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1,5-Benzodioxepin derivatives as a novel class of muscarinic M_3 receptor antagonists

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Abstract—The structure–activity relationships of novel 1,5-benzodioxepin derivatives as muscarinic M_1–M_3 receptor antagonists are reported. Some of these compounds were found to possess high binding affinity for the muscarinic M_3 receptor and potent effect on rhythmic increase in bladder pressure in unanesthetized rats following oral administration. These compounds displayed selectivity for the bladder over the salivary gland.

Muscarinic receptors are expressed in peripheral tissues and the central nervous system, which are classified into the subtypes M_1–M_5.[1] One of these receptor subtypes, the muscarinic M_3 receptor, which is distributed in the airway, small intestine, urinary bladder, and secretory glands, is believed to be a mediator of smooth muscle contraction and glandular secretion, and its antagonists are therefore thought to be potentially useful in the treatment of disorders such as urinary incontinence (UI), irritable bowel syndrome (IBS), and chronic obstructive pulmonary disease (COPD). Unfortunately, anti-cholinergic medication often brings about undesirable side effects such as tachycardia, dry mouth, constipation, and blurred vision. For example, oxybutynin, available for the treatment of overactive bladder, occasionally causes dry mouth due to antagonism of the muscarinic receptor in the salivary gland.

The muscarinic M_3 receptor antagonists currently known (oxybutynin, solifenacin,[2] darifenacin,[3] tolterodine,[4,5] tiotropium,[6] Banyu compound,[7–9] KRP-197)[10,11] have relatively similar chemical structures (diphenyl or phenylcycloalkyl) in their skeletons (Fig. 1). We performed random screening to explore a new class of compounds structurally different from these muscarinic M_3 receptor antagonists. We hypothesized that these new compounds would be useful as anti-muscarinic drugs and would display different characteristics from existing medicines for overactive bladder, IBS, and COPD.

As a result, we discovered 1,5-benzodioxepin derivatives have high affinity for the muscarinic M_3 receptor.

In the present study, we attempted to develop the 1,5-benzodioxepins as useful medicines for urinary incontinence. In the present paper, we report the preparation and structure–activity relationship (SAR) of 1,5-benzodioxepin derivatives, their muscarinic M_1–M_3 receptor binding affinities, and the effect of these derivatives on overactive bladder and the salivary gland.

The preparation of the 1,5-benzodioxepin key intermediates **5a**–**c** was performed from the starting materials catechol, 4-fluorocatechol, or 4,5-dichlorocatechol as shown in Scheme 1.[12–14] The alkylation of catechols with methyl chloroacetate gave compounds **1a**–**c**, which were then cyclized under basic conditions (60% NaH in DMSO) to give **2a**–**c**. Decarboxylation of these compounds by treatment with refluxing HCl/2-propanol

