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Synthesis of Novel Succinamide Derivatives Having a 5,11-Dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one Skeleton as Potent and Selective M_2 Muscarinic Receptor Antagonists. II[1)]

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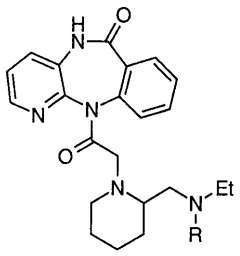
A series of succinamide derivatives containing the 5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one skeleton (6a—z) was prepared and evaluated for binding affinity to muscarinic receptors *in vitro* and for antagonism of bradycardia and salivation *in vivo* in comparison with AF-DX 116 (1a). Structure-activity relationships (SAR) studies *in vitro* indicated that the 4-(4-alkyl-1-piperazinyl)benzylamino moiety plays a crucial role in enhancing the affinity for M_2 muscarinic receptors. Compound 6y, containing a 4-(4-isopropyl-1-piperazinyl)benzylmethylamino moiety, exhibited the highest affinity for M_2 muscarinic receptors ($pK_i = 9.2$), being 200 times as potent as 1a, and compound 6u, containing a 4-(4-ethyl-1-piperazinyl)benzylethylamino moiety, showed the highest selectivity for M_2 over M_3 muscarinic receptors (M_3/M_2 ratio = 320). Both 6y and 6u antagonized the oxotremorine-induced bradycardia in rats after intravenous or oral administration. Oral evaluation in conscious dogs showed that the efficacy for increasing the heart rate was at least 3-fold greater than that of 1a.

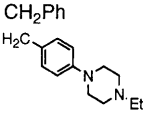
Key words succinamide derivative; M_2 muscarinic receptor; antagonism; M_2 selectivity; bradycardia

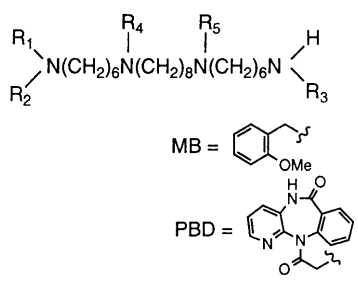
The neurotransmitter acetylcholine interacts with two different types of receptors, nicotinic and muscarinic receptors. The muscarinic receptor family is one of the G-protein coupled receptors (GPCRs), and molecular-biological studies have demonstrated that muscarinic receptors comprise at least five subtypes designated m_1—m_5. The m_1, m_3 and m_5 receptors are coupled to phosphatidylinositol (PI) turnover, whereas the m_2 and m_4 receptors show inhibitory coupling to adenylate cyclase. To date, the m_1—m_4 receptors have been pharmacologically correlated to the M_1—M_4 muscarinic receptors, respectively,[2)] and this pharmacological subclassification was made possible by the discovery of the corresponding selective antagonists, pirenzepine,[3,4)] AF-DX 116,[5,6)] 4-diphenyl-acetoxy-*N*-methylpiperidine methiodide (4-DAMP[7)]) and tropicamide.[8)]

We consider that the M_2 subtype has potential for the purpose of developing drugs for cardiac disorders. M_2 muscarinic receptors are abundant in peripheral effector organs, *e.g.*, heart and smooth muscle, and are also found in the central nervous system.[9—11)] In the heart, an excessive stimulation of M_2 muscarinic receptors, that is, an increase in parasympathetic tone, is thought to be a major factor of sick sinus syndrome and atrioventricular block, and this implies that M_2 muscarinic antagonists are promising candidates for antibradycardiac agents. Currently, atropine, a non-specific muscarinic receptor antagonist, is used to treat bradycardiac disease. However its use is limited by the short duration of action and the occurrence of undesirable side effects, such as dry mouth, mydriasis and gastrointestinal and urinary events caused by antagonism of other muscarinic receptor subtypes. This is the reason why a new, potent and selective M_2 muscarinic receptor antagonist is desired for the treatment of bradycardiac patients.

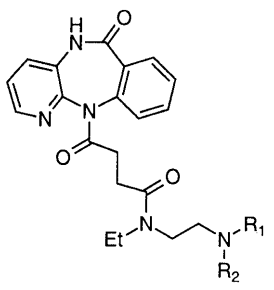
Engel *et al.* reported AF-DX 116 (11-[[2-(diethylaminomethyl)-1-piperidyl]acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one) (Otenzepad) (**1a**) as a selective M_2 muscarinic receptor antagonist in 1984 (Fig. 1).[5,6)] This compound was found by modification of pirenzepine, by moving the most basic nitrogen of the piperazine ring to a location attached to the piperidine ring *via* a methylene bridge. On the other hand, Melchiorre *et al.* presented polymethylenetetraamines such as



1a : R = Et (AF-DX 116)
1b : R = CH_2Ph
1c : R = 



2a : R_1=R_4=R_5=H, R_2=R_3=MB (methoctramine)
2b : R_1=R_2=R_3=PBD, R_4=R_5=Me (tripitramine)



3a : R_1=R_2=Et
3b : R_1=Et, R_2=CH_2Ph

Fig. 1

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methoctramine (**2a**)[12,13] and tripitramine (**2b**)[14] as pharmacological tools possessing high selectivity for M_2 muscarinic receptors. A terminal aromatic component and a nitrogen atom at an appropriate distance from the terminal aromatic component are significant for the affinity and selectivity of these compounds for M_2 muscarinic receptors.

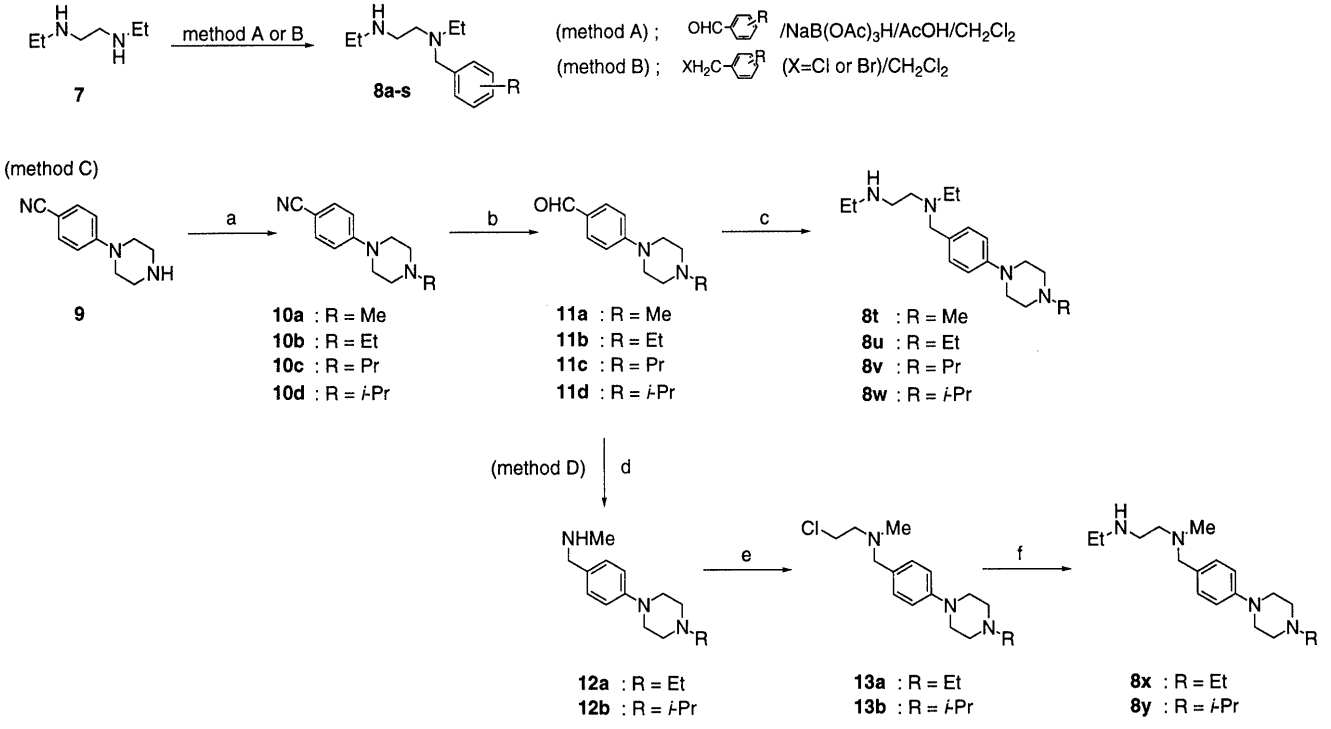
We reported succinamide derivatives containing structural features similar to those of **1a** and **2b**, such as **3b**, as novel selective M_2 muscarinic receptor antagonists having stronger anti-bradycardiac activity in *in vitro* and *in vivo* than **1a**.[1] Earlier structure–activity relationship (SAR) studies of succinamide derivatives indicated that the substituent on the nitrogen atom of the amide junction (the ethyl group in **3a** and **3b**) and the arylmethyl moiety of the amino side-chain (the benzyl group in **3b**) are essential pharmacophores for M_2 muscarinic receptors. The implication that the terminal benzylamino moiety plays a crucial role in enhancing the affinity and selectivity for M_2 muscarinic receptors prompted us to introduce several substituents into the phenyl ring. As a result, compounds **6u** (YM-43571) and **6y** (YM-47244), which contained a 4-(4-alkyl-1-piperazinyl)benzylamino moiety, were found to be superior in both *in vitro* and *in vivo* assays to **1a** and **3a, b**. In this paper, we describe the results of our work on the synthesis, biological activities and SAR of the succinamide series.

Chemistry

Synthetic routes to the intermediate diamines **8a—y** are shown in Chart 1. The diamines **8a—s** were prepared by the reductive amination of *N,N'*-diethylethylenediamine **7** with a substituted benzaldehyde in the presence of sodium triacetoxyborohydride ($NaB(OAc)_3H$),[15] a very mild and easy-to-handle reagent, and acetic acid (method A) or by the reaction between *N,N'*-diethylethylenediamine and benzyl halide (method B). The diamines containing the 4-alkyl-1-phenylpiperazinyl group **8t—y** were synthesized *via* methods C and D. 1-(4-Cyanophenyl)piperazine **9**[16] was subjected to reductive alkylation with alkylaldehyde in the presence of $NaB(OAc)_3H$ and acetic acid, and the resulting **10a—d**[17] were reduced with diisobutylaluminum hydride (DIBAH) to afford the benzaldehyde derivatives **11a—d**.[18] Reductive amination of **11a—d** using *N,N'*-diethylethylenediamine **7** gave the expected secondary amines **8t—w**. Compounds **12a** and **12b**, prepared from **11a** and **11d** according to method A, were subjected to reductive alkylation with chloroacetaldehyde to yield compounds **13a, b**. The resulting **13a, b** were heated with ethylamine to obtain the respective diamines **8x, y**.

