Synthesis and Muscarinic Activity of Novel Aniline Derivatives with a 1-Azabicyclo[3.3.0]octane Moiety[1)]

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In order to develop drugs effective against Alzheimer's disease, we synthesized a series of aniline derivatives having a characteristic cyclic amine, 1-azabicyclo[3.3.0]octane ring, and evaluated their binding affinity for the central muscarinic cholinergic receptor.

Among these compounds which showed high affinity to the M_1 receptor, *N*-[2-(1-Azabicyclo[3.3.0]octan-5-yl)ethyl]-2-nitroaniline (9f fumarate, SK-946) showed the highest affinity. The ability of this compound to improve cognitive function was assessed using the passive avoidance test in scopolamine-induced dementia mice.

Some anilines with a 1-azabicyclo[3.3.1]nonane ring were also synthesized by the ring expansion of the 1-azabicyclo[3.3.0]octane ring, and showed a high affinity for the central muscarinic cholinergic receptor.

Key Words Alzheimer's disease; 1-azabicyclo[3.3.0]octane; 1-azabicyclo[3.3.1]nonane; muscarinic receptor binding affinity; SK-946

Recently, medical science and technology have made remarkable advances and have prolonged the average human life span. As a result, a variety of diseases of aging have emerged. Alzheimer's disease (AD) is one of the senile dementias, and approximately 12 million people are estimated to suffer from AD in Japan, Europe and the U.S.A. A steady increase in the number of AD patients can be predicted with certainty in the near future.[2)] AD is characterized by mental deterioration caused by a progressive loss of neurons from particular regions of the brain, accompanied by the presence of neurofibrillary tangles in dead and dying neurons and numerous amyloid-containing senile plaques in affected brain regions.[3)]

Despite the fact that AD seems to be linked to an apparent deficiency of multiple neurotransmitters, evidence for a central role of the cholinergic system in cognitive impairment is very clear.[4)] This cholinergic hypothesis triggered research efforts aimed at restoring defective cholinergic transmission. Muscarine receptor agonist binds to an acetylcholine receptor to actuate the receptor. YM796[5)] and SR46559[6)] have been developed as this type of drug.

Muscarinic M_1 receptors are abundant in the cerebral cortex and hippocampus, and M_1 receptor activation is important for learning and memory.[7)] On the other hand, M_2 receptors are widely distributed in peripheral tissues, and M_2 receptor activation causes cholinergic side effects such as salivation.[5)] Therefore, muscarinic M_1 receptor agonists are expected to be cognition enhancers.

We have continued to develop drugs which can ameliorate AD for several years, and found a new compound, SK-946, which has highly efficacious and selective M_1 affinity.

SK-946 increased inositol phosphate production in primary cultured rat fetal hippocampal neuronal cells, and improved brain function in the scopolamine-induced dementia model mouse.[1)]

This paper describes the synthesis of SK-946 and a series of aniline derivatives having a characteristic cyclic amine, 1-azabicyclo[3.3.0]octane ring, and their biological evaluation for central muscarinic cholinergic receptor binding affinity and cognitive function.

Chemistry

The syntheses of 1-azabicyclo[3.3.0]octane derivatives were described in our previous report.[8)] The requisite 5-chloro$(CH_2)_n$-1-azabicyclo[3.3.0]octanes **3a** ($n=1$), **3b** ($n=2$) were prepared by chlorination of the corresponding alcohols, and 5-aminoalkyl-1-azabicyclo[3.3.0]octanes (**8a**—**d**) were synthesized by hydrogenating the corresponding cyano compounds.

Various aniline derivatives with the 1-azabicyclo[3.3.0]octane ring for biological tests were prepared by the five routes shown in Chart 2. The compounds **5a**—**d**, **f** were provided by procedure A, starting from the corresponding *N*-formylaniline derivatives **2**, which were converted to *N*-formyl-*N*-substituted anilines (**4a**—**e**) followed by reduction with BH_3–tetrahydrofuran (THF). When 2-fluoro-*N*-formylaniline (**2e**) was treated with **3a** in the presence of sodium hydride (NaH), deformylation induced alkylation to give *N*-(1-azabicyclo[3.3.0]octan-5-yl)methyl-2-fluoroaniline (**5e**) (54.6%) together with an *N*-formyl derivative **4e** (27.3%). *N*-(1-Azabicyclo[3.3.0]octan-5-yl)methyl-*N*-methyl-2-(trifluoromethyl)aniline (**5g**) could not be prepared *via* an *N*-formyl derivative **4g** by procedure A because of this deformylation. Therefore, compound **5g** was synthesized by the alkylation of *N*-methyl-2-(trifluoromethyl)aniline (**6**) which was derived by a reduction of *N*-formyl-2-(trifluoromethyl)aniline (**2g**)

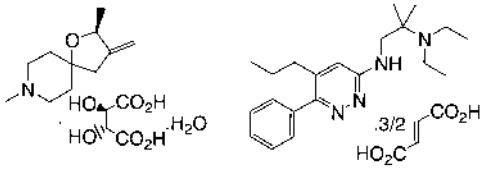

Chart 1

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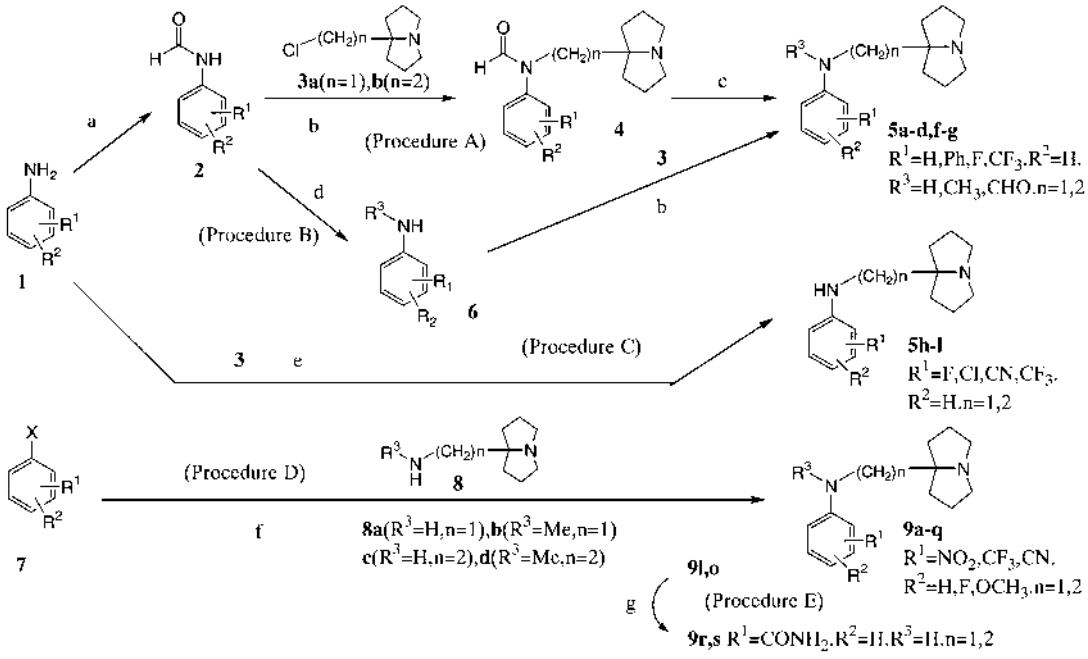


a) HCO_2H; b) NaH/DMF; c) BH_3/THF; d) $LiAlH_4$/THF; e) K_2CO_3/$PhNO_2$; f) pyridine; g) H_2SO_4

Chart 2

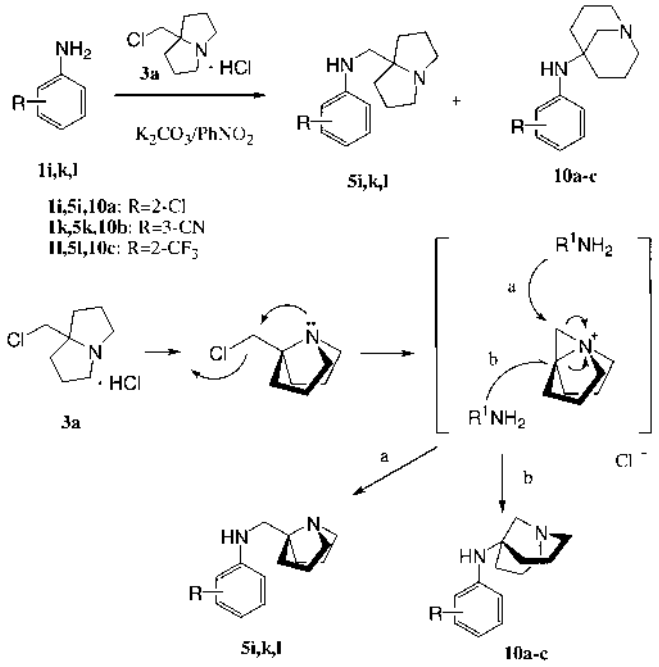
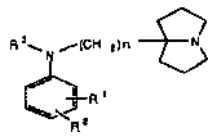


Chart 3

with **3a** in 7.7% yield (procedure B). Direct alkylation of anilines (**1**) with **3a**, **b** gave *N*-monosubstituted aniline derivatives (**5h**—**l**) in low yields (procedure C). Anilines with an electron-withdrawing group at the *ortho*- or *para*-position (**9a**—**q**) were provided by aromatic nucleophilic substitution of the corresponding halobenzenes (**7**) in good yield (procedure D). 2-Carbamoyl derivatives (**9r**, **s**) were prepared by hydrolysis of the corresponding cyano compounds (**9l**, **o**) with H_2SO_4 (procedure E).