Keywords: Muscarinic M_1–M_3 receptor; Antagonists; Urinary incontinence; 1,5-benzodioxepin derivatives.
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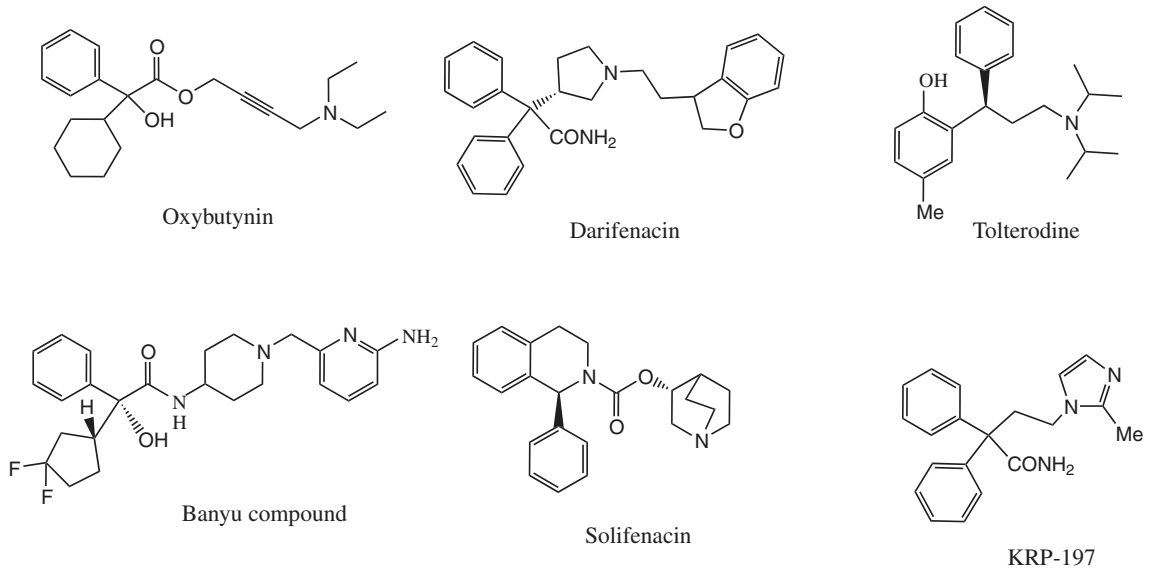
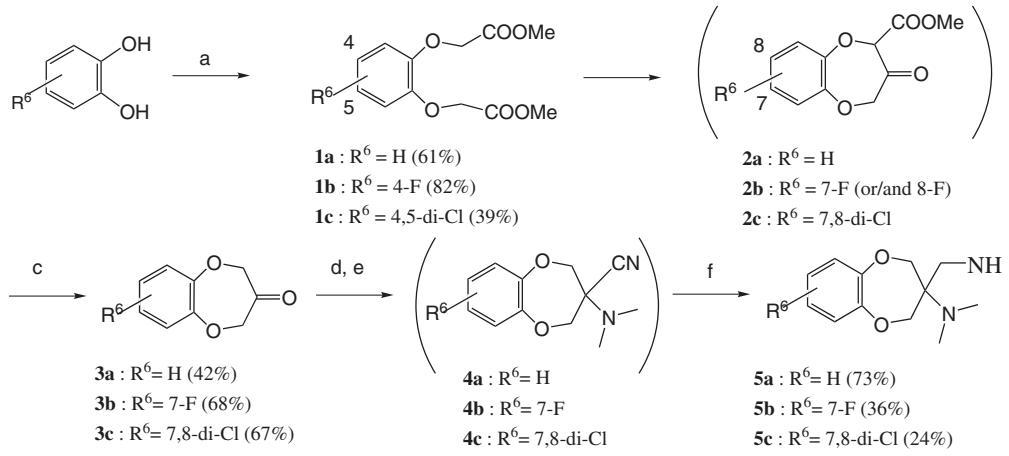


Figure 1.



Scheme 1. Reagents: (a) $ClCH_2COOCH_3$, K_2CO_3, KI, MeOH (or 2-butanone); (b) 60% NaH, DMSO/toluene; (c) HCl/2-propanol; (d) KCN, H_2O/MeOH; (e) MeNH–HCl; (f) Red-Al/toluene.

afforded the 1,5-benzodioxepin-3-one derivatives **3a**–**c**. These compounds were treated with KCN and the resulting cyanohydrines were reacted with Me_2NH hydrochloride to give compounds **4a**–**c**, reduction of which with Red-Al gave the desired key intermediates **5a**–**c**.

