Spiro-Substituted Piperidines as Neurokinin Receptor Antagonists. I. Design and Synthesis of (±)-N-[2-(3,4-Dichlorophenyl)-4-(spiro[isobenzofuran-1(3H),4'-piperidin]-1'-yl)butyl]-N-methylbenzamidine, YM-35375, as a New Lead Compound for Novel Neurokinin Receptor Antagonists

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Analysis of the structural requirements of compound 1 (SR48968), a potent NK2 receptor antagonist, revealed that the 4-phenyl group of the piperidine is essential for binding with the NK2 receptor and occupies an equatorial position. Energy calculation of a variety of substituted 4-phenyl piperidines revealed that spiro[isobenzofuran-1(3H), 4'-piperidine] possesses a conformationally restricted equatorial phenyl group. Our compound 12 (YM-35375) possessing this spiro-substituted piperidine bound to the NK2 receptor with an IC_{50} value of 84 nM and to the NK1 receptor with an IC_{50} value of 710 nM. It showed more potent inhibitory activity (ID_{50} 41 μg/kg i.v.) against [β-Ala^2]-NKA(4—10)-induced bronchoconstriction in guinea pigs than (±)-SR48968 (ID_{50} 68 μg/kg i.v.). YM-35375 may be a new lead compound for novel NK2 receptor antagonists or NK_{1}/NK_{2} dual antagonists.

Key words spiro[isobenzofuran-1(3H), 4'-piperidine]; NK2 antagonist; dual antagonist; YM-35375; SR48968

Neurokinins are peptides comprising ten or eleven amino acids and possess in common the sequence -Phe-X-Gly-Leu-Met-NH_{2} at their C-termini. Among them, substance P (SP), neurokinin A (NKA), and neurokinin B (NKB), isolated from mammals, exhibit a wide variety of biological responses through their receptors. Neurokinin receptors are now classified into the following three subtypes, NK_{1}, NK_{2} and NK_{3}, which have high affinity for SP, NKA and NKB, respectively. Recently, Barnes et al. proposed that SP and NKA may play important roles in the pathogenesis of asthma. Namely, SP and NKA are released from the endings of sensory nerves by a variety of stimuli and induce the pathological features of asthma, such as microvascular leakage, mucus hypersecretion and bronchoconstriction. In the airway, SP causes microvascular leakage and mucus hypersecretion, and NKA induces bronchoconstriction. Based on these mechanisms, NK_{1} and NK_{2} receptor antagonists could prevent these functions in asthmatic patients and may be of clinical benefit for treatment of asthma.

Recently, various potent and selective non-peptide NK_{1} receptor antagonists have been reported and their clinical efficacy has been evaluated. As regards non-peptide NK_{2} receptor antagonists, only SR48968 (Fig. 1) has been clinically evaluated to our knowledge. SR48968 has a characteristic structure, 4-acetamido-4-phenylpiperidine, which may be important for binding to the NK_{2} receptor.

In this paper, we report the molecular design, synthesis and pharmacological properties of (±)-N-[2-(3,4-dichlorophenyl)-4-(spiro[isobenzofuran-1(3H),4'-piperidin]-1'-yl)butyl]-N-methylbenzamide (12, YM-35375) as a new lead compound for novel antiasthmatic drugs.

Chemistry

(±)-N-[2-(3,4-Dichlorophenyl)-4-(4-phenylpiperidinobutyl)]-N-methylbenzamide (2) and (±)-N-[4-(4-acetamidopiperidino)-2-(3,4-dichlorophenyl)butyl]-N-methylbenzamide (3) were synthesized according to the literature. Spiro[isobenzofuran-1(3H),4'-piperidine] (8) was synthesized from 2-bromobenzyl alcohol (4) as shown in Chart 1. Compound 4 was converted to a diamin with n-butyl lithium (n-BuLi) in tetrahydrofuran (THF)—dichloromethane (CH_{2}Cl_{2}) and treated with 1-ethoxy carbonyl-4-piperidine (5) to give ethyl 4-hydroxy-4-(2-hydroxyethyl) piperidine-1-carboxylate (6). The primary hydroxy group of compound 6 was selectively tosylated by treatment with p-toluenesulfonyl chloride (TsCl), and

![Fig. 1. Structure of SR48968 (1)](chart1.png)

