Sch 217048: A Novel Cyclodepsipeptide with Neurokinin Antagonist Activity

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Neurokinins (NK), a family of 9−11 amino acid peptides, trigger a variety of inflammatory responses.[1,2] The most well characterized neurokinins, namely substance P, NKA, and NKB, are involved in a variety of responses including pain transmission, neurogenic inflammation, bronchoconstriction, and vasodilation. The peptides act principally through seven transmembrane domain G protein-coupled receptors that have been cloned and are termed NK_1, NK_2, and NK_3 and have selective affinity for substance P, NKA, and NKB, respectively.[3] A number of neurokinin antagonists have been identified, and these have been useful in clarifying the pathophysiologic roles of neurokinins in a variety of diseases including asthma.[4] During our search for novel neurokinin receptor inhibitors, we have isolated a cyclodepsipeptide (Sch 217048, **1**) from an unidentified fungal fermentation broth with selective NK_2 antagonist-type activity.[5] The fermentation was extracted with EtOAc at pH ∼6.5.[6] Purification of **1** was achieved by NK2 assay-guided fractionation using gel filtration (Sephadex LH-20/MeOH) and reverse phase preparative HPLC (Waters Deltapak C-18, 3.0 × 30 cm, ACN: 0.05% TFA (35:65)). Pure compound was white with end UV absorption and a mp of 192−194 °C. In this paper, we report the structure elucidation of **1**.

The molecular formula for **1** was determined to be $C_{57}H_{88}N_{10}O_{14}$ by HRFABMS [$(M + H)^+$ at *m*/*z* 1137.6560, calcd; 1137.6591, measured], and ^1H and ^{13}C NMR spectra (Table 1) were indicative of a peptide. **1** was ninhydrin-negative, indicating a blocked N-terminus or a cyclic peptide.

Data contained in the ^1H, ^{13}C, APT, and DEPT NMR spectra indicated 11 methyls (6 doublets, 2 triplets, 3 NMe singlets), 15 methylenes (12 CH_2, 3 NCH_2), 13 methines (8 CHN, 1 CHO, 4 $CH-CH_3$), 5 aromatic type carbons (=CH), and 13 quaternary carbons (12 CON(O), 1 =C) for $C_{57}H_{81}$ plus 7 heteroatom protons consisting of 4 NH, 1 amide NH_2, and 1 carboxylic acid proton.

The 19 degrees of unsaturation calculated from the molecular formula were divided as follows: 12 carbonyls, 3 trisubstituted double bonds, 1 proline, 1 pipecolic acid (pip), 1 benzene ring, and finally 1 due to cyclic peptide.

Extensive analysis of COSY, HOHAHA, HETCOR, HMBC, and HMQC-TOCSY NMR data revealed spin systems for the amino acids; Phe, Pro, Gly, MeVal, MeGln, Ile, Pip (pipecolic acid), Val, and MeGlu. In addition an acid containing a hydroxymethine function

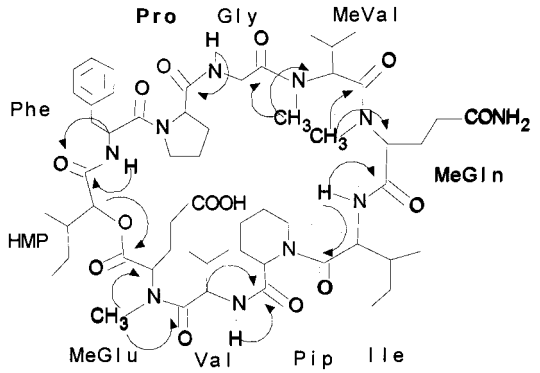

Figure 1. Sch 217048.

was assigned to 2-hydroxy-3-methylpentanoic acid (HMP) (see Table 1). The amino acid sequence of **1** was determined by analysis of HMBC data (Figure 1) and FABMS-MS data of the base hydrolysis product **2** and its derivatives.

Amino acid analysis indicated the presence of one mole each of glycine, valine, D-phenylalanine, proline, and D,L-isoleucine. From the CIMS of the acid hydrolysis products *m*/*z* $(M + 1)^+$ 76(Gly), 118(Val), 116(Pro), 132(Leu and/or MeVal), 166(Phe), and 130(Pip) were observed. A strong *m*/*z* 144 [$(M + 1)^+$; $C_6H_{10}NO_3$] was postulated to be *N*-methylglutamic acid anhydride.

FABMS analysis of the natural product, **1** , provided ion signals at *m*/*z* 1137 $(M + 1)^+$, 787, 399, 307, 268, and 238 and very little information about the amino acid sequence. However, basic hydrolysis[7] of the ester linkage of **1** produced two linear peptides, **2** and **2**a (Table 2). FABMS-MS analysis of **2** provided useful sequence information complimenting the HMBC NMR data. Specifically, MS-MS analysis of ions at *m*/*z* 895, 671, 627, and

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(5) The culture sample was isolated from mixed litter of humid forest near Kandy, Sri Lanka, and was described as a fungul with sterile, dematiaceous mycelium with low, dry, thin hyphae. The culture was deposited in Schering collection as SCF 1575.

