ONE-STEP SYNTHESIS AND ENZYME INHIBITING ACTIVITIES OF PYRIZINOSTATIN ANALOGS

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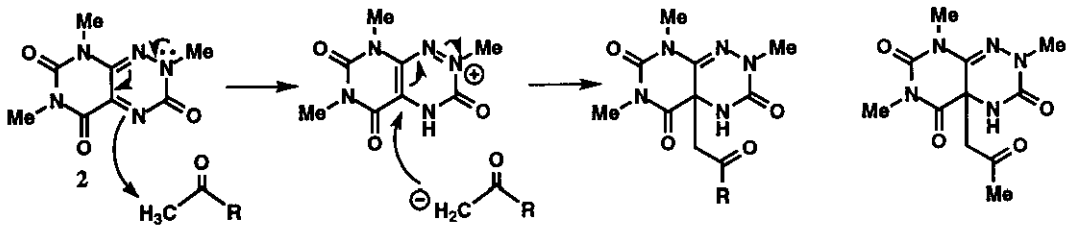
Abstract - Pyrizinostatin analogs were synthesized from 2-methylfervenulone and a variety of methyl ketones in only one step and showed stronger enzyme inhibiting activities than pyrizinostatin itself.

Pyrizinostatin [(-)-**1**] isolated from fermentation broth of *Streptomyces* sp. is a strong inhibitor against pyroglutamyl peptidase.[1,2] Recently, racemic pyrizinostatin (**1**) has been synthesized in our laboratories from an antibiotic, 2-methylfervenulone (**2**), in only one step and showed similar enzyme inhibiting activity with the natural product.[3]

Herein, we report one-step synthesis of a variety of racemic pyrizinostatin analogs, most of which displayed stronger enzyme inhibiting activitities than pyrizinostatin (**1**) itself.

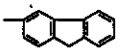
Pyrizinostatin analogs (**3** - **11**) were synthesized from 2-methylfervenulone[4,5](**2**: 2MF) and methyl ketones ($RCOCH_3$) with or without solvent as shown in Table 1. The reaction mechanism is rationalized to be due to the nucleophilic attack of the resulting anion ($RCOCH_2^-$) to **2** as shown below.



3 - 11 **Pyrizinostatin (1)**

A typical synthetic procedure is the following.
The starting fluorescent 2-methylfervenulone (**2**) was isolated from the fermentation broth of the microbial strain[4] and also readily prepared on large scale in 4 steps from 1,3-dimethyl-4-chlorouracil.[5] 2-Methylfervenulone (**2**: 96.2 mg) was dissolved in acetophenone (4 ml) and the solution was stirred at 70°C for 2 days. The reaction mixture was directly chromatographed on silica gel column (10 g) with EtOAc-hexane (1:2→2:1). The fractions having Rf-value 0.29 on tlc (EtOAc-hexane 2:1) were combined and evaporated to dryness *in vacuo*. Recrystallization from MeOH gave crystals of **5** (133 mg) in 90% yield. mp 177°C; EI-ms m/z 343 (M^+); ^1H nmr (90 MHz, $CDCl_3$) 3.20 (6H, s), 3.26 (3H, s), 3.36 (1H, d, J=15.0 Hz), 3.80 (1H, d, J=15.0 Hz), 5.83(1H, br s), 7.37-7.94 (5H, m).

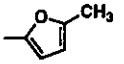
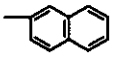
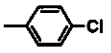
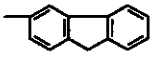
Table 1. Synthesis and physico-chemical properties of pyrizinostatin analogs

Compds	R	Solvent	Yield(%)	mp(°C)	Recrystallization solvent	FAB-ms(m/z)	Tlc(Rf value*)
3		CH_2ClCH_2Cl	67	158~159.5	EtOAc-hexane	310$(M+H)^+$	0.42
4		CH_2ClCH_2Cl	37	151.5~152.5	EtOAc-hexane	308$(M+H)^+$	0.24
5		——	90	177	MeOH	343(M^+)***	0.29
6		CH_2ClCH_2Cl	85	176.5~177.5	MeOH	377(M^+)***	0.34
7		——	61	184.5~186	MeOH	345$(M+H)^+$	0.21
8		CH_2ClCH_2Cl	87	180~181.5	EtOAc-hexane	350$(M+H)^+$	0.26
9		——	79	194~194.5	EtOAc-hexane	348$(M+H)^+$	0.11
10		CH_2Cl_2	86	221~222**	MeOH	395$(M+H)^+$	0.47
11		CH_2ClCH_2Cl	58	245~246	MeOH	432$(M+H)^+$	0.47

* On KGF_{254} 60 (Merck) with EtOAc-hexane (2:1), ** Decomposition, *** Measured by EI-ms.

Remarkably, all of the new pyrizinostatin analogs (**3** - **11**) showed enzyme inhibiting activities against pyroglutamyl peptidase as shown in Table 2.[1,2] The results indicated that the analogs having aromatic rings showed stronger activities than aliphatic analogs and a pyridine analog (**7**) was the strongest one. The further biological assay for medicinal use of these analogs will be reported in due course.

Table 2. Enzyme inhibiting activity against pyroglutamyl peptidase
(IC_{50}:μg/ml)

Compds	R	IC_{50}	Compds	R	IC_{50}
1	$-CH_3$ (Pyrizinostatin)	0.8	7		0.02
3		3.0	8		0.4
4		4.5	9		0.4
5		0.25	10		0.4
6		0.2	11		0.4

ACKNOWLEDGMENT

We are grateful to the Institute of Microbial Chemistry, Nippon Kayaku Co. Ltd., Yamanouchi Pharmaceutical Co. Ltd. and Shikoku Chemicals Co. for their generous support of our program.

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Received, 28th February, 1994