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J-104129, a Novel Muscarinic M_3 Receptor Antagonist with High Selectivity for M_3 over M_2 Receptors

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Abstract—A new class of 4-acetamidopiperidine derivatives has been synthesized and investigated for human muscarinic receptor subtype selectivity. Introduction of a hydrocarbon chain of appropriate length into the piperidine nitrogen of the racemic *N*-(piperidin-4-yl)-2-cyclobutyl-2-hydroxy-2-phenylacetamide platform conferred up to 70-fold selectivity for human muscarinic M_3 receptors over M_2 receptors. Subsequent synthetic derivatizations resulted in highly potent M_3 receptor antagonists with selectivity greater than two orders of magnitude for M_3 over M_2 receptors, from which the analogue **4r** was selected. Preparation of both enantiomers of **4r** led to the identification of (2*R*)-*N*-[1-(4-methyl-3-pentenyl)piperidin-4-yl]-2-cyclopentyl-2-hydroxy-2-phenylacetamide (J-104129, **(*R*)-4r**), which exhibited 120-fold selectivity for M_3 receptors ($K_i = 4.2$ nM) over M_2 receptors ($K_i = 490$ nM). In isolated rat trachea, **(*R*)-4r** potently and specifically antagonized acetylcholine (ACh)-induced responses with a K_B value of 3.3 nM. The highly subtype-selective profile was also seen in isolated rat tissue assays (50-fold) and in anesthetized rats (>250-fold). Oral administration of J-104129 (**(*R*)-4r**) antagonized ACh-induced bronchoconstriction with an ED_{50} value of 0.58 mg/kg in rats. Thus, J-104129 (**(*R*)-4r**) may effectively facilitate bronchodilation in the treatment of obstructive airway disease.

Introduction

Anti-cholinergic drugs have been used for a century in the treatment of obstructive airway diseases. Although classical muscarinic antagonists such as atropine are potent bronchodilators, their clinical utility is limited due to the high occurrence of both peripheral and central adverse effects such as tachycardia, blurred vision and dementia. Subsequent development of the quaternary derivatives of atropine such as ipratropium bromide resulted in the potential clinical use of muscarinic antagonists as aerosol agents. Although they are better tolerated than parenterally administered atropine, the quartenary derivatives may be not ideal anti-cholinergic bronchodilators because of their short duration and non-selective profile for muscarinic receptor subtypes.

In the last decade, five muscarinic receptor subtypes have been identified and cloned: they are classified as m1, m2, m3, m4 and m5.[1–5] Pharmacological criteria suggested the presence of four muscarinic receptors, denoted M_1, M_2, M_3 and M_4; the physiological role(s) of the m5 gene product remains to be identified.[6,7] The different localizations and functions of these receptor subtypes raised the possibility of designing antagonists that selectively interact with distinct subtypes, thus avoiding the occurrence of adverse effects.

The airways of several species including humans contain at least three pharmacological muscarinic receptor subtypes as follows:[8,9] neuronal M_1 receptors, which facilitate parasympathetic neurotransmission; neuronal presynaptic M_2 receptors, which inhibit acetylcholine (ACh) release as part of a negative feedback mechanism; and smooth muscle M_3 receptors, which mediate contraction and secretion. There are a number of reports indicating that non-selective muscarinic antagonists increase ACh release from isolated human and guinea pig bronchial tissues and potentiate bronchoconstriction in certain animals by blocking the neuronal M_2 receptors after vagal stimulation.[10–13] Furthermore, we recently demonstrated that the blockade of airway neuronal M_2 receptors stimulates SO_2-induced mucus hypersecretion in a rat bronchitis model.[14] Therefore,

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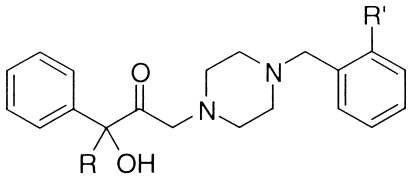
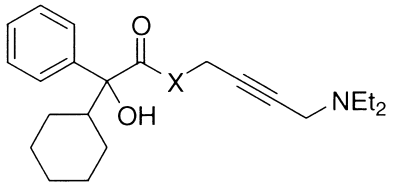
identification of M_3 antagonists with far greater selectivity for M_3 over airway neuronal M_2 receptors might lead to an ideal anti-cholinergic bronchodilator with greater efficacy than the existing non-selective antagonists.[8–10,15]

A number of studies in the muscarinic field have suggested that binding is initiated by a tight ion–ion interaction between the quarternary ammonium moiety of a ligand and the aspartic residue in the third transmembrane of the receptor proteins.[16,17] It has been very difficult to distinguish between receptor subtypes due to a high degree of sequence homology, especially in the close circumstance of the binding space around the aspartic (Asp) residue. In fact, only a few compounds such as revatropate,[10] darifenacin[10] and Scios Nova compounds **1**[18] have been reported to show subtype selectivity for M_3 over M_2 receptors. Accordingly, further exploration has been desired to develop a new class of muscarinic antagonists highly selective for M_3 over M_2 receptors.

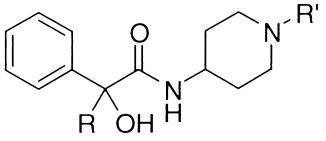
In the course of our program to develop a novel bronchodilator with fewer muscarinic side effects, we designed a new class of 4-acetamidopiperidine derivatives **4** as the lead structure based on the structural features of the following two compounds: the Scios Nova compounds **1** exhibiting good selectivity for M_3 over M_1 and M_2 receptors in isolated tissues assays[18] and the amide analogue **3** of oxybutynin (**2**),[19,20] which was reported not only to protect against hydrolysis by liver esterase but also to reduce several side effects, probably due to differential tissue distribution (Fig. 1).[21,22] The synthesized derivatives were tested for their binding affinities for human m1, m2 and m3 receptors expressed in CHO cells. Further evaluation of the selected compound was conducted in in vitro and in vivo functional assays.



Nova's compounds
1a R = cyclobutyl, R' = Cl
1b R = phenyl, R' = H



2 X = O oxybutynin
3 X = NH



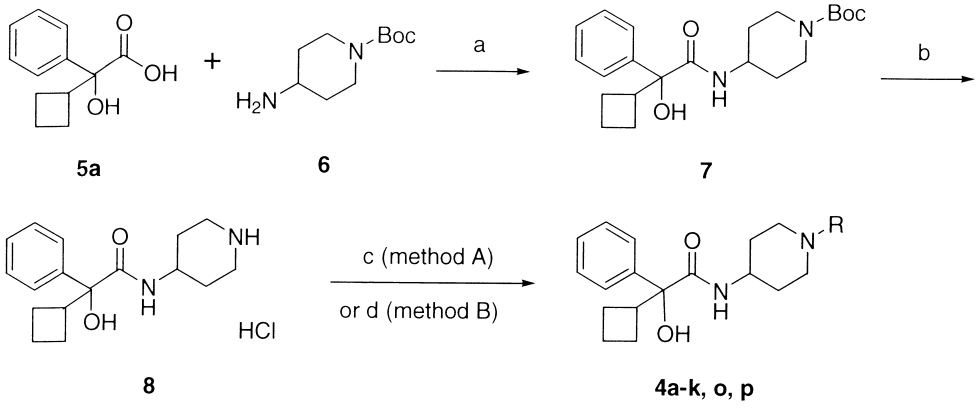
4 4-acetamidopiperidines

Figure 1.

Chemistry

The 4-acetamidopiperidine derivatives **4a–k**, **4o** and **4p** were generally synthesized as racemates by the methods described in Scheme 1. The usual coupling of racemic carboxylic acid **5a** with 4-amino-1-*tert*-butyloxycarbonylpiperidine (**6**) followed by acidic deprotection gave amide **8**. Subsequently, the amide **8** was alkylated with mesylates or halides in the presence of a base (method A) or was subjected to reductive alkylation with the corresponding aldehydes (method B).

The synthesis of **4q–t**, **14a–c**, **15a**, **15b** and **18** is outlined in Schemes 2 and 3. Hydroxy or aminopiperidine derivative **12**, **13** was prepared from commercially available 4-piperidone hydrochloride (**9**) and 5-bromo-2-methyl-2-pentene (**10**) in two steps involving alkylation and reduction or reductive amination, respectively. The coupling reaction of the compounds **12**, **13** with acids **5a–g** completed the synthesis of the derivatives **4q–t**, **14a–c** under similar conditions described for the preparation of the compound **7**.

