New 4-Hydroxypyridine and 4-Hydroxyquinoline Derivatives as Inhibitors of NADH-ubiquinone Reductase in the Respiratory Chain

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Inhibitors of NADH-UQ Reductase, Submitochondria, Respiratory Electron Transport, 4-Hydroxypyridine Derivatives, 4-Hydroxyquinoline Derivatives

Many derivatives of 2,3-dimethoxy-4-hydroxypyridine, which were designed from examination of the structure-activity relationship of piericidins, were tested for inhibition of NADH-UQ reductase. The lipophilic side chain of those compounds was indicated to be a key part for activity and its optimal length was conjectured. By the use of two different phases of assay material, intact mitochondria and submitochondria, the size of a membrane effect was shown to depend on the structure of the side chain. 4-Hydroxyquinoline derivatives were also tested for an analogous role in relation to the electron transport function of menaquinone, and they were proven to be inhibitors of NADH-UQ reductase as good as the pyridine derivatives.

Introduction

Since the discovery of piericidins potent insecticidal compounds have been discovered in metabolites of *Streptomyces mobaraensis* [1] and *pactum* [2], and their chemical structures [3, 4] and physiological properties [5, 6] extensively studied. The mode of action of piericidins was demonstrated to be specific and potent inhibition of ubiquinone (UQ, coenzyme Q) action at the NADH-UQ reductase site in the respiratory electron transport system in mitochondria [7, 8]. Although piericidin A_1 is significantly effective in killing many kinds of insects, application of these types of natural products has been abandoned for reason of their chemical instability and high toxicity to mammals.

The structure-activity relationship between piericidins and respiratory inhibition was investigated [9] using some synthetic analogs, and the essential structures for the inhibition of the mitochondrial respiratory chain were proposed as illustrated by Types A and B. The effective inhibition at the NADH-UQ reductase site induced by artificial compounds [9, 10] suggested a need for further synthetic investigation to look for more efficient structures in this class of chemicals applicable in pest control.

In a previous study [9] on the structure-activity correlation of piericidins, effects of their functionality on activity were summarized in terms of the following facts: 1) hydrogenation on all double bonds in side chains of piericidins causes remarkable loss of activity in the parent compounds; 2) piericidin analogs holding three isoprene units (farnesyl) as a side chain demonstrate activity as high as that of natural piericidins; 3) a free phenolic hydroxy group on the pyridine ring is necessary to maintain the high level of activity; 4) location of a lipophilic side chain at either α- (TYPE A) or β- (TYPE B) positions of the pyridine ring has a similar effect on activity.

In the earlier studies it was considered that the structural resemblance of piericidins to ubiquinone resulted in their showing competitive inhibition of NADH-UQ reductase in the respiratory electron transport system, where ubiquinone plays an impor-

Abbreviations: ETP, electron transport particles; NADH-UQ reductase, NADH-ubiquinone (oxido)reductase; Tris, Tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

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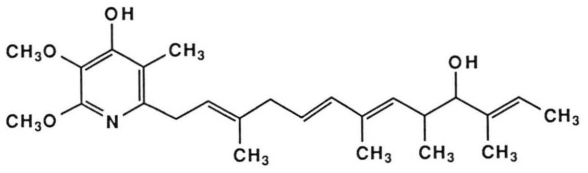

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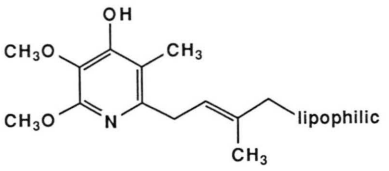

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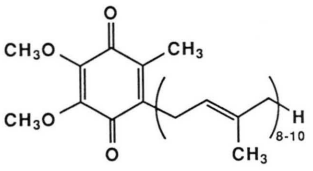

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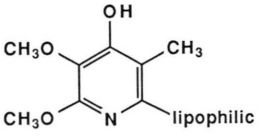
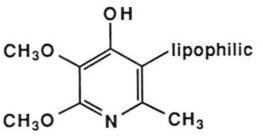


Type A



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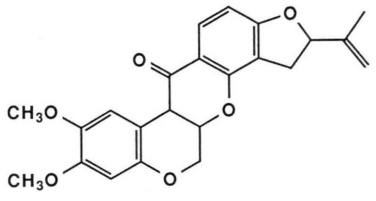

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tant role as an electron carrier substrate. Actually piericidins were recognized as specific inhibitors at that step in the mitochondria of insects [11] and mammals [7, 8] in very low concentration. The relation between piericidins and the active site of the enzyme was also demonstrated by means of extensive biochemical studies using radioactive compounds, and the results indicated that piericidins should bind at the ubiquinone reducing site, as is the case for rotenoids [12, 13]. Based on the above information two new series of inhibitors, classified as 4-hydroxy-pyridine and 4-hydroxyquinoline derivatives, were designed, synthesized and bioassayed. In this paper we report on the structural requisites of the lipophilic part in these molecules for inhibition of NADH-UQ reductase in the respiratory electron transport system of mitochondria and of submitochondria (ETP).