Compounds **6a—z** were synthesized *via* the routes illustrated in Chart 2. The condensation reaction between a carboxylic acid **5** obtained by hydrolysis of the ester derivative **4**[1] and the diamines **8a—y** in the presence of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (WSCD) and 1-hydroxybenzotriazole (HOBT) afforded compounds **6a—y**. Compound **16**, prepared from the *N*-protected piperazinylbenzaldehyde **14** and the diamine **7**, was condensed with **5**, followed by deprotection of the *tert*-butyloxycarbonyl group to afford **6z**. Physical data for compounds **6a—z** are given in Table 5.

NMR measurements demonstrated that compounds **6a—z** exist as mixtures of rotamers about the amide bond in dimethyl sulfoxide (DMSO)-d_6. The free energy of activation ($\Delta G^{\neq}$) of these compounds is low enough to allow free rotation of the two rotamers at room tem-



reagents: (a)$R_1R_2CO/NaB(OAc)_3H/AcOH/CH_2Cl_2$; (b)DIBAH/THF; (c)**7**/$NaB(OAc)_3H/AcOH/CH_2Cl_2$; (d)$MeNH_2/NaB(OAc)_3H/AcOH/CH_2Cl_2$; (e)$CHOCH_2Cl/NaB(OAc)_3H/AcOH/CH_2Cl_2$; (f)$EtNH_2$/EtOH.

Chart 1. Preparation of the Diamines **8a—y**

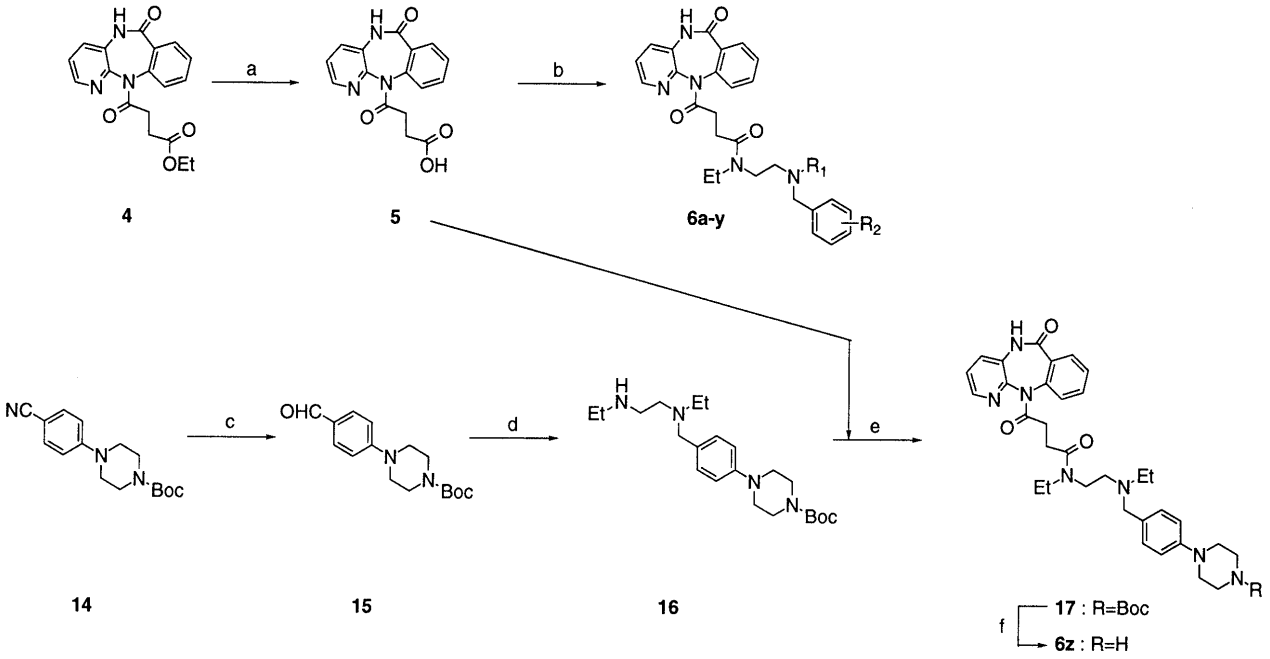


Chart 2. Preparation of Compounds **6a—z**

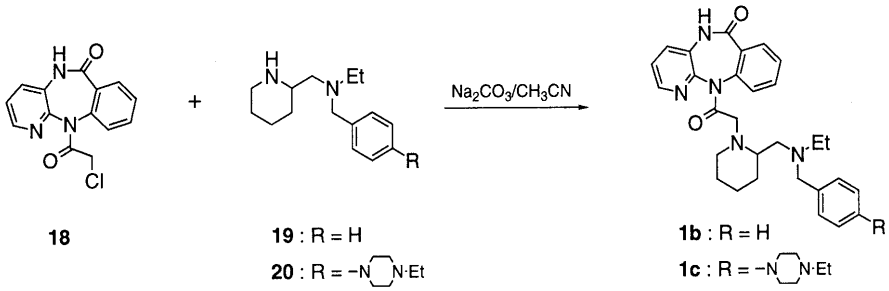


Chart 3. Preparation of Compounds **1b** and **1c**

perature (25 °C).[1)]

The AF-DX 116 derivatives **1b** and **1c** were prepared according to the method reported by Engel *et al.*[5)] Namely, compound **18** was reacted with *N*-ethylbenzylamine **19** or *N*-ethyl-4-(4-ethyl-1-piperazinyl)benzylamine **20** to obtain **1b** and **1c**, respectively (Chart 3).

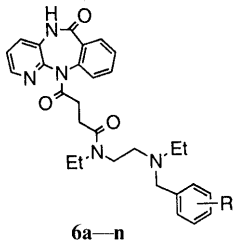
Pharmacological Results and Discussion

***In Vitro* Tests** The muscarinic receptor selectivity was assessed by employing receptor binding assays as previously described.[19)] The binding affinities for the compounds were obtained by using the 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*Ki* values, and the selectivity ratios for M_2 muscarinic receptors over 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 (**1a**) was used as the reference compound.

Initially, we investigated the electronic effect of a substituent by introduction of a methoxy or a chloro group. The comparison of **6a—f** demonstrated that the methoxy group at the *para* position of the phenyl ring (**6c**) gave the best result in terms of both the affinity and selectivity for M_2 muscarinic receptors. On the other hand, compounds that possessed other electron-withdrawing groups, such as bromo, nitro, trifluoromethyl and methoxycarbonyl, at the *para* position exhibited similar affinity for M_2 muscarinic receptors to **6f** (data not shown). These results prompted us to introduce several electron-donating groups at this position. Replacement of the methoxy group of **6c** by alkyl (**6g—i**), thiomethyl (**6j**), hydroxy (**6k**) and propoxy (**6l**) groups retained the affinity for M_2 muscarinic receptors.

Replacement of the methoxy group of **6c** by dialkylamino groups produced extremely interesting results. Although the affinity and selectivity for M_2 muscarinic receptors of compound **6n**, bearing a dimethylamino group, were equal to those of **6c**, compound **6p** containing the structurally hindered piperidine showed the same affinity for M_2 muscarinic receptors as **6c** ($pK_i = 8.3$) and an outstanding selectivity, especially over M_3 muscarinic receptors ($M_3/M_2 = 160$). A five- or seven-membered analog (**6o**, **6q**) displayed slightly less affinity and selectivity than **6p**. The morpholine analog **6r** was equipotent to **6p**, whereas the piperazine analog **6z** was found to be less selective for M_1 and M_3 muscarinic receptors with an

Table 1. The Binding Affinities of **6a**—**n** to M_1, M_2 and M_3 Muscarinic Receptors



6a—**n**

Compd. No.	R	Position	Yield[a] (%)	pK_i[b]			Selectivity ratio	
				M_1	M_2	M_3	M_1/M_2	M_3/M_2
1a				6.1	6.9	5.7	6.3	16
1b				6.3	6.9	6.2	4.0	5.0
1c				7.2	7.3	6.3	1.3	10
3a				6.7	7.6	6.5	7.9	13
3b	H			7.0	8.2	6.5	16	50
6a	OMe	2	60	6.8	7.7	6.5	7.9	16
6b	OMe	3	58	6.7	7.6	6.4	7.9	16
6c	OMe	4	74	7.1	8.3	6.5	16	63
6d	Cl	2	72	6.9	7.9	6.6	10	20
6e	Cl	3	81	6.9	7.7	6.6	6.3	13
6f	Cl	4	84	7.4	7.8	6.9	2.5	7.9
6g	Me	4	71	7.1	8.2	6.7	13	32
6h	Et	4	65	6.9	8.5	7.0	40	32
6i	iso-Pr	4	63	7.1	8.4	6.9	20	32
6j	SMe	4	60	7.0	8.1	6.8	13	20
6k	OH	4	40	7.2	8.4	6.6	16	63
6l	OPr	4	43	6.9	8.2	6.4	20	63
6m	$O(CH_2)_3NEt_2$	4	47	8.3	8.9	7.0	5.0	79
6n	NMe_2	4	65	6.8	8.2	6.5	25	50

a) Yield of condensation reaction between **5** and **8a**—**n**. *b*) pK_i values each represent an average of two or more determinations from separate assays.

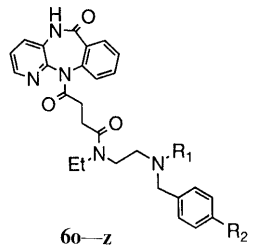
equal affinity for M_2 muscarinic receptors compared to **6p**. Surprisingly, the introduction of small alkyl groups into the piperazine nitrogen of **6z** enhanced the affinity for M_2 muscarinic receptors, with only a small change in affinity for M_1 and M_3 muscarinic receptors, consequently the M_1/M_2 and M_3/M_2 selectivities were improved (**6t**—**w** *vs.* **6z**). Additionally, the 4-methylpiperidine analog **6s** showed less affinity for M_1 and M_2 muscarinic receptors than the corresponding compound **6t**. These results suggested that the nitrogen atom at the 4-position of piperazine plays an important role in receptor affinity, especially for M_1 and M_2 subtypes, and a small alkyl moiety, such as methyl, ethyl or isopropyl, helps to enhance the binding affinity between the antagonist and M_2 muscarinic receptors. This finding might imply that the nitrogen atom of benzylamine and the additional nitrogen atom on the 4-position of piperazine in **6t**—**z**, which are protonated in the binding site, interact with different anionic groups located on M_2 or M_1 muscarinic receptors.[10]

The comparison between **6l** and **6m** also supports our speculation that two nitrogen atoms are important for the receptor–antagonist binding. Namely, the introduction of the second nitrogen atom by the addition of diethylamine to the *n*-propyl moiety of **6l** resulted in a considerable enhancement of the binding affinity, as we had expected. In addition, the fact that **6u** and **6m** exhibit a similar affinity for the M_2 muscarinic receptors, but the M_1/M_2 and M_3/M_2 ratios of the former are 4-fold more than those of the latter, further demonstrate that the bulky piperazine moiety participates in the appearance of the selectivity for M_2 muscarinic receptors.

