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A Family of Depsi-peptide Fungal Metabolites, as Selective and Competitive Human Tachykinin Receptor (NK_2) Antagonists: Fermentation, Isolation, Physico-chemical Properties, and Biological Activity

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Four tachykinin (NK_2) receptor inhibitors, SCH 378161 (**1**), SCH 217048 (**2**), SCH 378199 (**3**), and SCH 378167 (**4**) were isolated from the fermentation broth of a taxonomically unidentified fungus. These compounds were separated from the fermentation broth by ethyl acetate extraction. Purification and separation of the individual compounds were achieved by NK_2 assay-guided fractionation using gel filtration, reverse phase chromatography and HPLC. They were identified to be a family of depsipeptides by spectroscopic and degradation studies. Compounds **1** and **3** contain proline and differ as an amide and acid whereas **2** and **4** contain pipecolic acid and differ in being an amide and acid. All of these compounds contain an identical hydroxy acid. They are selective NK_2 inhibitors with *Ki* values ranging from 27~982 nM and demonstrate no activity at 10 μM in the NK_1 and NK_3 assays. In addition, compounds **1** and **2** inhibited NKA-induced increases in the concentration of intracellular Ca^{2+}, $[Ca^{2+}]_i$, in a CHO cell expressing the human NK_2 receptor; this inhibition was competitive in nature with pA2 values of 7.2 and 7.5, respectively. These data demonstrate that these natural products are selective and competitive receptor antagonists of the human NK_2 receptor.

The tachykinin peptides present in mammalian systems include substance P (Sub. P), neurokinin A (NKA) and neurokinin B (NKB). These structurally related peptides share a common carboxyl-terminal sequence, Phe-X-Gly-Leu-Met-NH_2[1]. Tachykinins are located in sensory neurons in both the peripheral and central nervous systems[2]. Upon stimulation of C-fibres, these peptides are released and exert their biological effects through specific receptors designated as NK_1, NK_2 and NK_3. Sub. P preferentially binds to the NK_1 receptor, NKA to the NK_2 receptor, and NKB to the NK_3 receptor[3].

Activation of the NK_1 receptor has been implicated in edema and neurogenic inflammation[4]. The NK_2 receptor has been demonstrated to induce contractile responses in a number of smooth muscle preparations[5]. The role of the NK_3 is unclear. Thus, there are several potential therapeutic areas where an NK_1, NK_2 or dual NK_1/NK_2 receptor antagonist may be beneficial. These include migraine, rheumatoid arthritis, asthma and bronchopulmonary disease, and emesis.[6,7] We screened many natural products to identify novel tachykinin receptor antagonists. During our search, we have isolated four depsi-peptides from an unidentified fungal fermentation broth with NK_2 antagonist activity.

In this report, we describe fermentation of the producing fungal culture, and the isolation, physico-chemical properties, and biological activity of these novel and selective NK_2 tachykinin receptor antagonists.

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Materials and Methods

The fungus that produces the active compounds was isolated from mixed litter from a humid forest area near Kandy, Sri Lanka. The microorganism is a fungal culture with sterile, dematiaceous mycellium with low, dry, thin hyphae. Taxonomic evaluation and classification of the fungus producing the neurokinin inhibitors has not been performed.

Fermentation Conditions

Fermentation studies were carried out in shake flasks. Stock cultures were maintained as frozen whole broths at −80°C in a final concentration of 10% glycerol. The inoculum medium for NK_2 inhibitor contained (g/liter) proteus peptone 5, NaCl 5, KH_2PO_4 5, yeast extract 3, cerelose 20, soybean grits 5, antifoam 1 ml, tap H_2O to 1 liter. 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 hours. Two and one half ml of this seed culture was used to inoculate another 250 ml Erlenmeyer flask containing 70 ml of the same seed medium. This flask was incubated as above for 96 hours.

Five percent of the second germination was used to inoculate the fermentation medium containing (g/liter) neopeptone (10), cerelose (40), $CaCO_3$ (4), and Tap H_2O to 1 liter. The pH was adjusted to 7.4 prior to autoclaving. The fermentation was carried out in 2 liter 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 hours.

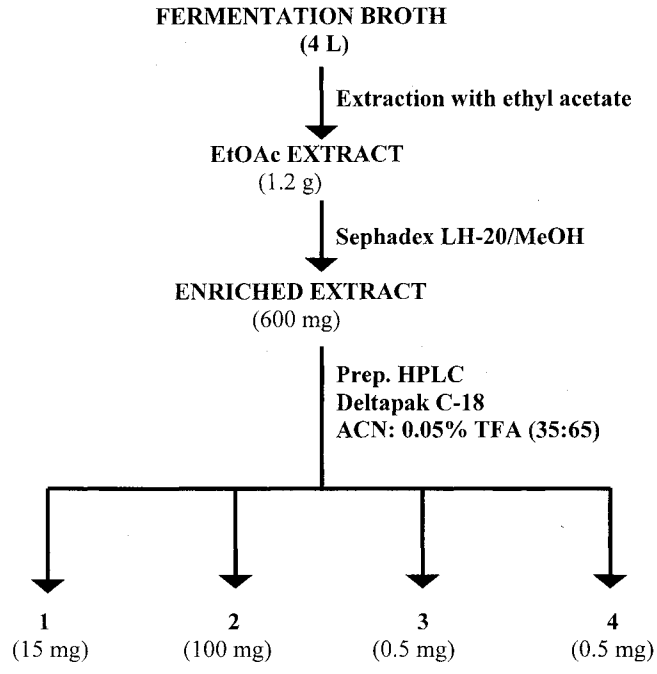
Fermentation Analysis

Production of the neurokinin receptor inhibitors was monitored over time by NK_2 binding assays.