Materials and Methods

Chemicals

Compounds carrying 2,3-dimethoxy-4-hydroxypyridine systems were synthesized by the modified method [14] of the previous paper [9], and 4-hydroxyquinoline derivatives were obtained by a modification [14] of the classical method [15, 16]. All compounds were checked by instrumental analyses as follows: IR, NMR and mass spectrum with a Shimadzu IR-435, a Bruker AM-400 (or a Jeol GX-400) and a Hitachi M80A (or a Finnigan INCOS-50) spectrometer respectively.

Rat liver mitochondria

Suspensions of rat liver mitochondria were prepared in the usual way as follows [17]. Male albino rats (5 week old) were decapitated to remove livers which were immersed in 50 ml ice-cold 0.25 M sucrose–0.1 mM EDTA in a tared beaker. The livers were rinsed with the buffer, sliced into several pieces with a pair of scissors and transferred into a 20 ml glass-Teflon homogenizer. The homogenate was centrifuged at $900 \times g$ for 10 min. The supernatant was decanted into a flask and the pellet was homogenized and centrifuged as for the first run. Both supernatants were combined and recentrifuged at $8000 \times g$ for 20 min. The pellet was suspended to repeat the same centrifugation. Finally the pellet was resuspended by gentle swirling with 0.25 M sucrose–10 mM Tris–0.1 mM EDTA (pH 7.4, 5 ml).

Bovine heart submitochondria (ETP)

ETP were obtained from bovine heart mitochondria which was prepared by an established method [18]. The sucrose mitochondrial suspension was adjusted to pH 8.5 at 0 °C with 0.1 N KOH and maintained at this pH value for 30 min by addition of alkali. The suspension was then treated with a 20 ml glass-Teflon homogenizer at 0 °C. The treated suspension was centrifuged at $19{,}000 \times g$ for 7 min under cooling conditions (0 °C). The supernatant was collected and the pellet was again homogenated by the above procedure. The combined supernatants were centrifuged for 30 min at $80{,}000 \times g$. The final pellet was taken up in 0.25 M sucrose and, after adjustment of the pH to 7.5 with acetic acid, was stored in liquid nitrogen.

Inhibition assay method

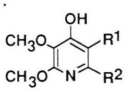
Respiratory inhibition of compounds was measured by an oxygen electrode of Clark type at 25 °C in 2 ml of a medium (pH 7.4) consisting of a mitochondrial suspension (0.2 ml), the phosphate buffer (1.8 ml), $MgCl_2$ (10 μmol), ADP (0.5 μmol) and L-glutamic acid (10 μmol), or of a ETP suspension (0.2 ml), the phosphate buffer (1.8 ml), $MgCl_2$ (10 μmol), cytochrome *c* (0.03 μmol), ADP (0.5 μmol) and NADH (2 μmol). The inhibitory activity of compounds is expressed as a pI_{50} value, the negative logarithm of inhibitor amount (mol/mg-protein) at 50% inhibition.

Results

A) Effects of lipophilic substituents on the 2,3-dimethoxy-4-hydroxypyridine system

As shown in Table I the derivatives carrying a saturated linear chain on the pyridine system (**5–10**) are much less active than piericidin A_1 with rat liver mitochondria. This is consistent with the previous conclusion obtained from the data on piericidin analogs [8]. Since both Types A and B carrying a farnesyl side chain show the highest inhibition level in the mitochondrial assay [9, 10] this was thought to indicate that some steric factors are required in the lipophilic side chain for piericidin-like inhibitors to penetrate from the outer membrane to the inner one. It is at least certain now that these 4-hydroxypyridine derivatives with a linear alkyl side chain need tenfold higher concentration for the inhibition of mitochondria than for inhibition of ETP. Apart from the effect of the alkyl side chain, results in Table I indicate that Type A is more effective than Type B.

Table I. Activity of 2,3-dimethoxy-4-hydroxypyridines against NADH-UQ reductase of intact mitochondria and of ETP.