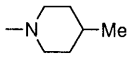
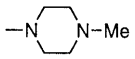
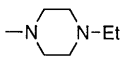
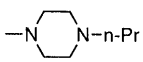
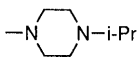
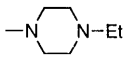
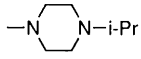
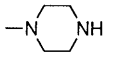
We also investigated the pharmaceutical properties of AF-DX 116 type compounds **1b** and **1c**, in which the diethylamine of **1a** is replaced with *N*-ethylbenzylamine and *N*-ethyl-4-(4-ethyl-1-piperazinyl)benzylamine, respectively (Table 1). A comparison of the activities of **3a**, **3b** and **6u** indicates that the introduction of a phenyl ring into **1a** did not influence the binding affinity for M_2 muscarinic receptors. Additionally, there was no significant difference in the selectivity for M_2 muscarinic receptors over M_3 muscarinic receptors between **1a** and **1c**, while **6u** had a 25-fold higher M_3/M_2 value than **3a**. These findings show that the 4-(4-ethyl-1-piperazinyl)-benzylamino moiety is not as important in AF-DX 116 derivatives as in the succinamide-type antagonists in terms of the affinity and selectivity for M_2 muscarinic receptors. In addition, viewed in the structural light, **1c** is more rigid than **6u** due to the piperidine ring. Based on these differences of the SAR and structural features, we speculate that the benzylamine nitrogen atoms of **1c** and **6u** may recognize different receptor regions and therefore the 4-(4-ethyl-1-piperazinyl)benzylamino moiety of **1c** does not play a crucial role in the receptor–antagonist binding.

Compounds **6x** and **6y**, in which the ethyl group of **6u** and **6v** is replaced with a methyl group, gave the same results in terms of the increase in affinity for all subtypes

Table 2. The Binding Affinities **6o**—**z** to M_1, M_2 and M_3 Muscarinic Receptors



6o—**z**

Compd. No.	R_1	R_2	Yield[a] (%)	pK_i[b]			Selectivity ratio	
				M_1	M_2	M_3	M_1/M_2	M_3/M_2
1a				6.1	6.9	5.7	6.3	16
3b	Et	H		7.0	8.2	6.5	16	50
6o	Et		58	7.3	8.2	6.4	7.9	63
6p	Et		70	7.0	8.3	6.1	20	160
6q	Et		40	6.7	8.0	6.3	20	50
6r	Et		67	6.9	8.3	6.2	25	130
6s	Et		70	6.9	8.3	6.5	25	63
6t	Et		44	7.4	8.7	6.4	20	200
6u	Et		67	7.5	8.8	6.3	20	320
6v	Et		58	7.6	8.9	6.6	20	200
6w	Et		75	7.8	9.1	6.8	20	200
6x	Me		56	8.0	9.0	7.1	10	79
6y	Me		58	8.2	9.2	7.3	10	79
6z	Et		18[c]	7.5	8.4	6.6	7.9	63

a) Yield of condensation reaction between **5** and **8o**—**y**. *b*) pK_i values each represent an average of two or more determinations from separate assays. *c*) Overall yield from **16**.

and the decrease in selectivity for the M_2 subtype, in accord with our previous findings.[1] As a result, we found that compound **6y** (YM-47244) was the strongest M_2 muscarinic receptor antagonist and compound **6u** (YM-43571) showed the highest selectivity for M_2 muscarinic receptors in this series.

***In Vivo* Tests** Among the succinamide analogs, **6u** and **6y** were evaluated *in vivo*. From the view point of side effects, we have to pay attention to the M_3 receptor antagonistic activities, because dry mouth or mydriasis caused by antagonism of the M_3 muscarinic receptors is the main problem in the administration of atropine. We first studied the oxotremorine-induced bradycardia in pithed rats and the oxotremorine-induced salivation in urethane-anesthetized rats to assess of the M_2 and M_3 muscarinic receptor antagonistic activities in comparison with those of **1a** and atropine, respectively. Test compounds were given by intravenous (i.v.) or oral (*p.o.*) administration, and the data are presented as pDR_{10} values against bradycardia and pID_{50} values against salivation as described in the experimental section. Compounds **6u** and **6y** behaved as noncompetitive-like antagonists in this oxotremorine-induced bradycardia model; the agonist dose–response curves were displaced to the right with a decrease in the maximum response of about 60%, and the pDR_{10} values were calculated from the ED_{30} values.[1] This behavior is different from that of **1a** as a competitive antagonist.[20] In Table 3, the M_2 and M_3 muscarinic receptor antagonistic activities and M_2-selectivity for the four compounds are given. The selectivity ratio (M_3/M_2) in i.v. experiments was calculated according to the following equation using the potencies of the compounds relative to those of atropine (selectivity ratio = 1).

Table 3. Muscarinic Receptor Antagonistic Activities and Selectivity Ratios of **6u**, **6y**, **1a** and Atropine in *in Vivo* Experiments in Rats

Compd.	Inhibitory effects in oxotremorine-induced bradycarida (M_2)				Inhibitory effects in oxotremorine-induced salivation (M_3)		Selectivity ratio (M_3/M_2)
	i.v.		*p.o.*		i.v.		
	pDR_{10}[a]	*n*	pDR_{10}[a]	*n*	pID_{50}[a]	*n*	
6u	7.34[b] (7.26—7.42)	8	5.52[b] (5.35—5.69)	12	4.62 (4.50—4.73)	8	1047
6y	7.67[b] (7.51—7.89)	8	5.79[b] (5.71—5.86)	5	5.32 (5.25—5.38)	9	447
1a	5.63 (5.56—5.70)	32	4.90 (3.68—6.02)	36	4.60 (4.52—4.69)	24	21
Atropine	6.94 (6.88—7.01)	21	NT[c]		7.24 (7.21—7.28)	14	1

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. See the experimental section. *c*) NT: Not tested.

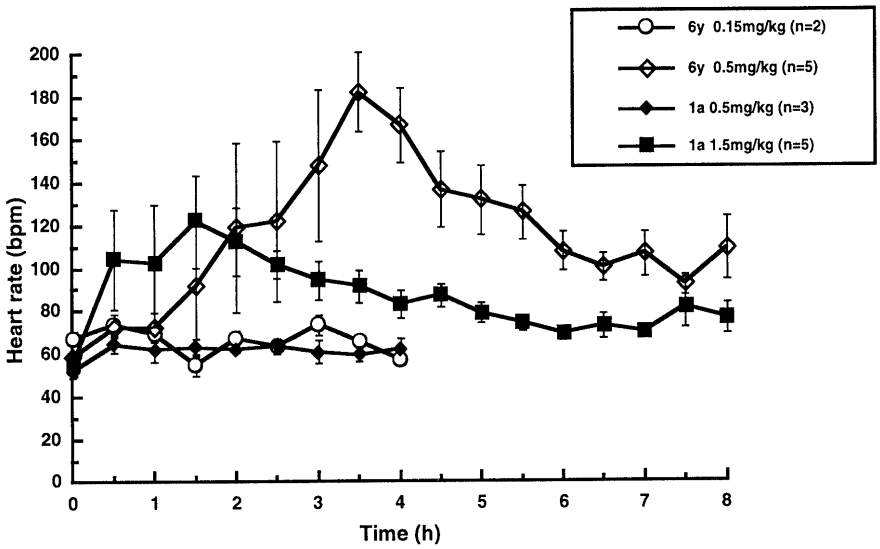


Fig. 2. Increase of the Heart Rate by **6y** and **1a** in Conscious Dogs

$$M_3/M_2=\left[\frac{ID_{50}(\text{compound})}{ID_{50}(\text{atropine})}\right]\Big/\left[\frac{DR_{10}(\text{compound})}{DR_{10}(\text{atropine})}\right]$$

In the i.v. experiments, the oxotremorine response in heart rate was antagonized by **6u** and **6y**, providing pDR_{10} values of 7.34 and 7.67, respectively. These activities were about 50- to 110- and 2.5- to 5-fold more potent than those of **1a** and atropine, respectively. In addition, **6u** and **6y** showed about 80—400 times weaker M_3 muscarinic receptor antagonism than atropine, and their activities were equipotent to that of **1a**. Thus, **6u** and **6y** displayed very much higher selectivity for M_2 muscarinic receptors (M_3/M_2 ratio of 1047 and 447, respectively) than **1a** and atropine. These results suggest that **6u** and **6y** might have superior activities and selectivities for M_2 muscarinic receptors not only *in vitro*, but also *in vivo*.

We performed a further study with **6u** and **6y** to evaluate the oral activity in the oxotremorine-induced bradycardia model, and the results are shown in Table 3. In the preliminary study using **6u** and **1a**, the maximal increase in the heart rate was observed at 180 min after oral administration. Compounds **6u** and **6y** had about 4 to 8 times greater inhibitory activity on the oxotremorine-induced bradycardia as compared with **1a**. However, these oral activities were weaker than expected, and metabolism studies indicated that this might be due to poor bioavailability and metabolism.

Next, the increasing effect on heart rate of **6y** and **1a** in conscious unrestrained dogs was assessed. Oral administration of **6y** at doses of 0.15 and 0.5 mg/kg was compared with doses of 0.5 and 1.5 mg/kg of **1a**. Measurements were made during the night when the muscarinic receptors were activated. These results are shown in Fig. 2. The control heart rate was between 50 and 70 beats per minute and 0.5 mg/kg of **6y** produced a smooth responce, giving a maximal increase of 130 beats per minute at 3.5 h after administration. On the other hand, the administration of **1a** at a dose of 1.5 mg/kg produced an increase of 70 beats per minute as a maximal effect and this response was rapid. Neither 0.15 mg/kg of **6y** nor 0.5 mg/kg of **1a** produced any effects. These results suggest that the oral activity of **6y** is at least 3 times higher than that of **1a** in conscious dogs, and there was no significant difference between the rat and dog models.