Isolation

The steps leading to isolation and purification of the neurokinin receptor inhibitors are shown in Figure 1. Four liters of fermentation broth was extracted with two volumes of ethyl acetate twice, the organic layer was removed from the broth, dried over anhydrous sodium sulfate and the solvent was removed to yield 1.2 g of organic extract. The extract was dissolved in a minimum amount of methanol and loaded on a Sephadex LH-20 column packed in methanol. The column was eluted with methanol and the fractions were monitored by the NK_2 receptor binding assay. The active fractions were combined and the solvent removed to yield 0.6 g of solids enriched with NK_2 inhibitors. The active compounds were purified on a preparative Deltapak C-18 column eluting with an acetonitrile: 0.05% triflouroacetic acid TFA (35 : 65). The acetonitrile was removed from the individual peak eluates under vacuum and the remaining aqueous solution on freeze drying yielded 15, 100, 0.5, and 0.5 mgs of **1**, **2**, **3** and **4** respectively.

Fig. 1. Isolation scheme for compounds **1**~**4**.



Physico-chemical Properties

The physico-chemical properties of the neurokinin receptor inhibitors **1**~**4** are summarized in the experimental section. As compound **2** was produced in large quantity, this was used for structural elucidation purposes. The structure of this compound is published elsewhere.[8] All compounds were isolated as amorphous white powders. These compounds showed only end absorption in UV spectrum. The amino acid residues were identified by acid hydrolysis, with 6 N HCl and subsequent TLC analysis of the hydrolysis products.[9]

Receptor Binding Assays

Chinese Hamster ovary (CHO) cells transfected with the coding regions for the human tachykinin receptors (NK_1, NK_2 and NK_3) were obtained from Dr. JIM KRAUSE

(Washington University, St. Louis, MO). Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum, 0.1 mM non-essential amino acids, 2 mM glutamine, 100 units/ml of penicillin and streptomycin, and 0.8 mg/ml of G418 at 37°C in a humidified atmosphere containing 5% CO_2. Cells were detached from T-175 flasks with a sterile solution containing 5 mM EDTA in phosphate buffered saline, harvested by centrifugation and washed in RPMI media at 4°C for 5 minutes. The pellet was resuspended in Tris-HCl (pH 7.4) containing 1 μM phosphoramidon and 4 μg/ml of chymostatin at a cell density of 30×10^6 cells/ml. The suspension was then homogenized in a Brinkman Polytron (setting 5) for 30~45 seconds. The homogenate was centrifuged at $800\times g$ for 5 minutes at 4°C to collect unbroken cells and nuclei. The supernatant was centrifuged in a Sorvall RC5C at 19,000 rpm ($44,000\times g$) for 30 minutes at 4°C. The pellet was resuspended (an aliquot was removed for a protein determination (*i.e.* BCA)) and washed again. The resulting pellet (10 mg/ml) was stored at −80°C.

To assay receptor binding, 50 μl of either [^{3}H]Sar-substance P (9-Sar, 11-Met [O_2]) (0.8 nM), [^{3}H]Neurokinin A (1.0 nM), and [^{125}I]MepheNKB (0.1 nM) was added to wells of a deep-well microplate containing buffer (*i.e.* 50 mM Tris-HCl (pH 7.4) with 1 mM $MnCl_2$ and 1 mM $MgCl_2$ and 0.2% bovine serum albumin) and either vehicle (DMSO) or various concentrations test compounds. Binding was initiated by the addition of 100 μl of membrane (10~20 μg) containing the human NK_1, NK_2, NK_3 receptor in a final volume of 200 μl. After 40 minutes at room temperature, the reaction was stopped by rapid filtration onto Whatman GF/B filters that had been presoaked in 0.3% polyethylenimine. Filtration was accomplished using a TOMTEC automated harvester. Filters were washed 3 times with cold 50 mM Tris-HCl (pH 7.4). Filter mats were dried in a microwave oven on high for 2×2.5 minutes. Meltilex solid scintillant was applied to filters and scintillation counting carried out in a Betaplate counter (Wallac, Gaithersburg, MD). Non-specific binding was determined by the addition of either CP 99994 (NK_1)[10], SR 48968 (NK_2)[11], or SR 142801 (NK_3)[12] at a concentration of 1 μM. CP 99994, SR 48968 and SR 142801 were synthesized by the Chemistry Dept. at Schering-Plough Research Institute. IC_{50} values were determined from competition binding curves and *Ki* values were determined according to CHENG and PRUSOFF[13] using the experimentally determined *Kd* values of 0.8 nM for the NK_1 receptor, 2.4 nM for the NK_2 receptor and 0.2 nM for the NK_3 receptor. *Kd* and Bmax values were determined by non-linear regression using the program PRISM (GraphPad Software, San Diego, CA)

Measurement of $[Ca^{2+}]_i$

Cells were seeded in clear, flat-bottomed, black-wall 96-well plates 1~3 days prior to assay for a final density of 50,000 cells/well. On the day of the assay, the medium was removed and cells were incubated in Hank's buffered saline solution, 20 mM HEPES, 0.4% BSA, 2.5 mM probenecid, 10% pluronic acid and 4 μM Fluo-3 AM for 1 hour at 37°. Cells were washed 4× with buffer utilizing a Labsystems Cellwash plate washer, leaving 100 μl of FLIPR buffer in each well. Solutions containing the appropriate concentration of test compound or buffer were added to the cells and incubated at 37° for 15 minutes. before addition of Sar-Sub. P, NKA, MepheNKB, which were added in 50 μl volume at a concentration 0.3 nM, 0.5 nM and 0.8 nM respectively. Changes in intracellular calcium were measured with a Flurometric Imaging Plate Reader (FLIPR) by excitation of the calcium sensitive fluorescent dye with an argon laser and measuring excitation at 488 nM and emission in the 500~560 nM range. Data is presented as a percent of maximum response of the peak heights standardized with the addition of 100 μM of Sar-Sub. P, NKA or MepheNKB. Schild analysis was used to calculate pA2 values in the program PRISM through linear regression of dose ratios of different antagonist concentrations.

