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Novel quinuclidinyl heteroarylcarbamate derivatives as muscarinic receptor antagonists

Shinya Nagashima *, Yuji Matsushima, Hisao Hamaguchi, Hiroshi Nagata †, Toru Kontani ‡, Ayako Moritomo, Tadatsura Koshika, Makoto Takeuchi

Drug Discovery Research, Astellas Pharma Inc, 21, Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan

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ABSTRACT

Herein, we describe the synthesis and pharmacological profiles of novel quinuclidinyl heteroarylcarbamate derivatives. Among them, the quinuclidin-4-yl thiazolylcarbamate derivative ASP9133 was identified as a promising long-acting muscarinic antagonist (LAMA) showing more selective inhibition of bronchoconstriction against salivation and more rapid onset of action in a rat model than tiotropium bromide.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by progressive airflow obstruction that is only partially reversible and inflammation in the airways, resulting in loss of lung function and chronic dyspnea.[1] The main causes of COPD are tobacco smoking, including passive exposure, and air pollution, and the World Health Organization predicts that this disease will become the third leading cause of death worldwide by 2030.[2] However, at present, no drugs have yet been developed to stop COPD progression.

The reversibility of airway obstruction in COPD is due in part to increased cholinergic tone, and muscarinic receptor antagonists are effective therapeutic drugs for ameliorating dyspnea. Two types of inhaled muscarinic receptor antagonists are currently used to manage COPD: ipratropium bromide, a short-acting compound requiring up to four doses per day; and tiotropium bromide, a long-acting compound which only needs to be taken once daily (Fig. 1). Neither agents exhibit any significant selective affinities toward M_1, M_2, or M_3 receptors.[3] While tiotropium is currently used as a first-line drug for the treatment of COPD, we believe two areas of the pharmacological profile of this compound may be improved upon.

One point that merits improvement is insufficient lung selectivity. The clinical dosage of tiotropium is already limited by the incidence of systemic-related side effects, such as dry mouth, which is likely mediated by antagonistic activity against M_1 and M_3 muscarinic receptors in submandibular gland.[4,5] In addition, a short-acting muscarinic antagonist administered to patients receiving chronic treatment with a clinical dose of tiotropium improved lung function to a greater extent than tiotropium alone.[6,7] These findings indicate that tiotropium does not exert the maximum bronchodilatory effect as a muscarinic receptor antagonist, and an agent showing greater lung selectivity in vivo may improve the therapeutic effect of this drug class.

Another point that merits improvement is the slow onset of action, as a rapid onset of action is desirable for achieving quick relief from symptoms and ensuring good compliance. Improvement in forced expiratory volume in 1 s (FEV_1) peaked at 1.5–2 h after inhalation of tiotropium, a relatively slow onset of action.[7] Recently, two inhaled long-acting muscarinic antagonists (LAMAs) were launched (Fig. 1): the twice-daily agent aclidinium bromide showed better improvement of bronchodilation and COPD symptoms at night than tiotropium with a low incidence of adverse effects in clinical trials,[8] and the once-daily agent glycopyrronium

* Corresponding author at present address: Medicinal Chemistry 4, Chemistry Research Labs, Drug Discovery Research, Astellas Pharma Inc, 21, Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan. Tel.: +81 (0)29 829 6176; fax: +81 (0)29 852 5378.
E-mail address: shinya.nagashima@astellas.com (S. Nagashima).
† Present address: Astellas Research Technologies Co. Ltd, 21, Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan.
‡ Present address: General Affairs, Astellas Pharma Inc, 2-5-1, Nihonbashi-Honcho, Chuo-ku, Tokyo 103-8411, Japan.

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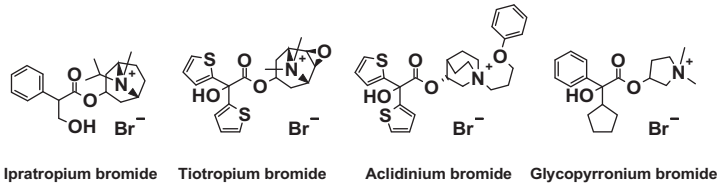


Figure 1. Structures of inhaled muscarinic receptor antagonists.

bromide also has a more rapid onset of action than tiotropium.[9] In these circumstances, it is expected that novel LAMA showing better improvement of efficacy and more rapid onset of action with lower side effects should be useful for maintenance treatment of COPD.

To identify a novel LAMA, we focused our attention on the muscarinic receptor antagonist YM-46303, which was investigated as a therapeutic agent for ameliorating urinary urge incontinence (Fig. 2). YM-46303 showed in vivo selective inhibitory activities on bladder pressure in reflexly-evoked rhythmic contraction against oxotremorine-induced salivary secretion.[10] In addition, the compound showed potent activity in a guinea pig model of methacholine-induced bronchospasm on intravenous administration.[11] While the reason for the in vivo selectivity of YM-46303 is still unclear, researchers suspected that modification of YM-46303 might lead to the identification of a novel LAMA with in vivo selectivity and rapid onset of action.

Herein, we report the synthesis and biological evaluation of a number of heteroarylcarbamate derivatives of YM-46303 and their pharmacological profiles. In addition, we describe the results of a docking study of a promising derivative (ASP9133) and rat M_3 receptor (rM_3R) based on the crystal structure of rM_3R and tiotropium.[12]

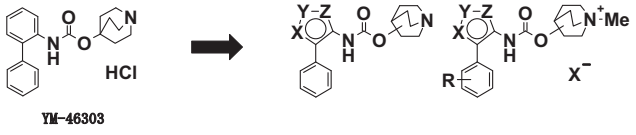
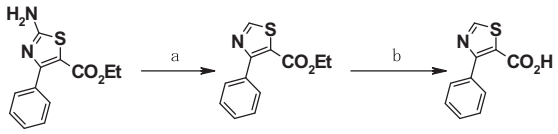


Figure 2. Strategy for producing a new LAMA.

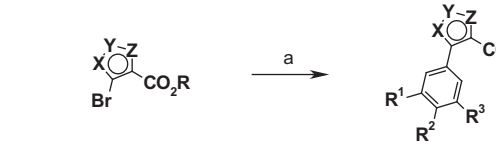


Scheme 2. (a) $NaNO_2$/50% aqueous H_2SO_4, below 0 °C, then 50% aqueous H_3PO_2, below 0 °C. (b) 1 M NaOH/MeOH–THF, 60 °C.

2. Chemistry

Our strategies for finding new LAMAs were described in Figure 2. At first, the benzene attached carbamate group of YM-46303 was converted into heterocycles such as thiophene which is contained in tiotropium and aclidinium. Secondly, quaternization of nitrogen of quinuclidine and introduction several substituents into the benzene attached the heterocycles were performed.[13] Scheme 1 shows the syntheses of heteroarylcarboxylic acid derivatives as intermediates. Suzuki–Miyaura coupling of methyl bromothiophenecarboxylate **1**–**3** and phenylboronic acid gave methyl phenylthiophenecarboxylate **5**–**7**, and hydrolysis of the esters provided corresponding carboxylic acid derivatives **13**–**15**. In the same manner, 5-phenyl-1,3-thiazole-4-carboxylic acid derivatives **16**–**20** were synthesized from ester derivatives **8**–**12** which were prepared from ethyl 2-bromothiazole-3-carboxylate **4** and corresponding boronic acid derivatives.

4-Phenyl-1,3-thiazole-4-carboxylic acid (**23**) was synthesized via deamination of ethyl 2-amino-4-phenyl-1,3-thiazole-4-carboxylate (**21**)[14] and hydrolysis of ethyl ester (Scheme 2). Quinuclidinyl carbamate derivatives **25**–**38** were prepared from synthesized



1– 4

No	X	Y	Z	R
1	CH	CH	S	Me
2	CH	S	CH	Me
3	S	CH	CH	Me
4	S	CH	N	Et

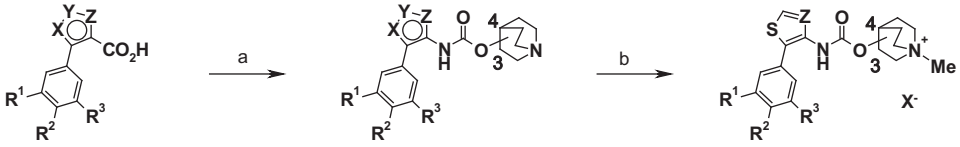
5– 12

No	X	Y	Z	R	R1	R2	R3
5	CH	CH	S	Me	H	H	H
6	CH	S	CH	Me	H	H	H
7	S	CH	CH	Me	H	H	H
8	S	CH	N	Et	H	H	H
9	S	CH	N	Et	Cl	H	H
10	S	CH	N	Et	H	F	H
11	S	CH	N	Et	CF_3	H	H
12	S	CH	N	Et	Cl	H	Cl

13– 20

No	X	Y	Z	R1	R2	R3
13	CH	CH	S	H	H	H
14	CH	S	CH	H	H	H
15	S	CH	CH	H	H	H
16	S	CH	N	H	H	H
17	S	CH	N	Cl	H	H
18	S	CH	N	H	F	H
19	S	CH	N	CF_3	H	H
20	S	CH	N	Cl	H	Cl

Scheme 1. (a) Phenylboronic acid or substituted phenylboronic acid, $Pd(PPh_3)_4$, 2 M Na_2CO_3/1,4-dioxane, 90 °C. (b) 1 M NaOH or 1 M KOH/EtOH or MeOH–THF, reflux.