Comp. Nr.	Type	Side Chain R1	Side Chain R2	pI_{50} Mitochondria	pI_{50} ETP
1	A	piericidin A_1		11.0	11.4
4	B	rotenone		10.8	10.8
5	A	$-CH_3$	$-(CH_2)_8CH_3$	9.4	10.5
6	B	$-(CH_2)_7CH_3$	$-CH_3$	7.8	8.8
7	A	$-CH_3$	$-(CH_2)_{10}CH_3$	9.7	10.6
8	B	$-(CH_2)_9CH_3$	$-CH_3$	7.8	8.9
9	A	$-CH_3$	$-(CH_2)_{12}CH_3$	8.4	9.1
10	B	$-(CH_2)_{11}CH_3$	$-CH_3$	7.5	8.4
11	B	$-CH_2$-Ph	$-CH_3$	<7.5	7.7
12	B	$-(CH_2)_2$-Ph	$-CH_3$	<7.5	7.8
13	B	$-(CH_2)_3$-Ph	$-CH_3$	<7.5	8.1
14	B	$-CH_2$-Ph-$(CH_2)_2CH_3$	$-CH_3$	<7.5	8.8
15	B	$-CH_2$-Ph-$(CH_2)_4CH_3$	$-CH_3$	8.1	9.3
16	B	$-CH_2$-Ph-$(CH_2)_8CH_3$	$-CH_3$	<7.5	8.7
17	B	$-CH_2$-Ph-$(CH_2)_{10}CH_3$	$-CH_3$	<7.5	<7.5
18	A	$-CH_3$	$-(CH_2)_2$-Ph	<7.5	9.1
19	A	$-CH_3$	$-(CH_2)_3$-Ph	8.4	9.5
20	A	$-CH_3$	$-(CH_2)_4$-Ph	10.0	10.6
21	A	$-CH_3$	$-(CH_2)_6$-Ph	10.4	10.8

Table 1 (cont.)

Comp. Nr.	Type	Side Chain R^1	Side Chain R^2	pI_{50} Mitochondria	pI_{50} ETP
22	A	$-CH_3$	$-(CH_2)_2$-Ph-$(CH_2)_2CH_3$	8.6	9.9
23	A	$-CH_3$	$-(CH_2)_2$-Ph-$(CH_2)_4CH_3$	8.8	10.4
24	A	$-CH_3$	$-(CH_2)_2$-Ph-$(CH_2)_6CH_3$	8.1	9.9
25	A	$-CH_3$	$-(CH_2)_2$-Ph-$(CH_2)_8CH_3$	<7.5	8.2
26	A	$-CH_3$	$-(CH_2)_2$-Ph-$(CH_2)_{10}CH_3$	-	<7.5
27	A	$-CH_3$	$-(CH_2)_2$-Ph-$(CH_2)_{12}CH_3$	-	<7.5
28	A	$-CH_3$	$-(CH_2)_2$-Ph-OCH_2CH_3	9.2	9.8
29	A	$-CH_3$	$-(CH_2)_2$-Ph-$O(CH_2)_3CH_3$	9.2	10.4
30	A	$-CH_3$	$-(CH_2)_2$-Ph-$O(CH_2)_5CH_3$	8.5	9.6
31	A	$-CH_3$	$-(CH_2)_2$-Ph-$O(CH_2)_7CH_3$	7.8	9.4
32	A	$-CH_3$	$-(CH_2)_2$-Ph-$O(CH_2)_9CH_3$	<7.5	8.5
33	A	$-CH_3$	$-(CH_2)_2$-Ph-$O(CH_2)_{11}CH_3$	<7.5	8.0
34	A	$-CH_3$	$-(CH_2)_3$-Ph-OCH_2CH_3	9.5	9.8
35	A	$-CH_3$	$-(CH_2)_4$-Ph-OCH_3	9.8	10.5
36	A	$-CH_3$	$-(CH_2)_4$-Ph-OCH_2CH_3	9.7	10.5
37	A	$-CH_3$	$-(CH_2)_4$-Ph-$O(CH_2)_2CH_3$	9.6	10.6
38	A	$-CH_3$	$-(CH_2)_4$-Ph-$O(CH_2)_4CH_3$	9.3	10.4
39	-	$-CH_2CH_3$	$-(CH_2)_6$-Ph	-	8.9
40	-	$-CH_2CH_3$	$-(CH_2)_2$-Ph-$O(CH_2)_3CH_3$	-	9.7
41	-	$-CH_2CH_3$	$-(CH_2)_4$-Ph-OCH_3	-	9.4
42	-	$-CH_2CH_3$	$-(CH_2)_4$-Ph-OCH_2CH_3	-	8.1

