Spiro-Substituted Piperidines as Neurokinin Receptor Antagonists. III.[1] Synthesis of (±)-*N*-[2-(3,4-Dichlorophenyl)-4-(spiro-substituted piperidin-1′-yl)butyl]-*N*-methylbenzamides and Evaluation of NK_1–NK_2 Dual Antagonistic Activities

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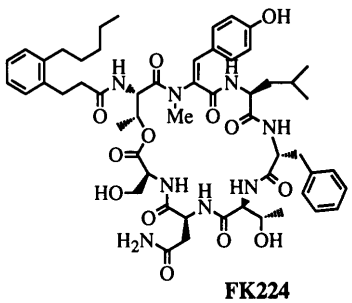
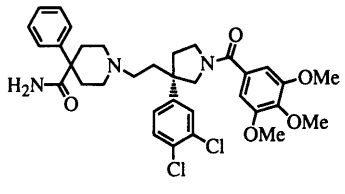
To discover a novel NK_1–NK_2 dual antagonist, we have synthesized a series of spiro-substituted piperidines utilizing YM-35375 as a lead compound, and evaluated affinities for NK_1 and NK_2 receptors. In the *N*-methylbenzamide moiety, introduction of methoxy groups increased affinity for the NK_1 receptor without a significant loss of affinity for the NK_2 receptor. We also found that a conformation in which the phenyl groups of the *N*-methylbenzamide and 3,4-dichlorophenyl moieties are close to each other through a cis-amide bond, may be favorable for showing high affinity for the NK_1 receptor and that a hydrogen bond-accepting group in the spiro-substituted piperidine moiety may be crucial for exhibiting high affinity for the NK_2 receptor. Among the compounds prepared, YM-44778 (31) showed high and well-balanced affinity for NK_1 and NK_2 receptors (IC_{50} values of 18 and 16 nM, respectively). This compound also exhibited potent antagonistic activities against both NK_1 and NK_2 receptors (IC_{50} values of 82 and 62 nM, respectively) in isolated tissues.

Key words NK_1–NK_2 dual antagonist; YM-44778; YM-35375; conformational analysis; NK_1 receptor; NK_2 receptor

Tachykinins[2] are peptides possessing closely homologous carboxy termini. The mammalian tachykinins, substance P (SP),[3] neurokinin A (NKA)[4] and neurokinin B (NKB),[4] have been classified into the neurokinin family and produce their physiological and pharmacological effects by binding to and activating their receptors. Neurokinin receptors are now classified into three subtypes, NK_1, NK_2 and NK_3, which have high affinity to SP, NKA and NKB, respectively.[5] Recently, it was reported that SP and NKA were released from the endings of sensory nerves by a variety of stimulants in the airway of asthmatic patients and caused the physiological features of asthma.[6] SP is thought to cause microvascular leakage and mucus hypersecretion,[7] and NKA induces bronchoconstriction.[7,8] Antagonizing both NK_1 and NK_2 receptors may be effective in preventing these functions in asthmatic patients, and an NK_1–NK_2 dual antagonist may be useful as an antiasthmatic drug. Indeed, the peptidic NK_1–NK_2 dual antagonist FK224 (Fig. 1)[9] has been evaluated for its clinical efficacy. In addition, the first non-peptide NK_1–NK_2 dual antagonist MDL-105212 (Fig. 1) was reported in 1996.[10]



FK224



MDL-105212

Fig. 1. Structures of FK224 and MDL-105212

In our previous studies on novel neurokinin receptor antagonists,[1] we designed the spiro[isobenzofuran-1(3*H*), 4′-piperidine] derivative, YM-35375 (**1**, Fig. 2), which showed moderate affinity for both NK_1 and NK_2 receptors (IC_{50} values of 710 and 84 nM, respectively). Structural modification of this compound led to discovery of the potent and selective NK_2 receptor antagonist YM-38336 (**2**).[1] During the course of this study,[1] we also found that some modifications resulted in a change in selectivities between the NK_1 and NK_2 receptors and that YM-35384 (**3**) showed high affinity for both of these receptors with IC_{50} values at the 10^{-8} M level. These results suggested that we may be able to find a more potent and well-balanced NK_1–NK_2 dual antagonist by further study of the structure–activity relationships of a series

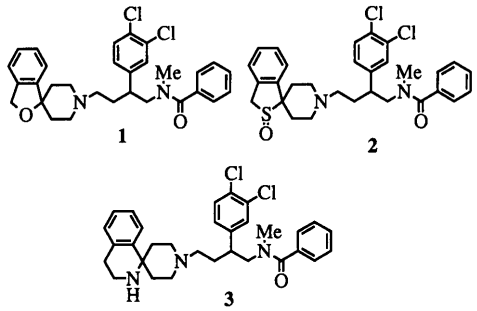


Fig. 2. Structures of YM-35375 (**1**), YM-38336 (**2**) and YM-35384 (**3**)

