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Selective Muscarinic Antagonists. II.[1] Synthesis and Antimuscarinic Properties of Biphenylylcarbamate Derivatives

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A novel series of biphenylylcarbamate derivatives were synthesized and evaluated for binding to M_1, M_2 and M_3 receptors and for antimuscarinic activities. Receptor binding assays indicated that biphenyl-2-ylcarbamate derivatives had high affinities for M_1 and M_3 receptors and good selectivities for M_3 receptor over M_2 receptor, indicating that the biphenyl-2-yl group is a novel hydrophobic replacement for the benzhydryl group in the muscarinic antagonist field. In this series, quinuclidin-4-yl biphenyl-2-ylcarbamate monohydrochloride (8l, YM-46303) exhibited the highest affinities for M_1 and M_3 receptors, and selectivity for M_3 over M_2 receptor. Compared to oxybutynin, YM-46303 showed approximately ten times higher inhibitory activity on bladder pressure in reflexly-evoked rhythmic contraction, and about 5-fold greater selectivity for urinary bladder contraction against salivary secretion in rats. Moreover, selective antagonistic activity was also observed *in vitro*. Further evaluation of antimuscarinic effects on bradycardia and pressor in pithed rats, and on tremor in mice, showed that YM-46303 can be useful for the treatment of urinary urge incontinence as a bladder-selective M_3 antagonist with potent activities and fewer side effects.

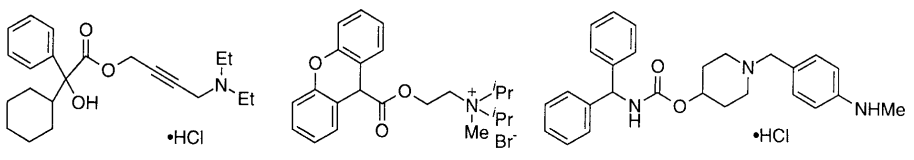
Key words YM-46303; biphenyl-2-ylcarbamate; muscarinic antagonist; urinary urge incontinence; urinary bladder contraction; salivary secretion

Treatment of urinary urge incontinence (UUI), associated with hyperactivity of the detrusor muscle, by muscarinic receptor antagonists is now widely used. Major drugs include oxybutynin (**1**) and propantheline (**2**) which show little selectivities among the various M_1, M_2 and M_3 subtypes (Fig. 1). However, it is well known that treatment with these agents is associated with a variety of systemic side effects such as dry mouth, tachycardia and mydriasis.[2] These side effects result from nonselective antimuscarinic effects. Tachycardia is caused by blockage of muscarinic M_2 receptor in the heart. Dry mouth and mydriasis are due to blockage of M_3 receptors in salivary glands and the pupil, respectively. Among these side effects, dry mouth most frequently limits the use of these agents. Consequently, muscarinic receptor antagonists with M_3 selectivity and bladder-selectivity would be useful for the treatment of UUI.

In the previous paper, we reported a new series of 1-benzyl-4-piperidyl benzhydrylcarbamate derivatives as potent and selective M_3 antagonists.[1] This study revealed that the urethane juncture can play an important role for linking the benzhydryl group and the piperidine ring to bind to M_3 receptor. In addition, one benzene ring in the benzhydryl group may be necessary for binding to muscarinic receptors and the other benzene ring may help fix the orientation of the phenyl ring for optimum interaction. In this series, 1-(4-methylaminobenzyl)-4-piperidyl benzhydrylcarbamate monohydrochloride (**3**, YM-58790) had greater than 6-fold selective inhibitory activity for urinary bladder contraction *versus* salivery secretion compared to oxybutynin in rats (Fig. 1). In the course of further research to develop more potent and bladder-selective M_3 antagonists for the treatment of UUI, screening of our chemical library uncovered the 2-phenoxybenzoate derivative (**4**), with moderate affinity for M_3 receptor with an IC_{50} value of 0.27 μM and 11-fold selectivity for M_3 over M_2 receptors (Chart 1 and Table 1). Based on the findings in the previous study, a series of substituted phenylcarbamate derivatives related to **4** were synthesized and evaluated for their affinities toward M_1, M_2 and M_3 receptors (Chart 1). Selected compounds with high affinity and selectivity for M_3 receptor were then examined for inhibitory activities against bladder pressure in reflexly-evoked rhythmic contraction and oxotremorine-induced salivary secretion in rats. In addition, further *in vitro* investigations of selectivities between bladder contraction and salivary secretion were performed. Herein, we report the synthesis, structure–activity relationships and pharmacological evaluation of this novel series of muscarinic antagonists.

Chemistry

Phenylcarbamate derivatives were prepared by the two general methods, Methods A and B shown in Chart 2. Conversion of benzoic acids (**5**) to the corresponding phenylisocyanates (**6**) *via* Curtius rearrangement[3] fol-



oxybutynin (**1**) propantherine (**2**) YM-58790 (**3**)

Fig. 1

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lowed by reaction with quinuclidinols (**7**) gave phenylcarbamates (**8**) (Method A). In the alternative method (Method B), phenylcarbamates (**8**) were prepared from anilines (**9**) as the starting materials. Treatment of **9** with methyl chloroformate in the presence of potassium carbonate gave the methyl phenylcarbamates (**10**). On the other hand, when triethylamine was used as the base, symmetrical ureas (**11**) were obtained as the main product. The methyl carbamate (**10**) and ureas (**11**) were converted to phenylcarbamates (**8c**, **8h**, **8i** and **8k**) by base-catalyzed replacement with the corresponding alcohols. Substitution at the 1-position of the piperidine ring in the 4-piperidyl biphenyl-2-ylcarbamate derivatives was performed as shown in Chart 3. Treatment of 1-benzyl-4-piperidyl biphenyl-2-ylcarbamate (**13**), prepared from biphenyl-2-carboxylic acid (**12**) *via* Curtius rearrangement, with 1-chloroethyl chloroformate[4] followed by methanolysis gave debenzylated piperidine (**14**). Alkylation with alkyl halides (Method C), or reductive alkylation with the corresponding aldehyde and sodium triacetoxyborohydride[5] (Method D) of **14** gave the desired 1-alkyl-4-piperidyl biphenyl-2-ylcarbamates (**15a—o**). Hydrogenation of the nitro group in compounds **15n** and **15o** with Raney-Ni gave the aminobenzyl derivatives (**16a** and **16b**).