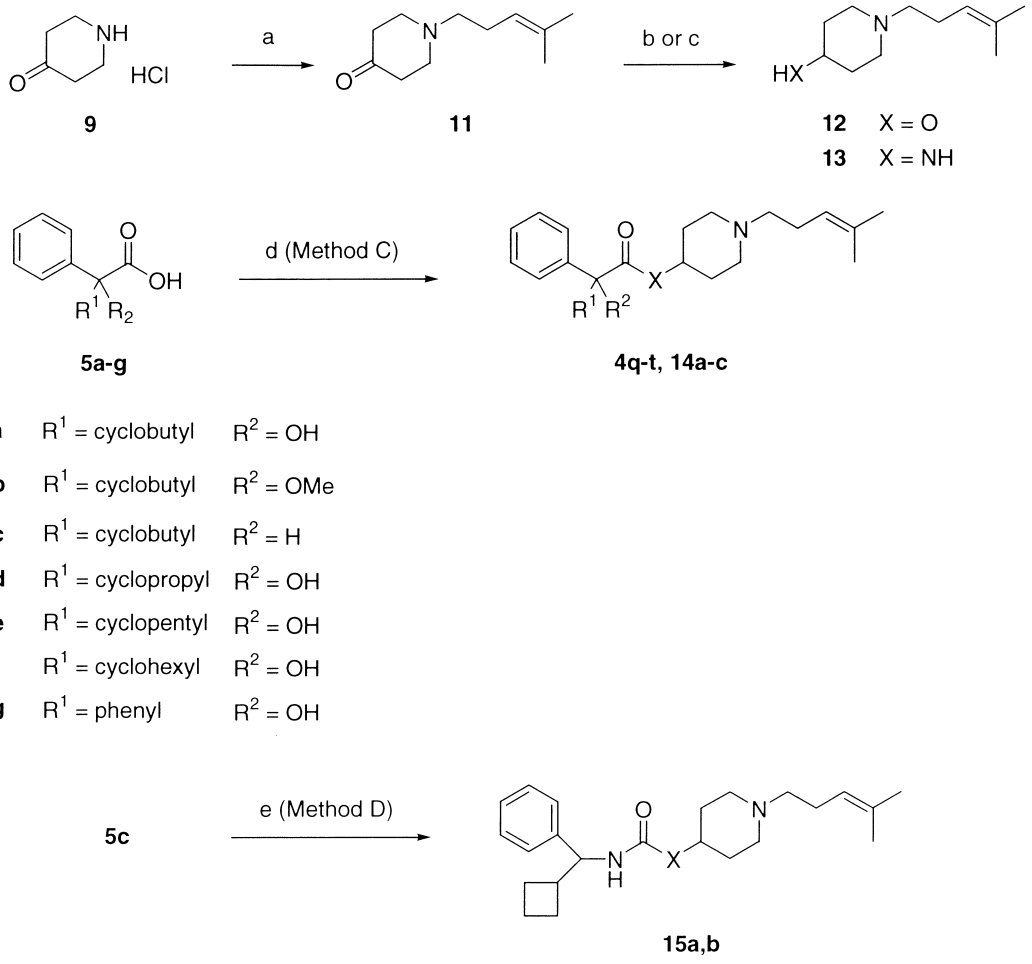


Scheme 1. Conditions: (a) WSC–HCl, HOBt, $CHCl_3$; (b) HCl–MeOH; (c) mesylate or halide, K_2CO_3, CH_3CN; (d) aldehyde, $NaB(OAc)_3H$, THF.

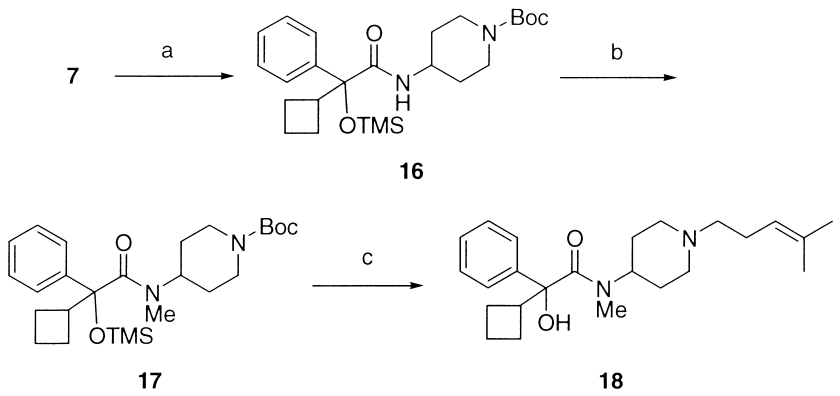
Carbamate **15a** and urea **15b** were prepared from acid **5c**. Curtius rearrangement of **5c** followed by treatment of the resulting isocyanate with **12** or **13** gave **15a** and **15b**, respectively.

N-Methylamide derivative **18** was obtained from the common intermediate **7**, in which the hydroxy group was protected as trimethylsilylether to give **16**. The amide moiety of **16** was alkylated by treatment with iodomethane in the presence of sodium hydride and *tetra-n*-butylammonium iodide to yield **17**. Deprotection of the trimethylsilyl and *tert*-butyloxycarbonyl groups by acid exposure and subsequent alkylation with the bromide **10** yielded the desired *N*-methylamide **18**.

As for **4r**, optically active compounds **(*R*)-4r** and **(*S*)-4r** were prepared to determine the biologically active enantiomer and for use in further biological evaluations.



Scheme 2. Conditions: (a) 5-bromo-2-methyl-2-pentene **10**, K_2CO_3, Kl, DMF; (b) $NaBH_4$, MeOH; (c) $AcONH_4$, $NaBCNH_3$, MeOH; (d) **12** or **13**, WSC–HCl, HOBt; (e) DPPA, toluene then **12** or **13**.



Scheme 3. Conditions: (a) TMSCl, imidazole, DMF; (b) NaH, Mel, *n*-Bu_4Nl, THF; (c) HCl–MeOH then **10**, K_2CO_3, Kl, DMF.

Optical resolution of racemic 2-cyclopentyl-2-hydroxy-2-phenylacetic acid **5e** was achieved by repetitive recrystallization of its cinchonidine salt, avoiding the use of amphetamine.[23] Subsequent treatment with hydrochloric acid afforded the chiral acid **(−)-5e** in >99% ee and 24% chemical yield. The absolute configuration of the acid **(−)-5e** was determined to be *R* by the X-ray crystallographic analysis of (*R*)-mandelic acid salt of **(*R*)-(−)-4r**.[24] The antipodal **(*S*)-4r** was obtained by preparative HPLC (98% ee).

Results and Discussion

This investigation was prompted by the observation that the *N*-benzyl and 1,1-diphenyl-3-piperazinylpropanone derivatives (Nova compounds, **1a**, **1b**) showed high selectivity for M_3 over M_2 receptors in isolated tissues assays.[18] We speculated that the substituents on the piperazine nitrogen and at the 1-position of the 1-phenyl-3-piperadinylpropan-2-one may contribute to differentiation among the receptor subtypes. In our acetamidopiperidine series **4**, an *N*-substituent (R′) on the piperidine ring would be one of the most effective moieties for improving the subtype selectivity for M_3 over M_2 receptors according to the above speculation. Therefore, extensive investigation of the substituent on the piperidine nitrogen of acetamidopiperidine series **4** was made a priority, whereas a cyclobutylmandelic acid moiety was fixed to interpret the correlation between Nova's and our compounds. After selecting a favorable *N*-substituent, further optimization of the other functional groups, e.g. the cyclobutyl, hydroxyl and amide moieties, was performed systematically to identify a compound with the best selectivity.

First, introduction of low alkyl groups such as methyl and butyl substituents into the piperidine nitrogen gave compounds **4a**, **4b** that showed less than 10-fold m3 over m2 selectivity, comparable to that of the corresponding piperazinylpropanone derivatives.[18] When a benzyl group was introduced, the m3/m2 selectivity of the resulting derivative **4o** did not improve despite good binding affinity. As the first example of a compound exhibiting more than 20-fold m3/m2 selectivity, the pentyl derivative **4c** with a low affinity for m3 receptors (K_i = 240 nM) was identified. This result prompted us to perform further derivatization by introducing various types of long hydrocarbon chains and branched chains to improve m3/m2 selectivity. Surprisingly, the 4-methylpentyl and 4-methylpentenyl derivatives **4e**, **4g** and **4h** prepared as representatives with a branched hydrocarbon side chain showed approximately 50-fold m3 selectivity over m2 receptors. This subtype selectivity was maximized in the case of the (4*R*)-4-methylhexyl derivative **4i** (m3/m2 = 71). In contrast, introduction of ether (**4k**), amide (**4l**), alcohol (**4m**) and carboxylic acid (**4n**) moieties into the side chain resulted in a considerable decrease in the binding affinity. Thus, we found that the nature of the *N*-substituent on the piperidine in this series significantly influenced not only receptor subtype selectivity but also affinity for m3 receptors. Based on the selectivities and affinities of the compounds shown in Table 1, the derivative **4g** was selected for further optimization of the other parts of the molecule (Table 2).

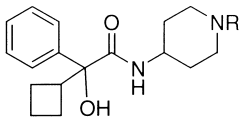
Removal of the *tert*-alcohol (**14b**) and methylation of the amide (**18**) or the alcohol (**14a**) reduced both selectivity and affinity, indicating that a hydrogen bonding interaction contributes to receptor binding and especially to selectivity for m3 over m2 receptors. On the other hand, the ester analogue **14c** exhibited remarkably high affinity (m3 = 0.28 nM) but low selectivity (m3/m2 = 13). This finding coincides with a number of reports that known ester classes of compounds potently bind to all receptor subtypes in a non-selective manner.[25,26] The carbamate analogue **15a** also enhanced binding affinity (m3 = 3.8 nM) despite a lack of *tert*-hydroxy function, while urea **15b** showed lower affinity (m3 = 86 nM). Among these compounds with a variety of linkage moieties, **4g** with the *tert*-alcohol and amide linkage displayed the best selectivity (m3/m2 = 50).

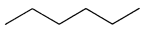
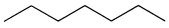
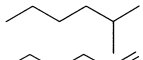
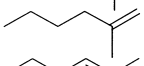
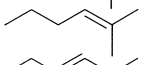
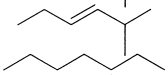
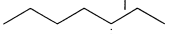
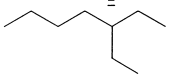
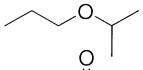
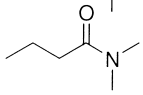
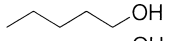
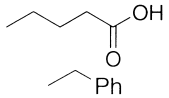
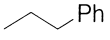
Subsequently, the cyclobutyl moiety of **4g** was optimized by replacing it with different ring sized cycloalkyls and a phenyl group. The ring expansion of the cyclobutyl moiety in **4g** gave cyclopentyl and cyclohexyl derivatives **4r**, **4s** which exhibited greatly improved subtype selectivity (**4r**: m3/m2 = 120, **4s**: m3/m2 = 110), whereas the ring size reduction (**4q**) or replacement with a phenyl group (**4t**) greatly reduced the selectivity (m3/m2 = 21 or 26). This result indicated that the substituent on the manderoyl group also played an important role in the improvement of subtype selectivity. Thus, the cyclopentyl group at this position was superior with respect to selectivity and affinity (Table 3).

