A New Nonpeptide Tachykinin NK_1 Receptor Antagonist Isolated from the Plants of Compositae

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To find new tachykinin NK_1 receptor antagonists from natural sources, we examined the tachykinin antagonist activity in the extracts of approximately 200 species of plants by the use of isolated guinea pig ileum. As a result, we discovered a novel and potent NK_1 receptor antagonist in the extract of dried flowers of *Matricaria chamomilla* L. (chamomile). The structure of the antagonist was established as *N*1,*N*5,*N*10,*N*14-tetrakis[3-(4-hydroxyphenyl)-2-propenoyl]-1,5,10,14-tetraazatetradecane (tetracoumaroyl spermine, 1a). The K_i values of 1a, estimated from the inhibitory action on the substance P (SP)-induced contraction of the guinea pig ileum and the inhibition of the binding of [^{3}H][Sar^9, Met(O_2)^{11}]SP to human NK_1 receptors, were 21.9 nM and 3.3 nM, respectively. 1a is the first potent NK_1 receptor antagonist from natural sources and it has a unique structure of a polyacylated spermine. 1a was concentrated in pollen of *Matricaria chamomilla* L. and was also found in the extracts of flowers of other four species of Compositae. In addition, we found *N*1,*N*5,*N*10-tris[3-(4-hydroxyphenyl)-2-propenoyl]-1,5,10,14-tetraazatetradecane (2) as a new compound in the extract of flowers of *Matricaria chamomilla* L., which did not exhibit any tachykinin antagonist activity. A number of related compounds were synthesized, and the structure–activity relationship was studied.

Key words tachykinin antagonist; NK_1; chamomile; *Matricaria chamomilla*; Compositae

Since the report of the first nonpeptide tachykinin receptor antagonist, CP-96,345,[1] a large number of nonpeptide tachykinin antagonists have been reported. Among these, tachykinin NK_1 receptor antagonists are most numerous and probably most important because the NK_1 receptor is predominant in the human central nervous system,[2] and because the selective endogenous ligand for NK_1 receptor, substance P (SP), is most abundant among tachykinin peptides in both central and peripheral nervous systems of various species. It was shown that many tachykinin NK_1 receptor antagonists block the neurotransmitter action of SP mediated by NK_1 receptors.[3] Clinical application of NK_1 antagonists for various diseases has been a target of many recent studies, and indeed a successful treatment of depression and anxiety by an NK_1 antagonist, MK 806, was reported.[4]

Most of the NK_1 antagonists so far reported are synthetic, and a few of them are of microbial origin but of low potencies.[5,6] No potent tachykinin NK_1 antagonist from natural sources has been reported. In this paper we report a new potent tachykinin NK_1 receptor antagonist obtained from the extracts of plants of Compositae. A preliminary report has been published as a patent application No. P2000-256293A.[7]

Results

Extraction, Isolation and Structure Determination Dried flower heads of *Matricaria chamomilla* L. were extracted with MeOH at room temperature. A small portion of the MeOH extract was subjected to octadecyl silica (ODS) column chromatography. The obtained fractions were dried and submitted to the bioassay for tachykinin NK_1 receptor antagonist activity using the isolated guinea pig ileum. Figure 1 shows the chromatogram monitored by UV (300 nm) and the activities of the fractions. The major peak of active fractions, exhibiting the NK_1 antagonist activity, was eluted with the retention time from 10.8 to 12.4 min. These fractions, however, did not show any anticholinergic activity.

The residual major part of the MeOH extract, therefore, was partitioned between *n*-hexane and H_2O. The H_2O layer was further partitioned between *n*-BuOH and H_2O. The *n*-BuOH layer was subjected to a silica gel column chromatography. The active compound (**1a**) was obtained in pure form as a white powder by repeated reversed phase HPLC.

The molecular formula of **1a** was determined to be $C_{46}H_{50}N_4O_8$ from the result of elemental analysis and the electrospray ionization liquid chromatography mass spectrometry (ESI-LC-MS) spectrum giving a pseudo-molecular ion peak at *m*/*z* 785.43 ([M−H]$^-$).

Because of the complicated signals in ^{1}H-NMR spectrum caused by the conformers of **1a**, several modification and derivatization reactions were needed for the structure analysis of **1a**. The ^{1}H-NMR spectrum of **1a** showed 6 protons, which

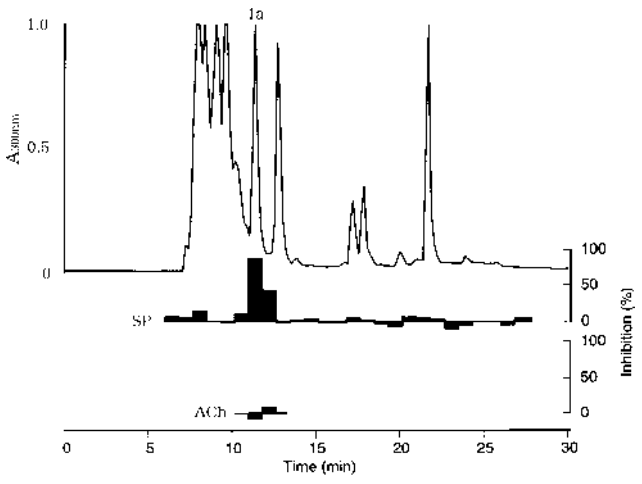

Fig. 1. Chromatogram of Methanol Extract of Flowers of *Matricaria Chamomilla* L.

Relative inhibitory activities of the eluted fractions on the contractile responses to SP (10 nM) and acetylcholine (ACh, 100 nM) of the guinea pig ileum are shown by black bars.

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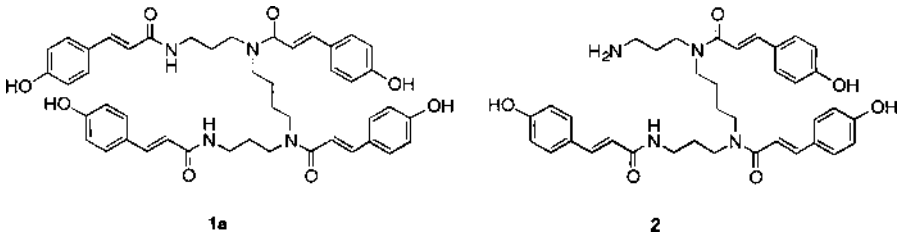


1a 2

Fig. 2. Chemical Structures of Coumaroyl Spermines, **1a** and **2**

were exchangeable with D_2O, at δ 9.69—9.92 (4H, m), and δ 7.94—7.98 and 8.05—8.11 (total 2H, m). Acetylation of **1a** with acetic anhydride in pyridine gave tetra-acetate (**4a**). In the ^{1}H-NMR spectrum of **4a**, signals at δ 9.69—9.92 (4H, m) of **1a** disappeared, whereas new signals at δ 2.24 (3H, s), 2.25 (3H, s), and 2.27 (6H, br s) were observed. The residual two protons of **4a** were at about δ 8, suggesting the presence of the amide protons.