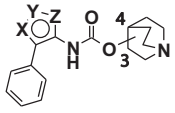
13– 20, 23, 24[c] 25– 38 39– 46, 43a

No	X	Y	Z	R1	R2	R3	quinclidinol
25	CH	CH	S	H	H	H	4
26	CH	S	CH	H	H	H	4
27	S	CH	CH	H	H	H	4
28	S	CH	CH	H	H	H	(*R*)-3
29	S	CH	CH	H	H	H	(*S*)-3
30	S	CH	N	H	H	H	4
31	S	CH	N	H	H	H	(*R*)-3
32	S	CH	N	Cl	H	H	4
33	S	CH	N	Cl	H	H	(*R*)-3
34	S	CH	N	H	F	H	4
35	S	CH	N	CF_3	H	H	4
36	S	CH	N	Cl	H	Cl	4
37	N	CH	S	H	H	H	(*R*)-3
38	O	CH	N	H	H	H	(*R*)-3

No	Z	R1	R2	R3	quinclidinol	X-
39	CH	H	H	H	4	I
40	N	H	H	H	4	I
41	N	H	H	H	(*R*)-3	I
42	N	Cl	H	H	(*R*)-3	I
43	N	Cl	H	H	4	I
43a	N	Cl	H	H	4	Br
44	N	H	F	H	4	I
45	N	CF_3	H	H	4	I
46	N	Cl	H	Cl	4	I

Scheme 3. (a) DPPA, Et_3N/toluene, room temperature for 40 min, then 90 °C for 1 h, then quinuclidin-4-ol, (*R*)-quinuclidin-3-ol, or (*S*)-quinuclidin-3-ol/toluene-DMF, reflux. (b) CH_3I or $CH_3Br/CHCl_3$-MeCN or AcOEt, room temperature. (c) Syntheses of compound **13**–**20** and **23** were described in this report. An oxazole intermediate (**24**; X = O, Y = CH, Z = N) is commercially available.

Table 1
Binding affinities of quinuclidine carbamate derivatives for M_1, M_2, and M_3 receptors



Compound	X	Y	Z	Quinuclidinol	K_i[a] (nM)		
					M_1	M_2	M_3
Tiotropium					0.16	0.15	0.30
Ipratropium					0.29	0.78	0.18
YM-46303					0.32	3.8	0.14
25	CH	CH	S	4-	0.33	1.4	0.12
26[b]	CH	S	CH	4-	0.51	3.6	0.21
27[b]	S	CH	CH	4-	0.095	0.46	0.056
28[b]	S	CH	CH	(*R*)-3-	0.19	0.82	0.19
29[b]	S	CH	CH	(*S*)-3-	4.6	13	4.0
30	S	CH	N	4-	0.39	0.80	0.20
31[b]	S	CH	N	(*R*)-3-	0.75	1.9	0.93
37[b]	N	CH	S	(*R*)-3-	8.7	39	12
38[b]	O	CH	N	(*R*)-3-	8.7	59	9.7

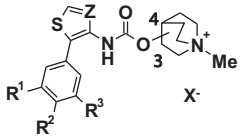
[a] K_i values are shown as the mean of duplicate experiments.
[b] HCl salt.

heteroarylcarboxylic acid derivatives **13**–**20**, **23**, and commercially available 5-phenyl-1,3-oxazole-4-carboxylic acid (**24**) with 4-quinuclidinol and either (*R*)- or (*S*)-3-quinuclidinol via Curtius rearrangement. Methylation of selected quinuclidinyl carbamate derivatives with iodomethane or bromomethane gave quaternized quinuclidine derivatives **39**–**46** and **43a** (Scheme 3).

3. Results and discussion

Affinities of the synthesized compounds and reference compounds for muscarinic M_1–M_3 receptor subtypes were measured

Table 2
Binding affinities of quaternized quinuclidine carbamate derivatives for M_1, M_2, and M_3 receptors



Compound	Z	R^1	R^2	R^3	Quinuclidinol	K_i[a] (nM)		
						M_1	M_2	M_3
Tiotropium						0.16	0.15	0.30
39	CH	H	H	H	4	0.020	0.027	0.020
40	N	H	H	H	4	0.055	0.12	0.064
41	N	H	H	H	(*R*)-3-	0.37	1.2	0.37
42	N	Cl	H	H	(*R*)-3-	0.31	1.2	0.25
43	N	Cl	H	H	4	0.041	0.053	0.049
44	N	H	F	H	4	0.073	0.21	0.10
45	N	CF_3	H	H	4	2.3	14	1.8
46	N	Cl	H	Cl	4	0.12	0.64	0.12

[a] K_i values are shown as the mean of duplicate experiments.

Table 3
Percent inhibition of carbachol-induced bronchoconstriction after intratracheal administration

Compound	% Inhibition of bronchoconstriction[a]			
	0.25 h	0.5 h	2 h	24 h
Ipratropium	70	85	83	15
Tiotropium	44	59	83	78
39	42	78	86	ND[b]
40	ND[b]	ND[b]	ND[b]	61
41	74	62	54	14
42	64	64	50	ND[b]
43	67	67	84	82

[a] Inhibitory effects of test compounds (0.5 μg/kg, i.t.) on bronchoconstriction are calculated as the mean percentage of the control value ($n = 3$).
[b] Not determined.

Table 4
ID_{50} values of ASP9133 and reference compounds for carbachol-induced bronchoconstriction and salivation 24 h after single i.t. administration

Compound	ID_{50}[a] (μg/kg)		Ratio [b]
	Bronchoconstriction [B]	Salivation [S]	[S/B]
ASP9133 (**43a**)	0.08 (0.06–0.1)	4.2 (2.8–7.6)	53
Tiotropium	0.12 (0.09–0.18)	1.9 (0.6–22.2)	16
Aclidinium	0.4 (0.3–0.6)	9.4 (7.2–14.3)	24
Glycopyrronium	3.4 (0.9–12.3)	26.7 (4.1–510)	17

[a] ID_{50} values are calculated from three experiments, and numbers in parentheses denote 95% confidence intervals.
[b] S/B ratio was calculated as the ID_{50} value for salivation divided by the ID_{50} value for bronchoconstriction.

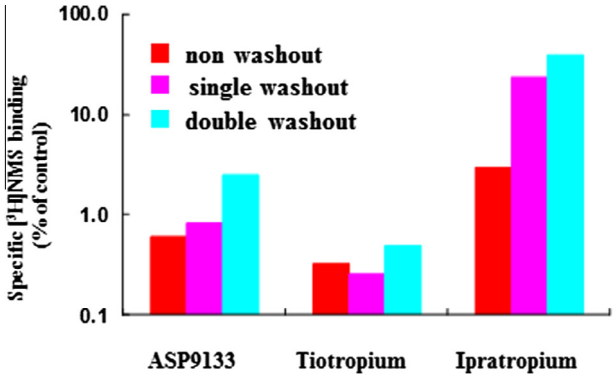


Figure 3. Specific [^{3}H]NMS binding to rat submandibular gland after incubation with test compounds and subsequent washout experiments. ASP9133, ipratropium, or tiotropium at concentrations 3000-fold each K_i value were incubated for 1 h each with cell membranes from rat submandibular gland. After washing the membranes once or twice, specific [^{3}H]NMS binding was assessed and calculated as the mean percentage of the control value from duplicate experiments. Red: non-washout, magenta: single washout, cyan: double washout.

based on [^{3}H]-*N*-methylscopolamine ([^{3}H]NMS) binding to the cortex (M_1), heart (M_2), and submandibular gland (M_3) in male Sprague–Dawley rats. Tiotropium and Ipratropium were evaluated as reference compounds.

The binding affinities of quinuclidinyl phenylheteroarylcarbamate derivatives are listed in Table 1. (3-Phenyl-2-thienyl)carbamate derivative **25** and (4-phenyl-3-thienyl)carbamate derivative **26** showed affinities comparable to that of YM-46303. Interestingly, (2-phenyl-3-thienyl)carbamate derivative **27** showed high affinities for all muscarinic receptors compared to the parent compound. (*R*)-3-Quinuclidinyl analogue **28** was more potent than the (*S*)-3-isomer **29**. This trends of **28/29** accorded with findings for the biphenylcarbamate derivatives.[10] 5-Phenyl-1,3-thiazol-4-yl analogues **30** and **31** also showed relatively good affinity, while (4-phenyl-1,3-thiazol-5-yl)carbamate derivative **37** and (5-phenyl-1,3-oxazol-4-yl)carbamate derivative **38** were less active than **31**.

