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Substituted Amides of Quinoline Derivatives: Preparation and Their Photosynthesis-inhibiting Activity

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Abstract: The series of nine amides of substituted 8-hydroxyquinolines were prepared. The synthetic procedures of compounds are presented. All the prepared quinoline derivatives were analyzed using RP-HPLC method for the lipophilicity measurement and their lipophilicity were determined. The prepared compounds were tested for the reduction of chlorophyll content in *Chlorella vulgaris* Beij. Several compounds showed biological activity comparable with or higher than the standard 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). The relationships between the lipophilicity and the chemical structure of the studied compounds are discussed as well as structure-activity relationships (SAR) between the chemical structure and the biological activities of the evaluated compounds.

Keywords: Quinoline derivatives; Lipophilicity; Reduction of chlorophyll content; *Chlorella vulgaris*; Structure-activity relationships.

INTRODUCTION

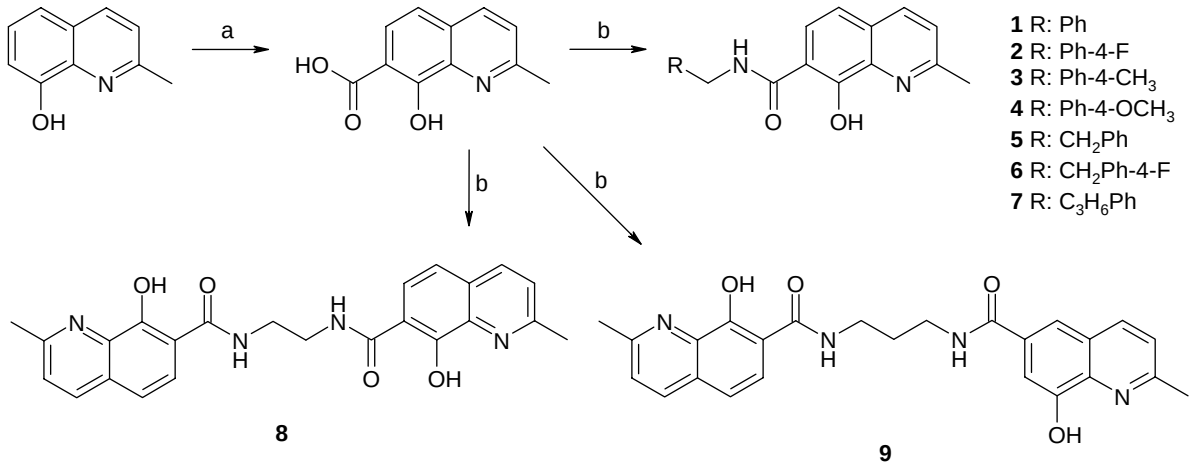
Quinoline moiety is present in many classes of biologically active compounds. A number of them have been clinically used as antifungal, antibacterial and antiprotozoic drugs [1,2] as well as antituberculotic agents [3,4]. Some quinoline based compounds showed also

antineoplastics activity [5]. Styrylquinoline derivatives have gained strong attention recently due to their activity as perspective HIV integrase inhibitors [6,7,8,9,10].
Our previous study dealing with styrylquinoline derivatives showed that they could possess also strong antifungal activity [11], the compounds containing 8-hydroxyquinoline pharmacophore seem especially interesting. According to the results reported recently some new 8-hydroxyquinoline derivatives possessed interesting antifungal and herbicidal activities [12,13,14,15].
The chemistry of quinoline has been described very well. On the other hand synthetic routes of quinoline derivatives are time consuming. Thus new efficient methods of microwave assisted organic synthesis for discussed quinoline derivatives were applied [16,17,18]. This interesting route to structurally diverse quinoline derivatives is now under optimisation. Wider discussion will be subsequently published. During our preliminary studies we have found that some up-to-date synthesized structures could be interesting to wider forum.
Various compounds possessing -NHCO- moiety were found to inhibit photosynthetic electron transport. Amides of the substituted pyridine-4-carboxylic acids [19] as well as anilides of the substituted pyrazine-2-carboxylic acids [20,21,22,23] inhibited oxygen evolution rate in *Chlorella vulgaris* and they showed some antialgal properties. Therefore a new series of amides of 8-hydroxyquinoline derivatives were prepared by means of the above-discussed pathways [16,17,18] and evaluated as potential herbicides. Synthesis, lipophilicity as well as structure-activity relationships are discussed in this paper.