On the other hand, catalytic hydrogenation of **1a** gave a single product (**6**) which showed pseudo-molecular ion at *m*/*z* 795 ($[M+H]^+$) in the secondary ion (SI)-MS spectrum. Since, as compared with **6**, **1a** possessed 8 more protons at δ 6.36—7.56 and 16 less protons at δ 2.24—3.02 in the ^{1}H-NMR spectrum, the presence of four olefins in **1a** was suggested. After acid hydrolysis of **6**, the hydrolysate was partitioned between H_2O and *n*-BuOH. The *n*-BuOH extract contained 3-(4-hydroxyphenyl)propanoic acid as a single compound, whose ^{1}H-NMR spectrum and retention time in HPLC analysis were identical with those of the authentic compound, whereas the H_2O extract contained spermine as a sole compound, which was identical with the authentic compound in ^{1}H-NMR spectra[5] and in HPLC analysis.[7]

The structure of **6** was, therefore, presumed to be *N*1,*N*5,*N*10,*N*14-tetrakis[3-(4-hydroxyphenyl)propanoyl]-1,5,10,14-tetraazatetradecane and confirmed by direct comparison of its *Rf* value in TLC (silica gel, MeOH–EtOAc, 1 : 9), retention time in HPLC, and ^{1}H-NMR chemical shifts with those of the synthetic material (see Experimental section).

Since **1a** possessed four olefins, the structure of **1a** was easily deduced to be *N*1,*N*5,*N*10,*N*14-tetrakis[3-(4-hydroxyphenyl)-2-propenoyl]-1,5,10,14-tetraazatetradecane (Fig. 2), which was confirmed by direct comparison with the synthetic sample in a manner similar to that for **6** (see Experimental section).

A ninhydrin positive analog of **1a**, designated as **2**, was also isolated from $CHCl_3$–MeOH–H_2O (10 : 5 : 1) eluates in silica gel column chromatography of the MeOH extract. The structure of **2** was deduced to be *N*1,*N*5,*N*10-tris[3-(4-hydroxyphenyl)-2-propenoyl]-1,5,10,14-tetraazatetradecane (Fig. 2) by instrumental analyses of trifluoroacetic acid (TFA) salt of **2**, its reduction product, and its acid-hydrolysates (see Experimental section).

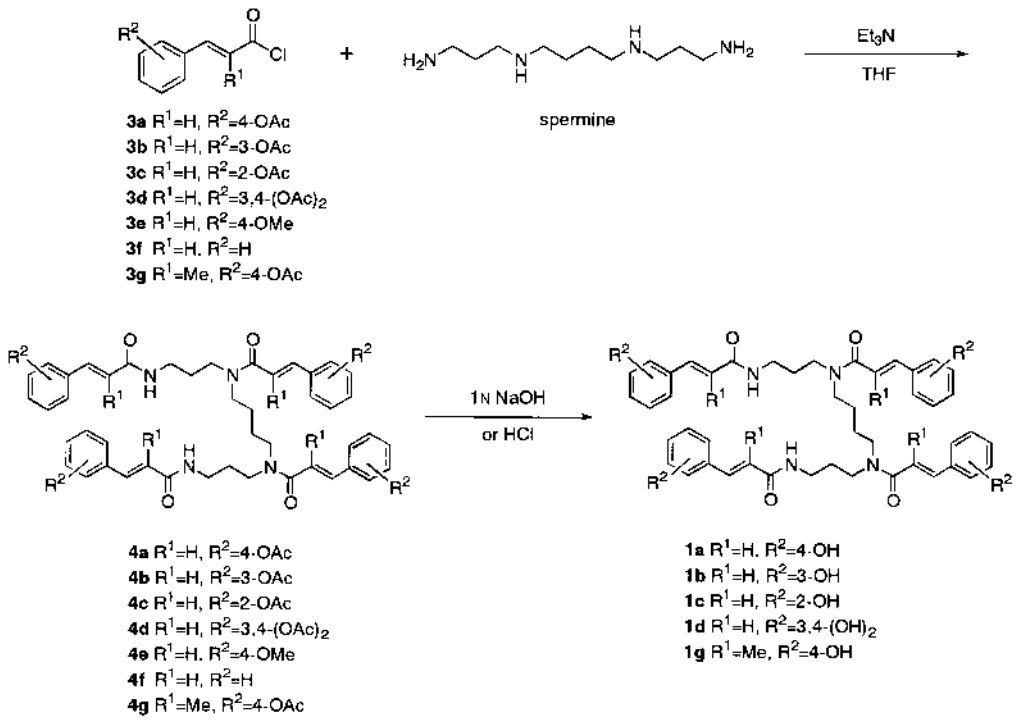
Distribution of Coumaroyl Spermines The extracts of about 200 species of plants were screened for NK_1 antagonist activity by the use of guinea pig ileum, and when the active extracts were found, the presence of **1a** was examined in the extracts by HPLC (see Experimental section). As a result, **1a** was found in flowers of 6 species of Compositae. In four species examined, **1a** was found in tubular but not in ligulate flowers. In both German and Roman chamomiles (*Matricaria chamomilla* L. and *Anthemis nobilis* L.), **1a** was specifically concentrated in pollen (Table 1). In addition, we found **2** in pollen and tubular flowers of both German and Roman chamomiles.

Table 1. Contents of **1a** and **2** in Flowers of the Plants of Compositae

Genus, species	Part	Source[a]	Contents (mg/g wet weight)	
			1a	**2**
Matricaria chamomilla L.	Pollen	A	8.92	8.60
	Tubular	A	0.49	0.55
	Ligulate	A	0.00	0.00
Anthemis nobilis L.	Pollen	A	16.31	28.15
	Tubular	A	0.48	0.85
	Ligulate	A	0.00	0.00
Cosmos bipinnatus Cav.	Tubular	A	1.48	0.00
	Ligulate	A	0.00	0.00
Erigeron annus L.	Tubular	B	0.20	n.d.[b]
	Ligulate	B	0.00	n.d.[b]
Inula britanica L.	Flower heads	C	0.86[c]	0.00
Tussilago farfara L.	Flower buds	C	1.05[c]	0.00

a) A: cultivated at the herb garden of Nippon Zoki Pharmaceutical Co., Ltd.; B: collected locally in Hyogo; C: purchased from Shigatensanbutsu Laboratory Co., Ltd. *b*) Not determined. *c*) mg/g dry weight.