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the resultant tosylate was cyclized in the presence of pyridine to give the protected spiro-substituted piperidine (7). Compound 7 was treated with sodium hydroxide to give compound 8. Synthesis of YM-35375 is shown in Chart 2. (±)-4-Amino-3-(3,4-dichlorophenyl)butanol (9) was prepared according to the literature. Compound 9 was treated with a mixture of formic acid and acetic anhydride (Ac₂O), followed by reduction with borane-THF complex to give the N-methyl derivative (10). Compound 10 was treated with benzoyl chloride in the presence of triethylamine (Et₃N), followed by hydrolysis with sodium hydroxide to give (±)-N-[2-(3,4-dichlorophenyl)-4-hydroxybutyl]-N-methylbenzamide (11). Treatment of compound 11 with methanesulfonyl chloride (MsCl), followed by substitution with compound 8 in the presence of Et₃N gave compound 12 (YM-35375).

**Results and Discussion**

Thus obtained compounds were evaluated for their binding affinities to hamster urinary bladder NK₂ receptor and guinea pig urinary bladder NK₁ receptor.

As shown in Table 1, (±)-SR48968 exhibited high affinity for the NK₂ receptor with an IC₅₀ value of 4.1 nm. The removal of the phenyl group of 4-acetamido-4-phenylpiperidine (2) caused complete loss of the potency in contrast to the analog without the acetamido group (3), which retained the potency with an IC₅₀ value of 180 nm. These facts suggested that the phenyl group at the

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**Table 1. NK₂ Receptor Binding Affinities and Antagonistic Activities in Vivo of N-[2-(3,4-Dichlorophenyl)-4-(4-substituted piperidin-1'-yl)butyl]-N-methylbenzamides**

<table>
<thead>
<tr>
<th>Compound</th>
<th>NK₂ binding IC₅₀ (nm)</th>
<th>NK₁ binding IC₅₀ (nm)</th>
<th>NK₂ selectivity index</th>
<th>Bronchoconstriction in guinea pigs ID₅₀ (ng/kg iv.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ([±]-SR48968)</td>
<td>4.1</td>
<td>&gt;1000</td>
<td>&gt;240</td>
<td>68</td>
</tr>
<tr>
<td>2</td>
<td>&gt;1000</td>
<td>N. T.</td>
<td>N. T.</td>
<td>N. T.</td>
</tr>
<tr>
<td>3</td>
<td>180</td>
<td>N. T.</td>
<td>N. T.</td>
<td>N. T.</td>
</tr>
<tr>
<td>12 (YM-35375)</td>
<td>84</td>
<td>710</td>
<td>8.5</td>
<td>41</td>
</tr>
</tbody>
</table>

* a) The binding affinities for hamster urinary bladder NK₂ receptor. See experimental section. b) The binding affinities for guinea pig urinary bladder NK₁ receptor. See experimental section. c) IC₅₀ to NK₂ receptor/IC₅₀ to NK₁ receptor. d) See experimental section. N. T.: Not tested.

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**Fig. 2. Putative 3-Dimensional Structures** of 4-Acetamido-1-methyl-4-phenylpiperidine (13) and 1'-Methylspiro[isobenzofuran-1(3H),4'-piperidine] (14)

* a) The 2-dimensional structures of these molecules were constructed using Sybyl (6.22) and converted to initial 3-dimensional structures with Concord 3.2.1. These initial structures were relaxed using the Tripos force field.

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4-position of the piperidine may be crucial to bind to the NK₂ receptor and that the acetamide group may be necessary for increasing the affinity for the NK₂ receptor. To analyze the conformation of the 4-acetamido-4-phenylpiperidine moiety of SR48968, a putative 3-dimensional structure of 4-acetamido-1-methyl-4-phenylpiperidine (13), which was adopted as a simplified molecule, was constructed by energy minimization. As shown in Fig. 2A, the phenyl group was placed in an equatorial position and the acetamide group in an axial position. This conformation was 1.68 kcal/mol more stable than the other one with an axial phenyl group and an equatorial acetamide group. We speculated that the phenyl group may be immobilized in the equatorial position by...
the axial acetamide group and that compounds with more restricted equatorial phenyl groups may show higher affinity for the NK₂ receptor. We designed 1-methyl-spiro[isobenzofuran-1(3H),4'-piperidine] (14) as a conformationally restricted 4-phenyldapiperidine. Conformational analysis of compound 14 suggested that the phenyl group lies in an equatorial position (Fig. 2B). This conformation was 2.51 kcal/mol more stable than the other one with an axial phenyl group. Based on these results, it was expected that YM-35375, which possesses this spiro-substituted piperidine instead of 4-acetamido-4-phenyldapiperidine of SR48968, would bind to the NK₂ receptor with high affinity. Unfortunately, it was found that YM-35375 showed affinity for the NK₂ receptor with an IC₅₀ value of only 84 nm, which was a 20-fold decrease in potency relative to (±)-SR48968. One of the reasons for this decreased affinity for the NK₂ receptor was considered to be the lack of the acetamide group, which would affect the angle of the equatorial phenyl group and interact with the NK₂ receptor, e.g., through hydrogen bonding.