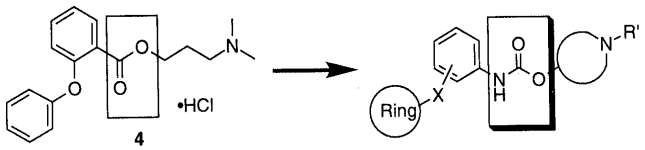

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Chart 1

Results and Discussion

Affinities of the synthesized compounds for muscarinic receptor subtypes were measured based on inhibition of [^{3}H]-pirenzepine binding to rat cortex (M_1), [^{3}H]-quinuclidinyl benzilate (QNB) binding to rat heart (M_2) and [^{3}H]-*N*-methylscopolamine binding to rat salivary glands (M_3).[6] These results are presented in Tables 1—3. Initially, we investigated conversion of dimethylaminopropyl ester (**4**) to quinuclidin-3-yl carbamate derivatives and optimization of the substituents on the phenyl ring of the

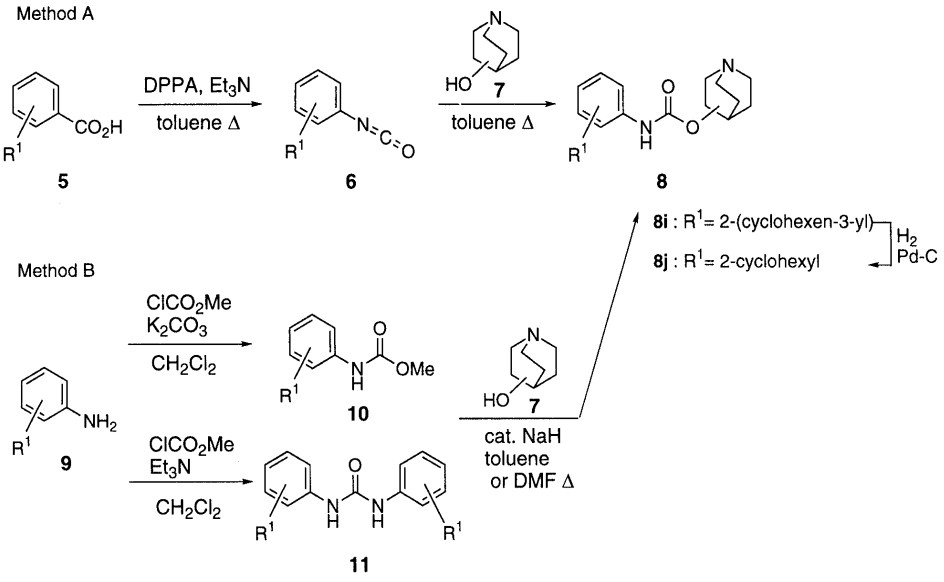


Chart 2

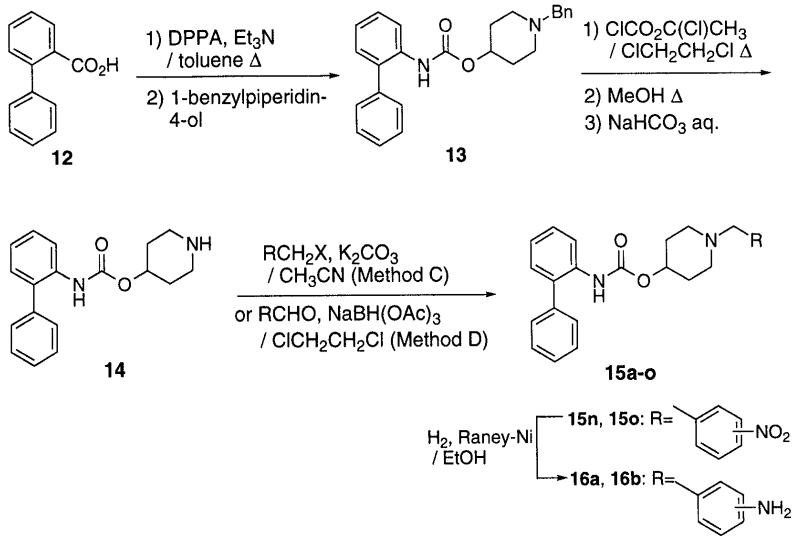
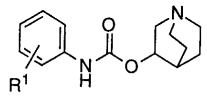


Chart 3

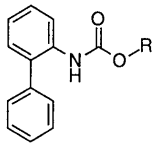
Table 1. Physical Data and Affinities for Muscarinic Receptors of Quinuclidin-3-yl Phenylcarbamate Derivatives (**8a—k**)



Compound	R^1	Method[a]	Yield (%)	mp (°C)	Recrystn. solvent	K_i (nM)			Selectivity ratio
						M_1[b]	M_2[b]	M_3[b]	M_2/M_3
8a	2-PhO	A	50	211—212	EtOH–Et_2O	11	170	18	9.4
8b	H	A	79	204—205	EtOH–Et_2O	690	4600	1700	2.7
8c	2-Et	B	69	182—183	EtOH–AcOEt	89	610	140	4.4
8d	2-Bn	A	88	170—172	MeCN–(iso-Pr)$_2$O	49	410	97	4.2
8e	2-PhS	B	92	231—233	EtOH–Et_2O	11	130	25	5.2
8f	2-Ph	A	77	137—138	iso-PrOH–Et_2O	1.2	26	0.94	28
8g	3-Ph	A	57	203—204	MeOH	3200	>10000	3700	>2.7
8h	4-Ph	A	91	281—283	EtOH–Et_2O	720	1800	570	3.1
8i	2-(Cyclohexen-3-yl)	B	54	98—100	MeCN	1.5	9.0	1.1	8.2
8j	2-Cyclohexyl	[a]	26	102—103	MeCN–Et_2O	4.1	62	8.3	7.5
8k	2-(1*H*-Pyrrol-1-yl)	B	61	183—185	EtOH–Et_2O	5.6	100	4.9	20
4						890[c]	3000[c]	270[c]	11
1						6.6	18	6.5	2.8

a) See experimental section, chemistry. *b*) See experimental section, pharmacology. *c*) Values were IC_{50}.

Table 2. Physical Data and Affinities for Muscarinic Receptors of Quinuclidinyl Biphenyl-2-ylcarbamates (**8f**, (*R*)-**8f**, (*S*)-**8f** and **8l**)

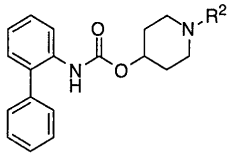


Compound	R	Method[a]	Yield (%)	mp (°C)	Recrystn. solvent	K_i (nM)			Selectivity ratio
						M_1[b]	M_2[b]	M_3[b]	M_2/M_3
8f		A	[c]	[c]	[c]	1.2	26	0.94	28
(*R*)-**8f**		A	Quant.	[d]		0.65	7.6	0.47	16
(*S*)-**8f**		A	Quant.	[d]		8.2	110	13	8.5
8l		A	37	249—251	EtOH–Et_2O	0.67	5.9	0.39	15

a) See experimental section, chemistry. *b*) See experimental section, pharmacology. *c*) See Table 1. *d*) Amorphous.

