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Quinolones and their *N*-oxides as inhibitors of photosystem II and the cytochrome b_6/f-complex

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Abstract

4(1H)-quinolones (2-alkyl- (**1**), 2-alkyl-3-methyl- (**2**), 2-methyl-3-alkyl- (**3**), 1-hydroxy-2-methyl-3-alkyl- (**4**) and 1-hydroxy-2-alkyl- (**5**)) with *n*-alkyl side chains varying from C_5 to C_{17} have been synthesized and tested for biological activity in photosystem II and the cytochrome b_6/f-complex. In photosystem II, quinolones **1** and **2** showed only moderate activity, whereas **3** < **5** < **4** (increasing activity) were potent inhibitors. Displacement experiments with [^{14}C]atrazine indicated that the quinolones share an identical binding site with other photosystem II commercial herbicides. In the cytochrome b_6/f-complex, only **3** < **4** showed enhanced activity. Maximal inhibitory potency was achieved at a carbon chain length of 12–14 Å. Further increase of the chain length decreased activity. In a quantitative structure–activity relationship inhibitory activity in photosystem II and the cytochrome b_6/f-complex could be correlated to the physicochemical parameters lipophilicity π and/or to STERIMOL *L*.

Keywords: Structure–activity relationship; Q_B-site; [^{14}C]Atrazine displacement; Heterocyclic ketone

1. Introduction

In photosynthetic electron transport, plastoquinone is reduced at the Q_B-site of photosystem II, and the reduced plastoquinol reoxidized at the cytochrome b_6/f-complex. From here, the electrons are forwarded to photosystem I by plastocyanin. Both the reduction of plastoquinone and the oxidation of plastoquinol can be inhibited. Plastoquinone reduction can be inhibited by a variety of compounds which are important commercial herbicides like *s*-triazines, triazinones and phenols, to quote only a few (for review, see [1]). Well known inhibitors of the cytochrome b_6/f-complex are DBMIB [2], DNP-INT [3], stigmatellin [4] and the aurachins [5]. All inhibitors can serve as useful tools to elucidate structural and mechanistic effects of photosynthetic reactions.

We have recently reported on the quantitative structure–activity relationship (QSAR) of quinolones and their *N*-oxides as inhibitors of mitochondrial electron transport. Depending on their substitution pattern, they serve as inhibitors of complex I and complex III as well [6]. It is long known that 2-*n*-heptyl-4-hydroxy-quinoline-*N*-oxide (1-hydroxy-2-*n*-heptyl-4(1H)-quinolone; HQNO) and 2-*n*-nonyl-4-

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hydroxy-quinoline-*N*-oxide (NQNO) are potent inhibitors of photosynthetic electron transport in isolated thylakoids [7]. They exert a dual mode of action since they inhibit electron transport in photosystem II at the Q_B-site before the site of diuron and, in addition, at the cytochrome b_6/f-complex [8–10]. A detailed analysis of the effect of quinolone derivatives on photosynthetic electron transport was, however, hampered by the fact that only HQNO and NQNO were available. We wish to report here on the effect of 43 different quinolone derivatives on photosynthetic electron transport through photosystem II and the cytochrome b_6/f-complex, and their QSAR.

2. Materials and methods

2.1. Chemicals

The synthesis of 2-alkyl-4(1H)-quinolones (**1**), 2-alkyl-3-methyl-4(1H)-quinolones (**2**), 3-alkyl-2-methyl-4(1H)-quinolones (**3**), 3-alkyl-1-hydroxy-2-methyl-4(1H)-quinolones (**4**), and 2-alkyl-1-hydroxy-4(1H)-quinolones (**5**) (Fig. 1) has been described recently [6].

2.2. Biochemical methods

Thylakoids from spinach were isolated as described by Nelson et al. [11]. Uncoupled electron flow rates from water to DCIP were measured spectrophotometrically at 600 nm in a Zeiss PMQII spectrophotometer, equipped for cross-illumination with actinic light (filter RG645, Schott, Mainz, Germany). Assays contained 10 μg chlorophyll in 30 mM HEPES-NaOH, pH 7.0, 10 mM $MgCl_2$, 5 mM DCPIP, 1 μg gramicidin and 1 μM DNP-INT in a total volume of 3 ml. DNP-INT was included to block reoxidation of plastohydroquinone at the cytochrome b_6/f-complex, and thus prevent reduction of DCPIP by photosystem I [3]. Thylakoids were preincubated with the inhibitors for 5 min in the dark. I_{50}-values (i.e. the concentration which inhibits electron transport by 50%) were determined graphically.

