Synthesis and Biological Evaluation of 1,2,3,4-Tetrahydroisoquinoline Derivatives as Potent and Selective M_2 Muscarinic Receptor Antagonists

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A series of 1,2,3,4-tetrahydroisoquinoline derivatives containing the 5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one skeleton were prepared and evaluated for their *in vitro* binding affinities to muscarinic receptors and for antagonism of bradycardia *in vivo*. Among them, compound 3f had the highest affinity for M_2 muscarinic receptors in the heart (p*K*i=9.1) with low affinity for M_3 muscarinic receptors in the submandibular gland. A structure–activity relationship (SAR) study suggested that the benzene ring fused piperidine and the alkyl linker chain length are crucially important for increased M_2 affinity.

Key words 1,2,3,4-tetrahydroisoquinoline derivative; M_2 muscarinic receptor; antagonism; M_2 selectivity; bradycardia

Muscarinic cholinergic receptors can presently be biologically categorized into at least five subtypes (m_1—m_5) and pharmacologically divided into four subtypes (M_1—M_4) by different selective antagonists[1—6] M_2 (m_2) muscarinic receptors are abundant in the heart, smooth muscle and the central nervous system and play a crucial role in the regulation of the heart rate mediated by the vagus nerve. In the heart, an increase in parasympathetic tone is thought to be a significant factor in sick sinus syndrome and atrioventricular block, and this implies that M_2 muscarinic receptor antagonists are promising candidates as antibradycardiac agents. Atropine, a non-specific muscarinic receptor antagonist, has been used for treatment of these diseases, though undesirable side effects such as dry mouth, mydriasis and gastrointestinal and urinary events caused by antagonism of the M_3 muscarinic receptors have appeared.[7]

To achieve an effective and safer therapy for such diseases, selective M_2 muscarinic receptor antagonists are required, and AF-DX 116 (**1**) was reported as the first selective M_2 muscarinic receptor antagonist by Engel *et al*.[8,9] AF-DX 116 includes a 5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one skeleton and two nitrogen atoms protonated at physiological pH, and the spatial orientation of the nitrogen atom in the side chain of the tricyclic ring system is thought to be the most important factor in determining M_2 selectivity over other muscarinic receptor subtypes, especially M_3 muscarinic receptors.[10] We previously designed succinamide derivatives based on the assumption that cleavage of the piperidine ring of **1** would give compounds with sufficient flexibility for interacting with M_2 muscarinic receptors and discovered YM-43571 (**2**), which showed a higher M_2 affinity than AF-DX 116 and significant M_2 selectivity over M_3 muscarinic receptors (p*K*i(M_2)=8.8, M_3/M_2 selectivity=320).[11] Furthermore, we discussed how the two amino nitrogen atoms of YM-43571, a benzylamino nitrogen atom and an alkyl-bearing nitrogen atom of a piperazine ring, play a crucial role in the interaction with M_2 muscarinic receptors, and the importance of the latter for M_3 muscarinic receptor affinity was less than that for M_2 receptors.[11] As another strategy for changing the spacial position of the terminal diethylamino group, we designed 1,2,3,4-tetrahydroisoquinoline derivatives **3a**—**f**, which have the piperidine ring shown in **1** and an alkyl side chain of the proper length (Fig. 1). Herein, we describe the synthesis and biological evaluation of these compounds.

Chemistry

1,2,3,4-Tetrahydroquinoline (**7**), 1,2,3,4-tetrahydroisoquinoline (**11**, **15**) and indoline (**16**) derivatives were prepared by the multi-step routes shown in Chart 1.[12] Diamines **6**, **10** and **14a**, **b** were synthesized from the corresponding acids (**4**, **8** and **12a**, **b**) by the respective methods indicated. Though reduction of 2-(diethylaminomethyl)quinoline with platinum oxide (PtO_2), $NiCl_2$–$NaBH_4$ or Ni–Al alloy resulted in recovery of starting material, the desired diamine **6** was obtained by reduction of quinoline-2-carboxylic acid diethylamide **5** with Ni–Al catalyst, followed by treatment with $LiAlH_4$.[13] Catalytic hydrogenation of **5** over PtO_2 resulted in over-reduction to decahydroquinoline, however, 1,2,3,4-tetrahydroisoquinoline derivative **10** was obtained from **9** under the same conditions, followed by reduction using $BH_3 \cdot THF$.[14] The resulting diamines **6**, **10** and **14a**, **b** were treated with 11-(chloroacetyl)-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one **17**, which was prepared according to the method reported by Schmidt,[15] in the presence of

"cleavage of piperidine ring" "side chain with proper length"

2 YM-43571 **1 AF-DX 116** **3a—f**

Fig. 1

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a) Et_2NH, WSCD, HOBT, CH_2Cl_2; b) Ni-Al alloy, KOH, MeOH; c) $LiAlH_4$, THF; d) **17**, Na_2CO_3, CH_3CN; e) H_2, PtO_2, c.HCl, EtOH; f) BH_3, THF; g) 4 *N* HCl/dioxane

Chart 1

a) H_2, PtO_2, c.HCl, MeOH; b) $(Boc)_2O$, Et_3N, H_2O/dioxane; c) $Br(CH_2)_nBr$, NaOHaq, *n*-Bu_4NHSO_4; d) Et_2NH, EtOH; e) 4*N* HCl/dioxane; f) **17**, Na_2CO_3, CH_3CN

Chart 2

sodium carbonate in acetonitrile to give the target compounds **7**, **11**, **15** and **16**, respectively.