quinuclidin-3-yl phenylcarbamate, as shown in Table 1. Converting the 3-dimethylaminopropyl benzoate (**4**) into the quinuclidin-3-yl phenylcarbamate (**8a**) resulted in increased affinity for M_1 and M_3 receptors, while maintaining selectivity for M_3 receptor over M_2 receptor. The increased affinities for M_1 and M_3 receptors can be attributed to introduction of the urethane bond as a more appropriate linker than an ester, and to increased basicity going from 3-dimethylaminopropyl to quinuclidine. Removal of the phenoxy substituent (**8b**) and exchange by ethyl (**8c**) or benzyl (**8d**), decreased affinity, although phenylsulfanyl derivative (**8e**) was as potent as **8a**. On the other hand, benzene (**8f**), cyclohexene (**8i**), cyclohexane (**8j**) and pyrrole (**8k**) rings at the *ortho*-position relative to the urethane bond as substituents on the benzene ring were preferable for binding to muscarinic receptors. Moreover, compounds **8f** and **8k** showed high selectivity for M_3 receptor over M_2 receptor. Amongst these compounds, biphenyl-2-yl derivative (**8f**) exhibited the highest affinity for M_3 receptor with a K_i value of 0.94 nM and 28-fold selectivity for M_3 over M_2 receptor. In addition, affinity and selectivity for M_3 receptor was higher than the corresponding benzhydrylcarbamate derivative (K_i value for M_3 receptor, 2.0 nM; selectivity, 2.3-fold).[1] On the other hand, the biphenyl-3- and 4-yl derivatives (**8g** and **8h**) showed low affinity for M_3 receptor. These results

Table 3. Physical Data and Affinities for Muscarinic Receptors of 4-Piperidyl Biphenyl-2-ylcarbamate Derivatives (**13**—**16**)



Compound	R^2	Method[a]	Yield (%)	mp (°C)	Recrystn. solvent	K_i (nM)			Selectivity ratio
						M_1[b]	M_2[b]	M_3[b]	M_2/M_3
13	Bn	A	97	183—184	EtOH–Et_2O	5.2	44	6.1	7.2
14	H	a)	71	147—149	MeCN	32	270	27	10
15a	$(CH_2)_2Ph$	C	82	137—139	EtOH–Et_2O	2.70	16	11	1.5
15b	CH_2-cyclo-Hex	C	12	180—181	EtOH–Et_2O	4.8	21	13	1.6
15c	CH_2Ph-2-Me	C	78	185—186	EtOH–Et_2O	51	320	100	3.2
15d	CH_2Ph-3-Me	C	Quant.	180—181	EtOH–Et_2O	7.2	59	11	5.4
15e	CH_2Ph-4-Me	C	89	103—107	MeCN–(iso-Pr)$_2O$	16	42	10	4.2
15f	CH_2Ph-3-OH	D	Quant.	174—175	EtOH–MeCN	2.0	21	1.8	12
15g	CH_2Ph-4-OH	D	95	140—142	Aq. EtOH	6.1	43	7.0	6.1
15h	CH_2Ph-3-OMe	D	Quant.	136—138	EtOH–Et_2O	5.6	68	7.3	9.3
15i	CH_2Ph-4-OMe	D	Quant.	131—134	MeCN–(iso-Pr)$_2O$	4.1	12	5.7	2.1
16a	CH_2Ph-3-NH_2	a)	66	189—193	EtOH–(iso-Pr)$_2O$	2.2	12	1.8	6.7
16b	CH_2Ph-4-NH_2	a)	60	111—114	MeCN–(iso-Pr)$_2O$	6.4	18	2.9	6.2
15j	CH_2Ph-4-NMe_2	D	77	149—152	MeCN–(iso-Pr)$_2O$	1.7	14	2.8	5.0
15k	CH_2Ph-2-Cl	C	80	197—198	Aq. EtOH	45	390	100	3.9
15l	CH_2Ph-3-Cl	C	Quant.	114—118	MeCN–(iso-Pr)$_2O$	22	190	56	3.4
15m	CH_2Ph-4-Cl	C	40	172—173	EtOH	18	210	72	2.9

a) See experimental section, chemistry. *b*) See experimental section, pharmacology.

indicate that the biphenyl-2-yl group is a novel hydrophobic replacement for the benzhydryl group in the muscarinic antagonist field.

Further investigation of the quinuclidine ring in **8f** was carried out, as shown in Table 2. With regards to stereochemistry, the (*R*)-isomer was more potent and selective for M_3 receptor than the (*S*)-isomer. Surprisingly, 4-quinuclidine derivative (**8l**) exhibited high affinity for M_3 receptor (K_i value, 0.39 nM), the same as (*R*)-**8f**. Moreover, compound **8l** had high selectivity for M_3 receptor over M_2 receptor, comparable to (*R*)-**8f**. These results are inconsistent with our previous study[1] and the reports[7,8] that quinuclidin-4-yl esters were less potent and selective than quinuclidin-3-yl ones. The high affinity and selectivity for M_3 receptor of compound **8l** may be attributed to the reduced distance between the phenyl ring in the hydrophobic region and the basic nitrogen in the quinuclidine ring compared to quinuclidin-4-yl benzhydrylcarbamate.

Next, the substituents at the 1-position of the piperidine ring of 1-substituted-4-piperidyl biphenyl-2-ylcarbamate derivatives were examined. These results are shown in Table 3. The 1-benzyl-4-piperidyl biphenyl-2-ylcarbamate derivatives also showed high affinity and selectivity for M_3 receptor. Unlike the benzhydrylcarbamates,[1] phenethyl (**15a**) and cyclohexylmethyl (**15b**) derivatives showed almost the same affinity for M_3 receptor as compound **13**, although they showed less selectivity for M_3 receptor over M_2 receptor. Substituents on the phenyl ring of the benzyl group were examined and electron-donating groups at the 3- or 4-position (**15d**—**h**, **j**, **16a** and **16b**) were preferred in terms of affinity and selectivity rather than electron-withdrawing groups (**15k**—**m**). In particular, the 3-hydroxy derivative (**15f**) showed high affinity for M_3 receptor with a K_i value of 1.8 nM and 12-fold selectivity for M_3 receptor over M_2 receptor.

Table 4. M_3 Antagonistic Activities *in Vivo*

Compound	Rhythmic contraction ED_{30} (mg/kg, i.v.)	Salivary secretion ID_{50} (mg/kg, i.v.)	Selectivity ratio[a]
(*R*)-**8f**	0.012	0.033	2.8
8l	0.014	0.064	4.6
15f	0.058	0.30	5.2
1	0.18	0.17	0.94

a) Selectivity ratios were calculated by dividing the IC_{50} values for salivary secretion by the ED_{30} values for bladder contraction.

Table 5. M_3 Antagonistic Activities *in Vitro*

Compound	Bladder contraction pA_2	Rb^+ efflux pIC_{50}	Selectivity ratio[a]
8l	9.2	7.1	4.0
15f	8.5	5.7	20
1	8.1	6.6	1.0

a) Selectivity ratios relative to oxybutynin were calculated from the antilogs of the differences between the pA_2 values for bladder contraction and the pIC_{50} values for Rb^+ efflux.

Selected compounds ((*R*)-**8f**, **8l** and **15f**) with high affinity and selectivity for M_3 receptor were examined for their inhibitory activities on bladder pressure in reflexly-evoked rhythmic contraction and oxotremorine-induced salivary secretion in rats as shown in Table 4. These compounds exhibited much more potent activities than

Table 6. Comparison of Compound **8l** (YM-46303) and Oxybutynin

Compound	M_3 antagonism		M_1 antagonism	M_2 antagonism	CNS effect
	Rhythmic contraction ED_{30} (mg/kg, i.v.)	Salivary secretion ID_{50} (mg/kg, i.v.)	Pressor ID_{50} (mg/kg, i.v.)	Bradycardia DR_{10} (mg/kg, i.v.)	Tremor
8l (YM-46303)	0.014	0.064	0.39	1.1	0/5[a]
1	0.18	0.17	1.1	1.6	4/5[b]

a) Inhibited/total tested at the dose of 0.1 mg/kg, i.v. *b*) Inhibited/total tested at the dose of 3 mg/kg, i.v.