Findings for the quaternized quinuclidine derivatives of **27**, **30**, and **31** are shown in Table 2. Quaternization of a nitrogen of 4- or (*R*)-3-quinuclidinol with a methyl group led to somewhat increased affinities (**27** vs **39**, **30** vs **40**, **31** vs **41**). Introduction of chlorine at the 3-position of the benzene maintained steady affinities (**41** vs **42**, **40** vs **43**). 4-Fluorophenyl derivative **44** showed comparable affinity to **40**, whereas substitution of the chlorine of **43** with a trifluoromethyl group reduced affinity (**45**). Introduction of an additional chlorine at the 5-position of the benzene of **43** was also slightly detrimental to binding affinity (**46**).

We performed rat in vivo screening for bronchodilatory effect to identify particularly promising compounds. The speed of onset and duration of action of selected compounds were investigated in a rat

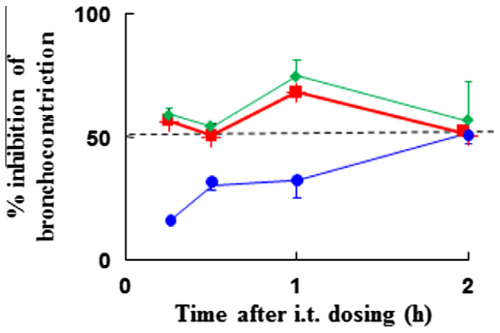


Figure 4. Comparison onset of action of ASP9133, tiotropium, and glycopyrronium. Test compounds were administered intratracheally at doses equivalent to ID_{50} values for CCh-induced bronchoconstriction at 24 h. Square: ASP9133, 0.1 μg/kg; circle: tiotropium, 0.1 μg/kg; diamond: glycopyrronium, 3.4 μg/kg. Inhibitory effects of test compounds are calculated as the mean percentage ± SE (n = 2–3).

model of carbachol-induced bronchoconstriction, with ipratropium and tiotropium also tested as reference compounds. All compounds tested were administrated intratracheally (i.t.) at a dose of 0.5 μg/kg, and their effects are described as percent inhibition of bronchoconstriction at 0.25, 0.5, 2, and 24 h after administration (Table 3). Ipratropium showed efficacy at 0.25 h, but the effect lasted less than 24 h. In contrast, tiotropium exerted an obvious effect at 2 h which persisted up to 24 h, reflecting clinical findings regarding the efficacy of tiotropium. Among our tested compounds, **39**–**42** proved less effective at 24 h than tiotropium, but **43** showed comparable activity at both 2 and 24 h. In addition, the activity of **43** at 0.25 and 0.5 h seemed to be actually slightly more potent than that of tiotropium—promising findings.

Further investigation of the pharmacological profiles of **43** was performed by changing the salt form of **43** from iodide to bromide, **43a** (ASP9133), and the effects of ASP9133 and other LAMAs on carbachol-induced bronchoconstriction and salivation via single i.t. administration were evaluated. Responses to both outcomes were measured simultaneously in the same rat at 24 h after administration. ASP9133 was found to be as potent as tiotropium, with the highest ratio of ID_{50} for salivation to ID_{50} for bronchoconstriction (S/B) among test compounds (Table 4). These data suggest that ASP9133 is superior to other LAMAs in lung selectivity.

Preliminary pharmacokinetic studies of ASP9133 and tiotropium after i.t. administration revealed that both compounds showed sufficient concentrations in lung to maintain inhibition of bronchoconstriction up to 24 h. However, in the submandibular gland, only marginal concentrations of ASP9133 and tiotropium remained at 24 h, indicating that the difference in lung selectivity between the two compounds was not due to differences in tissue distribution. Subsequent in vitro washout experiments indicated differing dissociation rates from the submandibular gland between ASP9133 and tiotropium. Briefly, after incubation of ASP9133, ipratropium, or tiotropium with cell membranes from the submandibular gland, the membranes were washed once or twice. Specific binding of [^{3}H]NMS to the membranes was then measured and presented as percent of control (Fig. 3). While percent specific [^{3}H]NMS binding of ipratropium- or ASP9133-treated membranes increased with number of times the procedure was carried out, specific [^{3}H]NMS binding in tiotropium-treated membranes was hardly affected. These data strongly suggest that tiotropium is more firmly associated with M_3 receptors than ASP9133 or ipratropium. We therefore speculated that the short duration of ASP9133's inhibitory effect on salivation was due to its more rapid dissociation from the submandibular gland compared to tiotropium.

Onset of action of ASP9133 was compared with those of tiotropium and glycopyrronium in a rat model of carbachol-induced

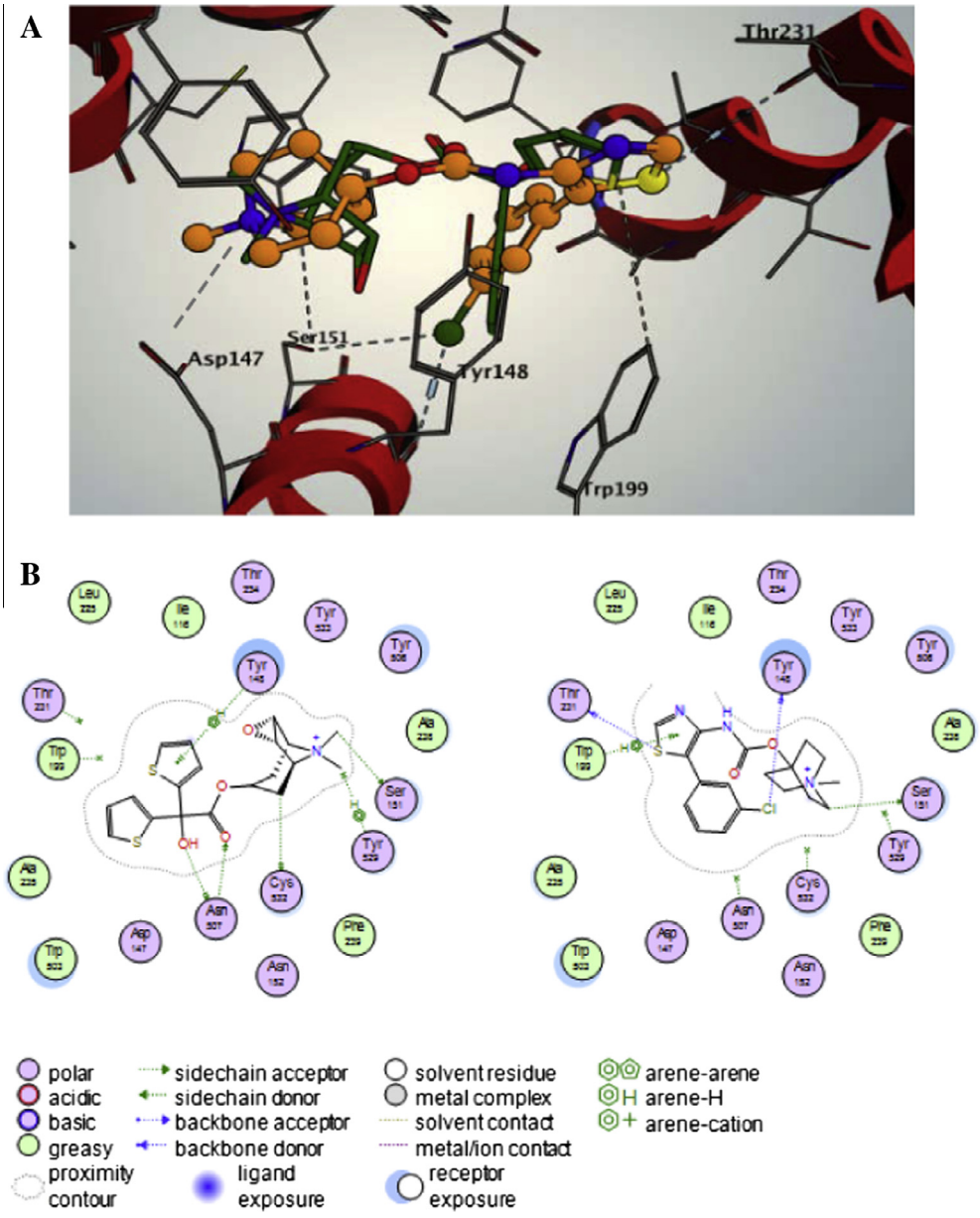


Figure 5. (A) Binding mode of ASP9133 to the rat M3 receptor superimposed on a tiotropium co-crystal structure. Orange: ASP9133, green: Tiotropium (X ray). (B) Two-dimensional diagram prepared using the ligand interactions application in MOE. Left: Tiotropium, right: ASP9133.

bronchoconstriction. The dose of each compound was set at the ID_{50} value for bronchoconstriction, listed in Table 4, and results are shown in Figure 4. ASP9133 and glycopyrronium both showed greater inhibition from 15 min to 1 h than tiotropium, suggesting that the onset of action of ASP9133 was quicker than that of tiotropium and comparable to that of glycopyrronium.