Finally, to determine the absolute configuration of the biologically active enantiomer, **(*R*)-4r** and **(*S*)-4r** were prepared and compared in binding assays as shown in Table 4. The (*R*)-form showed much higher affinity than did the antipode, as found in other antagonists with a similar structure.[21,27] Furthermore, **(*R*)-4r** retained high m3/m2 selectivity (120-fold), while the antipode **(*S*)-4r** showed moderate selectivity (28-fold). Thus, we identified **(*R*)-4r** as the best compound in the series of 4-acetamidopiperidine derivatives.

Next, we evaluated **(*R*)-4r** in isolated tissues assays to determine whether it is a highly selective M_3 receptor antagonist over M_2 receptors. Several functional assays in isolated tissues have been pharmacologically well-characterized.[27–29] Carbachol-induced contractile responses in isolated rat trachea are mediated through M_3 receptors. McN A-343-induced inhibition of twitch contraction in isolated rabbit vas deferens and carbachol-induced bradycardia in isolated rat right atria are mediated through M_1 and M_2 receptors, respectively. As shown in Table 5, in isolated rat trachea, **(*R*)-4r** potently and specifically antagonized the ACh-induced responses with a K_B value of 3.3 nM. In isolated rat right atria, **(*R*)-4r** showed less potent inhibition of carbachol-induced bradycardia with a K_B value of 170 nM. In isolated rabbit vas deferens, **(*R*)-4r** antagonized the inhibition of McN A-343 against the twitch response

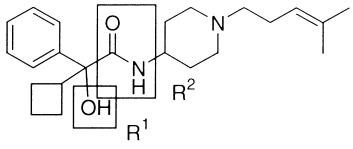
Table 1. Binding affinity to muscarinic receptors (**1**)



Compound	*R*	% Yield (method)	Binding affinity (K_i, nM)[a]			Selectivity	
			m1	m2	m3	m1/m3	m2/m3
4a	Me	59 (B)	180	1500	160	1.1	9.0
4b		84 (B)	2900	12,000	1500	1.9	7.8
4c		57 (B)	1400	6500	240	5.9	27
4d		59 (B)	1300	4400	130	10	35
4e		77 (A)	750	3700	73	10	51
4f		70 (A)	270	1900	52	5.2	37
4g		62 (A)	130	1300	26	4.9	50
4h		52 (A)	300	1600	30	9.7	54
4i		46 (A)	690	4800	68	10	71
4j		72 (A)	2300	6500	680	3.4	10
4k		63 (A)	2000	31,000	530	3.8	58
4l		[b]	1400	20,000	500	2.9	40
4m		[b]	> 3000	> 60,000	> 3000	—	—
4n		[b]	> 2900	> 59,000	> 2800	—	—
4o		80 (B)	4.8	35	2.1	2.3	14
4p		74 (B)	130	210	50	2.7	4.2

[a] Values are the mean of two or more independent assays.
[b] See Experimental.

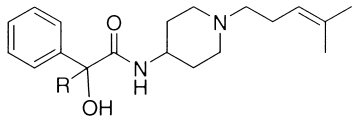
Table 2. Binding affinity to muscarinic receptors (**2**)



Compound	R^1	R^2	% Yield (method)	Binding affinity (K_i, nM)[a]			Selectivity	
				m1	m2	m3	m1/m3	m2/m3
4g	OH	CONH	62 (A)	130	1300	26	4.9	50
18	OH	CONMe	[b]	79	150	140	0.6	1.1
14a	OMe	CONH	50 (C)	> 3000	15,000	> 2900	—	—
14b	H	CONH	40 (C)	990	5100	250	4.0	21
14c	OH	COO	34 (C)	0.97	3.5	0.28	3.5	13
15a	H	NHCOO	23 (D)	14	110	3.8	3.8	30
15b	H	NHCONH	49 (D)	340	3000	86	4.0	34

[a] Values are the mean of two or more independent assays.
[b] See Experimental.

Table 3. Binding affinity to muscarinic receptors (**3**)



Compound	*R*	% Yield (method)	Binding affinity (K_i, nM)[a]			Selectivity	
			m1	m2	m3	m1/m3	m2/m3
4g		62 (A)	130	1300	26	4.9	50
4q		60 (C)	670	2700	130	5.1	21
4r		59 (C)	25	760	6.5	3.8	120
4s		50 (C)	16	940	8.7	1.8	110
4t		62 (C)	173	1700	67	2.6	26

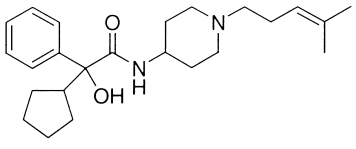
[a] Values are the mean of two or more independent assays.

induced by electrical field stimulation, with a K_B value of 14 nM, exhibiting modest tracheal selectivity over rabbit vas deferens. Accordingly, **(*R*)-4r** is a potent M_3 receptor antagonist with 50-fold selectivity for tracheal M_3 over cardiac M_2 receptors.

Furthermore, the in vivo selectivity of **(*R*)-4r** was examined in rats (Table 6). Intravenous administration of **(*R*)-4r** dose-dependently inhibited ACh-induced bronchoconstriction with an ED_{50} value of 0.012 mg/kg. In contrast, intravenous administration of **(*R*)-4r** up to 3 mg/kg did not affect the ACh-induced bradycardia that was mediated by M_2 receptors. These results indicated that **(*R*)-4r** showed >250-fold bronchial selectivity over heart.

Finally, we examined the oral activity of **(*R*)-4r** in anesthetized rats. Oral administration also potently antagonized ACh-induced bronchoconstriction with an ED_{50} value of 0.58 mg/kg, indicating that **(*R*)-4r** is a potent oral bronchodilator.

Table 4. Binding affinity of **(*R*)-4r** and **(*S*)-4r**



Compound	Binding affinity (K_i, nM)[a]			Selectivity	
	m1	m2	m3	m1/m3	m2/m3
(*R*)-4r	19	490	4.2	4.5	120
(*S*)-4r	1700	15,000	540	3.1	28
4r	25	760	6.5	3.8	120

[a] Values are the mean of two or more independent assays.

Table 5. The K_b values (nM) of **(*R*)-4r** and atropine in functional assays using isolated tissues[a]

Compound	Rabbit vas deferens (M_1)	Rat right atria (M_2)	Rat trachea (M_3)
(*R*)-4r	14	170	3.3
Atropine	0.11	1.5	0.49

[a] Values are the mean of more than three experiments.

Table 6. In vivo activity in rats

Compound	Bronchus (M_3)		Heart (M_2)
	ED_{50} (iv)[a]	ED_{50} (po)[a]	ED_{50} (iv)
(*R*)-4r fumarate	0.012 (1)[c]	0.58	<3[b] (>250)[c]
Atropine	0.0043 (1)[c]	0.27	0.0037 (0.9)[c]

[a] ED_{50} values (mg/kg) are the mean of three experiments.
[b] $52.7 \pm 2.2\%$ inhibition at 3 mg/kg, $n = 4$.
[c] Values in parentheses represent selectivity for M_3 (-fold).

Conclusion

For the purpose of developing a selective M_3 receptor antagonist as a bronchodilator, a new class of 4-acetamidopiperidine derivatives was prepared and evaluated for anti-muscarinic activities. Among these derivatives, (2*R*)-*N*-[1-(4-methyl-3-pentenyl)piperidin-4-yl]-2-cyclopentyl-2-hydroxy-2-phenylacetamide **(*R*)-4r** (J-104129), displayed 120-fold selectivity for m3 over m2 receptors with a K_i value of 4.2 nM for affinity in the binding assay. This highly subtype-selective profile was also seen in both in vitro (isolated tissues) and in vivo studies (antagonism for ACh-induced bronchoconstriction versus ACh-induced bradycardia in rats). Furthermore, it was

demonstrated that **(*R*)-4r** was an orally active M_3 receptor antagonist in rats. Since **(*R*)-4r** (J-104129) with high selectivity for M_3 over airway neuronal M_2 receptors should not induce M_2-mediated adverse effects such as tachycardia and paradoxical bronchoconstriction, it may provide more effective anti-cholinergic therapy than do non-selective muscarinic antagonists in obstructive airway diseases. Detailed pharmacological and pharmacokinetic profiles of this compound will be reported elsewhere.

Experimental

Melting points were determined with a Yanaco MP micromelting point apparatus and were not corrected. Proton NMR spectra were obtained on a Varian Gemini-300 with tetramethylsilane as an internal standard. IR spectra were recorded with Horiba FT-200 spectrometer. Mass spectrometry were performed with JEOL JMS-SX 102A. Optical rotations were measured with Jasco DIP-370 polarimeter. TLC were done with Merck Kieselgel F_{254} pre-coated plates.

***N*-(1-*tert*-Butyloxycarbonylpiperidin-4-yl)-2-cyclobutyl-2-hydroxy-2-phenylacetamide (7)**. To a stirred solution of 2-cyclobutyl-2-hydroxy-2-phenylacetic acid (**5a**)[30] (1.43 g, 6.94 mmol) and 4-amino-1-*tert*-butyloxycarbonylpiperidine (**6**)[31] (1.38 g, 6.90 mmol) in $CHCl_3$ (60 mL) were sequentially added 1-hydroxybenzotriazole (1.39 g, 10.29 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (1.50 g, 7.82 mmol) at room temperature, and the mixture was stirred for 15 h. The mixture was diluted with Et_2O and washed with satd $NaHCO_3$ and brine, and dried over $MgSO_4$. Evaporation of the solvent gave the crude product, which was purified by silica gel column chromatography to afford **7** (2.10 g,78%) as a white solid: mp 154.5–156°C (from *n*-hexane/$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.15–1.35 (2H, m), 1.44 (9H, s), 1.70–2.13 (8H, m), 2.75–2.91 (2H, m), 3.31 (1H, OH, s), 3.38 (1H, m), 3.75–4.07 (3H, m), 6.22 (1H, NH, brd, J = 7.8 Hz), 7.20–7.39 (3H, m), 7.49 (2H, brd, J = 8.4 Hz); MS m/z 389 $(M+H)^+$. Anal. calcd for $C_{22}H_{32}N_2O_4 \cdot 0.2H_2O$: C, 67.39; H, 8.33; N, 7.14. Found: C, 67.19; H, 8.37; N, 7.13.