All the compounds carrying an ω-phenylalkyl group as the side chain (**11**–**13**, **18**–**21**) showed an inhibitory effect at the ETP level and this was enhanced by increasing the number of methylene groups. Effects of *para*-alkyl substitution on the phenyl group were also measured through the series of benzyl [Type B] (**11**, **14**–**17**) and β-phenethyl [Type A] (**18**, **22**–**27**) derivatives. Activity of the benzyl derivatives was improved by an alkyl substituent, especially by *n*-pentyl (**15**), but the *n*-undecyl group (**17**) appeared to be oversize for the binding. Among the β-phenethyl derivatives *para*-*n*-pentyl-β-phenethyl compound (**23**) also showed an optimally high level of inhibition and, as in the benzyl case, the activity was lost beyond the point of the *n*-undecyl side chain (**26**). In the series of *para*-alkoxy-β-phenethyl derivatives (**28**–**33**) the same optimal length for activity of **29** is obvious as for the *para*-*n*-pentyl-β-phenethyl derivative **23**. Thus the range of suitable sizes (*ca.* eleven carbon-bond length) of the substituted β-phenethyl group appears to be limited to lengths less than those of side chains in natural piericidins which are of thirteen or fourteen carbon-bond length [4].

Further interesting results were obtained by varying the distance between the pyridine and the phenyl nuclei in the side chain. Insertion of four methylene caused the ω-phenylalkyl derivative (**20**) to reach the highest level of activity and this was unchanged by *para*-substitution on the phenyl ring (**35**–**38**) or by extending two further methylene units (**21**).

Comparison of the activity patterns between *para*-alkyl-β-phenethyl (**18**, **22**–**27**) and *para*-alkoxy-β-phenethyl (**28**–**33**) derivatives pointed up no significant difference in effects due to a methylene and an oxygen at the arylic position. It is noteworthy that the mitochondrial inhibition values of those compounds (**20**, **21**, **23**, **29** and **35**–**38**) are quite different (due presumably to the location of the phenyl group in the side chain) despite their similar potential in the ETP assay.

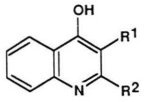
Although in the previous studies piericidin analogs lacking the methyl group on the pyridine ring system were proven to be inactive [9], the effect of an extension at that point had not been examined. So the methyl residues of the pyridine ring were modified into ethyl group for four derivatives. From those four sets of compound contrasts (**21** *vs.* **39**; **29** *vs.* **40**; **35** *vs.* **41**; **36** *vs.* **42**) it is obvious that an ethyl substituent is less effective than a methyl one in hihibiting the NADH-UQ reductase.

B) Effects due to structural modification of the 2,3-dimethoxy-4-hydroxypyridine system into the 4-hydroxyquinoline system

A ring system carrying a vicinal dimethoxy group is common for piericidins, rotenoids and ubiquinones. However, an interesting nucleus may be conceived from another group of natural electron carriers, menaquinones, which are widely distributed in both respiratory [19] and photosynthetic mechanisms [21, 22], and perform the function of ubiquinone. Thus the 4-hydroxyquinoline derivatives (**43**–**71**) were synthesized to measure their activity on NADH-UQ reductase as shown in Table II. The lipophilic side chains of these compounds were designed following the same idea as for the 2,3-dimethoxy-4-hydroxypyridine series.

As well as the pyridine series there are also two types of 4-hydroxyquinolines classified by the position of the lipophilic side chains, Types A and B.

Table II. Activity of 4-hydroxyquinoline derivatives against NADH-UQ reductase of ETP.