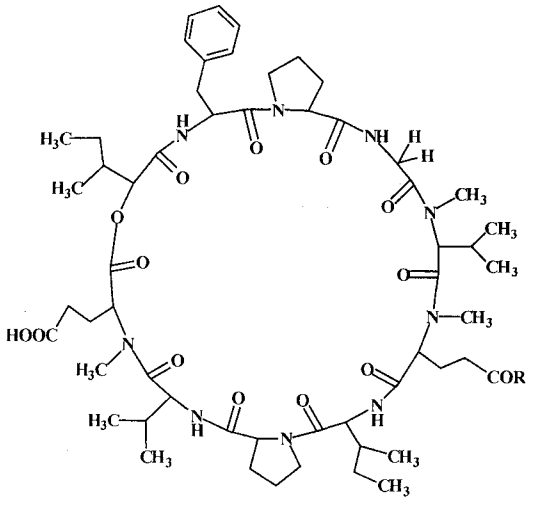
Results

Production of the neurokinin inhibitors in fermentation was monitored using ethyl acetate extracts of the aliquots obtained at different times during the fermentation process. The production of neurokinin inhibitors in the fermentation peaked at 120 hours.

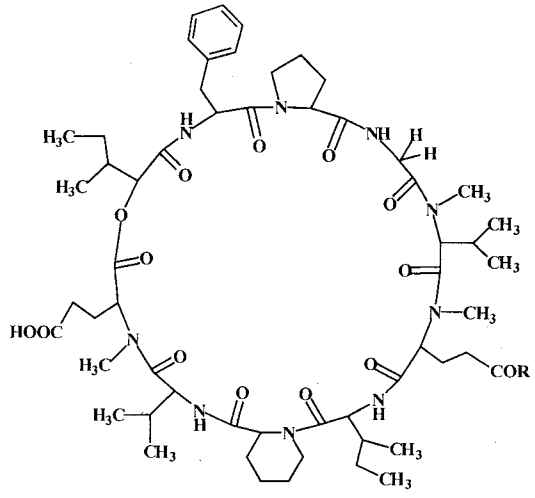
The ethyl acetate extract provided four tachykinin (NK_2) inhibitors that were similar in nature. They were further purified and separated by using reverse phase chromatography.

Structure Determination

These compounds were white amorphous solids, soluble in methanol, chloroform, ethyl acetate but insoluble in water. SCH 217048 (**2**) a major component, in Cs^+ ion liquid secondary ionization mass spectrum (SIMS), displayed an intense molecular ion at *m/z* 1137 $(M+H)^+$ revealing the molecular weight to be 1136. Peak matching



1. SCH 378161
R = -NH_2
3. SCH 378199
R = -OH



2. SCH 217048
R = -NH_2
4. SCH 378167
R = -OH

measurements using high resolution mass measurements showed the elemental composition to be $C_{57}H_{88}N_{10}O_{14}$ suggesting nineteen degrees of unsaturation. The IR spectrum displayed peaks at 3324, 2923, 1678, 1631, 1540, 1462, 1204 cm^{-1}. The ^{13}C NMR spectrum measured in DMSO-d_6 at 100.5 MHz, revealed the presence of 57 carbon atoms and are shown in Table 1. The structure of **2** was determined based on ^{1}H NMR, ^{13}C NMR, HMBC and degradation studies and has been published recently.[8]

SCH 378161 (**1**), displayed an molecular ion at *m/z* 1123 $(M+H)^+$ and a sodiated ion at 1145 $(M+Na)^+$ in Cs^+ ion liquid secondary ionization mass spectrum (SIMS), revealing the molecular weight to be 1122. High resolution mass measurements showed the elemental composition to be $C_{56}H_{86}N_{10}O_{14}$. Thus **1** is a lower homolog of **2**. ^{1}H and ^{13}C NMR chemical shifts were similar to those of **2**. A thorough spectral comparison of **1** with that of **2** suggested most of the amino acid residues are intact as in **2** except some changes in pipecolic acid. Compound **1**, on 6 N hydrochloric acid hydrolysis and the hydrolyzed product on amino acid analysis,[10] did not show the presence of pipecolic acid. The information from COSY, HMBC, HMQC, and HMQC-TOCSY spectra suggested the presence of proline (pro-2) in place of pipecolic acid as shown in Figure 2. The presence of an additional proline (pro-2) instead of pipecolic acid (Pip) was further confirmed by base hydrolysis. Hydrolysis of **1** with 1 N NaOH at room temperature for 20 hours gave hydrolyzed product **5**. Compound **5** on FAB MS/MS showed fragmentation pattern as shown in Figure 3. The fragmentation pattern for base hydrolyzed product of **2** has been previously established[8]. In case of compound **2** fragments 260 and 371 shows difference of 111 mass units for pipecolic acid but in case of **1**, fragments *m/z* 260 and 357 shows a difference of 97 mass units (Figure 3), in agreement with the presence of a second proline (pro-2) instead of pipecolic acid.