***N*-(Piperidin-4-yl)-2-cyclobutyl-2-hydroxy-2-phenylacetamide hydrochloride (8)**. A solution of **7** (2.00 g, 5.15 mmol) in 10% HCl–MeOH (60 mL) was stirred at room temperature for 15 h and then evaporated to dryness in vacuo to obtain the crude oily residue **8** (1.68 g) which was used in the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 1.24–1.35 (2H, m), 1.60–2.19 (8H, m), 2.57–2.71 (2H, m), 2.90–3.09 (2H, m), 3.38 (1H, m), 3.78 (1H, m), 6.38 (1H, m), 7.18–7.40 (3H, m), 7.50 (2H, brd, J = 7.2 Hz); MS m/z 289 $(M+H)^+$. Free base of **8**: mp 153–155°C (from hexane–AcOEt). Anal. calcd for $C_{17}H_{24}N_2O_2 \cdot 0.2H_2O$: C, 69.93; H, 8.42; N, 9.52. Found: C, 69.70; H, 8.62; N, 9.42.

General procedure (method A)

The experimental procedure of **4f** was described as a representative example.

***N*-[1-(4-Methyl-4-pentenyl)piperidin-4-yl]-2-cyclobutyl-2-hydroxy-2-phenylacetamide (4f)**. To a solution of 4-methyl-4-pentenol[32] (50 mg, 0.50 mmol) in AcOEt (5 mL) were added triethylamine (0.12 mL, 0.86 mmol) and mesylchloride (0.05 mL, 0.65 mmol) at 0°C. After stirring for 1 h, the mixture was quenched with sat. $NaHCO_3$ and further stirred for 1 h at room temperature. The organic layer was separated, washed with brine and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to give the crude mesylate which was used in the next step without purification. To a suspension of **8** (130 mg, 0.40 mmol), K_2CO_3 (200 mg, 1.45 mmol) and potassium iodide (10 mg, 0.06 mmol) in CH_3CN was added the crude mesylate, and the mixture was heated at 75°C for 4 h. After cooling to room temperature, the mixture was diluted with H_2O and extracted twice with $CHCl_3$. The solvent was evaporated under reduced pressure and the resulting residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 100:1) to yield **4f** (104 mg, 70%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.30–2.20 (16H, m), 1.71 (3H, s), 2.24–2.39 (2H, m), 2.69–2.88 (2H, m), 3.29–3.58 (2H, m), 3.71 (1H, m), 4.66 (1H, brd, J = 1.4 Hz), 4.69 (1H, brd, J = 1.4 Hz), 6.15 (1H, brd, J = 7.8 Hz), 7.21–7.41 (3H, m), 7.48 (2H, brd, J = 8.4 Hz); MS m/z 371 $(M+H)^+$. **4f**·fumarate: mp 170–173°C (from *i*-Pr_2O/*i*-PrOH). Anal. calcd for $C_{23}H_{34}N_2O_2 \cdot C_4H_4O_4$: C, 66.64; H, 7.87; N, 5.76. Found: C, 66.44; H, 7.73; N, 5.71.

The following compounds **4e**, **4g–k** and **19** were prepared from **8** and the appropriate alcohols or bromides in a similar method described for **4f**.

***N*-[1-(4-Methyl-1-pentyl)piperidin-4-yl]-2-cyclobutyl-2-hydroxy-2-phenylacetamide (4e)**. **4e** was obtained from **8** and 1-bromo-4-methylpentane (77%): ^{1}H NMR ($CDCl_3$) δ 0.87 (6H, d, J = 6.6 Hz), 1.10–1.20 (2H, m), 1.34–1.60 (5H, m), 1.70–2.15 (10H, m), 2.24–2.33 (2H, m), 2.72–2.86 (2H, m), 3.30–3.60 (2H, m), 3.72 (1H, m), 6.13 (1H, d, J = 8.1 Hz), 7.22–7.38 (3H, m), 7.49 (2H, brd, J = 8.3 Hz); MS m/z 373 $(M+H)^+$. **4e**·fumarate: mp 188–190°C (from *i*-Pr_2O/*i*-PrOH). Anal. calcd for $C_{23}H_{36}N_2O_2 \cdot C_4H_4O_4$: C, 66.37; H, 8.25; N, 5.73. Found: C, 66.43; H, 8.60; N, 5.75.

***N*-[1-(4-Methyl-3-pentenyl)piperidin-4-yl]-2-cyclobutyl-2-hydroxy-2-phenylacetamide (4g)**. **4g** was obtained from **8** and 5-bromo-2-methyl-2-pentene (62%): ^{1}H NMR ($CDCl_3$) δ 1.38–1.52 (2H, m), 1.60 (3H, s), 1.68 (3H, s), 1.65–2.22 (12H, m), 2.27–2.38 (2H, m), 2.70–2.88 (2H, m), 3.59 (1H, m), 3.46 (1H, brs), 3.73 (1H, m), 5.08 (1H, m), 6.18 (1H, brd, J = 8.0 Hz), 7.21–7.40 (3H, m), 7.49 (2H, brd, J = 8.4 Hz); MS m/z 371 $(M+H)^+$. **4g**·fumarate: mp 185–187°C (from *i*-Pr_2O/*i*-PrOH). Anal. calcd for $C_{23}H_{34}N_2O_2 \cdot C_4H_4O_4$: C, 66.64; H, 7.87; N, 5.76. Found: C, 66.24; H, 7.87; N, 5.64.

***N*-[1-(4-Methyl-2-pentenyl)piperidin-4-yl]-2-cyclobutyl-2-hydroxy-2-phenylacetamide (4h)**. **4h** was obtained from **8** and 4-methyl-2-pentenol[33] (52%): ^{1}H NMR ($CDCl_3$) δ 0.98 (6H, d, J = 6.9 Hz), 1.38–2.18 (12H, m), 2.28 (1H, m), 2.69–2.86 (2H, m), 2.82 (2H, brd, J = 6.6 Hz), 3.29–3.49 (2H, m), 3.71 (1H, m), 5.40 (1H, m), 5.56 (1H, m),

6.16 (1H, m), 7.20–7.40 (3H, m), 7.49 (2H, brd, $J=8.4$ Hz); MS m/z 371 $(M+H)^+$. **4h**·fumarate: mp 175–176.5°C (from *i*-Pr_2O/*i*-PrOH). Anal. calcd for $C_{23}H_{34}N_2O_2 \cdot C_4H_4O_4$: C, 66.64; H, 7.87; N, 5.76. Found: C, 66.48; H, 7.98; N, 5.73.

***N*-[1-[(4*S*)-4-Methyl-1-hexyl]piperidin-4-yl]-2-cyclobutyl-2-hydroxy-2-phenylacetamide (4i)**. **4i** was obtained from **8** and (*S*)-4-methyl-1-hexanol (46%). ^1H NMR ($CDCl_3$) δ 0.79–0.90 (6H, m), 1.00–1.54 (9H, m), 1.68–2.15 (10H, m), 2.27 (2H, brt, $J=7.8$ Hz), 2.70–2.85 (2H, m), 3.29–3.51 (2H, m), 3.71 (1H, m), 6.12 (1H, brd, $J=8.1$ Hz), 7.21–7.38 (3H, m), 7.44–7.51 (2H, m); MS m/z 387 $(M+H)^+$. A mixture of diastereomers (t_R 11.6 and 14.0 min, Daicel Chiralcel OJ 0.46×25 cm, *n*-hexane:AcOEt = 95:5, flow rate = 0.5ml/min, UV detection 220 nm).

***N*-[1-(4-Ethyl-1-hexyl)piperidin-4-yl]-2-cyclobutyl-2-hydroxy-2-phenylacetamide (4j)**. **4j** was obtained from **8** and 4-ethyl-1-hexanol[34] (72%): ^1H NMR ($CDCl_3$) δ 0.82 (6H, s), 1.10–1.54 (10H, m), 1.70–2.17 (11H, m), 2.23–2.35 (2H, m), 2.70–2.88 (2H, m), 3.62–3.80 (2H, m), 3.71 (1H, m), 6.16 (1H, brd, $J=8.1$ Hz), 7.21–7.39 (3H, m), 7.49 (2H, brd, $J=8.3$ Hz); MS m/z 401 $(M+H)^+$. **4j**·fumarate: mp 190–191°C (from *i*-Pr_2O/*i*-PrOH). Anal. calcd for $C_{25}H_{40}N_2O_2 \cdot C_4H_4O_4$: C, 67.42; H, 8.58; N, 5.42. Found: C, 67.08; H, 8.73; N, 5.39.

***N*-[1-(2-Isopropyloxyethyl)piperidin-4-yl]-2-cyclobutyl-2-hydroxy-2-phenylacetamide (4k)**. **4k** was obtained from **8** and 2-isopropyloxy-1-ethanol (63%): ^1H NMR ($CDCl_3$) δ 1.13 (6H, d, $J=6.0$ Hz), 1.40 (1H, m), 1.56–2.27 (10H, m), 2.55 (2H, t, $J=6.0$ Hz), 2.72–2.88 (2H, m), 3.29–3.78 (6H, m), 6.11 (1H, d, $J=7.5$ Hz), 7.21–7.47 (3H, m), 7.48 (2H, brd, $J=8.1$ Hz); MS m/z 375 $(M+H)^+$. **4k**·fumarate: mp 172–175°C (from *i*-Pr_2O/*i*-PrOH). Anal. calcd for $C_{22}H_{34}N_2O_3 \cdot C_4H_4O_4$: C, 63.65; H, 7.81; N, 5.71. Found: C, 63.63; H, 7.96; N, 5.72.

