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# Investigating biological activity spectrum for novel quinoline analogues

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**Abstract**—The lack of the wide spectrum of biological data is an important obstacle preventing the efficient molecular design. Quinoline derivatives are known to exhibit a variety of biological effects. In the current publication, we tested a series of novel quinoline analogues for their photosynthesis-inhibiting activity (the inhibition of photosynthetic electron transport in spinach chloroplasts (*Spinacia oleracea* L.) and the reduction of chlorophyll content in *Chlorella vulgaris* Beij.). Moreover, antiproliferative activity was measured using SK-N-MC neuroepithelioma cell line. We described the structure–activity relationships (SAR) between the chemical structure and biological effects of the synthesized compounds. We also measured the lipophilicity of the novel compounds by means of the RP-HPLC and illustrate the relationships between the RP-HPLC retention parameter $\log K$ (the logarithm of capacity factor $K$) and $\log P$ data calculated by available programs.


## 1. Introduction

Designing drugs is a complex issue that still lacks general approach. Although we usually do not realize that, the lack of the appropriate wide spectrum biological data is one of the important problems. In fact, this contributes to the low efficiency of the current molecular design. Thus, molecular modeling provides us with molecular data describing small molecule effectors. However, we cannot model so efficiently biological records. On the other side, only very few experimental information are available on such compounds with the exception of the certain activities under investigations. It has been suggested that QSAR efficiency could be significantly improved by the incorporation into the respective models of not only structural, physical or chemical parameters but also a spectrum of biological activities. PASS is a novel strategy aimed at closing this gap.[1] The appropriate software can be downloaded from the internet PASS site.[2] From the medicinal chemist point of view this strategy suggests that we should investigate and report the data for a variety of biological effects.

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 as well as antineoplastics.[3] Styrylquinoline derivatives have gained strong attention recently due to their activity as perspective HIV integrase inhibitors.[4–7] Our previous study dealing with styrylquinoline derivatives showed that they could show also strong antifungal activity,[8] the compounds containing 8-hydroxyquinoline pharmacophore seem especially interesting. According to the results reported recently some new 8-hydroxyquinoline derivatives possess interesting antifungal and herbicidal activity.[9] The lack of comprehensive studies in the field of styrylquinoline chemistry is due to com-

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plex and time-consuming synthetic routes. Thus, we have applied microwave-assisted organic synthesis to design new efficient methods recently.[10–12]

The elimination of chemical entities with inappropriate pharmacokinetics or physico-chemical properties is a major challenge in pharmaceutical profiling at an early stage of drug discovery and development. A general strategy to predict absorption, for example, the most frequently passive transport of a molecule through cellular membranes, is the measurement of the permeability of a drug candidate. One-step earlier the lipophilicity of a compound is determined, which is directly related to permeability and fraction absorbed.[13] Hydrophobicity is an important physical property determining biological activity of chemical compounds. Thus, we attempted to measure this by means of high-performance liquid chromatography (RP-HPLC) and calculate using commercially available computer programs.

The current study is a follow-up paper to the previous articles.[8–12] In this publication, we investigated herbicidal and antiproliferative activity of the novel series of quinoline derivatives. Structure–activity relationships (SAR) are discussed in this work as well as the relationships between the lipophilicity and the chemical structure of the studied compounds.

## 2. Results and discussion

### 2.1. Chemistry

Compounds **1**–**4** were obtained from 8-hydroxyquinoline or 8-hydroxyquinaldine as shown in Scheme 1.

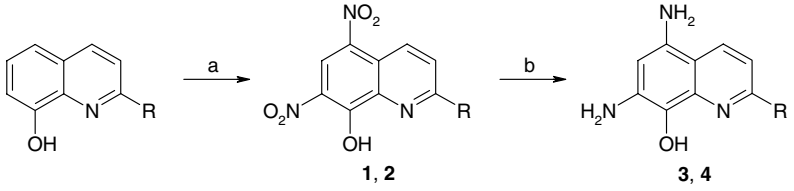
Microwave-assisted organic synthesis was used to obtain the group of styrylquinoline-like compounds, see Scheme 2. The appropriate quinaldine (1.0 equiv) was mixed with aldehyde (2.0 equiv) and irradiated in the microwave reactor for 2 min using pulse irradiation. A detailed information concerning synthesis of styrylquinoline derivatives is given elsewhere.[11] (Table 1).

Azaanalogues of styrylquinolines were obtained from quinoline-2-carbaldehyde and the appropriate aniline. This reaction was performed in dry benzene to generate the required product, see Scheme 3.

