SNA-4606-1, a New Member of Elaiophylins with Enzyme Inhibition Activity Against Testosterone 5 α-Reductase

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In the course of our screening program for testosterone 5α-reductase inhibitors, we have isolated a new member of elaiophylins[1~3] enzyme inhibitor from cultured broth of *Streptomyces* sp. SNA-4606 (FERM P-12912). This report is concerned with the isolation, structure and biological properties of a novel enzyme inhibitor.

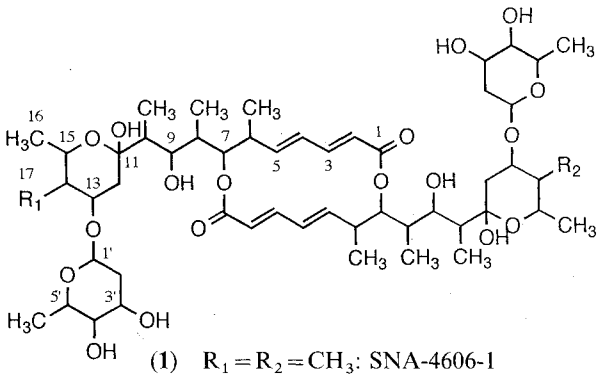
The microorganism was isolated from a soil sample collected in Towada-shi, Aomori Prefecture, Japan. The taxonomic studies indicated that it belonged to the genus *Streptomyces*. A loop of the producing strain SNA-4606 from a slant culture was inoculated into a 500-ml volume Erlenmeyer flask containing 70 ml of 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%, (pH 6.7), and incubated for 5 days at 27°C on rotary shaker (200 rpm). The seed culture (280 ml) were transferred into a 30 liter jar fermentor containing 18 liter of the medium and cultivated at 27°C for 3 days (final pH 7.5). The cultured broth was centrifuged and the mycellium (1.4 kg) was extracted with acetone. After removal of acetone, the active material was extracted with ethyl acetate. The ethyl acetate layer was dehydrated with anhydrous Na_2SO_4 and evaporated (2.7 g). It was subjected to silica gel column (Merck, 70~230 mesh, 3×50 cm) and washed with $CHCl_3$: MeOH=98:2. The crude SNA-4606 compounds were eluted with $CHCl_3$: MeOH=96:4. The active fractions were evaporated (1.2 g), dissolved in MeOH and purified by preparative HPLC (CAPCELL-Pak C_{18} (30×250 mm), CH_3OH: H_2O=80:20, 27 ml/minute). Finally, active compounds of SNA-4606-1 (71 mg), -2 (156 mg) and -3 (321 mg) were obtained in pure form as white amorphous powder.

The UV, IR, ^1H and ^{13}C NMR spectra of these compounds were similar with each other and suggested that they were elaiophylin group compounds. The molecular weights, molecular formulae and ^{13}C NMR data of SNA-4606-2 and -3 were identical with those of efomycin G (**2**)[4] and elaiophylin (**3**)[1~3]. But SNA-4606-1 (**1**) was distinct from all the known elaiophylin group antibiotics. The molecular related ion (MNa^+) in the SI-MS spectrum of SNA-4606-1 was 14 mass and 28 mass unit smaller than those of efomycin G and elaiophylin, respectively. It was suggested that the molecule of SNA-4606-1 lacks one methylene and two methylene groups in contrast with those of efomycin G and elaiophylin. Physico-chemical properties of three compounds were shown in Table 1.

The ^{13}C NMR spectrum of SNA-4606-1 (**1**) showed only half of the expected carbon signals (Table 2), suggesting that it had a symmetric molecular structure such as in elaiophylin (**3**). The ^1H and ^{13}C NMR spectra of (**1**) were similar to those of (**3**) except the absence of signals assigned to an ethyl group. Instead of that, an another methyl group was observed in the spectrum: δ_H 0.91 (H-17, d, J=6.4 Hz), 13.7 (C-17). The analyses of the H-H and C-H COSY spectra led to the structure (**1**) for SNA-4606-1. The assignment of the ^1H and ^{13}C signals exhibited the close similarity of (**1**) and (**3**), though there were small differences in the chemical shifts due to the signals at C-13, C-14 and C-15[4,5].

