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Ring-substituted 8-hydroxyquinoline-2-carboxanilides as photosystem II inhibitors

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ABSTRACT

Ring-substituted 8-hydroxyquinoline-2-carboxanilides inhibited photosynthetic electron transport (PET) through photosystem (PS) II. Their inhibitory efficiency depended on the compound lipophilicity, the electronic properties of the substituent R and the position of the substituent R on the benzene ring. The most effective inhibitors showing IC_{50} values in the range 2.3–3.6 μM were substituted in $C'_{(3)}$ by F, CH_3, Cl and Br. The dependence of the PET-inhibiting activity on the lipophilicity of the compounds was quasi-parabolic for 3-substituted derivatives, while for $C'_{(2)}$ ones a slight increase and for $C'_{(4)}$ derivatives a sharp decrease of the activity were observed with increasing lipophilicity. In addition, the dependence of PET-inhibiting activity on electronic Hammett's σ parameter of the substituent R was observed with optimum σ value 0.06 for $C'_{(4)}$ and 0.34 for $C'_{(3)}$ substituted derivatives, while the value of σ parameter did not significantly influence the PET-inhibiting activity of $C'_{(2)}$ substituted compounds. Interactions of the studied compounds with chlorophyll *a* and aromatic amino acids present in the pigment–protein complexes mainly in PS II were documented by fluorescence spectroscopy. The section between P680 and plastoquinone Q_B occurring on the acceptor side of PS II can be suggested as the site of action of the compounds.

8-Hydroxyquinoline scaffold represents an important type of 'privileged structure' possessing a rich diversity of biological properties.[1] Some quinoline derivatives and their analogues/isosteres also show noteworthy herbicidal activities. Quinclorac belongs to the class of highly selective auxin herbicides and is used primarily to control weeds in rice crops.[2] Some other quinoline derivatives could be used to control undesirable plant growth.[3–5] Also several recently described hydroxyquinoline-carboxamides reduced chlorophyll content in *Chlorella vulgaris* with IC_{50} values about 5.9–10.9 μM.[6] On the other hand, some quinoline derivatives originating from anthropogenic activities are environmental contaminants that are toxic to living organisms.[7–9]

Quinoline derivatives were found to inhibit photosynthetic electron transport (PET) in plant chloroplasts. 2-Heptyl-1-hydroxy-4(1*H*)-quinolone and 4-hydroxy-2-nonylquinoline-*N*-oxide are potent inhibitors of PET in isolated thylakoids acting in photosystem (PS) II at the Q_B-site before the site of diuron and, in addition, at the cytochrome b_6f-complex.[10,11] Also quinolone-*N*-oxides inhibited PET in PS II and in the cytochrome b_6f-complex. The most potent inhibitors were found to be 2-methyl-3-alkyl-, 1-hydroxy-2-methyl-3-alkyl- and 1-hydroxy-2-alkyl-(1*H*)-quinolones with *n*-alkyl side chains varying from C_5 to C_{17}.[12] Displacement experiments with [^{14}C]atrazine indicated that the quinolones share an identical binding site with other PS II commercial herbicides. Maximal inhibitory potency was achieved at the carbon chain length of 12–14 Å, and a further increase of the chain length resulted in a decreased activity. PET inhibition in spinach chloroplasts was also observed for several other quinoline derivatives.[6,13–15]

Ring-substituted 8-hydroxyquinoline-2-carboxanilides were prepared according to Scheme 1 and published recently.[16] Based on the above-mentioned observations,[6,13–15] they were tested for their PET-inhibiting activity in spinach (*Spinacia oleracea* L.) chloroplasts, where PET through PS II, from H_2O to plastoquinone Q_B, was monitored spectrophotometrically as photoreduction of the artificial electron acceptor 2,6-dichlorophenol indophenol (DCPIP) according to Kralova et al.[17] The studied compounds strongly inhibited photoreduction of DCPIP in spinach chloroplasts, and their activity was compared with the commercial PET inhibitor diuron. DMSO solutions of the studied compounds were added to

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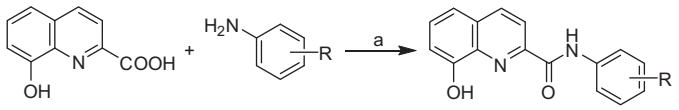

Scheme 1. Synthesis of ring-substituted 8-hydroxyquinoline-2-carboxanilides **1**–**7c**: (a) PCl_3, chlorobenzene, microwave-assisted synthesis.[16]

chloroplasts due to the limited solubility of the compounds in water. The applied DMSO concentration (up to 4 vol %) did not affect the photochemical activity of spinach chloroplasts. Relationships between the structure and the PET inhibition of the studied compounds are discussed.