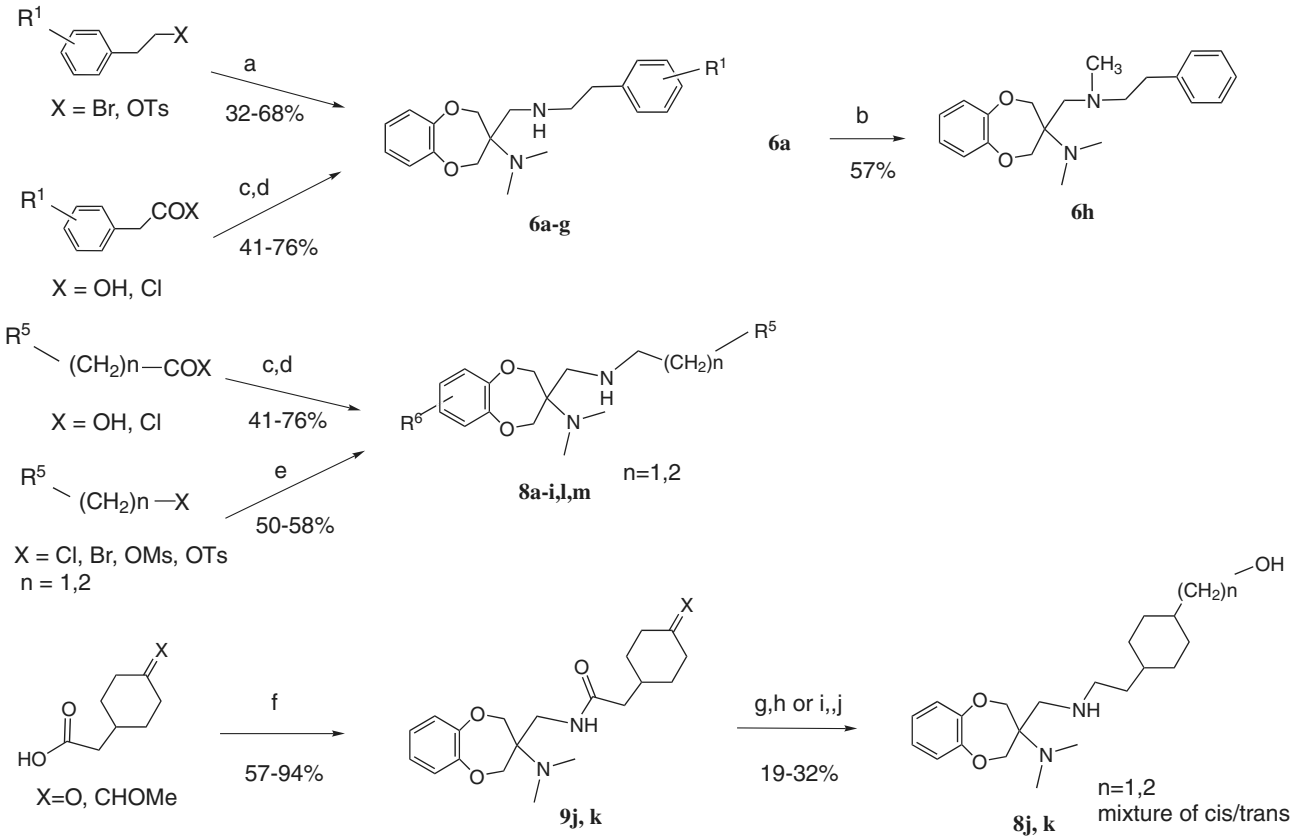
The intermediates **5a**, **5b** (racemates), **5c**, and **6a** were functionalized as shown in Scheme 2. The derivatives **6b**, **c**, **e**, and **8g**–**i** were synthesized by coupling of **5a** with aralkylhalide, alkylhalide in MeCN in the presence of K_2CO_3. Reductive amination of **6a** with HCHOaq–$NaBH_3CN$ afforded the tertiary amine **6h**.

The compounds **6a**, **d**, **f**, **g** and **8a**–**f**, **j**–**m** were synthesized by condensation of **5a**–**c** with a variety of carboxylates followed by reduction of the amide group using $NaBH_4$–BF_3Et_2O (or borane–THF).

