

ONE-STEP SYNTHESIS AND ENZYME INHIBITING ACTIVITIES OF PYRIZINOSTATIN ANALOGS

Kuniaki Tatsuta* and Masayuki Kitagawa

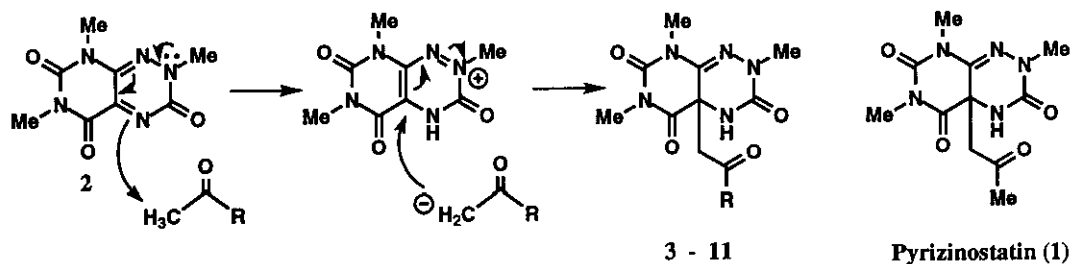
Graduate School of Science and Engineering, Waseda University,
3-4-1 Ohkubo, Shinjuku-ku, Tokyo 169, Japan

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 activities than pyrizinostatin (1) itself.

Pyrizinostatin analogs (3 - 11) were synthesized from 2-methylfervenulone^{4,5} (2: 2MF) and methyl ketones (RCOCH₃) 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 (RCOCH₂⁻) to 2 as shown below.



A typical synthetic procedure is the following.

The starting fluorescent 2-methylferavenulone (2) was isolated from the fermentation broth of the microbial strain⁴ and also readily prepared on large scale in 4 steps from 1,3-dimethyl-4-chlorouracil.⁵ 2-Methylferavenulone (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 R_f-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, CDCl₃) 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).

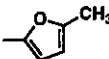
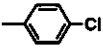
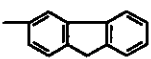
Table 1. Synthesis and physico-chemical properties of pyrizinostatin analogs

Compds	R	Solvent	Yield(%)	mp(°C)	Recrystallization solvent	FAB-ms(m/z)	Tlc(R _f value*)
3		CH ₂ ClCH ₂ Cl	67	158-159.5	EtOAc-hexane	310(M+H) ⁺	0.42
4		CH ₂ ClCH ₂ Cl	37	151.5-152.5	EtOAc-hexane	308(M+H) ⁺	0.24
5		—	90	177	MeOH	343(M ⁺)***	0.29
6		CH ₂ ClCH ₂ Cl	85	176.5-177.5	MeOH	377(M ⁺)***	0.34
7		—	61	184.5-186	MeOH	345(M+H) ⁺	0.21
8		CH ₂ ClCH ₂ Cl	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 ₂ Cl ₂	86	221-222**	MeOH	395(M+H) ⁺	0.47
11		CH ₂ ClCH ₂ Cl	58	245-246	MeOH	432(M+H) ⁺	0.47

* On KGF254 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
(IC₅₀:μg/ml)

Compds	R	IC ₅₀	Compds	R	IC ₅₀
1	 (Pyrizinostatine)	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.

REFERENCES

- 1 T. Aoyagi, M. Hatsu, C. Imada, H. Naganawa, Y. Okami, and T. Takeuchi, *J. Antibiot.*, 1992, **45**, 1795.
- 2 M. Hatsu, H. Naganawa, T. Aoyagi, and T. Takeuchi, *J. Antibiot.*, 1992, **45**, 1961.
- 3 K. Tatsuta and M. Kitagawa, *J. Antibiot.*, 1994, **47**, 925.
- 4 T. W. Miller, L. Chalet, B. Arison, R. W. Walker, N. R. Trenner, and F. J. Wolf, *Antimicrob. Agents Chemother.*, 1963, 58.
- 5 E. C. Taylor and F. Sowinski, *J. Org. Chem.*, 1975, **40**, 2321.

Received, 28th February, 1994