Structure-activity Relationships Study of Pyripyropenes: Reversal of Cancer Cell Multidrug Resistance

Sir:

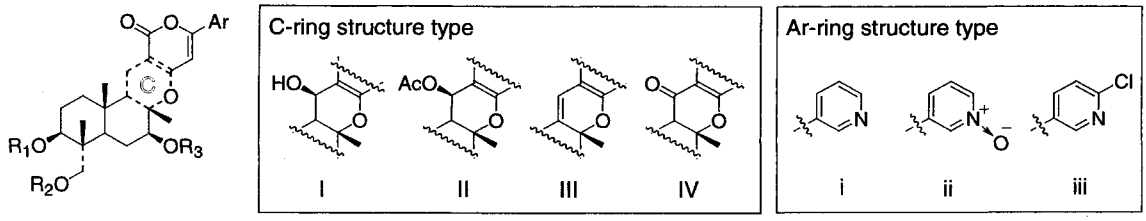
Fungal metabolite pyripyropenes[1~4] isolated from *Aspergillus fumigatus* FO-1289 were originally found to be potent acyl-CoA : cholesterol acyltransferase (ACAT) inhibitors. During studies on chemical modification and structure-activity relationships of pyripyropenes to develop potent ACAT inhibitors, more than 300 derivatives were synthesized[5~10]. Recently, WANG *et al.* reported that pyripyropene A (**1**) was obtained from a metabolite of *Eupenicillium reticulisporum* NRRL 3446 in the course of antiinsectan screening[11]. Furthermore, structurally related analogs of **1** were presented as microtuble-disrupting anticancer drugs[12]. Herein we describe the potential in reversing multidrug resistance (MDR) by pyripyropene derivatives in drug-resistant cells.

Sixteen structurally representative derivatives of pyripyropene and **1** were tested for the enhancement of sensitivity of adriamycin or vincristine in each resistant P388 cell by the method previously reported[13]. As shown in Table 1, none of the 16 derivatives or **1** showed cytotoxicity to drug-sensitive (P388/S) cells, but showed cytotoxicity to adriamycin-resistant (P388/ADR) and vincristine-resistant (P388/VCR) cells. Remarkably, compound **2** inhibited growth of P388/ADR and P388/VCR cells with IC_{50} values of 1.7 and 0.31 μM, respectively. **1** also showed the reverse effect on MDR; however, its activity was about 10 times less than that of **2**.

To determine the relationships between the reverse effect of MDR and ACAT inhibitory activity, because both **1** and **2** are potent ACAT inhibitors, cytotoxic activity of pyripyropenes in P388/ADR cells *versus* in P388/VCR cells (Fig. 1 A) and ACAT inhibitory activity (Fig. 1 B) were plotted. Direct proportion of the reverse effect of MDR was shown between P388/ADR and P388/VCR cells. On the other hand, relationships between the reverse effect of MDR in P388/ADR cells and ACAT inhibitory activity of pyripyropenes were not found.

When enhancement of the sensitivity effect to adriamycin-resistant cells was compared with derivatives