The inhibition activity of those compounds against rat prostate testosterone 5 α-reductase was investigated by the same method of LIANG *et al.*[6]. The IC_{50} values were shown in Table 3. They showed almost the same

Fig. 1. Structures of SNA-4606 compounds.



(**1**) $R_1=R_2=CH_3$: SNA-4606-1
(**2**) $R_1=CH_3$, $R_2=CH_2CH_3$: Efomycin G
(**3**) $R_1=R_2=CH_2CH_3$: Elaiophylin

Table 1. Physico-chemical properties of SNA-4606 compounds.

	SNA-4606-1	SNA-4606-2[a]	SNA-4606-3[b]
Appearance	White amorphous powder	White amorphous powder	White amorphous powder
MP	171.5~172.0°C	183.5~184.0°C	189.5~190.0°C
$[\alpha]_D^{25}$	−44.8° (*c* 0.5, MeOH)	−43.2° (*c* 1.0, MeOH)	−42.4° (*c* 1.0, MeOH)
SI-MS	1019 $(M+Na)^+$	1033 $(M+Na)^+$	1047 $(M+Na)^+$
MW	996	1010	1024
Molecular formula	$C_{52}H_{84}O_{18}$	$C_{53}H_{86}O_{18}$	$C_{54}H_{88}O_{18}$
UV λ_{max}^{MeOH} nm (log ε)	253 (4.59)	253 (4.54)	252 (4.56)
IR ν_{max} (KBr) cm^{-1}	3425, 2975, 1680, 1640, 1225, 1005, 985	3425, 2975, 1680, 1640, 1225, 1005, 985	3425, 2975, 1680, 1640, 1225, 1005, 985

[a] Efomycin G, [b] Elaiophylin.

Table 2. ^1H and ^{13}C NMR spectral data for SNA-4606-1 in $CDCl_3$ and in $CDCl_3$/CD_3OD.

Position	δ_H[a]	δ_C[b]
1	—	170.3
2	5.69 (2H, d, J=15.0)	121.7
3	6.98 (2H, dd, J=15.0, 11.1)	146.0
4	6.13 (2H, dd, J=15.0, 11.1)	132.2
5	5.63 (2H, dd, J=15.0, 9.5)	146.0
6	2.53 (2H, m)	41.8
7	4.74 (2H, dd, J=10.2, 1.9)	77.9
8	1.70 (2H, m)	43.0
9	4.11 (2H, dd, J=4.0, 1.9)	71.2
	4.06 (2H, d, J=4.0, OH)	
10	1.96 (2H, m)	36.8
11	—	100.1
12	2.37 (2H, ddd, J=11.8, 4.5, 1.0)	38.6
13	3.65 (2H, m)	73.1
14	1.20 (2H, m)	44.0
15	3.71 (2H, m)	70.3
16	1.10 (6H, d, J=6.2)	19.5
17	0.91 (6H, d, J=6.4)	13.7
18	1.01 (6H, d, J=6.9)	15.4
19	1.00 (6H, d, J=7.1)	7.0
20	0.81 (6H, d, J=6.9)	9.2
1′	5.05 (2H, d, J=1.9)	93.8
2′	1.80 (4H, m)	33.1
3′	4.00 (2H, m)	66.3
	2.03 (2H, d, J=8.5, OH)	
4′	3.59 (2H, dt, J=7.0, 7.0, 2.8)	71.5
	1.88 (2H, d, J=7.0, OH)	
5′	4.00 (2H, m)	67.0
6′	1.24 (2H, d, J=6.4)	16.8

[a] 500 MHz, $CDCl_3$ as solvent, [b] 125 MHz, $CDCl_3$/CD_3OD as solvent.

Table 3. Inhibition activity of SNA-4606 compounds against testosterone 5α-reductase derived from rat prostate.

	SNA-4606-1	SNA-4606-2[a]	SNA-4606-3[b]
IC_{50} (μM)	6.6	8.7	5.8

[a] Efomycin G, [b] Elaiophylin.

inhibition activity. Structurely, they are different from testosterone which is the substrate of the enzyme. The phenazine compounds (WS-9659 A and B)[7] and riboflavin[8] have been reported to be inhibitors of testosterone 5α-reductase, but this is the first report that the elaiophylin group compounds showed the inhibitory activities. Much interests will be taken in the inhibition mechanism by such macrodiolide antibiotics.

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