Comp. Nr.	Type	Side Chain R^1	Side Chain R^2	pI_{50} (ETP)
43	A	$-CH_3$	$-(CH_2)_2$-Ph	8.9
44	A	$-CH_3$	$-(CH_2)_3$-Ph	9.3
45	A	$-CH_3$	$-(CH_2)_4$-Ph	10.1
46	A	$-CH_3$	$-(CH_2)_6$-Ph	10.7
47	A	$-CH_3$	$-(CH_2)_2$-Ph-$(CH_2)_2CH_3$	9.9
48	A	$-CH_3$	$-(CH_2)_2$-Ph-$(CH_2)_4CH_3$	10.4
49	A	$-CH_3$	$-(CH_2)_2$-Ph-$(CH_2)_6CH_3$	9.7
50	A	$-CH_3$	$-(CH_2)_2$-Ph-$(CH_2)_8CH_3$	8.6
51	A	$-CH_3$	$-(CH_2)_2$-Ph-$(CH_2)_{10}CH_3$	8.4
52	A	$-CH_3$	$-(CH_2)_2$-Ph-OCH_2CH_3	8.5
53	A	$-CH_3$	$-(CH_2)_2$-Ph-$O(CH_2)_3CH_3$	10.1
54	A	$-CH_3$	$-(CH_2)_2$-Ph-$O(CH_2)_5CH_3$	10.2
55	A	$-CH_3$	$-(CH_2)_4$-Ph-OCH_3	10.1
56	A	$-CH_3$	$-(CH_2)_4$-Ph-OCH_2CH_3	10.2
57	A	$-CH_3$	$-(CH_2)_4$-Ph-$O(CH_2)_2CH_3$	10.1
58	A	$-CH_3$	$-(CH_2)_2$-Ph-Cl	8.9
59	A	$-CH_3$	$-(CH_2)_2$-Ph-Br	9.1
60	B	$-(CH_2)_7CH_3$	$-CH_3$	9.7
61	B	$-(CH_2)_{11}CH_3$	$-CH_3$	9.6
62	B	geranyl	$-CH_3$	10.1
63	B	H_4-geranyl	$-CH_3$	10.2
64	B	farnesyl	$-CH_3$	10.2
65	B	H_6-farnesyl	$-CH_3$	10.0
66	-	$-CH_2CH_3$	$-(CH_2)_6$-Ph	10.4
67	-	$-(CH_2)_2CH_3$	$-(CH_2)_6$-Ph	9.7
68	-	$-CH_2CH_3$	$-(CH_2)_4$-Ph-OCH_2CH_3	9.4
69	-	$-(CH_2)_2CH_3$	$-(CH_2)_4$-Ph-OCH_2CH_3	8.8
70	A	$-CH_3$	$-(CH_2)_5O$-Ph	9.8
71	-	$-CH_2CH_3$	$-(CH_2)_5O$-Ph	9.2

Compounds corresponding to Type B with a straight alkyl chain (**60** and **61**) were fairly active, as was the case for the pyridine derivatives, and those with an isoprenoid chain (**62–65**) also showed the same high level of activity. 4-Hydroxyquinoline derivatives with an ω-phenylalkyl (**43–46**), a *para*-alkyl-β-phenethyl (**47–51**), a *para*-alkoxy-β-phenethyl (**52–54**) or a *para*-alkoxy-δ-phenylbutyl (**55–57**) showed the characteristic patterns of activity which were observed in the 4-hydroxypyridine series. It is clearly indicated that heterocyclic systems of 4-hydroxyquinoline and 2,3-dimethoxy-4-hydroxypyridine are relevant structural requisites for inhibitors of the NADH-UQ reductase.

It is well known that halogen substitution on the phenylalkyl side chain in inhibitors of the photosynthetic electron transport system induces a great enhancement of activity at the site of the plastoquinone receptor (*cf.* [22]) and it was therefore thought worth trying to introduce a *para*-chloro-β-phenethyl or *para*-bromo-β-phenethyl side chain into the above quinoline derivatives. However, two halogenated compounds (**58** and **59**) exhibited nearly the same level of activity as the non-halogenated derivative (**43**).

The effect of the arylic methyl was also recognized in the case of 4-hydroxyquinoline derivatives (**46** *vs.* **66** and **67**; **56** *vs.* **68** and **69**; **70** *vs.* **71**) as well as the pyridine series, namely, an extension of the methyl into an ethyl and a propyl group reduced the activity gradually.

Discussion

We have here the first evidence that piericidin-like analogs with even the linear chain are effective in inhibiting NADH-UQ reductase, especially in the case of Type A compounds. In the light of research on photosynthetic electron transport inhibitors [22–24] a substituted phenylalkyl group (instead of an alkyl group) was regarded as a good template to examine sterical and functional limits for the inhibitors at the receptor site of the exposed NADH-UQ reductase, because of both its bulkiness and the π-electron clouds in the phenyl part. Effects with side chains carrying the phenylalkyl group suggest that the phenyl group might be accommodated in a binding niche of the NADH-UQ reductase.

We might have expected the results for the benzyl group to be attributable to the short distance between the two aromatic rings, with intramolecular torsion resulting from steric factors forcing the alkyl substituent in a particular direction. It is interesting however that the limit for the chain length is evident

not only for the benzyl series but for other series as well.