The pharmacophore features of muscarinic receptor antagonists are reported to be three-fold: positive-charged amine part, aromatic groups, and linker connecting the amine part and aromatic groups.[15] A rat M3 crystal structure bound to tiotropium (PDB code: 4DAJ)[12] was used to study the putative interactions between ASP9133 and the M3 receptor. A docking study of ASP9133 to rat M3 suggested that ASP9133 exerts its antagonistic activity via a number of different interactions (Fig. 5A). As a result, the positive-charged amine part of ASP9133 and tiotropium overlapped at several points, and the electrostatic interaction between the quaternized nitrogen of ASP9133 and carboxylate residue of Asp147 was the same as that noted with tiotropium and Asp147 (Fig. 5A). In aromatic groups of ASP9133, a π–π interaction between thiazole and Trp199, an S–O interaction between the sulfur of thiazole and carbonyl oxygen of Thr231, and a halogen bond between chlorine and carbonyl oxygen of Tyr148 were all observed (Fig. 5B).[16]

However, a marked difference was noted between ASP9133 and tiotropium with regard to the linker part of the two compounds. Specifically, while the hydroxyl group and ester carbonyl oxygen of tiotropium formed tight hydrogen bonds network with Asn507, no obvious interaction between the carbamate moiety of ASP9133 and Asn507 was observed (Fig. 5B). Tautermann et al. recently reported that the hydrogen bonds between tiotropium and Asn, which is highly conserved between rats and humans, prevents rapid dissociation of the compound from M_3R in humans.[17] These differences in binding mode seem to support our findings in washout experiments from the submandibular gland.

In summary, we identified ASP9133 as a promising LAMA expected to exert an improved bronchodilatory effect with little influence on salivation and a more rapid onset of action than tiotropium. These pharmacological features of ASP9133 may contribute to improved maintenance treatment of COPD. Studies on the binding mode of ASP9133 may help researchers design new muscarinic antagonists.

4. Experimental

4.1. Chemistry

^{1}H NMR spectra were measured using a Bruker BioSpin Avance 400, and chemical shifts are expressed in d units using tetramethylsilane as the standard (NMR peak description: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad peak). ^{13}C NMR spectra were measured using a Varian VSN400. Mass spectra were recorded using an Agilent HP1100 mass spectrometer, and elemental analyses were performed with a Yanako MT-5 microanalyzer (C, H, N) and a Yokogawa IC-7000S ion chromatographic analyzer (halogens). Melting points were determined on a Yanaco MP-500D melting point apparatus and are uncorrected. Specific rotations were measured with a HORIBA SEPA-300 polarimeter.

Organic solutions were dried over anhydrous $MgSO_4$ during work-up. Column chromatography was carried out on silica gel (Yamazen HI-FLASH™ COLUMN, 40 μm). Electrospray ionization-positive high-resolution mass spectra (HRMS) were obtained using a Waters LCT Premier. Unless otherwise noted, all commercial reagents and solvents were used without further purification.

4.1.1. Methyl 3-phenylthiophene-2-carboxylate (5)

Tetrakis(triphenylphosphine)palladium (5.49 g, 4.75 mmol) and 2 M aqueous disodium carbonate (71 mL) were added to a mixture of methyl 3-bromothiophene-2-carboxylate (**1**, 21 g, 95.0 mmol), phenylboronic acid (13.9 g, 114 mmol) and 1,4-dioxane (210 mL), and the mixture was stirred for 5 h at 90 °C. The reaction mixture was cooled to room temperature and then diluted with H_2O and extracted with AcOEt. The organic layer was washed successively with H_2O and saturated aqueous NaCl and then dried and concentrated in vacuo. The residue was chromatographed on silica gel with elution using hexane/AcOEt (9:1 to 4:1) to produce the desired compound **5** (14.4 g, 70%) as a colorless solid which was used in the next reaction without recrystallization. ^{1}H NMR (DMSO-d_6) δ 3.70 (3H, s), 7.22 (1H, d, J = 5.2 Hz), 7.36–7.47 (5H, m), 7.93 (1H, d, J = 5.2 Hz); FAB MS m/e $[M+H]^+$ 219.

4.1.2. Methyl 4-phenylthiophene-3-carboxylate (6)

Compound **6** was prepared from methyl 4-bromothiophene-3-carboxylate **2** in quantitative yield as a colorless oil, using an approach similar to that described for **5**, and was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 3.68 (3H, s), 7.32–7.39 (5H, m), 7.56 (1H, d, J = 3.2 Hz), 8.37 (1H, d, J = 3.2 Hz); FAB MS m/e $[M+Na]^+$ 241.

4.1.3. Methyl 2-phenylthiophene-3-carboxylate (7)

Compound **7** was prepared from methyl 2-bromothiophene-3-carboxylate **3** in quantitative yield as a colorless solid, using an approach similar to that described for **5**, and was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 3.67 (3H, s), 7.41–7.49 (6H, m), 7.64 (1H, d, J = 5.6 Hz); FAB MS m/e $[M+H]^+$ 219.

4.1.4. Ethyl 5-phenyl-1,3-thiazole-4-carboxylate (8)

Compound **8** was prepared from ethyl 2-bromothiazole-3-carboxylate **4**[18] in 94% yield as a yellow oil, using an approach similar to that described for **5**, and was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 1.12 (3H, t, J = 7.2 Hz), 4.17 (2H, q, J = 7.2 Hz), 7.44–7.52 (5H, m), 9.14 (1H, s); FAB MS m/e $[M+H]^+$ 234.

4.1.5. Ethyl 5-(3-chlorophenyl)-1,3-thiazole-4-carboxylate (9)

Compound **9** was prepared from **4** and (3-chlorophenyl)boronic acid in 94% yield as a yellow oil, using an approach similar to that described for **5**. ^{1}H NMR (DMSO-d_6) δ 1.13 (3H, t, J = 7.2 Hz), 4.18 (2H, q, J = 7.2 Hz), 7.46–7.55 (3H, m), 7.63–7.64 (1H, m), 9.17 (1H, s); FAB MS m/e $[M+Na]^+$ 290.

4.1.6. Ethyl 5-(4-fluorophenyl)-1,3-thiazole-4-carboxylate (10)

Compound **10** was prepared from **4** and (4-fluorophenyl)boronic acid in 80% yield as a yellow solid, using an approach similar to that described for **5**, and was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 1.13 (3H, t, J = 6.8 Hz), 4.17 (2H, q, J = 6.8 Hz), 7.27–7.33 (2H, m), 7.55–7.60 (2H, m), 9.14(1H, s); FAB MS m/e $[M+Na]^+$ 274.

4.1.7. Ethyl 5-(3-trifluoromethylphenyl)-1,3-thiazole-4-carboxylate (11)

Compound **11** was prepared from **4** and (3-trifluoromethylphenyl)boronic acid in 84% yield as a yellow solid, using an approach similar to that described for **5**, and was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 1.08 (3H, t, J = 7.2 Hz), 4.16 (2H, q, J = 7.2 Hz), 7.68–7.91 (5H, m), 9.21(1H, s); FAB MS m/e $[M+H]^+$ 302.

4.1.8. Ethyl 5-(3,5-dichlorophenyl)-1,3-thiazole-4-carboxylate (12)

Compound **12** was prepared from **4** and (3,5-dichlorophenyl)boronic acid in 63% yield as a yellow solid, using an approach similar to that described for **5**, and was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 1.14 (3H, t, J = 7.2 Hz), 4.18 (2H, q, J = 7.2 Hz), 7.65 (2H, d, J = 2.0 Hz), 7.74 (1H, t, J = 2.0 Hz), 9.20(1H, s); FAB MS m/e $[M+H]^+$ 302.

4.1.9. 3-Phenylthiophene-2-carboxylic acid (13)

To a mixture of **5** (4.0 g, 18.33 mmol) and EtOH (40 mL), 1 M NaOH (40 mL) was added, and the mixture was refluxed for 6 h and then cooled to room temperature and adjusted to pH 2 with 1 M HCl. The resulting precipitate was collected via filtration, washed with water, and dried in vacuo to produce the desired compound **13** (3.47 g, 93%) as a colorless solid, which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 7.17 (1H, d, J = 5.2 Hz), 7.35–7.47 (5H, m), 7.86 (1H, d, J = 5.2 Hz), 12.86 (1H, br); FAB MS m/e $[M-H]^-$ 203.

4.1.10. 4-Phenylthiophene-3-carboxylic acid (14)

Compound **14** was prepared from **6** in 87% yield as a colorless oil, using an approach similar to that described for **13**, and was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ7.32–7.37 (5H, m), 7.52 (1H, d, J = 3.6 Hz), 8.30 (1H, d, J = 3.6 Hz) 12.55 (1H, br); FAB MS m/e $[M+H]^+$ 205.

4.1.11. 2-Phenylthiophene-3-carboxylic acid (15)

Compound **15** was prepared from **7** in 95% yield as a colorless solid, using an approach similar to that described for **13**, and was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 7.38–7.43 (4H, m), 7.46–7.50 (2H, m), 7.59 (1H, d, J = 5.2 Hz), 12.62 (1H, br); FAB MS m/e $[M-H]^-$ 203.