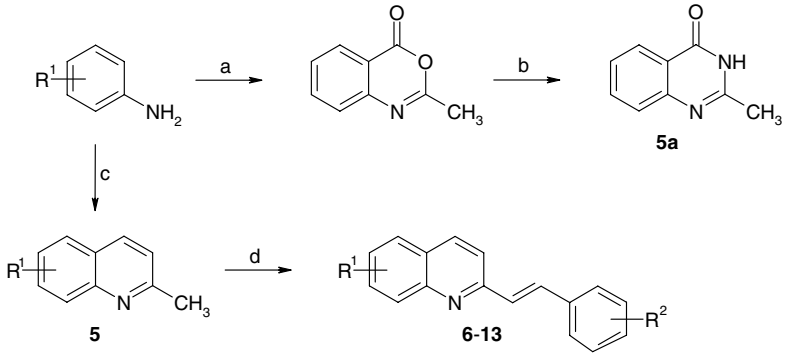
### 2.2. Hydrophobicity

Hydrophobicity (log *P*/*C*log *P* data) was calculated using two commercially available programs and measured by means of RP-HPLC by the determination of capacity factors *K* with a subsequent calculation of log *K*. The results are shown in Table 2 and illustrated in Figure 1.

log *P* is the logarithm of the partition coefficient in a biphasic system (e.g., *n*-octanol/water), defined as the ratio of a compound concentration in phase 1 and in phase 2. A value of log *P* is determined for the uncharged species of the drug. The application of log *P* in drug design has been widely reported.[14–16] Nowadays various software packages can be used for the estimation of hydrophobicity. They are based on the additive nature of partial hydrophobicity of structural moieties, atoms or functional groups gathered in internal databases. In particular, the *C*log *P* computer program was developed allowing chemists to calculate hydrophobicity using the fragmental constant approach of Leo and Hansch. This presents the logarithm of *n*-octanol/water partition coefficient based on established chemical interactions. However calculated values may differ for particular compounds, in general they correlate well and can be used as reliable QSAR parameters. As programs do not take into calculation specific structural effects like hydrogen bonding, tautomeric forms or charged groups, results for compounds bearing these effects are mostly

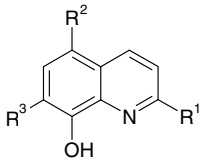
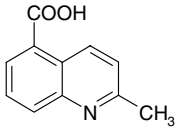
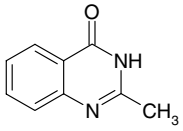
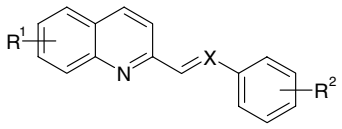


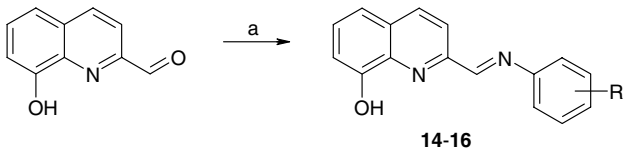

**Scheme 1.** Synthesis of quinoline derivatives **1**–**4**. Reagents and conditions: (a) $HNO_3/H_2SO_4$, 0 °C; (b) $H_2$/Pd.




**Scheme 2.** Preparation of styrylquinoline derivatives **5**–**13**. Reagents: (a) $Ac_2O$, μW; (b) $NH_{3(aq)}$, MeOH; (c) crotonaldehyde, HCl; (d) aldehyde, μW.

**Table 1.** Structures of synthesized compounds

| Compound | | | |
|---|---|---|---|
| |  | | |
| | $R^1$ | $R^2$ | $R^3$ |
| **1** | H | $NO_2$ | $NO_2$ |
| **2** | $CH_3$ | $NO_2$ | $NO_2$ |
| **3** | H | $NH_2$ | $NH_2$ |
| **4** | $CH_3$ | $NH_2$ | $NH_2$ |
| **5** |  | | |
| **5a** |  | | |
| |  | | |
| | X | $R^1$ | $R^2$ |
| **6** | C | 5-COOH | 4-Cl |
| **7** | C | 6-COOH | 2-Cl |
| **8** | C | 7-COOH | 3-Cl |
| **9** | C | 8-COOH | 2-OMe |
| **10** | C | 5,8-COOH | 3-Br |
| **11** | C | 8-OH | 3-Cl |
| **12** | C | 8-OH | 4-Cl |
| **13** | C | 8-OH | 4-Br |
| **14** | N | 8-OH | 2-OH |
| **15** | N | 8-OH | 3-OH |
| **16** | N | 8-OH | 4-OH |




**Scheme 3.** Synthesis of azaanalogues of styrylquinoline derivatives **14**–**16**. Reagents: (a) aniline, dry benzene.

uncertain. Some problems concerning these effects in the investigated compound group have been highlighted recently.[17] In turn, $\log K$ is the logarithm of the retention factors (e.g., capacity factor $K$) estimated by chromatographic approaches. Consequently, $\log K$ is related to the partitioning of a compound between a mobile and a (pseudo-)stationary phase. The procedure is most frequently performed under isocratic conditions with an organic modifier in the mobile phase using end-capped non-polar $C_{18}$ stationary RP column. $\log K$ can be used as the lipophilicity index converted to $\log P$ scale. $\log K$ does not need an additional reference and shows real lipophilicity enclosing specific molecular effects and interactions between molecules and environment.[18] This parameter could be successfully used as reference value when calculation fails due to their limitations.