1,2,3,4-Tetrahydroisoquinoline derivatives **3a**—**f** were synthesized from 5-hydroxyisoquinoline **18** as shown in Chart 2. Compound **18** was hydrogenated over PtO_2 and subsequently protected with a Boc group to afford **19**. After treatment of **19** with an excess amount of dibromoalkanes with different methylene chain lengths (n=2—7) in the presence of tetra-*n*-butylammonium hydrogensulfate in sodium hydroxide solution, the resulting compounds were reacted with an excess of diethylamine and then deprotected by acid to afford diamines **20a**—**f**. Treatment of these diamines with **17** gave the desired compounds **3a**—**f,** according to the above-mentioned method.

Pharmacological Results and Discussion

The muscarinic receptor binding affinity and selectivity were assessed by employing receptor-binding assays, as reported previously.[16] The binding affinities for synthesized compounds were obtained by using rat cerebral cortex (M_1), heart (M_2) and submandibular gland (M_3), and measuring the displacement of [^{3}H]pirenzepine (PZ), [^{3}H]quinuclidinyl benzilate (QNB) and [^{3}H]*N*-methylscopolamine (NMS), respectively. The results, expressed as p*K*i values, and the selectivity ratios for M_2 muscarinic receptors to M_1 and M_3 muscarinic receptors (M_1/M_2, M_3/M_2, respectively), are presented in Tables 1 and 2. AF-DX 116 (**1**) was used as the reference compound.

Before the biological evaluation of compounds **3a**—**f**, we focused our efforts on investigating the effect of a fused benzene ring by comparison of **7**, **11**, **15**, **16** and **1** (Table 1). Except for **16**, these compounds showed higher affinities for all three subtypes than AF-DX 116, and compounds **7** and **15** were found to have similar M_3/M_2 selectivity to that of AF-DX 116, in particular. On the other hand, compound **11** displayed a higher affinity for M_1 muscarinic receptors than M_2 and M_3 muscarinic receptors. In addition, we found that there was a significant difference in binding affinities between **7** and **16**, though it was reported that exchange of the piperidine ring in AF-DX 116 with the corresponding pyrrolidine lead to a compound with similar affinity for M_2 and M_3 muscarinic receptors.[8] These results suggest that a benzene ring attached to a piperidine ring might play an important role in increasing the affinity for muscarinic receptors probably by π–π and/or π–H interactions, and that its spatial position in relation to the tricyclic ring system or the terminal amino moiety influences both the affinity and subtype selectivity. Furthermore, from comparison of **7** and **15**, we obtained the

Table 1. Binding Affinities of **7**, **11**, **15** and **16** to M_1, M_2 and M_3 Muscarinic Receptors

Compd.	R	Binding affinity, p*Ki*[a]			Selectivity ratio	
		M_1	M_2	M_3	M_1/M_2	M_3/M_2
7		7.8	8.0	6.9	1.6	13
15		7.0	7.4	6.3	2.5	13
11		7.7	7.2	6.7	0.32	3.2
16		5.9	6.3	5.5	2.5	6.3
1		6.1	6.9	5.7	6.3	16

a) p*K*i values represent an average of two or more determinations from separate assays.

Table 2. Binding Affinities **3a**—**f** to M_1, M_2 and M_3 Muscarinic Receptors

Compd.	*n*	Binding affinity, p*Ki*[a]			Selectivity ratio	
		M_1	M_2	M_3	M_1/M_2	M_3/M_2
3a	2	7.7	8.2	7.2	3.2	10
3b	3	8.1	8.1	7.3	1.0	6.3
3c	4	7.2	8.0	6.7	6.3	20
3d	5	7.4	7.9	6.5	3.2	25
3e	6	8.2	8.7	7.1	3.2	40
3f	7	8.5	9.1	7.4	4.0	50
1		6.1	6.9	5.7	6.3	16

a) p*K*i values represent an average of two or more determinations from separate assays.

Table 3. M_2 Muscarinic Receptor Antagonistic Activities of **3f** and **1** in *in Vivo* Experiments in Rats

Compound	Inhibitory effects in oxotremorine-induced bradycardia	
	pDR_{10}[a]	*n*
3f	5.81 (5.69—5.87)[b]	8
1	5.63 (5.56—5.70)	32

a) Values are the means of the indicated number of experiments (*n*). Figures in parentheses represent 95% confidence limits. *b*) Values are calculated from the ED_{30} values.

information that the basicity of the nitrogen atom in the piperidine ring (p*K*a (**7**)=0.58±0.40 *vs*. p*K*a (**15**)=4.36±0.40)[17] does not greatly affect binding to muscarinic receptors.

Based on the results in Table 1, we evaluated the structure–activity relationships (SAR) of 5-[(diethylamino)-alkoxy]-1,2,3,4-tetrahydroisoquinoline derivatives **3a**—**f** by changing the alkyl length of the side chain. The results are shown in Table 2.

