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Biological activity of quinoline derivatives as inhibitors of NADH-ubiquinone oxidoreductase in the respiratory chain

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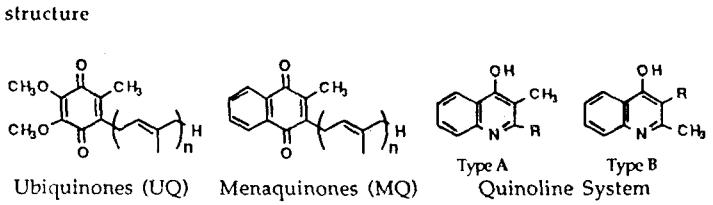
Abstract : New quinoline compounds were designed, synthesized, and examined with submitochondria. Most compounds showed high activity against NADH-ubiquinone oxidoreductase. Inhibition activity was mainly affected by the length of the lipophilic part, regardless of bulkiness or location of a phenyl group in the side chain. The β-methyl group was demonstrated to be the optimal functionality on the nuclei of the quinoline derivatives so that either deletion or insertion of a methylene on the group eliminated its activity(Received January 25, 1991, Accepted Mach 25,1991).

Respiratory electron transport(RET) inhibitors have been discovered in naturally occurring substances, but few series of synthetic compounds have been found from results of random screening research. Examples of the former are rotenone[1], piericidins[2], myxalamid[3], stigmatellin[4] and antimycin[5] and those of the latter are benzimidazoles[6], amytal[7], fenaminosulf[8] and carboxin[9]. Among these compounds, rotenone, piericidins, myxalamid, bezimidazoles and amytal have been classified as inhibitors of NADH-ubiquinone oxidoreductase in complex I of the respiratory chain(Fig. 1). Earlier study[2] has suggested that the structural resemblance between



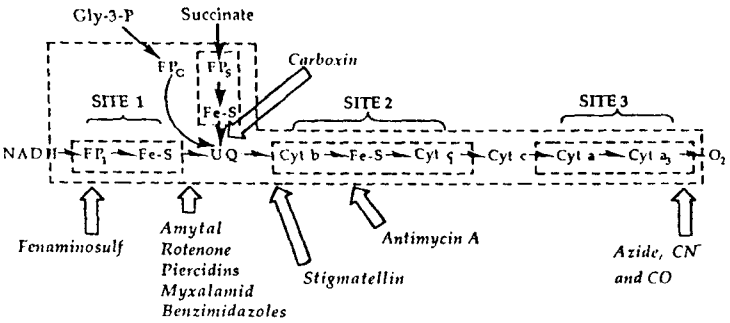


Fig. 1. Respiratory electron transport(RET) system and inhibitors

Key words : Respiratory electron transport system, rotenone, ubiquinone, menaquinone, NADH-ubiquinone oxidoreductase, submitochondria, inhibitor, quinoline derivatives

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inhibitor and ubiquinone(UQ) should correlate with the competitive behavior of the inhibitors at the site of NADH-UQ oxidoreductase, where UQ plays the role of an electron-carrying substrate[10].

Quinoline system is an attractive nucleus for a new type of inhibitors conceived from menaquinone(MQ) because MQ is widely distributed in both respiratory[11] and photosynthetic mechanisms[12,13], and perform the function of UQ. In order to elucidate further structural requisites which affect inhibition activity[14], we have synthesized more compounds and we describe, herein full details of inhibition activity of quinoline derivatives on NADH-UQ oxidoreductase in the RET system.

Materials and Methods

Chemicals

Reagents for bioassay were purchased from Sigma Co. Quinoline compounds were synthesized by a modification[15] of classical method[16] and identified by instrumental analyses. The lipophilic sidechains of these compounds were designed following the same idea as for the photosynthetic electron transport inhibitors[12,17,18].

Isolation of submitochondrial particles(SMP)

The SMP suspension was obtained from bovine heart mitochondria which was prepared by established methods [19, 20] as follows. The sucrose suspenion of bovine heart mitochondria was adjusted to pH 8.5 at 0℃ with 0.1N KOH and maintained at this pH value for 30 minutes. The suspension was then treated with a glass-Teflon homogenizer at 0℃ and was centrifuged at 19,000g for 7minutes at 0℃. The collected supernatants were centrifuged for 30 minutes at 80,000g. The final pellet was taken up in 0.25M sucrose and, after adjustment of pH 7.5 with acetic acid, was stored in liquid nitrogen. NADH-UQ oxidoreductase was characterized by using the method in literature[20,21] before use.