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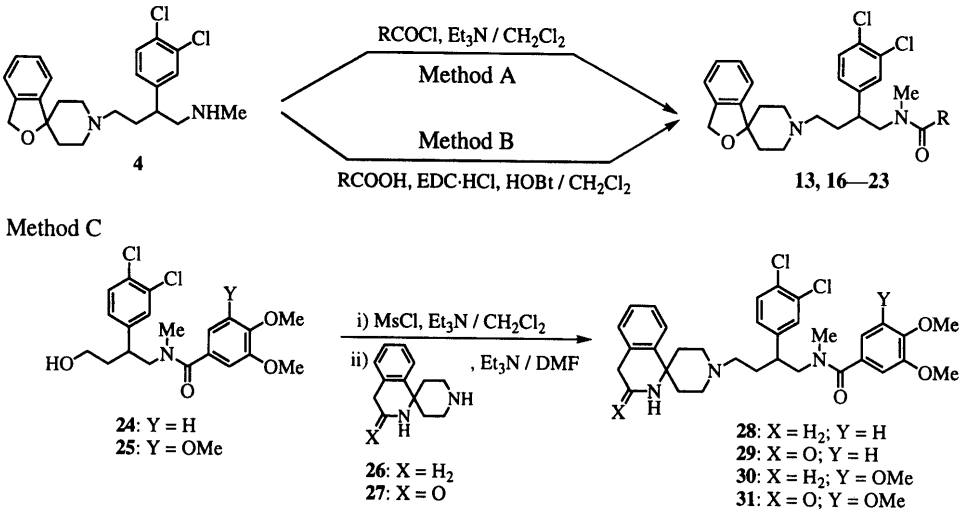


Chart 1

of 2-(3,4-dichlorophenyl)-4-(spiro-substituted piperidin-1′-yl)butylcarboxamides. In order to prepare a novel NK_1–NK_2 dual antagonist, we examined the structure–activity relationships of the *N*-methylbenzamide moiety of YM-35375, followed by structural modification of the spiro-substituted piperidine moiety. We describe here the synthesis, structure–activity relationships and pharmacological properties of these novel spiro-substituted piperidines (**5**—**23**, **28**—**31**).

Chemistry

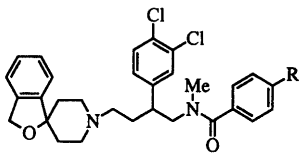
Some of the desired spiro-substituted piperidines (**5**—**12**, **14**, **15**) were prepared in the previous report.[1] Compounds **13**, **16**—**23** and **28**—**31** were synthesized as shown in Chart 1. (±)-1′-[3-(3,4-Dichlorophenyl)-4-methylaminobutyl]spiro-[isobenzofuran-1(3*H*),4′-piperidine] (**4**)[1] was treated with acid chlorides in the presence of triethylamine (Et_3N) to give compounds **13**, **16**—**20** and **23** (method A in Chart 1). Compound **4** was converted to compounds **21** and **22** with the respective carboxylic acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) and 1-hydroxybenzotriazole (HOBt) in dichloromethane (method B in Chart 1). (±)-*N*-[2-(3,4-Dichlorophenyl)-4-hydroxybutyl]-3,4-dimethoxy-*N*-methylbenzamide (**24**) and (±)-*N*-[2-(3,4-dichlorophenyl)-4-hydroxybutyl]-3,4,5-trimethoxy-*N*-methylbenzamide (**25**) were prepared according to the methods in the literature[1] and treatment of these compounds with methanesulfonyl chloride (MsCl) followed by reaction with the spiro-substituted piperidines (**26**, **27**),[1] in the presence of Et_3N gave the desired compounds **28**—**31** (method C in Chart 1). The synthetic details and physical properties of compounds **16**—**23** and **28**—**31** are summarized in Tables 2, 3 and 6.

Results and Discussion

The obtained compounds were evaluated for their binding affinities[11] to guinea pig urinary bladder NK_1 receptors and hamster urinary bladder NK_2 receptors.

In order to examine the substituent effects on the NK_1 and NK_2 receptor affinity for the *N*-methylbenzamide moiety, compounds substituted at the 4-position of the phenyl group were evaluated. As shown in Table 1, electron-donating groups (**5**—**7**), halogens (**10**—**12**) and electron-withdrawing groups (**13**—**15**) have no influence on affinity for both NK_1 and NK_2 receptors, except for dimethylamino (**7**) and trifluoromethyl groups (**13**), which had decreased affinity for these receptors. On the other hand, acetamido and amino groups (**8**, **9**), which could act as both electron-donating and hydrogen bond-donating groups, had increased affinity for the NK_2 receptor[1] and decreased affinity for the NK_1 receptor. Unfortunately, no substituents at this position increased affinity for both NK_1 and NK_2 receptors, as shown.

Table 1. Biological Properties of (±)-*N*-[2-(3,4-Dichlorophenyl)-4-(spiro-[isobenzofuran-1(3*H*), 4′-piperidin]-1′-yl)butyl]-*N*-methylbenzamides

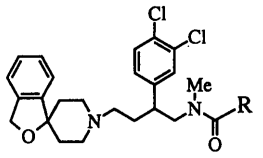


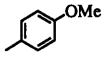
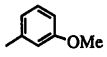
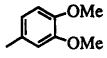
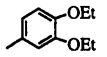
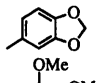
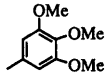
Compd. No.	R	Binding affinity IC_{50} (nM)	
		NK_1[a]	NK_2[b]
1	H	710	84
5[c]	Me	950	57
6[c]	OMe	1100	79
7[c]	NMe_2	4100	240
8[c]	NHAc	2100	24
9[c]	NH_2	1300	38
10[c]	F	540	110
11[c]	Cl	610	120
12[c]	Br	890	120
13	CF_3	1600	320
14[c]	NO_2	670	95
15[c]	CN	460	65

a) Binding affinities for the guinea pig urinary bladder NK_1 receptor. See experimental section. *b*) Binding affinities for the hamster urinary bladder NK_2 receptor. *c*) See ref. 1.