The first peak in affinity for the three muscarinic receptors appeared at 2 or 3 methylenes, and compound **3a** showed a 6-fold higher affinity for M_2 muscarinic receptors than that of **15**. An outstanding increase in M_1 and M_2 affinity was observed when the alkyl linker chain length was 7, while there was no great difference in M_3 affinity between **3a** and **3f**. This finding suggests two possibilities; the first is that the terminal amino moieties of **3a** and **3f** recognize the same site on the receptors, and the second is that they interact with different sites. In the former case, it may be inferred that the hydrophobic interaction of the alkyl linker chain with the M_2 muscarinic receptor contributes to the increase in the M_2 affinity. As a result, we discovered compound **3f** having a high affinity for M_2 muscarinic receptors (p*K*i=9.1), comparable to that of **2**, with M_3/M_2 selectivity (M_3/M_2=50) which was 3-fold larger than that of **1**, and our strategy was successful.

The *in vivo* M_2 muscarinic receptor antagonistic activity of compound **3f** was next evaluated. Oxotremorine-induced bradycardia in pithed rat as a marker of M_2 antagonistic activity was assessed in comparison with **1**.[16] Compound **3f** was given by intravenous administration, and the data are presented as pDR_{10} values, as shown in Table 3.

Compound **3f** acted as a noncompetitive antagonist, comparable to **2**, in this model, and this behavior was different from **1**, which exhibited competitive antagonism.[10] The pDR_{10} value of **3f**, which was calculated from the ED_{30} value, was almost equal to that of **1**. We believed that this might be due to plasma protein binding or differences in the distribution to tissues.

Conclusions

In conclusion, we designed novel M_2 muscarinic receptor antagonists **3a**—**f** having 1,2,3,4-tetrahydroisoquinoline units, based on the assumption that a change in spatial location of the terminal amino moiety might lead to compounds with higher affinity and selectivity for M_2 muscarinic receptors and evaluated them for binding affinities to M_1, M_2 and M_3 muscarinic receptors *in vitro*, and for antagonism of bradycardia *in vivo*. Furthermore, we examined the effect of the fused benzene ring in the 1,2,3,4-tetrahydroisoquinoline and 1,2,3,4-tetrahydroquinoline moieties on muscarinic receptor affinity and selectivity by comparison of **7**, **11**, **15**, **16** and **1**. As a result, compound **3f**, which has a 7-(diethylamino)heptyloxy moiety, without an "accessory portion" such as the 4-(4-alkylpiperazin-1-yl)benzylamino group in succinamide derivatives, showed the highest affinity for M_2 muscarinic receptors (p*K*i=9.1) with 50-fold M_2 selectivity

over M_3 receptors and comparable *in vivo* activity to AF-DX 116. In addition, we obtained SAR indicating that the benzene ring fused piperidine and alkyl linker chain length are crucially important for increasing M_2 affinity. We expect that this knowledge will be useful for the discovery of novel M_2 muscarinic receptor antagonists.

Experimental

All melting points were measured with a Yanaco MP-500D melting point apparatus without correction. ^{1}H-NMR spectra were obtained on a JEOL JNM-EX90 or JNM-A500 spectrometer and the chemical shifts are expressed in δ (ppm) values with tetramethylsilane as an internal standard. Abbreviations of ^{1}H-NMR signal patterns are as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; q,quartet; m,multiplet; br,broad. Mass spectra were obtained on a JEOL JMS-DX300 or Hitachi M-80 spectrometer. High-resolution mass spectra (HR-MS) were recorded on VG ZAB-VSE mass spectrometers. Column chromatography on silica gel was performed with Kieselgel 60 (E.Merck).

Quinoline-2-carboxylic Acid Diethylamide (5) A mixture of quinoline-2-carboxylic acid (**4**) (5.0 g, 28.9 mmol), 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide hydrochloride (WSCD) (6.1 g, 31.8 mmol), 1-hydroxybenzotriazole (HOBT) (1.95 g, 14.4 mmol) and diethylamine (3.3 ml, 31.8 mmol) in CH_2Cl_2 (100 ml) was stirred at room temperature for 5 h. After the mixture was washed with 5% citric acid, 5% $NaHCO_3$ aq. and brine, the organic layer was dried over $MgSO_4$, and the solvent was evaporated *in vacuo* to give 7.5 g of **5** as an oil in 99% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.23 (3H, t, J=7.2 Hz), 1.32 (3H, t, J=7.2 Hz), 3.34—3.75 (4H,m), 7.40—8.30 (6H,m). GC-MS *m/z*: 228 (M^+).

2-Diethylaminomethyl-1,2,3,4-tetrahydroquinoline (6) 1)A suspension of **5** (3.0 g, 13.2 mmol), 1 M KOH aq. (60 ml) and Ni–Al alloy (10 g) in methanol (60 ml) was stirred at room temperature for 2 h. The reaction mixture was filtered through Celite®, solvent evaporated off, and the residue purified on silica gel column chromatography (*n*-hexane–AcOEt, 5 : 1, v/v), to give 2.60 g of 1,2,3,4-tetrahydroquinoline-2-carboxylic acid diethylamide as colorless needles in 87% yield. mp 50—53 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.08—1.35 (6H, m), 1.40—2.25 (2H, m), 2.74—2.95 (2H, m), 3.15—3.70 (6H, m), 4.09 (1H, dd, J=3.4, 11.5 Hz), 4.40 (1H, br s), 6.60—6.75 (2H, m), 6.90—7.10 (2H, m). GC-MS *m/z*: 232 (M^+).

