



Preparation and Photosynthesis-Inhibiting Activity of *N*-(*n*-Alkoxy)phenylamides of 2-Hydroxynaphthalene-1-carboxylic acid

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Abstract: In this study a series of twelve *n*-alkoxy-substituted 2-hydroxynaphthalene-1-carboxanilides was prepared and characterized. The discussed compounds were prepared by microwave-assisted synthesis. The compounds were tested for their activity related to inhibition of photosynthetic electron transport (PET) in spinach (*Spinacia oleracea* L.) chloroplasts. The highest PET inhibition was observed for *meta*-substituted compounds, whereas 2-hydroxy-*N*-(3-propoxyphenyl)naphthalene-1-carboxamide showed the highest PET inhibition within the whole series. In spite of medium or moderate PET-inhibiting activity it was found that the compounds inhibit PET in photosystem II. The activity of all positional isomers is strongly influenced by lipophilicity and the length/bulkiness of the alkoxy chain.

Keywords: Hydroxyquinoline-1-carboxamides; PET inhibition; Spinach chloroplasts; Structure-activity relationships.

INTRODUCTION

Salicylanilides (*N*-substituted hydroxybenzamides) represent compounds with a wide range of pharmacological activities, including anti-inflammatory [1], anthelmintic [2] and antimicrobial [3–9] properties. The exact mechanisms of action are still under investigation, but these compounds are known to act as inhibitors of protein kinase epidermal growth factor receptor and they also interact with some other enzymatic system in a cell [9,10]. In addition, according to the results reported recently, salicylanilides and their analogues were found to be inhibitors of photosynthetic electron transport (PET). [5–8,11–15].

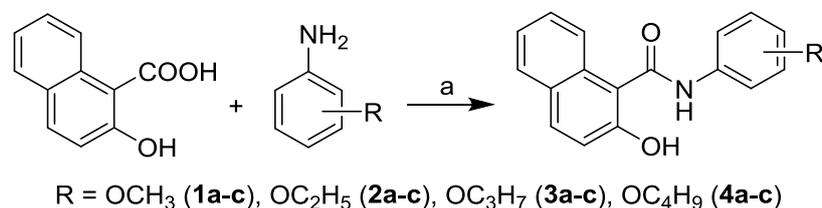
Although at present approximately 20 mechanisms of action of herbicides are known [16], over 50% of commercially available herbicides act by reversible binding to photosystem II (PS II), a membrane-protein complex in the thylakoid membranes, which catalyses the oxidation of water and the reduction of plastoquinone [17], and thereby inhibit photosynthesis [18–20]. Some organic compounds, possessing an amide (-NHCO-) group, e.g., substituted anilides [5–8,11–15,21,22], were found to interact with tyrosine radicals Tyr_Z and Tyr_D (or their surroundings) which are situated in D₁ and D₂ proteins on the donor side of PS II. Due to this interaction, interruption of the photosynthetic electron transport occurs.

In the context of the previously-described amides/carbamates [4–15,21,22], new *N*-(*n*-alkoxy)phenylamides of 2-hydroxynaphthalene-1-carboxylic acid were prepared and tested for their photosynthesis-inhibiting activity – the inhibition of photosynthetic electron transport in spinach chloroplasts (*Spinacia oleracea* L.). The 2-hydroxynaphthalene-1-carboxanilides can be considered as cyclic analogues of above discussed salicylanilides. The structure-activity relationships are discussed.

RESULTS AND DISCUSSION

All the studied compounds were prepared according to Scheme 1. Microwave-assisted synthesis [6–8] facilitated the process of obtaining ring-substituted 2-hydroxynaphthalene-1-carboxanilides, thus synthesis of the target compounds was carried out only by one step. The condensation of 2-hydroxy-1-naphthoic acid with *n*-alkoxy-substituted anilines using phosphorus trichloride in dry chlorobenzene under microwave conditions yielded a series of eleven *N*-substituted 2-hydroxynaphthalene-1-carboxanilides **1a–4c**. All the compounds were recrystallized from ethanol.

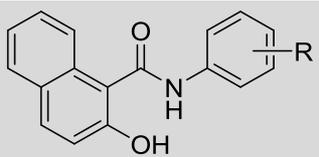
Scheme 1. Synthesis of ring-substituted 2-hydroxynaphthalene-1-carboxanilides **1a–4c**: (a) PCl₃, chlorobenzene, MW.