We found a very interesting ring expansion reaction in procedure C (Chart 3). Alkylation of aniline derivatives (**1i**, **k**, **l**) with **3a** formed the ring expanded products **10a**—**c** in addition to the desired substituted products **5i**, **k**, **l**. Miyano and his co-workers reported similar ring expansion reactions of 5-trichloromethyl- or 5-dichloromethyl-1-azabicyclo[3.3.0]octanes to dichloro- and chloro-1-azabicyclo [3.3.1]nonanes, respectively, and synthesized various azabicyclo[3.3.1]nonane analogs.[9] 2-Chloroaniline (**1i**) was allowed to react with **3a** to afford *N*-(1-azabicyclo[3.3.0] octan-5-yl)methyl-2-chloroaniline (**5i**) and *N*-(1-azabicyclo [3.3.1]nonan-5-yl)-2-chloroaniline (**10a**) in yields of 44.1% and 30.4%, respectively. The structure of the ring expansion product **10a** was characterized by ^{1}H-NMR, ^{13}C-NMR, and MS spectra. The ^{1}H-NMR spectrum of **10a** showed methylene signals of the [3.3.1]nonane ring: δ 3.01 (2H, s, 9-CH_2), 1.55—1.62, 2.00—2.14 (each 2H, m, 3,7-CH_2), 1.83—1.95, 2.27—2.33 (each 2H, m, 4,6-CH_2) and 2.97—3.07 (4H, m, 2,8-CH_2) in contrast to the signals of **5i**: δ 1.59—1.70 (2H, m, 3,7-CH_2), 1.70—1.92 (6H, m, 3,4,6,7-CH_2), 2.62—2.71 and 3.04—3.12 (each 2H, m, 2,8-CH_2), δ 2.98 (2H, d, J=5 Hz, N-CH_2-C). Characteristic ^{13}C-NMR peaks of **10a** were observed at δ 48.7 due to the bridgehead carbon (5-C) and 59.9 corresponding to the C-$\underline{C}H_2$-N (9-C) carbon in comparison with those of **5i**: δ 72.8 (5-C) and 52.1 (HN-$\underline{C}H_2$-C). The ring expansion products were given by the rearrangement of 5-chloromethyl-1-azabicyclo[3.3.0]octane (**3a**) *via* an aziridinium ion formed by the intramolecular nucleophilic attack of nitrogen on the chloromethyl group. An amine nucleophilically attacks at the methylene carbon of the aziridinium ring (path a) or at the bridgehead carbon (path b) to afford 5-aminomethyl-1-azabicyclo[3.3.0]octane at 5-amino-1-azabicyclo[3.3.1]nonane, respectively. *N*-(1-Azabicyclo[3.3.1]nonan-5-yl)-3-cyanoaniline (**10b**) and the 2-(trifluoromethyl)aniline congener (**10c**) were similarly obtained from the reactions of **1k**, **l** and **3a** in 19.6% and 33.2% yields, respectively (Table 2).

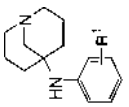
Table 1. Preparations and Affinities for M_1 and M_2 Receptors of Aniline Derivatives with a 1-Azabicyclo[3.3.0]octane Ring



Compd.	n	R^1	R^2	R^3	Procedure[a]	mp (°C)	Yield (%)	Formula	Analysis (%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N	Receptor affinities K_i(μM)[b] [^{3}H]Pirenzepine(M_1)	[^{3}H]QNB[c] (M_2)	Ratio of [^{3}H]QNB/[^{3}H]pirenzepine
4a	1	H	H	CHO	A	Oil	(95.1)	$C_{15}H_{20}N_2O$	73.74	8.25	11.47	73.55	8.40	11.28	0.36	0.95	2.6
4b	2	H	H	CHO	A	Oil	(88.1)	$C_{16}H_{22}N_2O$	74.38	8.58	10.84	74.42	8.73	10.63	1.6	9.0	5.6
5a	1	H	H	CH_3	A	Oil	88.8	$C_{15}H_{22}N_2$	78.21	9.63	12.16	78.44	9.69	11.97	1.8	8.6	4.8
5b	2	H	H	CH_3	A	Oil	75.1	$C_{16}H_{24}N_2$	78.64	9.90	11.46	78.69	9.97	11.25	0.48	10.4	21.7
5c	1	2-Ph	H	CH_3	A	Oil	86.9	$C_{21}H_{26}N_2$	82.31	8.55	9.14	82.06	8.50	8.86	0.12	0.30	2.5
5d	1	3-Ph	H	CH_3	A	Oil	45.3	$C_{21}H_{26}N_2$	82.31	8.55	9.14	82.23	8.58	8.92	2.8	2.4	0.86
5e	1	2-F	H	H	A[d]	Oil	54.6	$C_{14}H_{19}FN_2$	71.76	8.17	11.96	71.61	8.23	11.68	0.60	3.8	6.3
5f	1	2-F	H	CH_3	A	Oil	16.8	$C_{15}H_{21}FN_2$	72.55	8.52	11.28	72.83	8.39	11.24	0.23	1.9	8.3
5g	1	2-CF_3	H	CH_3	B	Oil	7.7	$C_{16}H_{21}F_3N_2$	64.41	7.09	9.39	64.29	7.02	9.27	0.33	1.8	5.5
5h	2	2-F	H	H	C	Oil	48.0	$C_{15}H_{21}FN_2$	72.55	8.52	11.28	72.45	8.28	11.13	1.9	17.9	9.4
5i	1	2-Cl	H	H	C	Oil	44.1	$C_{14}H_{19}ClN_2$	67.05	7.64	11.17	66.85	7.49	11.06	0.85	4.4	5.2
5j	2	2-Cl	H	H	C	Oil	40.1	$C_{15}H_{21}ClN_2$	68.04	7.99	10.58	67.83	8.05	10.80	0.52	15.1	29.0
5k	1	3-CN	H	H	C	Oil	41.4	$C_{15}H_{19}N_3$	74.65	7.94	17.41	74.45	7.96	17.48	2.0	7.0	3.5
5l	1	2-CF_3	H	H	C	Oil	37.7	$C_{15}H_{19}F_3N_2$	63.37	6.74	9.85	63.19	6.64	9.70	0.41	3.4	5.2
9a	1	4-CF_3	H	H	D	35—37	27.2	$C_{15}H_{19}F_3N_2$	63.37	6.74	9.85	63.02	6.59	9.68	1.2	6.1	5.1
9b	1	2-NO_2	H	H	D	69—70	80.9	$C_{14}H_{19}N_3O_2$	64.35	7.33	16.08	64.44	7.27	16.05	0.24	2.3	9.6
9c	1	4-NO_2	H	H	D	67—68	74.4	$C_{14}H_{19}N_3O_2$	64.35	7.33	16.08	64.32	7.26	16.12	3.5	9.9	2.8
9d	1	2-NO_2	H	CH_3	D	Oil	54.1	$C_{15}H_{21}N_3O_2$	65.43	7.69	15.26	65.17	7.98	15.30	0.24	0.96	4.0
9e	1	4-NO_2	H	CH_3	D	Oil	35.1	$C_{15}H_{21}N_3O_2$	65.43	7.69	15.26	65.54	7.84	15.09	1.1	2.5	2.3
9f	2	2-NO_2	H	H	D	Oil	93.5	$C_{15}H_{21}N_3O_2$	65.43	7.69	15.26	65.20	7.93	15.06	0.12	1.4	11.7
9g	2	4-NO_2	H	H	D	Oil	82.5	$C_{15}H_{21}N_3O_2$	65.43	7.69	15.26	65.56	7.91	15.15	1.7	11.7	6.9
9h	2	2-NO_2	H	CH_3	D	Oil	85.7	$C_{16}H_{23}N_3O_2$	66.41	8.01	14.52	66.03	8.18	14.26	0.24	2.1	8.8
9i	2	4-NO_2	H	CH_3	D	Oil	62.4	$C_{16}H_{23}N_3O_2$	66.41	8.01	14.52	66.46	7.98	14.51	0.96	2.5	2.6
9j	2	2-NO_2	4-F	H	D	Oil	99.8	$C_{15}H_{20}FN_3O_2$	61.42	6.87	14.32	61.62	6.83	14.30	0.83	5.3	6.4
9k	2	2-NO_2	4-OCH_3	H	D	38—41	63.9	$C_{16}H_{23}N_3O_3$	66.41	8.01	14.52	66.45	7.92	14.66	0.58	5.9	10.2
9l	1	2-CN	H	H	D	Oil	94.2	$C_{15}H_{19}N_3$	74.65	7.94	17.41	74.35	8.01	17.48	0.66	23.1	35.0
9m	1	4-CN	H	H	D	Oil	95.0	$C_{15}H_{19}N_3$	74.65	7.94	17.41	74.79	7.96	17.38	2.3	38.7	16.8
9n	1	2-CN	H	CH_3	D	Oil	37.4	$C_{16}H_{21}N_3$	75.26	8.29	16.46	75.32	8.40	16.31	0.16	1.6	10.0
9o	2	2-CN	H	H	D	Oil	95.8	$C_{16}H_{21}N_3$	75.26	8.29	16.46	75.34	8.32	16.32	0.38	7.5	19.7
9p	2	4-CN	H	H	D	Oil	99.1	$C_{16}H_{21}N_3$	75.26	8.29	16.46	75.03	8.25	16.42	5.5	>50	>9.1
9q	2	2-CN	H	CH_3	D	Oil	99.7	$C_{17}H_{23}N_3$	75.80	8.61	15.60	75.83	8.62	15.68	0.29	4.8	16.6
9r	1	2-$CONH_2$	H	H	E	163—165	71.5	$C_{15}H_{21}N_3O$	69.47	8.16	16.20	69.48	8.13	16.08	0.79	6.5	8.2
9s	2	2-$CONH_2$	H	H	E	145—147	67.0	$C_{16}H_{23}N_3O$	70.30	8.48	15.37	70.19	8.52	15.35	1.3	>50	>38.5
(−)-YM796	—	—	—	—	—	—	—	$(C_{17}H_{25}NO_7$	57.45	7.09	3.94	57.15	7.07	3.88)	1.8	7.7	4.3
SR46559	—	—	—	—	—	—	—	$(C_{27}H_{38}N_4O_6$	63.02	7.44	10.89	62.72	7.37	10.83)	0.12	0.98	8.2

a) Refer to the procedures in the Experimental Section. *b*) K_i value (μM) calculated from the respective IC_{50} using Cheng–Prusoff equation, $K_i = IC_{50}/1+[L]/K_d$, where [L] and K_d are, respectively, ligand concentration and dissociation constant. K_d values: [^{3}H] Pirenzepine, cortex, 7.1 nM; [^{3}H] QNB, cerebellum, 0.041 nM. *c*) [^{3}H] Quinuclidinyl benzilate. *d*) **5e** was obtained by a side reaction.