Next, we examined the effect of the position of the substituents in the *N*-methylbenzamide moiety, and the results are shown in Table 2. Among the compounds bearing chloro groups (**11**, **16**, **17**), the 3-chloro derivative (**16**) tended to show higher affinity for the NK_1 receptor and lower affinity for the NK_2 receptor than YM-35375. The 3,4-dichloro derivative (**18**) showed lower affinity for both receptors than the

Table 2. Physical and Biological Properties of (±)-*N*-[2-(3,4-Dichlorophenyl)-4-(spiro[isobenzofuran-1(3*H*), 4′-piperidin]-1′-yl)butyl]-*N*-methylcarboxamides



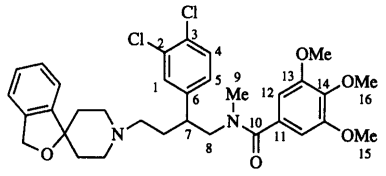
Compd. No.	R	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N	Method[a]	Yield (%)	Recrystn. solvent[b]	Binding affinity IC_{50} (nM) NK_1[c]	NK_2[d]
1	Ph									710	84
11[e]										610	120
16		Amorphous	$C_{30}H_{31}Cl_3N_2O_2 \cdot HCl \cdot 1.5H_2O$	57.98 (58.00	5.68 5.44	4.51 4.40)	A	74	—	310	260
17		Amorphous	$C_{30}H_{31}Cl_3N_2O_2 \cdot HCl \cdot 1.5H_2O$	57.98 (58.10	5.68 5.59	4.51 4.35)	A	79	—	640	150
18		185—186	$C_{30}H_{30}Cl_4N_2O_2 \cdot C_4H_4O_4$	57.64 (57.77	4.84 4.84	3.95 3.98)	A	33	M–A	1300	280
6[e]										1100	79
19		168—170	$C_{31}H_{34}Cl_2N_2O_3 \cdot C_4H_4O_4 \cdot 0.5H_2O$	61.95 (61.77	5.79 5.62	4.13 4.17)	A	40	A	420	83
20		172—174	$C_{32}H_{36}Cl_2N_2O_4 \cdot C_4H_4O_4$	61.80 (61.76	5.76 5.70	4.00 3.99)	A	42	A	73	95
21		182—183	$C_{34}H_{40}Cl_2N_2O_4 \cdot C_4H_4O_4$	62.72 (62.45	6.09 6.07	3.85 3.79)	B	45	A	160	250
22		213—214	$C_{31}H_{32}Cl_2N_2O_4 \cdot C_4H_4O_4$	61.50 (61.35	5.31 5.25	4.10 4.03)	B	51	M–A	260	56
23		183—185	$C_{33}H_{38}Cl_2N_2O_5 \cdot C_4H_4O_4$	60.91 (60.66	5.80 5.70	3.84 3.84)	A	46	A	33	180

a) See Chart 1. *b*) M=methanol, A=acetonitrile. *c*) Binding affinities for the guinea pig urinary bladder NK_1 receptor. See experimental section. *d*) Binding affinities for the hamster urinary bladder NK_2 receptor. See experimental section. *e*) See ref. 1.

monochlorinated compounds. In the case of compounds possessing methoxy groups (**6**, **19**), the *meta*-substituted derivative (**19**) also showed higher affinity for the NK_1 receptor than the *para*-substituted one (**6**). In contrast to the 3,4-dichloro derivative (**18**), introduction of two methoxy groups at the 3- and 4-positions (**20**) resulted in a 10-fold increase in affinity for the NK_1 receptor while retaining affinity for the NK_2 receptor relative to YM-35375. Compound **20** exhibited affinity for both NK_1 and NK_2 receptors at the 10^{-8} M level. Although other 3,4-dialkoxy compounds (**21**, **22**) showed less potent affinity for NK_1 receptors than compound **20**, substitution of the 3,4,5-trimethoxyphenyl group (**23**) for the 3,4-dimethoxyphenyl group caused a 2-fold increase in affinity for the NK_1 receptor (IC_{50} value of 33 nM), without a significant loss of affinity for the NK_2 receptor. These results indicated that methoxy groups at both the *meta*- and *para*-positions of the phenyl group in the *N*-methylbenzamide moiety were favorable for affinity for the NK_1 receptor.

