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Fungicidal activity of synthetic piericidin analogs as inhibitors of NADH-ubiquinone oxidoreductase on the respiratory chain

Kun Hoe Chung, Kwang Yun Cho, Nobutaka Takahashi* and Shigeo Yoshida**

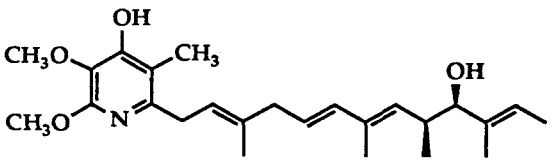
Organic Div. I, Korea Research Institute of Chemical Technology (KRICT), Daejeon, Korea, Department of Agricultural Chemistry, The University of Tokyo, Japan, **Chemical Regulation of Biomechanisms Lab., The Institute of Chemical and Physical Research (RIKEN), Japan.

Abstract : Representative synthetic piericidin-like compounds, such as hydroxypyridine and hydroxyquinoline derivatives, which showed high inhibition activity against NADH-ubiquinone oxidoreductase on the respiratory chain revealed good fungicide activity. Especially, hydroxypyridine ones showed high activity against rice blast (*Pyricularia oryzae*) and barley powdery mildew (*Erysiphe graminis*) (*Received August 13, accepted September 20, 1990*).

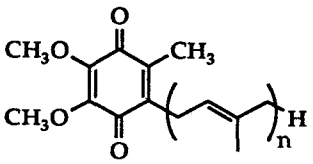
Earlier studies have suggested that the structural resemblance between piericidins (1) and ubiquinones (UQ 2) should correlate with the competitive behavior of the inhibitors at the site of NADH-UQ oxidoreductase, where UQ plays the role of an electron-carrying substrate[1, 2]. Although piericidins are extremely effective in killing many kinds of insects[3], application of these natural products has not been developed due to their chemical instability and high toxicity to mammals[4]. In the continuation of the research for structure-activity relationship between piericidin homologs and synthetic analogs, and respiratory electroon transport inhibition, we elucidted the structural requistites for the inhibition of NADH-UQ oxidoreductase using various bioassay methods[2~8].

We wish to report the result of fungicidal activity of representative synthetic compounds showing high inhibition on NADH-UQ oxidoreductase using various bioassay methods[2~8].

We wish to report the result of fungicidal activity of representative synthetic compounds showing high inhibition on NADH-UQ oxidoreductase (Table 1) and correlation between *in vitro* and *in vivo* activity.



1. Piericidin A1



2. Ubiquinones

Materials and Methods

Chemicals

All the compounds were synthesized by the modified method of previous papers and identified by instrumental analyses[9].

Submitochondrial particles(SMP) and mitochondria

Suspension of SMP were obtained from bovine heart by an established method[10] and rat liver mitochondrial suspension were prepared in the usual way[11]. NADH-UQ oxidoreductase was characterized by the literature[10, 12]

Key words : NADH-ubiquinone oxidoreductase, respiratory chain, inhibitor, piericidin, ubiquinone, hydroxypyridine, hydroxyquinoloine, respiratory electron transport, (sub)mitochondria
Corresponding auther : K.H. Chung

In vitro assays

Respiratory inhibition of compounds was measured by an oxygen electrode of Clark type at 25℃ in 2ml of a medium(pH 7.4) consisting of a mitochondrial suspension (0.2 ml), the phosphate buffer(1.8 ml), $MgCl_2$(10 μ mol), ADP(0.5 μ mol), and L-glutamic acid(10 μ mol), or of a SMP suspension(0.2 μ mol), the phosphate buffer (1.8㎖), $MgCl_2$(10 μ mol), cytochrome(0.03 μ mol), ADP(0.5 μ mol), and NADH(2 μ mol).

In vivo assays

Foliage disease assays were examined by the standard procedures[13] of the Korea Research Institute of Chemical Technology (KRICT) using plants inoculated by plant pathogenic fungi (Table 2), respectively, in the greenhouse.

Results and Discussion

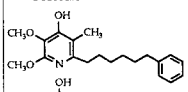
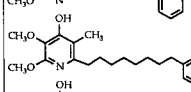
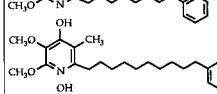
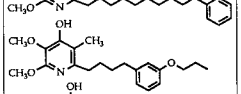
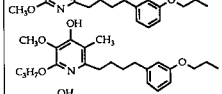
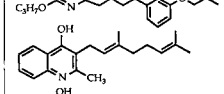
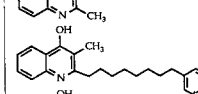
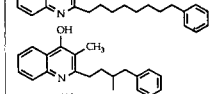
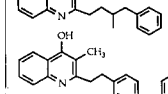
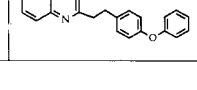
In the structure-activity study using piericidin-like compounds, some arylalkyls side chains were suggested as a factor to overcome barrier effects of membranes in the intact mitochondria. If it is allowed to extend those principles in to the case of single cell organisms, some new inhibitors of NADH-UQ oxidoreductase can be expected to show bactericidal and/or fungicidal activity. According to this idea, some highly active compounds found in the *in vitro* tests[8] (Table 1) were applied to the screening experiment for fungicide using several plant pathogenic fungi (Table 2).