Table 2. Preparations and Affinities for M_1 and M_2 Receptors of Aniline Derivatives with a 1-Azabicyclo[3.3.1]nonane Ring



Compd.	R^1	Procedure[a]	mp (°C)	Yield (%)	Formula	Analysis(%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N	Receptor affinities K_i (μM)[b] [^{3}H] Pirenzepine(M_1)	[^{3}H] QNB[c](M_2)	Ratio of [^{3}H] QNB/[^{3}H] pirenzepine
10a	2-Cl	B	59—61	30.4	$C_{14}H_{19}ClN_2$	67.05	7.64	11.17	66.81	7.62	10.93	0.059	0.47	8.0
10b	3-CN	B	119—120	19.6	$C_{15}H_{19}N_3$	74.65	7.94	17.41	74.39	7.71	17.20	2.2	3.8	1.7
10c	2-CF_3	B	Oil	33.2	$C_{15}H_{19}F_3N_2$	63.37	6.74	9.85	63.47	6.68	9.59	0.022	0.13	5.9

a—*c*) See footnotes *a*—*c*), respectively, in Table 1.

Table 3. Effect of the Compounds on Scopolamine-Induced Failure of Step-through Passive Avoidance Response in ddY Mice

Compd.	Dose (μg/kg)	*n*	R.T.[a] (s)	Criteria[b] (%)
Normal		20	288.4±10.22[c]	85[d]
Scopolamine control		30	95.9±23.54	5
9f	0.1 (*p.o.*)	20	124.6±24.66	20
	1.0 (*p.o.*)	20	176.8±26.89[e]	50[f]
	10 (*p.o.*)	20	224.7±24.66[c]	50[f]
	100 (*p.o.*)	20	179.9±25.63[e]	35[f]
9l	0.1 (*p.o.*)	20	136.1±26.24	20
	1.0 (*p.o.*)	20	191.7±25.10[g]	35[f]
	10 (*p.o.*)	20	186.1±28.25[e]	50[f]
	100 (*p.o.*)	20	204.9±26.12[g]	50[f]
10a	1.0 (*p.o.*)	20	110.3±21.54	10
	10 (*p.o.*)	20	111.9±26.32	20
	100 (*p.o.*)	20	116.6±27.59	10
Normal		20	283.5±9.34[c]	85[d]
Scopolamine control		30	86.5±17.41	10
(±)-YM796	1.0 (*p.o.*)	20	125.4±21.6	15
	10 (*p.o.*)	20	118.5±22.3	10
	100 (*p.o.*)	20	184.4±24.4[e]	30[f]

a) R.T.: The latency in retention trial. *b*) Criteria (%)=(the number of mice showing avoidance more than 300 s/total number of mice)×100. *c*) $p<0.001$, *e*) $p<0.05$, *g*) $p<0.01$ *vs.* control (Student's *t*-test). *d*) $p<0.001$, *f*) $p<0.05$ *vs.* control (Fisher's exact probability test).

Table 4. Concentration–Effect Relationship of **9f** Fumarate and Carbachol on IP Production in Primary Cultured Rat Fetal Hippocampal Cells Preloaded with [^{3}H]-*myo*-inositol

Concentration (M)	IP Production (%)	
	9f (fumarate)	Carbachol
Control	9.67±0.16	8.63±1.37
10^{-7}	11.61±0.45[a]	10.55±0.39
10^{-6}	12.45±0.49[a]	12.19±0.61
10^{-5}	13.60±0.50[b]	16.72±1.37[a]

Each value represents the mean±S.E. of 4 wells. *a*) $p<0.01$, *b*) $p<0.001$: significant difference from control (Fisher's protected least significant difference test).

Pharmacological Results and Discussion

We have already shown that quinoline and naphthalene derivatives with a 1-azabicyclo[3.3.0]octane ring had strong affinities for the muscarinic receptor.[8] Therefore, aniline derivatives can be expected to have a strong effect on the muscarinic receptor.

The compounds thus prepared were tested *in vitro* for their affinity to M_1 and M_2 receptors, and *in vivo* for behavioral efficacy to scopolamine-induced dementia models.

The affinity for the muscarinic receptor of aniline derivatives having a 1-azabicyclo[3.3.0]octane ring was examined and the results are shown in Table 1.

Initially, the effect of the substituent R^1 of the benzene ring was examined. The affinity of aniline derivatives **5** and **9** ($n=1$, $R^2=R^3=H$) for the M_1 receptor decreased in the order of **5n** (R^1=2-NO_2)>**5l** (2-CF_3)>**5e** (2-F)>**9l** (2-CN)>**9r** (2-$CONH_2$)>**5i** (2-Cl). Compounds with a substituent at the 3- or 4-position (**5k**, **5m**, **9c**, and **9m**) showed weaker M_1 activities than the 2-substituted derivatives. The ethylene-chained compounds ($n=2$, $R^2=R^3=H$) were ranked for M_1 affinity as follows: **9f** (R^1=2-NO_2, free base of SK-946)>**9o** (2-CN)>**5j** (2-Cl)>**9s** (2-$CONH_2$)>**5h** (2-F). These com-

pounds showed high selectivity to the M_1 receptor, and their M_1 receptor affinities were stronger than those of 4-substituted derivatives (**9g** and **9p**).

Among the *N*-methyl derivatives (($n=1$, $R^2=H$, $R^3=Me$), **5c** ($R^1=2$-Ph), **5f** (2-F), **5g** (2-CF_3), **9d** (2-NO_2) and **9n** (2-CN) had almost the same strong activities for the M_1 receptor, but the M_1/M_2 selectivity was low except for **9n**. The 3- or 4-substituted derivative (**5d** or **9e**) and unsubstituted derivative (**5a**) were much weaker than the *N*-methyl derivatives. The ethylene-chained compounds ($n=2$, $R^2=H$, $R^3=Me$) were ranked for M_1 affinity as follows: **9h** ($R^1=2$-NO_2)>**9q** (2-CN)>**5b** (H)>**9i** (4-NO_2).

The affinity of compounds with NO_2 substitution was compared. Under the conditions $R^1=2$-NO_2 in aniline derivatives, compound **9f** ($n=2$, $R^2=R^3=H$) showed the highest M_1 affinity and high M_1 selectivity in 2-nitroaniline derivatives. Some other aniline derivatives (**9b**, **9d**, and **9h**) also had high affinity for the M_1 receptor. The introduction of a substituent at the 4-position decreased the activity (**9j** and **9k**). In the 2-cyano compounds ($R^1=2$-CN), 2-cyano-*N*-methylaniline derivatives (**9n** and **9q**) had high binding activity to the M_1 receptor.

Similarly to the above result, the introduction of a strong electron-withdrawing group to the aniline derivative enhanced affinity for the M_1 receptor. Compounds which had a substituent at the 2-position exhibited stronger M_1 activities than 4-substituted derivatives. Some compounds which introduced a substituent at the 2-position on the aniline had strong affinity for the M_1 receptor, but less selectivity to the M_1 receptor. Two compounds were chosen as desirable samples for the *in vivo* test. Thus, compound **9f** had the most potent affinity for the M_1 receptor, and **9l** had the most M_1 selectivity of the compounds tested, having M_1 receptor selectivity more potent than 1.0 μM. **9f** was more potent and had higher selectivity than (−)-YM796 and SR46559, and **9l** had more M_1 selectivity than these. The compound **9f** ameliorated scopolamine-induced impairment in passive avoidance tasks at 1.0 μg/kg (*p.o.*) (Table 3). The agonistic property of **9f** was confirmed by receptor-stimulated phosphoinositide hydrolysis in primary cultured rat fetal hippocampal neuronal cells preloaded with [^{3}H]-*myo*-inositol (Table 4). Inositol phosphate (IP) production was expressed as [^{3}H] IP/([^{3}H] IP+incorporated [^{3}H]-*myo*-inositol)%. **9f** fumarate increased IP production at a concentration of more than 10^{-7} M.

The affinity for the muscarinic receptor of aniline derivatives with a 1-azabicyclo[3.3.1]nonane moiety was examined (Table 2). These compounds, **10a**—**c** had unexpectedly strong affinity for the muscarinic receptor. Compound **10a** had an affinity for the M_1 receptor with a K_i value of 59 nM, and high selectivity to the M_1 receptor. However, this compound was less active in ameliorating impairment in passive avoidance tasks than we expected (Table 3).

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Infrared (IR) spectra were taken with a Perkin–Elmer 1600 spectrometer. NMR spectra were recorded on a JEOL JNM-GSX270 spectrometer (270 MHz for ^{1}H and 68 MHz for ^{13}C). Chemical shifts are expressed in δ (ppm) values with tetramethylsilane as an internal standard and the following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad, dd=double doublet, ddd=double double doublet and dt=double triplet. Mass spectra (MS) were recorded on a JEOL JMS-DX300, or on a JEOL JMS-SX102.

Procedure A. *N*-(1-Azabicyclo[3.3.0]octan-5-yl)methyl-*N*-formylaniline (4a) and *N*-(1-azabicyclo[3.3.0]octan-5-yl)methyl-*N*-methylaniline (5a) To a solution of formanilide (**2a**) (1.90 g, 15.7 mmol) in *N*,*N*-dimethylformamide (DMF) (50.0 ml) was added 60% NaH (2.51 g, 62.7 mmol) at −60 °C, and the mixture was stirred at 10—15 °C for 1 h. 5-Chloromethyl-1-azabicyclo[3.3.0]octane hydrochloride (**3a**) (3.38 g, 17.2 mmol) was added to the mixture at −60 °C. After stirring at 15 °C for 3 h, the reaction mixture was poured into 200 ml of ice-water and extracted with AcOEt (600 ml). The extract was washed with brine (50 ml), dried and evaporated *in vacuo*. The residue was chromatographed on silica gel and eluted with AcOEt–triethylamine (70/1) to give 3.65 g (95.1%) of **4a** as a pale yellow oil. IR (neat) cm^{-1}: 3450, 2957, 1596, 1496. ^{1}H-NMR ($CDCl_3$) δ: 1.23—1.77 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.49 and 2.98 (each 2H, dt, J=10, 4 Hz, 2,8-CH_2 of azabicyclooctane), 3.83 (2H, s, CHO-N-C$\underline{H}_2$), 7.21 (2H, d, J=6 Hz, aromatic H), 7.29 (1H, t, J=6 Hz, aromatic H), 7.40 (2H, t, J=6 Hz, aromatic H), 8.35 (1H, s, CHO). CIMS *m/z*: 245 ((M+1)$^+$, base peak).