Conclusions

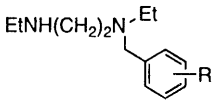
A series of succinamide derivatives was synthesized and evaluated in *in vitro* and *in vivo* experiments. Extensive

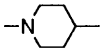
probing of the SAR in the *in vitro* assay resulted in the discovery of **6u** and **6y**, containing the 4-(4-alkyl-1-piperazinyl)benzylamino moiety. The former showed the highest M_2 selectivity and the latter had the highest M_2 affinity. This amino segment was not effective for the appearance of selectivity for M_2 muscarinic receptors in AF-DX 116 derivatives. Compounds **6u** and **6y** acted as noncompetitive-like antagonists in the *in vivo* study. The mechanism involved is under investigation. These compounds antagonized the oxotremorine-induced bradycardia in rats after both intravenous and oral administration. Moreover, the oral administration of **6y** produced an increase in the heart rate in conscious dogs. Our results indicate that these succinamide derivatives, such as **6u** and **6y**, are candidate antibradycardiac agents.

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 the internal standard. Abbreviations of the ^{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 was recorded on VG ZAB-VSE mass spectrometers. Column chromatography on silica gel was performed with Kieselgel 60 (E. Merck).

Table 4. Physical Data for Substituted Diamines **8a—s**



Compd. No.	R	Position	Method	Yield (%)	^{1}H-NMR δ (in $CDCl_3$, J in Hz)	MS m/z
8a	OMe	2	A	64	0.99 (3H, t, J=7.2), 1.11 (3H, t, J=7.2), 1.62 (1H, br s), 2.41—2.65 (8H, m), 3.59 (2H, s), 3.79 (3H, s), 6.77—7.38 (4H, m)	237 (M^++1)
8b	OMe	3	B	66	1.00 (3H, t, J=7.2), 1.16 (3H, t, J=7.2), 2.37—2.64 (8H, m), 3.54 (2H, s), 3.79 (3H, s), 6.80—7.31 (4H, m)	237 (M^++1)
8c	OMe	4	B	74	1.00 (3H, t, J=7.2), 1.10 (3H, t, J=7.2), 2.10 (1H, br s), 2.40—2.69 (8H, m), 3.50 (2H, s), 3.77 (3H, s), 6.86 (2H, d, J=7.9), 7.22 (2H, d, J=7.9)	237 (M^++1)
8d	Cl	2	B	60	1.03 (3H, t, J=7.2), 1.08 (3H, t, J=7.2), 1.75 (1H, br s), 2.53—2.58 (4H, m), 2.61—2.68 (4H, m), 3.53 (2H, s), 7.15—7.26 (2H, m), 7.33 (1H, d, J=6.9), 7.46 (1H, dd, J=7.2, 1.3)	241 (M^+)
8e	Cl	3	B	75	1.00—1.21 (6H, m), 2.36 (1H, br s), 2.45—2.71 (8H, m), 3.58 (2H, s), 7.24—7.40 (4H, m)	241 (M^+)
8f	Cl	4	B	88	0.94—1.16 (6H, m), 2.13 (1H, br s), 2.39—2.75 (8H, m), 3.52 (2H, s), 7.24 (4H, s)	241 (M^+)
8g	Me	4	B	69	1.03 (3H, t, J=7.2), 1.07 (3H, t, J=7.2), 1.59 (1H, br s), 2.33 (3H, s), 2.49—2.66 (8H, m), 3.52 (2H, s), 7.11 (2H, d, J=7.9), 7.18 (2H, d, J=7.9)	220 (M^+)
8h	Et	4	B	51	1.05 (3H, t, J=6.4), 1.07 (3H, t, J=6.4), 1.22 (3H, t, J=7.3), 2.51—2.61 (4H, m), 2.62—2.68 (6H, m), 3.52 (2H, s), 3.45 (1H, br s), 7.14 (2H, d, J=7.9), 7.20 (2H, d, J=7.9)	234 (M^+)
8i	iso-Pr	4	A	62	0.98—1.14 (6H, m), 1.24 (6H, d, J=6.8), 2.39—2.65 (8H, m), 2.70—3.01 (1H, m), 3.54 (2H, s), 3.59 (1H, br s), 7.22 (4H, s)	248 (M^+)
8j	SMe	4	A	47	1.05 (3H, t, J=7.3), 1.11 (3H, t, J=7.3), 2.47 (3H, s), 2.53—2.73 (8H, m), 3.54 (2H, s), 5.05 (1H, br s), 7.23 (4H, s)	252 (M^+)
8k	OH	4	A	33	1.08 (3H, t, J=6.8), 1.11 (3H, t, J=6.8), 2.54—2.65 (8H, m), 3.44 (2H, s), 5.55 (1H, br s), 6.54 (2H, d, J=7.9), 7.05 (2H, d, J=7.9)	221 (M^+)
8l	OPr	4	A	65	1.03 (3H, t, J=7.3), 1.08 (3H, t, J=7.3), 1.76—1.83 (2H, m), 3.53 (2H, s), 4.00 (2H, t, J=5.8), 6.82 (2H, d, J=7.3), 7.21 (2H, d, J=7.3)	336 (M^++1)
8m	$O(CH_2)_3NEt_2$	4	A	12	1.02—1.06 (12H, m), 1.09—2.02 (2H, m), 2.51—2.78 (14H, m), 3.53 (2H, s), 4.00 (2H, t, J=5.8), 6.82 (2H, d, J=7.3), 7.21 (2H, d, J=7.3)	336 (M^++1)
8n	NMe_2	4	A	15	1.04 (3H, t, J=7.2), 1.08 (3H, t, J=7.2), 2.52—2.69 (8H, m), 2.92 (6H, s), 3.49 (2H, s), 6.69 (2H, d, J=7.9), 7.14 (2H, d, J=7.9)	249 (M^+)
8o		4	A	58	1.02 (3H, t, J=7.3), 1.08 (3H, t, J=7.3), 1.82 (1H, br s), 1.97—2.00 (4H, m), 2.47—2.68 (8H, m), 3.27 (4H, t, J=6.8), 3.48 (2H, s), 6.52 (2H, d, J=8.8), 7.13 (2H, d, J=8.3)	276 (M^++1)
8p		4	A	79	1.00(3H, t, J=7.3), 1.11 (3H, t, J=7.3), 1.50—1.88 (6H, m), 2.09 (1H, br s), 2.05—2.66 (8H, m), 3.05—3.21 (4H, m), 3.48 (2H, s), 6.86 (2H, d, J=7.9), 7.16 (2H, d, J=7.9)	289 (M^+)
8q		4	A	74	1.03 (3H, t, J=7.3), 1.08 (3H, t, J=7.3), 1.53—1.55 (4H, m), 1.70—1.82 (4H, m), 2.50—2.67 (8H, m), 3.42—3.46 (6H, m), 6.62 (2H, d, J=78.6), 7.10 (2H, d, J=8.6)	304 (M^++1)
8r		4	A	37	1.03 (3H, t, J=6.7), 1.08 (3H, t, J=7.3), 1.92 (1H, br s), 2.48—2.68 (8H, m), 3.14 (4H, t, J=4.9), 3.50 (2H, s), 3.86 (4H, t, J=4.9), 6.86 (2H, d, J=8.6), 7.19 (2H, d, J=8.5)	291 (M^+)
8s		4	A	33	0.97 (3H, t, J=6.8), 1.06 (3H, t, J=7.3), 1.12 (3H, t, J=7.3), 1.30—1.38 (2H, m), 1.45—1.54 (1H, m), 1.73 (2H, d, J=14.2), 2.53—2.59 (4H, m), 2.64—2.71 (6H, m), 3.30 (1H, br s), 3.50 (2H, s), 3.62 (2H, d, J=12.0), 6.89 (2H, d, J=9.0), 7.16 (2H, d, J=9.5)	304 (M^++1)

General Procedure for the Preparation of Substituted Diamines 8a—s Physical data for **8a—s** are listed in Table 4.

***N,N'*-Diethyl-*N*-(2-methoxybenzyl)ethylenediamine (8a) [Method A]** A mixture of *N,N'*-diethylethylenediamine (4.3 g, 37 mmol), 2-methoxybenzaldehyde (1.0 g, 7.3 mmol), acetic acid (6.2 g, 104 mmol) and sodium triacetoxyborohydride ($NaB(OAc)_3H$) (4.7 g, 21 mmol) in CH_2Cl_2 (30 ml) was stirred for 8 h at room temperature. The mixture was made alkaline with 1 N aqueous NaOH and was extracted with CH_2Cl_2 (30 ml × 2) and the combined extract was washed successively with brine. After evaporation of the solvent, the residue was purified on a silica gel column ($CHCl_3$–MeOH–28% aqueous NH_4OH, 300 : 10 : 1, v/v/v) to give 1.1 g of **8a** as an oil in 64% yield. ^{1}H-NMR ($CDCl_3$) δ: 0.99 (3H, t, J=7.2 Hz), 1.11 (3H, t, J=7.2 Hz), 2.41—2.65 (8H, m), 3.59 (2H, s), 3.79 (3H, s), 6.77—7.38 (4H, m). FAB-MS *m/z*: 237 (M^++1).

***N,N'*-Diethyl-*N*-(3-methoxybenzyl)ethylenediamine (8b) [Method B]** 3-Methoxybenzyl chloride (1.5 g, 9.6 mmol) was added to a mixture of *N,N'*-diethylethylenediamine (5.6 g, 43 mmol) and CH_2Cl_2 (20 ml) below 10 °C. The mixture was stirred for 8 h at room temperature, and made alkaline with 1 N aqueous NaOH. The separated organic layer was washed with water and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was purified on a silica gel column ($CHCl_3$–MeOH–28% aqueous NH_4OH, 300 : 10 : 1, v/v/v) to give 1.5 g of **8b** as a yellow oil in 66% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.00 (3H, t, J=7.2 Hz), 1.16 (3H, t, J=7.2 Hz), 2.37—2.64 (8H, m), 3.54 (2H, s), 3.79 (3H, s), 6.80—7.31 (4H, m). FAB-MS *m/z*: 237 (M^++1).