Table 7. Analytical and Spectral Data for Compounds **4a—l**

Compound	Formula	Analysis (%) Calcd (Found)				^{1}H-NMR (DMSO-d_6) δ	FAB-MS *m/z*
		C	H	N	Other		
8a	$C_{20}H_{22}N_2O_3 \cdot HCl \cdot 0.2H_2O$	63.47 (63.48	6.23 6.23	7.40 7.38	Cl, 9.37 Cl, 9.40)	1.60—2.10 (4H, m), 2.20—2.30 (1H, m), 3.00—3.40 (5H, m), 3.55—3.70 (1H, m), 4.85—4.95 (1H, m), 6.85—7.05 (3H, m), 7.05—7.20 (3H, m), 7.35—7.45 (2H, m), 7.70—7.80 (1H, m), 9.10 (1H, br s), 10.95 (1H, s)	339 (MH^+)
8b	$C_{14}H_{18}N_2O_2 \cdot HCl$	59.47 (59.50	6.77 6.80	9.91 9.90	Cl, 12.54 Cl, 12.43)	1.70—2.00 (4H, m), 2.00—2.15 (1H, m), 3.10—3.40 (5H, m), 3.60—3.75 (1H, m), 4.90—5.05 (1H, m), 7.01 (1H, t, J= 7.3 Hz), 7.25—7.35 (2H, m), 7.47 (2H, d, J=7.8 Hz), 9.80 (1H, br s), 10.54 (1H, br s)	247 (MH^+)
8c	$C_{16}H_{22}N_2O_2 \cdot HCl \cdot 0.1H_2O$	61.47 (61.36	7.48 7.45	8.96 8.81	Cl, 11.34 Cl, 11.48)	1.12 (3H, t, J=7.3 Hz), 1.70—2.15 (4H, m), 2.20—2.50 (1H, m), 2.60 (2H, q, J=7.3 Hz), 3.10—3.30 (5H, m), 3.60—3.70 (1H, m), 4.85—5.00 (1H, m), 7.10—7.30 (3H, m), 7.32 (1H, d, J=7.3 Hz), 9.00 (1H, br s), 10.70 (1H, br s)	274 (M^+)[b]
8d	$C_{21}H_{24}N_2O_2 \cdot HBr$	60.44 (60.33	6.04 6.02	6.71 6.71	Br, 19.06 Br, 19.06)	1.60—2.10 (4H, m), 2.10—2.30 (1H, m), 2.95—3.40 (5H, m), 3.55—3.75(1H, m), 4.00 (2H, s), 4.75—4.90 (1H, m), 7.10—7.40 (9H, m), 9.10 (1H, br s), 9.67 (1H, br s)	337 (MH^+)
8e	$C_{20}H_{22}N_2O_2S \cdot HCl$	61.45 (61.32	5.93 5.90	7.17 7.14	S, 8.20 Cl, 9.07 S, 8.25 Cl, 9.33)	1.65—2.00 (4H, m), 2.15—2.25 (1H, m), 3.00—3.35 (5H, m), 3.55—3.65 (1H, m), 4.85—4.95 (1H, m), 7.15—7.40 (8H, m), 7.57 (1H, d, J=7.8 Hz), 9.05 (1H, br s), 10.66 (1H, br s)	355 (MH^+)
8f	$C_{20}H_{22}N_2O_2 \cdot HCl \cdot 0.8H_2O$	64.35 (64.38	6.64 6.62	7.50 7.36	Cl, 9.50 Cl, 9.50)	1.65—2.00 (4H, m), 2.05—2.15 (1H, m), 2.75—2.90 (1H, m), 2.95—3.30 (4H, m), 3.50—3.60 (1H, m), 4.70—4.85 (1H, m), 7.25—7.50 (9H, m), 8.93 (1H, br s), 10.55 (1H, br s)	323 (MH^+)
8g	$C_{20}H_{22}N_2O_6 \cdot C_4H_4O_4$[a]	65.74 (65.74	5.98 5.97	6.39 6.37)		1.50—1.85 (3H, m), 1.90—2.05 (1H, m), 2.10—2.20 (1H, m), 2.80—3.10 (5H, m), 3.35—3.50 (1H, m), 4.80—4.90 (1H, m), 6.55 (2H, s), 7.29 (1H, d, J= 7.9 Hz), 7.30—7.55 (5H, m), 7.59 (2H, d, J=7.3 Hz), 7.79 (1H, br s), 9.80 (1H, br s)	323 (MH^+)
8h	$C_{20}H_{22}N_2O_2 \cdot HCl$	66.94 (66.72	6.46 6.46	7.81 7.81	Cl, 9.88 Cl, 9.80)	1.75—2.00 (3H, m), 2.05—2.20 (1H, m), 2.25—2.35 (1H, m), 3.10—3.40 (5H, m), 3.60—3.75 (1H, m), 4.95—5.05 (1H, m), 7.32 (1H, t, J=7.3 Hz), 7.40—7.50 (2H, m), 7.35—7.70 (6H, m), 9,97 (1H, m), 10.90 (1H, br s)	323 (MH^+)
8i	$C_{20}H_{26}N_2O_2 \cdot CHO_2$[c] $\cdot H_2O$	64.76 (64.58	7.51 7.29	7.19 7.09)		1.35—2.15 (11H, m), 2.80—3.05 (5H, m), 3.40 (1H, m), 3.69 (1H, m), 4.78 (1H, m), 5.55 (1H, d, J=10 Hz), 5.89 (1H, d, J=10 Hz), 7.15—7.20 (3H, m), 7.25—7.30 (1H, m), 8.92 (1H, br s)	326 (M^+)[b]
8j	$C_{20}H_{28}N_2O_2 \cdot C_2H_2O_4$[d] $\cdot 0.75H_2O$	61.17 (61.29	7.35 7.33	6.48 6.50)		1.10—1.20 (6H, m), 1.65—2.05 (8H, m), 2.24 (1H, m), 2.77 (1H, m), 3.05—3.25 (5H, m), 3.62 (1H, m), 4.89 (1H, m), 7.10—7.30 (4H, m), 9.02 (1H, br s)	329 (MH^+)
8k	$C_{18}H_{21}N_3O_2 \cdot HCl \cdot 0.2H_2O$	61.52 (61.59	6.42 6.37	11.96 11.95	Cl, 10.09 Cl, 10.36)	1.65—2.00 (4H, m), 2.05—2.15 (1H, m), 2.80—3.30 (5H, m), 3.45—3.60 (1H, m), 4.70—4.85 (1H, m), 6.23 (2H, s), 6.99 (2H, s), 7.30—7.50 (4H, m), 9.01 (1H, br s), 10.92 (1H, br s)	312 (MH^+)
8l	$C_{20}H_{22}N_2O_2 \cdot HCl$	66.94 (66.70	6.46 6.52	7.81 7.84	Cl, 9.88 Cl, 9.89)	1.90—2.20 (6H, m), 3.20—3.45 (6H, m), 7.25—7.45 (9H, m), 8.81 (1H, br s), 10.49 (1H, br s)	323 (MH^+)

a) Fumarate. *b*) EI-MS. *c*) Hemioxalate. *d*) Oxalate.