A solution of **4a** (2.80 g, 11.5 mmol) in THF (45.0 ml) was added dropwise to BH_3–THF (1 M) (46.0 ml, 46.0 mmol) at 0 °C, and stirred at room temperature overnight and then at 40—50 °C for 1 h. To the reaction mixture was added 6 N HCl (6.70 ml) in an ice-bath. The organic solvent was removed by distillation under atmospheric pressure. The resulting mixture was made basic by NaOH pellet in an ice-bath, extracted with Et_2O (80 ml×4), dried, and then concentrated *in vacuo*. The residue was chromatographed on silica gel and eluted with AcOEt–triethylamine to give 2.45 g (92.5%) of **5a** as a colorless oil. IR (neat) cm^{-1}: 2956, 1599, 1506. ^{1}H-NMR ($CDCl_3$) δ: 1.47—1.90 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.59 and 3.01 (each 2H, dt, J=11, 6 Hz, 2,8-CH_2 of azabicyclooctane), 3.04 (3H, s, CH_3), 3.27 (2H, s, C$\underline{H}_2$-N-CH_3), 6.65 (1H, t, J=7 Hz, aromatic H), 6.77 (2H, d, J=7 Hz, aromatic H), 7.21 (2H, t, J=7 Hz, aromatic H). EIMS *m/z*: 230 (M$^+$), 110 (base peak).

***N*-[2-(1-Azabicyclo[3.3.0]octan-5-yl)ethyl]-*N*-formylaniline (4b) and *N*-[2-(1-Azabicyclo[3.3.0]octan-5-yl)ethyl]-*N*-methylaniline (5b)** In a similar manner to that described in procedure A, a reaction of **2a** (1.80 g, 14.9 mmol) with 60% NaH (2.38 g, 59.5 mmol) and 5-(2-chloroethyl)-1-azabicyclo[3.3.0]octane hydrochloride (**3b**) (3.43 g, 16.3 mmol), as for **4a**, gave 3.39 g (88.1%) of **5b** as a pale yellow oil. IR (neat) cm^{-1}: 3529, 2954, 1682, 1596, 1497. ^{1}H-NMR ($CDCl_3$) δ: 1.50—1.83 (10H, m, 3,4,6,7-CH_2 of azabicyclooctane and OHC-N-CH_2-C$\underline{H}_2$-), 2.56 and 2.95 (each 2H, dt, J=11, 5 Hz, 2-CH_2 of azabicyclooctane), 3.81—3.90 (2H, m, OHC-N-C$\underline{H}_2$-CH_2-), 7.18 (2H, dd, J=7, 2 Hz, aromatic H), 7.29 (1H, dt, J=7, 2 Hz, aromatic H), 7.40 (2H, dt, J=7, 2 Hz, aromatic H), 8.38 (1H, s, CHO). EIMS *m/z*: 258 (M$^+$), 110 (base peak).

In a similar manner to that described in procedure A, **4b** (2.60 g, 10.1 mmol) was treated with BH_3–THF (1 M) (40.0 ml, 40.0 mmol) to give 2.01 g (81.4%) of **5b** as a pale yellow oil. IR (neat) cm^{-1}: 2952, 1600, 1507. ^{1}H-NMR ($CDCl_3$) δ: 1.55—1.82 (10H, m, 3,4,6,7-CH_2 of azabicyclooctane and CH_3-N-CH_2-C$\underline{H}_2$-), 2.60 and 3.00 (each 2H, dt, J=10, 6 Hz, 2,8-CH_2 of azabicyclooctane), 2.90 (3H, s, CH_3), 3.35—3.42 (2H, m, CH_3-N-C$\underline{H}_2$-), 6.66 (1H, t, J=7 Hz, aromatic H), 6.72 (2H, d, J=7 Hz, aromatic H), 7.22 (2H, t, J=7 Hz, aromatic H). EIMS *m/z*: 244 (M$^+$), 110 (base peak).

***N*-(1-Azabicyclo[3.3.0]octan-5-yl)methyl-*N*-methyl-2-phenylaniline (5c)** In a similar manner to that described in procedure A, a reaction of *N*-formyl-2-phenylaniline (**2c**) (286 mg, 1.45 mmol) with 60% NaH (260 mg, 6.50 mmol) and **3a** (300 mg, 1.53 mmol), as for **4a**, gave 452 mg (97.3%) of *N*-(1-azabicyclo[3.3.0]octan-5-yl)methyl-*N*-formyl-2-phenylaniline (**4c**) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.17—1.73 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.39 and 2.86 (each 2H, dt, J=10, 7 Hz, 2,8-CH_2 of azabicyclooctane), 3.02—3.18 (2H, m, OHC-N-C$\underline{H}_2$), 7.18—7.45 (9H, m, aromatic H), 8.48 (1H, s, CHO). CIMS *m/z*: 321 ((M+1)$^+$, base peak).

In a similar manner to that described in procedure A, **4c** (450 mg, 1.40 mmol) was treated with BH_3–THF (1 M) (5.00 ml, 5.00 mmol) to give 383 mg (89.3%) of **5c** as a pale yellow oil. IR (neat) cm^{-1}: 2952, 1593, 1481, 1433. ^{1}H-NMR ($CDCl_3$) δ: 1.34—1.84 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.48 and 2.87 (each 2H, dt, J=11, 7 Hz, 2,8-CH_2 of azabicyclooctane), 2.60 (3H, s, CH_3), 2.85 (2H, s, CH_3-N-C$\underline{H}_2$), 7.03 (1H, t, J=7 Hz, aromatic H), 7.19—7.30 (4H, m, aromatic H), 7.37 (2H, t, J=7 Hz, aromatic H), 7.48 (2H, d, J=7 Hz, aromatic H). CIMS *m/z*: 307 ((M+1)$^+$), 110 (base peak).

***N*-(1-Azabicyclo[3.3.0]octan-5-yl)methyl-*N*-methyl-3-phenylaniline (5d)** In a similar manner to that described in procedure A, a reaction of *N*-formyl-3-phenylaniline (**2d**) (286 mg, 1.45 mmol) with 60% NaH (260 mg, 6.50 mmol) and **3a** (300 mg, 1.53 mmol), as for **4a**, gave 385 mg (82.7%) of *N*-(1-azabicyclo[3.3.0]octan-5-yl)methyl-*N*-formyl-3-phenylaniline (**4d**) as a

colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.28—1.76 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.50 and 2.99 (each 2H, dt, J=10, 6 Hz, 2,8-CH_2 of azabicyclooctane), 3.87 (2H, s, OHC-N-C$\underline{H}_2$), 7.18—7.60 (9H, m, aromatic H), 8.43 (1H, s, CHO). CIMS *m/z*: 321 ($(M+1)^+$, base peak).

In a similar manner to that described in procedure A, **4d** (370 mg, 1.15 mmol) was treated with BH_3–THF (1 M) (4.00 ml, 4.00 mmol) to give 193 mg (54.8%) of **5d** as a pale yellow oil. IR (neat) cm^{-1}: 2953, 1597, 1490. ^{1}H-NMR ($CDCl_3$) δ: 1.50—1.84 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.60 and 3.04 (each 2H, dt, J=10, 6 Hz, 2,8-CH_2 of azabicyclooctane), 3.11 (3H, s, CH_3), 3.33 (2H, s, CH_3-N-C$\underline{H}_2$), 6.76—7.02 (3H, m, aromatic H), 7.24—7.35 (2H, m, aromatic H), 7.42 (2H, t, J=7 Hz, aromatic H), 7.59 (2H, d, J=7 Hz, aromatic H). CIMS *m/z*: 307 ($(M+1)^+$), 110 (base peak).

***N*-(1-Azabicyclo[3.3.0]octan-5-yl)methyl-2-fluoroaniline (5e) and *N*-(1-azabicyclo[3.3.0]octan-5-yl)methyl-*N*-methyl-2-fluoroaniline (5f)** In a similar manner to that described in procedure A, a reaction of 2-fluoro-*N*-formylaniline (**2e**) (3.00 g, 21.6 mmol) with 60% NaH (3.46 mg, 86.5 mmol) and **3a** (4.66 g, 23.8 mmol) as for **4a** gave 1.55 g (27.3%) of *N*-(1-azabicyclo[3.3.0]octan-5-yl)methyl-*N*-formyl-2-fluoroaniline (**4e**) as a colorless oil and 2.76 g (54.6%) of **5e** as a colorless oil. **4e**: ^{1}H-NMR ($CDCl_3$) δ: 1.31—1.77 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.44—2.59 and 2.89—3.01 (each 2H, m, 2,8-CH_2 of azabicyclooctane), 3.78 (2H, m, OHC-N-C$\underline{H}_2$), 7.15—7.35 (4H, m, aromatic H), 8.38 (1H, s, CHO). CIMS *m/z*: 263 ($(M+1)^+$, base peak). **5e**: IR (neat) cm^{-1}: 3368, 2954, 1619, 1522. ^{1}H-NMR ($CDCl_3$) δ: 1.57—1.91 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.60—2.69 and 3.01—3.09 (each 2H, m, 2,8-CH_2 of azabicyclooctane), 2.97 (2H, s, N-CH_2), 4.36 (1H, br s, NH), 6.54—6.71 (2H, m, aromatic H), 6.91—7.00 (2H, m, aromatic H). CIMS *m/z*: 235 ($(M+1)^+$), 110 (base peak).