In the case of methyl substitution on the pyridine and the quinoline nuclei, the rather smaller influence of the extension for the quinoline case than for the pyridine one can be explained by a presumed shift of the binding position for the nuclei in the receptor, due to some difference in the size of the vicinal dimethoxyl groups on the pyridine ring from that of the fused benzene part in the quinoline.

There is in fact as yet very little information on the binding site of ubiquinone in NADH-UQ reductase, although a bound form of ubiquinone in the photoreaction center of photosynthetic bacteria, which evolutionary relates to the mitochondrial system [25], has been revealed by studies using X-ray crystallography [26–29]. The above facts on the various structures for effective inhibitors of the site may be more easily understood by allowing the introduction of such bacterial knowledge into a model of the ubiquinone binding domain of NADH-UQ reductase (Scheme). The size of the niche is presumably limited by the length of the sequential polypeptide which may involve various peptide units, as in the case of the photoreaction center [20] consisting of histidines for electron transfer, lipophilic amino acids to hold a side chain and a serine unit which binds with another particular part of the inhibitor. In this model, piericidin A_1 and rotenone suggest a suitable basic frame for the lipophilic part of the inhibitor (A and B in Scheme), and whether the active inhibitor has a proper fit is assumed to depend on the flexibility of the side chain. In any case these effects of the inhibitor series suggest a new approach to the study of binding at the NADH-UQ reductase site, although it is recognized that topological analyses employing such lipophilic side chains of piericidin-like inhibitors will require much more variation of those structures.

Although the piericidin analogs with a linear side chain were shown to be active in inhibiting NADH-UQ reductase, they were less effective against the intact mitochondria than against the ETP (submitochondria). The gap in the activity level between the mitochondrial and the ETP bioassays was very obvious. This contrast may be explained by a barrier effect at the outer membrane of mitochondria and similar results can be observed in the photosynthetic electron transport system where many inhibitors of the plastoquinone binding site carrying a saturated linear chain show higher activity in a thylakoid assay than in a chloroplast one [30]. The thylakoid suspension involves the unsealed plastoquinone receptor being exposed in the assay medium just as the NADH-UQ reductase is in the ETP suspension. If, as previously supposed, steric factors are involved in membrane penetration then the positions of a double bond and/or a branched structure in the side chain should be important factors in the design of new types of inhibitors at the mitochondrial level, but they may be less important for the exposed NADH-UQ reductase of ETP. This suggests that structure-activity studies on inhibitors of NADH-UQ reductase should be directed at ETP assays, in order to clarify the nature of side chain effects and avoid confusion of one factor for inhibition, like affinity with the binding site, with another such as permeability at the outer membrane.

Inhibition of NADH-UQ reductase due to piericidin-like compounds was mainly affected by the length of the lipophilic part, regardless of bulkiness or location of a phenyl group in the side chain. The vicinal dimethoxy functionality of the 4-hydroxypyridine ring system is replaceable with a fused benzene to form the 4-hydroxyquinolines as a novel class of inhibitors at the site. The methyl group was demonstrated to be the optimal functionality on the nuclei of the pyridine derivatives so that either deletion or insertion of a methylene on the group eliminated its activity. A methyl group on the nuclei of inhibitors belonging to the class of 4-hydroxyquinoline was also shown to be a functionality which optimized activity, but the sterical limit of the binding niche around the group appears to be more tolerant than in the case of the pyridine derivatives.

The finding of a large structural variation for synthetic inhibitors of NADH-UQ reductase is valuable for further investigations on the mitochondrial respiration system because these compounds are readily modified to introduce a functionality to probe the binding site; for example a radioisotope, a photoaffinity label and/or a chiral center *etc.* It will also be very interesting to confirm the side chain effects in other classes of inhibitors of NADH-UQ reductase such as benzimidazoles [31], mixalamids [32] and amytal [33].