$\log P$ is calculated for the uncharged molecules. Note that, in this particular case, the molecules can exist preferably in the ionic or zwitterionic form(s). Software calculates $\log P$ values as lipophilicity contributions/increments of individual atoms, fragments, and pair of interacting fragments in the chemical structure, that is, increments of carbon and heteroatoms, aromatic systems, and functional groups. Individual programs calculate lipophilicity contributions according to different internal databases/libraries. Therefore, the resulting values depend on the software applied. This fact as well as various ionic/zwitterionic forms and intramolecular interactions may cause differences between computed and experimentally determined lipophilicities.

The results obtained concerning the discussed compounds show that the experimentally determined lipophilicities ($\log K$ values) are generally lower than the calculated values of $\log P$/*C*$\log P$, see Figure 1. The experimentally determined $\log K$ values correlate approximately with $\log P$ values calculated either by the ChemOffice Ultra software or ACD program. *C*$\log P$ values calculated by ChemOffice software do not agree well with the measured ones.

Diamino-substituted compounds **3** and **4** indicate lower lipophilicity than dinitrosubstituted compounds **1** and **2**, and quinoline derivatives **1**, **3** show lower hydrophobicity than quinaldine derivatives **2** and **4**, as expected. Actually, the lipophilicity of the discussed 8-hydroxyquinoline derivatives may be modified by an intramolecular hydrogen bond between the quinoline nitrogen and the phenolic moiety. This keto–enol tautomerism of the quinoline derivatives possessing the phenol moiety is described in Ref. 19.

Compounds **14**–**16** are much less lipophilic than other styrylquinoline derivatives. This fact clearly results from the presence of the nitrogen atom in the olefinic linker. The experimentally measured values organize lipophilicity within individual series of compounds **14**–**16** (2-OH, 3-OH, 4-OH), as well as **11**–**13** (3-Cl, 4-Cl, 4-Br). 2-[(2-Hydroxyphenylimino)methyl]quinolin-8-ol (**14**) is much less lipophilic than indicated by the calculated lipophilicity. This fact is probably caused by the interaction of the imine nitrogen with the phenolic moiety.

As expected, compounds **9** and **10** are less hydrophobic than other carboxylic acid styrylquinoline derivatives. This is an effect of methoxy substitution in the phenyl ring (compound **9**). A presence of two carboxylic groups in quinoline (compound **10**) can further contribute to this effect.

### 2.3. Biological activity

Seventeen prepared compounds were used for biological assays. Some compounds could not be tested due to the

**Table 2.** Calculated lipophilicities (log *P*/Clog *P*) and determined log *K* of the studied compounds

| Compound | log *P*/Clog *P* | log *P* | log *K* | $IC_{50}$ (μmol/L) | | |
|---|---|---|---|---|---|---|
| | ChemOffice | ACD/log *P* | | Spinach chloroplasts | *Chlorella vulgaris* | SK-N-MC cell line |
| **1** | 1.80/1.9187 | 2.18 ± 0.34 | 0.7154 | 82 | [a] | >6.25 |
| **2** | 2.50/2.4177 | 2.64 ± 0.35 | 0.7292 | 26 | 95 | >6.25 |
| **3** | 0.12/1.3435 | −0.84 ± 0.34 | 0.0522 | 185 | 114 | >6.25 |
| **4** | 0.83/1.8425 | −0.38 ± 0.35 | 0.2707 | 142 | [a] | — |
| **5** | 2.38/2.5793 | 2.22 ± 0.72 | 0.3629 | 146 | [a] | >6.25 |
| **5a** | 0.98/0.8040 | −0.36 ± 0.59 | 0.1515 | [b] | [b] | >6.25 |
| **6** | 4.85/5.4853 | 4.97 ± 0.73 | 1.3976 | 487 | 96 | >6.25 |
| **7** | 4.85/5.4853 | 5.02 ± 0.32 | 1.4787 | 215 | 115 | >6.25 |
| **8** | 4.85/5.4853 | 4.97 ± 0.73 | 1.2858 | [a] | [a] | >6.25 |
| **9** | 4.16/4.6913 | 3.62 ± 0.35 | 1.1922 | [a] | [a] | — |
| **10** | 4.67/5.6501 | 4.49 ± 0.80 | 1.2171 | [a] | [a] | — |
| **11** | 4.90/5.4825 | 5.08 ± 0.32 | 1.5395 | 146 | 33 | >6.25 |
| **12** | 4.90/5.4825 | 5.08 ± 0.32 | 1.5558 | 448 | [a] | 0.77 |
| **13** | 5.17/5.6325 | 5.26 ± 0.38 | 1.5802 | 135 | [a] | >6.25 |
| **14** | 3.63/2.4315 | 1.09 ± 0.79 | 0.4308 | [a] | 11 | >6.25 |
| **15** | 3.63/2.4315 | 1.51 ± 0.79 | 0.8860 | [a] | 168 | — |
| **16** | 3.63/2.4315 | 1.32 ± 0.79 | 1.0911 | [a] | 17 | — |
| DCMU | 2.76/2.6910 | 2.78 ± 0.38 | — | 1.9 | 7.3 | — |
| Triapine | −0.02/0.7610 | 0.98 ± 0.38 | — | — | — | 0.31 |