Lipophilicity of all compounds **1a–4c** was calculated as log *P* using ACD/Percepta ver. 2012 (Advanced Chemistry Development, Inc., Toronto, ON, Canada). The results are shown in Table 1. Lipophilicity values of the compounds expressed as log *P* values ranged from 4.30 (compounds **1c**, R = 4-OCH₃) to 5.53/5.54 (compounds **4a/b**, R = 2- and 4-OC₄H₉). Logically lipophilicity increases with lengthening of the alkoxy chain. For individual substituents in the aniline part of the discussed compounds also electronic Hammett's σ parameters were predicted using the same software; they ranged from -0.29 to -0.27 for *ortho*- and *para*-substituted compounds and from 0.10 to 0.14 for *meta*-substituted compounds. Experience

has shown that a parameter representing the volume of the substituents on each compound relative to other members of the same series may often be correlated with biological measurement [23–25], therefore molar volume MV [cm³] was also calculated. The predicted molecular descriptors of the studied compounds are shown in Table 1.

Table 1. Structure of ring-substituted 2-hydroxynaphthalene-1-carboxanilides **1a–4c**, calculated values of log *P*, electronic Hammett's σ parameters, molar volume MV [cm³] (calculated using ACD/Percepta ver. 2012) and IC₅₀ [μmol/L] values related to PET inhibition in spinach chloroplasts of compounds **1a–4c** in comparison with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) standard. (ND = not determined)