The Cs^+ ion liquid secondary ionization mass spectrum (SIMS) of SCH 378199 (**3**) displayed an intense molecular ion at *m/z* 1124 $(M+H)^+$ and a sodiated ion at 1146 $(M+Na)^+$ revealing the molecular weight to be 1123. High resolution mass measurements of **3** suggested molecular formula $C_{56}H_{85}N_9O_{15}$ (obsd. 1124.6262 and calc. for $C_{56}H_{85}N_9O_{15}$ 1124.6243), with one nitrogen and hydrogen less but an additional oxygen compared to **1**. Compound **3** on esterification yielded a di-ester suggesting two free carboxylic acid groups. This led to the proposition that **3** might contain *N*-methyl glutamic acid in place of *N*-methyl glutamin. This diester on base hydrolysis gave a tri-carboxylic acid, which on methylation with diazomethane yielded compound **6**. The trimethyl ester **6**, on FAB MS/MS fragmentation (Figure 4) confirmed the presence of *N*-methyl glutamic acid in **3**. In compound **5** fragments *m/z* 470 and 612 (Figure 3) show a difference of 142 mass units indicating *N*-methyl glutamine residue, whereas in compound **6** the fragments *m/z* 498 and 655 (Figure 4)

Table 1. ^{1}H and ^{13}C NMR chemical shifts for **1** and **2**[a].

Amino Acids	Carbon #	^{1}H 1	^{13}C 1	^{1}H 2	^{13}C 2	Amino Acids	Carbon #	^{1}H 1	^{13}C 1	^{1}H 2	^{13}C 2
HMP	C=O		167.9		167.9	MeGln	C=O		168.1		168.4
	α	4.93(d, 1Hz)	74.9	5.00(d, 1Hz)	74.5		α	4.84(dd, 8&3Hz)	58.9	4.88(dd, 8&3Hz)	58.9
	β	1.95(m)	35.8	1.95(m)	35.6		β	2.06(m), 1.75(m)	23.8	2.05(m), 1.75(m)	23.8
	γ-Me	0.65(d, 6.5Hz)	14.4	0.65(d, 6.5Hz)	13.8		γ	2.08(m), 1.95(m)	30.9	2.08(m), 1.95(m)	30.8
	γ-CH_2	1.20(m)	25.4	1.20(m)	25.3		δ-$CONH_2$		173.1		173.1
	δ-Me	0.76(t, 6.5Hz)	11.4	0.76(t, 6.5Hz)	11.4		δ-$CONH_2$	7.34(bs), 6.81(bs)		7.29(bs), 6.8(bs)	
							NMe	2.64(s)	29.2	2.65(s)	29.1
Phe	C=O		169.8		169.7						
	α	4.71(dt, 8, 8&5Hz)	52.1	4.69(dt, 8, 8&4Hz)	52.2	Ileu	C=O		170.1		170.2
	β	2.93(m)	36.2	2.93(m)	36.3		α	4.61(dd, 8&3 Hz)	53.7	4.84(dd, 8 &3 Hz)	52.5
	γ-C_1		137.5		137.4		β	1.76(m)	37.7	1.76(m)	36.1
	C_2 , C_6	7.34(m)	129.2	7.32(m)	129.2		γ-Me	0.87(m)	14.9	0.86(d, 6.5Hz)	16.0
	C_3 , C_5	7.22(m)	128.2	7.22(m)	128.4		γ-CH_2	1.26(m)	23.6	1.26(m)	22.3
	- C_4	7.22(m)	126.3	7.22(m)	126.4		δ-Me	0.84	11.1	0.77(d, 6.5Hz)	11.3
	-NH	7.70(d, 8Hz)		7.60(d, 8Hz)			NH	6.22(d, 8Hz)		6.22(d, 8Hz)	
Pro	C=O		171.5		170.6	Pip (Pro)	C=O		168.1		170.2
	α	4.55(dd, 8&5Hz)	59.4	4.55(dd, 8&5Hz)	59.4		α	4.45(dd, 8&5 Hz)	59.5	5.12(dd, 4&2.5Hz)	52.4
	β	2.12(m), 1.74(m)	28.8	2.12(m), 1.74(m)	29.1		β	2.16(m)	29.7	1.75(m)	27.1
	γ	2.05(m), 1.92(m).	24.3	2.05(m), 1.92(m).	24.7		γ	1.87(m)	24.9	1.41(m), 1.15(m)	19.4
	δ	3.70(m)	47.0	3.70(m)	47.0		δ	3.50(m)	47.6	1.72(m)	24.4
							ε	--		3.81(m), 3.58(m)	43.1
Gly	C=O		170.7		170.4						
	α	4.44(dd, 17&8Hz) 4.24(d, 17Hz)	40.2	4.40(dd, 17&8Hz) 4.24(d, 17Hz)	41.0	Val	C=O		172.5		172.3
	-NH	8.00(d, 8Hz)					α	4.64(dt, 8&8Hz)	53.7	4.57(t, 8&9Hz)	54.1
							β	1.95(m)	31.5	1.95(m)	31.1
MeVal	C=O		169.8		169.7		γ-Me	0.80(d, 6.5Hz)	17.8	0.80(d, 6.5Hz)	17.7
	α	5.15(d, 10Hz)	57.1	5.14(d, 10Hz)	57.0		γ-Me	0.91(d, 6.5Hz)	19.3	0.82(d, 6.5Hz)	19.1
	β	2.29(m)	27.0	2.29(m)	27.3		-NH	8.46(d, 8.0Hz)		8.65(d, 8.0Hz)	
	γ-Me	0.74(d, 6.5Hz)	17.8	0.75(d, 6.5Hz)	17.9						
	γ-Me	0.84(d, 6.5Hz),	19.0	0.83(d, 6.5Hz)	19.1	MeGlu	C=O		169.4		169.4
	NMe	2.93(s)	28.2	2.9(s)	28.1		α	4.13(dd, 9&4Hz)	62.0	4.13(dd, 9&4Hz)	61.9
							β	2.25(m)	23.9	2.25(m)	23.8
							γ	2.33(m)	30.2	2.33(m)	30.2
							δ-COOH		173.9		174.0
							NMe	3.24(s)	38.4	3.22(s)	38.4