***N*-[1-(3-Ethoxycarbonylpropyl)piperidin-4-yl]-2-cyclobutyl-2-hydroxy-2-phenylacetamide (19)**. **19** was obtained from **8** and ethyl 4-bromobutyrate (90%): ^1H NMR ($CDCl_3$) δ 1.24–1.48 (2H, m), 1.26 (3H, t, $J=7.2$ Hz), 1.60–2.15 (12H, m), 2.21–2.39 (4H, m), 2.66–2.80 (2H, m), 3.36 (1H, m), 3.46 (1H, brs), 3.70 (1H, m), 4.12 (2H, d, $J=7.2$ Hz), 6.13 (1H, brd, $J=8.4$ Hz), 7.22–7.40 (3H, m), 7.50 (2H, brd, $J=8.4$ Hz); HRMS m/z calcd for $C_{23}H_{34}N_2O_4$ $(M+H)^+$: 403.2597. Found: 403.2589.

General procedure (method B)

The experimental procedure of **4b** was described as a representative example.

***N*-(1-Butylpiperidin-4-yl)-2-cyclobutyl-2-hydroxy-2-phenylacetamide (4b)**. To a solution of **8** (72 mg, 0.22 mmol) and *n*-butylaldehyde (40 mg, 0.55 mmol) in THF (3 mL) was added sodium triacetoxyborohydride (100 mg, 0.64 mmol) at room temperature and the mixture was stirred for 17 h. After the addition of saturated aqueous sodium bicarbonate soln, the mixture was extracted with $CHCl_3$. The organic layer was washed with brine and dried over anhyd Na_2SO_4. The solvent was evaporated under reduced pressure and the resulting residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 100:1) to obtain **4b** (64 mg, 84%) as an oil: ^1H NMR ($CDCl_3$) δ 0.90 (3H, t, $J=7.4$ Hz), 1.20–1.53 (6H, m), 1.55–2.19 (10H, m), 2.24–2.40 (2H, m), 2.69–2.90 (2H, m), 3.29–3.58 (2H, m), 3.71 (1H, m), 6.14 (1H, brd, $J=8.3$ Hz), 7.19–7.39 (3H, m), 7.49 (2H, brd, $J=8.4$ Hz); MS m/z 345 $(M+H)^+$. **4b**·fumarate: mp 174–176°C (from *i*-Pr_2O/*i*-PrOH). Anal. calcd for $C_{21}H_{32}N_2O_2 \cdot C_4H_4O_4$: C, 65.20; H, 7.88; N, 6.08. Found: C, 65.04; H, 8.01; N, 6.03.

The following compounds **4a, 4c, 4d, 4o** and **4p** were prepared from **8** and the appropriate aldehydes as described for **4b**.

***N*-(1-Methylpiperidin-4-yl)-2-cyclobutyl-2-hydroxy-2-phenylacetamide (4a)**. **4a** was obtained from **8** and paraformaldehyde (59%): ^1H NMR ($CDCl_3$) δ 1.30–1.51 (2H, m), 1.60–2.17 (10H, m), 2.25 (3H, s), 2.60–2.80 (2H, m), 3.36 (1H, m), 3.70 (1H, m), 6.20 (1H, brd, $J=7.8$ Hz), 7.20–7.40 (3H, m), 7.49 (2H, brd, $J=8.1$ Hz); MS m/z 302 $(M+H)^+$. **4a**·fumarate: mp 193–194°C (from *i*-PrOH). Anal. calcd for $C_{18}H_{26}N_2O_2 \cdot C_4H_4O_4$: C, 63.14; H, 7.23; N, 6.69. Found: C, 63.22; H, 7.38; N, 6.67.

***N*-(1-Pentylpiperidin-4-yl)-2-cyclobutyl-2-hydroxy-2-phenylacetamide (4c)**. **4c** was obtained from **8** and *n*-pentylaldehyde (57%): ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, $J=6.9$ Hz), 1.18–1.55 (8H, m), 1.68–2.18 (10H, m), 2.21–2.39 (2H, m), 2.70–2.90 (2H, m), 3.29–3.59 (2H, m), 3.70 (1H, m), 6.15 (1H, brd, $J=7.8$ Hz), 7.20–7.40 (3H, m), 7.48 (2H, brd, $J=8.3$ Hz); MS m/z 359 $(M+H)^+$. **4c**·fumarate: mp 158–160°C (from *i*-Pr_2O/*i*-PrOH). Anal. calcd for $C_{22}H_{34}N_2O_2 \cdot C_4H_4O_4$: C, 65.80; H, 8.07; N, 5.90. Found: C, 65.74; H, 8.43; N, 5.87.

***N*-(1-Hexylpiperidin-4-yl)-2-cyclobutyl-2-hydroxy-2-phenylacetamide (4d)**. **4d** was obtained from **8** and *n*-hexylaldehyde (59%): ^1H NMR ($CDCl_3$) δ 0.87 (3H, t, $J=6.8$ Hz), 1.21–1.50 (8H, m), 1.55–2.12 (12H, m), 2.24–2.31 (2H, m), 2.70–2.82 (2H, m), 3.25–3.60 (2H,m), 3.72 (1H, m), 6.11 (1H, d, $J=9.6$ Hz), 7.23–7.37 (3H, m), 7.48 (2H, brd, $J=8.4$ Hz); MS m/z 373 $(M+H)^+$. **4d**·fumarate: mp 164–166.5°C (from *i*-Pr_2O/*i*-PrOH). Anal. calcd for $C_{23}H_{36}N_2O_2 \cdot C_4H_4O_4$: C, 66.37; H, 8.25; N, 5.73. Found: C, 66.53; H, 8.52; N, 5.75.

***N*-(1-Benzylpiperidin-4-yl)-2-cyclobutyl-2-hydroxy-2-phenylacetamide (4o)**. **4o** was obtained from **8** and benzaldehyde (80%): ^1H NMR ($CDCl_3$) δ 1.26–1.50 (2H, m), 1.65–2.20 (10H, m), 2.64–2.81 (2H, m), 3.35 (1H, m), 3.46 (2H, s plus 1H, brs), 3.72 (1H, m), 6.08 (1H, brd, $J=8.7$ Hz), 7.19–7.41 (8H, m), 7.48 (2H, brd, $J=8.3$ Hz); MS m/z 379 $(M+H)^+$; mp 183–185°C (from *n*-hexane/$CHCl_3$). Anal. calcd for $C_{24}H_{30}N_2O_2$: C, 76.16; H, 7.99; N, 7.40. Found: C, 75.90; H, 8.26; N, 7.39.

***N*-[1-(Phenylethyl)piperidin-4-yl]-2-cyclobutyl-2-hydroxy-2-phenylacetamide (4p)**. **4p** was obtained from **8** and phenylacetaldehyde (74%): ^1H NMR ($CDCl_3$) δ 1.30–

2.24 (12H, m), 2.51–2.62 (2H, m), 2.71–2.92 (4H, m), 3.29–3.48 (2H, m), 3.73 (1H, m), 6.15 (1H, brd, $J=7.8$ Hz), 7.14–7.39 (8H, m), 7.49 (2H, brd, $J=8.3$ Hz); MS m/z 393 $(M+H)^+$; mp 143–145°C (from *n*-hexane/$CHCl_3$). Anal. calcd for $C_{25}H_{32}N_2O_2\cdot 0.2H_2O$: C, 75.74; H, 8.24; N, 7.07. Found: C, 75.73; H, 8.52; N, 7.07.

General procedure (method C)

The experimental procedure of **4r** was described as a representative example.

***N*-[1-(4-Methyl-3-pentenyl)piperidin-4-yl]-2-cyclopentyl-2-hydroxy-2-phenylacetamide (4r)**. To a solution of 2-cyclopentyl-2-hydroxy-2-phenylacetic acid (**5e**)[35] (150 mg, 0.68 mmol) and **13** (190 mg, 1.04 mmol) in $CHCl_3$ (20 mL) were added 1-hydroxybenzotriazole (220 mg, 1.63 mmol),1-(3-dimethylaminopropyl)-3-ethylcarbodiimide(160 mg, 0.83 mmol) at room temperature. The mixture was stirred overnight and concentrated under reduced pressure. The residue was shaken with Et_2O and sat. $NaHCO_3$. The Et_2O layer was separated, washed with H_2O and brine, and dried over $MgSO_4$. After removal of the solvent, the crude product was purified by silica gel column chromatography ($CHCl_3$:MeOH = 100:1) to give **4r** (154 mg, 59%) as an oil. **(*R*)-4r** was prepared by the same method. **(*S*)-4r** was obtained by preparative HPLC (98% ee, t_R of **(*S*)-4r**: 16.8 min, t_R of **(*R*)-4r**: 20.3 min, Daicel Chiralcel OD 0.46×25 cm, eluent *n*-hexane:EtOH:TFA = 92.5:7.5:0.1, flow rate = 0.5 ml/min, UV detection 230 nm): ^{1}H NMR ($CDCl_3$) δ 1.13–1.94 (12H, m), 1.60 (3H, s), 1.68 (3H, s), 2.00–2.20 (4H, m), 2.25–2.38 (2H, m), 3.02 (1H, m), 3.14 (1H, brs), 3.70 (1H, m), 5.07 (1H, m), 6.31 (1H, brd, $J=7.9$ Hz), 7.21–7.39 (3H, m), 7.59 (2H, brd, $J=8.3$ Hz); MS m/z 385 $(M+H)^+$; IR (KBr) 3400, 2950, 1710, 1655, 1520 cm^{-1}. **(*R*)-4r**·fumarate mp 203–205°C (from *i*-Pr_2O/*i*-PrOH). Anal. calcd for $C_{24}H_{36}N_2O_2\cdot C_4H_4O_4$: C, 67.18; H, 8.05; N, 5.60. Found: C, 66.98; H, 8.04; N, 5.56; $[\alpha]_D^{20}$ −7.1 ($c=1.0$, MeOH).

The following compounds **14a–c**, **4q**, **4s**, **4t** were prepared from **12** or **13**, and the appropriate acids as described for **4r**.