Inhibition bioassay method

Respiratory inhibition of compounds was measured by an oxygen electrode of Clark type(Rank Brothers) at 25 ℃ in 2ml of a medium(pH 7.4) consisting of a SMP suspension(0.2ml, 2mg protein), the phosphate buffer(1.8 ml), $MgCl_2$(10mmol), cytochrome(0.03mmol), ADP(0.5 mmol), and NADH(2mmol). The inhibitory activity of compounds is expressed as a pI_{50} value, the negative logarithm of inhibitor amount(moles/mg-protein) at 50% inhibition.

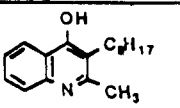
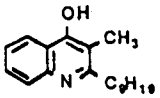
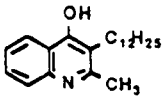
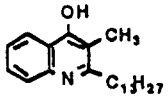
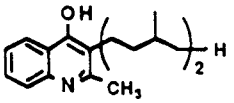
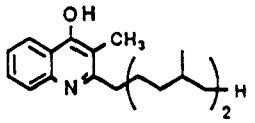
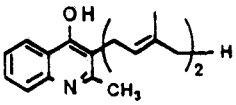
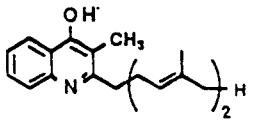
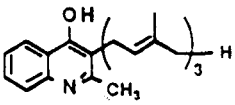
Results and Discussion

Since simple side chains are readily provided by commercial alkyl halides, it is useful to obtain information about a linear size of the lipophilic domain at the binding site. After obtaining some knowledge on an effective length of the side chain, steric factors of the lipophilic domain can be probed by varying arylalkyl side chains as a good templet to examine sterical and functional limits for the inhibitors at the receptor site of the NADH-UQ oxidoreductase, because of both its bulkiness and the π-electron clouds in the phenyl part[11,17,22,23].

As shown in Table 1, two types of 4-hydroxyquinolines were classified by the position of the lipophilic sidechains, "Types A and B". The compounds carrying a saturated linear chain(1, 2, 5, 6) were fairly active, however they were less active than rotenone with SMP. On the other hand, all the isopentanoid and isoprenoid sidechain ones(3, 4, 7. 8, 9) showed higher inhibition level than straight chain ones in the SMP. This was thought to indicate that some steric factors and double bond character are required in the lipophilic sidechain for the inhibitors to fit tightly to the binding niche. Especially, the activity of compound 4(Type A) which has one more additional methylene unit than 8 reached to the highest level(the same level as natural rotenone), therefore, it was confirmed that "Type A" should be a more preferable structure than "Type B" for the sake of this inhibition and further study was concentrated on "Type A" using various phenylalkyl series.

Interesting results were obtained by varying the distance between the quinoline and the phenyl nucleus in the sidechain(10~15), as shown in Table 2. Insertion of six and eight methylene units caused the ω-phenylalkyl derivatives(13, 14) to reach the highest level of activity.

Table 1. Effect of acyclic side chains in 4-hydroxyquinoline derivatives against NADH-ubiquinone oxidoreductase of SMP

Comp. No.	Structure Type A	pI_{50}[a]	Comp. No.	Structure Type B	pI_{50}[a]
	Rotenone[b]	10.8	5		9.4
1		9.4	6		9.6
2		9.3	7		10.2
3		10.5	8		10.1
4		11.1	9		10.2

[a]The negative logarithm of inhibitor amount(moles/mg-protein) at 50% inhibition. [b]Rotenone has been commonly used as a standard to compare the inhibitory activity of various compounds.