[a] Solvent DMSO-d_6.

show a difference of 157 mass units indicating the presence of *N*-methyl glutamic acid. Also **6** established the presence of a second proline instead of a pipecolic acid (fragments *m/z* 288 and 385 shows a difference of 97 mass units).

Mass spectrum (SIMS) of **4** displayed an intense molecular ion at *m/z* 1138 and high resolution mass measurements established a molecular formula $C_{57}H_{87}N_9O_{15}$ (obsd. 1138.6422 and calc. 1138.6400),

Fig. 2. HMBC correlation's for **1**.

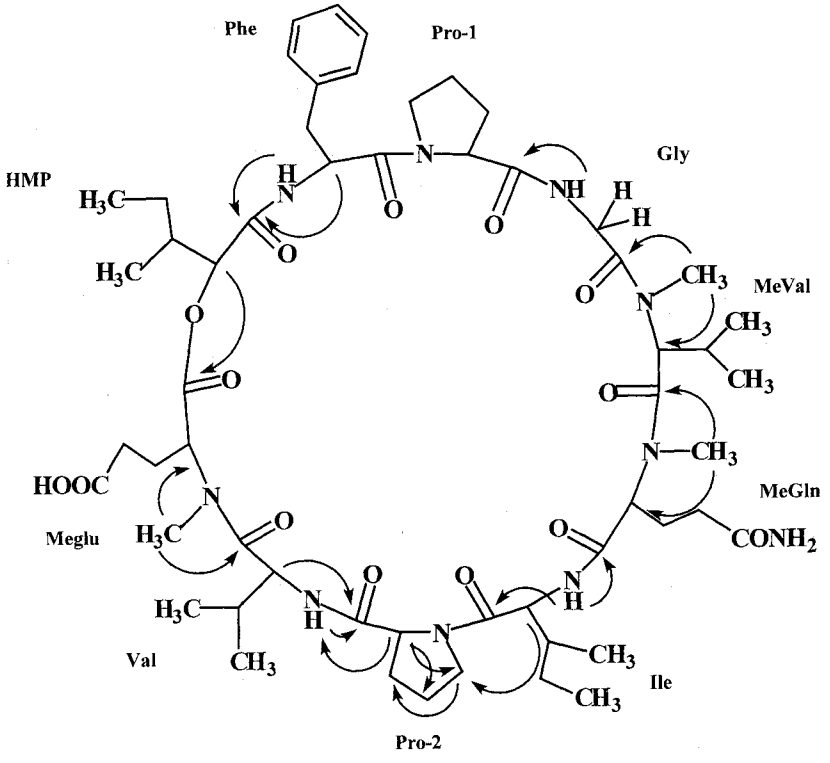
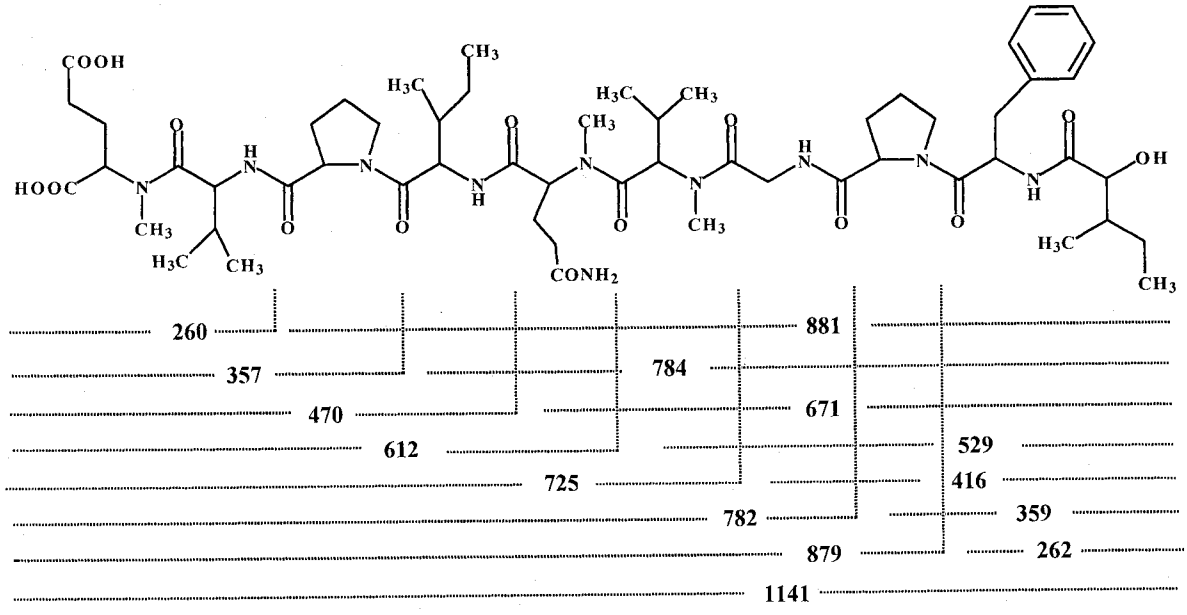
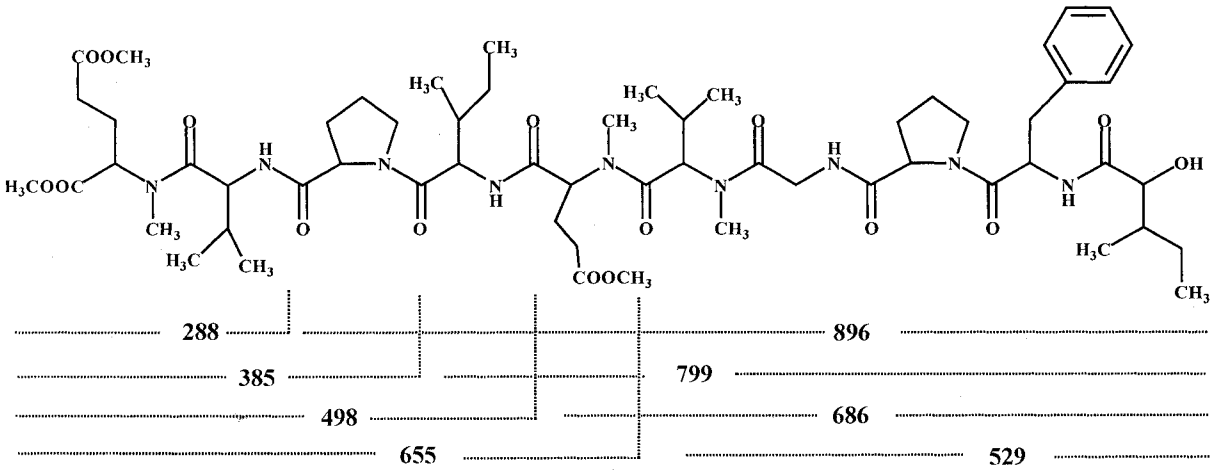