As mentioned above, introduction of three methoxy groups to the *N*-methylbenzamide moiety of YM-35375, which shows 8-fold higher affinity for NK_2 over NK_1 receptors, resulted in a reversal of receptor selectivity. The resultant compound **23** exhibited NK_1 receptor selectivity with an index of 5.5. We speculated that the conformation of compound **23** induced by the three methoxy groups may be favorable for showing high affinity for the NK_1 receptor. In order to ascertain this speculation, NMR analysis of this compound was performed. The ^{1}H- and ^{13}C-NMR spectral data are shown in Table 3. These NMR spectra indicated the presence of two conformers in a ratio of about 5 : 2 at 27 °C. Since peak doubling behavior was observed mainly around the amide bond, these conformers are presumed to be rotational isomers around the amide bond. The C-8 carbon in the major conformation (δ 51.3) shifted up-field relative to the minor one (δ 56.4). In contrast, the C-9 carbon resonated more down-field in the major conformation (δ 37.6) than in the minor one (δ 33.5). In *N*,*N*-dialkylbenzamides, the nitrogen-neighboring carbon is known to shift up-field by the γ-effect[12] when it is located on the same side as the phenyl group. Therefore, the major and minor conformations of compound **23** possessed the *cis*- and *trans*-configurations around the amide bond, respectively (Fig. 3). In order to examine the conformation further, a rotating frame nuclear Overhauser spectroscopy (ROESY)[13] experiment was performed. Long range nuclear Overhauser effect (NOE) was observed between the H-5 and H-12 protons in the major conformation. This NOE suggested that these two phenyl groups are close to each other in the major conformation possessing the *cis*-configuration around the amide bond (Fig. 4). Since no long range NOEs were observed in other compounds, it may be a special con-

Table 3. Selected ^{1}H- and ^{13}C-NMR Spectral Data for Compound **23** at 27 °C



Position No.	Major conformation[a] Chemical shift (δ, ppm) Proton	Major conformation[a] Chemical shift (δ, ppm) Carbon	Position No.	Minor conformation[a] Chemical shift (δ, ppm) Proton	Minor conformation[a] Chemical shift (δ, ppm) Carbon
1	7.68	[b]	1	7.30	[b]
2	—	[b]	2	—	[b]
3	—	[b]	3	—	[b]
4	7.59	130.2	4	7.52	[b]
5	7.39	128.8	5	7.10	128.5
6	—	144.1	6	—	[b]
7	3.35	40.8	7	3.03	41.5
8	[b]	51.3	8	[b]	56.4
9	2.74	37.6	9	2.90	33.5
10	—	169.8	10	—	[b]
11	—	[b]	11	—	131.7
12	6.27	103.6	12	6.38	104.3
13	—	152.6	13	—	[b]
14	—	138.0	14	—	[b]
15	3.76	55.8	15	[b]	[b]
16	3.69	60.0	16	[b]	[b]

a) Compound **23** possessed two conformers in a ratio of about 5 : 2 at this temperature. *b*) We could not assign these signals because of signal broadening and overlapping.

formational feature of compound **23** that the 3,4-dichlorophenyl group and 3,4,5-trimethoxyphenyl group are close to each other. This local structure may play an important role in exhibiting high affinity for the NK_1 receptor.

We have already found that 1,2,3,4-tetrahydroisoquinoline in the spiro-substituted piperidine moiety was favorable for showing affinity for both NK_1 and NK_2 receptors, and that 3-isoquinolone increased affinity for the NK_2 receptor.[1] In addition, modifications in the *N*-methylbenzamide moiety led us to discover that compound **20** exhibited affinity for both NK_1 and NK_2 receptors with IC_{50} values at the 10^{-8} M level, and that 3,4,5-trimethoxybenzamide (**23**) showed high affinity for the NK_1 receptor. Thus, derivatives possessing 3,4-dihydrospiro[isoquinoline-1(2*H*),4′-piperidine] or 3-oxo-3,4-dihydrospiro[isoquinoline-1(2*H*),4′-piperidine] instead of the spiro[isobenzofuran-1(3*H*),4′-piperidine] of compounds **20** and **23**, were evaluated for affinity to NK_1 and NK_2 receptors, and results are shown in Table 4. Introduction of 1,2,3,4-tetrahydroisoquinoline (**28**) to compound **20** resulted in an almost 3-fold increase in affinity for both NK_1 and NK_2 receptors. In 3,4,5-trimethoxy derivatives (**23**, **30**), this substitution was also effective for improving affinity for both receptors. Among the compounds prepared in this study, compound **30** showed the highest affinity for the NK_1 receptor, with an IC_{50} value of 6.5 nM, which was more than 100 times higher than YM-35375. Conversion of isobenzofuran to 3-isoquinolone increased affinity for the NK_2 receptor (**20** *vs.* **29** and **23** *vs.* **31**), as observed in the previous study,[1] however, this substitution was unfavorable for NK_1 receptor affinity with 3,4-dimethoxy derivatives (**20** *vs.* **29**). On the contrary, affinity of the 3,4,5-trimethoxy derivative (**31**) for the NK_1 receptor was as high as compound **23**. Thus, compound **31** showed high and well-balanced affinity for both NK_1 and NK_2 receptors with IC_{50} values of 18 and 16 nM, respectively. From these results, the spiro-substituted piperidine moiety may be crucial for binding with the NK_2 receptor, and the hydrogen bond-accepting group in this moiety may be required for high affinity for the NK_2 receptor. These speculations are consistent with the results described in the previous report.[1]

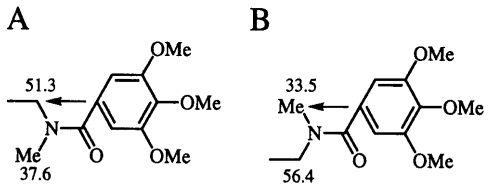


Fig. 3. The conformations around the amide bond of the major (A) and minor (B) conformers of compound **23**[a]

a) The numbers in the figures indicate the chemical shifts (δ) of the carbon atoms. The arrows indicate γ-effects due to the phenyl rings.