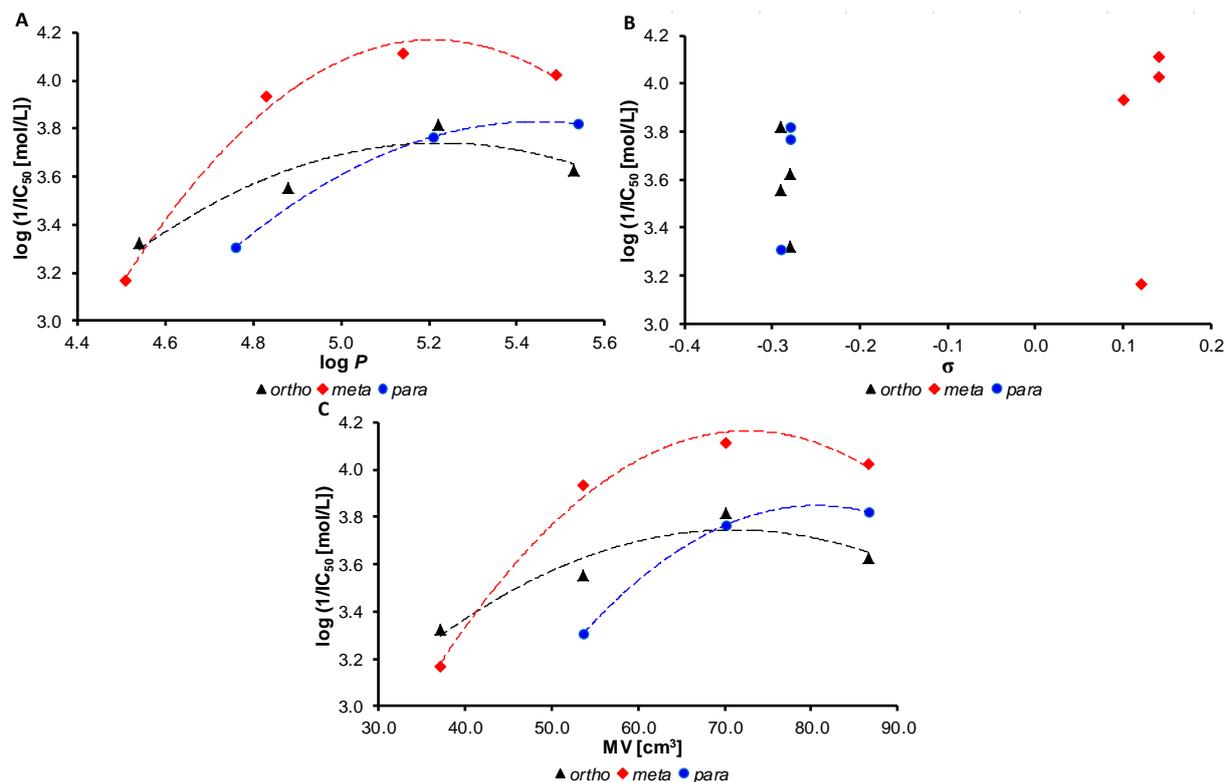
					
Comp.	R	log <i>P</i>	σ	MV [cm ³]	PET inhibition IC ₅₀ [μmol/L]
1a	2-OCH ₃	4.54	-0.28	37.15	477
1b	3-OCH ₃	4.51	0.12	37.15	681
1c	4-OCH ₃	4.30	-0.27	37.15	ND
2a	2-OC ₂ H ₅	4.88	-0.29	53.66	279
2b	3-OC ₂ H ₅	4.83	0.10	53.66	117
2c	4-OC ₂ H ₅	4.76	-0.29	53.66	493
3a	2-OC ₃ H ₇	5.22	-0.29	70.16	152
3b	3-OC ₃ H ₇	5.14	0.14	70.16	77.3
3c	4-OC ₃ H ₇	5.21	-0.28	70.16	171
4a	2-OC ₄ H ₉	5.53	-0.28	86.67	237
4b	3-OC ₄ H ₉	5.49	0.14	86.67	94.5
4c	4-OC ₄ H ₉	5.54	-0.28	86.67	151
DCMU	–	–	–		1.9

The evaluated 2-hydroxynaphthalene-1-carboxanilides derivatives showed a wide range of PET-inhibiting activity related to PET inhibition in spinach (*Spinacia oleracea* L.) chloroplasts, see Table 1. The PET-inhibiting activity was expressed by negative logarithm of IC₅₀ value (compound concentration in mol/L causing 50% inhibition of PET). It was not possible to determine IC₅₀ value for compound **1c** (R = 4-OCH₃) due to its interaction with 2,6-dichlorophenol-indophenol (DCPIP). 2-Hydroxy-*N*-(3-propoxyphenyl)naphthalene-1-carboxamide (**3b**) expressed the highest PET-inhibiting activity (IC₅₀ = 77.3 μmol/L), although it can be stated that all the compounds demonstrated rather moderate PET inhibition. With respect to these small but specifically substituted groups of compounds some structure-activity relationships (SAR) can be proposed.

Correlations between log(1/IC₅₀ [mol/L]) and lipophilicity (expressed as log *P*), electronic properties of the individual alkoxy substituents in compounds **1a–4c** (expressed as electronic Hammett's σ parameters) and the length (bulkiness) of the alkoxy tail (expressed as molar volume MV [cm³]) of individual alkoxy chains were performed, see Figure 1. Based on the obtained results it is evident that *meta* substitution of the aniline ring is preferred. Figure 1A (the dependences between PET inhibition and lipophilicity) and Figure 1C (the dependences between PET inhibition and molar volume) illustrate the general quasi-parabolic trend within all three positional isomers. The propoxy substituent in the *ortho* and *meta* positions of aniline

(compounds **3a**, **3b**) seems to be the most favourable. Among *para*-substituted compounds **2c**, **3c**, **4c** butyl derivative **4c** is the most effective PET inhibitor (see Figures 1A and 1C, blue line). The dependence of PET inhibition on electronic σ properties of the anilide substituents plays a secondary role, see Figure 1B. It can be stated that weak electron-withdrawing properties of a substituent in the *meta* position are more preferable than electron-donor properties of alkoxy moieties in the *ortho* and *para* positions.

Figure 1. Dependence of PET-inhibiting activity $\log(1/IC_{50}$ [mol/L]) of studied compounds **1a–4c** in spinach chloroplasts on lipophilicity expressed as $\log P$ (A), *N*-substituent electronic Hammett's σ parameters (B) and molar volume MV [cm^3] of individual alkoxy chains (C).