Structure–Activity Relationship A number of compounds with structures related to that of **1a** were synthesized by the methods shown in Charts 1—3. Thus, the acid chlorides **3a**—**g**[8—12] and **5**[13] prepared from the corresponding carboxylic acids were condensed with spermine or its analogs to give the amides **4a**—**g**, whose protecting groups were saponified with 1 N NaOH to afford **1a**—**d**, **g**, **6**, **10**—**12**. The activities of these amides as tachykinin NK_1 receptor antagonists were estimated by their inhibitory actions on SP-induced contraction of the guinea pig ileum and by their competitive inhibition of the binding of [^{3}H][Sar^9, Met(O_2)^{11}]SP to human tachykinin NK_1 receptors expressed on Chinese hamster ovary (CHO) cells. The results for the selected 13 compounds are shown in Table 2. Most of the other derivatives were inactive. Modification of the coumaroyl group (**1b**—**d**, **g**, **4a**, **e**, **f**, **6**) as well as the spermine skeleton (**10**—**12**) resulted in reduction of activity. The results suggest that the structure of **1a** is considerably optimized for the antagonist activity on the guinea pig and human NK_1 receptors. In contrast with the inactivity of **2**, the activity of **10** was noteworthy indicating that the lack of one coumaroylaminopropyl moiety of **1a** does not abolish the NK_1 antagonist activity completely. This difference may be due to the difference between their stable conformations. A hypothesis was postulated that two phenyl rings with specific relative spatial orientation may be an important factor for the NK_1 antagonist activity.[14] **1a** and **10**, however, were too flexible to pre-

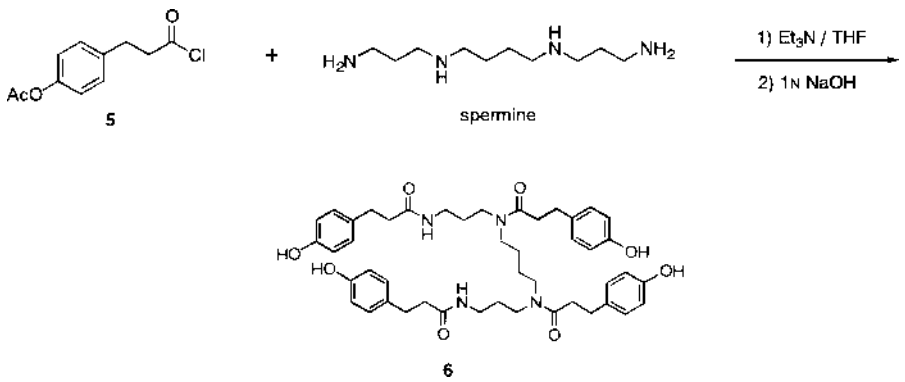


3a R^1=H, R^2=4-OAc
3b R^1=H, R^2=3-OAc
3c R^1=H, R^2=2-OAc
3d R^1=H, R^2=3,4-$(OAc)_2$
3e R^1=H, R^2=4-OMe
3f R^1=H, R^2=H
3g R^1=Me, R^2=4-OAc

4a R^1=H, R^2=4-OAc
4b R^1=H, R^2=3-OAc
4c R^1=H, R^2=2-OAc
4d R^1=H, R^2=3,4-$(OAc)_2$
4e R^1=H, R^2=4-OMe
4f R^1=H, R^2=H
4g R^1=Me, R^2=4-OAc

1a R^1=H, R^2=4-OH
1b R^1=H, R^2=3-OH
1c R^1=H, R^2=2-OH
1d R^1=H, R^2=3,4-$(OH)_2$
1g R^1=Me, R^2=4-OH

Chart 1



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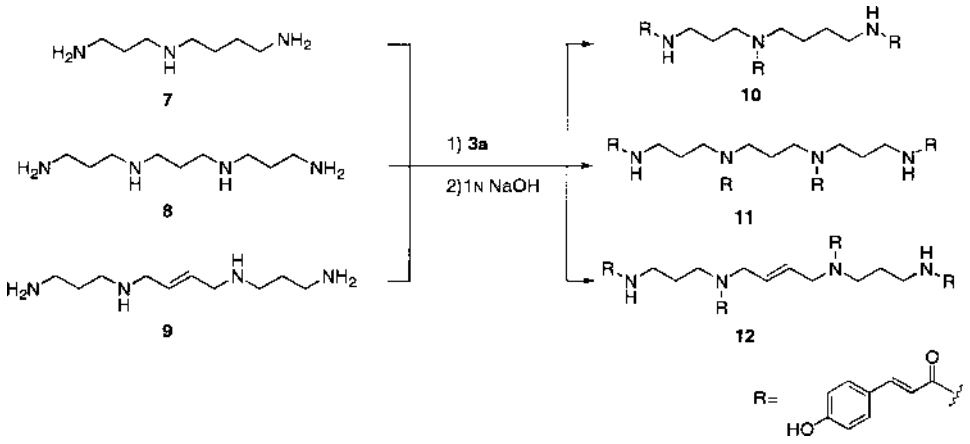
6

Chart 2



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8

9

10

11

12

Chart 3

Table 2. Tachykinin NK_1 Antagonist Activities of the Compounds

Compound	K_i (nM)[a]	
	Guinea pig ileum	Human NK_1 receptors on CHO cells
1a	21.9	3.3
1b	>1000.0	>1000.0
1c	>1000.0	>1000.0
1d	>1000.0	>1000.0
1g	367.1	53.3
2	>1000.0	>1000.0
4a	74.0	196.4
4e	>1000.0	>1000.0
4f	>1000.0	>1000.0
6	>1000.0	>1000.0
10	304.0	357.9
11	94.6	25.6
12	52.1	63.7

a) The concentration of the antagonist which displaces the log concentration response curve by log 2.

dict the active conformations by conformational analysis and to assign the roles of the coumaroyl groups.

Effects of 1a on the Receptors Other Than NK_1 In order to examine the specificity of the action of **1a** as an NK_1 receptor antagonist, its effects on other types of receptors were studied. **1a** at a concentration of 300 nM did not displace the concentration–contractile response curves on the guinea pig ileum to ACh, histamine and bradykinin. Moreover, **1a** altered neither the concentration–contractile response curves to neurokinin A (NK_2 receptor agonist) on the rat vas deferens, an NK_2 monoreceptor system,[15] nor the contraction of the rat portal vein, an NK_3 monoreceptor system,[15] evoked by senktide (NK_3 receptor agonist, 10 nM).