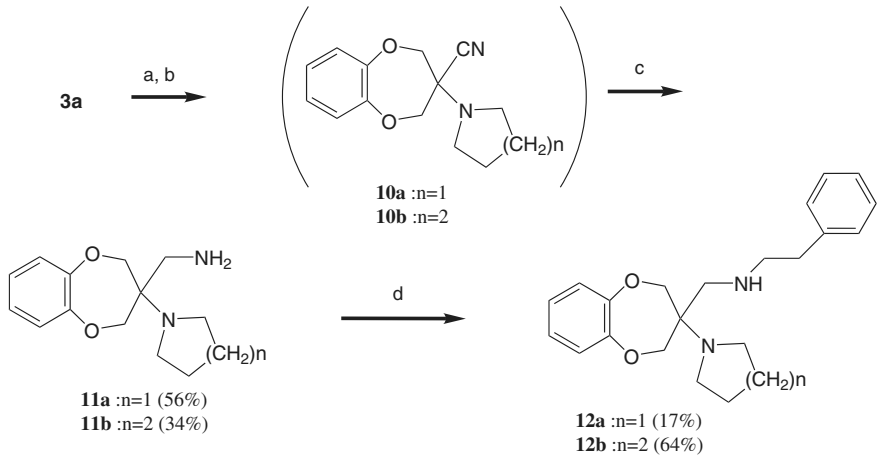
The preparation of compounds **10a** and **b** was performed by cyanation of the ketone **3a** followed by treatment with pyrrolidine or piperidine hydrochloride. The nitriles **10a** and **b** were reduced and the resulting primary amines **11a** and **b** were treated with a 2-phenylethylbromide to provide compounds **12a** and **b**, respectively (Scheme 3).

Compound **4a** was treated with Grignard reagents derived from 4-cyclohexylbutylbromide and cyclohexylmethyl bromide to give compounds **13a** and **13b**, respectively (Scheme 4).

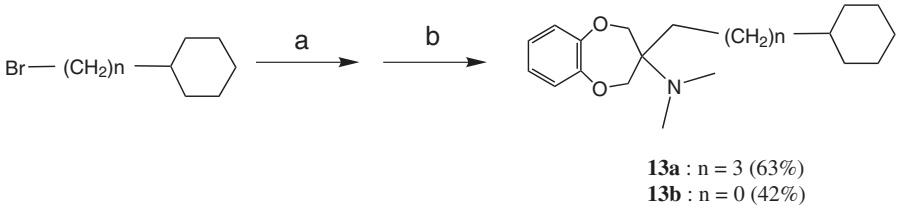
The binding affinity of 1,5-benzodioxepin derivatives for the human muscarinic receptor subtypes (M_1, M_2, and M_3) expressed in insect Sf 9 cells was determined by measuring their ability to inhibit the binding of 1-[*N*-methyl-^{3}H]scopolamine methyl chloride.[15]



Scheme 2. Reagents: (a) **5a**, K_2CO_3, (KI)/MeCN; (b) 37% HCHOaq, $NaBH_3CN$; (c) **5a**–**c**, Bop-reagent (or DEPC, EDC, none)/THF (or AcOEt, DMF); (d) $NaBH_4$, BF_3–Et_2O (or BH_3–THF)/THF, then HCl/EtOH; (e) **5a**, K_2CO_3, (KI)/MeCN; (f) **5a**, Bop-reagent/THF; (g) $NaBH_4$/EtOH; (h) $NaBH_4$, BF_3–Et_2O; (i) HCl/THF; (j) BH_3–THF, then HCl/EtOH.

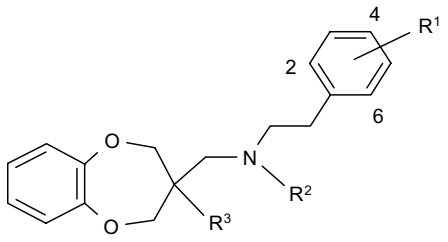


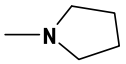
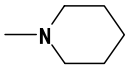
Scheme 3. Reagents: (a) KCN, H_2O, MeOH; (b) pyrrolidine–HCl or piperidine–HCl ; (c) Red-Al/toluene; (d) 2-phenylethylbromide, K_2CO_3/MeCN.



Scheme 4. Reagents: (a) Mg, I_2/THF; (b) **4a**/THF.