RESULTS AND DISCUSSION

The compounds **1**-**9** were synthesized according to the procedure showed below. Kolbe-Schmidt reaction was leading to carboxylic acids which further reacted with the appropriate amine in presence of DCC or ethyldimethylaminopropyl carbodiimid (EDCI) to afford an amide. In case of **8**, **9** diamine and twofold of quinaldic acid were used, see Scheme 1.

Scheme 1. Synthesis of compounds **1**-**9**: (a) KOH, CO_2; (b) amine, DCC or EDCI.



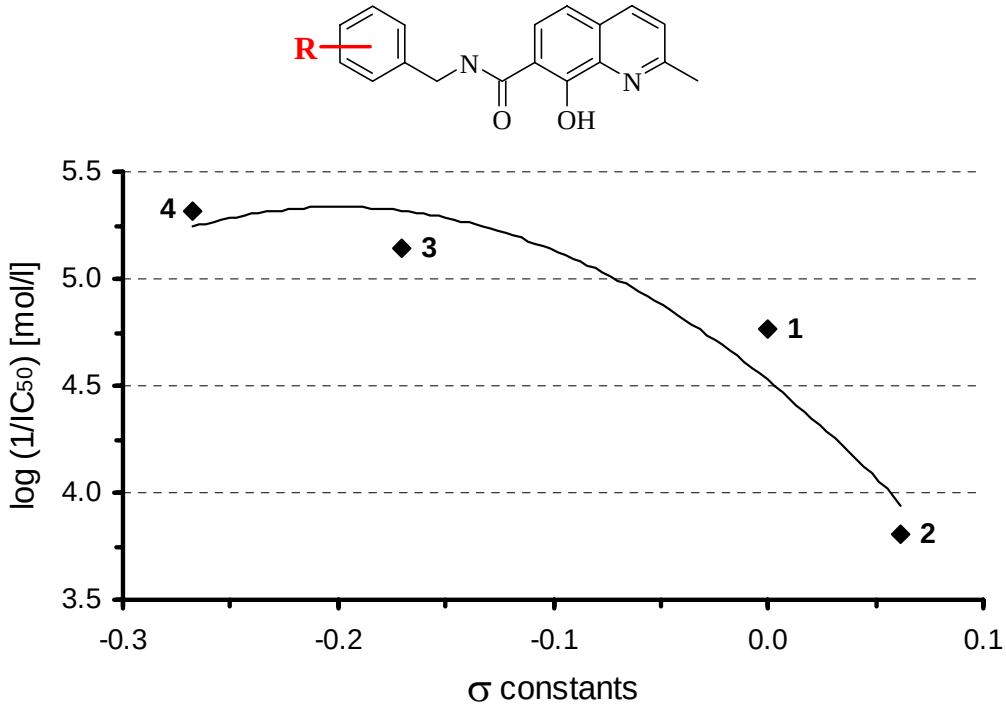
Hydrophobicities of the studied compounds **1**-**9** were measured by means of the reversed phase high performance liquid chromatography (RP-HPLC) method for the lipophilicity measurement. The procedure was performed under isocratic conditions with methanol as an organic modifier in the mobile phase using end-capped non-polar C_{18} stationary RP column.

The capacity factors *K* were determined and subsequent log *K* values were calculated. The results are shown in Table 1.