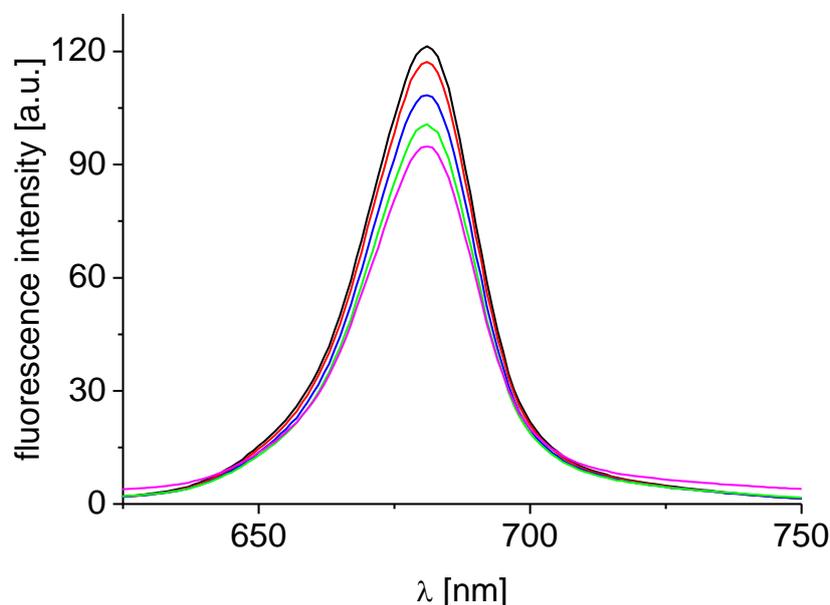
Application of artificial electron donors allows specifying the section in the photosynthetic electron transport chain in which PET is stopped by an inhibitor [26]. One of such suitable artificial electron donors is 2,5-diphenylcarbazide (DPC) which supplies electrons in the site of Z^{\bullet}/D^{\bullet} intermediate on the donor side of PS II [20]. Consequently, in the presence of DPC the PET which was inhibited in the section between the oxygen evolving complex and the Z^{\bullet}/D^{\bullet} intermediate can be restored. On the other hand, PET restoration by DPC does not occur if the site of PET inhibition is situated on the acceptor side of PS II, between P680 and secondary quinone acceptor Q_B . However, if PET inhibitors directly interact with the herbicide-binding niche they can be displaced from their binding site similarly as it was demonstrated for atrazine [27] or metribuzin [28], which is also connected with complete restoration of the photochemical activity of chloroplasts.

As application of DPC to chloroplasts, the activity of which was inhibited (up to 15% of the control), caused practically complete PET restoration, it can be concluded that the site of studied *n*-alkoxy-substituted 2-hydroxynaphthalene-1-carboxanilides is situated mainly on the donor side of PS II. However, for univocal confirmation whether the PET inhibition by the studied compounds occur also on the acceptor side of PS II further investigation would be necessary. 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-) [29], anilides of 2-alkylpyridine-4-carboxylic acids acting in the intermediates Z^{\bullet}/D^{\bullet} [30] and 2-alkylsulphonyl-4-pyridinecarbothioamides acting in the D^{\bullet} intermediate [31].

Studied compounds affected the chlorophyll *a* (Chl*a*) fluorescence in spinach chloroplasts. As shown in Figure 2, the intensity of the Chl*a* emission band at 686 nm belonging to the pigment–protein complexes in photosystem II decreased in the presence of compound **2b** [26]. This finding indicates a perturbation of the Chl*a*–protein complexes in the thylakoid membrane caused by the tested compound, which contributes to PET inhibition. A similar Chl*a* fluorescence decrease in spinach chloroplasts was observed previously with several PET inhibitors, e.g., ring-substituted 3-hydroxynaphthalene-2-carboxanilides [6], 2-hydroxynaphthalene-1-carboxanilides [7] and 1-hydroxynaphthalene-2-carboxanilides [8] or ring-substituted 4-arylamino-7-chloroquinolinium chlorides [32].

Figure 2. Fluorescence emission spectra of chlorophyll *a* in untreated spinach chloroplasts in presence of compound **2b**: 0, 102, 204, 408 and 612 $\mu\text{mol/L}$ (curves from top to bottom; $\lambda_{\text{ex}} = 436 \text{ nm}$; chlorophyll concentration 10 mg/L).



EXPERIMENTAL

General

All reagents were purchased from Aldrich (Sigma-Aldrich, St. Louis, MO, USA) and Merck (Merck, Darmstadt, Germany). TLC experiments were performed on alumina-backed silica gel 40 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 Electron Corporation, West Palm Beach, FL, USA). The spectra were obtained by accumulation of 256 scans with 2 cm^{-1} resolution in the region of $4000\text{--}600 \text{ cm}^{-1}$. All ^1H - and ^{13}C -NMR spectra were recorded on an Agilent 300 MHz VNMR spectrometer (299.96 MHz for ^1H and 75.43 MHz for ^{13}C , Agilent Technologies, Santa Clara, CA, USA) in $\text{DMSO-}d_6$. Chemical shifts (δ) are reported in ppm. Mass spectra were measured using a LTQ Orbitrap Hybrid Mass Spectrometer (Thermo Electron Corporation) with direct injection into an APCI source ($400 \text{ }^\circ\text{C}$) in the positive mode.