Table 1. Binding affinity of compounds to muscarinic M_1, M_2, and M_3 receptors



Compound	R_1	R_2	R_3	Binding affinity[a] K_i (nM)			Ratio	
				M_1	M_2	M_3	M_1/M_3	M_2/M_3
6a	H	H	$N(CH_3)_2$	220	90	20	11	4.5
6b	4-Me	H	$N(CH_3)_2$	220	48	18	12	2.7
6c	4-Cl	H	$N(CH_3)_2$	260	49	20	13	2.5
6d	4-*i*-Pr	H	$N(CH_3)_2$	200	44	56	3.6	0.8
6e	4-MeO	H	$N(CH_3)_2$	NT[b]	NT[b]	>100[c]	—	—
6f	2,6-Di-Cl	H	$N(CH_3)_2$	540	49	23	23	2.1
6g	2-F	H	$N(CH_3)_2$	380	41	19	20	2.1
6h	H	Me	$N(CH_3)_2$	NT[b]	NT[b]	>100[c]	—	—
12a	H	H		NT[b]	NT[b]	>100[c]	—	—
12b	H	H		NT[b]	NT[b]	>100[c]	—	—
Oxybutynin				1.0	8.1	0.78	1.3	10

[a] Each value is the mean from triplicate assays in a single experiment.
[b] Not tested.
[c] IC_{50} value.

The binding affinity of the parent compound **6a**, its derivatives **6b–h** and **12a**, **b** is presented in Table 1. Compound **6a** with the 2-phenylethylamine moiety at the 3-position of the 1,5-benzodioxepin ring showed moderate binding affinity for the muscarinic M_1, M_2, and M_3 receptors (K_i = 220, 90 nM, and M_3 = 20 nM, respectively).

At first, we examined the influence of the substituent group in the benzene ring of the 2-phenylethylamine moiety of **6a**. The introduction of a methyl group (**6b**) or a chlorine atom (**6c**) at the 4-position of the benzene ring had little effect on the binding affinity for the M_1, M_2, and M_3 receptors. Replacement with an isopropyl group at this position (compound **6d**) resulted in decreased affinity for the M_3 receptor compared with **6a**. This decrease was particularly dramatic when a methoxy group was incorporated at the 4-position of the benzene ring (compound **6e**; IC_{50} > 100 nM). These results suggest that the introduction of electron-donating groups at this position is unfavorable for muscarinic M_3 receptor binding.

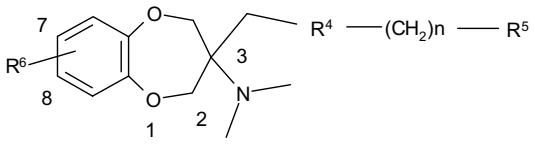
Transformation of the dimethylamino group to 1-pyrrolidinyl (**12a**) or 1-piperidino (**12b**) led to drastic decrease of the binding affinity for the M_3 receptor (IC_{50} > 100 nM). This finding suggests that the muscarinic M_3 receptor has an interaction with the dimethylamine part at the 3-position of 1,5-benzodioxepin derivatives and that the corresponding binding site of this receptor does not have enough space for bulkier alkylamines such as pyrrolidine or piperidine.

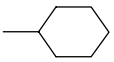
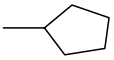
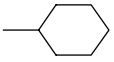
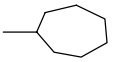
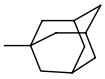
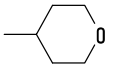
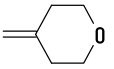
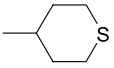
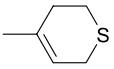
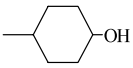
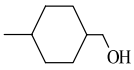
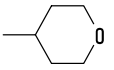
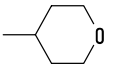
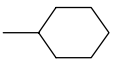
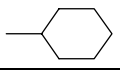
The M_1–M_3 receptor binding affinity of compounds with cycloalkyl amine at the 3-position of 1,5-benzodioxepin is summarized in Table 2. Cyclohexyl derivative **8c** obtained by saturation of the 2-phenylethylamine moiety of **6a** has 8-fold higher binding affinity for the M_3 receptor than the parent compound.