In vitro biological activities of the selected compounds in comparison with standards (DCMU and triapine). $IC_{50}$ values are related to photosynthesis inhibition in spinach chloroplasts, reduction of chlorophyll content in *C. vulgaris*, and antiproliferative activity.
[a] Not tested due to low solubility in the testing medium or interaction with DCPIP.
[b] Compound showed no activity as herbicide.

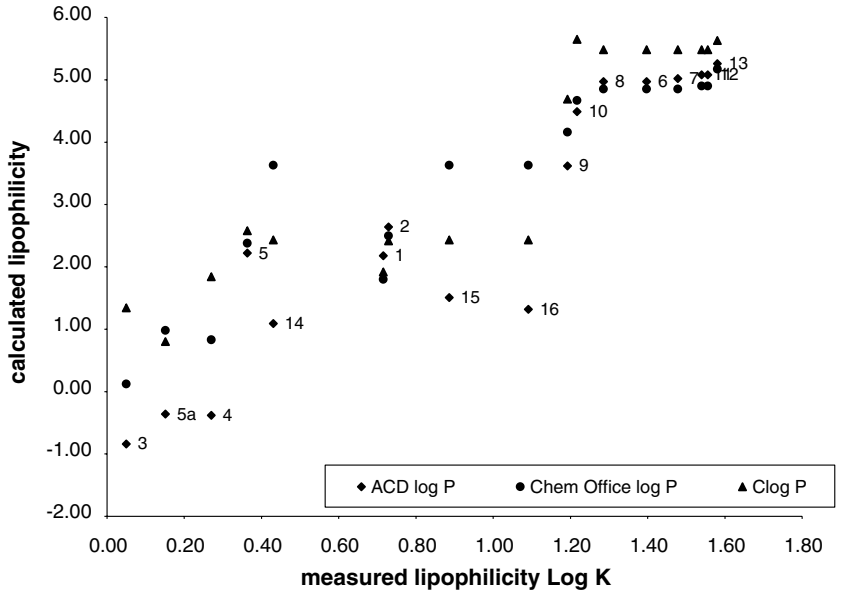



**Figure 1.** Calculated lipophilicity (log *P*/Clog *P*) versus measured (log *K*). The number of each structure is pointed at ACD log *P* value.

low solubility in a testing medium or their interaction with the applied artificial electron acceptor 2,6-dichlorophenol-indophenol (DCPIP), which caused discoloration of this reagent. Measured activities are shown in Table 2.

**2.3.1. PET inhibition in spinach chloroplasts.** Ten studied compounds inhibited photosynthetic electron transport in spinach chloroplasts, as shown in Table 2. The $IC_{50}$ values ranged from 26 to 487 μmol/L. The inhibitory activity of the studied compounds was relatively low; the most efficient inhibitor was compound **2** ($IC_{50}$ = 26 μmol/L) (Fig. 2).

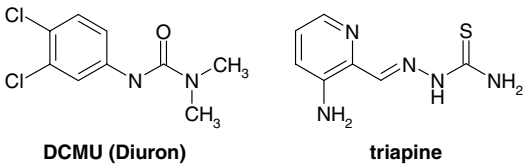



**Figure 2.** Chemical structures of the used standards.

The relationships between the PET inhibition {log (1/$IC_{50}$ [mmol/L])} in spinach chloroplasts and the logarithm of the retention factor (log *K*) of the studied compounds are shown in Figure 3. Figure 3 can be divided into two parts.

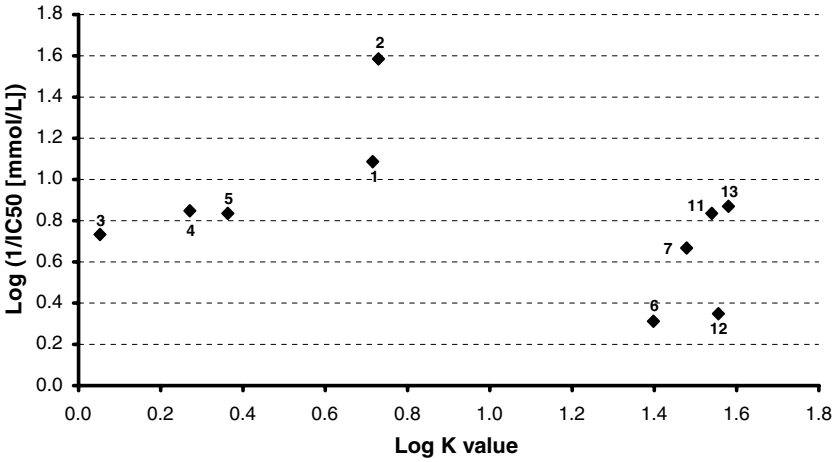



**Figure 3.** Relationships between the PET inhibition {log (1/$IC_{50}$ [mmol/L])} in spinach chloroplasts and the logarithm of the retention factor (log *K*) of the studied compounds.

