® 3000 (Thermo Scientific) coupled with a LTQ Orbitrap XL™ Hybrid Ion Trap-Orbitrap Fourier Transform Mass Spectrometer (Thermo Scientific) with injection into HESI II in the positive mode.

Synthesis

The synthetic pathway and characterization of *N*-(2-chlorophenyl)-2-hydroxynaphthalene-1-carboxamide (**1**) was described recently by Gonec *et al.* [14].

General procedure for synthesis of alkylcarbamates **2**–**9**: *N*-(2-chlorophenyl)-2-hydroxynaphthalene-1-carboxamide (**1**, 1.0 mmol) and triethylamine (1.1 mmol) were suspended in dry acetonitrile (10 mL). The solution of the appropriate alkyl isocyanate (1.2 mmol) in acetonitrile (5 mL) was added in four portions within 2 h, and the reacting mixture was stirred for 24 h at ambient temperature. The solvent was evaporated under reduced pressure, the solid residue washed with methanol and dichlormethan to give pure product. Studied compounds **2**–**9** are presented in Table 1.

1-[(2-Chlorophenyl)carbamoyl]naphthalen-2-yl ethylcarbamate (**2**). Yield 33%; Mp 137-139 °C; IR (cm^{-1}): 3264, 3214, 1718, 1675, 1540, 1523, 1510, 1481, 1435, 1300, 1256, 1229, 1006, 826, 741, 723, 688, 667; ^{1}H-NMR (DMSO-d_6) δ: 10.14 (s, 1H), 7.99-8.06 (m, 3H), 7.88 (t, *J* = 5.5 Hz, 1H), 7.82 (dd, *J* = 7.9 Hz, 1.6 Hz, 1H), 7.55-7.65 (m, 3H), 7.43 (td, *J* = 7.7 Hz, 1.6 Hz, 1H), 7.42 (d, *J* = 9.2 Hz, 1H), 7.30 (td, *J* = 7.7 Hz, 1.6 Hz, 1H), 3.08-3.19 (m, 2H), 1.08 (t, *J* = 7.7 Hz, 3H); ^{13}C-NMR (DMSO-d_6), δ: 164.33, 153.97, 142.20, 134.65, 130.54, 130.31, 129.77, 129.60, 128.40, 128.04, 127.58, 127.30, 127.43, 127.28, 126.60, 125.78, 124.67, 122.62, 35.42, 14.83; HR-MS: for $C_{20}H_{16}O_3N_2Cl$ $[M+H]^+$ calculated 367.08440 *m/z*, found 367.08545 *m/z*.

1-[(2-Chlorophenyl)carbamoyl]naphthalen-2-yl propylcarbamate (**3**). Yield 26%; Mp 132-134 °C; IR (cm^{-1}): 3259, 3224, 1716, 1659, 1546, 1526, 1510, 1484, 1441, 1298, 1259, 1232, 1060, 991, 809, 741, 694; ^{1}H-NMR (DMSO-d_6) δ: 10.13 (s, 1H), 7.99-8.07 (m, 3H), 7.90 (t, *J* = 5.5 Hz, 1H), 7.83 (dd, *J* = 7.9 Hz, 1.6 Hz, 1H), 7.54-7.68 (m, 3H), 7.43 (td, *J* = 7.7 Hz, 1.6 Hz, 1H), 7.41 (d, *J* = 9.2 Hz, 1H), 7.30 (td, *J* = 7.7 Hz, 1.6 Hz, 1H), 3.04 (q, *J* = 6.2 Hz, 2H), 1.48 (sx, *J* = 7.2 Hz, 2H), 0.86 (t, *J* = 7.3 Hz, 3H); ^{13}C-NMR (DMSO-d_6), δ: 164.33, 154.20, 145.23, 134.67, 130.56, 130.31, 129.81, 129.59, 128.33, 128.04, 127.48, 127.42, 127.30, 127.28, 126.60, 125.78, 124.67, 122.62, 42.35, 22.45, 11.17; HR-MS: for $C_{21}H_{18}O_3N_2Cl$ $[M+H]^+$ calculated 381.10005 *m/z*, found 381.10123 *m/z*.

1-[(2-Chlorophenyl)carbamoyl]naphthalen-2-yl isoproylcarbamate (**4**). Yield 39%; Mp 167-169 °C; IR (cm^{-1}): 3249, 3216, 1971, 1716, 1668, 1581, 1544, 1530, 1510, 1481, 1462, 1439, 1324, 1300, 1256, 1234, 1066, 1022, 962, 910, 820, 742, 697; ^{1}H-NMR (DMSO-d_6) δ: 10.09 (s, 1H), 7.98-8.06 (m, 3H), 7.84 (d, *J* = 8.0 Hz, 1H), 7.83 (dd, *J* = 7.9 Hz, 1.6 Hz, 1H), 7.53-7.68 (m, 3H), 7.43 (td, *J* = 7.7 Hz, 1.6 Hz, 1H), 7.40 (d, *J* = 9.2 Hz, 1H), 7.30 (td, *J* = 7.7 Hz, 1.6 Hz, 1H), 3.63-3.73 (m, 1H), 1.11 (d, *J* = 6.6 Hz, 6H); ^{13}C-NMR (DMSO-d_6), δ: 164.42, 153.41, 145.28, 134.70, 130.60, 130.37, 129.90, 129.65, 128.34, 128.10, 127.49, 127.48, 127.36, 127.35, 126.67, 125.85, 124.70, 122.71, 42.91, 22.40; HR-MS: for $C_{21}H_{18}O_3N_2Cl$ $[M+H]^+$ calculated 381.10005 *m/z*, found 381.10123 *m/z*.