Synthesis

Alkyloxy anilines (except commercially available *o*-, *m*- and *p*-anisidine) were prepared from corresponding aminophenols and alkyl bromides using the method described by De Marco *et al.* [33]. Freshly distilled crude products were used directly in the next step.

General procedure for synthesis of carboxamide derivatives (1a–4c): 2-Hydroxynaphthalene-1-carboxylic acid (5.3 mmol) and the corresponding substituted aniline (5.3 mmol) were suspended in dry chlorobenzene (30 mL). Phosphorous trichloride (2.65 mmol) was added dropwise, and the reacting mixture was heated in the microwave reactor at maximal allowed power 500 W and 130 °C, using infrared flask-surface control of temperature, for 15 min. The solvent was evaporated under reduced pressure, the solid residue washed with 2M HCl, and the crude product was recrystallized from aqueous ethanol. Studied compounds **1a–4c** are presented in Table 1.

2-Hydroxy-N-(2-methoxyphenyl)naphthalene-1-carboxamide (1a). Yield 76% [7].

2-Hydroxy-N-(3-methoxyphenyl)naphthalene-1-carboxamide (1b). Yield 77% [7].

2-Hydroxy-N-(4-methoxyphenyl)naphthalene-1-carboxamide (1c). Yield 74% [7].

N-(2-Ethoxyphenyl)-2-hydroxynaphthalene-1-carboxamide (2a). Yield 62%; Mp 116 °C; IR (cm⁻¹): 3318, 2970, 2880, 1623, 1611, 1601, 1579, 1539, 1490, 1464, 1452, 1391, 1338, 1284, 1254, 1222, 1203, 1118, 1043, 968, 926, 831, 809, 796, 745, 727; ¹H-NMR (DMSO-*d*₆) δ: 10.48 (br. s, 1H), 9.43 (s, 1H), 8.19 (d, 1H, *J* = 9.2 Hz), 8.15 (d, 1H, *J* = 9.2 Hz), 7.88 (d, 1H, *J* = 8.4 Hz), 7.84 (d, 1H, *J* = 7.0 Hz), 7.48 (td, 1H, *J* = 8.4 Hz, *J* = 1.1 Hz), 7.34 (td, 1H, *J* = 8.1 Hz, *J* = 1.1 Hz), 7.24 (d, 1H, *J* = 9.2 Hz), 7.13-6.96 (m, 3H), 4.10 (q, 2H, *J* = 7.0 Hz), 1.36 (t, 3H, *J* = 7.0 Hz); ¹³C-NMR (DMSO-*d*₆) δ: 165.06, 152.42, 148.91, 131.81, 131.05, 127.94, 127.73, 127.73, 126.85, 124.50, 124.12, 123.03, 121.74, 120.33, 118.25, 116.60, 112.21, 63.99, 14.57; HR-MS: for C₁₉H₁₇NO₃ [M+H]⁺ calculated 308.12812 *m/z*, found 308.12827 *m/z*.

N-(3-Ethoxyphenyl)-2-hydroxynaphthalene-1-carboxamide (2b). Yield 64%; Mp 133 °C; IR (cm⁻¹): 3323, 3053, 2966, 2916, 2872, 1623, 1614, 1594, 1578, 1536, 1510, 1466, 1448, 1387, 1315, 1284, 1243, 1230, 1208, 1158, 1052, 964, 866, 834, 758, 735, 681; ¹H-NMR (DMSO-*d*₆) δ: 10.34 (s, 1H), 10.05 (br. s, 1H), 7.85 (d, 2H, *J* = 8.8 Hz), 7.68 (d, 1H, *J* = 8.1 Hz), 7.53 (t, 1H, *J* = 2.0 Hz), 7.46 (td, 1H, *J* = 7.6 Hz, *J* = 1.3 Hz), 7.36-7.19 (m, 4H), 6.66 (ddd, 1H, *J* = 7.9 Hz, *J* = 2.2 Hz, *J* = 0.7 Hz), 4.02 (q, 2H, *J* = 7.0 Hz), 1.34 (t, 3H, *J* = 7.0 Hz); ¹³C-NMR (DMSO-*d*₆) δ: 165.68, 158.73, 151.55, 140.74, 131.36, 130.04, 129.34, 127.87, 127.35, 126.85, 123.35, 122.91, 118.58, 118.31, 111.53, 109.19, 105.66, 62.87, 14.60; HR-MS: for C₁₉H₁₇NO₃ [M+H]⁺ calculated 308.12812 *m/z*, found 308.12869 *m/z*.