The total lipophilicity of the studied compounds is affected by the lipophilicity of the substituents on the aromatic rings as well as by the lipophilicity of the CH_2 and other function groups (e.g. –NH– or –C=O) in the linker. Using the π-parameter the lipophilicity of the substituents on the benzene rings can be expressed [24]. On the other hand the lipophilicity of CH_2, –NH– and –C=O groups can be expressed by aliphatic lipophilic fragment constants (f) whereas for the lipophilicity of the substituents on aromatic ring, aromatic lipophilic fragment constants (π) can be used [25]. The lipophilicity of phenyl substituents in position 4 increased in the following order: H (**1**, π = 0) < OCH_3 (**4**, π = -0.03) < F (**2**, π = 0.15) < CH_3 (**3**, π = 0.60), which corresponds to the experimentally determined log *K* values (Table 1). The lipophilicity of substituents in amide part of molecules expressed by log *K* values increased in the following order: benzyl (**5**) < phenyl (**1**) < *n*-propylphenyl (**7**). Lower lipophilicity of benzyl substituent than that of phenyl substituent was also described in ref [26]. The compound **7** possessed the highest hydrophobicity within this series. Low lipophilicity of both dimmers **8** and **9** is connected with hydrophilic 8-hydroxy substituents, on both 2-methylquinoline rings as well as by two carbonyl and –NH– groups in the spacer linking of these two ring structures (the corresponding lipophilic fragment constants (f) are -0.44 for –OH, -1.09 for –C=O and -2.15 for –NH– [25]).

All the studied compounds were handed over for herbicidal evaluation. The compound **5** was not tested due to low solubility in the testing medium. Two studied compounds (**3**, **7**) inhibited chlorophyll production in *C. vulgaris* comparable with the standard DCMU and the inhibitory activity of compound **4** evenly exceeded the activity of DCMU (Table 1). The interesting IC_{50} values varied in the range from 4.8 (**4**) to 17.2 μmol/l (**1**). Compound **4** (IC_{50} = 4.8 μmol/l) was the most efficient inhibitor.

Figure 1. Dependence of IC_{50} values (log $1/IC_{50}$ [mol/l]) related to the reduction of chlorophyll content in the suspension of *Chlorella vulgaris* on the σ constants of R substituent of 8-hydroxy-2-methylquinoline-7-carboxylic acid 4-R-benzylamides (**1**-**4**).



The inhibitory activity of 8-hydroxy-2-methylquinoline-7-carboxylic acid 4-R-benzylamides (**1-4**) depended on the Hammett constants σ of R substituent (Fig. 1). The σ constants of the R substituent were taken from Hansch and Leo: 0 (H, **1**); 0.062 (4-F, **2**); -0.17 (4-CH_3, **3**) and -0.268 (4-OCH_3, **4**) [25]. From Fig. 1 it is evident that the biological activity decreased with increasing σ value, *i.e.* with the electron accepting the power of the substituent.
The compounds **1** and **7**, **2** and **6** as well as **8** and **9** differ from each other by the number of CH_2 groups in the linker connecting two ring structures in the molecule. According to Hansch and Leo the hydrophobic fragment constant (f) for CH_2 group (corresponding to the contribution of CH_2 group to the compound lipophilicity) is 0.66 [25]. The comparison of compound **1** and **7** showed that the increase of the compound lipophilicity caused by prolongation of the linker by two CH_2 groups led to moderate increase of inhibitory activity. On the other hand, the prolongation of the spacer in 8-hydroxy-2-methylquinoline-7-carboxylic acid 4-fluorobenzylamide (**2**) by one CH_2 group (**6**) did not practically affect the biological activity of the compound. The addition of one CH_2 group to the spacer of bis-[8-hydroxy-2-methylquinoline-7-carboxylic acid]-1,2-etylamide (**8**) led to moderately lower activity of (**9**).
It can be assumed that the biological activity of the studied compounds depends not only on the lipophilicity but also on the electron-releasing or electron-withdrawing power of the substituent on the benzene ring. Using suitable electron-withdrawing substituent on the benzene ring and/or choosing suitable lipophilicity of the linker by addition of CH_2 group(s) the biological activity of the compounds could be optimised.