***N*-[1-(4-Methyl-3-pentenyl)piperidin-4-yl]-2-cyclobutyl-2-methoxy-2-phenylacetamide (14a)**. Compound **14a** was obtained from **13** and 2-cyclopentyl-2-methoxy-2-phenylacetic acid (**20**) (50%): ^{1}H NMR ($CDCl_3$) δ 1.41–2.27 (13H, m), 1.64 (3H, s), 1.71 (3H, s), 2.30–2.48 (3H, m), 2.81–2.97 (2H, m), 3.14 (3H, s), 3.27 (1H, m), 3.87 (1H, m), 5.11 (1H, m), 6.81 (1H, brd, $J=7.8$ Hz), 7.23–7.44 (5H, m); MS m/z 385 $(M+H)^+$. **14a**·fumarate: mp 144–145°C (from *i*-Pr_2O/*i*-PrOH). Anal. calcd for $C_{24}H_{36}N_2O_2\cdot C_4H_4O_4$: C, 67.18; H, 8.05; N, 5.60. Found: C, 66.87; H, 8.33; N, 5.58.

***N*-[1-(4-Methyl-3-pentenyl)piperidin-4-yl]-2-cyclobutyl-2-phenylacetamide (14b)**. **14b** was obtained from **13** and α-cyclobutylphenylacetic acid (**5c**)[36] (40%): ^{1}H NMR ($CDCl_3$) δ 1.27–1.49 (2H, m), 1.60 (3H, s), 1.68 (3H, s), 1.51–1.98 (8H, m), 2.00–2.39 (6H, m), 2.71–3.06 (3H, m), 3.27 (1H, d, $J=10.8$ Hz), 3.76 (1H, m), 5.05 (1H, m), 5.23 (1H, brd, $J=8.4$ Hz), 7.19–7.39 (5H, m); MS m/z 355 $(M+H)^+$. Mp 132–133°C (from *i*-Pr_2O/*i*-PrOH). Anal. calcd for $C_{23}H_{34}N_2O$: C, 77.92; H, 9.67; N, 7.90. Found: C, 77.57; H, 9.98; N, 7.89.

***N*-(4-Methyl-3-pentenyl)piperidin-4-yl 2-cyclobutyl-2-hydroxy-2-phenylacetate (14c)**. **14c** was obtained from **12** and 2-cyclobutyl-2-hydroxy-2-phenylacetic acid (**5a**)[30] (34%): ^{1}H NMR ($CDCl_3$) δ 1.52–2.70 (18H, m), 1.64 (3H, s), 1.71 (1H, s), 3.33 (1H, m), 3.85 (1H, brs), 4.86 (1H, m), 5.10 (1H, m), 7.22–7.41 (3H, m), 7.60 (2H, brd, $J=8.4$ Hz); MS m/z 372 $(M+H)^+$. **14c**·fumarate: mp 138–140°C (from *i*-Pr_2O/*i*-PrOH). Anal. calcd for $C_{23}H_{33}NO_3\cdot C_4H_4O_4$: C, 66.51; H, 7.65; N, 2.87. Found: C, 66.25; H, 7.70; N, 2.90.

***N*-[1-(4-Methyl-3-pentenyl)piperidin-4-yl]-2-cyclopropyl-2-hydroxy-2-phenylacetamide (4q)**. **4q** was obtained from **13** and 2-cyclopropyl-2-hydroxy-2-phenylacetic acid (**5d**)[30] (60%): ^{1}H NMR ($CDCl_3$) δ 0.44–0.69 (4H, m), 1.32–1.99 (5H, m), 1.61 (3H, s), 1.68 (3H, s), 2.02–2.20 (4H, m), 2.25–2.38 (2H, m), 2.70–2.89 (2H, m), 3.39 (1H, m), 3.78 (1H, m), 5.06 (1H, m), 6.03 (1H, m), 7.20–7.42 (3H, m), 7.61 (2H, brd, $J=8.6$ Hz); MS m/z 357 $(M+H)^+$. **4q**·1/2fumarate: mp 75–78.5°C (from *i*-Pr_2O/*i*-PrOH). Anal. calcd for $C_{22}H_{32}N_2O_2\cdot 0.5C_4H_4O_4\cdot 1.5H_2O$: C, 65.28; H, 8.45; N, 6.34. Found: C, 65.31; H, 8.41; N, 6.00.

***N*-[1-(4-Methyl-3-pentenyl)piperidin-4-yl]-2-cyclohexyl-2-hydroxy-2-phenylacetamide (4s)**. **4s** was obtained from **13** and 2-cyclohexyl-2-hydroxy-2-phenylacetic acid (**5f**)[35] (50%): ^{1}H NMR ($CDCl_3$) δ 0.88 (1H, m), 1.01–1.95 (16H, m), 1.60 (3H, s), 1.68 (3H, s), 2.02–2.50 (4H, m), 2.25–2.38 (2H, m), 2.70–2.90 (2H, m), 3.71 (1H, m), 5.06 (1H, m), 6.57 (1H, brd, $J=8.6$ Hz), 7.20–7.40 (3H, m), 7.60 (2H, brd, $J=8.6$ Hz); MS m/z 399 $(M+H)^+$. **4s**·fumarate: mp 206–210°C (from *i*-Pr_2O/*i*-PrOH). Anal. calcd for $C_{25}H_{38}N_2O_2\cdot C_4H_4O_4$: C, 67.68; H, 8.23; N, 5.44. Found: C, 67.62; H, 8.52; N, 5.46.

***N*-[1-(4-Methyl-3-pentenyl)piperidin-4-yl]-2-hydroxy-diphenylacetamide (4t)**. **4t** was obtained from **13** and 2,2-diphenyl-2-hydroxyacetic acid **5g** (62%): ^{1}H NMR ($CDCl_3$) δ 1.41–1.80 (4H, m), 1.63 (3H, s), 1.71 (3H, s), 1.93–2.36 (6H, m), 2.69–2.88 (2H, m), 3.87 (1H, m), 5.07 (1H, m), 6.42 (1H, m), 7.26–7.88 (10H, m); MS m/z 393 $(M+H)^+$. **4t**·fumarate: mp 213–216°C (from *i*-Pr_2O/*i*-PrOH). Anal. calcd for $C_{25}H_{32}N_2O_2\cdot C_4H_4O_4$: C, 68.48; H, 7.13; N, 5.51. Found: C, 68.40; H, 7.11; N, 5.48.

***N*-[1-(2-Dimethylcarbamoyl-1-ethyl)piperidin-4-yl]-2-cyclobutyl-2-hydroxy-2-phenylacetamide (4l)**. A solution of free base of **8** (51 mg, 0.18 mmol) and *N*,*N*-dimethylacrylamide (50 mg, 0.50 mmol) in EtOH (3 mL) was heated at 80°C for 2 h. The mixture was cooled to room temperature and concentrated. H_2O and $CHCl_3$ were added to the residue. The organic layer was separated, dried over $MgSO_4$ and concentrated to afford the crude product which was purified by preparative TLC to give **4l** (49 mg, 72%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 1.30–1.50 (2H, m), 1.68–2.24 (10H, m), 2.49 (2H, brt, $J=7.4$ Hz), 2.64–2.84 (4H, m), 2.94 (3H, s), 3.01 (3H, s),

3.30–3.60 (2H, m), 3.72 (1H, m), 6.18 (1H, brd, $J=8.1$ Hz), 7.22–7.40 (3H, m), 7.49 (2H, brd, $J=8.1$ Hz); MS m/z 388 $(M+H)^+$. **4l**·hydrochloride: mp 204–206°C (from Et_2O/EtOH). Anal. calcd for $C_{22}H_{33}N_3O_3$·HCl: C, 62.32; H, 8.08; N, 9.91. Found: C, 62.16; H, 7.83; N, 9.86.

***N*-[1-(4-Hydroxy-1-butyl)piperidin-4-yl]-2-cyclobutyl-2-hydroxy-2-phenylacetamide (4m)**. To a solution of **19** (60 mg, 0.15 mmol) in THF (3 mL) was added lithium aluminum hydride (10 mg, 0.26 mmol) at ice-bath temperature. The mixture was stirred at the same temperature for 2 h. After addition of Na_2SO_4·$10H_2O$, the mixture was stirred for an additional 3 h at room temperature. Inorganic salts were filtered off and washed with THF and *i*-PrOH. The filtrate was concentrated and the residual oil was purified by preparative TLC ($CHCl_3$:MeOH = 9:1) to provide **4m** as an oil (39 mg, 73%): ^{1}H NMR ($CDCl_3$) δ 1.34–2.20 (16H, m), 2.26–2.44 (2H, m), 2.79–3.00 (2H, m), 3.35 (1H, m), 3.45–3.81 (4H, m), 6.22 (1H, brd, $J=7.8$ Hz), 7.21–7.40 (3H, m), 7.48 (2H, brd, $J=8.3$ Hz); MS m/z 361 $(M+H)^+$. **4m**·fumarate: mp 151–153°C (from Et_2O/EtOH). Anal. calcd for $C_{21}H_{32}N_2O_3$·$C_4H_4O_4$: C, 63.01; H, 7.61; N, 5.88. Found: C, 62.82; H, 7.83; N, 5.92.

***N*-[1-(3-Carboxylpropyl)piperidin-4-yl]-2-cyclobutyl-2-hydroxy-2-phenylacetamide (4n)**. **19** (63 mg, 0.16 mmol) was dissolved in EtOH (2 mL) and 1N NaOH (1 mL) was added at room temperature. After stirring for 16h, the mixture was adjusted to pH 4 with 1 N HCl and concentrated. The residue was purified by preparative TLC ($CHCl_3$:MeOH = 4:1) to obtain **4n** (30 mg, 51%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.49–2.19 (12H, m), 2.30–2.79 (6H, m), 3.00–3.21 (2H, m), 3.38 (1H, m), 3.80 (1H, m), 6.54 (1H, brd, $J=7.8$ Hz), 7.20–7.40 (3H, m), 7.42–7.57 (2H, m); MS m/z 375 $(M+H)^+$. Mp 98–102°C (from Et_2O/EtOH). Anal. calcd for $C_{21}H_{30}N_2O_4$·$1.5H_2O$: C, 62.82; H, 8.28; N, 6.98. Found: C, 63.14; H, 8.15; N, 7.01.