4.1.12. 5-Phenyl-1,3-thiazole-4-carboxylic acid (16)

Compound **16** was prepared from **8** in 86% yield as a yellow solid, using an approach similar to that described for **13**, and was

used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 7.42–7.46 (3H, m), 7.50–7.53 (2H, m), 9.11 (1H, s), 12.96 (1H, br); FAB MS m/e $[M-H]^-$ 204.

4.1.13. 5-(3-Chlorophenyl)-1,3-thiazole-4-carboxylic acid (17)

Compound **17** was prepared from ester in 99% yield as a colorless solid, using an approach similar to that described for **13**, and was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 7.46–7.53 (3H, m), 7.61–7.62 (1H, m), 9.15 (1H, s), 13.05 (1H, br); FAB MS m/e $[M-H]^+$ 238.

4.1.14. 5-(4-Fluorophenyl)-1,3-thiazole-4-carboxylic acid (18)

Compound **18** was prepared from **10** in 93% yield as a pale-brown solid, using an approach similar to that described for **13**, and was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 1.13 (3H, t, J = 6.8 Hz), 4.17 (2H, q, J = 6.8 Hz), 7.25–7.31 (2H, m), 7.55–7.61 (2H, m), 9.11(1H, s), 13.00 (1H, br); FAB MS m/e $[M-H]^-$ 222.

4.1.15. 5-(4-Trifluoromethylphenyl)-1,3-thiazole-4-carboxylic acid (19)

Compound **19** was prepared from **11** in 70% yield as a pale-yellow solid, using an approach similar to that described for **15**, and was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 7.67–7.71 (2H, m), 7.81–7.84 (2H, m), 7.90 (1H, s), 9.18(1H, s), 13.08 (1H, s); FAB MS m/e $[M-H]^-$ 272.

4.1.16. 5-(3,5-Dichlorophenyl)-1,3-thiazole-4-carboxylic acid (20)

Compound **20** was prepared from **12** in 83% yield as a colorless solid, using an approach similar that described for **15**, and was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 7.63 (2H, d, J = 2.0 Hz), 7.71 (1H, t, J = 2.0 Hz), 9.18(1H, s), 13.12 (1H, s); FAB MS m/e $[M-H]^-$ 272.

4.1.17. Ethyl 4-phenyl-1,3-thiazole-5-carboxylate (22)

To a mixture of ethyl 2-amino-4-phenyl-1,3-thiazole-5-carboxylate[14] (**21** 6.00 g, 24.2 mmol) and 50% aqueous H_2SO_4 (80 mL), $NaNO_2$ (2.0 g, 29.0 mmol) in H_2O (10 mL) was added dropwise below 0 °C. Subsequently, 50% aqueous H_3PO_2 (35.1 g, 266 mmol) was added to the mixture below 0 °C. The mixture was stirred for 1 h below 5 °C and then diluted with H_2O (100 mL) and adjusted to pH 9–10 with 10 M NaOH. The mixture was extracted with AcOEt, and the organic layer was dried and concentrated in vacuo. The residue was chromatographed on silica gel with elution using hexane/AcOEt (97:3 to 85:15) to produce the desired compound **22** (1.68 g, 30%) as an orange oil. ^{1}H NMR (DMSO-d_6) δ 1.21 (3H, t, J = 7.2 Hz), 4.23 (2H, q, J = 7.2 Hz), 7.43–7.47 (3H, m), 7.70–7.75 (2H, m), 9.33 (1H, s); FAB MS m/e $[M+H]^+$ 234.

4.1.18. 4-Phenyl-1,3-thiazole-5-carboxylic acid (23)

Compound **23** was prepared from **22** in 94% yield as an ivory solid, using an approach similar to that described for **15**, and was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 7.42–7.45 (3H, m), 7.72–7.75 (2H, m), 9.27 (1H, s) 13.41 (1H, br); FAB MS m/e $[M-H]^-$ 204.

4.1.19. 1-Azabicyclo[2.2.2]oct-4-yl (3-phenyl-2-thienyl)carbamate (25)

To a solution of **13** (300 mg, 1.47 mmol) in toluene (3 mL), triethylamine (0.24 mL, 1.69 mmol) and DPPA (0.36 mL, 1.69 mmol) in toluene (3 mL) were added dropwise at room temperature, and the mixture was stirred for 40 min. After stirring at 90 °C for 1 h, a suspension of quinuclidin-4-ol (280 mg, 2.20 mmol) and DMF (3 mL) was added to the solution, followed by heating under reflux for 15 h. The reaction mixture was cooled to room temperature, then diluted with H_2O and extracted with AcOEt. The organic layer was washed successively with H_2O and saturated aqueous NaCl and then dried and concentrated in vacuo. The residue was chromatographed on silica gel with elution using $CHCl_3$/MeOH (9:1), and collected fractions were concentrated in vacuo. The residue was triturated with AcOEt and diisopropyl ether to produce the desired compound **25** (37 mg, 8%) as a pale-brown solid. ^{1}H NMR (DMSO-d_6) δ 1.85 (6H, br), 2.84 (6H, br), 7.06 (1H, d, J = 5.6 Hz), 7.27–7.32 (2H, m), 7.41 (2H, t, J = 7.6 Hz), 7.46–7.47 (2H, m), 9.17 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 31.53, 49.45,78.74, 127.78, 127.91, 128.73, 129.42, 135.89; FAB MS m/e $[M+H]^+$ 329; HRMS calcd for $C_{18}H_{20}N_2O_2S$ $[M+H]^+$ 329.1324, found 329.1329. mp: 169–170 °C.

4.1.20. 1-Azabicyclo[2.2.2]oct-4-yl (5-phenyl-1,3-thiazol-4-yl)carbamate (30)

Compound **30** was prepared from **16** and quinuclidin-4-ol derivatives using an approach similar to that described for **25**. Yield: 10%, pale-yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.76 (6H, br s), 2.78–2.82 (6H, m), 7.36 (1H, t, J = 7.3 Hz), 7.44 (2H, t, J = 7.7 Hz), 7.52–7.54 (2H, m), 8.96 (1H, s), 9.16 (1H, br s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 31.50, 49.42,78.24, 128.70, 128.99, 129.73, 131.69, 143.58, 151.18, 154.15; FAB MS m/e $[M+H]^+$ 330; HRMS calcd for $C_{17}H_{19}N_3O_2S$ $[M+H]^+$ 330.1276, found 330.1276; mp: 186–187 °C.

4.1.21. 1-Azabicyclo[2.2.2]oct-4-yl (4-phenyl-3-thienyl)carbamate hydrochloride (26)

To a solution of **14** (240 mg, 1.18 mmol) in toluene (3 mL), triethylamine (0.235 mL, 1.69 mmol) and DPPA (0.304 mL, 1.41 mmol) in toluene (3 mL) were added dropwise at room temperature, and the mixture was stirred for 40 min. After stirring at 90 °C for 1 h, a suspension of quinuclidin-4-ol (280 mg, 2.20 mmol) and DMF (3 mL) was added to the mixture, followed by heating under reflux for 2 h. The reaction mixture was cooled to room temperature, then diluted with H_2O and extracted with AcOEt. The organic layer was washed successively with H_2O and saturated aqueous NaCl and then dried and concentrated in vacuo. The residue was chromatographed on silica gel with elution using $CHCl_3$/MeOH/28% aqueous ammonia (8:1:1), and collected fractions were concentrated in vacuo to give a pale-brown solid. To a solution of the solid in AcOEt/EtOH (1:1, 3 mL) was added 4 M HCl in AcOEt (1 mL), and the mixture was evaporated. The resulting residue was triturated with EtOH and diisopropyl ether to produce the desired compound **26** (173 mg, 40 %) as an ivory solid. ^{1}H NMR (DMSO-d_6) δ 1.80–2.26 (6H, m), 3.28–3.40 (6H, m), 7.31–7.44 (5H, m), 7.52–7.53 (1H, m), 8.85 (1H, s), 10.52 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.91, 56.91, 74.01, 123.83, 128.06, 128.52, 129.39; FAB MS m/e $[M+H]^+$ 329; HRMS calcd for $C_{18}H_{20}N_2O_2S$ $[M+H]^+$ 329.1324, found 329.1317; Anal. Calcd for $C_{18}H_{20}N_2O_2S$ HCl 0.2H_2O: C, 58.67; H, 5.85; N, 7.60; S, 8.70; Cl, 9.62. Found: C, 48.56; H, 6.03; N, 7.45; S, 7.88; Cl, 9.03, mp: 260–262 °C.

Compounds **27**–**29**, **31**–**35**, **37**, and **38** were prepared from corresponding carboxylic acid derivatives and appropriate quinuclidinol derivatives using an approach similar to that described for **26**.