Electron transport through the cytochrome b_6/f-complex was measured by oxygen uptake in a Clark type oxygen electrode using methyl viologen as an artificial electron acceptor and duroquinone as the electron donor. Electron flow through photosystem II was inhibited by diuron. The assay medium contained in a volume of 2 ml, 40 mM HEPES, pH 7.0, 40 mM $MgCl_2$, 4 mM methyl viologen, 4 mM NaN_3, 0.1 mg gramicidin, 1 μM diuron, 0.4 mM duroquinol and thylakoids corresponding to 20 μg chlorophyll/ml.

2.3. QSAR calculations

Regression equations were calculated on a Power Macintosh 7200/90 using the statistical package Minitab 10 Xtra from Minitab Inc., State College, PA,

Table 1
pI_{50}-values for inhibition of electron transport through photosystem II by quinolones[a]

pI_{50}-value					
4(1H)-quinolone	**1** 2-alkyl-	**2** 2-alkyl-3-methyl-	**3** 2-methyl-3-alkyl-	**4** 1-hydroxy-2-methyl-3-alkyl-	**5** 1-hydroxy-2-alkyl-
n-pentyl	< 4		< 4		4.02
n-hexyl			< 4	5.13	4.78
n-heptyl	< 4	< 4	< 4	5.62	5.03
n-octyl		4.08	4.36	5.97	5.57
n-nonyl	< 4	4.63	4.98	6.64	5.97
n-decyl		5.21	5.09	6.81	6.50
n-undecyl	< 4	5.25	5.49	6.91	6.57
n-dodecyl			4.96	7.03	6.79
n-tridecyl	4.66	4.69	4.35	6.93	6.59
n-tetradecyl			4.33	6.71	6.42
n-hexadecyl			< 4	6.37	
n-heptadecyl					6.25

[a]For comparison, the pI_{50}-value for 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU) is 6.70.

USA. The π-values were taken from [12], the *L*-values from [13]. Values for the longer alkyl chains had to be extrapolated.

2.4. Displacement of [^{14}C]atrazine

For displacement experiments, chloroplasts from spinach corresponding to 100 μg chlorophyll were suspended in 2 ml of a medium containing 20 mM tricine/NaOH-buffer, pH 8.0, and 20 mM $MgCl_2$. [^{14}C]atrazine (CIBA-Geigy; specific activity 210 MBq/mmol) was added as methanolic solution. The concentration of methanol never exceeded 1%. Samples were incubated for 5 min and then the quinolone was added and the sample incubated for another 5 min. Chloroplasts were removed by centrifugation at 22 000 × *g* for 10 min. The pellet was resuspended in 1 ml of buffer and pellet and supernatant assayed for radioactivity with Zinnser Quicksafe A in a LKB 1219 Rackbeta liquid scintillation counter. Counts were corrected for quenching.

3. Results

3.1. Photosystem II

The pI_{50}-values for inhibition of electron transport through photosystem II in spinach thylakoids are given in Table 1. As can be seen from Table 1, **1** proved to be almost inactive, whereas the **2** and **3** were only moderate inhibitors. In contrast, **4** and **5** exhibit high inhibitory potency. 1-Hydroxy-2-methyl-3-*n*-dodecyl-4(1H)-quinolone has a pI_{50}-value of 7.03 (Table 1) and is comparable to commercial photosystem II herbicides. It is noteworthy that the inhibitory activity of all quinolones increases with the chain length of the alkyl group until a maximum is reached at 12 carbon atoms, which corresponds to a length of about 14 Å (Table 1 and Fig. 2). Further increase of the chain length lowers the activity (Table 1 and Fig. 2).

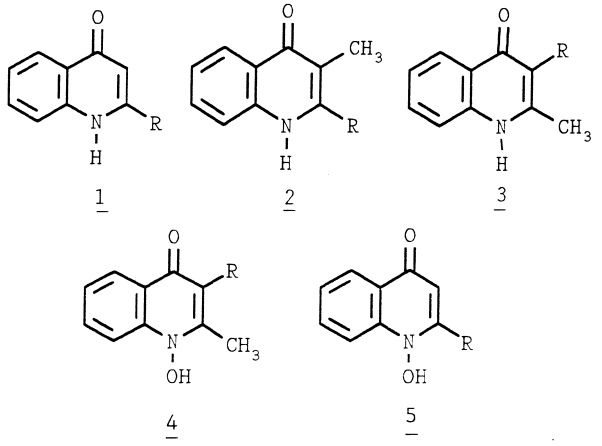

Fig. 1. Structures of **1**, **2**, **3**, **4**, and **5**.

