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Acridones and quinolones as inhibitors of ubiquinone functions in the mitochondrial respiratory chain

Walter Oettmeier, Klaus Masson, Michael Soll and Ellen Reil

Lehrstuhl Biochemie der Pflanzen, Ruhr-Universität, D-44780 Bochum, Germany

Introduction

Quinones and their reduced forms, quinols, play an important role as redox carriers in photosynthetic and respiratory electron transport chains. They mediate the transfer of both electrons and protons. In the reaction centre of photosynthetic bacteria, menaquinone and/or ubiquinone, respectively, constitute the primary and secondary electron-

acceptor sites Q_A and Q_B . Owing to the availability of X-ray crystallographic data, their structures are known in detail [1]. Similarly, through homology to the bacterial reaction centre, a three-dimensional structure of the plastoquinone Q_A and Q_B acceptor sites in photosystem II of higher plants and algae has been proposed [2]. Quinones reduced at the Q_B site are re-oxidized at the cytochrome bc_1 - or cytochrome b_6f complexes, respectively. These complexes also contain two quinone binding domains (Q_n and Q_p). In the mitochondrial respira-

Abbreviations used: QSAR, quantitative structure-activity relationship.

tory chain, quinone/quinol-binding sites are located at Complexes I, II and III. In addition, certain bacteria and some yeasts contain a soluble NADH dehydrogenase, which consists of a single protein entity with FAD serving as the prosthetic group. This soluble dehydrogenase reduces ubiquinone at the expense of NADH, but no proton translocation takes place (for review, see [3]).

For all of the above-mentioned quinone/quinol-binding sites, inhibitors are known (with the exception of the Q_A site). Inhibitors are extremely useful tools for the elucidation of structural and mechanistic aspects of quinone reactions. This has led to a very detailed knowledge of the Q_B -binding site of photosystem II (for review, see [4]). These photosystem II inhibitors have found widespread use as herbicides. Much less is known on inhibitors of mitochondrial complexes, though some naturally occurring substances are active in the nanomolar range. We wish to report here on the inhibitory properties of acridones and quinolones (Figure 1). Depending on their substitution pattern, they can function as inhibitors of mitochondrial Complexes I or III or of the soluble NADH dehydrogenase.

Materials and methods

The synthesis of the acridones has been described [5]; the synthesis of the quinolones will be reported elsewhere. Mitochondria and submitochondrial particles from beef heart were prepared according to Oettmeier et al. [5], the cytochrome bc_1 complex from beef heart according to Engel et al. [6], that of *Rhodospirillum rubrum* according to Oettmeier et al. [7] and the soluble NADH dehydrogenase from *Saccharomyces cerevisiae* according to de Vries and Grivell [8]. The assay systems for measurement of inhibitory activity are described in [5] and [6].

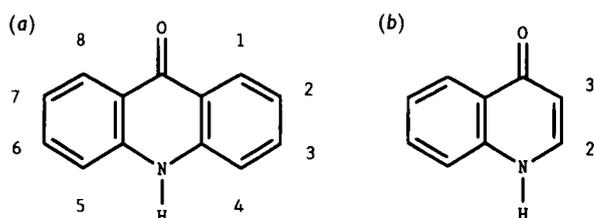
Results and discussion

NADH:ubiquinone oxidoreductase (Complex I)

The best-known inhibitors of Complex I are piericidin and rotenone [9], though there are others [5].

Figure 1

Structural formulae for acridones (a) and quinolones (b)



A detailed structure-activity relationship for inhibitory activity in Complex I was established for 4-hydroxypyridines and 4-hydroxyquinolines by Chung et al. [10]. However, until recently, no quantitative structure-activity relationship (QSAR) was available for Complex I inhibitors which correlates biological activity with physico-chemical parameters of the compound. We have recently developed acridones as new photosystem II inhibitors. They are highly active in photosystem II if they are substituted by several strongly electron-withdrawing halogen or nitro groups [11, 12]. Acridones can also be tailored to render active inhibitors of other quinone functions. This is especially true for acridones substituted by alkyl- or alkoxy groups in the 4-position, which have turned out to be effective inhibitors of Complex I. The pIC_{50} value ($-\log$ of the concentration where 50% inhibition is achieved) raises as the chain length increases. It reaches a maximum for 4-*n*-octyloxyacridone (pIC_{50} value = 6.38) and decreases if the chain length is further increased. Consequently, in the QSAR of 4-substituted acridones, the pIC_{50} value is parabolic, depending on Verloop's STERIMOL parameter L (length of the substituent) [5]. An additional substitution at the 5 or 6 position is unfavourable for biological activity; in contrast, substitution at the 7-position enhances activity. 7-Chloro-4-*n*-octyloxyacridone with a pIC_{50} value of 6.67 is the most potent inhibitor of the acridone series found so far [5]. We have also synthesized a 7-azido-4-*s*-butylacridone (pIC_{50} value = 6.00) which, after a radioactive synthesis, may serve as a photoaffinity label to identify the acridone-binding site within