Fig. 3. FABMS fragmentation analysis of **5**.



5

Fig. 4. FABMS fragmentation analysis of **6**.



6

revealing an additional oxygen for a –NH unit compared to **2**. Like **3**, compound **4** gave a dimethyl ester on esterification with diazomethane and this product, on base hydrolysis followed by esterification with diazomethane, yielded a tri-methyl ester, revealing that compound **4** contains *N*-methyl glutamic acid instead of *N*-methyl glutamine. Spectral data also established the presence of pipecolic acid as in **2**.

Receptor Binding and Functional Activity

Compounds **1** and **2** inhibited binding of [^{3}H]NKA to the human NK_2 receptor in a concentration dependent manner (Figure 5A and B). These compounds did not inhibit binding of [^{3}H]Sar-SP or [^{125}I]MepheNKB to the human NK_1 and NK_3 receptor respectively. These data demonstrated that compounds **1** and **2** are NK_2 receptor selective inhibitors.

The *Ki* values for compounds **1**~**4** in NK_1, NK_2 and NK_3 receptor binding assays are shown in Table 2. CP-99994, a selective NK_1 receptor antagonist, inhibited the binding of [^{3}H]Sar-SubP binding to the human NK_1 receptor with a *Ki* of 0.27 nM. In contrast, compound **1**~**4** were inactive. SR48968, a selective NK_2 receptor antagonist inhibited binding of [^{3}H]NKA to the human NK_2 receptor with a *Ki* of 0.37 nM. Compounds **1**~**4** had *Ki* values of 78.7 nM, 27.4 nM, 982 nM and 309 nM. These compounds are 212, 74, 2654, and 835 fold less potent than SR48968 at the human

Fig. 5. Inhibition of [^{3}H]Sar-SP, [^{3}H]NKA, and [^{125}I]MepheNKB binding to cloned human tachykinin receptors by compounds **1** (A) and **2** (B).