(6) Fermentation studies were carried out in shake flasks. The inoculum medium for NK2 production contained (g/L) proteus peptone (5), NaCl (5), KH_2PO_4 (5), yeast extract (3), cerelose (20), soybean grits (5), antifoam 1 mL, and tap water to 1 L. The pH was adjusted to 7.2 prior to autoclaving. A 250 mL Erlenmeyer flask containing 70 mL of this medium was inoculated with 2.0 mL of the stock culture. The flask was incubated at 24 °C on a rotary shaker at 250 rpm for 96 h. Second germination was prepared under the same conditions. Five percent of the second germination was used to inoculate the fermentation medium containing (g/L) neopeptone (10), cerelose (40), $CaCO_3$ (4), and tap water to 1 L. The pH was adjusted to 7.4 prior to autoclaving. The fermentation was carried out in 2 L Erlenmeyer flasks containing 350 mL of the fermentation medium. The flasks were incubated at 24 °C on a rotary shaker at 250 rpm for 120 h.

(7) Base hydrolysis of **1** was carried out with 1 N NaOH in MeOH at room temp for 50 h. Methylation of the resultant solid (mixture of **2** and **2**a obtained after ethyl acetate extraction) with diazomethane and subsequent separation on HPLC gave dimethyl ester **3** and trimethyl ester **4** in the ratio of 55:45. **4** was treated with acetic anhydride and pyridine to obtain **5**. Compounds **2**−**4** were characterized by ^1H, ^{13}C, APT, and MS data, while for **5** only MS data were obtained.

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Table 1. ^{1}H and ^{13}C NMR Data for Sch 217048 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]
MeGlu	CO	169.4		MeVal	CO	169.7	
	α	61.9	4.13 (dd, 9.0, 4.0)		α	57.0	5.14 (d, 10.0)
	β	23.8	2.25 m		*β*	27.3	2.29 m
	γ	30.2	2.33 m		*γ*-Me	17.9	0.75 (d, 6.5)
	δ-COOH	174.0			*γ*-Me	19.1	0.83 (d, 6.5)
	NMe	38.4	3.22 s		NMe	28.1	2.90 s
Val	CO	172.3		Gly	CO	170.4	
	α	54.1	4.57 (t, 8.0, 9.0)		α	41.0	4.40 (dd, 17.0, 8.0)
	β	31.1	1.95 m				4.24 (d, 17.0)
	γ-Me	17.7	0.80 (d, 6.5)				
	γ-Me	19.1	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	27.1	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	167.9	
	NH		6.22 (d, 8.0)		α	74.5	5.00 (d, 1.0)
					β	35.6	1.95 m
MeGln	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-*C*ONH$_2$	173.1					
	δ-CO*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.

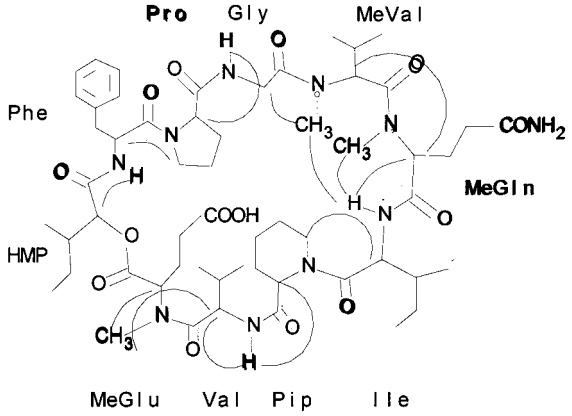


Figure 2. NOE interactions.

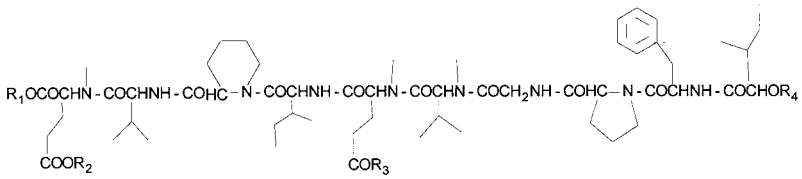
529 revealed the amino acid sequence of the linear peptide. Fragmentation of the ion at *m*/*z* 895 (Pip-Ile-MeGln-MeVal-Gly-Pro-Phe-HMP) produced major ions at *m*/*z* 741 (Pip-Ile-MeGln-MeVal-Gly-Pro-Phe), 634 (Pip-Ile-MeGln-MeVal-Gly-Pro), 367 (Pip-Ile-MeGln), 784 (Ile-MeGln-MeVal-Gly-Pro-Phe-HMP), 527 (MeVal-Gly-Pro-Phe-HMP), and 267 (MeVal-Gly-Pro).