Table 8. Analytical and Spectral Data for Compounds **13**—**16**

Compound	Formula	Analysis (%) Calcd (Found) C	H	N	Cl	^{1}H-NMR (DMSO-d_6) δ	FAB-MS m/z (MH^+)
13	$C_{25}H_{26}N_2O_2 \cdot HCl$	70.99 (70.86	6.43 6.46	6.62 6.59	8.38 8.49)	1.70—2.15 (4H, m), 2.70—3.10 (2H, m), 3.15—3.40 (2H, m), 4.15—4.30 (2H, m), 4,50—4.80 (1H, m), 7.20—7.65 (14H, m), 8.73 and 8.82 (total 1H, br s), 11.06 (1H, br s)	387
14	$C_{18}H_{20}N_2O_2$	72.95 (72.91	6.80 6.75	9.45 9.49)		1.20—1.35 (2H, m), 1.65—1.75 (2H, m), 2.35—2.55 (2H, m), 2.80—2.90 (2H, m), 3.33 (1H, br s), 4.40—4.55 (1H, m), 7.25—7.45 (9H, m), 8.58 (1H, br s)	297
15a	$C_{26}H_{28}N_2O_2 \cdot HCl \cdot 0.5H_2O$	70.02 (70.34	6.78 7.00	6.28 6.08	7.95 7.72)	1.70—2.20 (4H, m), 2.80—3.60 (8H, m), 4.60—4.80 (1H, m), 7.20—7.50 (14H, m), 8.79 and 8.85 (total 1H, br s), 11.03 and 11.10 (total 1H, br s)	401
15b	$C_{25}H_{32}N_2O_2 \cdot C_2H_2O_4$[a]	67.20 (66.99	7.10 6.97	5.80 5.82)		0.80—1.00 (2H, m), 1.00—1.20 (3H, m), 1.40—2.05 (10H, m), 2.60—3.20 (6H, m), 4.50—4.70 (1H, m), 7.25—7.50 (9H, m), 8.77 (1H, br s)	393
15c	$C_{26}H_{28}N_2O_4 \cdot C_2H_2O_4$[a]	68.56 (68.43	6.16 6.26	5.71 5.72)		1.55—1.70 (2H, m), 1.80—1.95 (2H, m), 2.35 (3H, s), 2.70—3.05 (4H. m), 3.94 (2H, s), 4.55—4.65 (1H, m), 7.15—7.50 (13H, m), 8.72 (1H, br s)	401
15d	$C_{26}H_{28}N_2O_4 \cdot C_2H_2O_4$[a]	68.56 (68.52	6.16 6.16	5.71 5.68)		1.55—1.75 (2H, m), 1.85—1.95 (2H, m), 2.32 (3H, s), 2.75—3.10 (4H. m), 4.00 (2H, s), 4.55—4.70 (1H, m), 7.20—7.45 (13H, m), 8.74 (1H, br s)	401
15e	$C_{26}H_{28}N_2O_4 \cdot C_2H_2O_4$[a] $\cdot 0.2H_2O$	68.06 (68.36	6.20 6.59	5.67 5.33)		1.60—1.75 (2H, m), 1.85—1.95 (2H, m), 2.32 (3H, s), 2.75-3.15 (4H, m), 4.02 (2H, s), 4.55—4.70 (1H, m), 7.23 (2H, d, J=7.9 Hz), 7.20—7.50 (11H, m), 8.74 (1H, s)	447
15f	$C_{25}H_{26}N_2O_3 \cdot C_2H_2O_4$[a]	65.84 (65.79	5.73 5.68	5.69 5.73)		1.55—1.75 (2H, m), 1.85—1.95 (2H, m), 2.80—3.10 (4H, m), 3.96 (2H, s), 4.50—4.65 (2H, m), 6.75—6.90 (3H, m), 7.21 (1H, t, J=7.8 Hz), 7.25—7.45 (9H, m), 8.76 (1H, s)	403
15g	$C_{25}H_{26}N_2O_3 \cdot CHO_2$[b] $\cdot 1.75H_2O$	65.19 (65.18	6.42 6.28	5.85 5.84)		1.45—1.65 (2H, m), 1.70—1.90 (2H, m), 2.50—2.65 (2H, m), 2.70—2.90 (2H, m), 3.69 (2H, m), 4.45—4.60 (1H, m), 6.75 (2H, d, J=8.6 Hz), 7.16 (2H, d, J=8.6 Hz), 7.25—7.50 (9H, m), 8.69 (1H, s)	403
15h	$C_{26}H_{28}N_2O_3 \cdot C_2H_2O_4$[a]	66.39 (66.25	5.97 5.94	5.53 5.47)		1.55—1.70 (2H, m), 1.85—1.95 (2H, m), 2.70—2.85 (2H, m), 2.90—3.00 (2H, m), 3.77 (3H, s), 3.95 (2H, s), 4.50—4.65 (1H, m), 6.95—7.05 (3H, m), 7.25—7.45 (10H, m), 8.71 (1H, br s)	417
15i	$C_{26}H_{28}N_2O_3 \cdot C_2H_2O_4$[a]	66.39 (66.06	5.97 5.92	5.53 5.48)		1.55—1.75 (2H, m), 1.85—1.95 (2H, m), 2.75—3.10 (4H, m), 3.77 (3H, s), 4.00 (2H, s), 4.50—4.65 (1H, m), 6.98 (2H, d, J=8.6 Hz), 7.30—7.45 (11H, m), 8.73 (1H, br s)	417
15j	$C_{27}H_{31}N_3O_2 \cdot 2C_2H_2O_4$[a]	61.08 (60.97	5.79 5.76	6.89 6.86)		1.60—1.80 (2H, m), 1.85—2.05 (2H, m), 2.80—3.30 (4H, m), 2.93 (6H, s), 4.09 (2H, s), 4.55—4.70 (1H, m), 6.74 (2H, d, J=8.3 Hz), 7.25—7.45 (11H, m), 8.79 (1H, br s)	430
15k	$C_{25}H_{25}ClN_2O_2 \cdot C_2H_2O_4$[a]	63.47 (63.55	5.33 5.31	5.48 5.51	6.94 6.88)	1.50—1.70 (2H, m), 1.75—1.90 (2H, m), 2.60—2.85 (2H, m), 2.85—2.95 (2H, m), 3.93 (2H, s), 4.50—4.65 (1H, m), 7.25—7.60 (13H, m), 8.70 (1H, br s)	421
15l	$C_{25}H_{25}ClN_2O_2 \cdot C_2H_2O_4$[a]	63.47 (63.60	5.33 5.36	5.48 5.45	6.94 7.17)	1.50—1.70 (2H, m), 1.75—1.95 (2H, m), 2.60—3.10 (4H, m), 3.97 (2H, s), 4.50—4.65 (1H, m), 7.20—7.55 (13H, m), 8.70 (1H, br s)	421
15m	$C_{25}H_{25}ClN_2O_2 \cdot C_2H_2O_4$[a] $\cdot 0.25H_2O$	62.91 (62.88	5.38 5.38	5.43 5.42	6.88 7.00)	1.50—1.70 (2H, m), 1.80—1.95 (2H, m), 2.60—3.05 (4H, m), 3.93 (2H, s), 4.50—4.65 (1H, m), 7.25—7.55 (13H, m), 8.71 (1H, br s)	421
15n	$C_{25}H_{25}N_3O_4$	69.59 (69.63	5.84 5.82	9.74 9.68)		1.40—1.55 (2H, m), 1.70—1.80 (2H, m), 2.10—2.25 (2H, m), 2.50—2.65 (2H, m), 3.61 (2H, s), 4.40—4.55 (1H, m), 7.25—7.45 (9H, m), 7.62 (1H, t, J=7.6 Hz), 7.75 (1H, d, J=7.9 Hz), 8.12 (1H, m), 8.14 (1H, s), 8.61 (1H, br s)	432
15o	$C_{25}H_{25}N_3O_4$	69.59 (69.46	5.84 5.82	9.74 9.70)		1.40—1.55 (2H, m), 1.70—1.80 (2H, m), 2.10—2.25 (2H, m), 2.50—2.65 (2H, m), 3.58 (2H, s), 4.40—4.50 (1H, m), 7.20—7.45 (9H, m), 7.58 (2H, d, J=8.5 Hz), 8.19 (2H, d, J=8.5 Hz), 8.60 (1H, br s)	432
16a	$C_{25}H_{27}N_3O_2 \cdot 2HCl \cdot H_2O$	60.98 (61.25	6.35 6.16	8.53 8.32	14.40 14.06)	1.70—2.15 (4H, m), 2.70—3.35 (4H, m), 3.90 (2H, br s), 4.25 (2H, s), 4.55—4.75 (1H, m), 7.20—7.60 (13H, m), 8.79 and 8.84 (total 1H, br s), 11.20 (1H, br s)	402
16b	$C_{25}H_{27}N_3O_2 \cdot 2C_2H_2O_4$[a] $\cdot 0.25H_2O$	59.43 (59.43	5.42 5.34	7.17 7.22)		1.50—2.05 (4H, m), 2.60—3.40 (6H, m), 4.01 (2H, s), 4.50—4.70 (1H, m), 6.58 (2H, d, J=8.6 Hz), 7.09 (2H, d, J=8.6 Hz), 7.20—7.45 (9H, m), 8.76 (1H, br s)	402