In a similar manner to that described in procedure A, **4e** (1.55 g, 5.91 mmol) was treated with BH_3–THF (1 M) (24.0 ml, 24.0 mmol) to give 1.22 g (85.9%) of **5f** as a colorless oil. IR (neat) cm^{-1}: 2952, 1611, 1501, 1213. ^{1}H-NMR ($CDCl_3$) δ: 1.46—1.98 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.56 and 2.98 (each 2H, dt, J=10, 6 Hz, 2,8-CH_2 of azabicyclooctane), 2.96 (3H, s, CH_3), 3.13 (2H, s, CH_3-N-C$\underline{H}_2$), 6.76—6.83 (1H, m, aromatic H), 6.93—7.03 (3H, m, aromatic H). CIMS *m/z*: 249 ($(M+1)^+$, base peak).

Procedure B. *N*-(1-Azabicyclo[3.3.0]octan-5-yl)methyl-*N*-methyl-2-(trifluoromethyl)aniline (5g) A reaction of *N*-methyl-2-(trifluoromethyl) aniline (**6**) (570 mg, 3.25 mmol) with 60% NaH (521 mg, 13.0 mmol) and **3a** (702 mg, 3.60 mmol) in DMF (10.0 ml) gave 74.7 mg (7.7%) of **5g** as a pale yellow oil. IR (neat) cm^{-1}: 2951, 2864, 1597, 1492. ^{1}H-NMR ($CDCl_3$) δ: 1.41—1.97 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.52—2.60 and 2.96—3.04 (each 2H, m, 2,8-CH_2 of azabicyclooctane), 2.70 (3H, s, CH_3), 2.97 (2H, s, CH_3-N-C$\underline{H}_2$), 6.97—3.04 (1H, m, aromatic H), 7.14—7.17 (3H, m, aromatic H). CIMS *m/z*: 299 ($(M+1)^+$), 110 (base peak).

Procedure C. *N*-[2-(1-Azabicyclo[3.3.0]octan-5-yl)ethyl-2-fluoroaniline (5h) A suspension of 2-fluoroaniline (**1f**) (400 mg, 3.60 mmol), **3b** (1.51 g, 7.20 mmol) and K_2CO_3 (2.99 g, 21.6 mmol) in nitrobenzene (12.5 ml) was stirred at 120 °C for 20 h. 10% NaOH (15 ml) was added to the resulting mixture and extracted with AcOEt (80 ml×3). The extract was washed with brine (150 ml), dried, and concentrated *in vacuo*. The residue was chromatographed on silica gel and eluted with AcOEt–triethylamine to give 429 mg (48.0%) of **5h** as a colorless oil. IR (neat) cm^{-1}: 3241, 2954, 1619, 1524. ^{1}H-NMR ($CDCl_3$) δ: 1.55—1.78 (10H, m, 3,4,6,7-CH_2 of azabicyclooctane and NH-CH_2-C$\underline{H}_2$), 2.54—2.60 and 2.97—3.04 (each 2H, m, 2,8-CH_2 of azabicyclooctane), 3.18 (2H, t, J=6 Hz, NH-C$\underline{H}_2$-CH_2), 5.79 (1H, br s, NH), 6.53—6.68 (2H, m, aromatic H), 6.87—6.95 (2H, m, aromatic H). EIMS *m/z*: 248 (M^+), 110 (base peak).

***N*-(1-Azabicyclo[3.3.0]octan-5-yl)methyl-2-chloroaniline (5i) and *N*-(1-Azabicyclo[3.3.1]nonan-5-yl)-2-chloroaniline (10a)** In a similar manner to that described in procedure C, a reaction of 2-chloroaniline (**1i**) (1.46 g, 11.4 mmol), **3a** (4.50 g, 22.9 mmol) and K_2CO_3 (4.74 g, 34.3 mmol) in nitrobenzene (80.0 ml) gave 1.27 g (44.1%) of **5i** as a brown oil and 870 mg (30.4%) of **10a** as a white powder. **5i**: IR (neat) cm^{-1}: 3352, 2954, 1599, 1510. ^{1}H-NMR ($CDCl_3$) δ: 1.59—1.70 (2H, m, 3,7-CH_2 of azabicyclooctane), 1.70—1.92 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.62—2.71 and 3.04—3.12 (each 2H, m, 2,8-CH_2 of azabicyclooctane), 2.98 (2H, d, J=5 Hz, NH-C$\underline{H}_2$), 4.82 (1H, br s, NH), 6.59 (1H, dt, J=2, 8 Hz, aromatic H), 6.63 (1H, dd, J=8, 2 Hz, aromatic H), 7.11 (1H, dt, J=2, 8 Hz, aromatic H), 7.23 (1H, dd, J=8, 2 Hz, aromatic H). ^{13}C-NMR ($CDCl_3$) δ: 25.1 (3,7-C of azabicyclooctane), 36.7 (4,6-C of azabicyclooctane), 52.1 (NH-C$\underline{H}_2$), 55.6 (2,8-C of azabicyclooctane), 72.8 (5-C of azabicyclooctane), 111.2 (6-C of aniline), 116.6 (4-C of aniline), 119.3 (2-C of aniline), 127.7 (5-C of aniline), 129.0 (3-C of aniline), 144.8 (1-C of aniline). EIMS *m/z*: 110 (base peak). CIMS *m/z*: 251 ($(M+1)^+$), 110 (base peak). **10a**: mp 59—61 °C. IR (KBr) cm^{-1}: 3388, 2929, 1594, 1342. ^{1}H-NMR ($CDCl_3$) δ: 1.55—1.62 (2H, m, 3,7-CH_2 of azabicyclononane), 1.83—1.95 (2H, m, 4,6-CH_2 of azabicyclononane), 2.00—2.14 (2H, m, 3,7-CH_2 of azabicyclononane), 2.27—2.33 (2H, m, 4,6-CH_2 of azabicyclononane), 2.97—3.07 (4H, m, 2,8-CH_2 of azabicyclononane), 3.01 (2H, s, 9-CH_2 of azabicyclononane), 4.03 (1H, br s, NH), 6.65 (1H, dt, J=2, 7 Hz, aromatic H), 6.95 (1H, dd, J=8, 2 Hz, aromatic H), 7.08 (1H, dt, J=2, 7 Hz, aromatic H), 7.25 (1H, dd, J=8, 2 Hz, aromatic H). ^{13}C-NMR ($CDCl_3$) δ: 23.7 (3,7-C of azabicyclononane), 35.6 (4,6-C of azabicyclononane), 48.7 (5-C of azabicyclononane), 51.7 (2,8-C of azabicyclononane), 59.9 (9-C of azabicyclononane), 116.5 (6-C-aniline), 118.1 (4-C-aniline), 121.5 (2-C-aniline),127.1 (5-C-aniline), 129.5 (3-C-aniline), 142.1 (1-C-aniline). EIMS *m/z*: 250 (M^+), 71 (base peak).

***N*-[2-(1-Azabicyclo[3.3.0]octan-5-yl)ethyl]-2-chloroaniline (5j)** In a similar manner to that described in procedure C, a reaction of **1i** (600 mg, 4.60 mmol), **3b** (1.98 g, 9.42 mmol) and K_2CO_3 (3.90 g, 28.2 mmol) in nitrobenzene (30.0 ml) gave 499 mg (40.1%) of **5j** as a colorless oil. IR (neat) cm^{-1}: 3217, 2954, 1598, 1516. ^{1}H-NMR ($CDCl_3$) δ: 1.59—1.81 (10H, m, 3,4,6,7-CH_2 of azabicyclooctane and NH-CH_2-C$\underline{H}_2$), 2.57—2.61 and 3.03—3.06 (each 2H, m, 2,8-CH_2 of azabicyclooctane), 3.20 (2H, t, J=6 Hz, NH-C$\underline{H}_2$-CH_2), 6.50 (1H, br s, NH), 6.54—6.60 (2H, m, aromatic H), 7.10 (1H, dt, J=2, 8 Hz, aromatic H), 7.21 (1H, dd, J=8, 2 Hz, aromatic H). EIMS *m/z*: 264 (M^+), 110 (base peak).

3-(1-Azabicyclo[3.3.0]octan-5-yl)methylaminobenzonitrile (5k) and 3-(1-Azabicyclo[3.3.1]nonan-5-yl)aminobenzonitrile (10b) In a similar manner to that described in procedure C, a reaction of 3-aminobenzonitrile (**1k**) (1.28 g, 10.8 mmol), **3a** (4.25 g, 21.7 mmol) and K_2CO_3 (4.50 g, 32.6 mmol) in nitrobenzene (80.0 ml) gave 1.08 g (41.4%) of **5k** as a brown oil and 510 mg (19.6%) of **10b**. **5k**: IR (neat) cm^{-1}: 3383, 2956, 2227, 1601. ^{1}H-NMR ($CDCl_3$) δ: 1.61—1.90 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.62—2.70 and 2.97—3.06 (each 2H, m, 2,8-CH_2 of azabicyclooctane), 2.93 (2H, d, J=6 Hz, NH-C$\underline{H}_2$), 4.51 (1H, br s, NH), 6.80 (1H, dt, J=2, 7 Hz, aromatic H), 6.81 (1H, d, J=2 Hz, aromatic H), 6.92 (1H, dt, J=2, 7 Hz, aromatic H), 7.19 (1H, dt, J=8, 2 Hz, aromatic H). CIMS *m/z*: 241 ($(M+1)^+$, base peak). **10b**: mp 119—120 °C. IR (KBr) cm^{-1}: 3269, 2937, 2228, 1600. ^{1}H-NMR ($CDCl_3$) δ: 1.58—1.65 (2H, m, 3,7-CH_2 of azabicyclononane), 1.77—1.90 (2H, m, 4,6-CH_2 of azabicyclononane), 2.04—2.15 (2H, m, 3,7-CH_2 of azabicyclononane), 2.25—2.32 (2H, m, 4,6-CH_2 of azabicyclononane), 2.93 (2H, s, 9-CH_2 of azabicyclononane), 3.00—3.07 (4H, m, 2,8-CH_2 of azabicyclononane), 3.45 (1H, br s, NH) 6.84—6.99 (3H, m, aromatic H), 7.19 (1H, t, J=9 Hz, aromatic H). CIMS *m/z*: 241 ($(M+1)^+$), 71 (base peak).