N-(4-Ethoxyphenyl)-2-hydroxynaphthalene-1-carboxamide (2c). Yield 53%; Mp 202 °C; IR (cm⁻¹): 3267, 1623, 1530, 1506, 1460, 1414, 1392, 1338, 1288, 1244, 1229, 1203, 1171, 1143, 1117, 1047, 825, 802, 705; ¹H-NMR (DMSO-*d*₆) δ: 10.22 (s, 1H), 10.06 (s, 1H), 7.84 (d, 2H, *J* = 8.4 Hz), 7.71 (d, 2H, *J* = 9.2 Hz), 7.69 (d, 1H, *J* = 6.6 Hz), 7.46 (td, 1H, *J* = 7.6 Hz, *J* = 1.3 Hz), 7.32 (td, 1H, *J* = 7.3 Hz, *J* = 1.1 Hz), 7.24 (d, 1H, *J* = 9.2 Hz), 6.91 (d, 2H, *J* = 9.2 Hz), 4.01 (q, 2H, *J* = 7.0 Hz), 1.33 (t, 3H, *J* = 7.0 Hz); ¹³C-NMR (DMSO-*d*₆) δ: 165.06, 154.44, 151.92, 132.75, 131.45, 129.89, 127.84, 127.37, 126.78, 123.45, 122.86, 120.71, 118.69, 118.32, 114.33, 63.08, 14.60; HR-MS: for C₁₉H₁₇NO₃ [M+H]⁺ calculated 308.12812 *m/z*, found 308.12866 *m/z*.

2-Hydroxy-N-(2-propoxyphenyl)naphthalene-1-carboxamide (3a). Yield 80%; Mp 125 °C; IR (cm⁻¹): 3307, 2963, 2934, 2869, 1622, 1614, 1600, 1578, 1537, 1488, 1451, 1390, 1336, 1286, 1255, 1221, 1201, 1117, 977, 826, 795, 751, 741, 725; ¹H-NMR (DMSO-*d*₆) δ: 10.49 (s, 1H), 9.40 (s, 1H), 8.20 (d, 1H, *J* = 9.2 Hz), 8.15 (d, 1H, *J* = 9.2 Hz), 7.88 (d, 1H,

$J = 8.8$ Hz), 7.84 (d, 1H, $J = 7.0$ Hz), 7.48 (td, 1H, $J = 7.3$ Hz, $J = 1.3$ Hz), 7.34 (td, 1H, $J = 7.9$ Hz, $J = 1.1$ Hz), 7.28 (d, 1H, $J = 9.2$ Hz), 7.16-6.95 (m, 3H), 4.00 (t, 2H, $J = 6.4$ Hz), 1.76 (sx, 2H, $J = 6.8$ Hz), 0.97 (t, 3H, $J = 7.3$ Hz); $^{13}\text{C-NMR}$ (DMSO- d_6), δ : 165.04, 152.45, 149.03, 131.81, 131.10, 127.96, 127.75, 127.63, 126.85, 124.52, 124.11, 123.04, 121.71, 120.27, 118.23, 116.53, 112.09, 69.76, 21.98, 10.35; HR-MS: for $\text{C}_{20}\text{H}_{19}\text{NO}_3$ $[\text{M}+\text{H}]^+$ calculated 322.14377 m/z , found 322.14438 m/z .