Discussion

The original purpose of the present study was to find in the plant kingdom clinically efficacious antagonists for tachykinin NK_1 receptors. In this respect, although **1a** is a potent NK_1 receptor antagonist, this compound appears unlikely to serve as a clinically useful drug for the following reasons. Firstly, the solubility of **1a** in water and human plasma is extremely low. The estimated maximal concentration of **1a** in the human plasma after deproteination was around 10 nM, and when **1a** was dissolved in non-deproteinated human plasma, it did not display any inhibitory action on the binding of [^{3}H][Sar^9, Met(O_2)^{11}]SP to human NK_1 receptors expressed on CHO cells. Secondly, when **1a**, dissolved in 0.5% carboxymethyl cellulose sodium salt solution, was orally administered in rats, **1a** was not detected in the blood. It is noteworthy that several commercially available preparations of chamomile were found to contain significant amounts of **1a**, which, if it were absorbed efficiently from gastrointestinal tract, could afford a significant concentration in the blood. However, when these preparations were orally administered in rats, again **1a** was not detected in the blood (unpublished observation). Therefore, it seems unlikely that the effects of the chamomile preparations administered orally in humans, such as anti-inflammatory and anti-asthmatic effects, involve the inhibition of tachykinin NK_1 receptors induced by **1a**.

In conclusion, we have discovered a new nonpeptide tachykinin NK_1 antagonist, **1a**, in the extracts of the plants of Compositae. Compound **1a** is the first alkaloid with a potent NK_1 receptor antagonist activity from natural sources and has a unique structure, which is entirely different from those of the previously reported synthetic NK_1 antagonists.

Experimental

General Melting points were determined on a Yamato MP-21 melting point apparatus and are uncorrected. UV spectra were recorded on a Beckman DU 650 spectrophotometer and IR spectra on a Horiba FT-200 Fourier transform (FT) IR spectrophotometer. ^{1}H-NMR spectra were taken on a Bruker ARX-500 (500 MHz) spectrometer. Chemical shifts are reported in δ units (parts per million downfield from internal tetramethylsilane). MS were measured on a Micromass Q-Tof or a Hitachi M-80B mass spectrometer. High resolution (HR)-ESI-MS was measured with Bruker Daltonics Matrix-Assisted Laser Desorption Ionization (MALDI)-Time-of-Flight (TOF)-MS: reflex III. The elemental analyses (C, H, N) were performed on a Yanaco CHN Corder MT-5 elemental analyzer. TLC was performed by using Silica gel 60F_{254} (Merck). Column chromatography was performed with Silica gel 60 (70—230 mesh, Merck). HPLC was performed on a Jasco system (pump, PU-920; detectors, UV-970 and FP-970; auto-sampler, 851-AS; controller, 802-SC; column oven, 860-CO; solvent mixing module, HG-980-31). Evaporation of the solvent was carried out under reduced pressure. Anhydrous Na_2SO_4 was employed as the drying agent.

Plant Materials Dried plants, *Inula britanica* L. and *Tussilago farfara* L., were obtained from commercial sources (Shigatensanbutsu Laboratory Co., Ltd., Shiga, Japan; Kishida Chemical Co., Ltd.). On the other hand, the fresh plants were obtained as follows; *Erigeron annus* L. was collected in Hyogo in June, *Matricaria chamomilla* L. and *Anthemis nobilis* L. were grown in the garden of our institute. They were seeded in September 1999 and were harvested between June and July 2000. *Cosmos bipinnatus* Cav. was also grown in the garden of our institute. It was seeded in April 2000 and was harvested in September 2000.

Extraction and Isolation The dried flower heads of *Matricaria chamomilla* L. (400 g) were extracted with MeOH (3 l×3 times) at room temperature. After filtration, the MeOH solution was evaporated to give the residue (78.8 g).

A portion of the residue (20 mg) was subjected to preparative HPLC [column, STR PREP-ODS, 20×250 mm; eluent, 0.1% TFA–MeOH (30 : 70) (0—6 min), 0.1% TFA–MeOH (30 : 70)→MeOH (6—16 min, linear gradient), MeOH (16—30 min); flow rate, 8 ml/min; column temperature, ambient; detection, 300 nm] and fractionated. 2% aliquot of each fraction was dried and dissolved in 10 μl dimethylsulfoxide (DMSO), and added to 5 ml Tyrode solution followed by application to the ileum (Fig. 1).

A residual part of the MeOH extract (78 g) was suspended in water (1 l) and extracted with *n*-hexane (0.5 l). The obtained aqueous layer was further extracted with *n*-BuOH (0.5 l). The *n*-BuOH extract (20.5 g) was subjected to silica gel column chromatography (5×40 cm), and elution was carried out in steps with a mobile phase of $CHCl_3$–MeOH (10 : 1, yield 5.8 g), $CHCl_3$–MeOH (4 : 1, yield 13.1 g), and $CHCl_3$–MeOH–H_2O (10 : 5 : 1, yield 1.8 g).

The $CHCl_3$–MeOH (4 : 1) eluate (13.0 g) was further separated repeatedly by HPLC [STR PREP-ODS, 20×250 mm, Shimadzu Techno Research Inc., MeOH–0.1% TFA (6 : 4)] and gave an active fraction (299 mg). The active fraction was concentrated and then crystallized from MeOH–H_2O to give **1a** (270 mg) as white powder: mp 139—141 °C (dec.). ^{1}H-NMR (DMSO-d_6) δ: 1.45—1.80 (8H, m), 3.12—3.55 (12H, m), 6.36—6.44 (2H, m), 6.68—6.94 (10H, m), 7.29—7.56 (12H, m), 7.94—7.98, 8.05—8.11 (total 2H, m), 9.69—9.92 (4H, m). UV λ_{max} (MeOH) nm (log ε): 227 (4.70), 299 (4.93), 311 (4.94). IR (KBr) cm^{-1}: 3300, 1650, 1606, 1514, 1440, 1234, 1169. Negative ion ESI-MS: *m/z* 785 $(M-H)^-$, 665 $(M-H-120)^-$, 639 $(M-H-146)^-$. Positive ion ESI-MS: *m/z* 787 $(M+H)^+$, 642, 623. *Anal.* Calcd for $C_{46}H_{50}N_4O_8\cdot H_2O$: C, 68.64; H, 6.51; N, 6.96. Found: C, 68.56; H, 6.47; N, 6.97.