The PET-inhibiting activity was expressed by the negative logarithm of IC_{50} value (compound concentration in mol/L causing 50% inhibition of PET). The activity of the most potent compound **4b** (R = 3-F; IC_{50} = 2.3 μM) was comparable with that of the standard DCMU (IC_{50} = 1.9 μM). However, also other 3-substituted compounds **3b** (R = 3-CH_3; IC_{50} = 2.7 μM), **6b** (R = 3-Br; IC_{50} = 3.4 μM) and **5b** (R = 3-Cl; IC_{50} = 3.6 μM) were very effective PET inhibitors. The dependences of log(1/IC_{50}) on the lipophilicity of the compounds expressed as log *k*[16] are presented in Figure 1. The lipophilicity of the compounds is additionally expressed and listed in Table 1 as log *P* values predicted by sw. ACD/Percepta ver. 2012 for comparison. Below discussed relationships are valid both for log *k* and log *P* values, therefore the dependence of log(1/IC_{50}) on log *P* values is not illustrated. Based on the obtained results it can be stated that the $C'_{(3)}$ substituted compounds expressed the highest PET-inhibiting activity, and the dependence of their PET-inhibiting activity on lipophilicity was quasi-parabolic (Fig 1). The lipophilicity of the most active compounds (IC_{50} range 2.3–3.6 μM) varied in the range from 0.9420 to 1.2357 for log *k* (from 2.76 to 3.31 for log *P*). On the other hand, while the PET-inhibiting activity of $C'_{(2)}$ substituted compounds increased slightly with increasing compound lipophilicity, the activity of $C'_{(4)}$ substituted compounds showed a strong decrease (Fig. 1).

Electronic properties of individual anilide substituents expressed as Hammett's σ constants (predicted by sw. ACD/Percepta ver. 2012, see Table 1) were determined as another parameter that could influence PET-inhibiting activity. For $C'_{(3)}$ and $C'_{(4)}$ substituted derivatives increasing σ values of halogen substituents caused a gradual decrease of activity expressed as IC_{50}, namely from 2.3 μM (**4b**, R = 3-F; σ = 0.34) to 21.3 μM (**7b**, R = 3-CF_3; σ = 0.43) and from 5.6 μM (**4c**, R = 4-F; σ = 0.06) to 477.6 μM (**7c**, R = 4-CF_3; σ = 0.51), respectively, whereby anilides

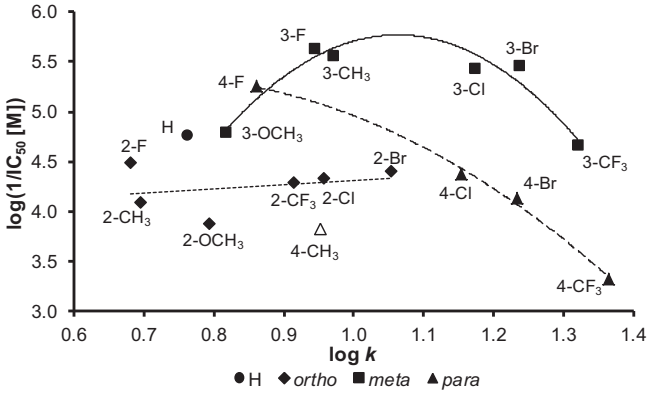

Figure 1. Relationships between PET inhibition log(1/IC_{50}) [M] in isolated spinach chloroplasts and lipophilicity expressed as log *k*. (Compound **3c** (4-CH_3) not included in SAR discussion is marked by empty symbol.).