EXPERIMENTAL

General

All reagents were purchased from Aldrich. Kieselgel 60, 0.040-0.063 mm (Merck, Darmstadt, Germany) was used for column chromatography. 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. Melting points were determined on Boetius PHMK 05 (VEB Kombinat Nagema, Radebeul, Germany) and are uncorrected. Elemental analyses were carried out on an automatic Perkin-Elmer 240 microanalyser (Boston, USA). All ^{1}H NMR spectra were recorded on a Bruker AM-500 (499.95 MHz for ^{1}H), Bruker BioSpin Corp., Germany. Chemicals shifts are reported in ppm (δ) to internal $Si(CH_3)_4$, when diffused easily exchangeable signals are omitted.

Synthesis of compounds **1-9**

General procedure for synthesis discussed compounds is as follows. 8-hydroxy-quinaldine-7-carboxylic acid, starting material for all synthesized amides was obtain according to known procedure.
To solution 8-hydroksyquinaldine-7-carboxylic acid (1.02 g, 5 mmol) in dry CH_2Cl_2 with 0.6 mmol of DCC was added 5.3 mmol of appropriate amine in dry CH_2Cl_2 during 4 h. After the reaction was completed, white solid was filtered, washed with 5% $NaHCO_3$, water and diethyl ether.

8-Hydroxy-2-methylquinoline-7-carboxylic acid benzylamide (**1**). Product was obtained with 86.4% yield as white solid mp 215-218 ^{o}C, ^{1}H NMR (DMSO-d_6, 500MHz) δ: 2.57 (s, 3H), 6.94 (d, 1H, *J*=8.4Hz), 7.3 (d, 1H, *J*=8.4Hz), 7.35-7.37 (m, 5H), 7.45 (d, 2H, *J*=7.3Hz), 7.74 (d, 1H, *J*=8.3Hz), 8.0 (d, 1H, *J*=8.3Hz).

8-Hydroxy-2-methylquinoline-7-carboxylic acid 4-fluorobenzylamide (**2**). Product was obtained according to ref [27].

8-Hydroxy-2-methylquinoline-7-carboxylic acid 4-methylbenzylamide (**3**). Product was obtained with 36.4% yield, as white solid mp 192-200°C (decomp.); ^{1}H NMR (DMSO-d_6, 500MHz) δ: 2.3 (s, 3H), 2.6(s, 3H), 4.02 (s, 2H), 6.9 (d, 1H, *J*=8.4Hz), 7.2 (d, 2H, *J*=7.5Hz), 7.31-7.35 (m, 3H), 7.75 (d, 1H, *J*=8.3Hz), 8.04 (d, 1H, *J*=8.2Hz).

8-Hydroxy-2-methylquinoline-7-carboxylic acid 4-methoxybenzylamide (**4**). Product was obtained with 34.5% yield as white solid, mp 180-190°C (decomp.); ^{1}H NMR (DMSO-d_6, 500MHz) δ: 2.6 (s, 3H), 3.7 (s, 3H), 3.9 (s, 2H), 6.93-694 (m, 3H), 7.3 (d, 1H, *J*=8.3Hz), 7.4 (d, 2H, *J*=8.3Hz), 7.7 (d, 1H, *J*=8.3Hz), 8.0 (d, 1H, *J*=8.3Hz).
21. ^{1}H NMR (DMSO-d_6, 500MHz) δ: 7.08 (d, 1H, *J*=7.3Hz), 7.71 (d, 2H, *J*=8.44Hz), 7.75 (d, 1H, *J*=8.54), 7.33-7.4 (m, 2H), 7.46-7.49 (m, 3H), 8.1 (d, 1H, *J*=16.1Hz), 8.27 (d, 1H, *J*=8.54).