4-4-Isopropyl-1-piperazinyl)benzonitrile (10d) A mixture of 4-(1-piperazinyl)benzonitrile **9** (4.80 g, 25.6 mmol), acetone (1.65 g, 28.1 mmol), acetic acid (3.85 g, 64.1 mmol) and $NaB(OAc)_3H$ (8.57 g, 38.4 mmol) in CH_2Cl_2 (60 ml) was stirred for 8 h at room temperature. The mixture was made alkaline with 1 N aqueous NaOH and extracted with CH_2Cl_2 (50 ml × 2). The combined extract was washed successively with brine. After evaporation of the solvent, the residue was purified on a silica gel column ($CHCl_3$–MeOH, 100 : 1, v/v), followed by recrystallization from *n*-hexane to give 4.38 g of **10d** as colorless needles in 75% yield. mp 95—96 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.08 (6H, d, J=6.3 Hz), 2.65 (4H, t, J=4.8 Hz), 2.73 (1H, dt, J=13.2, 6.3Hz), 3.33 (4H, t, J=4.8 Hz), 6.85 (2H, d, J=8.8 Hz), 7.48 (2H, d, J=8.8 Hz). GC-MS *m/z*: 229 (M^+).

4-(4-Isopropyl-1-piperazinyl)benzaldehyde (11d) A solution of **10d** (3.0 g, 13.1 mmol) in toluene (20 ml) was treated dropwise with 0.95 M DIBAH in *n*-hexane solution (21 ml, 20.0 mmol), and stirred for 1.5 h at 50 °C. The reaction mixture was cooled and quenched by the addition of MeOH (10 ml) and H_2O (10 ml). The precipitate was removed by filtration, and the filtrate was evaporated *in vacuo*. The residue was purified through a silica gel column with $CHCl_3$, followed by recrystallization from diisopropyl ether to give 2.46 g of **11d** as colorless needles in 81% yield. mp 65—66 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.09 (6H, d, J=6.3 Hz), 2.66 (4H, t, J=5.4 Hz), 2.73 (1H, dt, J=13.2, 6.3 Hz), 3.41 (4H, t, J=5.4 Hz), 6.91 (2H, d, J=8.8 Hz), 7.75 (2H, d, J=8.8 Hz), 9.77 (1H, s). GC-MS *m/z*: 232 (M^+).

***N,N'*-Diethyl-*N*-[4-(4-isopropyl-1-piperazinyl)benzyl]ethylenediamine (8w)** A mixture of *N,N'*-diethylethylenediamine (1.85 g, 16 mmol), **11d** (740 mg, 3.2 mmol), acetic acid (670 mg, 11.1 mmol) and $NaB(OAc)_3H$ (2.02 g, 9.1 mmol) in CH_2Cl_2 (20 ml) was stirred for 8 h at room temperature. The mixture was made alkaline with 1 N aqueous NaOH and extracted with CH_2Cl_2 (20 ml × 2), and the combined extract was washed successively with brine. After evaporation of the solvent, the residue was purified on a silica gel column ($CHCl_3$–MeOH, 50 : 1, v/v) to give 870 mg of **8w** as an oil in 82% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.02 (3H, t, J=6.8 Hz), 1.07 (3H, t, J=6.8 Hz), 1.09 (6H, d, J=6.4 Hz), 1.74 (1H, br s), 2.50—2.73 (12H, m), 3.18—3.20 (4H, m), 3.32—3.36 (1H, m), 3.49 (2H, s), 6.87 (2H, d, J=8.8 Hz), 7.18 (2H, d, J=8.8 Hz). GC-MS *m/z*: 332 (M^+).

Other diamines **8t—v** were prepared in the same fashion as described for **8w**.

N,N'-Diethyl-*N*-[4-(4-methyl-1-piperazinyl)benzyl]ethylenediamine (**8t**): ^{1}H-NMR ($CDCl_3$) δ: 0.96—1.10 (6H, m), 2.35 (3H, s), 2.52—2.68 (12H, m), 3.19 (4H, t, J=4.9 Hz), 3.50 (2H, s), 6.88 (2H, d, J=8.5 Hz), 7.18 (2H, d, J=8.5 Hz). GC-FAB *m/z*: 305 (M^++1).

N,N'-Diethyl-*N*-[4-(4-ethyl-1-piperazinyl)benzyl]ethylenediamine (**8u**): ^{1}H-NMR ($CDCl_3$) δ: 0.98—1.14 (9H, m), 2.45—2.68 (14H, m), 3.20 (4H, t, J=4.9 Hz), 3.49 (2H, s), 6.88 (2H, d, J=8.5 Hz), 7.18 (2H, d, J=8.5 Hz). GC-MS *m/z*: 318 (M^+).

N,N'-Diethyl-*N*-[4-(4-propyl-1-piperazinyl)benzyl]ethylenediamine (**8v**): ^{1}H-NMR ($CDCl_3$) δ: 0.88—1.38 (9H, m), 1.50—1.70 (2H, m), 2.28—2.79 (14H, m), 3.19 (4H, t, J=4.9 Hz), 3.52 (2H, s), 6.86 (2H, d, J=8.8 Hz), 7.17 (2H, d, J=8.8 Hz). GC-FAB *m/z*: 333 (M^++1).

***N*-Methyl-[4-(4-isopropyl-1-piperazinyl)]benzylamine (12b)** A mixture of methylamine (40% in MeOH) (7.96 g, 103 mmol), **11d** (6.80 g, 29.3 mmol), acetic acid (6.20 g, 100 mmol) and $NaB(OAc)_3H$ (9.80 g, 44.0 mmol) in CH_2Cl_2 (50 ml) was stirred for 2 h at room temperature. It was then made alkaline with 1 N aqueous NaOH and extracted with CH_2Cl_2 (50 ml × 2), and the combined extract was washed successively with brine. The solvent was evaporated to give 7.21 g of **12b** as an oil in 99% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.08 (6H, d, J=6.7 Hz), 2.34 (1H, br s), 2.44 (3H, s), 2.62—2.73 (5H, m), 3.14—3.25 (4H, m), 3.69 (2H, s), 6.88 (2H, d, J=8.8 Hz), 7.20 (2H, d, J=8.8 Hz). GC-MS *m/z*: 247 (M^+).

***N*-(2-Chloroethyl)-*N*-methyl-[4-(4-isopropyl-1-piperazinyl)]benzylamine (13b)** A mixture of chloroacetaldehyde (40% in H_2O) (5.75 g, 29.3 mmol), **12b** (7.24 g, 29.3 mmol), acetic acid (3.52 g, 58.6 mmol) and $NaB(OAc)_3H$ (9.80 g, 44.0 mmol) in CH_2Cl_2 (50 ml) was stirred for 1 h at room temperature. The mixture was made alkaline with 1 N aqueous NaOH and extracted with CH_2Cl_2 (50 ml × 2), and the combined extract was washed successively with brine. After evaporation of the solvent, the residue was purified on a silica gel column ($CHCl_3$–MeOH, 30 : 1, v/v) to give 6.21 g of **13b** as an oil in 68% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.09 (6H, d, J=6.8 Hz), 2.26 (3H, s), 2.68—2.74 (4H, m), 2.73 (2H, t, J=6.8 Hz), 3.19—3.22 (4H, m), 3.49 (2H, s), 3.55 (2H, t, J=6.8 Hz), 6.88 (2H, d, J=8.3 Hz), 7.18 (2H, d, J=8.3 Hz). GC-MS *m/z*: 309 (M^+).

***N'*-Ethyl-*N*-[4-(4-isopropyl-1-piperazinyl)benzyl]-*N*-methylethylenediamine (8y)** A solution of **13b** (5.94 g, 19.2 mmol) in EtOH (40 ml) was treated with $EtNH_2$ (70% in H_2O) (6.25 g, 96 mmol), and the mixture was heated for 2 h at 70 °C. The solvent was evaporated, and the residue was made alkaline with 1 N aqueous NaOH, then extracted with $CHCl_3$ (40 ml × 2). The combined extract was washed successively with brine. After evaporation of the solvent, the residue was purified on a silica gel column ($CHCl_3$–MeOH–28% aqueous NH_4OH, 300 : 10 : 1, v/v/v) to obtain 3.72 g of **8y** as an oil in 61% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.08—1.17 (9H, m), 1.96 (1H, br s), 2.17 (3H, s), 2.51 (2H, t, J=5.8 Hz), 2.60 (2H, dd, J=14.2, 7.3 Hz), 2.67—2.74 (7H, m), 3.18—3.20 (4H, m), 3.42 (2H, s), 6.87 (2H, d, J=8.8 Hz), 7.17 (2H, d, J=8.8 Hz). FAB-MS *m/z*: 319 (M^++1).

N'-Ethyl-*N*-[4-(4-ethyl-1-piperazinyl)benzyl]-*N*-methylethylenediamine (**8x**): The title compound was prepared in the same manner as described for **8y**. ^{1}H-NMR ($CDCl_3$) δ: 1.18 (3H, t, J=7.3 Hz), 1.34 (3H, t, J=7.3 Hz), 2.30 (3H, s), 2.55—2.59 (2H, m), 2.69—2.71 (4H, m), 2.79—2.86 (4H, m), 2.97—2.99 (2H, m), 3.26—3.28 (4H, m), 3.52 (2H, s), 6.89 (2H, d, J=8.5 Hz), 7.21 (2H, d, J=8.5 Hz). GC-MS *m/z*: 304 (M^+).

4-(4-*tert*-Butyloxycarbonyl-1-piperazinyl)benzaldehyde (**15**): The title compound was prepared in the same manner as described for **10d**. ^{1}H-NMR ($CDCl_3$) δ: 1.49 (9H, s), 3.33—3.43 (4H, m), 3.54—3.65 (4H, m), 6.90 (2H, d, J=8.8 Hz), 7.75 (2H, d, J=8.8 Hz). 9.80 (1H, s). FAB-MS *m/z*: 291 (M^++1).

N,N'-Diethyl-*N*-[4-(4-*tert*-butyloxycarbonyl-1-piperazinyl)benzyl]-ethylenediamine (**16**): The title compound was prepared in the same manner as described for **8w**. ^{1}H-NMR ($CDCl_3$) δ: 1.08 (3H, t, J=6.8 Hz), 1.14 (3H, t, J=6.8 Hz), 1.48 (9H, s), 2.55—2.64 (4H, m), 2.69—2.72 (2H, m), 2.78—2.80 (2H, m), 3.10—3.12 (4H, m), 3.53 (2H, s), 3.56—3.59 (4H, m), 6.88 (2H, d, J=8.8 Hz), 7.19 (2H, d, J=8.8 Hz). FAB-MS *m/z*: 391 (M^++1).