2) A solution of 1,2,3,4-tetrahydroquinoline-2-carboxylic acid diethylamide (1.0 g, 4.3 mmol) in dry THF (10 ml) was treated with $LiAlH_4$ (250 mg, 6.5 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 1 h and then partitioned between brine (10 ml) and $CHCl_3$ (15 ml). The organic solution was dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified on a silica gel column ($CHCl_3$–MeOH, 50 : 1, v/v) to give 740 mg of **6** as a yellow oil in 79% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.01 (6H, t, J=7.8 Hz), 1.20—2.05 (2H, m), 2.20—2.90 (8H, m), 3.15—3.50 (1H, m), 4.62 (1H, br s), 6.40—6.62 (2H, m), 6.80—7.05 (2H, m). GC-MS *m/z*:218 (M^+).

(±)-11-{[2-(Diethylaminomethyl)-1,2,3,4-tetrahydro-2-quinolyl]acetyl}-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (7) A mixture of 11-(chloroacetyl)-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (**17**)[15] (600 mg, 2.1 mmol), **6** (500 mg, 2.3 mmol), sodium carbonate (245 mg, 2.3 mmol) and acetonitrile (20 ml) was refluxed for 5 h with stirring. After cooling, the mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was purified on a silica gel column ($CHCl_3$–MeOH–NH_4OH, 30 : 1 : 0.1, v/v/v), and the product was crystallized from 1-propanol to give 170 mg of **7** as colorless needles in 17% yield. mp 214—215 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.90—0.97 (6H, m), 1.70—1.90 (2H, m), 2.20—2.80 (7H, m), 3.90—4.55 (4H, m), 6.49—6.59 (2H, m), 6.90—7.04 (2H, m), 7.31—7.34 (1H, m), 7.38—7.52 (2H, m), 7.55—7.70 (2H, m), 7.64—7.80 (1H, m), 8.07—8.38 (1H, m). FAB-MS *m/z*: 470 (M^++1). *Anal.* Calcd for $C_{28}H_{31}N_5O_2 \cdot 0.7H_2O$: C, 69.74; H, 6.77; N, 14.52. Found: C, 69.53; H, 6.46; N, 14.50.

Isoquinoline-1-carboxylic Acid Diethylamide (9) The title compound was prepared in the same manner as described for **5** in 68% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.05 (3H, t, J=7.9 Hz), 1.39 (3H, t, J=7.9 Hz), 3.15 (2H, q, J=7.9 Hz), 3.73 (2H, q, J=7.9 Hz), 7.50—8.10 (5H, m), 8.51 (1H, d, J=6.7 Hz). GC-MS *m/z*:228 (M^+).

1-Diethylaminomethyl-1,2,3,4-tetrahydroisoquinoline (10)[18] 1) A solution of **9** (3.0 g, 13.1 mmol) in EtOH (120 ml) was acidified with conc. HCl (1.37 ml) , and was hydrogenated over PtO_2 (300 mg) at 3.0 kgf/cm^2 for 1.5 h. The reaction mixture was filtered through Celite®, solvent evaporated off, and the product crystallized from acetone to give 1.22 g of 1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid diethylamide hydrochloride as colorless needles in 40% yield. mp 220—223 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.18 (3H, t, J=7.9 Hz), 1.37 (3H, t, J=7.9 Hz), 3.00—3.85 (8H, m), 5.32—5.60 (1H, m), 6.95—7.50 (4H, m), 8.40 (1H, br s). GC-MS *m/z*: 232 (M^+).

2) A suspension of 1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid diethylamide hydrochloride (800 mg, 2.98 mmol) in THF (10 ml) was added to $BH_3 \cdot THF$ (20.8 ml, 17.9 mmol) and refluxed for 7 h. After cooling, conc. HCl (1.5 ml) was added and the mixture was refluxed for another 1 h. After cooling to room temperature, the solvent was evaporated *in vacuo*. The residue was diluted with H_2O (10 ml) and then poured into KOH aq. The mixture was extracted with $CHCl_3$ (20 ml×3), dried over $MgSO_4$ and evaporated. The residue was purified on a silica gel column ($CHCl_3$–MeOH–NH_4OH, 20 : 1 : 0.1, v/v/v) to give 310 mg of **10** as a yellow oil in 48% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.05 (6H, t, J=7.8 Hz), 2.30—3.25 (11H, m), 7.05—7.20 (4H, m). GC-MS *m/z*: 218 (M^+).

(±)-11-{[1-(Diethylaminomethyl)-1,2,3,4-tetrahydro-2-isoquinolyl]-acetyl}-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (11) The title compound was prepared in the same manner as described for **7** in 63% yield. mp 174—175 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, t, J=7.2 Hz), 0.95 (3H, t, J=7.2 Hz), 2.20—3.10 (10H, m), 3.63 (1H, d, J=15.2 Hz), 3.76 (1H, d, J=15.2 Hz), 3.90—4.20 (1H, m), 6.90—7.00 (1H, m), 7.00—7.15 (4H, m), 7.15—7.25 (1H, m), 7.40—7.45 (1H, m), 7.66—7.68 (2H, m), 7.88—7.92 (1H, m), 8.20—8.25 (1H, m). FAB-MS *m/z*: 470 (M^++1). *Anal.* Calcd for $C_{28}H_{31}N_5O_2$: C, 71.62; H, 6.65; N, 14.91. Found: C, 71.46; H, 6.69; N, 14.66.