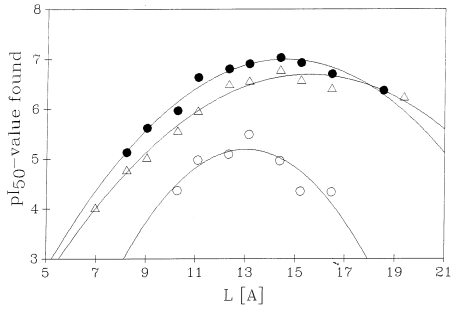

Fig. 2. Plot of Verloop's STERIMOL parameter *L* of the alkyl substituent versus the pI_{50}-value of various quinolones in photosystem II. ○ **3**, ● **4**, △ **5**.

In order to gain more insight into the mode of action of quinolones at the Q_B-site of photosystem II, the displacement of [^{14}C]atrazine by 1-hydroxy-2-methyl-3-*n*-dodecyl-4(1H)-quinolone was studied in more detail. The double reciprocal plots of 1/nmol free atrazine versus mg Chl/nmol bound atrazine in the control and at three different concentrations of the quinolone reveals a common ordinate intercept, but different abscissa intercepts, which indicates a competitive displacement reaction (Fig. 3). In this respect, the behavior of the quinolone is identical to that of the other photosystem II herbicides, and hence, exhibits an identical binding site [14,15].

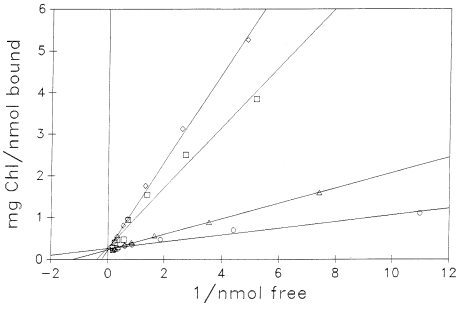

Fig. 3. Double reciprocal plot of the binding of [^{14}C]atrazine in the presence of 0 (○), 20 (△), 40 (□) and 60 (◆) nmol of 1-hydroxy-2-methyl-3-dodecyl-4(1H)-quinolone.

3.2. Cytochrome b_6/f-complex

Table 2 lists the pI_{50}-values for inhibition of electron transport through the cytochrome b_6/f-complex by various quinolones (Fig. 4). Whereas **1**, **2**, and **5** showed only moderate activity, the highest activity was observed for **3** and **4**. In the respective series, 2-methyl-3-*n*-undecyl- and 1-hydroxy-2-methyl-3-*n*-dodecyl-4(1H)-quinolone showed the highest activity and exhibited pI_{50}-values of 5.73 and 5.90, respectively (Table 2). Obviously, the presence of the hydroxyl group in position 1 enhances activity. Like in photosystem II, the maximal biological activity of the quinolones is reached at a chain length of 11 or 12 carbon atoms. Further increase in chain length decreases inhibitory potency (Table 2 and Fig. 2).

4. Discussion

Quinolones are produced by the bacteria *Pseudomonas aeruginosa* and *Stigmatella aurantiaca* (aurachins) and were shown to be inhibitors of respiratory [16] and photosynthetic electron transport chains as well [4,5,7–10]. They were taken as 'lead substances' for synthesis and evaluation of biological activity of a series of **1**, **2**, **3**, **4**, **5**. The length of the alkyl side chain was varied from 5–17 carbon atoms.

In photosystem II, **1** and **2** showed only moderate activity. If the alkyl side chain was positioned in the 3-position (**3**) instead, the biological activity could be slightly increased. The highest inhibitory potency have quinolones which bear a hydroxy moiety in position 1 (**4** and **5**). They reached a maximal activity at a chain length of 12 carbon atoms, which corresponds to about 14 Å. Thus, the long known inhibitors HQNO and NQNO [7–10] are inferior in their activity as compared to the *n*-undecyl quinolone derivatives. As judged from the competitive displacement of [^{14}C]atrazine, the quinolones share an identical binding site with other photosystem II commercial herbicides.