Table I

pIC_{50} values for inhibition of electron transport through the cytochrome bc_1 complex from beef heart or *R. rubrum* by 2-alkyl-acridones

	Acridone	Beef heart	<i>R. rubrum</i>
1	2-methyl-	5.26	6.84
2	2-ethyl-	4.40	6.96
3	2- <i>n</i> -propyl-	4.66	7.35
4	2- <i>n</i> -butyl-	5.38	7.43
5	2- <i>n</i> -pentyl-	5.62	7.77
6	2- <i>n</i> -hexyl-	5.98	8.04
7	2- <i>n</i> -heptyl-	6.22	8.15
8	2- <i>n</i> -octyl-	6.10	8.20
9	2- <i>n</i> -decyl-	4.75	7.87
10	2- <i>n</i> -dodecyl-	3.94	7.09

Complex I. By competition experiments with the highly fluorescent 4-*s* butylacridone and rotenone, it has been established that both compounds share an identical binding site [5].

Like 4-alkylacridones, 2-alkylquinolones (Figure 1) are also efficient inhibitors of electron transport through Complex I. Again, inhibitory potency increases as the alkyl side-chain gets larger. Maximum activity is achieved with 2-undecylquinolone (pIC_{50} value = 6.66) and further lengthening of the alkyl chain leads to a decrease in activity. The 3-methyl-2-alkylquinolones are even better inhibitors. The inhibitory potency peaks for 2-undecyl-3-methylquinolone (pIC_{50} value = 7.70). Thus, the presence of a methyl group in the 3-position raises biological activity by one order of magnitude. Further increase in the length of the alkyl side-chain again leads to a decrease in activity.

Ubihydroquinone:cytochrome-*c* oxidoreductase (Complex III)

Another ubiquinone-binding site within the mitochondrial respiratory chain is located at the ubihydroquinone:cytochrome-*c* oxidoreductase (Complex III). As for Complex I, several naturally occurring inhibitors of ubihydroquinone oxidation are known (for review, see [13]). Inhibitors can bind to two different centres, known as Q_n (antimycin) and Q_p (myxothiazol) [13]. 4-Substituted acridones are only moderate inhibitors of Complex III, but inhibition increases dramatically if an alkyl group is located in position 2 (Table 1). 2-Alkyloxyacridones exhibit similar activity (data not shown). 2-Substituted acridones have been assayed in two different systems, either in the cytochrome bc_1 complex from beef heart or from the photosynthetic bacterium *R. rubrum*. As is evident from Table 1, the inhibitory potency of 2-substituted acridones is much higher in the *R. rubrum* complex than in the beef heart complex. In *R. rubrum* Complex III, the 2-alkylacridones reach pIC_{50} values which come close to those of antimycin (8.38) or myxothiazol (8.51). Inhibitory activity in both systems reaches its maximum where alkyl is *n*-heptyl or *n*-octyl and decreases if the chain length is further increased. The QSAR for the inhibitory activity of 2-substituted acridones in Complex III from *R. rubrum* is governed by the lipophilicity constant π and the square of Verloop's STERIMOL parameters L and B_2 .

To decide which quinone-binding site is inhibited by 2-alkylacridones, i.e. the Q_n or Q_p site, the influence of 2-alkylacridones on the 'oxidant-induced reduction' of cytochrome *b* in the cytochrome bc_1 -complex from *R. rubrum* has been

investigated. When in the complex, cytochrome c_1 and the Rieske iron-sulphur protein are reduced, and the Q_n site is inhibited by antimycin, reduction of cytochrome *b* by ubiquinol is not possible. In contrast, when cytochrome c_1 and the Rieske iron-sulphur protein are kept oxidized by ferricyanide, cytochrome *b* will be rapidly reduced [13]. Our results clearly indicate that 2-alkylacridones do not behave like antimycin and do not occupy the Q_n site (data not shown). On the other hand, the 'oxidant-induced reduction' of cytochrome *b* in the presence of ferricyanide is blocked by inhibitors of the Q_p site, like myxothiazol [13]. It could be clearly demonstrated that 2-alkylacridones resemble myxothiazol in their mode of action and can be classified as Q_p -site inhibitors (data not shown).