Fragmentation of *m*/*z* 671 (HMP-Phe-Pro-Gly-MeVal-MeGln) gave rise to major ions at *m*/*z* 529 (HMP-Phe-Pro-Gly-MeVal), 556 (Phe-Pro-Gly-MeVal-MeGln), and 410 (Pro-Gly-MeVal-MeGln). Fragmentation of *m*/*z* 627 (MeGlu-Val-Pip-Ile-MeGln) provided 466 (Val-Pip-Ile-MeGln), 367 (Pip-Ile-MeGln), and 255 (Ile-MeGln). Fragmentation of *m*/*z* 529 (HMP-Phe-Pro-Gly-MeVal) gave 416 (HMP-Phe-Pro-Gly), 359 (HMP-Phe-Pro), 261 (HMP-Phe), and 267 (Pro-Gly-MeVal). The location of Ile in this sequence rather than MeVal (isomeric unit) was based on NMR analysis. Further mass spectral studies were carried out for dimethyl and trimethyl ester derivatives **3** and **4** prepared from **2** and **2**a, respectively.[7] In addition **4** was converted to trimethyl ester acetate **5** for MS analysis.[7] Fragmentation data are provided in Table 2.

The loss of 260 amu from $(M + 1)^+$ for **2** was assigned to MeGlu-Val, whereas for **3**−**5**, this loss increased to 288 due to diesterification of MeGlu [$(CH_3O)_2$MeGlu-Val = 288]. Similarly a loss 157 amu was observed from *m*/*z* 686 for **4** and was assigned to esterification of MeGln [MeGluOCH$_3$ = 157]. It is interesting to note that during hydrolysis, MeGln was converted to MeGlu and then esterified. Finally in the case of **5**, 2-hydroxy-3-methylpentanoic acid (HMP), it was acetylated as shown by the loss of 174 amu [AcHMPN = $C_8H_{13}NO_3$] from *m*/*z* 571 as compared to the loss of 129 amu [HMPN = $C_6H_{11}NO_2$] from *m*/*z* 529 for **2**−**4**. Amino acid positions of HMP, MeGln, and MeGlu in the sequence were thus confirmed.

HMBC and NOESY experiments for **1** afforded all the sequences relevent for the assignment of cyclic structure (Figures 1 and 2). Correlations between CO and *β*-protons (HMBC) and between α- and *β*-protons (NOESY) were also observed. *N*-Methyl singlets played an important role in the correlation assignments. Because of serious overlap in the upfield region of *β*-protons resonances, HOHAHA, HMQC, and HMQC-TOCSY experiments were utilized.

Table 2. Mass Spectral Data of 2−5[a]



2 R_1, R_2, R_4 = H, R_3 = NH_2

$$1155\ (M+1)^+ \xrightarrow{-260} 895 \xrightarrow{-111} 784 \xrightarrow{-113} 671 \xrightarrow{-142} 529 \xrightarrow{-129} 400$$

MeGlu-Val; Pip; Ile; MeGln; $C_6H_{11}NO_2$

$$529 \xrightarrow{-113} 416 \xrightarrow{-57} 359 \xrightarrow{-97} 262\ \text{(HMP-Phe)}$$

MeVal; Gly; Pro

2a R_1, R_2, R_4 = H, R_3 = OH

3 R_1,R_2 = CH_3, R_4 = H, R_3 = NH_2

$$1183(M+1)^+ \xrightarrow{-288} 895 \xrightarrow{-111} 784\ \text{(rest is same as scheme above)}$$

$(MeO)_2$MeGlu-Val; Pip

4 R_1,R_2 = CH_3, R_4 = H, R_3 = OCH_3

$$1198(M+1)^+ \xrightarrow{-288} 910 \xrightarrow{-111} 799 \xrightarrow{-113} 686 \xrightarrow{-157} 529\ \text{(rest is same as above)}$$

$(MeO)_2$MeGlu-Val; Pip; Ile; MeOMeGlu

5 R_1,R_2 = CH_3, R_4 = $COCH_3$, R_3 = OCH_3

$$1240(M+1)^+ \xrightarrow{-288} 952 \xrightarrow{-111} 841 \xrightarrow{-113} 728 \xrightarrow{-157} 571 \xrightarrow{-171} 400$$

(MeO)2MeGlu-Val; Pip; Ile; MeOGlu; $C_8H_{13}NO_3$ (AcHMPN)

$$571 \xrightarrow{-267} 304\ (CH_3COHMP\text{-}Phe)$$

MeVal-Gly-Pro

[a] All compounds gave sodiated ions. Both B and Y fragments as well as fragments from the hydroxy terminus were also observed.

The α-Pip resonance (δ 5.12 dd, 4.0, 2.5 Hz) was assigned as β-equatorial. Sch 217048 is a unique structure containing pipecolic and 2-hydroxy-3-methylpentanoic acids.

Natural products with similar structures have been identified with other biological activities.[8,9] Many cyclodepsipeptides displaying a variety of biological activities, e.g., antiviral,[10] and cytotoxicity toward leukemia cells,[11] have been reported. In the present study, compound **1** showed selectivity for NK_2 receptor with an IC_{50} value of 50 nM. For NK_1 receptor, the IC_{50} was >1000 nM.

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