11-[3-[*N*-Ethyl-*N*-[4-(4-isopropyl-1-piperazinyl)benzyl]-*N*-methylamino]ethyl]carbamoyl]propionyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (6y) A solution of ethyl 4-oxo-4-(6-oxo-5,6-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-11-yl)butylate **4** (3.90 g, 11.5 mmol), 1 N aqueous NaOH (40 ml) and EtOH (40 ml) was stirred for 25 min at room temperature. After neutralization of the solution with 1 N aqueous HCl (40 ml) and removal of the solvent under reduced pressure, the residue was dissolved in *N,N*-dimethylformamide (60 ml) and the solution was filtered. Next, **8y** (3.67 g, 11.5 mmol), WSCD (2.43 g, 12.7 mmol) and HOBT (770 mg, 5.7 mmol) were added to the filtrate and the mixture was stirred for 8 h at room temperature. After removal of the solvent under reduced pressure, the residue was diluted with 1 N aqueous NaOH and extracted with $CHCl_3$ (10 ml × 3). The organic layer was washed with water, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified on a silica gel column ($CHCl_3$–MeOH–28% aqueous NH_4OH, 300 : 10 : 1, v/v/v), followed by crystallization from Et_2O to give 4.36 g of **6y** in 62% yield. Recrystallization from 2-propanol afforded

Table 5. Physical Data for **6a**—**y**

Compd. No.	^{1}H-NMR δ (in DMSO-d_6, J in Hz)	MS m/z ($M^+ + 1$)	Formula	Analysis (%) Calcd (Found)		
				C	H	N
6a	0.88 (1.5H, t, J=7.2), 0.94 (1.5H, t, J=7.2), 0.99 (1.5H, t, J=7.2), 1.03 (1.5H, t, J=7.2), 2.00—2.15 (1H, m), 2.43—2.49 (6H, m), 2.70—2.76 (1H, m), 3.10—3.14 (1H, m), 3.20—3.28 (3H, m), 3.51 (1H, s), 3.54 (1H, s), 3.73 (1.5H, s), 3.75 (1.5H, s), 6.83—6.88 (1H, m), 6.91—6.94 (1H, m), 7.16—7.21 (1H, m), 7.27— 7.29 (1H, m), 7.41—7.48 (3H, m), 7.64—7.72 (2H, m), 7.79—7.81 (1H, m), 8.30—8.31 (1H, m), 10.81 (1H, brs)	530	$C_{30}H_{35}N_5O_4$	68.03 (67.75	6.66 6.68	13.22 13.11)
6b	0.87 (1.5H, t, J=6.8), 0.94 (1.5H, t, J=6.8), 0.99 (1.5H, t, J=6.8), 1.04 (1.5H, t, J=6.8), 2.00—2.15 (1H, m), 2.40—2.48 (6H, m), 2.73—2.77 (1H, m), 3.10—3.12 (1H, m), 3.22—3.28 (3H, m), 3.50 (1H, s), 3.53 (1H, s), 3.68 (1.5H, s), 3.71 (1.5H, s), 6.76 (1H, d, J=4.0), 6.82—6.85 (2H, m), 7.18 (1H, dt, J=8.2, 4.0), 7.38—7.50 (3H, m), 7.62—7.72 (2H, m), 7.79—7.81 (1H, m), 8.30—8.31 (1H, m), 10.81 (1H, brs)	530	$C_{30}H_{35}N_5O_4 \cdot 0.5H_2O$	66.90 (66.86	6.74 6.57	13.00 13.12)
6c	0.87 (1.5H, t, J=7.2), 0.93 (1.5H, t, J=7.2), 0.97 (1.5H, t, J=7.2), 1.03 (1.5H, t, J=7.2), 2.08—2.13 (1H, m), 2.39—2.48 (6H, m), 2.73—2.76 (1H, m), 3.08—3.14 (1H, m), 3.20—3.26 (3H, m), 3.46 (1H, s), 3.49 (1H, s), 3.71 (3H, s), 6.83 (2H, d, J=8.8), 7.16—7.20 (2H, m), 7.38—7.50 (3H, m), 7.62—7.72 (2H, m), 7.79—7.82 (1H, m), 8.30—8.31 (1H, m), 10.81 (1H, brs)	530	$C_{30}H_{35}N_5O_4 \cdot 0.1H_2O$	67.80 (67.70	6.68 6.54	13.18 13.25)
6d	0.87 (1.5H, t, J=7.2), 0.95 (1.5H, t, J=7.2), 0.98 (1.5H, t, J=7.2), 1.03 (1.5H, t, J=7.2), 2.00—2.16 (1H, m), 2.39—2.48 (6H, m), 2.72—2.77 (1H, m), 3.09—3.12 (1H, m), 3.20—3.26 (3H, m), 3.61 (1H, s), 3.65 (1H, s), 7.20—7.26 (2H, m), 7.36—7.39 (2H, m), 7.42—7.50 (3H, m), 7.62—7.71 (2H, m), 7.79—7.82 (1H, m), 8.30—8.31 (1H, m), 10.80 (1H, brs)	534	$C_{29}H_{32}N_5O_3Cl \cdot 0.6H_2O$	63.93 (63.72	6.14 5.78	12.85 13.10)
6e	0.87 (1.5H, t, J=7.2), 0.95 (1.5H, t, J=7.2), 0.98 (1.5H, t, J=7.2), 1.03 (1.5H, t, J=7.2), 2.00—2.16 (1H, m), 2.39—2.48 (6H, m), 2.72—2.77 (1H, m), 3.09—3.12 (1H, m), 3.20—3.26 (3H, m), 3.61 (1H, s), 3.65 (1H, s), 7.20—7.26 (2H, m), 7.36—7.39 (2H, m), 7.42—7.50 (3H, m), 7.62—7.71 (2H, m), 7.79—7.82 (1H, m), 8.30—8.31 (1H, m), 10.80 (1H, brs)	534	$C_{29}H_{32}N_5O_3Cl \cdot 0.2H_2O$	64.78 (64.68	6.07 5.96	13.03 13.06)
6f	0.88 (1.5H, t, J=6.8), 0.93 (1.5H, t, J=6.8), 0.98 (1.5H, t, J=6.8), 1.03 (1.5H, t, J=6.8), 2.06—2.14 (1H, m), 2.40—2.50 (6H, m), 2.72—2.77 (1H, m), 3.08—3.15 (1H, m), 3.20—3.28 (3H, m), 3.52 (1H, s), 3.55 (1H, s), 7.26—7.34 (4H, m), 7.40—7.50 (3H, m), 7.63—7.75 (2H, m), 7.78—7.81 (1H, m), 8.29—8.32 (1H, m), 10.81 (1H, brs).	534	$C_{29}H_{32}N_5O_3Cl \cdot 0.1H_2O$	65.00 (64.90	6.06 6.03	13.07 13.12)
6g	0.87 (1.5H, t, J=6.8), 0.93 (1.5H, t, J=6.8), 0.97 (1.5H, t, J=6.8), 1.03 (1.5H, t, J=6.8), 2.08—2.10 (1H, m), 2.09 (3H, s), 2.40—2.48 (6H, m), 2.68—2.76 (1H, m), 3.10—3.12 (1H, m), 3.21—3.25 (3H, m), 3.47 (1H, s), 3.51 (1H, s), 7.07 (2H, d, J=8.0), 7.13—7.16 (2H, m), 7.38—7.50 (3H, m), 7.61—7.73 (2H, m), 7.77— 7.82 (1H, m), 8.30—8.31 (1H, m), 10.81 (1H, brs)	514	$C_{30}H_{35}N_5O_3 \cdot 0.2H_2O$	69.66 (69.55	6.90 6.75	13.54 13.55)
6h	0.87 (1.5H, t, J=7.2), 0.93 (1.5H, t, J=7.2), 0.98 (1.5H, t, J=7.2), 1.02 (1.5H, t, J=7.2), 1.15 (3H, t, J=7.8), 2.01—2.10 (1H, m), 2.39—2.48 (6H, m), 2.55 (2H, q, J=7.8), 2.72—2.78 (1H, m), 3.09—3.13 (1H, m), 3.21—3.27 (3H, m), 3.48 (1H, s), 3.52 (1H, s), 7.10 (2H, d, J=7.8), 7.15—7.19 (2H, m), 7.40—7.50 (3H, m), 7.62—7.71 (2H, m), 7.79—7.82 (1H, m), 8.29—8.31 (1H, m), 10.80 (1H, brs)	528	$C_{31}H_{37}N_5O_3 \cdot 0.1H_2O$	70.32 (70.18	7.08 7.09	13.23 13.20)
6i	0.86 (1.5H, t, J=6.8), 0.94 (1.5H, t, J=6.8), 0.98 (1.5H, t, J=6.8), 1.03 (1.5H, t, J=6.8), 1.16 (6H, d, J=6.8), 2.00—2.15 (1H, m), 2.39—2.48 (6H, m), 2.74—2.78 (1H, m), 2.80—2.87 (1H, m), 3.08—3.13 (1H, m), 3.19—3.26 (3H, m), 3.48 (1H, s), 3.52 (1H, s), 7.12—7.19 (4H, m), 7.38—7.50 (3H, m), 7.60—7.73 (2H, m), 7.77—7.81 (1H, m), 8.30—8.31 (1H, m), 10.80 (1H, brs)	542	$C_{32}H_{39}N_5O_3 \cdot 0.2H_2O$	70.48 (70.37	7.28 7.14	12.84 12.80)
6j	0.88 (1.5H, t, J=7.2), 0.93 (1.5H, t, J=7.2), 0.98 (1.5H, t, J=7.2), 1.04 (1.5H, t, J=7.2), 2.01—2.15 (1H, m), 2.38—2.48 (6H, m), 2.47 (3H, s), 2.72—2.78 (1H, m), 3.09—3.15 (1H, m), 3.21—3.28 (3H, m), 3.49 (1H, s), 3.52 (1H, s), 7.16—7.24 (4H, m), 7.40—7.50 (3H, m), 7.63—7.71 (2H, m), 7.80—7.81 (1H, m), 8.30—8.31 (1H, m), 10.79 (1H, brs)	546	$C_{30}H_{35}N_5O_3S$	66.03 (65.73	6.46 6.44	12.83 12.69)
6k	0.87 (1.5H, t, J=7.2), 0.92 (1.5H, t, J=7.2), 0.96 (1.5H, t, J=7.2), 1.03 (1.5H, t, J=7.2), 2.00—2.16 (1H, m), 2.39—2.48 (6H, m), 2.71—2.80 (1H, m), 3.08—3.13 (1H, m), 3.21—3.28 (3H, m), 3.41 (1H, s), 3.44 (1H, s), 6.66 (2H, d, J=8.3), 7.04 (2H, dd, J=8.3, 4.4), 7.40—7.50 (3H, m), 7.62—7.71 (2H, m), 7.80—7.82 (1H, m), 8.30—8.31 (1H, m), 9.20 (1H, brs), 10.80 (1H, brs)	516	$C_{29}H_{33}N_5O_4$	67.55 (67.28	6.45 6.50	13.58 13.39)
6l	0.86—1.05 (9H, m), 1.66—1.75 (2H, m), 2.18—2.22 (1H, m), 2.38—2.48 (6H, m), 2.72—2.78 (1H, m), 3.08—3.14 (1H, m), 3.20—3.26 (3H, m), 3.45 (1H, s), 3.48 (1H, s), 3.87 (2H, t, J=6.8), 6.82 (2H, d, J=8.4), 7.14—7.18 (2H, m), 7.39—7.50 (3H, m), 7.62—7.72 (2H, m), 7.79—7.81 (1H, m), 8.30—8.31 (1H, m), 10.81 (1H, brs)	558	$C_{32}H_{39}N_5O_4$	68.92 (68.85	7.05 7.00	12.56 12.50)
6m	0.85—1.04 (12H, m), 1.74—1.80 (2H, m), 2.18—2.23 (1H, m), 2.38—2.48 (6H, m), 2.71—2.80 (1H, m), 3.08—3.15 (1H, m), 3.20—3.29 (3H, m), 3.45 (1H, s), 3.48 (1H, s), 3.95 (2H, t, J=6.3), 6.82 (2H, d, J=8.3), 7.16 (2H, dd, J=8.3, 4.4), 7.40—7.50 (3H, m), 7.62—7.70 (2H, m), 7.80—7.82 (1H, m), 8.29—8.31 (1H, m), 10.80 (1H, brs)	629	$C_{36}H_{48}N_6O_4$	68.76 (68.52	7.69 7.72	13.36 13.44)