As shown in Table 1, (±)-SR48968 inhibited [β-Ala³]-NKA(4–10)-induced bronchoconstriction in guinea pigs¹⁴,¹⁵ with an ID₅₀ value of 68 μg/kg (i.v.). In contrast to the binding assay, YM-35375 showed more potent inhibitory activity with an ID₅₀ value of 41 μg/kg (i.v.) than (±)-SR48968 in this model. These results suggested that the substitution of spiro[isobenzofuran-1(3H),4'-piperidine] for 4-acetamido-4-phenyldapiperidine was favorable for NK₂ receptor-antagonistic activity in vivo and that YM-35375 may be a new lead compound for further research to find a novel NK₂ receptor antagonist. Furthermore, YM-35375 also showed weak NK₁ receptor affinity with an IC₅₀ value of 710 nm, and its NK₂ receptor selectivity index was 8.5 in contrast to over 240 of (±)-SR48968. This result suggested that the substitution of spiro[isobenzofuran-1(3H),4'-piperidine] for 4-acetamido-4-phenyldapiperidine caused a change in selectivity and that the discovery of a novel NK₂–NK₃ dual antagonist¹⁶ might be possible by suitable modifications of YM-35375. In conclusion, spiro[isobenzofuran-1(3H),4'-piperidine] was designed as a conformationally restricted piperidine with an equatorial phenyl group with the aim of obtaining a potent NK₂ receptor antagonist. Although YM-35375 which possessed this spiro-substituted piperidine was less potent than (±)-SR48968 in binding assay, it was more potent than (±)-SR48968 in vivo. As YM-35375 also showed weak NK₁ receptor affinity, we anticipate that further structural modifications of this compound may alter the selectivity between the NK₁ and NK₂ receptors and give rise to not only highly selective NK₂ receptor antagonists, but also NK₂–NK₃ dual antagonists. We are currently searching for novel neurokinin antagonists by utilizing YM-35375 as a lead compound. The abbreviations of signal patterns are as follows: s, singlet; brs, broad singlet; d, doublet; t, triplet; dd, double doublet; q, quartet; m, multiplet. Column chromatography was carried out on silica gel (Wakogel C-200 or Merck Silica gel 60). FAB-MS 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.

Ethyl 4-Hydroxy-4-(2-hydroxyethyl)phenyl)-1-piperidinecarboxylate (6) A 1.0-solution of n-BuLi (705 ml, 1.15 mol) in hexane was added dropwise to a solution of 2-bromobenzyl alcohol (4) (101 g, 537 mmol) in THF (500 ml) and Et₂O (1000 ml) at −78 °C under an argon atmosphere. The mixture was stirred for 1 h at −78 °C, then 1-ethoxy-carbonyl-4-piperidine (5) (101 g, 591 mmol) and THF (100 ml) were added at the same temperature. The mixture was stirred for 16 h at room temperature, H₂O was added and the whole was extracted with ethyl acetate (AcOEt). The extract was washed with saturated brine, dried over anhydrous MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (hexane:AcOEt=1:1) to give the diol (6, 69.3 g, 46%) as a pale yellow oil. 1H-NMR (CDCl₃) δ: 1.27 (3H, t, J=7.1 Hz), 1.85–1.91 (2H, m), 1.96–2.04 (2H, m), 3.23–3.38 (2H, m), 3.98–4.07 (2H, m), 4.13 (2H, q, J=7.1 Hz), 4.89 (2H, s), 7.20–7.30 (4H, m). EI-MS m/z: 279 (M⁺).