Table 1
Predicted values of lipophilicity log *P*, experimentally determined values of lipophilicity log *k* and electronic Hammett's σ parameters of substituents R, IC_{50} values related to PET inhibition in spinach chloroplasts in comparison with diuron (DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea) standard

Compd	R[1]	log *k*	log *P*[a]	σ[a]	PET IC_{50} [μM]
1	H	0.7600	2.55	0	16.6
2a	2-OCH_3	0.7935	2.67	−0.28	134.6
2b	3-OCH_3	0.8164	2.61	0.12	16.0
2c	4-OCH_3	0.7129	2.51	−0.27	b
3a	2-CH_3	0.6944	2.90	−0.17	81.6
3b	3-CH_3	0.9686	2.90	−0.07	2.7
3c	4-CH_3	0.9521	2.90	−0.17	150.0
4a	2-F	0.6806	2.59	0.06	32.1
4b	3-F	0.9420	2.76	0.34	2.3
4c	4-F	0.8598	2.59	0.06	5.6
5a	2-Cl	0.9566	3.07	0.22	46.0
5b	3-Cl	1.1718	3.28	0.37	3.6
5c	4-Cl	1.1543	3.05	0.23	42.5
6a	2-Br	1.0536	3.16	0.22	38.9
6b	3-Br	1.2357	3.31	0.39	3.4
6c	4-Br	1.2347	3.19	0.23	72.7
7a	2-CF_3	0.9147	3.36	0.51	51.2
7b	3-CF_3	1.3206	3.44	0.43	21.3
7c	4-CF_3	1.3653	3.27	0.51	477.6
DCMU	—	—			1.9

[a] Predicted using sw. ACD/Percepta ver. 2012.
[b] Not determined due to precipitation during experiment.

4b and **7c** were the most active compounds among $C'_{(3)}$ and $C'_{(4)}$ substituted derivatives. On the other hand, the value of σ parameter did not significantly influence the PET-inhibiting activity of $C'_{(2)}$ substituted compounds.

From the above-mentioned results it is evident that beside lipophilicity and electronic properties, the PET-inhibiting activity of the studied compounds is significantly affected by the position of substituents R on the phenyl ring. For example, the lower PET-inhibiting activity of $C'_{(2)}$ substituted derivatives as compared to $C'_{(3)}$ and $C'_{(4)}$ substituted ones was observed previously for several esters of 2-, 3- and 4-substituted alkoxyphenylcarbamic acids.[18,19] In these series, the lower inhibitory activity of 2-alkoxy substituted derivatives in comparison with their 3- and 4-substituted analogues can be explained by a secondary steric effect, which is induced due to interactions between the alkoxy substituent and the carbamate group.[20] The lower activity of the tested $C'_{(2)}$ substituted 8-hydroxyquinoline-2-carboxanilides could be connected with intramolecular interactions of the substituent R with the NH group resulting in reduced interaction of these compounds with photosynthetic proteins embedded in thylakoid membranes. On the other hand, the strong activity decrease with increasing lipophilicity of 4-substituted compounds could be caused by the limited solubility of more lipophilic compounds. Summarizing, it could be concluded that for PET-inhibiting activity, sufficient (but not too high) lipophilicity enabling easier penetration of the compounds into the lipids of photosynthetic membranes is necessary. On the other hand, the increasing electronegativity of halogen substituents in positions $C'_{(3)}$ and $C'_{(4)}$ was reflected in a gradual activity decrease.

The detection of PET through PS II (from the intermediate $Z^{\cdot}$ situated on the donor side of PS II to Q_B located on the acceptor side of PS II) was performed according to Xiao et al.[21] and Sersen at al.[22] using the artificial electron donor 1,5-diphenylcarbazide (DPC) acting in the $Z^{\cdot}/D^{\cdot}$ intermediate.[23] The application of DPC to chloroplasts, the activity of which was inhibited by compounds **4b** (R = 3-F) or **6b** (R = 3-Br) to 85%, resulted in a gradual restoration of PET with increasing DPC concentration. The complete restoration of PET occurred only when the concentration of DPC was higher by more than one order of magnitude than the concentration of the applied inhibitor. Therefore it could be assumed that

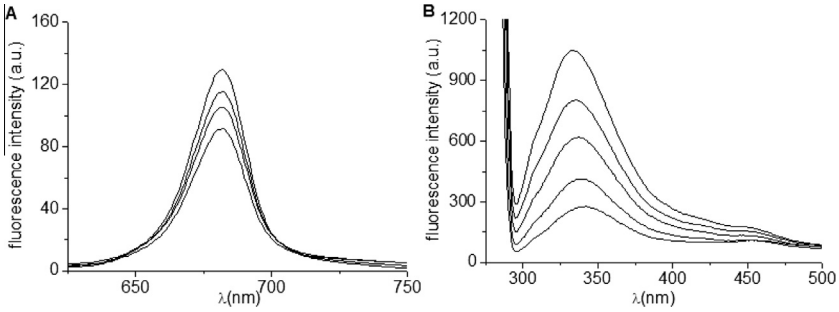

Figure 2. Fluorescence emission spectra of chlorophyll *a* in suspension of spinach chloroplasts without and with compound **4b** (R = 3-F; *c* = 0, 55, 110 and 220 μM, the curves from top to bottom; excitation wave length λ = 436 nm) (A) and fluorescence emission spectra of aromatic amino acids in suspension of spinach chloroplasts without and with compound **4b** (*c* = 0, 11, 22, 44 and 66 μM, the curves from top to bottom; excitation wave length λ = 275 nm) (B). Chlorophyll concentration in chloroplast suspension: 10 mg/L.

