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 $K_2HPO_4$ 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 $CHCl_3$ : MeOH = 5 : 1 and 1 : 1. The crude SNA-60-367 compounds were eluted with MeOH and lyophilyzed (1.7 g). This powder was dissolved in isobutylic alcohol : TFA : $H_2O$ = 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 $C_{18}$ (20$\phi$ × 250 mm), $CH_3CN$ : 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 $^{13}C$ NMR spectra of these compounds suggested that they were peptide enzyme inhibitors. A typical UV spectrum of SNA-60-367-13 in MeOH and $^{13}C$ NMR spectrum in DMSO-$d_6$ are shown in Figs. 1 and 2. Thus, all compounds were hydrolyzed by 6 N 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 $A_2$ 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 $A_1$, $A_2$, $B_1$ and $B_2$, 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 previously[4]. Inhibition % of the compounds at 100 $\mu$g/ml were shown to be between 30% to 80% as listed

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

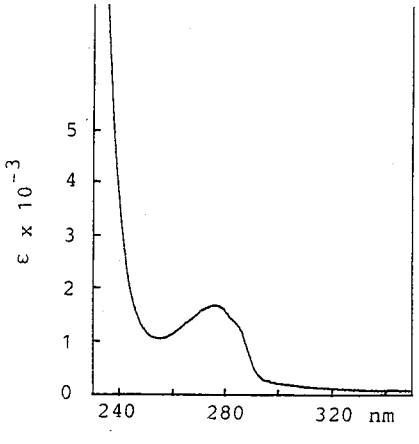


Fig. 2. $^{13}C$ NMR spectrum of SNA-60-367-13 (DMSO-$d_6$, 125 MHz).

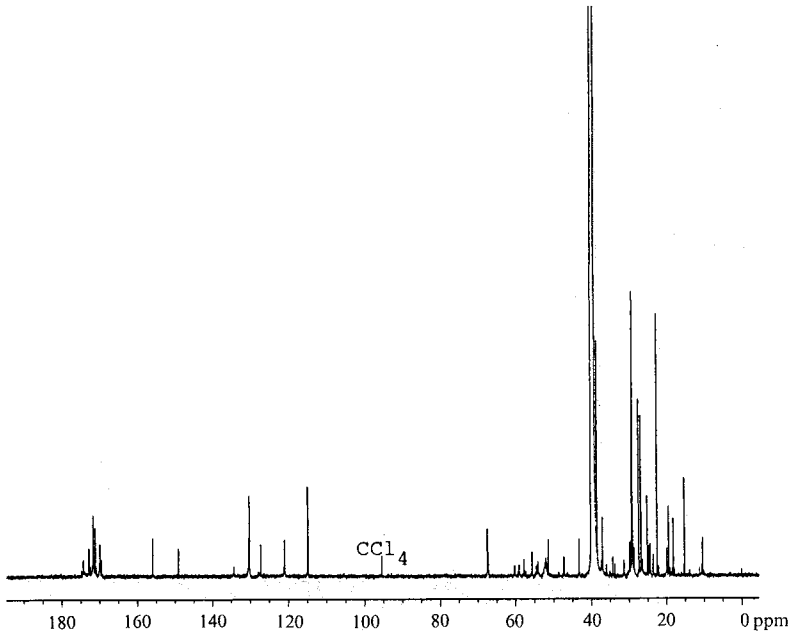
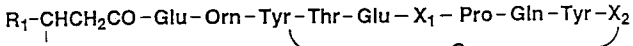



Fig. 3. Structures of SNA-60-367 compounds.




| 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 |

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

| No. | HR-FABMS ($MH^+$) | Composition | | | | Inhibition (%, 100 $\mu$g/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 |

—: Not measured.

in Table 1. The $IC_{50}$ values of SNA-60-367-2, 10 and 13 were 63, 42 and 66 $\mu$M, respectively. The original report on these compounds shows phospholipase inhibitory activity at approximately 50 times greater potency (1 to 3 $\mu$M)[1] 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 $\mu$g/disc).

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