a) Oxalate. *b*) Hemioxalate.

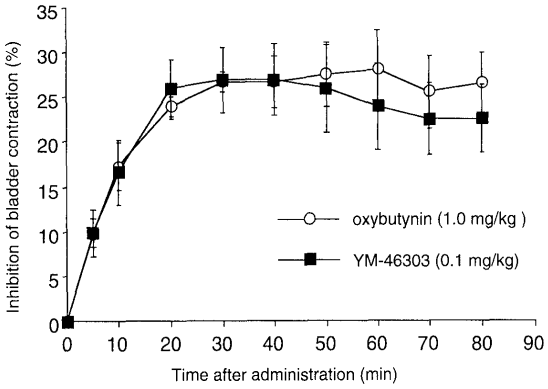


Fig. 2. Effects of YM-46303 and Oxybutynin on Bladder Pressure in Rat Reflexly-evoked Rhythmic Contraction by Intraduodenal Administration

oxybutynin in reflexly-evoked rhythmic contraction. Among them, **8l** and **15f** showed about a 5-fold greater selectivity for urinary bladder contraction *versus* salivary secretion than oxybutynin. Moreover, compared to oxybutynin, **8l** and **15f** showed more selective antagonistic activities *in vitro* for rat urinary bladder contraction against Rb^+ efflux from rat salivary glands, which is an indication of salivary secretion, as shown in Table 5.

Compound **8l** (YM-46303), with the most potent activity in rhythmic contraction, was selected for further pharmacological evaluation (Table 6). YM-46303 had a much weaker effect on McN-A343-induced pressor (M_1 receptor antagonism) and oxotremorine-induced bradycardia (M_2 receptor antagonism) in pithed rat than that on rhythmic contraction. In oxotremorine-induced tremor in mice, as an indicator of central nervous system (CNS) effects, YM-46303 showed little effect, thus demonstrating its poor penetration of the blood–brain barrier. These results indicate that YM-46303 is potentially a bladder-selective M_3 antagonist with few side effects. Moreover, the potent activity of YM-46303 during intraduodenal administration was 10-fold grater than that of oxybutynin (Fig. 2).

In conclusion, a new series of biphenyl-2-ylcarbamate derivatives were synthesized and evaluated for muscarinic antagonistic activities. High affinities for M_3 receptor, and good selectivities for M_3 over M_2 receptor indicated that the biphenyl-2-yl group is a novel hydrophobic replacement of the benzhydryl group in the muscarinic antagonist field. Amongst the compounds in this series, quinuclidin-4-yl biphenyl-2-ylcarbamate monohydrochloride (**8l**, YM-46303) exhibited the most potent activity on bladder pressure in reflexly-evoked rhythmic contraction and selective antagonistic activity for urinary bladder contraction *versus* salivary secretion, both *in vivo* and *in vitro*. Further evaluation of antimuscarinic effects on bradycardia, pressor and tremor showed that YM-46303 can be useful for treatment of urinary urge incontinence as a bladder-selective M_3 antagonist with potent activities and fewer side effects.

Experimental

Melting points were determined using a Yanaco micro melting apparatus and are uncorrected. Proton magnetic resonance (^{1}H-NMR) spectra were obtained in $CDCl_3$ or dimethyl sulfoxide-d_6 (DMSO-d_6) with a JEOL JNM-EX90, JNM-EX400, JNM-GX500 or JNM-A500 spectrometer. Chemical shifts are recorded in parts per million (δ), downfield relative to tetramethylsilane as the internal standard. Mass spectra (MS) were recorded on a JEOL JMS-DX300 or a Hitachi M-80 mass spectrometer. Elemental analyses were carried out on a Yanaco MT-3 or MT-5 CHN analyzer and a Yokogawa IC 7000S Ion Chromatoanalyzer. Optical rotations were measured on a Horiba SEPA-200 polarimeter. Chromatographic separations were performed on a silica gel column [Wakogel C-200 or Merck Kieselgel 60 (30—400 mesh)]. Analytical thin-layer chromatography (TLC) was carried out on precoated glass plates (Merck Kieselgel 60 F_{254}).

Authentic Materials Oxybutynin hydrochloride was purchased from Sigma Co.