The $CHCl_3$–MeOH–H_2O (10 : 5 : 1) eluate was chromatographed by HPLC [STR PREP-ODS, 20×250 mm, Shimadzu Techno Research Inc., MeOH–0.1% TFA (55 : 45)]. The fraction containing the main UV peak was concentrated and then precipitated with ether to give TFA salt of **2** (230 mg) as an yellow powder: mp 109—111 °C (dec.). ^{1}H-NMR (DMSO-d_6) δ: 1.43—1.86 (8H, m), 2.71—2.92 (2H, m), 3.13—3.56 (10H, m), 6.37—6.46 (1H, m), 6.70—6.97 (8H, m), 7.23—7.60 (9H, m), 7.68—7.76 (3H, br s), 8.00—8.13 (1H, m), 9.83—9.96 (3H, m). UV λ_{max} (MeOH) nm (log ε): 209 (4.41), 304 (4.55), 312 (4.58). IR (KBr) cm^{-1}: 3409, 1641, 1606, 1514, 1447, 1202, 1171. Positive ion SI-MS: (*m/z*) 641 $(M+H)^+$. Positive ion HR-

ESI-MS: (*m*/*z*) 641.3355 $(M+H)^+$, Calcd for $C_{37}H_{44}N_4O_6$+H, 641.3334.

Modification and Decomposition Reaction of 1a and 2. Acetylation of 1a A solution of **1a** (40 mg) was stirred with Ac_2O (5 ml) in pyridine (5 ml) at room temperature for 2 h. The reaction solution was concentrated and crystallized from MeOH–pyridine to give **4a** (26 mg) as a white powder: ^{1}H-NMR (DMSO-d_6) δ: 1.49—1.62 (4H, m), 1.70—1.81 (4H, m) , 2.24 (3H, s), 2.25 (3H, s), 2.27 (6H, br s), 3.14—3.54 (12H, m), 6.57—6.63 (2H, m), 7.00—7.06 (4H, m), 7.09—7.18 (6H, m), 7.40—7.53 (4H, m), 7.57—7.62 (4H, m), 7.64—7.79 (4H, m), 8.09—8.14 (1H, m), 8.21—8.26 (1H, m). UV λ_{max} (MeOH) nm: 218, 280. IR (KBr) cm^{-1}: 3400, 2900, 1760, 1639, 1623. Negative ion ESI-MS: *m*/*z* 953 $(M-H)^-$.

Catalytic Hydrogenation of 1a A solution of **1a** (80 mg) in dimethylformamide (DMF) (40 ml) was stirred in the presence of 10% Pd/C (5 mg) at room temperature for 4 h under an H_2 atmosphere (1 atm). The reaction mixture was filtered, and the filtrate was concentrated. The residue was purified by HPLC (Chemcosorb I-7C18, Chemco, 20×250 mm, 60% MeOH) to give **6** (65 mg) as a white amorphous solid: ^{1}H-NMR (DMSO-d_6) δ: 1.29—1.37 (4H, m), 1.48—1.59 (4H, m), 2.24—2.31 (4H, m), 2.43—2.51 (4H, m), 2.64—2.72 (8H, m), 2.94—3.08 (4H, m), 3.11—3.21 (8H, m), 6.61—6.66 (8H, m), 6.93—7.01 (8H, m), 7.71—7.75 (1H, m), 7.78—7.82 (1H, m), 9.14 (4H, br s). Positive ion SI-MS: *m*/*z* 795 $(M+H)^+$.

Acid Hydrolysis of 6 The compound **6** (25 mg) in 6 N HCl (1 ml) was heated at 110 °C for 24 h. The reaction mixture was concentrated and partitioned between H_2O (5 ml) and *n*-BuOH (5 ml). The H_2O layer and the *n*-BuOH layer were concentrated to yield an H_2O extract (21 mg) and *n*-BuOH extract (11 mg), respectively.

The H_2O Extract: ^{1}H-NMR (D_2O) δ: 1.79—1.86 (4H, m), 2.09—2.17 (4H, m), 3.12—3.22 (12H, m).

The *n*-BuOH Extract: ^{1}H-NMR (CD_3OD) δ: 2.53 (2H, t, *J*=7.5 Hz), 2.80 (2H, t, *J*=7.5 Hz), 6.69 (2H, dd, *J*=2.0, 8.0 Hz), 7.02 (2H, dd, *J*=2.0, 8.0 Hz).

HPLC Analysis of Hydrolysate of 6 The analysis of spermine was performed with the on-column method of Saito *et al.*[16] with minor modification. Briefly, a 1 μl aliquot of the H_2O layer was injected into a reverse phase column (YMC-Pack Polymer C18, YMC, 4.6×250 mm). The mobile phase was a mixture of 50 mM sodium borate buffer (pH 9.9)–acetonitrile (77 : 23, v/v) containing 2 mM *o*-phthalaldehyde and 2 mM *N*-acetyl-L-cysteine. The flow rate was adjusted to 0.8 ml/min and the peaks were detected at excitation and emission wavelengths of 330 and 430 nm, respectively. The separation was performed at a temperature of 40 °C. The water-soluble derivative thus obtained showed a single peak at 10.5 min, identical to that of authentic spermine.

The analysis of 3-(4-hydroxyphenyl)propanoic acid was carried out on YMC-Pack Polymer C18 (CH_3CN–10 mM phosphate buffer pH 2.0, 22 : 78) at a temperature of 30 °C. The mobile phase was delivered isocratically at a flow rate of 1.0 ml/min. The detection wavelength was set to 280 nm and a 4 μl aliquot of the *n*-BuOH layer was injected. A single peak at a retention time of 13.8 min was identical to that of authentic 3-(4-hydroxyphenyl)-propanoic acid.

Catalytic Hydrogenation of 2 and Acid Hydrolysis of Its Reduction Product A solution of **2** (17 mg) in MeOH (15 ml) was stirred with 10% Pd/C (5 mg) at room temperature for 4 h under an H_2 atmosphere. The reaction mixture was filtered, and the filtrate was concentrated. The residue gave a pseudo-molecular ion at *m*/*z* 647 $(M+H)^+$ in the SI-MS spectrum. A portion of the material was hydrolyzed in 6 N HCl (1 ml) at 110 °C for 24 h. The hydrolysate was concentrated and partitioned between H_2O (4 ml) and *n*-BuOH (4 ml). The analysis of each layer was performed by HPLC with the same conditions as for **6**. The H_2O layer was found to contain spermine, and the *n*-BuOH layer contained 3-(4-hydroxyphenyl)propanoic acid.