First including the series of small quinoline derivatives **1**–**5**, and the second of styrylquinoline derivatives **6**–**13**.

The highest biological activity is indicated by smaller quinoline molecules, compounds **1**–**4**. In general, the inhibitory activity of 5,7-dinitrosubstituted compounds (**1**, **2**) was higher than that of comparable 5,7-diamino-substituted derivatives (**3**, **4**). An interesting activity level was also observed for acid **5**.

Styrylquinolines and their analogues indicated rather moderate effect on PET inhibition in spinach chloroplasts. It could be assumed that, the optimum lipophilicity (log *K*) in the context of the PET-inhibiting activity ranges around 0.7. The biological activity decreases with the considerable log *K* increase (styrylquinolines) or decrease (amino-substituted quinoline derivatives). Compound **2** whose log *P* value amounts to ca. 0.7 has the highest PET-inhibiting activity. The activity versus lipophilicity relationship, shown in Figure 3, is in good agreement with the results of our previous observations for other activities of quinolines.[8,9] The high importance of phenolic moiety in the $C_{(8)}$ quinoline position was again confirmed.

The addition of diphenylcarbazide (an artificial electron donor acting in the intermediate $Z^+/D^+$ on the donor side of photosystem II) to spinach chloroplasts inhibited by **2** caused the complete restoration of the photosynthetic electron transport.[20] This indicates that the primary donor of PS II (P680) was not damaged by this compound. Previous EPR experiments showed that the site of action of the related compounds in the photosynthetic apparatus of spinach chloroplasts was intermediate $D^+$, that is, tyrosine radical situated in the 161st position of the protein $D_2$ located on the donor side of photosystem II.[9,21]

**2.3.2. Reduction of chlorophyll content in *Chlorella vulgaris*.** Eight studied compounds inhibited chlorophyll production in *C. vulgaris*, see Table 2. The $IC_{50}$ values ranged from 11 to 168 μmol/L. The inhibitory activity of the majority of the studied compounds was relatively low, the most efficient inhibitor was compound **14** ($IC_{50}$ = 11 μmol/L). Four tested compounds (**2**, **3**, **6**, and **7**) showed only moderate effect on chlorophyll content in *C. vulgaris*.

The highest biological activity is indicated by styrylquinoline analogues, especially compounds **14** and **16**. The substitution at $C_{(8)}$ of quinoline and $C_{(2)}$ or $C_{(4)}$ of benzene by the phenolic groups (compounds **14**, **16**) was more advantageous from the viewpoint of biological activity than substitution at $C_{(3)}$ (compound **15**). The substitution of phenyl ring by halogens results in the activity decrease. The presence of the nitrogen atom in the olefinic linker increases the activity. Apparently, the higher activity of compound **14** in comparison to that of compound **16** explains the interaction of the phenolic moiety in $C_{(2)}$ of benzene (compound **14**) with the nitrogen atom in the olefinic linker. The inhibition of chlorophyll production in *C. vulgaris* is not influenced by lipophilicity.

**2.3.3. Antiproliferative activity of selected compounds.** Twelve prepared compounds were tested for their in vitro antitumor activity. Cellular proliferation was determined using the MTT assay. The results are shown in Table 2.

Eleven compounds did not show any antiproliferative activity under the tested concentration of 6.25 μmol. Only 2-[2-(4-chlorophenyl)vinyl]quinoline-8-ol (**12**) indicates a significant effect which is similar to the activity of triapine used as the standard. This thiosemicarbazone derivative is used as a potent inhibitor of ribonucleotide reductase activity. Triapine is an inhibitor of leukemia cells as well as of nasopharyngeal, lung, and ovarian carcinoma.[22]

## 3. Conclusion

Seventeen compounds were prepared and tested for photosynthesis inhibiting activity. Their lipophilicity (log *K*) was determined by means of RP-HPLC and the measured values were compared to the calculated log *P* parameters. 2-Methyl-5,7-dinitroquinolin-8-ol (**2**)

appeared to be the most efficient PET inhibitor in spinach chloroplasts; $IC_{50}$ = 26 μmol/L. The optimal parameter of lipophilicity (log *K*) in the context of the PET-inhibiting activity ranges about 0.73. The most intensive reduction of chlorophyll content in the green algae *C. vulgaris* showed 2-[(2-hydroxyphenylimino)methyl]quinolin-8-ol (**14**); $IC_{50}$ = 11 μmol/L. Antiproliferative activity of the novel compounds was also determined. Their $IC_{50}$ are rather high when compared to the active antiproliferative agents. However, the activity level of compound **12** ($IC_{50}$ = 0.77 μmol/L) indicates possible structure for further optimization. Last but not least, the results reported increase the activity spectrum data for styrylquinoline-like compounds.