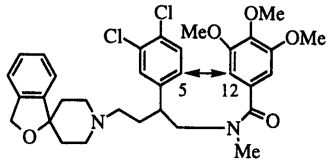


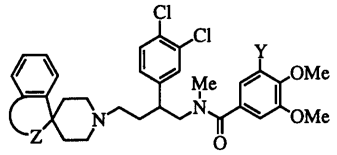
Fig. 4. Long range NOE (↔) observed in the major conformation[a] of compound **23**

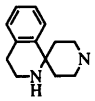
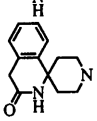
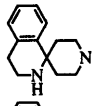
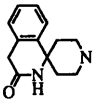
a) See Table 3.

Selected compounds (**20**, **23**, **28**, **30**, **31**) were evaluated for their inhibitory activities against SP-induced contraction of guinea pig ileum (GPI) and NKA-induced contraction of hamster trachea (HT), and results are summarized in Table 5. Isobenzofuran derivatives **20** and **23** inhibited both contractions with almost the same IC_{50} values observed in the binding assays, however, the 1,2,3,4-tetrahydroisoquinoline derivatives (**28**, **30**) showed only weak inhibitory activity against both NK_1 and NK_2 receptors, compared with their binding affinity. From these results, we speculated that the isoquinoline ring in the spiro-substituted piperidine moiety may be unfavorable for penetration into the tissues, compared with the isobenzofuran ring. In contrast, the 3-isoquinolone derivative (**31**, YM-44778) showed potent and well-balanced antagonistic activity against both NK_1 and NK_2 receptors with IC_{50} values of 82 and 62 nM, respectively.

In conclusion, we designed novel spiro-substituted piperidine derivatives based on our lead compound YM-35375 and evaluated them for NK_1 and NK_2 receptor antagonistic activities. In this study, we found that the conformation in which the two phenyl groups of the 3,4-dichlorophenyl and *N*-methylbenzamide moieties are close to each other through a *cis*-amide bond, may be favorable for high affinity to the NK_1 receptor. It was also found that a hydrogen bond-accepting group in the spiro-substituted piperidine moiety may be necessary for high affinity to the NK_2 receptor. Among these compounds, YM-44778 (**31**) inhibited contractions mediated through both NK_1 and NK_2 receptors in isolated tissues, with IC_{50} values at the 10^{-8} M level. It should be noted that all of the compounds reported here are racemic mixtures. Many neurokinin receptor antagonists reported as optically pure

Table 4. Physical and Biological Properties of (±)-*N*-[2-(3,4-Dichlorophenyl)-4-(spiro-substituted-4′-piperidin-1′-yl)butyl]-3,4,5-trimethoxy-*N*-methylbenzamides



Compd. No.		Y	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N	Method[a] Yield (%)	Recrystn. solvent[b]	Binding affinity IC_{50} (nM) NK_1[c]	NK_2[d]
20[e]		H								73	95
28		H	215—217	$C_{33}H_{39}Cl_2N_3O_3 \cdot 2HCl \cdot 0.75H_2O$	58.03 (58.22)	6.27 5.99	6.15 6.15)	C 2	M–P	27	36
29		H	219 (dec.)	$C_{33}H_{37}Cl_2N_3O_4 \cdot C_4H_4O_4$	61.16 (60.95	5.69 5.61	5.78 5.75)	C 54	M–EA	140	13
23[e]		OMe								33	180
30		OMe	210—212	$C_{34}H_{41}Cl_2N_3O_4 \cdot 2HCl$	58.38 (58.21	6.20 6.11	6.01 6.01	C 7	M–EA–E	6.5	54
31		OMe	138—142	$C_{34}H_{39}Cl_2N_3O_5 \cdot C_4H_4O_4 \cdot 0.5H_2O$	59.61 (59.56	5.79 5.62	5.49 5.47)	C 66	P–E	18	16

a) See Chart 1. *b*) M=methanol, EA=ethyl acetate, E=diethyl ether, P=2-propanol. *c*, *d*) See the corresponding footnotes in Table 2. *e*) See Table 2.

Table 5. Binding Affinities and Inhibitory Activities in Functional Assays of Compounds **20**, **23**, **28**, **30** and **31**

Compd. No.	Binding affinity NK_1[a] IC_{50} (nM)	Binding affinity NK_2[b] IC_{50} (nM)	Inhibitory activity NK_1[c] IC_{50} (nM)	Inhibitory activity NK_2[d] IC_{50} (nM)
20	73	95	75	140
23	33	180	19	200
28	27	36	140	500
30	6.5	54	53	570
31	18	16	82	62

a, *b*) See the corresponding footnotes in Table 1. *c*) SP-induced contraction of GPI. See experimental section. *d*) NKA-induced contraction of HT. See experimental section.

compounds are far more potent than their optical antipodes. From these facts, the optically pure isomer of YM-44778 should demonstrate more potent inhibitory activity against both NK_1 and NK_2 receptors. YM-44778 is a novel, potent and well-balanced NK_1–NK_2 dual antagonist possessing a unique spiro-substituted piperidine. We expect that these studies will lead to discovery of a clinical effective antiasthmatic drug.