HPLC Analysis of Coumaroyl Spermines (1a, 2) Plant materials were powdered and extracted by percolation with 10 volume of MeOH at room temperature in the dark. The MeOH solution was concentrated, and the residue was dissolved in DMSO. The quantification of **1a** and **2** in the extracts was performed by HPLC using **1a** and **2** isolated from *Matricaria chamomilla* L. as standards (Develosil ODS-HG-5, 4.6×250 mm, Nomura Chemical, 50 mM phosphate buffer (pH 2.5)–MeOH (48 : 52), 1 ml/min, 40 °C, UV at 300 nm, t_R of **1a** and **2**: 10.4 and 4.1 min, respectively).

Synthesis. Preparation of the Acid Chlorides The acid chlorides **3a**,[8] **3b**,[9] **3c**,[10] **3d**,[11] **3e**,[12] and **5**[13] were prepared from the corresponding carboxylic acids by the reported procedures. 3-Phenyl-2-propenoyl chloride (**3f**) was obtained from Aldrich.

(*E*)-3-(4-Acetoxyphenyl)-2-methyl-2-propenoyl Chloride (3g) A mixture of 4-hydroxybenzaldehyde (9.3 g, 76.2 mmol), methylmalonic acid (18.0 g, 150 mmol), and piperidine (15 ml) in pyridine (10 ml) was stirred at 100 °C for 25 h. The reaction mixture was poured into 2 N HCl and extracted with EtOAc. The extract was washed with brine, dried, and condensed to give a solid, which was recrystallized from EtOAc to give (*E*)-3-(4-hydroxyphenyl)-2-methyl-2-propenoic acid (4.2 g, 27%) as white crystals: mp 210—213 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.02 (3H, s), 6.83 (2H, d, *J*=8.6 Hz), 7.35 (2H, d, *J*=8.6 Hz), 7.51 (1H, s), 9.82 (1H, s), 12.12 (1H, s). The acid obtained was acetylated with acetic anhydride (10 ml) in pyridine (50 ml) at room temperature for 5 h. The mixture was condensed and partitioned between EtOAc and water. The organic layer was washed with brine, dried, and subjected to silica gel column chromatography ($CHCl_3$–MeOH, 9 : 1) to give (*E*)-3-(4-acetoxyphenyl)-2-methyl-2-propenoic acid (3.6 g, 70%) as crystals: mp 142—144 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.03 (3H, s), 2.28 (3H, s), 7.20 (2H, d, *J*=8.2 Hz), 7.52 (2H, d, *J*=8.2 Hz), 7.59 (1H, s), 12.52 (1H, s). A mixture of the propenoic acid (3.4 g, 15.6 mmol) and thionyl chloride (5 ml) in CH_2Cl_2 was stirred at room temperature for 24 h. The reaction mixture was condensed to give quantitatively the acid chloride **3g** as a pale yellow oil, which was applied to the next step without further purification.

Condensation of Acid Chlorides with Spermine. *N*1,*N*5,*N*10,*N*14-Tetrakis[3-(4-acetoxyphenyl)-2-propenoyl]-1,5,10,14-tetraazatetradecane (4a) The acid chloride **3a** (4.7 g, 20.9 mmol) was added to a solution of spermine (1.0 g, 5.0 mmol) and Et_3N (2.1 g, 21.0 mmol) in dry tetrahydrofuran (THF) (80 ml) at 0 °C under an argon atmosphere. The mixture was stirred at an ambient temperature overnight and diluted with 10-fold volume of water. The precipitated solid was filtered, washed with water, and dried to give **4a** as a white solid (4.0 g, 83% yield): mp 171—173 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.49—1.62 (4H, m), 1.70—1.81 (4H, m), 2.24 (3H, s), 2.25 (3H, s), 2.27 (6H, 2s), 3.14—3.54 (12H, m), 6.57—6.63 (2H, m), 7.00—7.06 (4H, m), 7.09—7.18 (6H, m), 7.40—7.53 (4H, m), 7.57—7.62 (4H, m), 7.64—7.79 (4H, m), 8.09—8.14 (1H, m), 8.21—8.26 (1H, m). *Anal.* Calcd for $C_{54}H_{58}N_4O_{12}$: C, 66.35; H, 6.24; N, 5.73. Found: C, 66.42; H, 6.18; N, 5.75.

*N*1,*N*5,*N*10,*N*14-Tetrakis[3-(3-acetoxyphenyl)-2-propenoyl]-1,5,10,14-tetraazatetradecane (**4b**): mp 85—90 °C. Yield 58%. ^{1}H-NMR (DMSO-d_6) δ: 1.44—1.62 (4H, m), 1.64—1.80 (4H, m), 2.26 (6H, s), 2.28 (6H, s), 3.16—3.25 (4H, m), 3.39—3.45 (4H, m), 3.47—3.55 (4H, m), 6.60—6.66 (2H, m), 7.05—7.21 (6H, m), 7.29—7.33 (3H, m), 7.39—7.61 (13H, m), 8.11—8.20 (2H, m). *Anal.* Calcd for $C_{54}H_{58}N_4O_{12}\cdot H_2O$: C, 66.65; H, 6.22; N, 5.76. Found: C, 66.45; H, 6.22; N, 5.77.

*N*1,*N*5,*N*10,*N*14-Tetrakis[3-(2-acetoxyphenyl)-2-propenoyl]-1,5,10,14-tetraazatetradecane (**4c**): mp 94—96 °C. Yield 31%. ^{1}H-NMR (DMSO-d_6) δ: 1.46—1.64 (m, 4H), 1.67—1.82 (m, 4H), 2.29 (s, 3H), 2.30—2.38 (m, 9H), 3.13—3.27 (m, 4H), 3.34—3.44 (m, 4H), 3.46—3.58 (m, 4H), 6.61—6.68 (m, 2H), 7.06—7.56 (m, 18H), 7.61—7.69 (m, 2H), 7.83—8.02 (m, 2H), 8.17—8.31 (m, 2H). *Anal.* Calcd for $C_{54}H_{58}N_4O_{12}\cdot H_2O$: C, 66.65; H, 6.22; N, 5.76. Found : C, 66.67; H, 6.24; N, 5.94.

*N*1,*N*5,*N*10,*N*14-Tetrakis[3-(3,4-diacetoxyphenyl)-2-propenoyl]-1,5,10,14-tetraazatetradecane (**4d**): mp 89—94 °C. Yield 87%. ^{1}H-NMR (DMSO-d_6) δ: 1.48—1.60 (4H, m), 1.66—1.79 (4H, m), 2.26—2.31 (24H, m), 3.16—3.54 (12H, m), 6.56—6.63 (2H, m), 7.07—7.66 (18H, m), 8.08—8.13 (1H, m), 8.16—8.22 (1H, m). *Anal.* Calcd for $C_{62}H_{66}N_4O_{20}\cdot 0.65H_2O$: C, 62.11; H, 5.66; N, 4.67. Found: C, 62.14; H, 5.69; N, 4.65.