1-[(2-Chlorophenyl)carbamoyl]naphthalen-2-yl butylcarbamate (**5**). Yield 55%; Mp 156-159 °C; IR (cm^{-1}): 3258, 3228, 1724, 1665, 1553, 1526, 1506, 1479, 1439, 1232, 1007, 818, 751; ^{1}H-NMR (DMSO-d_6) δ: 10.09 (s, 1H), 7.98-8.06 (m, 3H), 7.88 (t, *J* = 5.6 Hz, 1H), 7.84 (dd, *J* = 7.9 Hz, 1.6 Hz, 1H), 7.54-7.67 (m, 3H), 7.43 (td, *J* = 7.7 Hz, 1.6 Hz, 1H), 7.41 (d, *J* = 9.2 Hz, 1H), 7.30 (td, *J* = 7.7 Hz, 1.6 Hz, 1H), 3.08 (q, *J* = 6.2 Hz, 2H), 1.44 (qi,

J = 7.0 Hz, 2H), 1.28 (sx, *J* = 7.0 Hz, 2H), 0.85 (t, *J* = 7.3 Hz, 3H); ^{13}C-NMR (DMSO-d_6), δ: 164.31, 154.19, 145.23, 134.67, 130.57, 130.31, 129.84, 129.60, 128.20, 128.05, 127.41, 127.32, 127.31, 127.22, 126.59, 125.79, 124.65, 122.60, 40.26, 31.32, 19.37, 13.63; HR-MS: for $C_{22}H_{20}O_3N_2Cl$ $[M+H]^+$ calculated 395.11570 *m/z*, found 395.11639 *m/z*.

1-[(2-Chlorophenyl)carbamoyl]naphthalen-2-yl pentylcarbamate (**6**). Yield 36%; Mp 137-138 °C; IR (cm^{-1}): 3227, 3215, 1724, 1669, 1550, 1521, 1508, 1480, 1434, 1233, 1037, 997, 910, 817, 746, 686; ^{1}H-NMR (DMSO-d_6) δ: 10.10 (s, 1H), 7.97-8.07 (m, 3H), 7.88 (t, *J* = 5.5 Hz, 1H), 7.84 (dd, *J* = 7.9 Hz, 1.6 Hz, 1H), 7.54-7.67 (m, 3H), 7.42 (td, *J* = 7.7 Hz, 1.6 Hz, 1H), 7.40 (d, *J* = 9.2 Hz, 1H), 7.30 (td, *J* = 7.7 Hz, 1.6 Hz, 1H), 3.07 (q, *J* = 6.2 Hz, 2H), 1.45 (qi, *J* = 6.6 Hz, 2H), 1.24-1.28 (m, 4H), 0.84 (t, *J* = 7.0 Hz, 3H); ^{13}C-NMR (DMSO-d_6), δ: 164.39, 154.24, 145.26, 134.68, 130.62, 130.36, 129.90, 129.65, 128.27, 128.10, 127.46, 127.40, 127.37, 127.31, 126.64, 125.87, 124.70, 122.65, 40.59, 28.91, 28.42, 21.87, 13.92; HR-MS: for $C_{23}H_{22}O_3N_2Cl$ $[M+H]^+$ calculated 409.13135 *m/z*, found 409.13303 *m/z*.

1-[(2-Chlorophenyl)carbamoyl]naphthalen-2-yl hexylcarbamate (**7**). Yield 50%; Mp 103-106 °C; IR (cm^{-1}): 2954, 2924, 1725, 1673, 1587, 1525, 1516, 1463, 1440, 1301, 1241, 1210, 1037, 995, 813, 756, 747, 684; ^{1}H-NMR (DMSO-d_6) δ: 10.09 (s, 1H), 7.97-8.07 (m, 3H), 7.88 (t, *J* = 5.5 Hz, 1H), 7.84 (dd, *J* = 7.9 Hz, 1.6 Hz, 1H), 7.54-7.67 (m, 3H), 7.42 (td, *J* = 7.7 Hz, 1.6 Hz, 1H), 7.40 (d, *J* = 9.2 Hz, 1H), 7.30 (td, *J* = 7.7 Hz, 1.6 Hz, 1H), 3.06 (q, *J* = 6.2 Hz, 2H), 1.35-1.47 (m, 2H), 1.12-1.32 (m, 6H), 0.84 (t, *J* = 6.6 Hz, 3H); ^{13}C-NMR (DMSO-d_6), δ: 164.39, 154.26, 145.26, 134.70, 130.63, 130.37, 129.93, 129.66, 128.25, 128.13, 127.46, 127.39, 127.36, 127.30, 126.66, 125.88, 124.71, 122.65, 40.64, 31.03, 29.21, 25.92, 22.07, 13.96; HR-MS: for $C_{24}H_{24}O_3N_2Cl$ $[M+H]^+$ calculated 423.14700 *m/z*, found 423.14767 *m/z*.