3-Diethylaminomethyl-1,2,3,4-tetrahydroisoquinoline (14a) The title compound was prepared in the same manner as described for **10** in 55% yield from **13a**. ^{1}H-NMR ($CDCl_3$) δ: 1.03 (6H, t, J=7.8 Hz), 2.25—3.10 (9H, m), 4.07 (2H, s), 7.05—7.15 (4H, m). GC-MS *m/z*: 218 (M^+).

(2-Diethylaminomethyl)indoline (14b) The title compound was prepared in the same manner as described for **10** in 36% yield from **13b**. ^{1}H-NMR ($CDCl_3$) δ: 0.97 (6H, t, J=7.8 Hz), 2.30—2.80 (9H, m), 3.00—4.05 (2H, m), 6.55—6.75 (2H, m), 6.90—7.15 (2H, m). GC-MS *m/z*: 204 (M^+).

(±)-11-{[3-(Diethylaminomethyl)-1,2,3,4-tetrahydro-2-isoquinolyl]-acetyl}-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (15) The title compound was prepared in the same manner as described for **7** in 86% yield. mp 180—181 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.87—0.96 (6H, m), 1.90—2.25 (2H, m), 2.30—2.80 (7H, m), 3.30—4.20 (4H, m), 6.90—7.02 (2H, m), 7.07—7.11 (2H, m), 7.15—7.25 (2H, m), 7.35—7.45 (1H, m), 7.57—7.62 (2H, m), 7.80—7.90 (1H, m), 8.25—8.28 (1H, m). FAB-MS *m/z*:470 (M^++1). *Anal.* Calcd for $C_{28}H_{31}N_5O_2$: C, 71.62; H, 6.65; N, 14.91. Found: C, 71.38; H, 6.72; N, 14.61.

(±)-11-{[2-(Diethylaminomethyl)-1-indolinyl]acetyl}-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (16) The title compound was prepared in the same manner as described for **7** as colorless needles in 12% yield. mp 190—191 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.78—0.95 (6H, m), 2.30—2.65 (6H, m), 2.90—3.10 (1H, m), 3.60—4.60 (4H, m), 6.10—6.30 (1H, m), 6.47—6.55 (1H, m), 6.80—7.00 (2H, m), 7.30—7.80 (6H, m), 8.25—8.40 (1H, m). FAB-MS *m/z*: 456 (M^++1). *Anal.* Calcd for $C_{27}H_{29}N_5O_2 \cdot 0.1H_2O$: C, 70.91; H, 6.44; N, 15.31. Found: C, 70.80; H, 6.35; N, 15.31.

2-*tert*-Butyloxycarbonyl-5-hydroxy-1,2,3,4-tetrahydroisoquinoline (19) A suspension of **18** (2.0 g, 12.4 mmol), conc. HCl (1.5 ml) and platinum oxide (500 mg) in methanol (100 ml) was hydrogenated at 3.0 kgf/cm^2 for 3.5 h. The reaction mixture was filtered through Celite® and the solvent was evaporated to afford 2.42 g of 5-hydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride as a yellow solid in 99% yield. A mixture of this salt (2.40 g, 12.9 mmol), triethylamine (1.31 g, 12.9 mmol), $(Boc)_2O$ (3.0 g, 13.7 mmol) in 1,4-dioxane–H_2O (25 ml−25 ml) was stirred for 18 h at room temperature, then concentrated *in vacuo*. The residue was dissolved in $CHCl_3$ and washed with 5% citric acid and brine. The extract was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$–MeOH, 40 : 1, v/v) to give 2.60 g of **19** as an amorphous solid in 80% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.49 (9H, s), 2.76 (2H, t, J=7.3 Hz), 3.65 (2H, t, J=7.3 Hz), 4.55 (2H, s), 6.55—6.80 (2H, m), 6.92—7.15 (1H, m). FAB-MS *m/z*: 250 (M^++1).

5-[(7-Diethylamino)heptyloxy]-1,2,3,4-tetrahydroisoquinoline (20f) 1) A mixture of **19** (500 mg, 2.0 mmol), 1,7-dibromoheptane (5.2 g, 20 mmol), tetra-*n*-butylammonium hydrogensulfate (34 mg, 0.1 mmol) and 1 N NaOH aq. (6 ml) was heated at 60 °C for 2 h. After cooling, the mixture was extracted with $CHCl_3$ (10 ml×3) and then washed with brine. The extract was dried over $MgSO_4$ and evaporated *in vacuo* and the residue was purified by silica gel column chromatography (*n*-hexane–AcOEt, 10 : 1, v/v) to give 800 mg of *N*-*tert*-butyloxycarbonyl-5-(7-bromoheptyloxy)-1,2,3,4-tetrahydroisoquinoline as an oil in 94% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (9H, s),

1.50—2.00 (10H, m), 2.65—2.87 (2H, m), 3.41 (2H, t, J=6.8 Hz), 3.65 (2H, t, J=6.8 Hz), 3.95 (2H, t, J=6.8 Hz), 4.55 (2H, s), 6.60—6.80 (2H, m), 6.95—7.20 (1H, m). FAB-MS m/z: 426 (M^++1).