*N*1,*N*5,*N*10,*N*14-Tetrakis[3-(4-methoxyphenyl)-2-propenoyl]-1,5,10,14-tetraazatetradecane (**4e**): mp 145—149 °C. Yield 66%. ^{1}H-NMR (DMSO-d_6) δ: 1.36—1.76 (8H, m), 3.17—3.51 (12H, m), 3.65 (3H, s), 3.75—3.82 (9H, m), 6.49—6.63 (2H, m), 6.79—7.03 (10H, m), 7.38—7.64 (12H, m), 8.01—8.04 (1H, m), 8.16—8.18 (1H, m).

*N*1,*N*5,*N*10,*N*14-Tetrakis(3-phenyl-2-propenoyl)-1,5,10,14-tetraazatetradecane (**4f**): mp 173—182 °C. Yield 86%. ^{1}H-NMR (DMSO-d_6) δ: 1.51—1.61 (4H, m), 1.71—1.79 (4H, m), 3.18—3.54 (12H, m), 6.60—6.63 (2H, m), 7.08—7.19 (2H, m), 7.29—7.72 (24H, m), 8.14—8.16 (1H, m), 8.22—8.24 (1H, m). *Anal.* Calcd for $C_{46}H_{50}N_4O_4\cdot 0.5H_2O$: C, 75.60; H, 7.05; N, 7.45. Found: C, 75.49; H, 7.02; N, 7.65.

*N*1,*N*5,*N*10,*N*14-Tetrakis[3-(4-acetoxyphenyl)-2-methyl-2-propenoyl]-1,5,10,14-tetraazatetradecane (**4g**): An amorphous solid. Yield 98%. ^{1}H-NMR (DMSO-d_6) δ: 1.48—2.10 (8H, m), 2.26 (6H, s), 3.08—3.42 (12H, m), 6.41 (2H, br s), 7.02—7.45 (18H, m), 8.06 (2H, br s).

***N*1,*N*5,*N*10,*N*14-Tetrakis[3-(4-hyroxyphenyl)-2-propenoyl]-1,5,10,14-tetraazatetradecane (1a)** The compound **4a** (954 mg, 1.0 mmol) was hydrolyzed with 1 N NaOH (4.8 ml) at room temperature for 1 h. The reaction mixture was neutralized with 1 N HCl (4.8 ml) and the solid precipitated was filtered, washed with water, and dried to give **1a** (676 mg, 86%) as a white solid: mp 138—140 °C (dec). ^{1}H-NMR (DMSO-d_6) δ: 1.45—1.80 (8H, m), 3.12—3.55 (12H, m), 6.36—6.44 (2H, m), 6.68—6.94 (10H, m), 7.29—7.56 (12H, m), 7.94—7.98 (1H, m), 8.05—8.11 (1H, m), 9.69—9.92 (4H, m).

Anal. Calcd for $C_{46}H_{50}N_4O_8 \cdot H_2O$: C, 68.64; H, 6.51; N, 6.96. Found: C, 68.83; H, 6.47; N, 6.90.

*N*1,*N*5,*N*10,*N*14-Tetrakis[3-(3-hyroxyphenyl)-2-propenoyl]-1,5,10,14-tetraazatetradecane (**1b**): mp 106 °C (dec.). Yield 88%. ^{1}H-NMR (DMSO-d_6) δ: 1.50—1.77 (8H, m), 3.17—3.52 (12H, m), 6.54 (2H, d, J=15.8 Hz), 6.74—6.78 (4H, m), 6.96—7.20 (14H, m), 7.32—7.42 (4H, m), 8.11—8.21 (2H, m), 9.51—9.55 (4H, m). *Anal.* Calcd for $C_{46}H_{50}N_4O_8 \cdot H_2O$: C, 68.64; H, 6.51; N, 6.96. Found: C, 68.61; H, 6.60; N, 6.89.

*N*1,*N*5,*N*10,*N*14-Tetrakis[3-(2-hyroxyphenyl)-2-propenoyl]-1,5,10,14-tetraazatetradecane (**1c**): mp 106 °C (dec.). Yield 82%. ^{1}H-NMR (DMSO-d_6) δ: 1.56—1.77 (8H, m), 3.17—3.57 (12H, m), 6.20 (2H, br s), 6.64—7.41 (22H, m), 7.67—7.81 (2H, m), 10.65 (4H, br s). *Anal.* Calcd for $C_{46}H_{50}N_4O_8 \cdot 5H_2O$: C, 63.00; H, 6.90; N, 6.39. Found: C, 62.79; H, 6.74; N, 6.08.

*N*1,*N*5,*N*10,*N*14-Tetrakis[3-(3,4-dihyroxyphenyl)-2-propenoyl]-1,5,10,14-tetraazatetradecane (**1d**): mp 159—163 °C (dec.). Yield 74%. ^{1}H-NMR (DMSO-d_6) δ: 1.49—1.76 (8H, m) , 3.16—3.53 (12H, m), 6.24—6.46 (2H, m), 6.70—7.43 (18H, m), 7.66—7.98 (1H, m), 7.99—8.02 (1H, m), 9.08 (4H, br s), 9.47 (4H, br s). *Anal.* Calcd for $C_{46}H_{50}N_4O_{12} \cdot 1.25H_2O$: C, 63.26; H, 6.06; N, 6.41. Found: C, 63.26; H, 6.14; N, 6.32.

*N*1,*N*5,*N*10,*N*14-Tetrakis[3-(4-hyroxyphenyl)-2-methyl-2-propenoyl]-1,5,10,14-tetraazatetradecane (**1g**): mp 158—162 °C. Yield 99%. ^{1}H-NMR (DMSO-d_6) δ: 1.51 (4H, br s), 1.73 (4H, br s), 1.93 (12H, br s), 3.14 (4H, br s), 3.34 (8H, br s), 6.30 (4H, br s), 6.75—6.81 (8H, m), 7.05—7.19 (12H, m), 7.93 (2H, br s). *Anal.* Calcd for $C_{50}H_{58}N_4O_8$: C, 71.24; H, 6.93; N, 6.65. Found: C, 70.45; H, 7.18; N, 5.64.