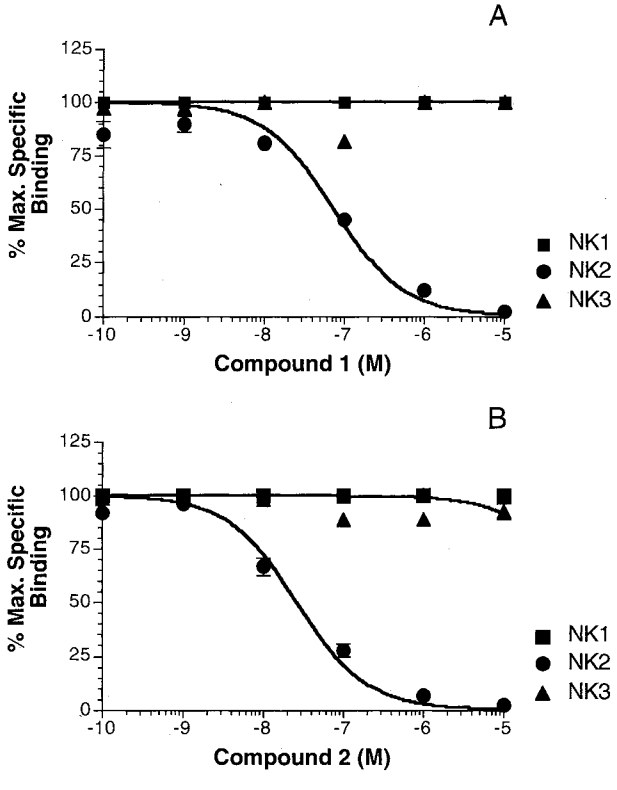
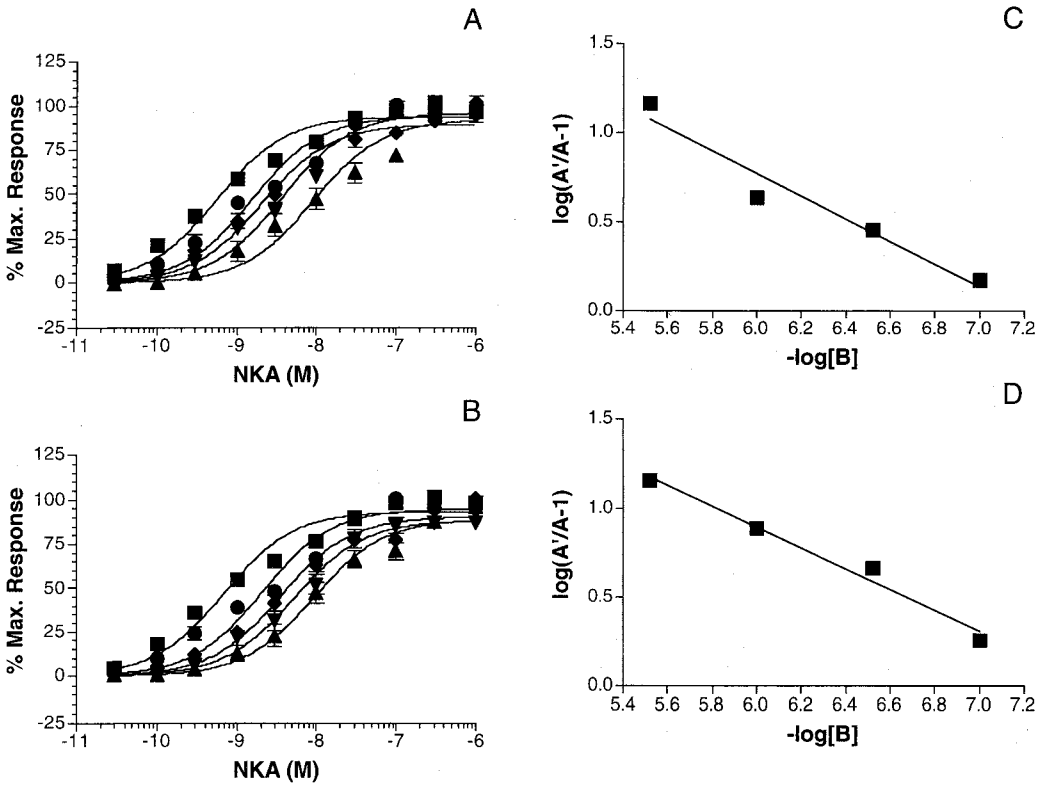


Table 2. Receptor binding affinity of compounds **1** and **2** and selective tachykinin antagonists for cloned human tachykinin receptors (*Ki* values were obtained from 3 independent experiments performed in duplicate).

COMPOUNDS	K_i (nM) NK_1	NK_2	NK_3
1	>10000	78.74±17.65	>10000
2	>10000	27.43±4.26	>10000
3	>10000	982.86±424.88	>10000
4	>10000	309.87±89.47	>10000
CP99994	0.27±0.07	>10000	>10000
SR48968	273.6±16.1	0.37±0.11	1282.3±565.7
SR142801	291.1±18.1	20.4±5.69	1.71±0.73

Fig. 6. Effect of compound **1** and **2** on NKA concentration curves for $[Ca^{2+}]_i$ flux in CHO cells expressing the human NK_2 receptor.

Increasing concentrations of NKA were added to CHO cells expressing human receptor in the absence (■) or presence of 3 μM (▲), 1 μM (▼), 300 nM (◆), and 100 nM (●) compound **1** (A) or compound **2** (B). Schild analysis of compounds **1** (C) and **2** (D). Curves plotted from **2** independent experiments performed in triplicate.



NK_2 receptor. Compound **1**~**4** were inactive at the human NK_3 receptor for the concentration ranges tested, although SR142801, a selective NK_3 receptor antagonist inhibited the binding of [^{125}I]MePheNKB with a *Ki* of 1.7 nM. These results demonstrate that compounds **1**~**4** are selective inhibitors of the human NK_2 receptor although less potent than SR48968.

Receptor binding assays do not yield information on the intrinsic activity of a compound, which could be either an agonist or an antagonist. In order to determine if compounds **1** and **2** were NK_2 receptor antagonists, the activities of these compounds were determined in an assay that measures NKA-induced $[Ca^{2+}]_i$ flux in CHO cells expressing the human NK_2 receptor. NKA induced a concentration-dependent increase in $[Ca^{2+}]_i$ in CHO cells expressing the human NK_2 receptor with an EC_{50} of 0.5 nM (Figure 6A and B). In the presence of increasing concentrations of compound **1** or **2**, the NKA response curves shift to the right in a parallel fashion with no decrease in the maximum response (Figure 6A and B). These data demonstrate that compound **1** and **2** are functional antagonists of the human NK_2 receptor. Schild analysis of the data demonstrates a competitive antagonism with pA2 values of 7.2 and 7.5 for compounds **1** and **2**, respectively (Figure 6C and D).