Chemistry. General Methods Method A: Quinuclidin-4-yl Biphenyl-2-ylcarbamate Monohydrochloride (**8l**): A solution of diphenylphosphoryl azide (9.57 g, 35 mmol) in toluene (10 ml) was added dropwise to a mixture of biphenyl-2-carboxylic acid (6.00 g, 30 mmol) and triethylamine (3.52 g, 35 mmol) in toluene (60 ml). The mixture was stirred at room temperature for 20 min, then heated at 95 °C for 20 min. To the mixture was added a solution of quinuclidin-4-ol (4.62 g, 36 mmol) in *N*,*N*-dimethylformamide (DMF, 15 ml), and the mixture stirred at 110 °C for 3.6 h. After cooling to room temperature, the resulting mixture was diluted with ethyl acetate (100 ml) and washed twice with water. The organic layer was extracted with 1 N hydrochloric acid (50 ml × 3) and the combined aqueous layer basified to pH 8—9 with solid potassium carbonate followed by extraction with chloroform. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The residual yellow solid was stirred at room temperature in a mixture of diethyl ether (30 ml) and hexane (30 ml), collected, washed with hexane and dried to give quinuclidin-4-yl biphenyl-2-ylcarbamate (3.61 g, 37%) as a pale yellow solid. This compound (3.61 g) was converted into the corresponding hydrochloride (3.84 g) and 2.04 g of the crystals were recrystallized from ethanol–diethyl ether to give 1.99 g of **8l** as colorless crystals.

(*R*)-Quinuclidin-3-yl Biphenyl-2-ylcarbamate Monohydrochloride [(*R*)-**8f**]: Compound (*R*)-**8f** was prepared from biphenyl-2-carboxylic acid and (*R*)-quinuclidin-3-ol[9] according to method A as a colorless amorphous solid, $[\alpha]_D^{25}$ −5.1° (c=0.93, MeOH). ^{1}H-NMR (DMSO-d_6) δ: 1.65—2.00 (4H, m), 2.05—2.15 (1H, m), 2.70—2.90 (1H, m), 2.95—3.30 (4H, m), 3.45—3.60 (1H, m), 4.70—4.85 (1H, m), 7.25—7.50 (9H, m), 8.94 (1H, br s), 10.79 (1H, br s). FAB-MS m/z: 323 (MH^+). *Anal.* Calcd for $C_{20}H_{23}ClN_2O_2 \cdot 1.1H_2O$: C, 63.44; H, 6.71; Cl, 9.36; N, 7.40. Found: C, 63.55; H, 6.94; Cl, 9.46; N, 7.13.

(*S*)-Quinuclidin-3-yl Biphenyl-2-ylcarbamate Monohydrochloride [(*S*)-**8f**]: Compound (*S*)-**8f** was prepared from biphenyl-2-carboxylic acid and (*S*)-quinuclidin-3-ol[9] according to method A as a colorless amorphous solid, $[\alpha]_D^{25}$ +6.1° (c=1.2, MeOH). The ^{1}H-NMR and mass spectra of (*S*)-**8f** were the same as those of (*R*)-**8f**. *Anal.* Calcd for $C_{20}H_{23}ClN_2O_2 \cdot H_2O$: C, 64.35; H, 6.64; Cl, 9.50; N, 7.50. Found: C, 64.38; H, 6.91; Cl, 9.14; N, 7.26.

Method B: Quinuclidin-3-yl 2-Phenylsulfanylphenylcarbamate Monohydrochloride (**8e**): Methyl chloroformate (4.6 ml, 60 mmol) was added dropwise to an ice-cooled mixture of 2-phenylsulfanylaniline[10] (5.44 g, 27 mmol), saturated aqueous sodium hydrogencarbonate ($NaHCO_3$) solution (100 ml), and chloroform (50 ml). The mixture was then stirred at room temperature for 3 h. The organic layer was separated, washed with water and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (20 : 1) as eluent to give methyl 2-phenylsulfanylphenylcarbamate (**10**, 5.18 g, 74%) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 3.72 (3H, s), 6.95—7.80 (9H, m), 8.25—8.30 (1H, m). EI-MS m/z: 259 (M^+). Sodium hydride (60% in mineral oil, 0.12 g, 3 mmol) was added to a mixture of methyl 2-phenylsulfanylphenylcarbamate (**10**, 3.11 g, 12 mmol) and quinuclidin-3-ol (1.98 g, 16 mmol) in toluene (90 ml) and the mixture heated under reflux with removal of the resulting methanol using a MS 4A column which was attached between the flask and the reflux condenser, for 20 h. After cooling, the mixture was poured into brine, extracted with ethyl acetate, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHDCl_3$–MeOH (10 : 1) as eluent to give quinuclidin-3-yl 2-phenylsulfanylphenylcarbamate (3.93 g, 92%) as a yellow oil. This compound (0.60 g) was converted into the corresponding hydrochloride and recrystallized from ethanol–diethyl ether to give 0.49 g of **8e** as colorless crystals.

Quinuclidin-3-yl 2-(1*H*-Pyrrol-1-yl)phenylcarbamate Monohydrochloride (**8k**): Methyl chloroformate (3.28 g, 35 mmol) was added

dropwise to an ice-cooled solution of 2-(1*H*-pyrrol-1-yl)aniline (5.02 g, 32 mmol) and triethylamine (3.83 g, 38 mmol) in dichloromethane (50 ml). The mixture was stirred at room temperature overnight, then methyl chloroformate (3.28 g, 35 mmol) and triethylamine (3.83 g, 38 mmol) were added dropwise to the ice-cooled solution. After stirring at room temperature for 6 h, further methyl chloroformate (3.28 g, 35 mmol) and triethylamine (3.83 g, 38 mmol) were added. The resulting mixture was stirred at room temperature overnight, washed with water, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel with hexane–ethyl acetate (4 : 1) as eluent to give 1,3-bis[2-(1*H*-pyrrol-1-yl)phenyl]urea (**11b**, 1.34 g, 26%) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 6.30 (4H, dd, J=2.5, 1.8 Hz), 6.46 (2H, s), 6.71 (4H, dd, J=2.4, 1.9 Hz), 7.10—7.16 (2H, m), 7.20—7.26 (2H, m), 7.32—7.38 (2H, m), 8.04 (2H, d, J=7.3 Hz). EI-MS *m/z*: 342 (M^+). Sodium hydride (60% in mineral oil, 0.12 g, 3 mmol) was added to a mixture of 1,3-bis[2-(1*H*-pyrrol-1-yl)phenyl]urea (**11b**, 1.00 g, 3.1 mmol) and quinuclidin-3-ol (1.05 g, 8.3 mmol) in toluene (30 ml) and DMF (1 ml) and the mixture was heated under reflux for 6 h. After cooling, the mixture was poured into brine, extracted with ethyl acetate, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (9 : 1) as eluent to give quinuclidin-3-yl 2-(1*H*-pyrrol-1-yl)phenylcarbamate (3.93 g, 92%) as a yellow oil. This compound (0.60 g) was converted into the corresponding hydrochloride and recrystallized from ethanol–diethyl ether to give 0.35 g of **8k** as colorless crystals.

Method C: 1-Phenethyl-4-piperidyl Biphenyl-2-ylcarbamate Monohydrochloride (**15a**): A mixture of **14** (0.50 g, 1.7 mmol), phenethyl bromide (0.42 ml, 1.7 mmol) and potassium carbonate (0.23 g, 1.7 mmol) in acetonitrile (10 ml) was stirred under reflux for 6 h. The mixture was cooled, concentrated *in vacuo*, and the residue partitioned between chloroform and water, and the organic layer washed with water, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue was chromatographed on silica gel with $CHCl_3$–MeOH (50 : 1) as eluent to give 1-phenethyl-4-piperidyl biphenyl-2-ylcarbamate (0.56 g, 82%) as a yellow oil which was converted into the corresponding hydrochloride and recrystallized from ethanol–diethyl ether to give 0.33 g of **15a** as colorless crystals.