## 4. Experimental

### 4.1. General

All reagents were purchased from Aldrich. Kieselgel 60, 0.040–0.063 mm (Merck, Darmstadt, Germany) was used for column chromatography. Microwave-assisted syntheses were performed in RM800PC microwave laboratory reactor from Plazmatronika (Wroclaw, Poland). During all syntheses monomode cavity was applied and temperature control was performed below boiling point of the used aldehyde. 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 vapor. 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. Chemical shifts are reported in ppm ($\delta$) to internal $Si(CH_3)_4$, when diffused easily exchangeable signals are omitted. The signals are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet; br s, broad singlet.

### 4.2. Synthesis

**4.2.1. 5,7-dinitro-8-hydroxyquinoline (1) and 5,7-dinitro-8-hydroxy-2-methylquinoline (2).** The appropriate quinoline (2.0 g) was added slowly in small quantities to the mixture $HNO_3/H_2SO_4$ 7:3 (20 mL) in an ice bath. After 2 h, the mixture was poured on crushed ice. A yellow powder was filtered, washed with hot EtOH, and crystallized from nitrobenzene.

(**1**) Yield 70%. Mp 315 °C; Ref.: mp 314–315 °C.[8]

(**2**) Yield 75%. Mp 260 °C; Ref.: mp 260 °C.[8]

**4.2.2. 5,7-diamino-8-hydroxyquinoline (3) and 5,7-diamino-8-hydroxy-2-methylquinoline (4).** Both compounds were obtained by reduction of **1** or **2**, respectively. Reductions were performed according to the described procedure.[23,24]

(**3**) Yield 60%. Mp 274 °C; Ref.: mp 274 °C.[25]

(**4**) Yield 65%. (As hydrochloride) Mp 200 °C; Ref.: mp 200 °C (decomp).[24]

**4.2.3. Quinaldine-5-carboxylic acid (5).** Quinaldine-5-carboxylic acid (**5**) and other carboxylic acids of quinaldine were obtained in Skraup reaction from the appropriate amine as described earlier.[7] Other derivatives of quinaldine, that is, quinaldine-6-carboxylic acid, quinaldine-7-carboxylic acid, quinaldine-8-carboxylic acid and quinaldine-5,8-dicarboxylic acid, were used in further steps of synthesis without thorough purification.

**4.2.4. 2-Methyl-3*H*-quinazolin-4-one (5a).** 2-Methyl-3*H*-quinazolin-4-one (**5a**) was obtained according to a two-step procedure. Benzoxazinone was obtained in reaction of acetic anhydride and 2-aminobenzoic acid in microwave reactor as described in Ref. 26. Then it was transformed into **5a** in reaction with $NH_{3(aq)}$ according to described procedure. Mp 240 °C; Ref.: Mp 240 °C.[27]

### 4.3. General procedure of styrylquinoline derivatives' 6–13 synthesis

Quinaldine derivative (10 mmol) and the appropriate aldehyde (20 mmol) were mixed thoroughly using mortar and put in an open vessel. Then the mixture was exposed to microwave irradiation for 4 min. After the reaction, the mixture was allowed to cool down and $Et_2O$ (10 mL) was added. The crude product was filtered, washed with $Et_2O$ (15 mL), and purified by crystallization in EtOH.[11]

**4.3.1. 2-[2-(4-Chlorophenyl)vinyl]quinoline-5-carboxylic acid (6).** A light yellow crystalline compound. Yield 71%. Mp 295 °C. Anal. Calcd for $C_{18}H_{12}ClNO_2 \cdot 1/2H_2O$ (318.75): C, 67.83%; H, 4.11%. Found: C, 68.02%; H, 4.52%. $^1$H NMR (DMSO-$d_6$), $\delta$: 7.46 (d, $J$ = 8.24 Hz, 2H), 7.51 (d, $J$ = 16.30 Hz, 1H); 7.75 (d, $J$ = 8.30 Hz, 2H), 7.90 (d, $J$ = 16.20 Hz, 1H), 7.94 (d, $J$ = 8.60 Hz, 1H), 8.20 (d, $J$ = 8.70 Hz, 1H), 8.18 (d, $J$ = 8.60 Hz, 1H), 8.55 (d, $J$ = 8.50 Hz, 1H), 8.60 (s, 1H).