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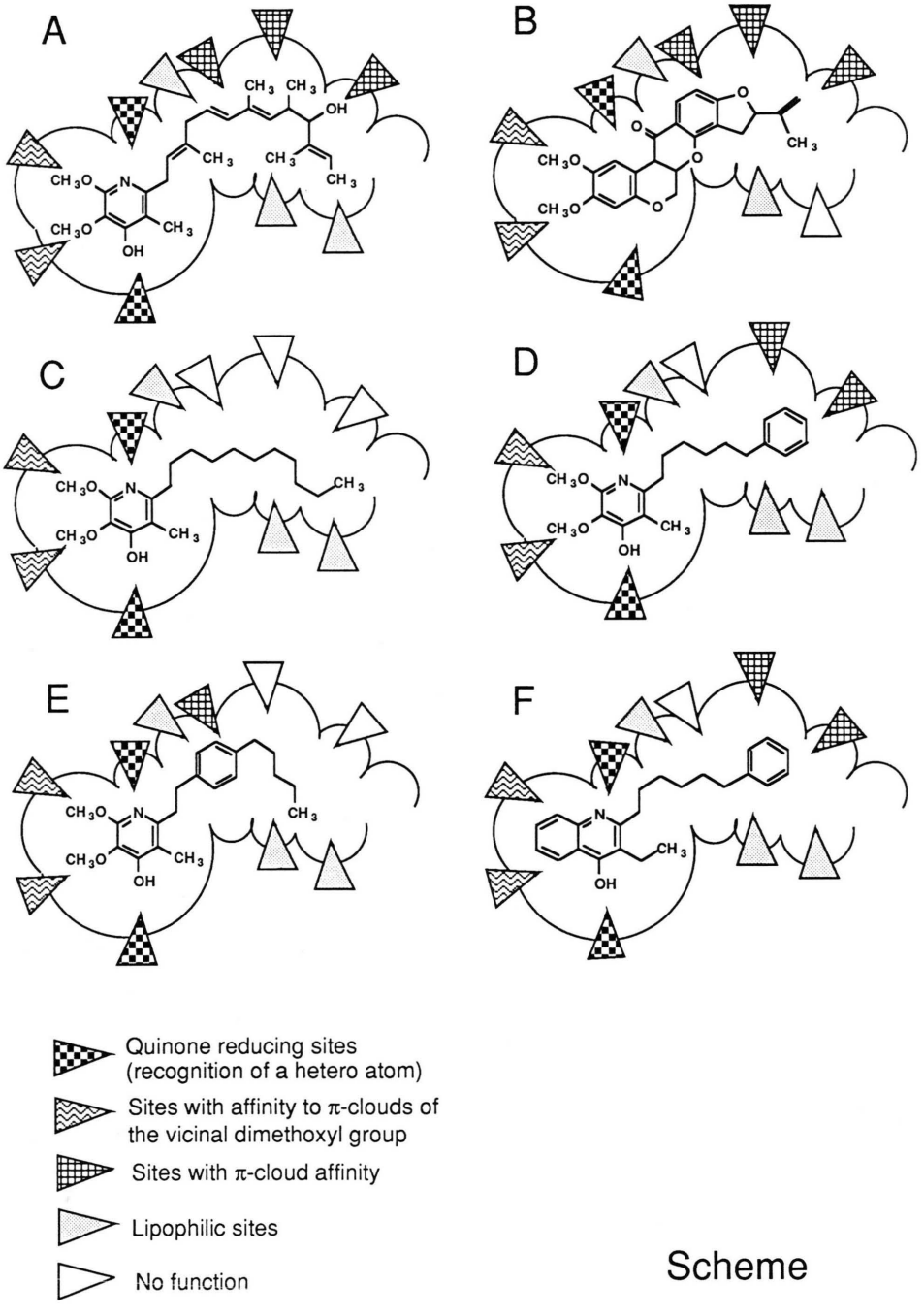

Scheme

Scheme. Hypothetical binding manners of piericidin-like inhibitors at the ubiquinone reducing site of NADH-UQ reductase. A) Piericidin A_1, $pI_{50} = 11.4$; B) rotenone, $pI_{50} = 10.8$; C) compound **7**, $pI_{50} = 10.6$; D) compound **21**, $pI_{50} = 10.8$; E) compound **23**, $pI_{50} = 10.4$; F) compound **66**, $pI_{50} = 10.4$.