2) A mixture of *N-tert*-butyloxycarbonyl-5-(7-bromoheptyloxy)-1,2,3,4-tetrahydroisoquinoline (800 mg, 1.88 mmol) and diethylamine (300 mg, 4.14 mmol) in EtOH (20 ml) was refluxed for 3 h and solvent was evaporated *in vacuo*. The residue was dissolved in $CHCl_3$ (15 ml) and washed with brine. The extract was dried over $MgSO_4$ and evaporated *in vacuo*, and the residue purified by silica gel column chromatography (*n*-hexane–AcOEt, 10 : 1, v/v) to give 410 mg of *N-tert*-butyloxycarbonyl-5-[(7-diethylamino)-heptyloxy]-1,2,3,4-tetrahydroisoquinoline as an oil in 52% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.05—1.95 (25H, m), 2.65—3.20 (8H, m), 3.65 (2H, t, J=6.8 Hz), 3.95 (2H, t, J=6.8 Hz), 4.55 (2H, s), 6.60—6.85 (2H, m), 6.95—7.20 (1H, m). FAB-MS m/z: 419 (M^++1).

3) 4 N Hydrochloric acid in 1,4-dioxane (4 ml) was added to *N-tert*-butyloxycarbonyl-5-[(7-diethylamino)heptyloxy]-1,2,3,4-tetrahydroisoquinoline (640 mg, 1.53 mmol) in 1,4-dioxane (10 ml) at 5 °C and the mixture was stirred at room temperature for 1 h. The solvent was evaporated *in vacuo* and the resulting residue was dissolved in $CHCl_3$ (15 ml) and washed with 1 N NaOH and brine. The extract was dried over $MgSO_4$ and evaporated *in vacuo* to give 350 mg of **20f** as a yellow oil in 72% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (6H, t, J=7.8 Hz), 1.25—2.00 (11H, m), 2.30—2.85 (8H, m), 3.09 (2H, t, J=6.9 Hz), 3.80—4.05 (4H, m), 6.50—6.90 (2H, m), 6.95—7.15 (1H, m). FAB-MS m/z: 319 (M^++1).

5-[(2-Diethylamino)ethoxy]-1,2,3,4-tetrahydroisoquinoline (**20a**): The title compound was prepared in the same manner as described for **20f** as a yellow oil in 61% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.07 (6H, t, J=7.8 Hz), 1.67 (1H, br s), 2.53—2.77 (6H, m), 2.90 (2H, t, J=6.8 Hz), 3.06 (2H, t, J=6.8 Hz), 3.98 (2H, s), 4.04 (2H, t, J=6.8 Hz), 6.53—6.90 (2H, m), 7.00—7.20 (1H, m). GC-MS m/z: 248 (M^+).

5-[(3-Diethylamino)propoxy]-1,2,3,4-tetrahydroisoquinoline (**20b**): The title compound was prepared in the same manner as described for **20f** as a yellow oil in 45% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.03 (6H, t, J=7.8 Hz), 1.70—2.10 (3H, m), 2.40—2.80 (8H, m), 3.00—3.30 (2H, m), 3.93—4.10 (4H, m), 6.55—6.75 (2H, m), 7.00—7.20 (1H, m). GC-MS m/z: 262 (M^+).

5-[(4-Diethylamino)butyloxy]-1,2,3,4-tetrahydroisoquinoline (**20c**): The title compound was prepared in the same manner as described for **20f** as a yellow oil in 51% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (6H, t, J=7.8 Hz), 1.60—2.00 (4H, m), 2.30 (1H, br s), 2.42—2.80 (8H, m), 3.10 (2H, t, J=6.9 Hz), 3.80—4.10 (4H, m), 6.55—6.70 (2H, m), 6.95—7.20 (1H, m). FAB-MS m/z: 277 (M^++1).

5-[(5-Diethylamino)pentyloxy]-1,2,3,4-tetrahydroisoquinoline (**20d**): The title compound was prepared in the same manner as described for **20f** as a yellow oil in 44% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.01 (6H, t, J=7.8 Hz), 1.35—2.00 (7H, m), 2.30—2.80 (8H, m), 3.08 (2H, t, J=6.4 Hz), 3.80—4.05 (4H, m), 6.50—6.90 (2H, m), 6.90—7.15 (1H, m). FAB-MS m/z: 291 (M^++1).

5-[(6-Diethylamino)hexyloxy]-1,2,3,4-tetrahydroisoquinoline (**20e**): The title compound was prepared in the same manner as described for **20f** as a yellow oil in 37% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.03 (6H, t, J=7.8 Hz), 1.30—1.95 (9H, m), 2.35—2.85 (8H, m), 3.10 (2H, t, J=6.9 Hz), 3.80—4.05 (4H, m), 6.55—6.95 (2H, m), 6.90—7.15 (1H, m). FAB-MS m/z: 305 (M^++1).