Preparation of 6, 10, 11, and 12. *N*1,*N*5,*N*10,*N*14-Tetrakis[3-(4-hydroxyphenyl)propanoyl]-1,5,10,14-tetraazatetradecane (6) The acid chloride (**5**) (3.4 g, 15.0 mmol) was added to a solution of spermine (606 mg, 3.0 mmol) and Et_3N (1.5 g, 15.0 mmol) in THF (20 ml) . The mixture was stirred at room temperature overnight and filtered to remove insoluble solid. The filtrate was condensed to give a residue, which was purified by silica gel column chromatography ($CHCl_3$–MeOH, 24 : 1) to give *N*1,*N*5,*N*10,*N*14-tetrakis[3-(4-acetoxyphenyl)propanoyl]-1,5,10,14-tetraazatetradecane as a colorless oil in quantitative yield.

The oil obtained above was stirred with 1 N NaOH (14.4 ml) in MeOH (20 ml) at room temperature for 1 h. The reaction mixture was neutralized with 1 N HCl and concentrated. After addition of MeOH to the residue, insoluble NaCl was filtered and the filtrate was purified by HPLC (Chemcosorb I-7C18, 20×250 mm, 60% MeOH) to give **6** (1.45 g, 61%) as a white amorphous solid. ^{1}H-NMR (DMSO-d_6) δ: 1.29—1.37 (4H, m), 1.48—1.59 (4H, m), 2.24—2.31 (4H, m), 2.43—2.51 (4H, m), 2.64—2.72 (8H, m), 2.94—2.30 (4H, m), 3.11—3.21 (8H, m), 6.61—6.66 (8H, m), 6.93—7.01 (8H, m), 7.71—7.75 (1H, m), 7.78—7.82 (1H, m), 9.14 (4H, br s).

*N*1,*N*5,*N*10-Tris[3-(4-hydroxyphenyl)-2-propenoyl]-1,5,10-triazadecane (**10**): mp 138—140 °C. Yield 95%. ^{1}H-NMR (DMSO-d_6) δ: 1.39—1.79 (6H, m), 3.14—3.50 (8H, m), 6.36—6.44 (2H, m), 6.71 (1H, d, J=8.3 Hz), 6.75—6.91 (7H, m), 7.29—7.40 (6H, m), 7.49 (1H, d, J=8.3 Hz), 7.54 (1H, d, J=8.3 Hz), 7.96—8.11 (2H, m), 8.79—9.83 (3H, m). *Anal.* Calcd for $C_{34}H_{37}N_3O_6 \cdot 0.8H_2O$: C, 68.25; H, 6.42; N, 6.99. Found: C, 68.28; H, 6.51; N, 7.03.

*N*1,*N*5,*N*9,*N*13-Tetrakis[3-(4-hydroxyphenyl)-2-propenoyl]-1,5,9,13-tetraazatridecane (**11**): mp 123 °C (dec.). Yield 78%. ^{1}H-NMR (DMSO-d_6) δ: 1.69—1.89 (6H, m), 3.15—3.51 (12H, m), 6.38—6.43 (2H, m), 6.71—6.86 (10H, m), 7.30—7.50 (12H, m), 7.97—8.10 (2H, m), 9.83 (4H, br s). *Anal.* Calcd for $C_{45}H_{48}N_4O_8 \cdot 1.5H_2O$: C, 67.57; H, 6.43; N, 7.00. Found: C, 67. 67; H, 6.43; N, 7.01.

*N*1,*N*5,*N*10,*N*14-Tetrakis[3-(4-hydroxyphenyl)-2-propenoyl]-1,5,10,14-tetraaza-7-tetradecene (**12**): mp *ca.* 126 °C (dec.). Yield 99%. ^{1}H-NMR (DMSO-d_6) δ: 1.67—1.74 (4H, m), 3.15—3.39 (8H, m), 3.99—4.13 (4H, m), 5.56—5.70 (2H, m), 6.38—6.44 (2H, m), 6.70—6.85 (10H, m), 7.30—7.49 (12H, m), 7.95—8.07 (2H, m), 9.84 (4H, m). *Anal.* Calcd for $C_{46}H_{48}N_4O_8 \cdot 2H_2O$: C, 67.30; H, 6.38; N, 6.82. Found: C, 67.56; H, 6.23; N, 6.89.

Bioassays The antagonist activities for tachykinin NK_1 receptor of chromatographic fractions of plant extracts and synthetic compounds were determined using the isolated ileum of the guinea pig as described previously.[17] Samples to be tested were added to the bath as a DMSO solution 5 min before the application of SP. Contractile responses were recorded isotonically and were expressed as percentages of the maximal response to 10 nM SP. In order to eliminate the anticholinergic and anti-histamine activities as well as the interference of prostaglandins, the Tyrode solution used for the experiments contained atropine 4 μM, mepyramine 4 μM, and indomethacin 3.6 μM.

To examine the specificity of the NK_1 antagonist action of **1a**, its effects on the concentration–contractile response curve produced by acetylcholine in the absence of atropine, that produced by histamine in the absence of mepyramine and that produced by bradykinin in normal Tyrode solution were evaluated in the guinea pig ileum.

The rat vas deferens and the rat portal vein were used to examine the receptor subtype selectivity of **1a**.[15] The methods used for experiments are the same as previously described.[17]

Binding Assay with Human Tachykinin NK_1 Receptors A CHO cell line expressing human tachykinin NK_1 receptors was established according to the procedures described previously[18] and was a generous gift from Amersham Pharmacia Biotech (Tokyo). The CHO cells were cultured in alpha minimum essential medium (α-MEM) supplemented with 100 IU/ml penicillin, 100 μg/ml streptomycin and 10% fetal bovine serum. Cultures were kept at 37 °C in a humidified atmosphere of 5% CO_2. Binding assays were carried out on whole cells. Cells grown to confluence in culture flask were detached with trypsin–EDTA (0.25% trypsin, 1 mM EDTA·Na) solution (GIBCO). Collected cells were washed twice with 50 mM Tris–Cl buffer (pH 7.5) containing 150 mM NaCl and 0.02% bovin serum albumin (BSA). The pellet was then resuspended in 50 mM Tris–Cl buffer containing 150 mM NaCl, 0.02% BSA, 40 μg/ml bacitracin (Sigma), 4 μg/ml leupeptin (Sigma), 4 μg/ml chymostatin (Sigma) and 4 μg/ml phosphoramidon (Sigma). CHO cells (10^6 cells) were incubated in 500 μl of buffer containing [^{3}H]-[Sar^9,Met(O$_2$)^{11}]SP (NEN Life Science Products, Inc.; final conc. 0.3 nM, 39.5 Ci/mmol) and one of the test compounds at room temperature for 60 min and then filtered over GF/B filters (Whatman) that had been presoaked in 0.1% polyethylenimine (Wako). The radioactivities in the filters were determined after addition of scintillator (AL-1, Dojindo).

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