Aurachins C and D, produced by the myxobacterium *Stigmatella aurantiaca* were recently found by us to be inhibitors of the cytochrome bc_1 complex from *R. rubrum* [7]. Chemically, the aurachins are quinolones (aurachin C being the *N*-oxide) and were used as lead substances to develop new synthetic inhibitors for Complex III. It turned out that the complex isoprenoidal side-chain in position 3 of the quinolone, which includes three double-bonds, is not necessary for biological activity. It can be replaced by an *n*-alkyl side-chain. In the 2-*n*-alkylquinolone-*N*-oxide series, weak activity in Complex III from beef heart was found for the pentyl derivative (pIC_{50} value = 4.47). The pIC_{50} value increased for each methylene group by which the alkyl chain was lengthened (for example: heptyl, 5.82; nonyl, 6.88; undecyl, 7.25). Maximum activity was found for 2-*n*-tridecylquinolone-*N*-oxide, which exhibited a pIC_{50} value of 7.57. As was the case for acridones, a further increase in chain length leads to a decrease in biological activity. A similar parabolic dependence of inhibitory potency on the chain length was observed for 2-methyl-3-*n*-alkylquinolone-*N*-oxides. Activity peaked for 2-methyl-3-*n*-undecylquinolone-*N*-oxide at a pIC_{50} value of 7.26. Work to characterize the quinolone-binding site, i.e. whether it is Q_n or Q_p , is in progress.

Soluble NADH:ubiquinone dehydrogenase

Contrary to mammalian mitochondria, mitochondria from plants, fungi and yeast can oxidize externally added NADH. This oxidation of cytosolic NADH via the mitochondrial respiratory chain is not coupled with proton translocation, and hence phosphorylation, nor is it inhibited by piericidin and rotenone [8]. This indicates that the soluble NADH dehydrogenase is structurally different from

Complex I. The soluble NADH dehydrogenase from *S. cerevisiae* has recently been purified to homogeneity and found to be a single protein with an apparent molecular mass of 53 kDa with FAD as the prosthetic group [8]. As already stressed, this NADH-dehydrogenase activity is insensitive to rotenone and piericidin. Little is known about inhibitors of external NADH dehydrogenases and the few that have been found all belong to the class of flavonoids [14]. The most efficient inhibitor known so far is platanetin (6-dimethylallyl-3,5,7,8-tetrahydroxyflavone). It was isolated from a natural source and exhibits a pIC_{50} value of 5.7 [14], which is low compared with the inhibitory potency of piericidin or rotenone.

Acridones can also function as inhibitors of external NADH dehydrogenases, provided they are substituted by a carboxylic acid function. The requirements for the position of the carboxylic group are very specific; it must be in the 4-position. Thus, acridone-4-carboxylic acid exhibits a pIC_{50} value of 4.7 in an assay system, where UQ-1 is utilized as the electron acceptor. Acridones, substituted in other positions by the carboxylic group, are inactive. Furthermore, the carboxylic group needs to be unsubstituted. Introduction of an amide or an ester function leads to a loss of activity. Inhibitory activity of the acridone-4-carboxylic acid can be enhanced by the introduction of a halogen substituent in the 5- or 7-position. The pIC_{50} value of 5-chloro-acridone-4-carboxylic acid is raised to 4.96 and that of 7-chloro- or 7-bromo-acridone-4-carboxylic acid to 5.01. 7-Iodo-acridone-4-carboxylic acid, with a pIC_{50} -value of 5.10, is the most efficient inhibitor for external NADH dehydrogenase that has been found so far. Whether acridone-4-carboxylic acid derivatives interfere with NADH or ubiquinone binding remains to be elucidated.

Future research will be devoted to the aim of developing even more effective inhibitors of soluble NADH dehydrogenases in order to get more insight into their mechanism of action.

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