Conclusion

Compounds **2** and **4** containing pipecolic acid are about two-fold more active than compounds **1** and **3** containing proline. Also compound **1** and **2** containing *N*-methyl glutamine are about eight-fold more active than compounds **3** and **4** containing *N*-methyl glutamic acid. All of these compounds exhibited no inhibition against NK_1 and NK_3 receptors.

There is substantial evidence that activation of NK_1 and NK_2 receptors is an important step in a variety of physiologic responses including pain transmission, smooth muscle contraction and neurogenic inflammation.[14] Appropriate neurokinin antagonists may therefore be therapeutically useful for several diseases. A variety of natural product structural types have been shown to inhibit NK ligand binding including the benzomalvins,[15] fiscalins,[16] anthrotainin,[17] dimerized Trp-Phe condensates,[18] polyhydroxy isoprenoids[19] and cyclic peptides.[20,21] The novel cyclic peptides reported in this report constitute a new structural class of NK_2 receptor antagonists with selectivity towards NK_2 receptors. These depsi-peptides might represent specific therapeutic agents for ailments caused by increased activity of human NK_2 receptors.

Experimental

General Procedures

Solvents employed for chromatography were obtained from Fisher Scientific, Fair Lawn, NJ, 07410. Sephadex LH-20 for GPC was obtained from Pharmacia LKB Piscataway, NJ 08854. The reverse phase column packing CHP-20 was obtained from Mitsubishi Kasei Corporation, Tokyo, Japan. The preparative reverse phase HPLC was carried out on a C-18 silica column (Deltapak, 2.5×30 cm) obtained from Waters Corporation, Milford, MA 01757.

IR spectra were determined on a Nicolet FTIR model 10-MX instrument. Ultraviolet spectra were obtained by using a Hewlett Packard '8450 A' UV-vis spectrophotometer equipped with HP-9872B plotter. All Cs^+ ion liquid secondary ion mass spectra (SIMS) and high resolution mass measurements were obtained on a VG-ZAB-SE mass spectrometer using a glycerol-thioglycerol or m-nitrobenzyl alcohol matrix with the sample dissolved in dimethyl sulfoxide. NMR spectra were measured on Varian instruments, XL-300 and XL-400. HMBC, HMQC, HMQC-TOCSY, spectra were obtained on GE-400 NMR spectrometer.

SCH 378161 (**1**): UV (MeOH) λ_{max}, nm: End absorption; IR (KBr) ν_{max}: 3298, 2965, 1747, 1631, 1540, 1446, 1204 cm^{-1}; FAB MS: *m/z* 1123 $(M+H)^+$; HRFABMS: Measured 1123.6384 $(M+H)^+$, Calcd. for $C_{56}H_{87}N_{10}O_{14}$ 1123.6403.

SCH 217048 (**2**): UV (MeOH) λ_{max}, nm: End absorption; IR (KBr) ν_{max}: 3324, 2923, 1678, 1631, 1540, 1462, 1204 cm^{-1}; FAB MS: *m/z* 1137 $(M+H)^+$; HRFABMS: Measured 1137.6560 $(M+H)^+$, Calcd. for $C_{57}H_{89}N_{10}O_{14}$ 1137.6591.

SCH 378199 (**3**): UV (MeOH) λ_{max}, nm: End absorption; FAB MS: *m/z* 1124 $(M+H)^+$; HRFABMS: Measured 1124.6262 $(M+H)^+$, Calcd. for $C_{56}H_{86}N_9O_{15}$ 1124.6243.

SCH 378167 (**4**): UV (MeOH) λ_{max}, nm: End absorption; FAB MS: *m/z* 1138 $(M+H)^+$; HRFABMS: Measured 1138.6422 $(M+Na)^+$, Calcd. for $C_{57}H_{88}N_9O_{15}$ 1138.6400.

Hydrolysis of **1**: SCH 378161 (**1**) (5 mg) was dissolved in 2 ml of 6 N HCl and the solution was heated at 95°C for 20 hours. The solution was diluted with water and lyophilized and the solids obtained were dissolved in 0.5 ml water, which was used for amino acid analysis[9].

Base hydrolysis of **1**: SCH 378161 (**1**) (2 mg) was dissolved in about 2 ml of 1 N NaOH and stirred for 20

hours at room temperature. Acidified the reaction mixture with dilute HCl and extracted with ethyl acetate. The organic extract was dried and the product (**5**) obtained was used for MS/MS fragmentation analysis

Esterification of **3**: SCH 378199 (**3**) (0.75 mg) was dissolved in about 0.5 ml MeOH. To this stirring solution was added excess diazomethane and stirred overnight at room temperature. Excess diazomethane was quenched using acetic acid and the reaction mixture was dried. The product was purified over HPLC (acetonitrile : water 65 : 35). FABMS of this compound showed it to be dimethyl ester 1153 $(M+H)^+$. This compound was dissolved in about 2 ml of 1 N NaOH and stirred for 20 hours. Acidified the reaction mixture with dilute HCl and extracted with ethyl acetate. The organic extract was dried, esterified with diazomethane and the product obtained was purified to yield 0.2 mg (**6**) and was used for MS/MS fragmentation analysis.

Esterification of **4**: SCH 378167 (**4**), (0.5 mg) was dissolved in about 0.5 ml MeOH. To this stirring solution was added excess diazomethane and stirred overnight at room temperature. Excess diazomethane was quenched using acetic acid and the reaction mixture was dried. The product was purified over HPLC (as above). FABMS of this compound showed it to be dimethyl ester.

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