On the other hand, this transformation had almost no effect on the binding affinity ratio for the M_3 versus M_1–M_2 receptors. The high affinity for the muscarinic M_1–M_3 receptors was retained after replacement of the cyclohexyl moiety of compound **8c** with a five-membered ring (**8b**), a seven-membered ring (**8d**), or a 1-adamantyl group (**8e**). This suggests that the corresponding hydrophobic binding region of the muscarinic M_1–M_3 receptors has a common space favorable for a five- to seven-membered cycloalkyl or 1-adamantyl group in the aminomethyl moiety at the 3-position of the 1,5-benzodioxepin and that this binding region prefers an aliphatic cycloalkyl ring to an aromatic ring (compounds **8a–e** vs **6a**).

Meanwhile, insertion of an oxygen atom (**8f**) or a sulfur atom (**8h**) at the 4-position of the cyclohexyl ring decreased the binding affinity for the muscarinic M_3 receptor compared with compound **8c**.

Table 2. Binding affinity of compounds to muscarinic M_1, M_2, and M_3 receptors



Compound	*n*	R_4	R_5	R_6	Binding affinity K_i[a] (nM)			Ratio	
					M_1	M_2	M_3	M_1/M_3	M_2/M_3
8a	1	NH		H	31	12	4.9	6.3	2.4
8b	2	NH		H	21	2.4	3.3	6.4	0.7
8c	2	NH		H	18	6.2	2.5	7.2	2.5
8d	2	NH		H	12	2.0	2.7	4.4	0.7
8e	2	NH		H	5.9	0.66	1.5	3.9	0.4
8f	2	NH		H	58	6.0	11	5.3	0.5
8g	2	NH		H	86	8.8	14	6.1	0.6
8h	2	NH		H	52	7.2	6.8	7.6	1.1
8i	2	NH		H	140	12	5.8	24	2.1
8j[b]	2	NH		H	19	1.9	2.9	6.6	0.7
8k[b]	2	NH		H	18	1.8	2.1	8.6	0.9
8l[c]	2	NH		7-F	36	5.1	4.2	8.6	1.2
8m	2	NH		7,8-Di-Cl	1.8	0.12	0.22	8.2	0.55
13a	2	CH_2		H	NT[d]	NT[d]	>100[e]	—	—
13b	0	None		H	NT[d]	NT[d]	>100[e]	—	—

[a] Each value is the mean from triplicate assays in a single experiment.
[b] A mixture of *cis*/*trans* isomers.
[c] A mixture of enantiomers.
[d] Not tested.
[e] IC_{50} value.

Additionally, the presence of a double bond at the 3,4-position of the tetrahydrothiopyran (**8i**) caused a 2.7-fold loss in binding affinity for the muscarinic M_1 receptor compared with **8h** and increased the binding affinity ratio for the M_3 versus M_1 receptor ($M_1/M_3 = 24$). Likewise, the 2,6-dichlorophenyl (**6f**)

Table 3. Inhibitory effects of oxybutynin and the compounds **8g**, **j**, **k**, and **l** on rhythmic contraction of bladder and methacholine-induced salivation

Compound	ID_{50} (mg/kg, iv)		Salivation/rhythmic contraction
	Rhythmic contraction[a]	Salivation[b]	
Oxybutynin	0.038	0.11	2.9
8g	0.012	0.25	20
8j	0.0088	0.18	20
8k	0.015	0.16	10
8l	0.010	0.20	20

[a] The ID_{50} values represent the doses to reduce the atropine-sensitive rhythmic contraction by 50% in unanesthetized rats ($n = 4$).
[b] The ID_{50} values represent the 50% inhibitory doses on methacholine-induced salivation in anesthetized rats ($n = 5$).