11-({5-[(2-Diethylamino)ethoxy]-1,2,3,4-tetrahydro-2-isoquinolyl}-acetyl)-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (3a) The title compound was prepared in the same manner as described for **7** as an amorphous solid in 64% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.09 (6H, t, J=7.2 Hz), 2.20—2.70 (8H, m), 2.83—2.94 (2H, m), 3.15—3.20 (1H, m), 3.27 (1H, d, J=14.8 Hz), 3.56 (1H, d, J=14.8 Hz), 3.80—3.90 (1H, m), 3.95—4.03 (2H, m), 6.51 (1H, d, J=7.6 Hz), 6.62 (1H, d, J=7.6 Hz), 7.05 (1H, d, J=7.6 Hz), 7.10—7.20 (2H, m), 7.39—7.44 (1H, m), 7.59—7.65 (2H, m), 7.91 (1H, d, J=7.6 Hz), 8.20—8.25 (1H, m). FAB-MS m/z: 500 (M^++1). *Anal.* Calcd for $C_{29}H_{33}N_5O_3 \cdot 0.5H_2O$: C, 68.48; H, 6.74; N, 13.77. Found: C, 68.66; H, 6.69; N, 13.78.

11-({5-[(3-Diethylamino)propoxy]-1,2,3,4-tetrahydro-2-isoquinolyl}-acetyl)-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (**3b**): The title compound was prepared in the same manner as described for **7** as an amorphous solid in 66% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (6H, t, J=7.2 Hz), 1.85—1.92 (2H, m), 2.45—2.64 (10H, m), 3.30—3.40 (1H, m), 3.38 (1H, t, J=14.8 Hz), 3.62 (1H, d, J=14.8 Hz), 3.65—3.72 (1H, m), 3.90—3.97 (2H, m), 6.51 (1H, d, J=8.0 Hz), 6.56 (1H, d, J=8.0 Hz), 6.96 (1H, d, J=8.0 Hz), 7.20—7.23 (1H, m), 7.32—7.45 (2H, m), 7.60—7.67 (2H, m), 7.89 (1H, d, J=7.6 Hz), 8.25—8.28 (1H, m). FAB-MS m/z: 514 (M^++1). *Anal.* Calcd for $C_{30}H_{35}N_5O_3 \cdot 0.5H_2O$: C, 68.94; H, 6.94; N, 13.40. Found: C, 68.84; H, 6.77; N, 13.45.

11-({5-[(4-Diethylamino)butoxy]-1,2,3,4-tetrahydro-2-isoquinolyl}-acetyl)-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (**3c**): The title compound was prepared in the same manner as described for **7** as colorless needles in 83% yield. mp 128—129 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.10 (6H, t, J=7.2 Hz), 1.60—1.80 (4H, m), 2.40—2.70 (10H, m), 3.30—3.40 (1H, m), 3.40 (1H, t, J=13.6 Hz), 3.60 (1H, d, J=14.8 Hz), 3.85—3.92 (2H, m), 6.50 (1H, d, J=8.0 Hz), 6.53 (1H, d, J=8.0 Hz), 6.95 (1H, d, J=8.0 Hz), 7.18—7.22 (1H, m), 7.35—7.50 (2H, m), 7.59—7.66 (2H, m), 7.88 (1H, d, J=7.6 Hz), 8.23—8.26 (1H, m). FAB-MS m/z: 528 (M^++1). *Anal.* Calcd for $C_{31}H_{37}N_5O_3 \cdot 0.4H_2O$: C, 69.61; H, 7.12; N, 13.09. Found: C, 69.55; H, 6.93; N, 13.18.

11-({5-[(5-Diethylamino)pentyloxy]-1,2,3,4-tetrahydro-2-isoquinolyl}-acetyl)-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (**3d**): The title compound was prepared in the same manner as described for **7** as an amorphous solid in 70% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.23 (6H, t, J=7.2 Hz), 1.40—1.58 (2H, m), 1.70—1.95 (4H, m), 2.40—3.00 (10H, m), 3.30—3.40 (1H, m), 3.37 (1H, t, J=14.8 Hz), 3.54 (1H, d, J=14.8 Hz), 3.65—3.80 (1H, m), 3.85—3.95 (2H, m), 6.48 (1H, d, J=7.6 Hz), 6.53 (1H, d, J=7.6 Hz), 6.96 (1H, d, J=7.6 Hz), 7.15—7.19 (1H, m), 7.36—7.40 (1H, m), 7.58—7.65 (3H, m), 7.87 (1H, d, J=8.0 Hz), 8.22—8.24 (1H, m). HR-MS (FAB) Found m/z: 542.3136. $C_{32}H_{40}N_5O_3$ Calcd m/z: 542.7000.

11-({5-[(6-Diethylamino)hexyloxy]-1,2,3,4-tetrahydro-2-isoquinolyl}-acetyl)-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (**3e**): The title compound was prepared in the same manner as described for **7** as colorless needles in 94% yield. mp 104—106 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.05 (6H, t, J=7.6 Hz), 1.30—1.38 (2H, m), 1.38—1.58 (4H, m), 1.65—1.75 (2H, m), 2.30—2.70 (10H, m), 3.35—3.50 (2H, m), 3.60—3.75 (2H, m), 3.80—3.90 (2H, m), 6.50—6.54 (2H, m), 6.95 (2H, t, J=8.0 Hz), 7.20—7.24 (1H, m), 7.35—7.50 (2H, m), 7.60—7.70 (2H, m), 7.89 (1H, d, J=7.2 Hz), 8.26—8.28 (1H, m). FAB-MS m/z: 556 (M^++1). *Anal.* Calcd for $C_{33}H_{41}N_5O_3$: C, 71.32; H, 7.44; N, 12.60. Found: C, 71.22; H, 7.44; N, 12.62.