As shown in Table 3, some compounds selected for the primary screening of fungicides indicated strong effect on BPM, WLR, RCB, CGM and TLB, although those did not affect on RSB. Surprisingly, obvious difference in the fungicidal spectra was recognized for those compounds in spite of their same mode of action, and this phenomenon presumably represents a sort of chemical function due to different structures of their lipophilic side chains. Hydroxypyridine and hydroxyquinoline derivatives which showed high level of inhibition in *in vitro* test using mitochondria (3~8) showed higher fungicidal activity.

The secondary screening results listed in Table 4 reveals this trend in activity more clearly, namely, hydroxypyridine compounds 3, 4 and 7 showed as much activity against RCB amd RPM as natural piericidins[3, 4].

In the case of hydroxyquinoline derivatives, the iiso prenoid side chain compound is thought to be more effective than arylalkyl side chain one because the highly ac-

Table 1. Inhibition activity of synthetic piericidin analogs on submitochondrial particles (SMP) and mitochondria (MIT)

Compound No.	Structure	pI_{50}* SMP	MIT
1	Piericidin A1 	11.4	11.0
3		10.8	10.4
4		10.4	10.0
5		10.2	9.7
6		10.6	9.6
7		10.9	10.0
8		10.1	10.0
9		10.7	9.3
10		10.3	9.0
11		10.4	9.1

*) The negative logarithm of inhibitors amount (moles/mg protein)at 50% inhibition.

Table 2. Plant pathogenic fungi tested in fungicide screening for new respiratory inhibitors

Abbreviation	Common name	Scientific name
RCB	Rice blast	*pyricularia oryzae*
RSB	Rice sheath blight	*Rhizoctionia solani*
CGM	Cucumber gray mold	*Botrytis cinerea*
TLB	Tomato late blight	*Phytophthora infestans*
WLR	Wheat leaf rust	*Puccinia recondita*
BPM	Barley powdery mildew	*Erysiphe graminis*

Table 3. Fungicidal activity of new respiratory inhibitors in the primary screening

Compound No.	Conc. (ppm)	Activity value (%)					
		RCB	RSB	CGM	TLB	WLR	BPM
3	250	100	18	0	37	100	100
4	250	99	19	0	71	88	100
5	100	75	8	0	16	100	97
6	78	89	8	23	79	86	100
7	250	99	18	28	34	88	100
8	250	40	11	93	57	100	63
9	250	45	1	28	8	0	38
10	100	0	18	0	27	4	55
11	250	85	8	71	18	0	17

tive one (9 : pI_{50}=10.7 for SMP) *in vitro* test showed lower fungicidal activity than geranyl side chain compound (8 : pI_{50}=10.1).

In addition, the fungicidal activity of hydroxypyridine compound 3 was higher than that of hydroxyquinoline one 9, however, they showed almost same level of SMP inhibition. In considering the gap in the degree of inhibition between mitochondria and SMP it may be explained by a barrier effect at the outer membrane of mitochondria. In other words, if there is a big gap in the activity level between the SMP and the mitochondrial bioassays, an *in vivo* activity can be no longer expected for this compound. However, it is clear that chemicals in the binding niche gives fatal damage to the organelle[8].

Although the activity of selected compounds does not reach that of commercial pesticide this result suggest that inhibitors of a specific bioreaction can be used as a lead for a new pesticide. The finding of fungicidal activity for synthetic inhibiors of NADH-UQ oxidoreductase is valuable for further investigation with consciousness of the biological parameters such as binding affinity to the receptor, mobility and stability in the living entity.

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Table 4. Results of the secondary fungicide screening for new respiratory inhibitors and standards

Compound No.	Fungus	Concentration(ppm)				
		250	100	50	10	1
3	RCB	100	100	97	85	9
	CGM	0	0	0	0	0
	WLR	100	67	56	16	2
	BPM	100	98	98	49	0
4	RCB	99	94	94	45	0
	CGM	19	0	0	0	0
	WLR	88	66	6	0	0
	BPM	100	100	99	83	42
7	RCB	99	94	90	25	0
	CGM	18	0	0	0	0
	WLR	88	54	0	0	0
	BPM	100	100	99	80	50
8	RCB	40	0	0	0	0
	CGM	93	—	35	0	0
	WLR	100	99	82	21	17
	BPM	63	53	3	1	13
9	RCB	45	0	0	0	0
	CGM	28	0	0	0	0
	WLR	—	45	25	0	0
	BPM	38	21	29	0	24
Beam	RCB	—	—	—	100	95
Vinclozolin	CGM	—	—	100	85	—
Flusilazol	WLR	—	—	—	96	60
Flusilazol	BPM	—	—	—	99	82

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호습쇄의 NADH-ubiquinone oxidoreductase 저해제인 합성 piericidin유사체들의 살균활성

정근회 · 조광연 · 다까하시 노부다까* · 요시다 시게오**
(한국화학연구소, *동경대학교, **이화학연구소)

초록 : 호습쇄의 NADH-ubiquinone oxidoreductase를 강력히 저해하는 합성 piericidin유사체로써 hydroxypyridine 및 hydroxyquinoline 유도체들이 전반적으로 좋은 살균활성을 보였다. 특히, hydroxypyridine 유도체들은 벼도열병(*Pyricularia oryzae*)과 보리흰가루병(*Erysiphe graminis*)에 대해서 높은 살균활성을 나타냈다.