2-Hydroxy-N-(3-propoxyphenyl)naphthalene-1-carboxamide (3b). Yield 87%; Mp 125 °C; IR (cm^{-1}): 3352, 2962, 2926, 2874, 1629, 1608, 1578, 1538, 1513, 1493, 1437, 1321, 1276, 1243, 1182, 1003, 988, 819, 777, 742, 684; $^1\text{H-NMR}$ (DMSO- d_6) δ : 10.34 (s, 1H), 10.10 (br. s, 1H), 7.85 (d, 2H, $J = 8.8$ Hz), 7.68 (d, 1H, $J = 8.4$ Hz), 7.55 (t, 1H, $J = 1.3$ Hz), 7.46 (td, 1H, $J = 7.1$ Hz, $J = 1.1$ Hz), 7.38-7.18 (m, 4H), 6.67 (dd, 1H, $J = 8.1$ Hz, $J = 2.2$ Hz), 3.92 (t, 2H, $J = 6.6$ Hz), 1.74 (sx, 2H, $J = 7.0$ Hz), 0.99 (t, 3H, $J = 7.3$ Hz); $^{13}\text{C-NMR}$ (DMSO- d_6), δ : 165.69, 158.91, 151.55, 140.74, 131.36, 130.04, 129.33, 127.87, 127.35, 126.87, 123.35, 122.91, 118.58, 118.31, 111.50, 109.22, 105.70, 68.83, 21.98, 10.32; HR-MS: for $\text{C}_{20}\text{H}_{19}\text{NO}_3$ $[\text{M}+\text{H}]^+$ calculated 322.14377 m/z , found 322.14420 m/z .

2-Hydroxy-N-(4-propoxyphenyl)naphthalene-1-carboxamide (3c). Yield 71%; Mp 153 °C; IR (cm^{-1}): 3328, 3185, 2962, 2935, 2875, 1627, 1599, 1577, 1531, 1507, 1464, 1435, 1236, 1169, 1043, 968, 818, 754, 742; $^1\text{H-NMR}$ (DMSO- d_6) δ : 10.23 (s, 1H), 10.00 (br. s, 1H), 7.84 (d, 2H, $J = 8.4$ Hz), 7.71 (d, 2H, $J = 8.8$ Hz), 7.68 (d, 1H, $J = 7.0$ Hz), 7.45 (td, 1H, $J = 7.0$ Hz, $J = 1.1$ Hz), 7.32 (td, 1H, $J = 8.0$ Hz, $J = 2.0$ Hz), 7.24 (d, 1H, $J = 9.2$ Hz), 6.92 (d, 2H, $J = 8.8$ Hz), 3.91 (t, 2H, $J = 6.6$ Hz), 1.73 (sx, 2H, $J = 7.0$ Hz), 0.99 (t, 3H, $J = 7.3$ Hz); $^{13}\text{C-NMR}$ (DMSO- d_6), δ : 165.07; 154.62, 151.51, 132.75, 131.45, 129.89, 127.84, 127.37, 126.76, 123.45, 122.85, 120.71, 118.69, 118.32, 114.38, 69.09, 22.02, 10.32; HR-MS: for $\text{C}_{20}\text{H}_{19}\text{NO}_3$ $[\text{M}+\text{H}]^+$ calculated 322.14377 m/z , found 322.14429 m/z .

N-(2-Butoxyphenyl)-2-hydroxynaphthalene-1-carboxamide (4a). Yield 70%; Mp 108 °C; IR (cm^{-1}): 3301, 2938, 2872, 1621, 1613, 1603, 1578, 1536, 1492, 1454, 1392, 1337, 1287, 1255, 1220, 1200, 1115, 971, 827, 751, 739, 724, 709; $^1\text{H-NMR}$ (DMSO- d_6) δ : 10.45 (s, 1H), 9.37 (s, 1H), 8.17 (d, 1H, $J = 8.4$ Hz), 8.12 (d, 1H, $J = 9.5$ Hz), 7.88 (d, 1H, $J = 8.8$ Hz), 7.84 (d, 1H, $J = 7.0$ Hz), 7.47 (td, 1H, $J = 7.0$ Hz, $J = 1.1$ Hz), 7.34 (td, 1H, $J = 7.9$ Hz, $J = 2.0$ Hz), 7.27 (d, 1H, $J = 9.2$ Hz), 7.13-6.95 (m, 3H), 4.04 (t, 2H, $J = 6.4$ Hz), 1.73 (qi, 2H, $J = 6.9$ Hz), 1.42 (sx, 2H, $J = 7.4$ Hz), 0.88 (t, 3H, $J = 7.3$ Hz); $^{13}\text{C-NMR}$ (DMSO- d_6), δ : 165.04, 152.42, 149.08, 131.75, 131.05, 127.94, 127.72, 127.61, 126.82, 124.55, 124.06, 123.03, 121.77, 120.27, 118.22, 116.62, 112.10, 67.95, 30.64, 18.58, 13.57; HR-MS: for $\text{C}_{21}\text{H}_{21}\text{NO}_3$ $[\text{M}+\text{H}]^+$ calculated 336.15942 m/z , found 336.15982 m/z .