11-({5-[(7-Diethylamino)heptyloxy]-1,2,3,4-tetrahydro-2-isoquinolyl}-acetyl)-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (**3f**): The title compound was prepared in the same manner as described for **7** as colorless needles in 73% yield. mp 133—134 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.03 (6H, t, J=7.2 Hz), 1.30—1.50 (6H, m), 1.55—1.75 (4H, m), 2.32—2.82 (8H, m), 3.25—3.40 (2H, m), 3.30—3.35 (2H, m), 3.39 (1H, d, J=14.1 Hz), 3.61 (1H, d, J=14.1 Hz), 3.65—3.75 (1H, m), 3.80—3.90 (2H, m), 6.54 (1H, d, J=7.5 Hz), 6.62 (1H, d, J=7.5 Hz), 7.08 (2H, t, J=7.5 Hz), 7.20—7.22 (1H, m), 7.38—7.45 (2H, m), 7.60—7.67 (2H, m), 7.89 (1H, d, J=7.5 Hz), 8.25—8.26 (1H, m). FAB-MS m/z: 570 (M^++1). *Anal.* Calcd for $C_{34}H_{43}N_5O_3 \cdot 0.5H_2O$: C, 68.48; H, 6.74; N, 13.77. Found: C, 68.66; H, 6.69; N, 13.78.

Biological Methods The following chemicals were obtained commercially: oxotremorine (Sigma, U.S.A.), atropine sulfate (Tanabe, Japan), [^{3}H]PZ, [^{3}H]QNB and [^{3}H]NMS (Du Pont-New England Nuclear, U.K.).

Receptor Binding Assay Male Wistar rats (350—400 g) were decapitated, and the cerebral cortex, heart and submandibular gland removed and homogenized in ice-cold HEPES buffer (20 mM HEPES, 100 mM NaCl, 10 mM $MgCl_2$; pH 7.5). The homogenates were filtered through two layers of cloth gauze and centrifuged at 50000×***g*** for 10 min. The pellets thus obtained were washed twice in HEPES buffer by resuspension and recentrifugation. The resulting pellets were resuspended in HEPES buffer to give final protein concentrations of approximately 0.47 mg/ml (cerebral cortex), 1.0 mg/ml (heart) and 0.83 mg/ml (submandibular gland) as determined by the method of Bradford.[19] Membrane suspensions were stored at −80 °C until required.

The membrane suspensions (volume of 150 ml) were incubated with approximately 1.0 nM [^{3}H]PZ (K_D=9.30±0.28 nM) for cerebral cortex , 0.1 M [^{3}H]QNB (K_D=0.128±0.004 nM) for heart and 0.3 nM [^{3}H]NMS (K_D=0.162±0.006 nM) for submandibular gland at 25 °C for 45 min. In the displacement studies, the inhibition of specific binding was examined in the presence of nonlabeled drugs in a total volume of 0.5 ml of HEPES buffer. Nonspecific binding was determined using 10 μM atropine. Assays were terminated by rapid filtration under vacuum through a Whatman GF/B filter. The filters were washed immediately three times with approximately 3 ml portions of ice-cold HEPES buffer, then solubilized in 5 ml of Scintillation cocktail (Aquasol-2; Packard) and counted for radioactivity using a Packard TR1-CARB 2200 CA liquid scintillation counter. Competition binding data were analyzed with a nonlinear least-squares program, "GraphPad PRISM ver.1.0" (GraphPad Software) to obtain the IC_{50} values. The IC_{50} values were corrected for receptor occupancy by [^{3}H]PZ, [^{3}H]QNB and [^{3}H]NMS, as described by Cheng and Prusoff[20] to give Ki values (concentrations of nonlabeled ligand that cause half-maximal receptor occupancy in the absence of [^{3}H]PZ, [^{3}H]QNB and [^{3}H]NMS, respectively).

Heart Rate Male Wistar rats (300—350 g) were anesthetized with pentobarbital (60 mg/kg i.p.). A tracheal cannula was inserted to allow artificial respiration with room air. A jugular vein was cannulated for i.v. administration of drugs. Rats were pithed by the introduction of a blunt steel rod *via* the orbit into the spinal canal and were pretreated with atenolol (10 mg/kg i.v.) to exclude catecholamine-induced tachycardia. The test compound or saline was administered i.v.. At 15 min thereafter, a cumulative administration of oxotremorine was carried out. Log dose–response curves were constructed by plotting the decrease in heart rate (percentage of the initial value) *vs*. the logarithm of the dose (moles per kilogram). The ED_{50} values, doses of oxotremorine required to produce a 50% decrease in heart rate, were calculated from the log dose–response curves, and the dose-ratio was calculated. The antagonism for M_2 muscarinic receptors was expressed as the pDR_{10} value, the negative logarithm of the DR_{10} value, which is the dose of the test compound required to produce the oxotremorine dose-ratio of 10. In the case of compound **3f**, the maximum decrease in heart rate of oxotremorine was about 60%. Therefore, the dose-ratio was calculated from ED_{30} values, *i.e*., the doses of oxotremorine required to produce a 30% decrease in heart rate.

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