COMMUNICATIONS TO THE EDITOR

**SNA-60-367, New Peptide Enzyme
Inhibitors against Aromatase**

Sir:

In the course of our screening program for new aromatase inhibitors, we have isolated new plipastatin^{1~3)} group peptide enzyme inhibitors from the broth of soil bacterium SNA-60-367. This report is concerned with the isolation, structures and biological properties of these novel enzyme inhibitors.

The producing microorganism was cultivated at 27°C for 120 hours (final pH 8.4) in a 500 ml volume Erlenmeyer flask containing 70 ml of the medium composed of glucose 2%, soluble starch 1%, meat extract 0.1%, dried yeast 0.4%, soybean flour 2.5%, NaCl 0.2%, and K₂HPO₄ 0.005%, adjusted to pH 6.7. The active material was extracted into butanol from both the filtered broth (4.7 liter) and the aqueous acetone extract of the mycelium. The butanol layer was evaporated and the extract was suspended in water. It was extracted with ethyl acetate and the water layer was subjected to a MCI GEL CHP 20P (Mitsubishi Chemical Industries, 1.4 × 20 cm). After washing with water and 50% MeOH for removing impurities, the active material was eluted with MeOH. The eluate was subjected to silica gel column (Merck, 70~230 mesh, 3.6 × 50 cm) and washed with CHCl₃:MeOH=5:1 and 1:1. The crude SNA-60-367 compounds were eluted with MeOH and lyophilized (1.7 g). This powder was dissolved in isobutylic alcohol: TFA:H₂O=120:1:160 and subjected to centrifugal partition chromatography (CPC). After active fractions were collected and adjusted to pH 7, it was subjected to a LH-20 column (Pharmacia, 3 × 120 cm) developed with MeOH to give active fractions. Finally, pure SNA-60-367 compounds were isolated by 2 times preparative HPLC (CAPCELL-Pak C₁₈ (20φ × 250 mm), CH₃CN:0.1% TFA=50:50, 11 ml/minute). Over 23 peaks were detected in the preparative HPLC profile. Finally, 17 compounds were obtained in pure form as white amorphous powder and used for their structure determination.

The UV, IR, ¹H and ¹³C NMR spectra of these compounds suggested that they were peptide enzyme inhibitors. A typical UV spectrum of SNA-60-367-13 in MeOH and ¹³C NMR spectrum in DMSO-*d*₆ are shown in Figs. 1 and 2. Thus, all compounds were hydrolyzed

by 6N HCl at 110°C for 24 hours and Thr(1), Glu(3), Tyr(2), Pro(1) and Orn(1) were detected as common components of all of the SNA-60-367 compounds. In the 3 molecules of Glu, 1 molecule was identified Gln by using FAB-MS and linked scan spectra. Some of the amino acids, Ile, Val, Ala and amino butyric acid (Aba), were observed as variable components in each compound as shown in Fig. 3. HRFAB-MS and the elemental composition data of the 17 compounds are shown in Table 1. These results suggested that SNA-60-367 compounds were plipastatin group enzyme inhibitors which were reported as phospholipase A₂ inhibitors. The structures of the peptide part were determined by using FAB-MS and linked scan spectra. Structures of fatty acid moieties were determined by using EI-MS of the fatty acid methyl esters and negative FAB/linked scan spectra of the intact compounds with the analyses of the charge remote fragmentations. From these data, structures of SNA-60-367 compounds are listed in Fig. 3. SNA-60-367-3, 6, 7 and 12 were identical with plipastatins A₁, A₂, B₁ and B₂, respectively. Retention time of SNA-60-367-3 and 6 were in accord with authentic samples on HPLC. The rest of compounds were new substances different in their the amino acids composition and fatty acid structure compared to the known plipastatins. Detailed mass spectrometric analyses of the structures will be reported elsewhere.

Inhibition activity against human placental aromatase was measured by the same method as described previously⁴⁾. Inhibition % of the compounds at 100 μg/ml were shown to be between 30% to 80% as listed

Fig. 1. UV spectrum of SNA-60-367-13 (in MeOH).

¹³C NMR spectrum of compound 1. The x-axis represents chemical shift in ppm, ranging from 0 to 180. The spectrum shows several peaks: a small peak at ~175 ppm, a cluster of peaks between 120-140 ppm, a small peak at ~95 ppm, a very large solvent peak at ~40 ppm labeled 'CCl₄', and a complex set of peaks between 20-40 ppm.

Table 1. HR-FABMS data, elemental composition and aromatase inhibition activity of SNA-60-367 compounds.

No.	X ₁	X ₂	R ₁	R ₂
2	Ala	Ile		OH
3	Ala	Ile		OH
4	Aba	Ile		OH
5	Val	Ile		OH
6	Ala	Ile		OH
8	Val	Val		OH
9	Val	Val		OH
10	Aba	Ile		OH
11	Aba	Ile		OH
12	Val	Ile		OH
13	Val	Ile		OH
14	Ala	Ile		H
17	Val	Ile		H
18	Val	Ile		H
19	Ala	Ile		H
21	Aba	Ile		H
23	Val	Ile		H

No.	HR-FABMS (MH ⁺)	Composition				Inhibition (%, 100 µg/ml)
		C	H	O	N	
2	1463.8064	72	111	20	12	60
3	1463.8024	72	111	20	12	—
4	1477.8220	73	113	20	12	65
5	1491.8357	74	115	20	12	63
6	1477.8198	73	113	20	12	74
8	1491.8323	74	115	20	12	61
9	1491.8353	74	115	20	12	55
10	1491.8348	74	115	20	12	68
11	1491.8357	74	115	20	12	72
12	1505.8461	75	117	20	12	60
13	1505.8524	75	117	20	12	50
14	1447.8087	72	111	19	12	31
17	1475.8404	74	115	19	12	48
18	1475.8396	74	115	19	12	49
19	1461.8280	73	113	19	12	49
21	1475.8411	74	115	19	12	36
23	1489.8595	75	117	19	12	32

activity at approximately 50 times greater potency (1 to $3\text{ }\mu\text{M}$)¹¹ than the aromatase activity reported here. But the structural similarity of aromatase substrate (androstenedione) and these compounds was not recognized. This is the first report to show that the peptide enzyme inhibitors, plipastatins, inhibit human aromatase activity. We are now interested in the inhibition mechanism of these compounds against aromatase. Antimicrobial

activities against *Pseudomonas aeruginosa* N-10 (L-form), *Pyricularia oryzae* IFO 5994 and *Botrytis cinerea* IFO 5365 were shown to be between 10 to 13 mm inhibition zone (30 µg/disc).

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