**4.3.2. 2-[2-(2-Chlorophenyl)vinyl]quinoline-6-carboxylic acid (7).** A yellow crystalline compound. Yield 72%. Mp 265 °C. Anal. Calcd for $C_{18}H_{12}ClNO_2 \cdot H_2O$ (327.75): C, 69.89%; H, 4.89%. Found: C, 70.00%; H, 5.10%. $^1$H NMR (DMSO-$d_6$), $\delta$: 6.91 (t, $J$ = 7.50 Hz, 1H), 7.04 (d, $J$ = 8.10 Hz, 1H), 7.29 (t, $J$ = 7.80 Hz, 1H), 7.62 (d, $J$ = 7.80 Hz, 1H), 7.94 (d $J$ = 16.30 Hz, 1H), 8.10 (s, 1H), 8.32–8.35 (m, 2H), 8.50 (d, $J$ = 9.10 Hz, 1H), 8.55 (d, $J$ = 8.40 Hz, 1H), 9.61 (d, $J$ = 9.30 Hz, 1H), 10.80 br s 1H.

**4.3.3. 2-[2-(3-Chlorophenyl)vinyl]quinoline-7-carboxylic acid (8).** A yellow crystalline compound. Yield 55%. Mp 325 °C; Ref.: mp 325 °C.[11]

**4.3.4. 2-[2-(2-Methoxyphenyl)vinyl]quinoline-8-carboxylic acid (9).** A yellow crystalline compound. Yield 82%. Mp 190 °C. Anal. Calcd for $C_{19}H_{15}NO_3$ (305.31): C,

68.76%; H, 4.33%. Found: C, 68.81%; H, 4.29%. $^1$H NMR (DMSO-$d_6$), $\delta$: 3.93 (s, 3H), 7.04 (t, $J$ = 7.50 Hz, 1H), 7.12 (d, $J$ = 8.30 Hz, 1H), 7.40 (t, $J$ = 6.90 Hz, 1H), 7.54 (d, $J$ = 16.50 Hz, 1H), 7.83 (d, $J$ = 7.70 Hz, 1H), 8.04 (d, $J$ = 16.50 Hz, 1H), 8.12 (d, $J$ = 9.20 Hz, 1H), 8.27 (d, $J$ = 7.70 Hz, 1H), 8.56 (d, $J$ = 7.70 Hz, 1H), 9.37 (d, $J$ = 9.10 Hz, 1H).

**4.3.5. 2-[2-(3-Bromophenyl)vinyl]quinoline-5,8-dicarboxylic acid (10).** A yellow crystalline compound. Yield 69%. Mp 275–280 °C. Anal. Calcd for $C_{19}H_{12}BrNO_4 \cdot H_2O$ (416.20): C, 65.96%; H, 4.31%. Found: C, 66.11%; H, 4.31%. $^1$H NMR (DMSO-$d_6$), $\delta$: 7.40–7.50 (m, 2H), 7.60 (d, $J$ = 16.50 Hz, 1H), 7.75 (d, $J$ = 7.50 Hz, 1H), 7.82–7.90 (m, 3H), 7.98 (d, $J$ = 9.00 Hz, 1H), 8.20–8.28 (m, 2H), 9.30 (d, $J$ = 9.00 Hz, 1H), 13.20 br s 1H.

**4.3.6. 2-[2-(3-Chlorophenyl)vinyl]quinoline-8-ol (11).** A yellow crystalline compound. Yield 40%. Mp 120–123 °C. Anal. Calcd for $C_{17}H_{12}ClNO$ (281.74): C, 72.47%; H, 4.29%. Found: C, 72.51%; H, 4.10%. $^1$H NMR (DMSO-$d_6$), $\delta$: 6.90 (t, $J$ = 7.40 Hz, 1H), 6.96 (d, $J$ = 7.40 Hz, 1H), 7.10 (d, $J$ = 7.00 Hz, 1H), 7.20 (t, $J$ = 7.10 Hz, 1H), 7.33–7.41 (m, 2H), 7.54 (d, $J$ = 16.50 Hz, 1H), 7.63 (d, $J$ = 7.90 Hz, 1H), 7.80 (d, $J$ = 8.70 Hz, 1H), 8.13 (d, $J$ = 16.40 Hz, 1H), 8.26 (d, $J$ = 8.50 Hz, 1H), 8.33 br s 1H.

**4.3.7. 2-[2-(4-Chlorophenyl)vinyl]quinoline-8-ol (12).** A light yellow crystalline compound. Yield 30%. Mp 150 °C. Anal. Calcd for $C_{17}H_{12}ClNO$ (281.74): C, 72.47%; H, 4.29%. Found: C, 72.11%; H, 4.50%. $^1$H NMR (DMSO-$d_6$), $\delta$: 7.08 (d, $J$ = 7.30 Hz, 1H), 7.71 (d, $J$ = 8.40 Hz, 2H), 7.75 (d, $J$ = 8.50 Hz, 1H), 7.33–7.40 (m, 2H), 7.46–7.49 (m, 3H), 8.10 (d, $J$ = 16.10 Hz, 1H), 8.27 (d, $J$ = 8.50 Hz, 1H).