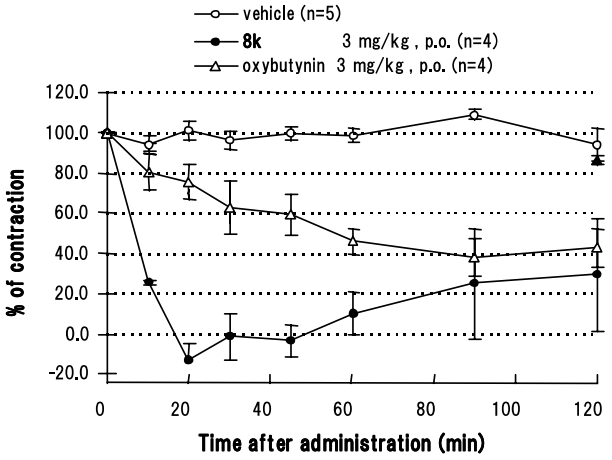


Figure 2. Effects of **8k** (3 mg/kg, po) and oxybutynin (3 mg/kg, po) on the rhythmic increase in bladder pressure of unanesthetized rats. Each symbol and bar indicates the mean ± SEM.

and 2-fluorophenyl (**6g**) derivatives showed some selectivity between the M_3 and M_1 receptors (M_1/M_3 = 20–23) as shown in Table 1. These results suggest that factors at the *ortho*-position of a six-membered ring such as thiopyran bearing a double bond or a 2,6-dichlorophenyl group of derivatives may be unfavorable for muscarinic M_1 receptor binding affinity.

In the next step, SAR development was focused on variation of the secondary amine moiety. Methylation of the secondary amine (**6h**) caused a drastic loss in the binding affinity for the M_3 receptor as shown in Table 1. Similarly, transformation of this amino group into a methylene chain (**13a** and **13b**) produced a notable decrease in the binding affinity (IC_{50} >100 nM) for the muscarinic M_3 receptor. These findings support the hypothesis that the secondary amino group is a moiety essential for binding affinity and acts as a hydrogen bond donor to the corresponding binding site of the muscarinic M_3 receptor.

Finally, we performed introduction of halogen atoms into the 1,5-benzodioxepin moiety. The introduction of a fluorine atom at the 7-position of 1,5-benzodioxepin (**8l**) somewhat increased the binding affinity for the muscarinic M_3 receptor (K_i = 4.2 nM) compared with the unsubstituted compound **8f**. The 7,8-dichloro derivative **8m** possessed 50-fold higher affinity (K_i = 0.22 nM) than **8f**. Meanwhile, the incorporation of these halogen substituents did not affect the binding affinity ratio for the muscarinic M_3 versus M_1–M_2 receptors.

We examined the inhibitory effect of intravenous injection of oxybutynin and compounds **8g**, **j**, **k**, **l** on rhythmic bladder contraction in unanesthetized rats[16] and methacholine-induced salivation in anesthetized rats[17] (Table 3). The inhibitory effect of compounds **8g**, **j**, **k**, and **l** on bladder contraction was 10- to 20-fold more potent than on the salivary gland (oxybutynin: 2.9-fold). This result suggests that these compounds possess tissue selectivity for the bladder over the salivary gland in rat. The effect of oral administration (3 mg/kg) of compound **8k** on rhythmic increase in the bladder pressure of unanesthetized rats is presented in Figure 2. In oral administration, this compound was found to have more potent effect than oxybutynin.

In summary, we prepared a novel class of 1,5-benzodioxepin derivatives with high affinity for the muscarinic M_1–M_3 receptors. These compounds were structurally different from the previously known typical muscarinic receptor M_1–M_3 antagonists.

SAR studies established that the secondary amine group at the 3-position on 1,5-benzodioxepin was essential moiety for muscarinic M_1–M_3 receptor binding affinity. Specifically, it was observed that a number of 1,5-benzodioxepin derivatives with a cycloalkyl group in the secondary amine moiety possessed high affinity for the muscarinic M_1–M_3 receptor. One of these, the 1,5-benzodioxepin derivative **8k**, displayed high affinity for the muscarinic M_1–M_3 receptors, had potent effect on rhythmic increase in the bladder pressure of unanesthetized rats, and displayed selectivity for the bladder over the salivary gland.

In addition, 1,5-benzodioxepin derivatives should provide useful anti-muscarinic drugs for disorders such as irritable bowel syndrome (IBS) and chronic obstructive pulmonary disease (COPD).

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References and notes

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