4.1.22. 1-Azabicyclo[2.2.2]oct-4-yl (2-phenyl-3-thienyl)carbamate hydrochloride (27)

Yield: 23%, colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.26 (6H, br), 3.36 (6H, br), 7.07 (1H, br), 7.31–7.34 (2H, m), 7.41–7.51 (4H, m), 9.12 (1H, br), 10.42 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.00, 47.37, 74.15, 124.41, 128.44, 128.49, 129.71, 132.05, 132.98, 133.86; FAB MS m/e $[M+H]^+$ 329; HRMS calcd for $C_{18}H_{20}N_2O_2S$ $[M+H]^+$ 329.1324, found 329.1324; Anal. Calcd for $C_{18}H_{20}N_2O_2S$ HCl: C, 59.25; H, 5.80; N, 7.68; S, 8.79; Cl, 9.72. Found: C, 59.11; H, 5.82; N, 7.70; S, 8.58; Cl, 9.68, mp: 238–239 °C

4.1.23. (3*R*)-1-Azabicyclo[2.2.2]oct-3-yl (2-phenyl-3-thienyl) carbamate hydrochloride (28)

Yield: 49%, colorless amorphous solid. ^{1}H NMR (DMSO-d_6) δ 1.79–1.90 (3H, m), 2.00 (1H, br), 2.23 (1H, br), 3.12–3.20 (5H, m), 3.60 (1H, br), 4.87 (1H, br), 7.15 (1H, d, J = 5.2 Hz), 7.32–7.36 (1H, m), 7.42–7.46 (2H, m), 7.51–7.55 (3H, m), 9.25(1H, br), 10.23 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 17.43, 20.64, 24.73, 45.51, 46.29, 53.43, 68.21, 124.51, 128.53, 128.60, 129.82, 132.07, 133.72; FAB MS m/e [M+H]$^+$ 329; HRMS calcd for $C_{18}H_{20}N_2O_2S$ [M+H]$^+$ 329.1324, found 329.1330; Anal. Calcd for $C_{18}H_{20}N_2O_2S$ HCl 1.5H_2O 0.1$C_4H_8O_2$: C, 55.15; H, 6.24; N, 6.99; S, 8.00; Cl, 8.85. Found: C, 55.42; H, 6.20; N, 6.81; S, 7.03; Cl, 8.55; $[\alpha]_D^{26}$ −5.3 (c 0.2, MeOH).

4.1.24. (3*S*)-1-Azabicyclo[2.2.2]oct-3-yl (2-phenyl-3-thienyl) carbamate hydrochloride (29)

Yield: 20%, colorless amorphous solid. ^{1}H NMR (DMSO-d_6) δ 1.79–1.90 (3H, m), 2.00 (1H, br), 2.23 (1H, br), 3.12–3.20 (5H, m), 3.60 (1H, br), 4.87 (1H, br), 7.15 (1H, d, J = 5.2 Hz), 7.32–7.36 (1H, m), 7.42–7.46 (2H, m), 7.51–7.55 (3H, m), 9.26 (1H, br), 10.55 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 17.43, 20.64, 24.73, 45.51, 46.29, 53.43, 68.20, 124.51, 128.53, 128.60, 129.81, 132.07, 133.71; FAB MS m/e [M+H]$^+$ 329; Anal. HRMS calcd for $C_{18}H_{20}N_2O_2S$ [M+H]$^+$ 329.1324, found 329.1330; calcd for $C_{18}H_{20}N_2O_2S$ HCl H_2O 0.1$C_4H_8O_2$: C, 56.42; H, 6.12; N, 7.15; S, 8.19; Cl, 9.05. Found: C, 56.61; H, 6.28; N, 7.06; S, 7.47; Cl, 8.75; $[\alpha]_D^{26}$ +6.3 (c 0.23, MeOH).

4.1.25. (3*R*)-1-Azabicyclo[2.2.2]oct-3-yl (5-phenyl-1,3-thiazol-4-yl)carbamate hydrochloride (31)

Yield: 14%, pale-yellow amorphous solid. ^{1}H NMR (DMSO-d_6) δ 1.72–1.91 (4H, m), 2.14 (1H, br s), 2.93–3.21 (5H, m), 3.56–3.61 (1H, m), 4.83–4.85 (1H, m), 7.39 (1H, t, J = 7.3 Hz), 7.46 (2H, t, J = 7.6 Hz), 7.56 (2H, d, J = 7.4 Hz), 9.02 (1H, s), 9.65 (1H, br), 10.21 (1H, br); FAB MS m/e [M+H]$^+$ 330; HRMS calcd for $C_{17}H_{19}N_3O_2S$ [M+H]$^+$ 330.1276, found 330.1284; $[\alpha]_D^{26}$ −7.0 (c 0.1, MeOH).

4.1.26. 1-Azabicyclo[2.2.2]oct-4-yl [5-(3-chlorophenyl)-1,3-thiazol-4-yl]carbamate hydrochloride (32)

Yield: 19%, pale-brown amorphous solid. ^{1}H NMR (DMSO-d_6) δ 2.17 (6H, br s), 3.34–3.39 (6H, m), 7.44–7.49 (3H, m), 7.58–7.58 (1H, m), 9.04 (1H, s), 9.64 (1H, br), 10.2 (1H, br); FAB MS m/e [M+H]$^+$ 364.

4.1.27. (3*R*)-1-azabicyclo[2.2.2]oct-3-yl [5-(3-chlorophenyl)-1,3-thiazol-4-yl]carbamate hydrochloride (33)

Yield: 49%, pale-brown amorphous solid. ^{1}H NMR (DMSO-d_6) δ1.73–1.91 (4H, m), 2.16 (1H, br), 2.93–3.23 (5H, m), 3.57–3.63 (1H, m), 4.85–4.87 (1H, m), 7.44–7.51 (3H, m), 7.61 (1H, br), 9.07 (1H, s), 9.79 (1H, br s), 10.47 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 17.37, 20.55, 24.75, 45.53, 46.34, 53.27, 68.46, 127.65, 127.86, 128.05, 129.03, 131.80, 133.49, 134.49, 143.63, 152.51, 154.49; FAB MS m/e [M+H]$^+$ 364; HRMS calcd for $C_{17}H_{18}N_3ClO_2S$ [M+H]$^+$ 364.0887, found 364.0886; $[\alpha]_D^{26}$ −5.0 (c 0.02, MeOH).

4.1.28. 1-Azabicyclo[2.2.2]oct-4-yl [5-(4-fluorophenyl)-1,3-thiazol-4-yl]carbamate hydrochloride (34)

Yield: 12%, colorless amorphous solid. ^{1}H NMR (DMSO-d_6) δ 2.14 (6H, br s), 3.35–3.37 (6H, br), 7.28–7.34 (2H, m), 7.53–7.58 (2H, m), 8.99 (1H, s), 9.51 (1H, br), 10.35 (1H, br); FAB MS m/e [M+H]$^+$ 348, HRMS calcd for $C_{17}H_{18}N_3FO_2S$ [M+H]$^+$ 348.1182, found 348.1187.

4.1.29. 1-Azabicyclo[2.2.2]oct-4-yl {5-[3-(trifluoromethyl) phenyl]-1,3-thiazol-4-yl}carbamate hydrochloride (35)

Yield: 21%, pale-brown amorphous solid. ^{1}H NMR (DMSO-d_6) δ 2.14 (6H, br s), 3.35–3.43 (6H, br), 7.68–7.84 (4H, m), 9.08 (1H, s), 9.72 (1H, br), 10.62 (1H, br); FAB MS m/e [M+H]$^+$ 398; HRMS calcd for $C_{18}H_{18}N_3F_3O_2S$ [M+H]$^+$ 398.1150, found 398.1150.

4.1.30. (3*R*)-1-Azabicyclo[2.2.2]oct-3-yl (4-phenyl-1,3-thiazol-5-yl)carbamate hydrochloride (37)

Yield: 8%, pale-brown solid. ^{1}H NMR (DMSO-d_6) δ 1.80 (3H, br), 2.05 (1H, br), 2.29 (1H, br s), 3.16–3.23 (5H, m), 3.64 (1H, br s), 4.97 (1H, br s), 7.37 (1H, t, J = 7.4 Hz), 7.47 (2H, t, J = 7.7 Hz), 7.78 (2H, d, J = 7.4 Hz), 8.92 (1H, s), 10.92 (1H, br), 10.4 (1H, br); FAB MS m/e [M+H]$^+$ 330. mp: 147–148 °C, $[\alpha]_D^{26}$ −10.8°(c 0.12, MeOH); HRMS calcd for $C_{17}H_{19}N_3O_2S$ [M+H]$^+$ 330.1276, found 330.1275.

4.1.31. (3*R*)-1-Azabicyclo[2.2.2]oct-3-yl (5-phenyl-1,3-oxazol-4-yl)carbamate hydrochloride (38)

Yield: 6%, pale-yellow amorphous solid. ^{1}H NMR (DMSO-d_6) δ 1.81–1.87 (4H, m), 2.24 (1H, br s), 3.14 (5H, br s), 3.64 (1H, br s), 4.91 (1H, br s), 7.4 (1H, t, J = 7.6 Hz), 7.5 (2H, t, J = 7.6 Hz), 7.67 (2H, d, J = 7.6 Hz), 8.4 (1H, s), 9.62 (1H, br s), 9.87 (1H, br s); FAB MS m/e [M+H]$^+$ 314, $[\alpha]_D^{26}$ −5.7 (c 0.07, MeOH); HRMS calcd for $C_{17}H_{19}N_3O_3$ [M+H]$^+$ 314.1505, found 314.1504.