***N*-(1-Azabicyclo[3.3.0]octan-5-yl)methyl-2-(trifluoromethyl)aniline (5l) and *N*-(1-Azabicyclo[3.3.1]nonan-5-yl)methyl-2-(trifluoromethyl) aniline (10c)** In a similar manner to that described in procedure C, a reaction of 2-(trifluoromethyl)aniline (**1l**) (150 mg, 0.931 mmol), **3a** (365 mg, 1.86 mmol) and K_2CO_3 (900 mg, 5.59 mmol) in nitrobenzene (5.00 ml) gave 99.8 mg (37.7%) of **5l** as a brown oil and 87.8 mg (33.2%) of **10c**. **5l**: IR (neat) cm^{-1}: 3376, 2956, 1615, 1515. ^{1}H-NMR ($CDCl_3$) δ: 1.61—1.87 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.61—2.69 and 3.02—3.10 (each 2H, m, 2,8-CH_2 of azabicyclooctane), 2.97 (2H, d, J=5 Hz, NH-C$\underline{H}_2$), 5.04 (1H, br s, NH), 6.66 (1H, t, J=7 Hz, aromatic H), 6.68 (1H, d, J=7 Hz, aromatic H), 7.35 (1H, t, J=7 Hz, aromatic H), 7.42 (1H, d, J=7 Hz, aromatic H). CIMS *m/z*: 285 ($(M+1)^+$, base peak). **10c**: IR (neat) cm^{-1}: 3458, 2936, 1614, 1588. ^{1}H-NMR ($CDCl_3$) δ: 1.56—1.62 (2H, m, 3,7-CH_2 of azabicyclononane), 1.82—1.94 (2H, m, 4,6-CH_2 of azabicyclononane), 2.04—2.10 (2H, m, 3,7-CH_2 of azabicyclononane), 2.31—2.37 (2H, m, 4,6-CH_2 of azabicyclononane), 2.98—3.07 (4H, m, 2,8-CH_2 of azabicyclononane), 2.99 (2H, s, 9-CH_2 of azabicyclononane), 4.03 (1H, br s, NH), 6.71 (1H, t, J=8 Hz, aromatic H), 6.98 (1H, d, J=8 Hz, aromatic H), 7.29 (1H, t, J=8 Hz, aromatic H), 7.42 (1H, d, J=8 Hz, aromatic H). CIMS *m/z*: 285 ($(M+1)^+$, base peak).

Procedure D. *N*-(1-Azabicyclo[3.3.0]octan-5-yl)methyl-4-(trifluoromethyl)aniline (9a) A suspension of 4-fluorobenzotrifluoride (**7a**) (1.50 g, 9.14 mmol), 5-aminomethyl-1-azabicyclo[3.3.0]octane (**8a**) (3.20 g, 22.9 mmol), and sodium acetate (500 mg) in pyridine (8.0 ml) was stirred at 170 °C for 20 h in a sealed tube and then concentrated *in vacuo*. To the residue was added 1 N NaOH (30 ml), and the mixture was extracted with AcOEt (80 ml×3). The extract was washed with brine (200 ml), dried, and concentrated *in vacuo*. The residue was chromatographed on silica gel eluting with AcOEt–triethylamine (20/1) to give 708 mg (27.2%) of **9a** as a yellow powder, mp 35—37 °C. IR (KBr) cm^{-1}: 3348, 2951, 1618, 1329. ^{1}H-NMR ($CDCl_3$) δ: 1.60—1.87 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.62—2.70 and 3.01—3.07 (each 2H, m, 2,8-CH_2 of azabicyclooctane), 2.97

(2H, d, J=5 Hz, NH-C$\underline{H}_2$), 4.58 (1H, br s, NH), 6.61 (2H, d, J=9 Hz, aromatic H), 7.37 (2H, d, J=9 Hz, aromatic H). CIMS *m/z*: 285 ((M+1)$^+$), 110 (base peak).

***N*-(1-Azabicyclo[3.3.0]octan-5-yl)methyl-2-nitroaniline (9b)** In a similar manner to that described in procedure D, a solution of *o*-chloronitrobenzene (**7b**) (3.16 g, 20.1 mmol) and **8a** (5.60 g, 39.9 mmol) in EtOH was stirred in a sealed tube at 120 °C for 19 h to give 4.02 g (80.9%) of **9b** as a yellow powder, mp 69—70 °C. IR (KBr) cm^{-1}: 3362, 2950, 1622, 1513. ^{1}H-NMR ($CDCl_3$) δ: 1.64—1.90 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.63—2.73 and 3.09—3.18 (each 2H, m, 2,8-CH_2 of azabicyclooctane), 3.16 (2H, d, J=5 Hz, C$\underline{H}_2$-NH), 6.60 (1H, t, J=9 Hz, aromatic H), 6.83 (1H, d, J=9 Hz, aromatic H), 7.40 (1H, t, J=9 Hz, aromatic H), 8.16 (1H, d, J=9 Hz, aromatic H), 8.36 (1H, br s, NH). EIMS *m/z*: 261 (M$^+$), 110 (base peak).

***N*-(1-Azabicyclo[3.3.0]octan-5-yl)methyl-4-nitroaniline (9c)** In a similar manner to that described in procedure D, a solution of *p*-chloronitrobenzene (**7c**) (3.16 g, 20.1 mmol) and **8a** (5.60 g, 39.9 mmol) in EtOH was stirred in a sealed tube at 120 °C for 24 h to give 3.70 g (74.4%) of **9c** as a yellow powder, mp 67—68 °C. IR (KBr) cm^{-1}: 3368, 2952, 1602, 1502. ^{1}H-NMR ($CDCl_3$) δ: 1.63—1.89 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.62—2.72 and 2.96—3.06 (each 2H, m, 2,8-CH_2 of azabicyclooctane), 3.04 (2H, d, J=5 Hz, C$\underline{H}_2$-NH), 5.27 (1H, br s, NH), 6.53 (2H, d, J=9 Hz, aromatic H), 8.06 (2H, d, J=9 Hz, aromatic H). EIMS *m/z*: 261 (M$^+$), 110 (base peak).

***N*-(1-Azabicyclo[3.3.0]octan-5-yl)methyl-*N*-methyl-2-nitroaniline (9d)** In a similar manner to that described in procedure D, a suspension of **7b** (1.27 g, 8.06 mmol), 5-(methylaminomethyl)-1-azabicyclo[3.3.0]octane (**8b**) (2.50 g, 16.2 mmol) and NaI (50.0 mg) in pyridine was refluxed for 12 h to give 1.20 g (54.1%) of **9d** as a yellow oil. IR (neat) cm^{-1}: 2955, 1604, 1514. ^{1}H-NMR ($CDCl_3$) δ: 1.51—1.90 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.55 and 2.99 (each 2H, dt, J=11, 6 Hz, 2,8-CH_2 of azabicyclooctane), 2.90 (3H, s, CH_3), 3.26 (2H, s, CH_3-N-C$\underline{H}_2$), 6.83 (1H, ddd, J=8, 7, 1 Hz, aromatic H), 7.25 (1H, dd, J=7, 1 Hz, aromatic H), 7.36 (1H, ddd, J=8, 7, 1 Hz, aromatic H), 7.71 (1H, dd, J=7, 1 Hz, aromatic H). CIMS *m/z*: 276 ((M+1)$^+$), 110 (base peak).

***N*-(1-Azabicyclo[3.3.0]octan-5-yl)methyl-*N*-methyl-4-nitroaniline (9e)** In a similar manner to that described in procedure D, a suspension of **7c** (1.27 g, 8.06 mmol), **8b** (2.50 g, 16.2 mmol) and NaI (50.0 mg) in pyridine was refluxed for 12 h to give 780 mg (35.1%) of **9e** as a yellow oil. IR (neat) cm^{-1}: 2956, 1595, 1310. ^{1}H-NMR ($CDCl_3$) δ: 1.52—1.80 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.60 and 2.96 (each 2H, dt, J=11, 6 Hz, 2,8-CH_2 of azabicyclooctane), 3.20 (3H, s, CH_3), 3.41 (2H, s, CH_3-N-C$\underline{H}_2$), 6.73 (2H, d, J=9 Hz, aromatic H), 8.09 (2H, d, J=9 Hz, aromatic H). CIMS *m/z*: 276 ((M+1)$^+$), 110 (base peak).

***N*-[2-(1-Azabicyclo[3.3.0]octan-5-yl)ethyl]-2-nitroaniline (9f)** In a similar manner to that described in procedure D, a mixture of **7b** (2.04 g, 12.9 mmol), 5-(2-aminoethyl)-1-azabicyclo[3.3.0]octane (**8c**) (4.00 g, 25.9 mmol) and pyridine (40.0 ml) was refluxed for 12 h to give 3.32 g (93.5%) of **9f** as a yellow oil. IR (neat) cm^{-1}: 3382, 2954, 1619, 1514. ^{1}H-NMR ($CDCl_3$) δ: 1.61—1.85 (10H, m, 3,4,6,7-CH_2 of azabicyclooctane and NH-$CH_2C\underline{H}_2$), 2.62 and 3.07 (each 2H, dt, J=11, 6 Hz, 2,8-CH_2 of azabicyclooctane), 3.33—3.40 (2H, m, NH-C$\underline{H}_2$), 6.58 (1H, ddd, J=9, 7, 2 Hz, aromatic H), 6.84 (1H, dd, J=9, 2 Hz, aromatic H), 7.40 (1H, ddd, J=9, 7, 2 Hz, aromatic H), 8.16 (1H, dd, J=9, 2 Hz, aromatic H), 9.19 (1H, br s, NH). EIMS *m/z*: 275 (M$^+$), 110 (base peak).

