Structure of Sch 218157, a Cyclodepsipeptide with Neurokinin Activity

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(Received for publication March 10, 2000)

Neurokinins (NKs), also known as tachykinins (TKs), are members of a family of 9~11 amino acid peptides which are involved in various pathological conditions including inflammation[1,2], pain transmission, cancer, anxiety, asthma, and vasodilation[3]. The peptides act mainly through specific 7-transmemberane G-protein-coupled receptor domains named NK_1, NK_2, and NK_3. In the course of our screening program for novel neurokinin receptor inhibitors, we have isolated a cyclodepsipeptide (**1**) from an unidentified fungal fermentation culture broth (MYCO-2838)[4] with selective NK_2 antagonist activity.

The fermentation broth was extracted with EtOAc at harvest (pH ~6.5). The crude extract was purified by high speed centrifugal partition chromatography (CPC) with solvent system of hexane : EtOAc : MeOH : H_2O (3 : 5 : 3 : 5, v/v/v/v). The combined bioactive fractions were further purified by normal phase HPLC (semi-preparative YMC PVA-Sil column, 20×250 mm, S-5, 5~25% MeOH in CH_2Cl_2 with a linear gradient in 30 minutes, UV=218 nm, 12 ml/minute). Pure compound **1** was obtained as a white/pale yellow solid.

The molecular formula was established as $C_{57}H_{89}N_{11}O_{13}$ by HRFABMS [$(M+H)^+$ at *m/z* 1136.6720, calcd.; 1136.6740, measured] and ^1H and ^{13}C NMR data (Table 1) were indicative of a peptide with a blocked *N*-terminus (ninhydrin-negative).

Amino acid analysis indicated the presence of one mole each of glycine, valine, D-phenylalanine, proline, and D,L-isoleucine. The CIMS of the acid hydrolysis products supported the presence of the above amino acids and also indicated the presence of pipecolic acid (*m/z* 130) and *N*-methylglutamine (a strong *m/z* 144 $(M+1)^+$; *N*-methylglutamic acid anhydride).

Extensive analysis of the COSY, HMBC, HMQC, HMQC-TOCSY NMR data revealed the spin systems of the amino acids; Phe, Pro, Gly, MeVal, two MeGln, Ile, Pip (pipecolic acid), and Val. In addition an acid containing component was assigned as 2-hydroxy-3-methylpentanoic acid (HMP).

The proton NMR data in $CDCl_3$ and DMSO-d_6 (25° and 50°C) indicated the presence of an extra pair of amide protons at δ 7.30 and 6.80 when compared with the data of Sch 217048[5]. Further analysis indicated an upfield shift of ~0.2 ppm for the γCH_2 of MeGlu. Similarly minor changes were observed for the carbon-13 chemical shifts around the MeGlu moiety. The NMR data suggested that the MeGlu moiety in Sch 217048 is transformed to MeGln (MeGlu→ MeGln). Thus compound **1** contains two units of MeGln as shown in Figure 1. Attempts to hydrolyze the ester bond under basic condition failed. The rationale behind this failure is the lack of δCOO^- group in MeGln that was

Fig. 1. Sch 218157

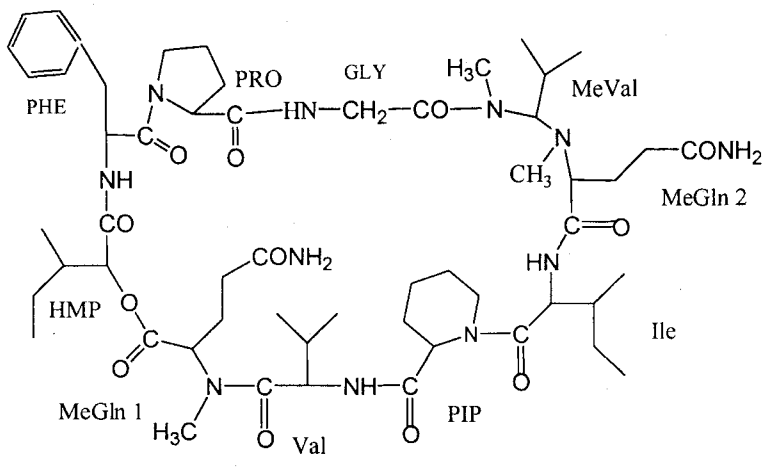

Table 1. ^{1}H and ^{13}C NMR data for Sch 218157 in DMSO-d_6[a].