4.1.32. 1-Azabicyclo[2.2.2]oct-4-yl [5-(3,5-dichlorophenyl)-1,3-thiazol-4-yl]carbamate fumarate (36)

Free base of **36** was prepared using an approach similar to that described for **25** to give a colorless amorphous solid (yield: 21%). To a mixture of the free amine of **36** (45 mg, 0.113 mmol) in MeOH (2 mL) was added fumaric acid (13 mg), and the mixture was concentrated in vacuo. The resulting residue was triturated with EtOH to give **36** (51 mg) as a colorless amorphous solid. ^{1}H NMR (DMSO-d_6) δ 1.87–1.91 (6H, br), 2.94–2.98 (6H, br), 8.52 (1.5H, s, fumaric acid), 7.54 (2H, d, J = 2.0 Hz), 7.64 (1H, t, J = 2.0 Hz), 9.06 (1H, s), 9.55 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 30.18, 48.44, 77.27, 125.68, 127.01, 128.32, 135.34, 135.39, 144.73, 152.99, 153.63, 168.28; FAB MS m/e [M+H]$^+$ 398; HRMS calcd for $C_{17}H_{17}N_3O_2Cl_2S$ [M+H]$^+$ 398.0497, found 398.0503.

4.1.33. 1-Methyl-4-{[(5-phenyl-1,3-thiazol-4-yl)carbamoyl] oxy}-1-azoniabicyclo[2.2.2]octane iodide (40)

To a solution of **30** (100 mg, 0.304 mmol) in $CHCl_3$ (3 mL) was added iodomethane (0.076 mL, 1.22 mmol) at room temperature and stirred. After 1 h, iodomethane (0.076 mL, 1.22 mmol) was added to the mixture and stirred for a further 1 h. A solid precipitated during stirring, and after the reaction mixture was diluted with AcOEt, the resulting precipitate was collected via filtration and washed with AcOEt to produce the desired compound **40** (133 mg, 93 %) as a pale-yellow solid. ^{1}H NMR (DMSO-d_6) δ 2.15–2.30 (6H, m), 2.92 (3H, s), 3.56–3.63 (6H, m), 7.36–7.41 (1H, m), 7.44–7.48 (2H, m), 7.51–7.54 (2H, m), 9.00 (1H, s), 9.53 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.74, 51.91, 57.88, 73.61, 128.66, 129.14, 129.86, 131.48, 142.90, 151.60, 153.64; FAB MS m/e [M]$^+$ 344; HRMS calcd for $C_{18}H_{22}N_3O_2S$ [M]$^+$ 344.1433, found 344.1428; Anal. Calcd for $C_{18}H_{22}N_3O_2S$.I.0.7H_2O.0.1$C_4H_8O_2$: C, 44.85; H, 4.95; N, 8.53; S, 6.51; I, 25.75. Found: C, 44.84; H, 4.71; N, 8.73; S, 6.61; I, 25.79, mp: 194–195 °C.

4.1.34. 1-Methyl-4-{[(2-phenyl-3-thienyl)carbamoyl]oxy}-1-azoniabicyclo[2.2.2]octane iodide (39)

Compound **39** was prepared from free base of **27** using an approach similar to that described for compound **40**. Yield: 97%, colorless amorphous solid. ^{1}H NMR (DMSO-d_6) δ 2.32 (6H, br), 2.93 (3H, br), 3.61 (6H, br), 7.05 (1H, br), 7.34 (1H, t, J = 7.3 Hz), 7.44 (2H, t, J = 7.6 Hz), 7.49–7.51 (2H, m), 9.14 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.85, 51.94, 57.93, 73.43, 124.48, 128.49, 129.75, 131.93, 133.03; FAB MS m/e [M]$^+$ 343; HRMS calcd for $C_{19}H_{23}N_2O_2S$ [M]$^+$ 343.1480, found 343.1488; Anal. Calcd for

$C_{19}H_{23}N_2O_2S$.I: C, 48.52; H, 4.93; N, 5.96; S, 6.83; I, 26.98. Found: C, 48.56; H, 5.01; N, 6.00; S, 6.73; I, 26.94, mp: 223–224 °C.

4.1.35. (3*R*)-1-Methyl-3-{[(5-phenyl-1,3-thiazol-4-yl) carbamoyl]oxy}-1-azoniabicyclo[2.2.2]octane iodide (41)

To a solution of **31** (103 mg, 0.282 mmol) in water (2 mL), 1 M NaOH (0.5 mL) was added and the mixture was stirred for 15 min. After the mixture was extracted with AcOEt, the organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo to give colorless oil. To the oil in AcOEt (5 mL) was added iodomethane (0.035 mL, 0.563 mmol) at room temperature, and the solution was stirred for 1 h. The reaction mixture was diluted with AcOEt, and the resulting precipitate was collected via filtration and washed with AcOEt to produce the desired compound **41** (143 mg, 98 %) as a pale-yellow amorphous solid. ^{1}H NMR (DMSO-d_6) δ 1.81–1.93 (4H, m), 2.16 (1H, br), 2.93 (3H, s), 3.26–3.42 (5H, m), 3.77–3.82 (1H, m), 4.88–4.90 (1H, m), 7.39 (1H, t, J = 7.4 Hz), 7.47 (2H, t, J = 7.6 Hz), 7.57 (2H, d, J = 7.6 Hz), 9.02 (1H, s), 9.68 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 18.73, 21.50, 24.41, 51.76, 55.93, 62.92, 68.67, 128.77, 129.23, 129.94, 131.35, 142.81, 151.72, 154.56; FAB MS m/e $[M]^+$ 344; HRMS calcd for $C_{18}H_{22}N_3O_2S$ $[M]^+$ 344.1433, found 344.1433; $[\alpha]_D^{26}$ −8.8 (c 0.13, MeOH), Anal. Calcd for $C_{18}H_{22}N_3O_2S$.I.0.6H_2O.0.1$C_4H_8O_2$: C, 45.01; H, 4.93; N, 8.56; S, 6.53; I, 25.85. Found: C, 45.21; H, 4.97; N, 8.86; S, 6.72; I, 25.42.

4.1.36. (3*R*)-3-({[5-(3-Chlorophenyl)-1,3-thiazol-4-yl] carbamoyl}oxy)-1-methyl-1-azoniabicyclo[2.2.2]octane iodide (42)

Compound **42** was prepared from **33** using an approach similar to that described for **41**. Yield: 95%, pale-brown amorphous solid. ^{1}H NMR (DMSO-d_6) δ 1.83–1.95 (4H, m), 2.19 (1H, br), 2.95 (3H, s), 3.27–3.44 (5H, m), 3.79–3.85 (1H, m), 4.9–4.93 (1H, m), 7.45–7.51 (3H, m), 7.62 (1H, s), 9.07 (1H, s), 9.8 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 18.75, 21.48, 24.46, 51.78, 55.95, 56.62, 62.84, 68.77, 127.67, 128.13, 129.03, 131.81, 133.48, 134.47, 143.51, 152.55, 154.41; FAB MS m/e $[M]^+$ 378, HRMS calcd for $C_{18}H_{21}N_3ClO_2S$ $[M]^+$ 378.1043, found 378.1049; $[\alpha]_D^{26}$ −11.2 (c 0.12, MeOH), Anal. Calcd for $C_{18}H_{21}N_3ClO_2S$.I.0.5H_2O: C, 41.99; H, 4.31; N, 8.16; Cl, 6.89; S, 6.23; I, 24.65. Found: C, 41.91; H, 4.41; N, 8.22; Cl, 7.06; S, 6.33; I, 24.47.

4.1.37. 4-({[5-(3-Chlorophenyl)-1,3-thiazol-4-yl]carbamoyl} oxy)-1-methyl-1-azoniabicyclo[2.2.2]octane iodide (43)

Compound **43** was prepared from **32** using an approach similar to that described for **41**. Yield: 80%, pale-yellow solid. ^{1}H NMR (DMSO-d_6DMSO-d_6) δ 2.25 (6H, br), 2.92 (3H, s), 3.59–3.63 (6H, m), 7.44–7.51 (3H, m), 7.57 (1H, s), 9.05 (1H, s), 9.68 (1H, br); FAB MS m/e $[M]^+$ 378. Anal. Calcd for $C_{18}H_{21}N_3ClO_2S$.I: C, 42.74; H, 4.18; N, 8.31; Cl, 7.01; S, 6.99; I, 25.09. Found: C, 42.74; H, 4.29; N, 8.34; Cl, 6.99; S, 6.36; I, 24.82, mp: 184–185 °C.