***N*-[2-(1-Azabicyclo[3.3.0]octan-5-yl)ethyl]-4-nitroaniline (9g)** In a similar manner to that described in procedure D, a solution of **7c** (2.04 g, 12.9 mmol) and **8c** (4.00 g, 25.9 mmol) in pyridine (40.0 ml) was stirred in a sealed tube at 120 °C for 36 h to give 2.93 g (82.5%) of **9g** as a yellow oil. IR (neat) cm^{-1}: 3374, 2955, 1602, 1310. ^{1}H-NMR ($CDCl_3$) δ: 1.61—1.83 (10H, m, 3,4,6,7-CH_2 of azabicyclooctane and NH-$CH_2C\underline{H}_2$), 2.63 and 3.00 (each 2H, dt, J=11, 6 Hz, 2,8-CH_2 of azabicyclooctane), 3.27 (2H, t, J=6 Hz, NH-C$\underline{H}_2$), 6.44 (2H, d, J=9 Hz, aromatic H), 7.76 (1H, br s, NH), 8.06 (2H, d, J=9 Hz, aromatic H). EIMS *m/z*: 275 (M$^+$), 110 (base peak).

***N*-[2-(1-Azabicyclo[3.3.0]octan-5-yl)ethyl]-*N*-methyl-2-nitroaniline (9h)** In a similar manner to that described in procedure D, a solution of **7b** (1.87 g, 11.9 mmol) and 5-(2-methylaminoethyl)-1-azabicyclo[3.3.0]octane (**8d**) (4.00 g, 23.8 mmol) in pyridine (40.0 ml) was stirred in a sealed tube at 125 °C for 18 h to give 2.95 g (85.7%) of **9h** as a yellow oil. IR (neat) cm^{-1}: 2953, 1606, 1514. ^{1}H-NMR ($CDCl_3$) δ: 1.51—1.81 (10H, m, 3,4,6,7-CH_2 of azabicyclooctane and CH_3-NH-$CH_2C\underline{H}_2$), 2.58 and 2.96 (each 2H, dt, J=10, 6 Hz, 2,8-CH_2 of azabicyclooctane), 2.81 (3H, s, CH_3), 3.18—3.24 (2H, m, NH-C$\underline{H}_2$), 6.83 (1H, t, J=8 Hz, aromatic H), 7.11 (1H, d, J=8 Hz, aromatic H), 7.38 (1H, t, J=8 Hz, aromatic H), 7.70 (1H, d, J=8 Hz, aromatic H). EIMS *m/z*: 289 (M$^+$), 110 (base peak).

***N*-[2-(1-Azabicyclo[3.3.0]octan-5-yl)ethyl]-*N*-methyl-4-nitroaniline (9i)** In a similar manner to that described in procedure D, a solution of **7c** (1.87 g, 11.9 mmol) and **8d** (4.00 g, 23.8 mmol) in pyridine (40.0 ml) was stirred in a sealed tube at 125 °C for 30 h to give 2.15 g (62.4%) of **9i** as a yellow oil. IR (neat) cm^{-1}: 2953, 1595, 1290. ^{1}H-NMR ($CDCl_3$) δ: 1.59—1.85 (10H, m, 3,4,6,7-CH_2 of azabicyclooctane and CH_3-N-$CH_2C\underline{H}_2$), 2.63 and 3.01 (each 2H, dt, J=11, 6 Hz, 2,8-CH_2 of azabicyclooctane), 3.05 (3H, s, CH_3), 3.48—3.54 (2H, m, CH_3-NH-C$\underline{H}_2$), 6.63 (2H, d, J=10 Hz, aromatic H), 8.10 (2H, d, J=10 Hz, aromatic H). EIMS *m/z*: 289 (M$^+$), 110 (base peak).

***N*-[2-(1-Azabicyclo[3.3.0]octan-5-yl)ethyl]-4-fluoro-2-nitroaniline (9j)** In a similar manner to that described in procedure D, a reaction of 2,5-difluoronitrobenzene (**7d**) (1.00 g, 6.28 mmol) and **8c** (2.42 g, 15.7 mmol) in pyridine (15.0 ml) at 45 °C for 1 h gave 2.00 g (99.8%) of **9j** as a yellow oil. IR (neat) cm^{-1}: 3380, 1520, 1180. ^{1}H-NMR ($CDCl_3$) δ: 1.63—1.84 (10H, m, 3,4,6,7-CH_2 of azabicyclooctane and NH-$CH_2C\underline{H}_2$), 2.61—2.65 and 3.02—3.08 (each 2H, m, 2-CH_2 of azabicyclooctane), 3.33—3.36 (2H, m, NH-C$\underline{H}_2$), 6.82 (1H, dd, J=9, 5 Hz, aromatic H), 7.22 (1H, ddd, J=9, 4, 3 Hz, aromatic H), 7.87 (1H, dd, J=9, 3 Hz, aromatic H), 9.25 (1H, br s, NH). EIMS *m/z*: 293 (M$^+$), 110 (base peak).

***N*-[2-(1-Azabicyclo[3.3.0]octan-5-yl)ethyl]-4-methoxy-2-nitroaniline (9k)** In a similar manner to that described in procedure D, a solution of 2-chloro-5-methoxynitrobenzene (**7e**) (1.00 g, 5.33 mmol) and **8c** (1.65 g, 10.7 mmol) in pyridine (15.0 ml) was stirred in a sealed tube at 150 °C for 2 d to give 1.04 g (63.9%) of **9k** as a red powder, mp 38—41 °C. IR (KBr) cm^{-1}: 3278, 2950, 1523. ^{1}H-NMR ($CDCl_3$) δ: 1.59—1.85 (10H, m, 3,4,6,7-CH_2 of azabicyclooctane and NH-$CH_2C\underline{H}_2$), 2.58—2.67 and 3.02—3.11 (each 2H, m, 2,8-CH_2 of azabicyclooctane), 3.33—3.40 (2H, m, NH-C$\underline{H}_2$), 3.79 (3H, s, CH_3), 6.83 (1H, d, J=9 Hz, aromatic H), 7.13 (1H, dd, J=9, 3 Hz, aromatic H), 7.62 (1H, d, J=3 Hz, aromatic H), 9.04 (1H, br s, NH). EIMS *m/z*: 305 (M$^+$), 110 (base peak).

2-(1-Azabicyclo[3.3.0]octan-5-yl)methylaminobenzonitrile (9l) In a similar manner to that described in procedure D, a solution of 2-fluorobenzonitrile (**7f**) (671 mg, 5.54 mmol) and **8a** (1.94 g, 13.9 mmol) in pyridine (8.00 ml) was stirred in a sealed tube at 180 °C for 15 h to give 1.26 g (94.2%) of **9l** as a colorless oil. IR (neat) cm^{-1}: 3346, 2955, 1606, 1511. ^{1}H-NMR ($CDCl_3$) δ: 1.63—1.86 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.62—2.68 and 2.99—3.11 (each 2H, m, 2,8-CH_2 of azabicyclooctane), 2.99 (2H, d, J=5 Hz, NH-C$\underline{H}_2$), 5.25 (1H, br s, NH), 6.59—6.65 (2H, m, aromatic H), 7.34—7.37 (2H, m, aromatic H). CIMS *m/z*: 242 ((M+1)$^+$, base peak).

4-(1-Azabicyclo[3.3.0]octan-5-yl)methylaminobenzonitrile (9m) In a similar manner to that described in procedure D, a solution of 4-fluorobenzonitrile (**7g**) (1.00 g, 8.26 mmol) and **8a** (2.31 g, 16.5 mmol) in pyridine (10.0 ml) was stirred in a sealed tube at 180 °C for 5 h to give 1.89 g (95.0%) of **9m** as a colorless oil. IR (neat) cm^{-1}: 3368, 2956, 2211, 1606, 1526. ^{1}H-NMR ($CDCl_3$) δ: 1.60—1.89 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.62—2.68 and 2.97—3.06 (each 2H, m, 2,8-CH_2 of azabicyclooctane), 2.97 (2H, d, J=5 Hz, NH-C$\underline{H}_2$), 4.85 (1H, br s, NH), 6.56 (2H, d, J=9 Hz, aromatic H), 7.39 (2H, d, J=9 Hz, aromatic H). CIMS *m/z*: 242 ((M+1)$^+$, base peak).

2-[*N*-(1-Azabicyclo[3.3.0]octan-5-yl)methyl-*N*-methylamino]benzonitrile (9n) In a similar manner to that described in procedure D, a solution of **7f** (1.00 g, 8.26 mmol) and **8b** (2.55 g, 16.5 mmol) in pyridine (10.0 ml) was stirred in a sealed tube at 180 °C for 5 h to give 790 mg (37.4%) of **9n** as a colorless oil. IR (neat) cm^{-1}: 2954, 2209, 1597, 1500. ^{1}H-NMR ($CDCl_3$) δ: 1.55—1.88 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.53—2.62 and 2.95—3.03 (each 2H, m, 2,8-CH_2 of azabicyclooctane), 3.20 (3H, s, C$\underline{H}_3$), 3.37 (2H, d, J=5 Hz, NH-C$\underline{H}_2$), 6.80 (1H, t, J=8 Hz, aromatic H), 7.03 (1H, d, J=8 Hz, aromatic H), 7.37 (1H, t, J=8 Hz, aromatic H), 7.47 (1H, d, J=8 Hz, aromatic H). CIMS *m/z*: 256 ((M+1)$^+$, base peak).

2-[2-(1-Azabicyclo[3.3.0]octan-5-yl)ethylamino]benzonitrile (9o) In a similar manner to that described in procedure D, a solution of **7f** (1.00 g, 8.26 mmol) and **8c** (3.15 g, 20.4 mmol) in pyridine (10.0 ml) was stirred in a sealed tube at 180 °C for 10.5 h to give 2.02 mg (95.8%) of **9o** as a colorless oil. IR (neat) cm^{-1}: 3170, 2955, 2209, 1606, 1517. ^{1}H-NMR ($CDCl_3$) δ: 1.59—1.80 (10H, m, 3,4,6,7-CH_2 of azabicyclooctane and NH-$CH_2C\underline{H}_2$), 2.58—2.65 and 3.07—3.13 (each 2H, m, 2,8-CH_2 of azabicyclooctane), 3.22 (2H, t, J=6 Hz, NH-C$\underline{H}_2$), 6.53—6.58 (2H, m, aromatic H), 7.31—7.35 (2H, m, aromatic H), 8.00 (1H, br s, NH). EIMS *m/z*: 255 (M$^+$), 110 (base peak).