***N*-(1-*tert*-Butyloxycarbonylpiperidin-4-yl)-2-cyclobutyl-2-trimethylsilyloxy-2-phenylacetamide (16)**. To a stirred solution of **7** (330 mg, 0.86 mmol) and imidazole (215 mg, 3.16 mmol) in DMF (8 mL) was added trimethylsilylchloride (0.30 mL, 2.36 mmol) at room temperature, and the mixture was stirred for 18 h. After addition of H_2O, the mixture was extracted with Et_2O. The organic extract was washed with H_2O and brine, and dried over $MgSO_4$. Evaporation of the solvent gave the crude product which was purified by silica gel column chromatography (*n*-hexane:AcOEt = 4:1) to afford **16** (380 mg, 96%) as an oil: ^{1}H NMR ($CDCl_3$) δ −0.05(9H, s), 1.31–1.50 (2H, m), 1.49 (9H, s), 1.66–2.29 (8H, m), 2.88–3.02 (2H, m), 3.31 (1H, m), 3.90–4.11 (3H, m), 7.02(1H, brd, $J=8.4$), 7.22–7.48 (5H, m); HRMS m/z calcd for $C_{25}H_{41}N_2O_4Si$ $(M+H)^+$: 461.2836. Found: 461.2863.

***N*-Methyl-*N*-(1-*tert*-butyloxycarbonylpiperidin-4-yl)-2-cyclobutyl-2-trimethylsilyloxy-2-phenylacetamide (17).** To a stirred solution of **16** (350 mg, 0.76 mmol) in THF (6 mL) were sequentially added sodium hydride (60% in oil, 50 mg, 1.25 mmol), *tetra-n*-butylammonium iodide (18 mg, 0.049 mmol) at 0°C. After 10 min, iodomethane (0.090 mL, 1.45 mmol) was added. The mixture was allowed to warm to room temperature and stirred for 19h. The reaction was quenched with satd NH_4Cl solution and the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane: AcOEt = 20:1–5:1) to give **17** as a oil (95 mg, 26%): ^{1}H NMR ($CDCl_3$) δ 0.21 (9H, s), 1.02–2.20 (11H, m), 1.40 (9H×1/4, s), 1.44 (9H×3/4, s), 2.40 (3H×3/4, s), 2.41–2.54 (2H×1/4, m), 2.70 (3H×1/4, s), 2.71–2.88 (2H×3/4, m), 3.20 (1H, m), 3.59–3.71 (1H×1/4, m), 3.87–4.30 (2H, m), 4.49–4.62 (1Hx3/4, m), 7.21–7.41 (5H, m); HRMS m/z calcd for $C_{26}H_{43}N_2O_4Si$ $(M+H)^+$: 475.2992. Found: 475.3023.

***N*-Methyl-*N*-[1-(4-methyl-3-pentenyl)piperidin-4-yl]-2-cyclobutyl-2-hydroxy-2-phenylacetamide (18)**. **17** (85 mg, 0.18 mmol) was dissolved in 10% HCl–MeOH (2 mL), and the mixture was stirred for 17 h at room temperature. The solvent was evaporated to obtain the crude oil (61 mg) which was used without purification. To a solution of the above amine hydrochloride in CH_3CN (3 mL) were added K_2CO_3 (80 mg, 0.58 mmol) and 5-bromo-2-methyl-2-pentene (**10**) (35 mg, 0.21 mmol) at room temperature. The mixture was heated for 4h at 80°C and then cooled to room temperature. After dilution with H_2O, the mixture was extracted three times with $CHCl_3$. The combined organic extract was dried over $MgSO_4$ and concentrated. Purification by preparative TLC ($CHCl_3$:MeOH = 19:1) afforded **18** (55 mg, 79%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.30–2.67 (17H, m), 1.62 (3H, s), 1.69 (3H, s), 2.49 (3H×1/3, brs), 2.81 (3H×2/3, brs), 2.98 (1H, m), 3.31 (1H, m), 3.53 (1H×2/3, m), 4.48 (1H×1/3, m), 5.04 (1H, m), 5.57 (1H, m), 7.11–7.43 (5H, m); MS m/z 385 $(M+H)^+$. **18**·fumarate: mp 81–83°C (from Et_2O/EtOH). Anal. calcd for $C_{24}H_{36}N_2O_2$·$0.5C_4H_4O_4$·$0.5H_2O$: C, 69.15; H, 8.70; N, 6.20. Found: C, 69.12; H, 8.67; N, 6.20.

1-(4-Methyl-3-pentenyl)-4-piperidone (11). To a suspension of 4-piperidone monohydrate hydrochloride **9** (2.00 g, 14.75 mmol), potassium iodide (80 mg, 0.48 mmol) and K_2CO_3 (6.01 g, 43.5 mmol) in CH_3CN (100 mL) was added 5-bromo-2-methyl-2-pentene (**10**) (2.58 g, 15.8 mmol) at room temperature. The mixture was heated for 7 h at 80°C and cooled to room temperature. After dilution with H_2O, the mixture was extracted three times with $CHCl_3$. The combined organic extract was dried over $MgSO_4$ and concentrated. The residual oil was purified by silica gel column chromatography ($CHCl_3$:MeOH = 50:1) to obtain **11** (2.60 g, 97%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.63 (3H, s), 1.71 (3H, s), 2.13–2.30 (2H, m), 2.39–2.54 (6H, m), 2.77 (4H, brt, $J=6.0$ Hz), 5.12 (1H, m); HRMS m/z calcd for $C_{11}H_{19}NO$ (M^+): 181.1467. Found: 181.1464.

4-Hydroxy-1-(4-methyl-3-pentenyl)piperidine (12). To a stirred solution of **11** (450 mg, 2.48 mmol) in MeOH (20 mL) was added $NaBH_4$ (150 mg, 3.97 mmol) at 0°C. The mixture was stirred for 1 h at the same temperature. After addition of H_2O, MeOH was evaporated and the mixture was extracted twice with $CHCl_3$. The combined

organic extract was dried over $MgSO_4$ and concentrated. The residual oil was purified by silica gel column chromatography ($CHCl_3$:MeOH = 50:1–20:1) to obtain **12** (445 mg, 98%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.50–1.78 (2H, m), 1.62 (3H, s), 1.69 (3H, s), 1.84–1.98 (2H, m), 2.06–2.39 (6H, m), 2.71–2.88 (2H, m), 3.62–3.78 (1H, m), 5.08 (1H, m). **12**·fumarate: mp 122–124°C (from *i*-Pr_2O/*i*-PrOH). Anal. calcd for $C_{11}H_{21}NO\cdot C_4H_4O_4$: C, 60.18; H, 8.42; N, 4.68. Found: C, 60.28; H, 8.73; N, 4.99.

***N*-[1-(4-Methyl-3-pentenyl)piperidin-4-yl]-*N'*-(α-cyclobutylbenzyl)carbamate (15a)**. To a solution of 2-cyclobutylphenylacetic acid (**5c**)[36] (55 mg, 0.29 mmol) and Et_3N (50 μL, 0.36 mmol) in toluene (2 mL) was added diphenylphosphoryl azide (98 mg, 0.36 mmol) at room temperature. The mixture was heated for 40 min at 100°C and cooled to room temperature. Alcohol **12** (57 mg, 0.31 mmol) was added and the resultant mixture was heated at 100°C for 5 h. After cooling, the mixture was diluted with AcOEt and washed with satd $NaHCO_3$, H_2O and brine, and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the residue was purified by preparative TLC ($CHCl_3$:MeOH = 9:1) to afford **15a** (25 mg, 23%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.35–2.37 (17H, m), 1.61 (3H, s), 1.69 (3H, s), 2.40–2.81 (2H, m), 4.50–4.70 (2H, m), 4.83 (1H, m), 5.06 (1H, m), 7.10–7.39 (5H, m); MS *m*/*z* 371 $(M+H)^+$. **15a**·fumarate: mp 125–128°C (from *i*-Pr_2O/*i*-PrOH). Anal. calcd for $C_{23}H_{34}N_2O_2\cdot C_4H_4O_4$: C, 66.64; H, 7.87; N, 5.76. Found: C, 66.45; H, 7.96; N, 5.90.

4-Amino-1-(4-methyl-3-pentenyl)piperidine (13). To a stirred solution of **11** (210 mg, 1.16 mmol) and NH_4OAc (253 mg, 3.28 mmol) in MeOH (10 mL) was added $NaBH_3CN$ (183 mg, 2.91 mmol) at room temperature. The mixture was stirred for 10 h at the same temperature. After addition of satd $NaHCO_3$, MeOH was evaporated and the mixture was extracted three times with $CHCl_3$. The combined $CHCl_3$ extract was dried over $MgSO_4$. The solvent was evaporated to obtain the crude oil **13** (190 mg) which was used in the next step without purification: ^{1}H NMR ($CDCl_3$) δ 1.30–1.50 (2H, m), 1.62 (3H, s), 1.69 (3H, s), 1.74–1.89 (2H, m), 1.94–2.09 (2H, m), 2.11–2.38 (4H, m), 2.65 (1H, m), 2.80–2.95 (2H, m), 5.09 (1H, m); MS *m*/*z* 183 $(M+H)^+$.