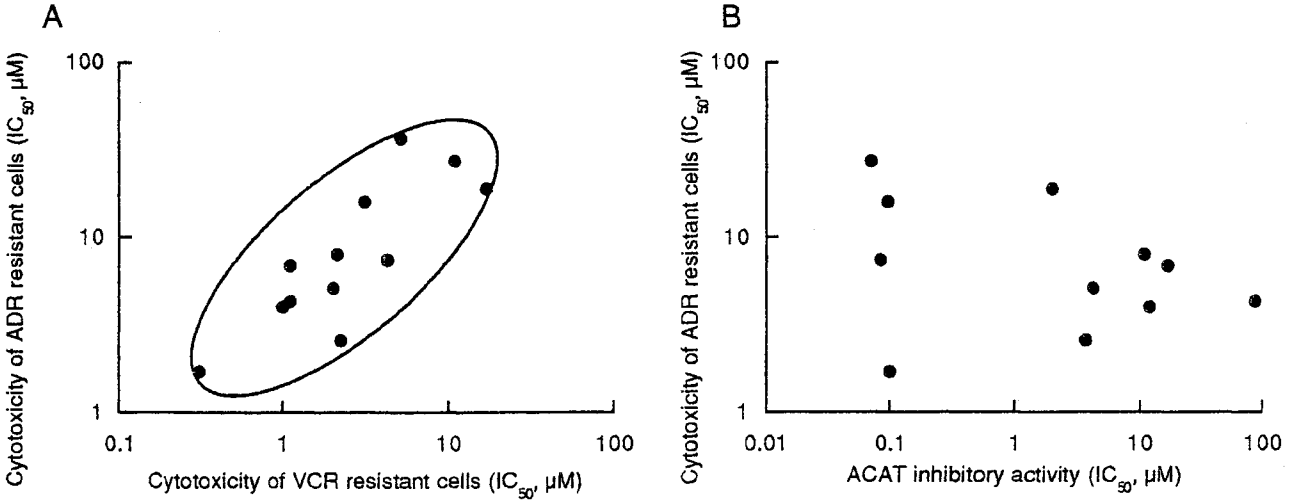
Table 1. Structures and biological activities of pyripyropene derivatives.



	Structure					Cytotoxicity (μM)			ACAT inhibitory activity (IC_{50}, μM)
	C-ring	R_1	R_2	R_3	Ar	P388/ADR[a]	P388/VCR[b]	P388/S	
1	I	Ac	Ac	Ac	i	16	3.1	>43	0.096
2	I	Ac	Ac	COPh	i	1.7	0.31	>39	0.10
3	II	Ac	Ac	Ac	i	2.6	2.2	>40	3.7
4	III	Ac	Ms[c]	Ac	i	4.0	1.0	>42	12
5	II	Ac	*n*Bu[d]	Ac	i	4.4	1.1	>38	87
6	I	Ac	*n*Bu	Ac	i	5.2	2.0	>41	4.2
7	III	-CHPh-		*n*Val[e]	i	6.9	1.1	>41	17
8	I	-CHPh-		*n*Val	i	7.5	4.3	>40	0.083
9	III	Ac	Ac	Ac	i	8.1	2.1	>44	11
10	IV	Ac	Ac	Ac	i	19	17	>43	2.0
11	I	Ste[f]	Ste	Ste	i	>20	>20	>20	>80
12	I	Ac	Ms	Ac	i	27	11	>40	0.069
13	I	-CH($CHMe_2$)-		H	i	37	5.1	>49	200
14	I	Ac	Ac	Ac	ii	>42	>42	>42	2.5
15	I	H	H	Ac	i	>50	>50	>50	100
16	IV	H	H	H	iii	>51	>51	>51	>100
17	I	H	H	H	i	>55	>55	>55	>220

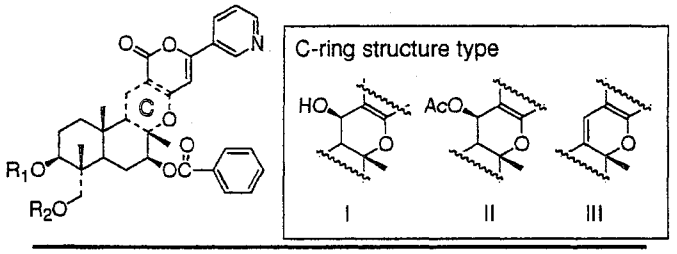
a: with added adriamycin 0.1 μg/ml, which did not affect the growth of the P388/ADR cells. b: with added vincristine 0.04 μg/ml, which did not affect the growth of the P388/VCR cells. c: Ms = SO_2CH_3; d: *n*Bu = $CO(CH_2)_2CH_3$; e: *n*Val = $CO(CH_2)_3CH_3$; f: Ste = $CO(CH_2)_{16}CH_3$

Fig. 1. Relationships between biological activities.