N-(3-Butoxyphenyl)-2-hydroxynaphthalene-1-carboxamide (4b). Yield 46%; Mp 118 °C; IR (cm^{-1}): 3350, 2956, 2929, 2870, 1628, 1608, 1578, 1537, 1493, 1437, 1321, 1276, 1241, 1208, 1182, 1159, 982, 819, 777, 742, 684; $^1\text{H-NMR}$ (DMSO- d_6) δ : 10.33 (s, 1H), 10.10 (br. s, 1H), 7.85 (d, 2H, $J = 8.8$ Hz), 7.67 (d, 1H, $J = 8.1$ Hz), 7.54 (t, 1H, $J = 1.8$ Hz), 7.46 (td, 1H, $J = 7.2$ Hz, $J = 1.1$ Hz), 7.36-7.18 (m, 4H), 6.69-6.65 (m, 1H), 3.96 (t, 2H, $J = 6.4$ Hz), 1.72 (qi, 2H, $J = 7.3$ Hz), 1.45 (sx, 2H, $J = 7.5$ Hz), 0.94 (t, 3H, $J = 7.3$ Hz); $^{13}\text{C-NMR}$ (DMSO- d_6), δ : 165.68, 158.91, 151.55, 140.72, 131.36, 130.02, 129.33, 127.87, 127.34, 126.85, 123.35, 122.89, 118.57, 118.31, 111.51, 109.21, 105.67, 67.01, 30.68, 18.67, 13.60; HR-MS: for $\text{C}_{21}\text{H}_{21}\text{NO}_3$ $[\text{M}+\text{H}]^+$ calculated 336.15942 m/z , found 336.15963 m/z .

N-(4-Butoxyphenyl)-2-hydroxynaphthalene-1-carboxamide (4c). Yield 43%; Mp 158 °C; IR (cm^{-1}): 3325, 3180, 2955, 2931, 2871, 1619, 1600, 1577, 1532, 1507, 1464, 1435, 1238, 1170, 968, 819, 742; $^1\text{H-NMR}$ (DMSO- d_6) δ : 10.22 (s, 1H), 10.07 (br. s, 1H), 7.84 (d, 2H, $J = 8.8$ Hz), 7.71 (d, 2H, $J = 9.2$ Hz), 7.68 (d, 1H, $J = 7.3$ Hz), 7.46 (td, 1H, $J = 7.3$ Hz, $J = 1.1$ Hz), 7.32 (td, 1H, $J = 8.1$ Hz, $J = 1.1$ Hz), 7.24 (d, 1H, $J = 9.2$ Hz), 6.92 (d, 2H,

$J = 9.2$ Hz), 3.95 (t, 2H, $J = 6.4$ Hz), 1.70 (qi, 2H, $J = 6.2$ Hz), 1.44 (sx, 2H, $J = 6.6$ Hz), 0.94 (t, 3H, $J = 7.1$ Hz); ^{13}C -NMR (DMSO- d_6), δ : 165.07, 154.63, 151.51, 132.74, 131.45, 129.89, 127.84, 127.37, 126.76, 123.45, 122.85, 120.71, 118.69, 118.32, 114.38, 67.27, 30.75, 18.67, 13.62; HR-MS: for $\text{C}_{21}\text{H}_{21}\text{NO}_3$ $[\text{M}+\text{H}]^+$ calculated 336.15942 m/z , found 336.15990 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 [34]. 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.* [35], 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/m² 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 1.9 $\mu\text{mol/L}$. The results are summarized in Table 1.

Study of fluorescence of chlorophyll a in spinach chloroplasts

The fluorescence emission spectra of chlorophyll *a* (Chla) in spinach chloroplasts were recorded on fluorescence spectrophotometer F-2000 (Hitachi, Tokyo, Japan) using excitation wavelength $\lambda_{\text{ex}} = 436$ nm for monitoring fluorescence of Chla, excitation slit 20 nm and emission slit 10 nm. The samples were kept in the dark for 2 min before measuring. The phosphate buffer used for dilution of the chloroplast suspension was the same as described above. Due to low aqueous solubility the compounds were added to the chloroplast suspension in DMSO solution. The DMSO concentration in all samples was the same as in the control (10% (v/v)). The chlorophyll concentration in the chloroplast suspension was 10 mg/L.

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