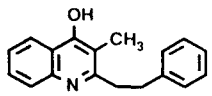
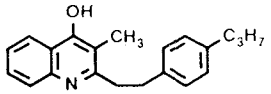
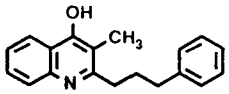
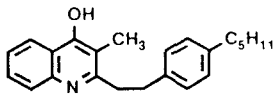
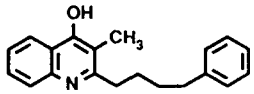
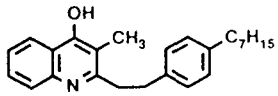
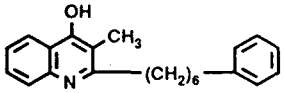
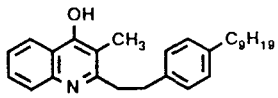
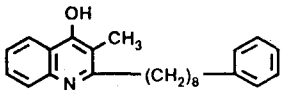
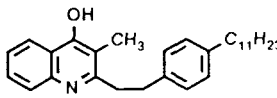
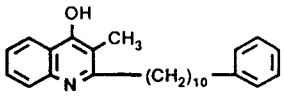
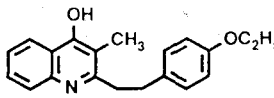
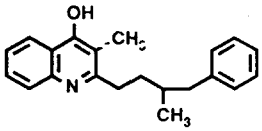
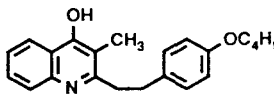
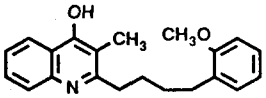
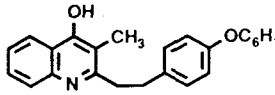
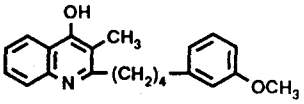
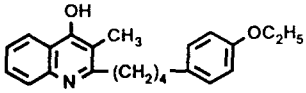
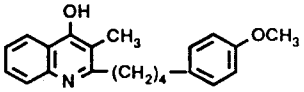
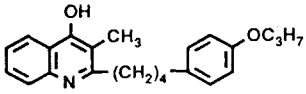
Effect of methoxyphenyl isomers(17~19) and of a branched methyl one(16) were examined. All of those showed similar potential to inhibit the electron flow of the RET system, therefore chemical parameters concerning those functionalities may be unlikely limiting factors for the binding of those 4-hydroxyquinolines in the receptor.

Consequently, 4-hydroxyquinoline derivatives with a substituted phenylalkyl(19~29) showed the characteristic patterns of activity depending on size of the side chain, which were observed in the photosynthetic inhibitors[17,18]. In the series of para-alkyl- and para-alkoxy-β-phenethyl derivatives, the same optimal length for activity of 21 and 26 is obvious as for the ω-phenylalkyl derivatives(13, 14), and this was unchanged by extending further methylene units(19). Thus the range of suitable sizes of the sidechain is around eleven carbon-bond length in synthetic inhibitors, whereas in natural inhibitors the range is around thirteen to fourteen carbon-bond length[24]. It is noteworthy that the inhibition values of those compounds(1, 2, 5, 6 in Table 1) are quite different(presumably due to the electron rich group in the side chain) despite their similar length of side chains. This result clearly indicated that the phenyl group might be accommodated well in the binding niche of the NADH-UQ oxidoreductase.

In Table 3, a bulky side chain, para-phenoxy-β-phenethyl(32) was also indicated to induce as strong inhibition as phenylalkyl compounds(13~15), so that the lipophilic domain for 4-hydroxyquinoline derivatives should have large spatial tolerance to accept the rigid structure. This idea is presumably understandable by referring the structure of D1 protein in chloroplast where a large lipophilic group can fit into the herbicide binding domain[12, 17, 18]. And hydroxyl group in the side chain(31~33) caused to reduce the activity compared to their precursors, however piericidin A which has a terminal hydroxyl group showed higher activity[10,24].