Experimental

All melting points were determined on a Yanagimoto MP-3 melting point apparatus without correction. ^{1}H-NMR spectra were taken on a JEOL JNM-EX400 spectrometer or a JEOL JNM-A500 spectrometer. Chemical shifts are given in ppm relative to Me_4Si (δ=0) in dimethylsulfoxide-d_6 (DMSO-d_6) as an internal standard. The abbreviations of signal patterns are as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; dd, double doublet; m, multiplet. Column chromatography was carried out on silica gel (Wakogel C-200 or Merck Silica gel 60). FAB-MS spectra were obtained with a JEOL JMS-DX300 mass spectrometer, and electron impact (EI)-MS with a Hitachi M-80 mass spectrometer or a Hewlett-Packard 5890 GC-5970 MSD.

(±)-*N*-[2-(3,4-Dichlorophenyl)-4-(spiro[isobenzofuran-1(3*H*),4′-piperidin]-1′-yl)butyl]-*N*-methyl-4-trifluoromethylbenzamide (13, Method A) 4-Trifluoromethylbenzoyl chloride (0.113 ml, 0.758 mmol) was added to a mixture of (±)-1′-[3-(3,4-dichlorophenyl)-4-methylaminobutyl]spiro[isobenzofuran-1(3*H*),4′-piperidine] (**4**, 212 mg, 0.505 mmol), Et_3N (0.141 ml, 1.01 mmol) and CH_2Cl_2 (5 ml) at 0 °C. The mixture was then stirred for 4 h at room temperature, diluted with H_2O and extracted with $CHCl_3$. The extract was washed with saturated $NaHCO_3$, saturated brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resulting residue was purified by column chromatography ($CHCl_3$: MeOH=49 : 1) and crystallized from diethyl ether (Et_2O) to give the benzamide (**13**, 220 mg, 74%) as a colorless powder, mp 113—116 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.66—2.04 (6H, m), 2.18—2.40 (4H, m), 2.64—3.89 (8H, m), 5.05 (2H, s), 6.80—7.34 (8H, m), 7.42—7.45 (1H, m), 7.62—7.64 (2H, m). FAB-MS *m/z*: 591 [$(M+H)^+$]. *Anal.* Calcd for $C_{31}H_{31}Cl_2F_3N_2O_2$: C, 62.95; H, 5.28; N, 4.74. Found: C, 63.04; H, 5.29; N, 4.77.

(±)-*N*-[2-(3,4-Dichlorophenyl)-4-(spiro[isobenzofuran-1(3*H*),4′-piperidin]-1′-yl)butyl]-3,4-diethoxy-*N*-methylbenzamide Monofumarate (21, Method B) EDC·HCl (110 mg, 0.572 mmol) and Et_3N (0.080 ml, 0.57 mmol) were added to a mixture of compound **4** (200 mg, 0.477 mmol), 3,4-diethoxylbenzoic acid (120 mg, 0.57 mmol), HOBt (97 mg, 0.72 mmol) and CH_2Cl_2 (5 ml) at 0 °C. The mixture was then stirred for 6 h at room temperature, diluted with brine and extracted with AcOEt. The extract was washed with 0.5 N NaOH, saturated brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography ($CHCl_3$: MeOH=49 : 1). The purified amine was dissolved in MeOH and treated with fumaric acid (42 mg, 0.36 mmol), and the mixture was concen-

Table 6. ^{1}H-NMR[a] and Mass Spectral Data for Spiro-substituted Piperidines

Compd. No.	NMR (δ)	MS *m/z*
16	1.81—2.35 (6H, m), 2.69—2.73 (2H, m), 2.86—3.26 (6H, m), 3.45—3.74 (4H, m), 5.03 (2H, s), 6.81—7.91 (11H, m), 10.84 (1H, br s)	556 (M^+) (EI)
17	1.77—2.39 (6H, m), 2.56—2.59 (2H, m), 2.85—3.28 (6H, m), 3.41—3.75 (4H, m), 5.02, 5.04 (2H, each s), 7.14—7.79 (1H, m), 10.79 (1H, br s)	556 (M^+) (EI)
18	1.62—1.74 (3H, m), 1.85—1.97 (3H, m), 2.20—2.43 (3H, m), 2.69—3.01 (6H, m), 3.17—3.80 (3H, m), 4.96 (2H, s), 6.59 (2H, s), 7.05—7.17 (2H, m), 7.22—7.39 (6H, m), 7.50—7.66 (2H, m)	591 [$(M+H)^+$] (FAB)
19	1.58—1.65 (2H, m), 1.70—1.96 (4H, m), 2.20—2.45 (4H, m), 2,69—3.00 (5H, m), 3.16—3.25 (1H, m), 3.52—3.82 (5H, m), 4.96 (2H, s), 6.54—6.68 (4H, m), 6.96 (1H, dd, J=8.3, 2.4 Hz), 7.05—7.39 (6H, m), 7.50—7.65 (2H, m)	553 [$(M+H)^+$] (FAB)
20	1.60—1.62 (2H, m), 1.82—1.95 (4H, m), 2,20—2.45 (4H, m), 2.62—3.22 (6H, m), 3.53—3.77 (8H, m), 4.96 (2H, s), 6.58—6.68 (4H, m), 6.92 (1H, d, J=8.6 Hz), 7.10—7.65 (7H, m)	583 [$(M+H)^+$] (FAB)
22	1.60—1.63 (2H, m), 1.83—1.96 (4H, m), 2.20—2.50 (4H, m), 2.68—3.22 (6H, m), 3.50—3.75 (2H, m), 4.96 (2H, s), 6.05 (2H, d, J=1.2 Hz), 6.58—6.65 (4H, m), 6.88—6.90 (1H, m), 7.21—7.65 (7H, m)	567 [$(M+H)^+$] (FAB)
23	1.60—1.63 (2H, m), 1.72—1.96 (4H, m), 2.20—2.50 (4H, m), 2.71—3.25 (6H, m), 3.47—3.51 (1H, m), 3.66 (3H, s), 3.73 (6H, s), 3.85—3.93 (1H, m), 4.96 (2H, s), 6.25—6.35 (2H, m), 6.60 (2H, s), 7.21—7.66 (7H, m)	613 [$(M+H)^+$] (FAB)
28	1.94—2.46 (5H, m), 2.75—2.91 (6H, m), 3.02—3.10 (3H, m), 3.37—3.70 (8H, m), 3.73 (3H, s), 3.77 (3H, s), 6.57—6.79 (2H, m), 6.92 (1H, d, J=7.8 Hz), 7.20—7.76 (7H, m), 10.10 (1H, br s), 10.20 (1H, br s), 11.38 (1H, br s)	596 [$(M+H)^+$] (FAB)
29	1.65—1.78 (3H, m), 1.84—2.11 (4H, m), 2,22—2.38 (1H, m), 2.53—3.26 (9H, m), 3.50—3.58 (3H, m), 3.71 (3H, s), 3.77 (3H, s), 6.58—6.68 (4H, m), 6.92 (1H, d, J=7.9 Hz), 7.19 (1H, d, J=6.7 Hz), 7.23—7.68 (6H, m), 7.81 (2H, br s)	610 [$(M+H)^+$] (FAB)
30	1.95—2.42 (4H, m), 2.66—3.19 (10H, m), 3.35—3.42 (2H, m), 3.51—3.67 (8H, m), 3.75 (6H, s), 3.80—3.93 (1H, m), 6.31 (2H, s), 7.07—7.78 (7H, m), 10.17 (1H, br s), 10.30 (1H, br s), 11.62 (1H, br s)	626 [$(M+H)^+$] (FAB)