**4.3.8. 2-[2-(4-Bromophenyl)vinyl]quinoline-8-ol (13).** A beige crystalline compound. Yield 31%. Mp 145 °C. Anal. Calcd for $C_{17}H_{12}BrNO$ (326.20): C, 62.60%; H, 3.71%. Found: C, 62.61%; H, 3.80%. $^1$H NMR (DMSO-$d_6$), $\delta$: 7.08 (d, $J$ = 7.20 Hz, 1H), 7.49 (d, $J$ = 8.40 Hz, 2H), 7.75 (d, $J$ = 8.50 Hz, 1H), 7.34–7.39 (m, 2H), 7.50–7.60 (m, 4H), 8.10 (d, $J$ = 16.20 Hz, 1H), 8.27 (d, $J$ = 8.53 Hz, 1H).

### 4.4. General procedure of styrylquinoline analogues 14–16 synthesis

Compounds **14–16** were synthesized from quinalidic aldehyde and the appropriate aniline in dry benzene according to described procedure in Ref. 8. Their physical properties were in agreement with Refs. 8,28.

**4.4.1. 2-[(2-Hydroxyphenylimino)methyl]quinolin-8-ol (14).** Yield 65%. Mp 168–169 °C.[28]

**4.4.2. 2-[(3-Hydroxyphenylimino)methyl]quinolin-8-ol (15).** Yield 66%. Mp 240 °C (decomp.).[8]

**4.4.3. 2-[(4-Hydroxyphenylimino)methyl]quinolin-8-ol (16).** Yield 75%. Mp 243 °C.[8]

### 4.5. 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, USA) were used. The chromatographic column Symmetry® $C_{18}$ 5 μm, 4.6 × 250 mm, Part No. WAT054275 (Waters Corp., Milford, MA, USA) was used. The HPLC separation process was monitored by Millennium32® Chromatography Manager Software, Waters 2004 (Waters Corp., Milford, MA, USA). The mixture of MeOH p.a. (55.0%) and $H_2O$-HPLC—Mili-Q Grade (45.0%) was used as a mobile phase. The total flow of the column was 0.9 mL/min, injection 30 μL, column temperature 25 °C, and sample temperature 10 °C. The detection wavelength 240 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. The log $K$ values of the individual compounds, calculated from the capacity factor $K$, are shown in Table 2.

### 4.6. Lipophilicity calculations

log $P$ was calculated using the programs CS ChemOffice Ultra ver. 7.0 (CambridgeSoft, Cambridge, MA, USA) and ACD/log $P$ ver. 1.0 (Advanced Chemistry Development Inc., Toronto, Canada). *C*log $P$ values were generated by means of CS ChemOffice Ultra ver. 7.0 (CambridgeSoft, Cambridge, MA, USA) software. The results are shown in Table 2.

### 4.7. Biological activities

**4.7.1. Study of photosynthetic electron transport inhibition in spinach chloroplasts.** Chloroplasts were prepared by the procedure of Walker[29] from spinach (*Spinacia oleracea* L.). The inhibition of photosynthetic electron transport (PET) in spinach chloroplasts was determined spectrophotometrically (Kontron Uvikon 800, Kontron, Muenchen, Germany) 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), $MgCl_2$ (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$^2$) 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 (PET). The inhibitory efficiency (concentration) of the studied compounds

has been expressed by $IC_{50}$ values, that is, by molar concentration of the compounds causing 50% decrease in the oxygen evolution 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 μmol/L. The results are summarized in Table 2.

**4.7.2. Study of chlorophyll content reduction in *C. vulgaris* Beij.** The green algae *C. vulgaris* Beij. was cultivated statically at room temperature according to Kralova et al.[31] (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 7-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.[32] The Chl content in the suspensions at the beginning of the cultivation was 0.01 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 the compounds was expressed as $IC_{50}$. The comparable $IC_{50}$ value for a selective herbicide DCMU was about 7.3 μmol/L. The results are summarized in Table 2.

**4.7.3. Antiproliferative activity.** The human SK-N-MC neuroepithelioma cell line was seeded in 96-well microtiter plates at $1.5 \times 10^4$ cells/well in the medium containing unlabeled human diferric transferrin (0.06 mg/mL) and compounds at a range of concentrations (0.0–6.25 μmol). Control samples contained the medium with diferric transferrin (0.06 mg/mL) without any ligands. The cells were incubated at 37 °C in a humidified atmosphere containing 5% $CO_2$ and 95% air for 96 h. After the incubation, 10 μL (5 mg/mL) of 1-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium (MTT) was added to each well and further incubated at 37 °C for 2 h. After solubilization of the cells with 100 μL of 10% SDS-50% isobutanol in 0.01 mol HCl, the plates were read at 570 nm using a scanning multi-well spectrophotometer. The inhibitory concentration ($IC_{50}$) was defined as the concentration necessary to reduce the absorbance to 50% of the untreated control. The comparable $IC_{50}$ value for the standard chelator 3-aminopyridine-2-carbaldehyde-thiosemicarbazone (triapine) was about 0.31 μmol/L. The results are shown in Table 2.

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