4-[2-(1-Azabicyclo[3.3.0]octan-5-yl)ethylamino]benzonitrile (9p) In a similar manner to that described in procedure D, a solution of **7g** (1.00 g, 8.26 mmol) and **8c** (2.55 g, 16.5 mmol) in pyridine (10.0 ml) was stirred in a

sealed tube at 180 °C for 5 h to give 2.09 mg (99.1%) of **9p** as a colorless oil. IR (neat) cm^{-1}: 3371, 2955, 2210, 1608, 1527. ^{1}H-NMR ($CDCl_3$) δ: 1.55—1.84 (10H, m, 3,4,6,7-CH_2 of azabicyclooctane and NH-$CH_2C\underline{H}_2$), 2.55—2.63 and 2.92—3.02 (each 2H, m, 2,8-CH_2 of azabicyclooctane), 3.20 (2H, t, J=6 Hz, NH-$C\underline{H}_2$), 6.49 (2H, d, J=9 Hz, aromatic H), 7.13 (1H, br s, NH) 7.38 (2H, d, J=9 Hz, aromatic H). EIMS *m/z*: 255 (M^+), 110 (base peak).

2-{*N*-[2-(1-Azabicyclo[3.3.0]octan-5-yl)ethyl]-*N*-methylamino}benzonitrile (9q) In a similar manner to that described in procedure D, a solution of **7f** (800 mg, 6.61 mmol) and **8d** (2.78 g, 16.5 mmol) in pyridine (8.00 ml) was stirred in a sealed tube at 130 °C for 5.5 h to give 2.16 g (99.7%) of **9q** as a colorless oil. IR (neat) cm^{-1}: 2954, 2212, 1597, 1492. ^{1}H-NMR ($CDCl_3$) δ: 1.55—1.78 (10H, m, 3,4,6,7-CH_2 of azabicyclooctane and N-$CH_2C\underline{H}_2$), 2.56—2.60 and 2.95—3.05 (each 2H, m, 2,8-CH_2 of azabicyclooctane), 2.98 (3H, s, CH_3), 3.38—3.44 (2H, m, CH_3-N-$C\underline{H}_2$), 6.81 (1H, t, J=9 Hz, aromatic H), 6.93 (1H, d, J=9 Hz, aromatic H), 7.48 (1H, t, J=9 Hz, aromatic H), 7.50 (1H, d, J=9 Hz, aromatic H). CIMS *m/z*: 270 ($(M+1)^+$, base peak).

Procedure E. 2-[(1-Azabicyclo[3.3.0]octan-5-yl)methylamino]benzamide (9r) A solution of **9l** (1.80 g, 7.46 mmol) in 93% H_2SO_4 (125 g) was stirred at 60 °C for 3 h. After being cooled at −78 °C, the resulting mixture was alkalized with 25% ammonia–water (500 ml), and extracted with AcOEt (750 ml). The solution was washed with brine (500 ml), dried, and concentrated *in vacuo*. The residue was recrystallized with AcOEt to give 1.38 g (71.5%) of **9r** as colorless crystals, mp 163—165 °C. IR (KBr) cm^{-1}: 3320, 2830, 1624, 1522. ^{1}H-NMR ($CDCl_3$) δ: 1.59—1.97 (8H, m, 3,4,6,7-CH_2 of azabicyclooctane), 2.61—2.69 and 3.08—3.16 (each 2H, m, 2,8-CH_2 of azabicyclooctane), 3.03 (2H, d, J=5 Hz, NH-$C\underline{H}_2$), 5.78 (2H, br s, $CONH_2$), 6.54 (1H, t, J=7 Hz, aromatic H), 6.68 (1H, d, J=8 Hz, aromatic H), 7.30 (1H, t, J=7 Hz, aromatic H), 7.36 (1H, d, J=8 Hz, aromatic H), 7.99 (1H, br s, NH). CIMS *m/z*: 260 ($(M+1)^+$, base peak).

2-[2-(1-Azabicyclo[3.3.0]octan-5-yl)ethyl]aminobenzamide (9s) A reaction of **9o** (1.34 g, 5.25 mmol) in 93% H_2SO_4 (106 g) at 110 °C for 2 h gave 961 mg (67.0%) of **9s** as colorless crystals, mp 145—147 °C. IR (KBr) cm^{-1}: 3314, 3148, 1676, 1521. ^{1}H-NMR ($CDCl_3$) δ: 1.56—1.82 (10H, m, 3,4,6,7-CH_2 of azabicyclooctane and NH-$CH_2C\underline{H}_2$), 2.55—2.64 and 3.02—3.06 (each 2H, m, 2,8-CH_2 of azabicyclooctane), 3.20 (2H, t, J=8 Hz, NH-$C\underline{H}_2$-CH_2), 5.96 (2H, br s, $CONH_2$), 6.54 (1H, t, J=7 Hz, aromatic H), 6.70 (1H, d, J=8 Hz, aromatic H), 7.29 (1H, t, J=7 Hz, aromatic H), 7.37 (1H, d, J=8 Hz, aromatic H), 7.80 (1H, br s, NH). EIMS *m/z*: 273 (M^+), 110 (base peak).

Preparation of Rat Brain Homogenate Sprague–Dawley male rats were sacrificed by decapitation, and their brains were excised. The cerebral cortex and the cerebellum were homogenized, respectively, in 10 volumes of ice-cold buffer (0.32 M sucrose) in a Potter–Elvehjem glass homogenizer. The resulting homogenate was centrifuged at 1000×***g*** for 10 min at 4 °C, the precipitate was removed, and the supernatant was recentrifuged at 40000×***g*** for 15 min at 4 °C. The pellets thus obtained were washed with each assay buffer by resuspension and recentrifugation. The membrane preparation was stored at −70 °C until required.

[^{3}H]Pirenzepine Binding Inhibition An assay for M_1 receptors was performed according to the method of Flynn and Mash.[10] Frozen rat cerebral cortex membrane was resuspended in an assay buffer (50 mM phosphate buffer, pH 7.4). The membrane suspension, corresponding to 0.6 mg of protein determined by the method of Lowry with bovine serum albumin as the standard, was incubated with approximately 1.0 nM [^{3}H]pirenzepine at 25 °C for 60 min. Test compounds were added in a volume of 1ml to give a final assay volume of 2.0 ml. Nonspecific binding was determined using 1 μM atropine. Assays were terminated by the addition of 3 ml of the chilled assay buffer and by rapid filtration under vacuo through Whatman GF/B filter paper which had been previously impregnated with a 0.1% polyethyleneimine solution for 60 min. The filters were washed immediately two times with 3 ml each of the assay buffers. The filter was placed in a scintillation vial, to which 4 ml of ACSII cocktail was added. Radioactivity retained on the filter paper was determined by liquid scintillation counter. All assays were performed in duplicate or triplicate. Competition binding data were analyzed by logic-log analysis to provide the inhibitory concentration (IC_{50}) value of the test compound on ^{3}H-pirenzepine binding to M_1 receptor.

[^{3}H]Quinuclidinyl benzilate (QNB) Binding Inhibition The assay for M_2 receptors was performed according to the method of Yamamura and Snyder.[11] A frozen rat cerebellum membrane was resuspended in the assay buffer (50 mM phosphate buffer, pH 7.4). The membrane preparation, corresponding to 0.6 mg of protein determined by the method of Lowry et al.,[12] was incubated with approximately 1.0 nM [^{3}H]QNB at 25 °C for 60 min. Test compounds were added in a volume of 1 ml to give a final assay volume of 2.0 ml. Non-specific binding was determined using 1 μM QNB. The estimation of filter-bound radioactivity and the data analysis to obtain the IC_{50} of the test compound on [^{3}H]QNB binding to M_2 receptors were similar to those in the case of [^{3}H]pirenzepine binding.

Reference Compounds (−), (±)-YM796 and SR56559 were synthesized at our laboratory. YM796 was prepared as a fumarate salt.

Passive Avoidance Performance in Scopolamine-Treated Mice A passive avoidance learning test using mice was conducted to examine whether a scopolamine-induced passive avoidance deficit can be improved by the compounds of the present invention.

A training box composed of a light room and a dark room, which both had the same structure, was used. The dark room was designed so that a foot shock is given to a test animal *via* grids of the floor. An opening is provided on the partitioning wall of the two rooms to let animals in and out freely.

The animal was put in the light room. Immediately after the animal moved into the dark room, a foot shock was given until the animal returned to the light room (acquisition trial). After 24 h from the training, the animal was again put in the light room, and the time required for the animal's moving to the dark room was measured up to 300 s (retention test).

Scopolamine hydrobromide dissolved in physiological saline was administered i.p. to a mouse at a dose of 0.25 mg/kg 15 min before the acquisition trial. Five minutes after the scopolamine administration, a test compound was administered *p.o.* to the mouse.

The data from the retention test were analyzed to obtain a percent prolongation of the avoidance of the test group based on that of the saline-treated control group.

Biochemical Assays Using Rat Primary Cultured Cells Hippocampal neurons were isolated from rat embryos of day 18 gestation by a trypsin treatment following the protocol of Banker and Cowan,[13] and were maintained for 5—7 d in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. To suppress any growth of the glial cells, the culture was treated with 10 μM cytosine arabinofuranoside for 24 h on the 5th day.

The rate of hydrolysis of inositol phospholipids (PI break down) was assayed in hippocampal neuronal cells, as described previously.[14] Neuronal cells were incubated with Hank's balanced salt solution containing 1 μCi/ml [^{3}H]-*myo*-inositol for 24 h. After washing the inositol-loaded cells, cultures were incubated with 10 mM LiCl and test drugs (all in Kreb's Henseleit bicarbonate buffer containing 20 mM KCl) for 60 min. Inositol phosphate (IP) production was expressed as [^{3}H]IP/([^{3}H]IP+incorporated [^{3}H]-*myo*-inositol)%.

[^{3}H]-*myo*-inositol incorporation in hippocampal neuronal cells was estimated in cultures incubated with [^{3}H]-*myo*-inositol and the drug in Kreb's Henseleit bicarbonate buffer containing 5 mM KCl without LiCl for 24 h.

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