Table 5. (continued)

Compd. No.	^{1}H-NMR δ (in DMSO-d_6, J in Hz)	MS m/z ($M^+ + 1$)	Formula	Analysis (%) Calcd (Found)		
				C	H	N
6n	0.88 (1.5H, t, J=7.2), 0.92 (1.5H, t, J=7.2), 0.97 (1.5H, t, J=7.2), 1.03 (1.5H, t, J=7.2), 2.02—2.13 (1H, m), 2.39—2.49 (6H, m), 2.70—2.80 (1H, m), 2.84 (6H, s), 3.08—3.15 (1H, m), 3.20—3.28 (3H, m), 3.40 (1H, s), 3.44 (1H, s), 6.63 (2H, d, J=8.3), 7.04—7.08 (2H, m), 7.40—7.50 (3H, m), 7.62—7.71 (2H, m), 7.80—7.83 (1H, m), 8.29—8.31 (1H, m), 10.79 (1H, brs)	543	$C_{31}H_{38}N_6O_3 \cdot 0.2H_2O$	68.16 (67.75	7.09 7.02	15.38 15.38)
6o	0.88 (1.5H, t, J=6.8), 0.92 (1.5H, t, J=6.8), 0.97 (1.5H, t, J=6.8), 1.03 (1.5H, t, J=6.8), 1.90—1.94 (4H, m), 2.00—2.10 (1H, m), 2.37—2.48 (6H, m), 2.71—2.81 (1H, m), 2.80—3.30 (8H, m), 3.39 (1H, s), 3.44 (1H, s), 6.44 (2H, d, J=8.3), 7.01—7.07 (2H, m), 7.40—7.50 (3H, m), 7.60—7.70 (2H, m), 7.80—7.82 (1H, m), 8.29—8.31 (1H, m), 10.80 (1H, brs)	569	$C_{33}H_{40}N_6O_3 \cdot 0.3H_2O$	69.04 (69.08	7.13 7.00	14.64 14.68)
6p	0.87 (1.5H, t, J=6.8), 0.93 (1.5H, t, J=6.8), 0.97 (1.5H, t, J=6.8), 1.02 (1.5H, t, J=6.8), 1.48—1.53 (2H, m), 1.55—1.61 (4H, m), 2.00—2.15 (1H, m), 2.38—2.48 (6H, m), 2.71—2.81 (1H, m), 3.04—3.08 (4H, m), 3.09—3.15 (1H, m), 3.20—3.30 (3H, m), 3.41 (1H, s), 3.45 (1H, s), 6.82 (2H, d, J=8.5), 7.06—7.10 (2H, m), 7.40—7.50 (3H, m), 7.60—7.70 (2H, m), 7.80—7.82 (1H, m), 8.29—8.31 (1H, m), 10.79 (1H, brs)	583	$C_{34}H_{42}N_6O_3 \cdot 0.1H_2O$	69.86 (69.67	7.28 7.17	14.38 14.33)
6q	0.87 (1.5H, t, J=6.8), 0.93 (1.5H, t, J=6.8), 0.98 (1.5H, t, J=6.8), 1.03 (1.5H, t, J=6.8), 1.40—1.44 (4H, m), 1.66—1.70 (4H, m), 2.05—2.15 (1H, m), 2.37—2.49 (6H, m), 2.71—2.81 (1H, m), 2.72—2.78 (1H, m), 3.09—3.28 (3H, m), 3.38—3.41 (6H, m), 6.57 (2H, d, J=8.5), 7.01—7.04 (2H, m), 7.38—7.48 (3H, m), 7.60—7.70 (2H, m), 7.80—7.81 (1H, m), 8.30—8.31 (1H, m), 10.79 (1H, brs)	597	$C_{35}H_{44}N_6O_3$	70.44 (70.20	7.43 7.45	14.08 13.97)
6r	0.88 (1.5H, t, J=6.8), 0.93 (1.5H, t, J=6.8), 0.97 (1.5H, t, J=6.8), 1.03 (1.5H, t, J=6.8), 2.00—2.13 (1H, m), 2.39—2.48 (6H, m), 2.70—2.80 (1H, m), 3.05 (4H, t, J=4.8), 3.08—3.15 (1H, m), 3.20—3.30 (3H, m), 3.43 (1H, s), 3.47 (1H, s), 3.72 (4H, t, J=4.8), 6.84 (2H, d, J=8.7), 7.10—7.14 (2H, m), 7.40—7.50 (3H, m), 7.60—7.70 (2H, m), 7.80—7.82 (1H, m), 8.30—8.32 (1H, m), 10.80 (1H, brs)	585	$C_{33}H_{40}N_6O_4 \cdot 0.4H_2O$	66.96 (66.82	6.95 6.77	14.20 14.22
6s	0.87 (1.5H, t, J=7.2), 0.92 (3H, d, J=5.5), 0.93 (1.5H, t, J=7.3), 0.99 (1.5H, t, J=7.2), 1.02 (1.5H, t, J=7.2), 1.17—1.24 (2H, m), 1.40—1.50 (1H, m), 1.63—1.69 (2H, m), 2.03—2.13 (1H, m), 2.39—2.50 (6H, m), 2.53—2.60 (2H, m), 2.65—2.76 (1H, m), 3.01—3.12 (1H, m), 3.19—3.28 (3H, m), 3.41 (1H, s), 3.45 (1H, s), 3.57—3.60 (2H, m), 6.82 (2H, d, J=8.6), 7.06—7.10 (2H, m), 7.40—7.50 (3H, m), 7.62—7.72 (2H, m), 7.79—7.81 (1H, m), 8.30—8.32 (1H, m), 10.81 (1H, brs)	597	$C_{35}H_{44}N_6O_3$	70.44 (70.23	7.43 7.38	14.08 14.12)
6t	0.88 (1.5H, t, J=7.2), 0.93 (1.5H, t, J=7.2), 0.97 (1.5H, t, J=7.2), 1.03 (1.5H, t, J=7.2), 2.03—2.13 (1H, m), 2.23 (3H, s), 2.38—2.60 (10H, m), 2.65—2.75 (1H, m), 3.03—3.10 (4H, m), 3.09—3.15 (1H, m), 3.19—3.28 (3H, m), 3.42 (1H, s), 3.45 (1H, s), 6.83 (2H, d, J=8.5), 7.07—7.12 (2H, m), 7.40—7.50 (3H, m), 7.62—7.72 (2H, m), 7.79—7.81 (1H, m), 8.29—8.31 (1H, m), 10.83 (1H, brs)	598	$C_{34}N_{43}N_7O_3$	68.32 (68.03	7.25 7.29	16.40 16.25)
6u	0.88 (1.5H, t, J=7.2), 0.93 (1.5H, t, J=7.2), 0.95—1.06 (6H, m), 2.03—2.15 (1H, m), 2.30—2.50 (10H, m), 2.68—2.78 (1H, m), 3.05—3.15 (5H, m), 3.20—3.26 (3H, m), 3.42 (1H, s), 3.46 (1H, s), 6.83 (2H, d, J=8.6), 7.08—7.12 (2H, m), 7.40—7.50 (3H, m), 7.60—7.70 (2H, m), 7.78—7.81 (1H, m), 8.30—8.31 (1H, m), 10.82 (1H, brs)	612	$C_{35}H_{45}N_7O_3 \cdot 0.2H_2O$	68.31 (68.22	7.44 7.44	15.93 15.98)
6v	0.86—0.89 (4.5H, m), 0.93 (1.5H, t, J=6.8), 0.97 (1.5H, t, J=6.8), 1.03 (1.5H, t, J=6.8), 1.42—1.52 (2H, m), 2.03—2.13 (1H, m), 2.27 (2H, t, J=7.2), 2.38—2.43 (4H, m), 2.45—2.52 (6H, m), 2.73—2.77 (1H, m), 3.04—3.08 (4H, m), 3.14—3.18 (1H, m), 3.28—3.34 (3H, m), 3.42 (1H, s), 3.45 (1H, s), 6.83 (2H, d, J=8.4), 7.07—7.12 (2H, m), 7.39—7.50 (3H, m), 7.60—7.70 (2H, m), 7.78—7.80 (1H, m), 8.30—8.31 (1H, m), 10.82 (1H, brs)	626	$C_{36}H_{47}N_7O_3$	69.09 (68.94	7.57 7.66	15.67 15.54)
6w	0.88 (1.5H, t, J=7.2), 0.92 (1.5H, t, J=7.2), 0.97 (1.5H, t, J=7.2), 1.00 (1.5H, t, J=7.2), 1.03 (1.5H, t, J=7.3), 2.03—2.15 (1H, m), 2.38—2.50 (4H, m), 2.55—2.58 (6H, m), 2.65—2.68 (1H, m), 2.73—2.77 (1H, m), 3.05—3.08 (4H, m), 3.14—3.18 (1H, m), 3.28—3.33 (3H, m), 3.42 (1H, s), 3.45 (1H, s), 6.82 (2H, d, J=8.5), 7.08—7.11 (2H, m), 7.40—7.50 (3H, m), 7.60—7.70 (2H , m), 7.78—7.80 (1H, m), 8.30—8.31 (1H, m), 10.81 (1H, brs)	626	$C_{36}H_{47}N_7O_3 \cdot 0.4H_2O$	68.31 (68.17	7.61 7.49	15.49 15.47)
6x	0.91 (1.5H, t, J=7.2), 1.01—1.07 (4.5H, m), 2.09 (1.5H, s), 2.14 (1.5H, s), 2.32—2.37 (1H, m), 2.40—2.60 (8H, m), 2.65—2.72 (1H, m), 3.05—3.08 (4H, m), 3.15—3.18 (1H, m), 3.28—3.32 (3H, m), 3.33 (1H, s), 3.38 (1H, s), 6.83 (2H, d, J=8.6), 7.06—7.10 (2H, m), 7.40—7.50 (3H, m), 7.60—7.70 (2H, m), 7.79—7.81 (1H, m), 8.31—8.32 (1H, m), 10.81 (1H, brs)	598	$C_{34}H_{43}N_7O_3$	68.32 (68.07	7.25 7.22	16.40 16.37)
6y	0.91 (1.5H, t, J=7.2), 1.00 (6H, d, J=6.1), 1.06 (1.5H, t, J=7.2), 2.08 (1.5H, s), 2.14 (1.5H, s), 2.32—2.34 (1H, m), 2.42—2.48 (4H, m), 2.54—2.56 (4H, m), 2.64—2.69 (1H, m), 2.73—2.77 (1H, m), 3.05—3.07 (4H, m), 3.14—3.17 (1H, m), 3.29—3.32 (3H, m), 3.33 (1H, s), 3.38 (1H, s), 6.83 (2H, d, J=8.5), 7.07—7.09 (2H, m), 7.41—7.46 (3H, m), 7.64—7.70 (2H, m), 7.79—7.81 (1H, m), 8.30—8.31 (1H, m), 10.81 (1H, brs)	612	$C_{35}H_{45}N_7O_3$	68.71 (68.51	7.41 7.39	16.03 15.92)