a) ^{1}H-NMR spectra were taken in DMSO-d_6.

trated *in vacuo*. The residue was crystallized from 2-propanol–Et_2O and recrystallized from acetonitrile (MeCN) to give (**21**, 155 mg, 45%) as a colorless powder, mp 182—183 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.31 (6H, t, J=6.8 Hz), 1.58—1.61 (2H, m), 1.80—1.92 (4H, m), 2.20—2.46 (4H, m), 2.68—2.85 (5H, m), 3.17—3.32 (1H+H_2O, m), 3.53 (1H, dd, J=13.2, 5.4 Hz), 3.72—3.84 (1H, m), 3.90—3.96 (2H, m), 4.02 (2H, q, J=6.8 Hz), 4.95 (2H, s), 6.51—6.66 (4H, m), 6.91 (1H, d, J=8.3 Hz), 7.21—7.56 (7H, m). EI-MS *m/z*: 610 (M^+). *Anal.* Calcd for $C_{34}H_{40}Cl_2N_2O_4 \cdot C_4H_4O_4$: C, 62.72; H, 6.09; N, 3.85. Found: C, 62.45; H, 6.07; N, 3.79.

(±)-*N*-[2-(3,4-Dichlorophenyl)-4-(3-oxo-3,4-dihydrospiro[isoquinoline-1(2*H*),4′-piperidin]-1′-yl)butyl]-3,4,5-trimethoxy-*N*-methylbenzamide Monofumarate Hemihydrate (31, Method C) A mixture of compound **25** (828 mg, 1.87 mmol), Et_3N (0.391 ml, 2.81 mmol) and CH_2Cl_2 (10 ml) was treated with MsCl (0.173 ml, 2.24 mmol) at 0 °C. The mixture was then stirred for 2 h at room temperature, diluted with AcOEt, then washed with H_2O and saturated brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give (±)-*N*-[2-(3,4-dichlorophenyl)-4-methanesulfonyloxybutyl]-3,4,5-trimethoxy-*N*-methylbenzamide (955 mg, 98%) as a colorless oil.

A mixture of 3-oxo-3,4-dihydrospiro[isoquinoline-1(2*H*),4′-piperidine] monohydrochloride (**27**, 457 mg, 1.81 mmol) and brine was basified with 1 N NaOH, and the free amine of compound **27** was extracted with $CHCl_3$. The extract was washed with saturated brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was added to a mixture of the mesylate (615 mg, 1.20 mmol), Et_3N (0.504 ml, 3.61 mmol) and *N*,*N*-dimethylformamide (DMF, 4 ml). The mixture was stirred for 4.5 h at 70 °C, diluted with H_2O and extracted with AcOEt. The extract was washed with H_2O and saturated brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography ($CHCl_3$: MeOH = 49 : 1). The purified amine was dissolved in MeOH and treated with fumaric acid (116 mg, 1.00 mmol), and the mixture was concentrated *in vacuo*. The residue was crystallized from 2-propanol–Et_2O and recrystallized from the same solvent system to give the fumarate (**28**, 599 mg, 66%) as colorless crystals, mp 138—142 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.76—2.41 (7H, m), 2.60—3.23 (8H, m), 3.47—3.52 (1H, m), 3.56 (2H, s), 3.67 (3H, s), 3.73 (6H, s), 3.78—3.91 (2H, m), 6.25—6.33 (2H, m), 6.59 (2H, s), 7.18—7.20 (1H, m), 7.23—7.29 (2H, m), 7.37—7.39 (2H, m), 7.51—7.67 (2H, m), 7.91 (1H, br s). FAB-MS *m/z*: 640 [$(M+H)^+$]. *Anal.* Calcd for $C_{34}H_{39}Cl_2N_3O_5 \cdot C_4H_4O_4 \cdot 0.5H_2O$: C, 59.61; H, 5.79; N, 5.49. Found: C, 59.56; H, 5.62; N, 5.47.