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[1] S. Tamura, N. Takahashi, S. Miyamoto, R. Mori, S. Suzuki, and J. Nagatsu, Agric. Biol. Chem. **27,** 576–580 (1963).
[2] S. Yoshida, K. Yoneyama, S. Shiraishi, A. Watanabe, and N. Takahashi, Agric. Biol. Chem. **41,** 849–854 (1977).
[3] S. Yoshida, S. Shiraishi, and N. Takahashi, Agric. Biol. Chem. **41,** 587–591 (1977).
[4] S. Yoshida, K. Yoneyama, S. Shiraishi, A. Watanabe, and N. Takahashi, Agric. Biol. Chem. **41,** 855–862 (1977).
[5] S. Tamura and N. Takahashi, in: Naturally Occurring Insecticides (M. Jacobson and D. G. Crosby, eds.), pp. 513–539, Marcel Dekker Inc., New York 1971.
[6] S. Yoshida and N. Takahashi, Heterocycles **10,** 425–467 (1978).
[7] C. Hall, M. Wu, F. L. Crane, N. Takahashi, S. Tamura, and K. Folkers, Biochem. Biophys. Res. Commun. **25,** 373–377 (1966).
[8] M. Jeng, C. Hall, F. L. Crane, N. Takahashi, S. Tamura, and K. Folkers, Biochemistry **7,** 1311–1322 (1968).
[9] S. Yoshida, Y. Nagao, A. Watanabe, and N. Takahashi, Agric. Biol. Chem. **44,** 2921–2924 (1980).
[10] M. Gutman and S. Kliatchko, FEBS Lett. **67,** 348–353 (1976).
[11] T. Mitsui, J. Fukami, K. Fukunaga, T. Sagawa, N. Takahashi, and S. Tamura, Botyu-Kagaku **34,** 126–134 (1969).
[12] T. P. Singer and M. Gutman, Adv. Enzymol. **34,** 79–153 (1971).
[13] M. Gutman, T. P. Singer, and J. E. Casida, J. Biol. Chem. **245,** 1992–1997 (1970).
[14] K. H. Chung, K. Y. Cho, Y. Asami, N. Takahashi, and S. Yoshida, Agric. Biol. Chem., submitted.
[15] N. J. Loenard, H. F. Herbrandson, and E. M. V. Heyningen, J. Am. Chem. Soc. **68,** 1279–1281 (1946).
[16] C. C. Price and W. G. Jackson, J. Am. Chem. Soc. **68,** 1282–1283 (1946).
[17] S. Fleischer, J. O. McIntyre, and J. C. Vidal, in: Methods in Enzymology (S. Fleischer and L. Packer, eds.), **Vol. 55,** pp. 32–39, Academic Press, New York, San Francisco, London 1979.
[18] F. L. Crane, J. L. Glenn, and D. E. Green, Biochim. Biophys. Acta **22,** 475–487 (1956).
[19] A. Kröger and G. Unden, in: Coenzyme Q (G. Lenaz, ed.), pp. 285–300, John Wiley & Sons Ltd., New York 1985.
[20] H. Michel, O. Epp, and J. Deisenhofer, EMBO J. **5,** 2445–2451 (1986).
[21] R. E. Blankenship and W. W. Parson, Ann. Rev. Biochem. **47,** 635–653 (1979).
[22] J. L. Huppatz and J. N. Phillips, Z. Naturforsch. **42c,** 679–683 (1987).
[23] O. Kirino and C. Takayama, J. Syn. Org. Chem. Japan **45,** 1107–1118 (1987).
[24] J. L. Huppatz and J. N. Phillips, Z. Naturforsch. **42c,** 674–678 (1987).
[25] B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson, Molecular biology of the cell, Garland Publisher Inc., New York 1983.
[26] J. Deisenhofer, O. Epp, K. Miki, R. Huber, and H. Michel, Nature **318,** 618–623 (1985).
[27] J. P. Allen, G. Feher, T. O. Yeates, H. Komiya, and D. C. Rees, Proc. Natl. Acad. Sci. U.S.A. **84,** 5730–5734 (1987).
[28] J. P. Allen, G. Feher, T. O. Yeates, H. Komiya, and D. C. Rees, Proc. Natl. Acad. Sci. U.S.A. **84,** 6162–6166 (1987).
[29] T. O. Yeates, H. Komiya, D. C. Rees, J. P. Allen, and G. Feher, Proc. Natl. Acad. Sci. U.S.A. **84,** 6438–6442 (1987).
[30] G. Hauska, in: Photosynthesis I, Encyclopedia of Plant Physiology, New series (A. Trebst and M. Avron, eds.), **Vol. 5,** pp. 253–265, Springer Verlag, Berlin, Heidelberg, New York 1977; *cf.* T. Asami, N. Takahashi and S. Yoshida, Z. Naturforsch. **41c,** 751–757 (1986).
[31] Y. Nakagawa, E. Kuwano, M. Eto, and T. Fujita, Agric. Biol. Chem. **49,** 3569–3573 (1985).
[32] K. Gerth, R. Jansen, G. Reifenstahl, G. Höfle, H. Irschik, B. Kunze, H. Reichenbach, and G. Thierbach, J. Antibiotics **36,** 1150–1156 (1983).
[33] D. J. Horgan and T. P. Singer, J. Biol. Chem. **243,** 834–843 (1968).