pure **6y** as colorless needles. mp 178—180 °C. ^{1}H-NMR (DMSO-d_6) (25 °C) δ: 0.91 (1.5H, t, J=7.3 Hz), 1.00 (6H, d, J=6.1 Hz), 1.06 (1.5H, t, J=7.3 Hz), 2.08 (1.5H, s), 2.14 (1.5H, s), 2.32—2.34 (1H, m), 2.42—2.48 (4H, m), 2.54—2.56 (4H, m), 2.64—2.69 (1H, m), 2.73—2.77 (1H, m), 3.05—3.07 (4H, m), 3.14—3.17 (1H, m), 3.29—3.32 (3H, m), 3.33 (1H, s), 3.38 (1H, s), 6.83 (2H, d, J=8.5 Hz), 7.07—7.09 (2H, t, m), 7.41—7.46 (3H, m), 7.64—7.70 (2H, m), 7.79—7.81 (1H, m), 8.30—8.31 (1H, m), 10.81 (1H, br s). *Anal.* Calcd for $C_{35}H_{45}N_7O_3$: C, 68.71; H, 7.41; N, 16.03. Found: C, 68.51; H, 7.39; N, 15.92. FAB-MS m/z: 612 (M^++1).

Compounds **6a**—**x** and **17** were prepared in the same fashion as described for **6y**.

11-[3-[*N*-Ethyl-*N*-[2-[*N*-ethyl-*N*-[4-(1-piperazinyl)benzyl]amino]-ethyl]carbamoyl]propionyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (6z) A solution of 290 mg (0.42 mmol) of **17** in dioxane (3 ml) was treated with 3 ml of 4 N HCl in dioxane. After 3 h the solvent was removed under vacuum, and the residue was dissolved in $CHCl_3$. The organic phase was washed with 5% $NaHCO_3$ solution and brine. After evaporation of the solvent, the residue was purified on a silica gel column ($CHCl_3$–MeOH–28% aqueous NH_4OH, 300 : 10 : 1, v/v/v) to give 130 mg of **6z** as an amorphous solid in 53% yield. ^{1}H-NMR (DMSO-d_6) δ: 0.88 (1.5H, t, J=7.3 Hz), 0.93 (1.5H, t, J=7.3 Hz), 0.97 (1.5H, t, J=7.3 Hz), 1.02 (1.5H, t, J=7.3 Hz), 2.10—2.14 (1H, m), 2.38—2.48 (6H, m), 2.73—2.82 (5H, m), 2.96—2.98 (4H, m), 3.10—3.12 (1H, m), 3.20—3.28 (3H, m), 3.41 (1H, s), 3.45 (1H, s), 6.81 (2H, d, J=8.3 Hz), 7.09 (2H, t, J=8.3 Hz), 7.41—7.46 (3H, m), 7.64—7.70 (2H, m), 7.78—7.79 (1H, m), 8.31 (1H, s), 10.81 (1H, s). HR-MS (FAB) Found m/z=584.3352, $C_{33}H_{42}N_7O_3$ Calcd m/z 584.3349.

11-[[2-[[*N*-Ethyl-*N*-(4-ethyl-1-piperazinyl)benzylamino]methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (1c) The title compound was prepared according to Engel *et al.*[5] from 11-(chloroacetyl)-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one **18** and *N*-ethyl-4-(4-ethyl-1-piperazinyl)benzylamine **20** as an amorphous solid in 68% yield. ^{1}H-NMR (DMSO-d_6) δ: 0.85—0.99 (3H, m), 1.00—1.04 (3H, m), 1.20—1.57 (6H, m), 2.03—2.06 (2H, m), 2.22—2.29 (2H, m), 2.33—2.38 (2.5H, m), 2.48—2.51 (5H, m), 2.85—2.88 (0.5H, m), 3.08—3.11 (4H, m), 3.23—3.39 (2.5H, m), 3.58—3.63 (0.5H, m), 4.13—4.17 (0.5H, m), 6.82—6.88 (2H, m), 7.02—7.08 (2H, m), 7.32—7.46 (3H, m), 7.56—7.65 (2H, m), 7.76—7.79 (1H, m), 8.12—8.19 (1H, m), 10.83—10.88 (1H, m). HR-MS (FAB) Found m/z=596.3712, $C_{35}H_{46}N_7O_2$ Calcd m/z 596.3713.

11-[[2-[(*N*-Ethyl-*N*-ethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (1b) Yield: 26% from **18**. mp 152—153 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.87—0.91 (3H, m), 1.06—1.12 (2H, m), 1.22—1.30 (3H, m), 1.50—1.60 (1H, m), 2.00—2.32 (7H, m), 3.30—3.46 (4H, m), 7.21—7.31 (5H, m), 7.38—7.47 (3H, m), 7.58—7.66 (2H, m), 2.76—2.78 (1H, m), 8.12—8.21 (1H, m), 10.80—10.87 (1H, m). *Anal.* Calcd for $C_{29}H_{33}N_5O_2$: C, 72.02; H, 6.88; N, 14.48. Found: C, 71.73; H, 6.94; N, 14.20. FAB-MS m/z: 484 (M^++1).

Biological Methods The following chemicals were commercially obtained: oxotremorine (Sigma, U.S.A.), atropine sulfate (Tanabe, Japan), and [^{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, then the cerebral cortex, heart and submandibular gland were each 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 the filtrate was 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/kg (cerebral cortex), 1.0 mg/ml (heart) and 0.83 mg/kg (submandibular gland) as determined by method of Bradford.[21] 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 the cerebral cortex, 0.1 nM [^{3}H]QNB (K_D=0.128±0.004 nM) for the heart and 0.3 nM [^{3}H]NMS (K_D=0.162±0.006 nM) for the submandibular gland at 25 °C for 45 min. In the displacement studies, the inhibition of the specific binding was examined in the presence of nonlabeled drugs in a total volume of 0.5 ml 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 immediately washed three times with approximately 3 ml portions of ice-cold HEPES buffer, then solubilized in 5ml of scintillation cocktail (Aquasol-2; Packard) and counted for radioactivity using a Packard TRI-CARB 2200 CA liquid scintillation counter. Competition binding data were analyzed with 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[22] 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 (Rat). General Procedure 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 common carotid artery cannula was used for monitoring blood pressure, and the heart rate was measured with a tachometer triggered by the pulse wave of blood pressure. A femoral vein was also cannulated for i.v. administration of the drugs. Rats were pithed by introducing a blunt steel rod *via* the orbit into the spinal canal and then treated with atenolol (10 mg/kg i.v.) to exclude catecholamine-induced tachycardia. The pithed preparation were allowed to equilibrate for at least 15 min before experiments.

i.v. Study After the general procedure, test compounds or saline were administered i.v. At 15 min after dosing, 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 then 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. On the other hand, in the case of compounds **6u** and **6y**, the maximum decrease in heart rate of oxotremorine was about 60%. Therefore, their dose-ratio was calculated from their ED_{30} values, the doses of oxotremorine required to produce a 30% decrease in heart rate.

***p.o.* Study** A test compound or 0.5% methylcellulose solution was administered *p.o.* 30 min before the assay, and the rats were treated as previously described. Three hours after the administration of a test compound, 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). Data were expressed as the negative logarithm of the DR_{10} value as described in the i.v. study.

Heart Rate (Conscious Dog) Experiments were performed on 2—6 male beagle dogs weighing to 9 to 14 kg. Electrocardiogram (ECG) leads were attached to the shaved chest, and a zippered dig jacket was applied. An ambulatory ECG Holter monitoring (SLB-90208, SpaceLabs Medical. Inc.) was placed in a pocket of the jacket, and the dog's activities were unrestricted. The ECG was recorded under conscious conditions for 24 h. Cassette tapes with two channels of ECG recordings were analyzed using a computer-assisted Holter analysis system (FT2000, SpaceLabs Medical. Inc.) to determine the heart rates. The test drugs were administered at 19 : 00. Measurements were performed at 30-min intervals during from 30 min before dosing to 8 h after dosing. Changes in heart rate after dosing were calculated with respect to the basal heart rate before dosing.

Salivation Male Wistar rats (300—350 g) were anesthetized with urethane (1.2 g/kg i.p.) and the femoral vein was cannulated for i.v. administration. After 10 min, a test compound or saline was administered i.v. 15 min after dosing, and 0.8 μmol/kg of oxotremorine was administered i.v. Saliva was collected for 5 min on filter paper according to Lavy and Mulder.[23] The average dose reducing salivary secretion to 50% of the control value was graphically determined (ID_{50} (moles per kilogram)) and the antagonism of the M_3 muscarinic receptors was expressed as the negative logarithm of the ID_{50} value, pID_{50}.

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