Spiro[isobenzofuran-1(3H)-4'-piperazine]-1'-carboxylic acid (7) A solution of TSCI (516.6 g, 271.0 mmol) in CH₂Cl₂ (160 ml) was added to a solution of compound 6 (6.87 g, 246 mmol), pyridine (43.7 ml, 541 mmol) and CH₂Cl₂ (800 ml) at 0°C. The mixture was stirred for 18 h at room temperature, then pyridine (19.9 ml, 246 mmol) and TSCI (46.9 g, 246 mmol) were added at 0°C. The whole was stirred for 2 h at room temperature. Further pyridine (19.9 ml, 246 mmol) and TSCI (46.9 g, 246 mmol) were added to the reaction mixture at 0°C, and stirring was continued for an additional 18 h at room temperature. The mixture was diluted with CH₂Cl₂ and washed with H₂O, 5% aqueous NaHCO₃, 10% aqueous citric acid and saturated brine. The organic layer was dried over anhydrous MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (hexane:AcOEt=4:1) to give the carboxylate (7, 52.2 g, 81%) as a pale yellow solid. 1H-NMR (CDCl₃) δ: 1.29 (3H, t, J=7.4 Hz), 1.70–1.76 (2H, m), 1.79–1.89 (2H, m), 3.16–3.29 (2H, m), 4.06–4.22 (4H, m), 5.08 (2H, s), 7.07–7.10 (1H, m), 7.20–7.24 (1H, m), 7.26–7.30 (2H, m). EI-MS m/z: 261 (M⁺).

Spiro[isobenzofuran-1(3H)-4'-piperidine] (8) A mixture of compound 7 (51.6 g, 197 mmol), 5% NaOH (100 ml) and EtOH (500 ml) was heated to reflux for 20 h, then concentrated in vacuo. The residue was diluted with brine and extracted with EtO. The organic layer was washed with saturated brine, dried over anhydrous MgSO₄ and concentrated in vacuo to give the piperidine (8, 33.3 g, 89%) as a colorless solid. 1H-NMR (CDCl₃) δ: 1.71–1.77 (2H, m), 1.80–1.89 (2H, m), 1.99 (1H, s), 2.99–3.10 (4H, m), 5.07 (2H, s), 7.11–7.16 (1H, m), 7.19–7.22 (1H, m), 7.25–7.30 (2H, m). EI-MS m/z: 189 (M⁺).

A 4N HCl solution in 1,4-dioxane (0.476 ml, 1.90 mmol) was added to a mixture of compound 8 (300 mg, 1.59 mmol), AcOEt (3 ml) and MeOH (0.3 ml) at 0°C, and the whole was stirred for 20 min at the same temperature. The resulting precipitate was collected by filtration, washed with AcOEt and recrystallized from MeOH:AcOEt to give the hydrochloride of compound 8 (230 mg, 64%) as colorless crystals. mp 191–193°C. 1H-NMR (DMSO-d₆) δ: 1.74–1.81 (2H, m), 2.16–2.27 (2H, m), 3.04–3.12 (2H, m), 3.27–3.36 (2H, m), 5.04 (2H, s), 7.15–7.20 (1H, m), 7.31–7.37 (3H, m). EI-MS m/z: 189 (M⁺).

(±)-N-(2-(3,4-Dichlorophenyl)-4-hydroxybutyl)-N'-methylbenzamide (11) A mixture of Ac₂O (10.0 ml, 112 mmol) and HCOOH (10.0 ml, 265 mmol) was stirred for 30 min at 60°C, and then a solution of (±)-4-amino-3-(3,4-dichlorophenyl)butan-1-ol (9, 2.99 g, 12.8 mmol) in THF (30 ml) was added at 0°C. The mixture was stirred for 2.5 h at room temperature, poured into H₂O and neutralized with K₂CO₃, and the product was extracted with CHCl₃. The extract was washed with saturated brine, and the organic layer was dried over anhydrous MgSO₄ and concentrated in vacuo. The residue was diluted with THF (30 ml), then added to BH₃/THF complex (1.0 mol solution in THF, 38 ml, 0.38 mol) at 0°C, and the mixture was heated to reflux for 5 h. Next, EtOH (30 ml) and diethanolamine (2.69 g, 25.6 mmol) were added at 0°C, and the mixture was heated again to reflux for 14 h and concentrated in vacuo. The residue was diluted with brine, and extracted with AcOEt. The extract was washed with saturated brine, dried over anhydrous MgSO₄ and concentrated in vacuo. CH₂Cl₂ (70 ml) and EtN (4.46 g, 32.0 mmol) were added to the residue, and the mixture was cooled to...
route 15 min before challenge with the agonist, and lung resistance was measured using a whole-body plethysmograph. The doses required to reduce the responses by 50% (ID$_{50}$) were determined by probit analysis.

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References