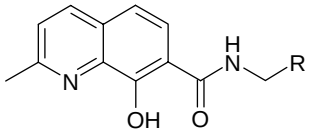
8-Hydroxy-2-methylquinoline-7-carboxylic acid phenethylamide (**5**).
8-Hydroxy-2-methylquinoline-7-carboxylic acid [2-(4-fluorophenyl)-ethyl]-amide (**6**).
8-Hydroxy-2-methylquinoline-7-carboxylic acid (3-phenylpropyl)-amide (**7**).
Detailed discussion on synthesis and biological activity of compounds **5**-**7** will be published elsewhere.
bis-[8-Hydroxy-2-methylquinoline-7-carboxylic acid]-1,2-etylamide (**8**). Product was obtained according to ref [27].
bis-[8-Hydroxy-2-methylquinoline-7-carboxylic acid]-1,3-propylamide (**9**). Product was obtained according to ref [27].

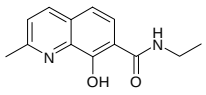
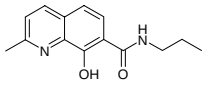
Lipophilicity HPLC determination (capacity factor K / calculated log K)
The HPLC separation module Waters Alliance 2695 XE and Waters Photodiode Array Detector 2996 (Waters Corp., Milford, MA, U.S.A.) were used. The chromatographic column Symmetry® C_{18} 5 μm, 4.6×250 mm, Part No. WAT054275, (Waters Corp., Milford, MA, U.S.A.) was used. The HPLC separation process was monitored by Millennium32® Chromatography Manager Software, Waters 2004 (Waters Corp., Milford, MA, U.S.A.). The mixture of MeOH p.a. (50.0%) and H_2O-HPLC – Mili-Q Grade (50.0%) was used as a mobile phase. The total flow of the column was 0.9 ml/min, injection 30 μl, column temperature 30 °C and sample temperature 10 °C. The detection wavelength 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 the 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. The log *K* values of the individual compounds are shown in Table 1.

Study of chlorophyll content reduction in Chlorella vulgaris Beij.
The green algae *C. vulgaris* Beij. was cultivated statically at room temperature according to Kralova et al. [28] (photoperiod 16 h light/8 h dark; photosynthetic active radiation 80 μmol/m^2.s; pH 7.2). The effect of the compounds on algal chlorophyll (Chl) content was determined after 4-day cultivation in the presence of the tested compounds. The Chl content in the algal suspension was determined spectrophotometrically (Kontron Uvikon 800, Kontron, Muenchen, Germany) after extraction into methanol according to Wellburn [29].

The Chl content in the suspensions at the beginning of the cultivation was 0.1 mg/l. Because of the low solubility of the studied compounds in water, these were dissolved in DMSO. DMSO concentration in the algal suspensions did not exceed 0.25% and the control samples contained the same DMSO amount as the suspensions treated with the tested compounds. The antialgal activity of compounds was expressed as IC_{50}. Comparable IC_{50} value for a selective herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea, DCMU (DIURON) was about 7.3 µmol/l. The results are summarized in Table 1.

Table 1. Experimentally found hydrophobicity (log *K*) and IC_{50} values related to reduction of chlorophyll content in *C. vulgaris* of the compounds **1-9** in comparison with standard (DCMU).



Compound	R	log *K*	IC_{50} [µmol/l]
1	-Ph	0.3019	17.2
2	-Ph-4-F	0.3603	158
3	-Ph-4-CH_3	0.3983	7.2
4	-Ph-4-OCH_3	0.3142	4.8
5	-CH_2Ph	0.2954	*a*
6	-CH_2Ph-4-F	0.3721	15.9
7	-C_3H_6Ph	0.6021	8.5
8		0.2151	93.7
9		0.2388	149
DCMU	–	–	7.3

[a] not tested due to precipitation of a dissolved drug.

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