Method D: 1-(3-Hydroxybenzyl)-4-piperidyl Biphenyl-2-ylcarbamate Monooxalate (**15f**): A mixture of **14** (0.55 g, 1.9 mmol), 3-hydroxybenzaldehyde (0.23 g, 1.9 mmol) and sodium triacetoxyborohydride (0.79 g, 3.7 mmol) in 1,2-dichloroethane (10 ml) was stirred at room temperature for 3 h. The mixture was diluted with chloroform, washed with saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$ and concentrated *in vacuo*. The resulting oily residue was chromatographed on silica gel with $CHCl_3$–MeOH (20 : 1) as eluent to give 1-(3-hydroxybenzyl)-4-piperidyl biphenyl-2-ylcarbamate (0.75 g, quant.) as a colorless amorphous material which was converted into the corresponding oxalate and recrystallized from acetonitrile–ethyl acetate to give 0.29 g of **15f** as colorless crystals.

1,3-Bis[2-(cyclohexen-3-yl)phenyl]urea (11a) Compound **11a** was prepared in the same manner as **11b**. ^{1}H-NMR ($CDCl_3$) δ: 1.30—1.50 (2H, m), 1.50—1.70 (4H, m), 1.90—2.10 (6H, m), 3.65—3.75 (2H, m), 5.63 (2H, dd, J=10, 2.2 Hz), 5.94 (2H, m), 7.06 (2H, t, J=7.7 Hz), 7.10—7.20 (4H, m), 7.60 (2H, t, J=7.7 Hz), 8.18 (2H, s). FAB-MS *m/z*: 373 (MH^+).

Quinuclidin-3-yl 2-Cyclohexylphenylcarbamate Monooxalate (8j) A mixture of quinuclidin-3-yl 2-(cyclohexen-3-yl)phenylcarbamate (0.23 g, 0.70 mmol), 10% palladium on carbon (0.20 g) and 4 N hydrogen chloride in 1,4-dioxane solution (0.2 ml) in ethanol (5 ml) was stirred at room temperature under a hydrogen atmosphere for 3 h. After the catalyst was filtered off, the filtrate was concentrated *in vacuo*, and the residue basified with saturated $NaHCO_3$ solution and extracted with chloroform. The organic layer was dried over Na_2SO_4, concentrated *in vacuo*, and the resulting residue converted into the corresponding oxalate and recrystallized from acetonitrile–diethyl ether to give **8j** (0.08 g, 26%) as colorless crystals.

4-Piperidyl Biphenyl-2-ylcarbamate (14) 1-Chloroethyl chloroformate (9.2 ml, 85 mmol) was added to a solution of **13** (11.0 g, 29 mmol) in 1,2-dichloroethane (70 ml) and the solution refluxed for 3 h. After cooling to room temperature, the solvent was evaporated under reduced pressure. The residue was then dissolved in methanol (70 ml) and refluxed overnight. After evaporation, the residue was partitioned between chloroform and saturated $NaHCO_3$ solution and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue was crystallized from chloroform–diethyl ether to give 4-piperidyl biphenyl-2-ylcarbamate (**14**, 6.26 g, 71%). This compound (0.76 g) was recrystallized from acetonitrile to give 0.40 g of **14** as colorless crystals.

1-(3-Aminobenzyl)-4-piperidyl Biphenyl-2-ylcarbamate Dihydrochloride (16a) A mixture of 1-(3-nitrobenzyl)-4-piperidyl biphenyl-2-ylcarbamate (**15n**, 1.10 g, 2.5 mmol) and Raney-Ni in methanol (50 ml) was stirred under a hydrogen atmosphere at room temperature for 1 d. After the catalyst was filtered off, the filtrate was concentrated *in vacuo*, and the residue chromatographed on silica gel using $CHCl_3$–MeOH (10 : 1) as eluent to give 1-(3-aminobenzyl)-4-piperidyl biphenyl-2-ylcarbamate (0.68 g, 66%), which was converted into the corresponding hydrochloride and recrystallized from acetonitrile–diethyl ether to give **16a** (0.15 g) as colorless crystals.

Pharmacology. Muscarinic Receptor Binding Assays Muscarinic receptor binding assays were performed using the reported method[6] with rat cortex, heart, and salivary glands using [^{3}H]-pirenzepine, [^{3}H]-quinuclidinyl benzilate and [^{3}H]-*N*-methylscopolamine as ligands for M_1, M_2 and M_3 receptors, respectively.

Rhythmic Contraction Female Wistar rats were anesthetized with urethane (1.0 g/kg, s.c.). The bilateral ureters were ligated, then the urinary bladder was trans-urethrally catheterized and filled with saline to evoke reflex rhythmic contractions. After recording the peak intravesical pressure, compounds were cumulatively administered *via* the femoral vein. The pressure drop was measured in the period 5—10 min after each administration. A dose–response curve was obtained in each rat and the dose required to reduce the peak intravesical pressure by 30% (ED_{30}) was determined.

Salivary Secretion Male Wistar rats were anesthetized with urethane (1.2 g/kg, i.p.). Compounds were intravenously administered 15 min prior to the injection of oxotremorine (0.8 μmol/kg, i.v.). Saliva was collected by absorbent paper for 5 min after the injection of oxotremorine.

Pressor The effect on pressor response caused by McN-A343 was determined in pithed rats.[6] Compounds were intravenously administered 15 min before the injection of McN-A343 (3 μmol/kg, i.v.).

Bradycardia Bradycardia was evoked in pithed rats by the intravenous injection of oxotremorine.[6] Compounds were administered 15 min prior to the challenge of oxotremorine. A dose shifting the dose–response curve of oxotremorine to the right by 10-fold (DR_{10}) was calculated.

Tremor Test compounds were intravenously administered to male ICR mice 5 min prior to injection of oxotremorine (1 mg/kg s.c.). Tremor was observed in periods of 5—10 min after the injection of oxotremorine.

Rat Urinary Bladder Contraction Excised rat bladder was suspended in an organ bath containing gassed Kreb's–Henseleit buffer at 37 °C and the isometric tension was measured. After construction of two dose–response curves to cumulatively added carbachol, compounds were added to the bath, and a dose–response curve to carbachol was established again.

Rb^+ Efflux from Rat Salivary Glands The effect on Rb^+ efflux, a reflection of water outflow, from rat salivary glands was determined according to the reported method[11] with minor modifications. Chopped submandibular glands were loaded with ^{86}RbCl (100 mCi) in 5 ml of Kreb's–Henseleit buffer for 40 min at 37 °C. The glands were then placed in a flow cell perfused with the buffer solution (2 ml/min). After perfusion for 3 min, the perfusate was replaced by the buffer solution containing the compounds and 10 μM of carbachol. The perfusate was collected and its radioactivity was determined.

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