Compounds **16—20**, **22**, **23** and **28—30** were prepared according to the methods previously described (methods A—C). Melting points, elemental analyses and yields are summarized in Tables 2—4, and NMR and MS data are shown in Table 6.

Binding Assays Binding studies were carried out according to the method described by Burcher and Buck.[11] To determine the NK_1 receptor binding affinity of the compounds, ^{125}I-Bolton–Hunter-SP and guinea pig urinary bladder were used, while ^{125}I-NKA and hamster urinary bladder were employed to test the NK_2 binding affinity.

NMR Analysis of Compound 23 NMR spectra were taken on a JMN-A500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C) in DMSO-d_6. The assignments of the protons and carbons are summarized in Table 3.

The ROESY spectrum[13] was recorded with 1024 points in t_2 and 256 points in t_1 with a mixing time of 250 ms at 27 °C. The sweep width was 4527 Hz in both dimensions, and 32 scans were acquired per t_1 value. The data sets were zero-filled to 512 data points along the t_1 axis and multiplied by the Lorentz–Gaussian window function before Fourier transformation. The digital resolutions in t_2 and t_1 were 4.42 and 8.84 Hz/point after zero-filling, respectively.

***In Vitro* Assay for NK_1 Receptors** Guinea pig ileal strips were suspended with an initial tension of 1.0 g in organ baths filled with oxygenated Tyrode's solution, containing atropine (5 μM), mepyramine (5 μM) and indomethacin (5 μM), at 37 °C. After obtaining three reproducible contractions evoked by SP (1 nM), a compound was added to the bath. The contraction was induced by the agonist again 15 min after the addition of the compound, and the reduction of the peak-contraction was determined. The IC_{50} values were determined by log-logit linear regression.

***In Vitro* Assay for NK_2 Receptors** Rings of hamster trachea were suspended with an initial tension of 0.5 g in 10 ml organ baths filled with oxygenated Tyrode's solution at 37 °C. Contractions were induced by NKA (100 nM), and the effect of the compounds was determined as previously mentioned.

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References

1) Part I: Kubota H., Fujii M., Ikeda K., Takeuchi M., Shibanuma T., Isomura Y., *Chem. Pharm. Bull.*, **46**, 351—354 (1998); part II: Kubota H., Kakefuda A., Nagaoka H., Yamamoto O., Ikeda K., Takeuchi M., Shibanuma T., Isomura Y., *Chem. Pharm. Bull.*, **46**, 242—254 (1998).
2) Erspamer V., *Trends Neurosci.*, **4**, 267 (1981).
3) Von Euler U. S., Gaddum J. H., *J. Physiol.*, **72**, 74—86 (1931); Chang M. M., Leeman S. E., Niall H. D., *Nature New Biol.*, **232**, 86—87 (1971).
4) Kimura S., Okada M., Sugita Y., Kanazawa I., Munekata E., *Proc. Jpn.*

Acad. (*B*), **59**, 101—104 (1983).
5) Nakanishi S., *Annu. Rev. Neurosci.*, **14**, 123—136 (1991); Gerard N. P., Bao L., Ping H. X., Gerard C., *Regul. Pept.*, **43**, 21—35 (1993).
6) Barnes P. J., *Lancet*, **1**, 242—245 (1986); *idem*, *Trends Pharmacol. Sci.*, **8**, 24—27 (1987); *idem*, *Arch. Int. Pharmacodyn.*, **303**, 67—82 (1990); Barnes P. J., Belvisi M. G., Rogers D. F., *Trends Pharmacol. Sci.*, **11**, 185—189 (1990).
7) Sekizawa K., Tamaoki J., Graf P. D., Basaum C. B., Borson D. B., Nadel J. A., *J. Pharm. Exp. Ther.*, **243**, 1211—1217 (1987).
8) Uchida Y., Nomura A., Ohtsuka M., Hasegawa S., *Am. Rev. Respir. Dis.*, **136**, 718—721 (1987).
9) Murai M., Morimoto H., Maeda Y., Kiyotoh S., Nishikawa M., Fujii T., *J. Pharm. Exp. Ther.*, **262**, 403—408 (1992).
10) Burkholder T. P., Kudlacz E. M., Le T.-B., Knippenberg R. W., Shatzer S. A., Maynard G. D., Webster M. E., Horgan S. W., *Bioorg. Med. Chem. Lett.*, **6**, 951—956 (1996).
11) Burcher E., Buck S. H., *Eur. J. Pharmacol.*, **128**, 165—177 (1986).
12) Grant D. M., Cheney B. V., *J. Am. Chem. Soc.*, **89**, 5315—5318 (1967).
13) Bax A., Davis D. G., *J. Magn. Reson.*, **63**, 207—213 (1985); Kessler H., Griesinger C., Kerssebaum R., Wagner K., Erust R. R., *J. Am. Chem. Soc.*, **109**, 607—609 (1987).