AA		^{13}C [b]	^{1}H (mult, J in Hz) [b]	AA		^{13}C [b]	^{1}H (mult, J in Hz)[b]
MeGln 1	CO	169.5		MeVal	CO	169.7	
	α	62.0	4.13 (dd, 9.0, 4.0)		α	57.0	5.14 (d, 10.0)
	β	23.9	2.25 m		β	27.3	2.29 m
	γ	30.7	2.15 m		γ-Me	17.9	0.75 (d, 6.5)
	δ-$\underline{CO}NH_2$	173.4			γ-Me	19.1	0.83 (d, 6.5)
	δ-CO$\underline{NH_2}$		7.30 bs, 6.80 bs		NMe	28.1	2.90 s
	NMe	38.5	3.22 s				
Val	CO	172.2		Gly	CO	170.4	
	α	54.2	4.57 (t, 8.0, 9.0)		α	41.1	4.40 (dd, 17.0, 8.0)
	β	31.0	1.95 m				4.24 (d, 17.0)
	γ-Me	17.7	0.80 (d, 6.5)				
	γ-Me	19.2	0.82 (d, 6.5)	Pro	CO	170.6	
	NH		8.65 (d, 8.0)		α	59.4	4.55 (dd, 8.0, 5.0)
					β	29.1	2.12 m, 1.74 m
Pip	CO	170.2			γ	24.7	2.05 m, 1.92 m
	α	52.4	5.12 (dd, 4.0, 2.5)		δ	47.0	3.70 m
	β	27.0	1.75 m				
	γ	19.4	1.41 m, 1.15 m	Phe	CO	169.7	
	δ	24.4	1.72 m		α	52.2	4.69 (dt, 8.0, 8.0, 4.0)
	ε	43.1	3.81 m, 3.58 m		β	36.3	2.93 m
					γ-C_1	137.4	
Ile	CO	170.2			-C_2, C_6	129.2	7.32 m
	α	52.5	4.84 (dd, 8.0, 3.0)		-C_3, C_5	128.4	7.22 m
	β	36.1	1.76 m		-C_4	126.4	7.22 m
	γ-Me	16.0	0.86 (d, 6.5)		NH		7.60 (d, 8.0)
	γ-CH_2	22.3	1.26 m				
	δ-Me	11.3	0.77 (d, 6.5)	HMP	CO	168.0	
	NH		6.22 (d, 8.0)		α	74.4	5.00 (d, 1.0)
					β	35.6	1.95 m
MeGln 2	CO	168.4			γ-Me	13.8	0.65 (d, 6.5)
	α	58.9	4.88 (dd, 8.0, 3.0)		γ-CH_2	25.3	1.20 m
	β	23.8	2.05 m, 1.75 m		δ-Me	11.4	0.76 (d, 6.5)
	γ	30.8	2.08 m, 1.95 m				
	δ-$\underline{CO}NH_2$	173.1					
	δ-CO$\underline{NH_2}$		7.29 bs, 6.80 bs				
	NMe	29.1	2.65 s				

a Instruments: Varian XL-400, GE-400.
b The chemical shifts are in ppm with reference to internal TMS and coupling constants, J, are in Hertz.

envisioned to participate intramolecularly in the hydrolysis of the ester bond.

Compound **1** showed selective binding to the NK_2 receptor with an IC_{50} value of 68 nM and an IC_{50} value was >1000 nM for the NK_1 receptor. These values are comparable to those reported for Sch 217048.

References

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