A QSAR could be performed for the inhibitory activity of the quinolones **4** and **5** in electron transport through photosystem II. As physicochemical parameter Verloop's STERIMOL parameter L (length of the substituent) [13] of the alkyl side chain in positions 2 (R_2) and 3 (R_3) have been used. The following equation has been obtained:

$$pI_{50} = -4.83 + 1.21\ L\ (R_2) - 0.04\ L^2\ (R_2) + 1.18\ L\ (R_3) - 0.04\ L^2\ (R_3) \quad (1)$$

$$n = 21;\ r = 0.95;\ s = 0.19;\ F = 87.57$$

n represents the number of compounds, r the correlation coefficient, s the standard deviation and F denotes the F-test of significance. Eq. 1 again demonstrates the dependency of the inhibitory activity from the chain length in a parabolic fashion as is also

Table 2
pI_{50}-values for inhibition of electron transport through the cytochrome b_6/f-complex by quinolones[a]

pI_{50}-value					
4(1H)-quinolone	**1** 2-alkyl-	**2** 2-alkyl-3-methyl-	**3** 2-methyl-3-alkyl-	**4** 1-hydroxy-2-methyl-3-alkyl-	**5** 1-hydroxy-2-alkyl-
n-pentyl	< 4		< 4		< 4
n-hexyl			< 4	4.51	< 4
n-heptyl	4.35	4.48	< 4	4.76	< 4
n-octyl		4.57	4.50	5.14	4.05
n-nonyl	4.24	4.67	5.34	5.60	4.19
n-decyl		4.50	5.46	5.79	4.15
n-undecyl	4.58	4.72	5.73	5.89	3.73
n-dodecyl			5.58	5.90	4.17
n-tridecyl	4.43	4.40	5.55	5.55	4.22
n-tetradecyl			4.71	5.34	4.15
n-hexadecyl			< 4	4.98	
n-heptadecyl					< 4

[a]For comparison, the pI_{50}-values for DBMIB and stigmatellin are 6.15 [2] and 7.22 [4], respectively.

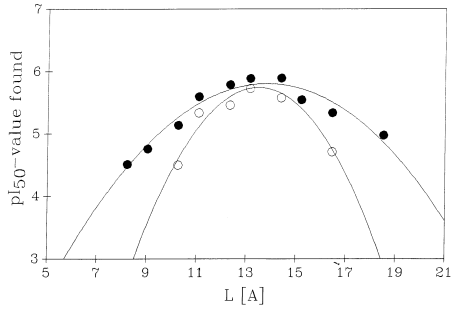

Fig. 4. Plot of Verloop's STERIMOL parameter L of the alkyl substituent versus the pI_{50}-value of quinolones in the cytochrome b_6/f-complex. ○ **3**, ● **4**.

obvious from Fig. 2. The second order terms in Eq. 1 are responsible for this parabola.

In contrast to photosystem II, where **5** are excellent inhibitors, they show only mediocre activity in the cytochrome b_6/f-complex. Here, the **3** and **4** are the best inhibitors. For them, a QSAR could be calculated:

$$\mathrm{p}I_{50} = -1.89 + 1.68\pi\ (\mathrm{R}_2) - 0.17\ \pi^2\ (\mathrm{R}_2) + 0.98\ L\ (\mathrm{R}_3) - 0.04\ L^2\ (\mathrm{R}_3) \quad (2)$$

$$n = 17;\ r = 0.90;\ s = 0.23;\ F = 37.64$$

Like in Eq. 1, Verloop's STERIMOL parameter L serves as one variable, whereas the other one is the lipophilicity π. It should be noted, however, that L and π are closely intercorrelated. This is due to the fact that the lipophilicity π increases linearly with the chain length. If instead of $\pi\ L(\mathrm{R}_2)$ is used, r slightly drops to 0.88.

So far, the quinolones have proven themselves as versatile inhibitors of quinone functions, which also include ubiquinone and plastoquinone. We have recently demonstrated that quinolones efficiently block ubiquinone reduction at the mitochondrial complex I (NADH:ubiquinone oxidoreductase) and simultaneously ubiquinol reoxidation at complex III (cytochrome b/c_1-complex) [6]. Here we report on the inhibition of plastoquinone reduction at photosystem II and plastoquinol reoxidation at the cytochrome b_6/f-complex by quinolones.

A similar situation is found for the acridones (9-azaanthracen-10-ones) which differ from the quinolones only by the presence of an additional aromatic moiety. The acridones are also inhibitors of mitochondrial complexes I and III, depending on their substitution pattern [17–19]. Provided the acridones are substituted by four strong electron withdrawing substituents, they are also efficient inhibitors of photosystem II [20]. In addition, acridone-4-carboxylic acids are inhibitors of the soluble, non-proton pumping NADH-dehydrogenases (NDHII) [19].

A motif for quinone binding in the protein sequences has not been identified yet. However, the fact that heterocyclic ketones can inhibit entirely different quinone binding sites located at photosystem II, the cytochrome b_6/f-complex, the cytochrome b/c_1-complex or the NADH-reductase indicates that these sites must share similarities which have not been recognized yet.

Acknowledgements

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