***N*-(α-Cyclobutylbenzyl)-*N'*-[1-(4-methyl-3-pentenyl)piperidin-4-yl]urea (15b)**. To a solution of α-cyclobutylphenylacetic acid (**5c**)[36] (148 mg, 0.779 mmol) and Et_3N (140 μL, 1.01 mmol) in toluene (5 mL) was added diphenylphosphoryl azide (280 mg, 1.02 mmol) at room temperature. The mixture was heated for 30 min at 100°C and cooled to room temperature. A solution of the crude amine **13** (190 mg, ca. 1.04 mmol) in toluene (2 mL) was added and the resultant mixture was heated at 100°C for 3 h. After cooling, the mixture was diluted with AcOEt and washed with satd $NaHCO_3$, H_2O and brine, and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the residue was purified by preparative TLC ($CHCl_3$:MeOH = 9:1) to afford **15b** (140 mg, 49%) as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 1.08–1.41 (2H, m), 1.60 (3H, s), 1.68 (3H, s), 1.49–2.37 (14H, m), 2.43–2.78 (3H, m), 3.55 (1H, m), 4.08 (1H, m), 4.36 (1H, dd, *J* = 9.2, 5.6 Hz), 4.57 (1H, m), 5.06 (1H, m), 7.14–7.42 (5H, m); MS *m*/*z* 370 $(M+H)^+$. Mp 123–124.5°C (from *n*-hexane/AcOEt). Anal. calcd for $C_{23}H_{35}N_3O$: C, 74.75; H, 9.55; N, 11.37. Found: C, 74.59; H, 9.77; N, 11.27.

Methyl 2-cyclobutyl-2-methoxy-2-phenylacetate (20). To a stirred solution of methyl 2-cyclobutyl-2-hydroxy-2-phenyl acetate (**5a**)[30] (230 mg, 1.05 mmol) in DMF (8 mL) was added sodium hydride (60% in oil, 75 mg, 1.88 mmol) at 0°C. After 10 min, iodomethane (0.15 mL, 2.41 mmol) was added, and the mixture was stirred for 1 h. The reaction was quenched with satd NH_4Cl solution and the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane:AcOEt = 20:1) to give **20** (230 mg, 94%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.51–1.96 (4H, m), 2.00–2.21 (2H, m), 3.05 (1H, m), 3.27 (3H, s), 3.82 (3H, s), 7.23–7.41 (5H, m); HRMS *m*/*z* calcd for $C_{14}H_{18}O_3$ (M^+): 234.1256. Found: 234.1230.

2-Cyclobutyl-2-methoxy-2-phenylacetic acid (5b). **20** (230 mg, 0.982 mmol) was dissolved in MeOH (6 mL) and 2N NaOH solution (2 mL) was added at room temperature. The mixture was heated at 60°C for 4 h and cooled to room temperature. After evaporation of MeOH, the mixture was washed with Et_2O, acidified with 1 N HCl and extracted twice with $CHCl_3$. The combined extract was concentrated to give the acid **5b** (210 mg) as an oil which was used without purification: ^{1}H NMR ($CDCl_3$) δ 1.69–1.97 (2H, m), 2.00–2.18 (3H, m), 2.41 (1H, m), 3.25 (1H, m), 3.23 (3H, s), 7.23–7.41 (5H, m).

Optical resolution of 2-cyclopentyl-2-hydroxy-2-phenylacetic acid (5e). Racemic 2-cyclopentyl-2-hydroxy-2-phenylacetic acid (**5e**)[35] (77.5 g, 0.352 mol) in toluene (15 L) was treated with cinchonidine (100.9 g, 0.343 mol). The suspension was dissolved by heating at 130°C and the solution was gently cooled to 55°C. The formed precipitate was collected, washed with toluene and dried. This process was repeated additional two times to afford **(−)-5e** cinchonidine salt (46.3 g) as a white solid: ^{1}H NMR (CD_3OD) δ 1.20–1.77 (9H, m), 1.88 (1H, m), 2.00–2.24 (3H, m), 2.72 (1H, m), 3.03 (1H, m), 3.10–3.29 (2H, m), 3.47 (1H, dd, *J* = 13.2 and 10.5 Hz), 3.59 (1H, m), 4.17 (1H, m), 4.85–5.11 (2H, m), 5.73 (1H, m), 6.03 (1H, brd, *J* = 2.1 Hz), 7.13 (1H, m), 7.17–7.31 (2H, m), 7.61 (1H, m), 7.64–7.80 (4H, m), 8.07 (1H, d, *J* = 8.4 Hz), 8.20 (1H, d, *J* = 8.1 Hz), 8.86 (1H, d, *J* = 4.5 Hz). Mp 223–225°C (from toluene). $[\alpha]_D^{20}$ −87.4 (*c* = 1.0, MeOH). This was partitioned with 1 N HCl and AcOEt. The aq layer was separated, extracted with AcOEt and the combined AcOEt layers were washed with brine, dried over Na_2SO_4 and concentrated to obtain **(−)-5e** (18.6 g, 24%, >99% ee): $[\alpha]_D^{20}$ −1.9 (*c* = 3.0, MeOH). The enantiomeric excess was determined by HPLC analysis (t_R of (+)-acid: 10.2 min, t_R of (−)-acid: 11.3 min, Daicel Chiralcel OJ 0.46×25 cm, eluent *n*-hexane:EtOH:TFA = 7:3:0.1, flow rate = 0.5 ml/min, UV detection 220 nm).

Binding assay

The binding affinities (K_i) to five subtypes were determined by inhibition of specific binding of [^{3}H]-NMS using the human receptor membranes. In competitive experiments, membranes from CHO cells stably expressing cloned human m1–m5, purchased from Receptor Biology, Inc., Baltimore, MD were incubated with 0.2 nM [^{3}H]-NMS in the medium consisted of a buffer containing 50 mM Tris–HCl, 10 mM $MgCl_2$ and 1 mM EDTA at pH 7.4 and 25°C for 2 h. Non-specific binding was determined in the presence of 1 μM NMS. Incubations were terminated by rapid filtration over Packard UniFilter-GF/C, using cell harvester Packard Filtermate™ 196. Then, the filter was rinsed four times with 1 mL buffer each. The radioactivity was counted by Packard TopCount™ liquid scintillation counter. IC_{50} values were converted to apparent K_i values using the Cheng–Prusoff equation.[37]

Functional assay using isolated tissues

The pK_B value, as an index of potency, was determined for each individual curve by the equation $pK_B = -\log\{[B]/(\text{concentration ratio} - 1)\}$, where concentration ratio is the ratio of ED_{50} values with and without the antagonist and [B] is the concentration of the antagonist.

Rat trachea (M_3)

Male Sprague–Dawley rats (250–400 g) were exsanguinated. The trachea was prepared free of serosal connective tissue and single open ring preparations were placed in 5 mL organ baths containing modified Krebs–Henseleit solution maintained at 32°C and continuously aerated with 95% O_2 and 5% CO_2, and connected to isometric transducers (model TB-651T, Nihon-Kohden) with sutures. Mechanical responses were recorded isometrically by a multi-channel polygraph (model RMP-6018, Nihon-Kohden). The tissue was equilibrated for at least 60 min with initial tension of 1 g, and then contracted by adding 1 μM carbachol twice. The second contraction with carbachol was taken as a reference. The concentration–contraction curves for carbachol were obtained by cumulative addition of carbachol to the organ bath. The test compound or vehicle was added 10 min before the addition of carbachol.

Rabbit vas deferens (M_1)

Experiments were conducted according to the described method.[29]

Rat right atria (M_2)

Male Sprague–Dawley rats (250–400 g) were exsanguinated and right atria were isolated. Right atria were placed in 20 mL of organ baths containing modified Krebs–Henseleit solution maintained at 32°C and continuously aerated with 95% O_2 and 5% CO_2, and connected to isometric transducers (model TB-651T, Nihon-Kohden) with sutures. Mechanical responses were recorded isometrically by a multi-channel polygraph (model RMP-6018, Nihon-Kohden). Following the stabilization of the right atria beat, cumulative concentration response curves to carbachol were obtained before and after addition of the test compounds. Responses were expressed as a percentage of inhibition induced by carbachol.

Acetylcholine-induced bronchoconstriction in rats

Male Sprague–Dawley rats (380–420 g) were anesthetized (0.75 or 1.0 g/kg, respectively) and α-chloralose (37.5 or 50 mg/kg, respectively) injected intraperitoneally. A tracheal cannula was inserted for ventilated respiration. A cannula was inserted into the jugular vein for intravenous administration. In the case of the experiments for oral activity, the animals were fasted overnight before oral drug administration. The anesthetized animals were paralysed with succinylcholine (10 mg/kg s.c.) and placed in the Plethysmograph-Box and respired with positive pressure, constant rate and volume ventilation (6 mL/kg, 90 strokes/min for rats) from a Harvard rodent respirator. Air flow was measured by placing a pneumotachograph, which was connected to a Validyn differential pressure transducer (model MP45-14), between the tracheal cannula and the ventilation. Transpulmonary pressure was measured with a Validyn differential pressure transducer (model MP45-28).

Acetylcholine-induced bradycardia in rats

Male Sprague–Dawley rats (300–350 g) were used. The animals were anesthetized with urethane (1.0 g/kg) and α-chloralose (0.05 g/kg) injected intraperitoneally. The trachea, carotid artery and jugular vein were cannulated after a midline neck incision. The animals were pretreated with succinylcholine (10 mg/kg s.c.) and then ventilated with room air at a constant rate and volume (8 mL/kg, 90 strokes/min) by a Harvard Apparatus rodent ventilator. Arterial blood pressure was measured with a pressure transducer (Model 041-500-503, COBE) and the heart rate was integrated from the blood pressure signal. Output signal from the transducer was amplified through a blood pressure amplifier (AP-641G, Nihon-Kohden) and Heart rate counter (AT-601G, Nihon-Koden). The above parameters were all recorded on a heat recorder (Nihon-Koden). Bradycardia was induced by an administration of acetylcholine (5 mg/kg), delivered into the saphenous vein, at 5 min intervals. Once three similar responses (control response) were obtained, the compound was administered 5 min before the following acetylcholine administration. The ED_{50} values for the reversal of acetylcholine-induced bradycardia were calculated from dose–response curves with a probit analysis, after changes of heart rate induced by acetylcholine at pre- or post-drug-administration were expressed as the percent control for each dose of the compound.

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