the section between P680 (primary donor of PS II) and plastoquinone Q_B occurring on the acceptor side of PS II was damaged by these PET inhibitors. The complete restoration of the photochemical activity of chloroplasts treated with **4b** or **6b** at higher DPC concentrations could be connected with the replacement of these inhibitors from their binding site by *sym*-diphenylcarbazide due to their direct interaction with the herbicide-binding niche, similarly as it was demonstrated for atrazine[24] or metribuzin.[25] Inhibition of electron transport in PS II at the Q_B site before the site of diuron by 4-hydroxyquinoline-*N*-oxides[10] or quinolones and quinolone *N*-oxides[12] was reported previously.

The effect of the studied compounds on the fluorescence of chlorophyll *a* (Chl*a*) and aromatic amino acids (AAA) in spinach chloroplasts was investigated as well, applying a published method.[26] The DMSO concentration in all samples was the same as in the control (10% (v/v)). The studied 8-hydroxyquinoline-2-carboxanilides affected the chlorophyll *a* (Chl*a*) fluorescence in spinach chloroplasts. As shown in Figure 2A, the intensity of the Chl*a* emission band at 686 nm belonging to the pigment–protein complexes in PS II decreased in the presence of compound **4b**, indicating a perturbation of the Chl*a*–protein complexes in the thylakoid membrane[27] caused by the this compound. A similar Chl*a* fluorescence decrease in spinach chloroplasts was observed previously for several PET inhibitors, namely ring-substituted 3-hydroxynaphthalene-2-carboxanilides,[28] 1-hydroxynaphthalene-2-carboxanilides,[29] 2-hydroxynaphthalene-1-carboxanilides[30] and ring-substituted 4-arylamino-7-chloroquinolinium chlorides.[15] The tested 8-hydroxyquinoline-2-carboxanilides also interacted with residues of AAA, mainly tryptophan and tyrosine occurring in photosynthetic proteins situated mainly in PS II. This was documented by the quenching of AAA fluorescence at 334 nm. Figure 2B presents fluorescence emission spectra of AAA of untreated spinach chloroplasts and of chloroplasts treated with increasing concentrations of compound **4b**. As shown in Figure 2B, the quenching of the fluorescence of aromatic amino acids at 334 nm increased with the increasing concentration of the tested compound. The quenching of the fluorescence of Chl*a* as well as of aromatic amino acids in the presence of 5-bromo- and 3,5-dibromo-2-hydroxy-*N*-phenylbenzamides and ring-substituted 2-hydroxynaphthalene-1-carboxanilides was observed previously.[30,31]

The studied ring-substituted 8-hydroxyquinoline-2-carboxanilides were tested previously for their in vitro antimycobacterial activity against *Mycobacterium tuberculosis* and clinical isolates of *Mycobacterium avium* complex and *M. avium* subsp. *paratuberculosis*, and some of them showed the antimycobacterial activity against *M. avium* subsp. *paratuberculosis* comparable with or higher than that of rifampicin. However, it could be noted that with the exception of some compounds with lower solubility, the antitubercular activity of compounds mainly against *M. tuberculosis* was comparable, independently of compound lipophilicity, electronic properties of R substituent or its position of substitution.[16] Similarly to the present results in relation to PET-inhibiting activity, compounds with potency against all three mycobacterial strains were obtained by the substitution of $C'_{(3)}$ position of aniline; however, also the hydroxyl moiety in $C_{(8)}$ of quinoline seems to play a significant role in antimycobacterial activity, because its absence in quinoline-2-carboxanilides led to an activity decrease.[32] An essential contribution of the hydroxyl moiety for amplifying antimycobacterial potency was observed previously also for 1-hydroxynaphthalene-2-carboxanilides[29] and 6-hydroxynaphthalene-2-carboxanilides[33] contrary to naphthalene-2-carboxanilides.[32]

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