4.1.38. 4-({[5-(3-Chlorophenyl)-1,3-thiazol-4-yl]carbamoyl} oxy)-1-methyl-1-azoniabicyclo[2.2.2]octane iodide (43a)

A solution of free base of **32** (450 mg, 1.24 mmol) in $CHCl_3$ (15 mL) and MeCN (3 mL) was bubbled with bromomethane at room temperature until **32** had disappeared on TLC analysis. The precipitated solid was collected, washed with $CHCl_3$, and recrystallized from EtOH to give **43a** (445 mg, 78 %) as a colorless crystal. ^{1}H NMR (DMSO-d_6) δ 2.25 (6H, br s), 2.93 (3H, s), 3.59–3.64 (6H, m), 7.44–7.51 (3H, m), 7.57–7.58 (1H,m), 9.05 (1H, s), 9.68 (1H, br s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.77, 51.93, 57.85, 73.80, 127.59, 127.87, 128.93, 131.75, 133.63, 134.42, 143.56, 152.46, 153.51; FAB MS m/e $[M]^+$ 378; HRMS calcd for $C_{18}H_{21}N_3ClO_2S$ $[M]^+$ 378.1043, found 378.1045; Anal. Calcd for $C_{18}H_{21}N_3ClO_2S$.Br: C, 47.12; H, 4.61; N, 9.16; Cl, 7.73; S, 6.99; Br, 17.42. Found: C, 47.01; H, 4.60; N, 9.04; Cl, 7.81; S, 6.87; Br, 17.41, mp: 230–231 °C.

4.1.39. 4-({[5-(3,5-Dichlorophenyl)-1,3-thiazol-4-yl]carbamoyl} oxy)-1-methyl-1-azoniabicyclo[2.2.2]octane iodide (46)

To a solution of free base of **36** (45 mg, 0.113 mmol) in AcOEt (5 mL) was added iodomethane (0.07 mL, 1.13 mmol) at room temperature. The mixture was stirred for 1 h and then diluted with AcOEt, and the resulting precipitate was collected via filtration and washed with $CHCl_3$ to produce the desired compound **46** (60 mg, 98%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.26 (6H, br), 2.92 (3H, s), 3.59–3.65 (6H, m), 7.54–7.66 (3H, m), 9.09 (1H, s), 9.62 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.77, 51.98, 57.88, 73.95, 125.95, 127.07, 128.46, 135.16, 135.42, 144.23, 152.26, 153.34; FAB MS m/e $[M]^+$ 412; HRMS calcd for $C_{18}H_{20}N_3Cl_2O_2S$. $[M]^+$ 412.0653, found 412.0656; Anal. Calcd for $C_{18}H_{20}N_3Cl_2O_2S$.I.0.4$CHCl_3$: C, 37.58; H, 3.50; N, 7.15; S; 5.45, Cl, 19.29; I, 21.58. Found: C, 37.35; H, 3.66; N, 7.09; S; 5.38, Cl, 19.03; I, 21.56, mp: 271–273 °C.

4.1.40. 4-({[5-(4-Fluorophenyl)-1,3-thiazol-4-yl]carbamoyl} oxy)-1-methyl-1-azoniabicyclo[2.2.2]octane iodide (44)

Compound **44** was prepared from **34** using an approach similar to that described for **46**. Yield: 99%, pale-yellow solid. ^{1}H NMR (DMSO-d_6) δ 2.23 (6H, br s), 2.92 (3H, s), 3.57–3.61 (6H, m), 7.28–7.34 (2H, m), 7.53–7.58 (2H, m), 9.00 (H, s), 9.55 (1H, br s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.75, 51.93, 57.89, 73.66, 116.73, 116.95, 127.95, 127.98, 130.82, 130.90, 142.98, 151.65, 153.59, 161.51, 163.95; FAB MS m/e $[M]^+$ 362; HRMS calcd for $C_{18}H_{21}N_3FO_2S$ $[M]^+$ 362.1339, found 362.1346; Anal. Calcd for $C_{18}H_{21}N_3FO_2S$.I.0.2$CHCl_3$: C, 42.30; H, 4.21; N, 8.13; S; 6.20, Cl, 4.12; F, 3.68; I, 24.55. Found: C, 42.20; H, 4.53; N, 7.83; S; 6.12, Cl, 4.42; F, 3.67; I, 24.35, mp: 146–148 °C.

4.1.41. 1-Methyl-4-[({5-[3-(trifluoromethyl)phenyl]-1,3-thiazol-4-yl}carbamoyl)oxy]-1-azoniabicyclo[2.2.2]octane iodide (45)

Compound **45** was prepared from **35** using an approach similar to that described for **40**. Yield: 94%, colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.23 (6H, br s), 2.93 (3H, s), 3.59–3.63 (6H, m), 7.69–7.84 (4H, m), 9.02 (1H, s), 9.75 (1H, br s); FAB MS m/e $[M]^+$ 412; HRMS calcd for $C_{19}H_{21}N_3F_3O_2S$ $[M]^+$ 412.1306, found 412.1303; Anal. Calcd for $C_{19}H_{21}N_3F_3O_2S$.I: C, 42.31; H, 3.92; N, 7.79; S; 5.95, F, 10.57; I, 23.53. Found: C, 42.30; H, 4.10; N, 7.74; S; 5.96, F, 10.57; I, 23.27, mp: 199–200 °C.

5. Biology

5.1. Muscarinic receptor binding

5.1.1. Preparation of a membrane specimen

The submandibular gland, heart, and cerebral cortex were harvested from male Sprague–Dawley rats (Japan SLC, Inc., Hamamatsu), and a 10-fold volume of 25 mM Tris buffer (pH 7.4) containing 3.75 mM magnesium chloride was added to the samples, followed by homogenization under ice-cooled conditions. Centrifugation was performed at 1000 g, 4 °C, for 30 min. The resultant membrane precipitate was stored at −80 °C. Membranes were suspended in Tris buffer until use in the binding assay.

5.1.2. Binding assay

Membrane specimens from the submandibular gland, heart, and cerebral cortex were separately incubated with [^{3}H]NMS and a test compound in 0.3 mL of Tris buffer at 25 °C for 2 h and then suction-filtered through a glass filter (Whatman GF/B), which was then washed 8 times with 0.3 mL of Tris buffer. The radioactivity of [^{3}H]NMS adsorbed on the filter was measured using a Top Count device (PerkinElmer, Waltham, MA). Receptor-specific binding was determined by adding 1 μM NMS. The affinity of the test

compound for muscarinic receptors was determined as a dissociation constant (K_i) from the concentration of test compound which inhibited the binding of [^{3}H]NMS as a labeled ligand by 50%.

5.2. In vivo bronchodilatory effect by intratracheal (i.t.) administration

Male Sprague–Dawley rats (250 to 400 g) were anesthetized via intraperitoneal administration of pentobarbital sodium (Nembutal®; 50 mg/kg), and the main respiratory tract was dissected. An airway cannula was intubated into the trachea, and the pressure in the airway was measured using a pressure transducer. After intravenous administration of pancuronium bromide (0.2 mg/kg), stable pressure in the airway was obtained. The test compounds were administered intratracheally at a dose of 0.5 μg/kg. At 0.25, 0.5, 2 and 24 h after dosing, carbachol (CCh; 30 μg/kg) was administered and airway pressure measured using a pressure transducer. Effects of test compounds on CCh-induced bronchoconstriction are shown as percent inhibition in Table 3.

5.3. Effects of ASP9133, tiotropium bromide, aclidinium bromide, or glycopyrronium bromide on carbachol-induced bronchoconstriction and salivation in rats

CCh-induced bronchoconstriction and salivation were evaluated following single i.t. administration of ASP9133, tiotropium bromide, aclidinium bromide, or glycopyrronium bromide. At 24 h after dosing, CCh (30 μg/kg)-induced bronchoconstriction and salivation were simultaneously evaluated. Airway pressure was measured using a pressure transducer, and salivation was assessed based on net weight increase of cotton balls placed in the oral cavity after CCh administration. The inhibitory effects of test compounds on both bronchoconstriction and salivation were described as ID_{50} values, shown in Table 4.

5.4. Comparison of dissociation from rat submandibular gland

ASP9133, ipratropium bromide, and tiotropium bromide at concentrations 3000-fold each K_i value were incubated for 1 h each with cell membranes from rat submandibular gland. Test tubes were centrifuged, and cell membranes were washed once or twice. [^{3}H]NMS was added, and specific binding was assessed as percent of total specific [^{3}H]NMS binding without test compounds (percent specific [^{3}H]NMS binding). Percent specific [^{3}H]NMS binding without washing was calculated as a control.

5.5. Docking study

Crystal structures of rat M_3 and inhibitor have been published in the Protein Data Bank (PDB ID: 4DAJ).[13] Protein structures were prepared in MOE,[19] and the protonate 3D tool was used to ionize amino acids, add hydrogens, calculate charges, and remove the ligand. The ligand molecule was sketched in Maestro and energy-minimized using Confgen with OPLS_2005 force field.[20] The docking study was performed using GOLD software (version 5.1).[21,22] The ligand molecule was docked 10 times, and the top-scoring pose, as assessed by its ChemPLP value, was employed for discussions.

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