Although in the previous studies piericidin analogs lacking the methyl group on the pyridine ring system were proven to be inactive[24], the effect of an extension at that point had not been examined. So the methyl residue of the quinoline ring was modified into ethyl and propyl groups. From those two sets of compounds(13 vs 34

Teble 2. Effect of various phenylalkyl side chains in 4-hydroxyquinoline derivatives against NADH-ubiquinone oxidoreductase of SMP

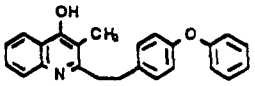
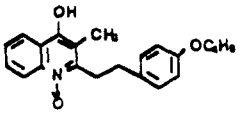
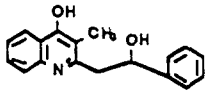
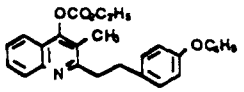
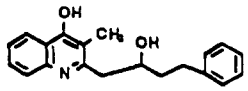
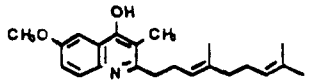
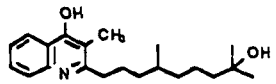
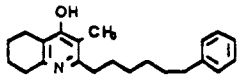
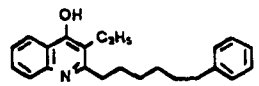
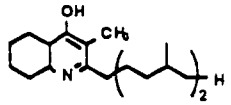
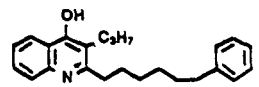
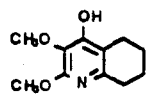
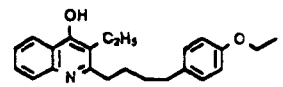
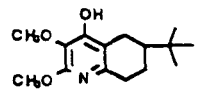
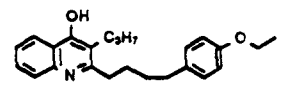
Comp. No.	Structure Type A	pI_{50}[a]	Comp. No.	Structure Type A	pI_{50}[a]
10		8.9	20		9.9
11		9.3	21		10.4
12		10.1	22		9.7
13		10.7	23		8.6
14		10.7	24		8.4
15		10.5	25		9.5
16		10.3	26		10.4
17		10.2	27		10.2
18		10.1	28		10.2
19		10.5	29		10.1

[a]Refer to Table 1.

vs 35; 19 vs 36 vs 37) it is obvious that ethyl and propyl substituents are less effective than methyl ones in inhibiting the NADH-UQ oxidoreductase. It is considered that the size of binding niche around β-methyl group is not so wide that large alkyl group in β-position interferes with strong binding to NADH-UQ oxidoreductase.

Also, the last part of Table 3 shows the activity of compounds of which nucleus is modified(38~44). Typical examples of N-oxide(38), carboethoxy(39), 6-methoxy(40), tetrahydrogenated quinolines(41, 42), showed lower inhibitory activity than their precursors, respectively. The compounds(43~44) which were synthesized as simple rotenone-type showed almost no activity, even though rotenone is a highly active polycyclic compound. This fact is similar to the effect of β-alkyl group in the A type compounds.

Table 3. Effect of miscellaneous 4-hydroxyquinoline derivatives against NADH-ubiquinone oxidoreductase of SMP

Comp. No.	Structure Type A	pI_{50}[a]	Comp. No.	Structure Type A	pI_{50}[a]
30		10.4	38		9.5
31		<7.5	39		8.5
32		8.2	40		10.0
33		10.0	41		10.1
34		10.4	42		10.1
35		9.7	43		<7.5
36		9.4	44		<7.5
37		8.8			

[a]Refer to Table 1.

Conclusion

There is in fact as yet very little information on the binding site of ubiquinone in NADH-UQ oxidoreductase, 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 X-ray crystallography studies[24,26]. In this sense, finding of a large structural variation of quinolines as NADH-UQ oxidoreductase inhibitors is valuable for further investigation on the respiratory electron transport system because these compounds are readily modified to introduce a functionality to probe the binding site; for example a radio-isotope, a photo-affinity label and/or a chiral center etc.

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NADH-ubiquinone oxidoreductase 저해제인 quinoline 유도체들의 생리활성

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초록: Menaquinone과 비슷한 구조로써 새로운 quinoline 화합물들은 design하고 합성하여 submitochondria를 이용하여 생리활성을 검정하였다. NADH-ubiquinone oxidoreductase를 저해하는 생리활성은 주로 친유성 부분인 측쇄의 길이에 의존되었다. Quinoline핵의 3위치는 methyl group일 때가 가장 높은 저해활성을 나타냈다.