1-[(2-Chlorophenyl)carbamoyl]naphthalen-2-yl heptylcarbamate (**8**). Yield 66%; Mp 89-90 °C; IR (cm^{-1}): 3279, 3227, 2956, 2933, 2923, 1720, 1662, 1538, 1520, 1479, 1463, 1438, 1296, 1266, 1253, 1234, 1192, 1060, 986, 911, 816, 750; ^{1}H-NMR (DMSO-d_6) δ: 10.09 (s, 1H), 7.97-8.06 (m, 3H), 7.87 (t, *J* = 5.5 Hz, 1H), 7.84 (dd, *J* = 7.9 Hz, 1.6 Hz, 1H), 7.54-7.67 (m, 3H), 7.41 (td, *J* = 7.7 Hz, 1.6 Hz, 1H), 7.39 (d, *J* = 9.2 Hz, 1H), 7.29 (td, *J* = 7.7 Hz, 1.6 Hz, 1H), 3.06 (q, *J* = 6.2 Hz, 2H), 1.37-1.46 (m, 2H), 1.14-1.35 (m, 8H), 0.85 (t, *J* = 6.6 Hz, 3H); ^{13}C-NMR (DMSO-d_6), δ: 164.42, 154.29, 145.28, 134.71, 130.65, 130.39, 129.96, 129.68, 128.27, 128.14, 127.48, 127.41, 127.39, 127.31, 126.67, 125.90, 124.73, 122.67, 40.65, 31.26, 29.26, 28.48, 26.22, 22.11, 14.02; HR-MS: for $C_{25}H_{26}O_3N_2Cl$ $[M+H]^+$ calculated 437.16265 *m/z*, found 437.16431 *m/z*.

1-[(2-Chlorophenyl)carbamoyl]naphthalen-2-yl octylcarbamate (**9**). Yield 52%; Mp 115-116 °C; IR (cm^{-1}): 3297, 3286, 2924, 2851, 1724, 1673, 1584, 1548, 1540, 1509, 1467, 1441, 1434, 1299, 1246, 1215, 1160, 993, 827, 756, 747, 734, 677; ^{1}H-NMR (DMSO-d_6) δ: 10.09 (s, 1H), 7.97-8.06 (m, 3H), 7.87 (t, *J* = 5.5 Hz, 1H), 7.84 (dd, *J* = 7.9 Hz, 1.6 Hz, 1H), 7.53-7.68 (m, 3H), 7.42 (td, *J* = 7.7 Hz, 1.6 Hz, 1H), 7.40 (d, *J* = 9.2 Hz, 1H), 7.29 (td, *J* = 7.7 Hz, 1.6 Hz, 1H), 3.06 (q, *J* = 6.2 Hz, 2H), 1.38-1.47 (m, 2H), 1.15-1.30 (m, 10H), 0.86 (t, *J* = 6.6 Hz, 3H); ^{13}C-NMR (DMSO-d_6), δ: 164.33, 154.19, 145.23, 134.68, 130.57, 130.33, 129.87, 129.60, 128.22, 128.07, 127.40, 127.35, 127.33, 127.22, 126.61, 125.81, 124.67, 122.60, 40.59, 31.25, 29.20, 28.73, 28.64, 26.21, 22.10, 13.96; HR-MS: for $C_{26}H_{28}O_3N_2Cl$ $[M+H]^+$ calculated 451.17830 *m/z*, found 451.18018 *m/z*.

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

Chloroplasts were prepared from spinach (*Spinacia oleracea* L.) according to Masarovicova and Kralova [46]. The inhibition of photosynthetic electron transport (PET) in spinach chloroplasts was determined spectrophotometrically (Genesys 6, Thermo Scientific), using an

artificial electron acceptor 2,6-dichlorophenol-indophenol (DCPIP) according to Kralova *et al*. [47], 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), $MgCl_2$ (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/m^2 with 10 cm distance) with a halogen lamp (250 W) using a 4 cm water filter to prevent warming of the samples (suspension temperature ~4 °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 IC_{50} values, i.e. by molar concentration of the compounds causing 50% decrease in the oxygen evolution rate relative to the untreated control. The comparable IC_{50} value for a selective herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea, DCMU (Diuron®) was about 0.002 mmol/L. The results are summarized in Table 1.

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