Cytotoxic activity of pyripyropenes in P388/ADR cells *versus* in P388/VCR cells (A) and ACAT inhibitory activity (B).

Table 2. Structures and biological activities of pyripyropene derivatives.

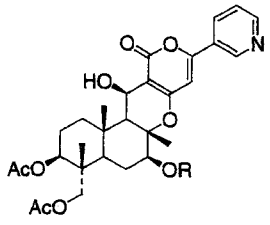


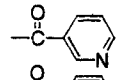
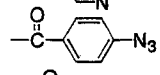
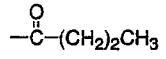
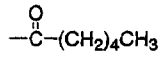
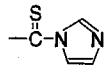
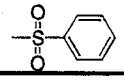
	Structure			Cytotoxicity (μM)	
	C-ring	R_1	R_2	P388/ADR	P388
18	III	Ac	Ac	1.6	>40
19	III	-CMe_2-		12	21
20	II	Ac	Ac	13	17
21	I	H	Ac	17	~41
22	I	-CHPh-		~38	38
23	I	H	H	>45	>45

with C-ring structures, Type II structures showed similar or better activity than Type I structures (**1** to **3** and **6** to **5**). Similarly, Type III structures showed equivalent or better activity than Type I structures (**12** to **4**, **8** to **7** and **1** to **9**). On the other hand, Type IV structures resulted in decreases of the activity in comparison to Type I structures (**10** to **1**). Oxidation of the pyridine moiety caused loss of activity (**14** to **1**). Since the potency of derivatives **11**, **13**, **15**, **16** and **17** was low or absent, an appropriate substitute should be used at R_1, R_2 and R_3 to show potent activity.

To determine structure-activity relationships of position R_1, R_2, and C-ring structures, several derivatives (**18**~**23**) were tested. As shown in Table 2, compound **18**, which was obtained by dehydroxylation of **2**, showed equivalent potent cytotoxicity to **2**, and compound **20**, which was obtained by acetylation of **2**, showed less potency than **2**. Among these derivatives, R_1=R_2=acetyl showed the most potent activity (**2** and **18**).

Table 3. Structures and biological activities of pyripyropene derivatives.



	R	Cytotoxicity (μM) P388/ADR	P388
24		0.6	>39
25		1.3	>36
26		1.6	>40
27		5.1	>41
28		9.4	>39
29		19	>38
30	—H	24	>46
31		37	>37

For further modification, the benzoyl moiety of **2** was substituted with several substituents (**24**~**31**). As shown in Table 3, 3-pyridyl (**24**), *p*-azido-benzoyl (**25**) and tetrahydropyranyl (**26**) substitute showed as potent cytotoxicity as **2**.

In summary, pyripyropene derivatives **2**, **18**, **24**, **25**, and **26** showed cytotoxicity to adriamycin-resistant P388 cells with IC_{50} values less than 2 μM, and did not show cytotoxicity to drug-sensitive P388 cells. A more detailed study will be published elsewhere in due course.

RIKA OBATA
TOSHIAKI SUNAZUKA
YOSHIHIRO HARIGAYA
MASAHIKO HAYASHI
MUN-CHUAL RHO
HIROSHI TOMODA
SATOSHI ŌMURA*

Research Center for Biological Function,
The Kitasato Institute and Kitasato University,
Minato-ku, Tokyo 108-8642, Japan

(Received December 9, 1999)

References

1) ŌMURA, S.; H. TOMODA, Y. K. KIM & H. NISHIDA: Pyripyropenes, highly potent inhibitors of acyl-CoA: cholesterol acyltransferase produced by *Aspergillus fumigatus*. J. Antibiotics 46: 1168~1169, 1993
2) TOMODA, H.; Y. K. KIM, H. NISHIDA, R. MASUMA & S. ŌMURA: Pyripyropenes, novel inhibitors of acyl-CoA: cholesterol acyltransferase produced by *Aspergillus fumigatus* I. Production, isolation, and biological properties. J. Antibiotics 47: 148~153, 1994
3) KIM, Y. K.; H. TOMODA, H. NISHIDA, T. SUNAZUKA, R. OBATA & S. ŌMURA: Pyripyropenes, novel inhibitors of acyl-CoA: cholesterol acyltransferase produced by *Aspergillus fumigatus* II. Structure elucidation of pyripyropenes A, B, C and D. J. Antibiotics 47: 154~162, 1994
4) TOMODA, H.; H. NISHIDA, Y. K. KIM, R. OBATA, T. SUNAZUKA, S. ŌMURA, J. BORDNER, M. GUADLLANA, P. G. DORMER & A. B. SMITH III: Relative and absolute stereochemistry of pyripyropene A, a potent, bioavailable inhibitor of acyl-CoA: cholesterol acyltransferase (ACAT). J. Am. Chem. Soc. 116: 12097~12098, 1994
5) OBATA, R.; T. SUNAZUKA, Z. LI, H. TOMODA & S. ŌMURA: Structure-activity relationships of pyripyropenes, fungal acyl-CoA : cholesterol acyltransferase inhibitors. J. Antibiotics 48: 749~750, 1995
6) OBATA, R.; T. SUNAZUKA, H. TOMODA, Y. HARIGAYA & S. ŌMURA: Chemical modification and structure-activity relationships of pyripyropenes; Potent orally bioavailable inhibitors of acyl-CoA: cholesterol *O*-acyltransferase (ACAT). Bioorg. Med. Chem. Lett. 5: 2683~2688, 1995
7) OBATA, R.; T. SUNAZUKA, Z. LI, Y. HARIGAYA, N. TOBATA, H. TOMODA & S. ŌMURA: Chemical modification and structure-activity relationships of pyripyropenes. 1. Modification at the four hydroxy group. J. Antibiotics 49: 1133~1148, 1996
8) OBATA, R.; T. SUNAZUKA, Y. KATO, H. TOMODA, Y. HARIGAYA & S. ŌMURA: Chemical modification and

structure-activity relationships of pyripyropenes. 2. 1,11-Cyclic analogs. J. Antibiotics 49: 1149~1156, 1996

9) OBATA, R.; T. SUNAZUKA, Z. TIAN, H. TOMODA, Y. HARIGAYA & S. ŌMURA: Chemical modification and structure-activity relationships of pyripyropenes. 3. Synthetic conversion of pyridine-pyrone moiety. J. Antibiotics 50: 229~236, 1997

10) OBATA, R.; T. SUNAZUKA, Z. TIAN, H. TOMODA, Y. HARIGAYA, S. ŌMURA & A. B. SMITH III: New analogs of pyripyropene family of ACAT inhibitors *via* α-pyrone fragmentation and γ-acylation/cyclization. Chem. Lett. 935~937, 1997

11) WANG, H-. J.; J. B. GLOER, D. T. WICKLOW & P. F. DOWD: Aflavinines and other antiinsectan metabolites from the ascostromata of *Eupenicillium crustaceum* and related spacies. Appl. Environ. Microbiol. 61: 4429~4435, 1995

12) NEWELL, S. W.; E. M. PERCHLLET, J. B. LADESICH, J. A. FREEMAN, Y. CHEN, L. LIU, D. H. HUA, S. L. KRAFT, R. J. BASARABA & J-. P. PERCHLLET: Tricyclic pyrone analogs: A new class of microtuble-disrupting anticancer drugs effective against murine leukemia cells *in vitro*. Int. J. Oncol. 12: 433~442, 1998

13) MUN-CHUAL, R.; M. TOYOSHIMA, M. HAYASHI, R. UCHIDA, K. SHIOMI, K. KOMIYAMA & S. ŌMURA: Enhancement of drug accumulation by andrastin A produced by *Penicillium* sp. FO-3929 in vincristine-resistant KB cells. J. Antibiotics 51: 68~72, 1998