Synthesis and Structure–Activity Studies of a Series of 1-Oxa-2,8-diazaspiro[4.5]decan-3-ones and Related Compounds as M_1 Muscarinic Agonists

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A series of novel 2,8-dialkyl-1-oxa-2,8-diazaspiro[4.5]decan-3-ones and 2,8-dimethyl-1,2,8-triazaspiro[4.5]-decan-3-one (13), related to M_1 muscarinic agonists YM796 and RS86, were synthesized by using Michael addition reaction of hydroxyurea or methylhydrazine to α,β-unsaturated esters followed by cyclization reaction. These compounds were assessed for binding affinities for M_1 and M_2 receptors and *in vivo* muscarinic activity: namely, amelioration of scopolamine-induced impairment in rat passive avoidance tasks and induction of hypothermia, tremor, and salivation. 2,8-Dimethyl-1-oxa-2,8-diazaspiro[4.5]decan-3-one (6a) exhibited high affinities for both M_1 and M_2 receptors, showed antiamnesic activity (0.1 mg/kg, s.c.) and induced hypothermia (3 mg/kg, s.c.). In addition, 6a stimulated phosphoinositide hydrolysis in rat hippocampal slices, indicating partial agonistic activity for M_1 muscarinic receptors. The alteration of the methyl group at N2 of 6a increased the selectivity in binding affinities for M_1 over M_2 receptors, but resulted in loss of M_1 agonistic activity or antiamnesic activity. Compound 13 exhibited only low affinity for M_1 receptors, suggesting that a basic nitrogen atom is not tolerated in M_1 receptor binding as a substitute for an oxygen atom or a carbonyl group at the 1-position of 6a or RS86. None of these derivatives exhibited high selectivity for antiamnesic effect over induction of hypothermia compared to YM796.

Key words 1-oxa-2,8-diazaspiro[4.5]decane; 1,2,8-triazaspiro[4.5]decane; muscarinic M_1 agonist; scopolamine-induced amnesia; structure–activity relationship

Much interest has been focused in the last decade on the development of M_1-selective muscarinic receptor agonists as drugs for symptomatic treatment of senile dementia of Alzheimer type (SDAT).[1] Stimulation of cholinergic transmission in cerebral cortex and hippocampus by M_1-selective agonists would ameliorate the characteristic cholinergic hypofunction in SDAT, without inducing muscarinic side effects mediated by M_2 or M_3 subtype muscarinic receptors.[2]

We have already reported YM796 (I),[3] (−)-(*S*)-2,8-dimethyl-3-methylene-1-oxa-8-azaspiro[4.5]decane L-tartrate monohydrate, as a partial M_1 muscarinic agonist with high functional selectivity for M_1 over M_2 subtype, and it is currently under clinical study. Compound I was discovered in structure–activity studies of a series of spiro-cyclic amine derivatives including 1-oxa-3,8-diazaspiro[4.5]decane-2,4-dione derivatives such as II,[1g] 1-oxa-8-azaspiro[4.5]decane derivatives such as III,[3e] and a putative M_1 agonist RS86 (IV).[1a]

In our continuing studies, we designed 1-oxa-2,8-diazaspiro[4.5]decan-3-one derivatives (V). This structure seemed to be interesting not only as a 1-oxa analogue of IV, but also as a 2-aza analogue of III, which is a potent but nonselective muscarinic agonist and a mother compound of I.[3e] In addition, some difficulty[3e] in the preparation of I also prompted us to search for an achiral M_1 agonist such as II or IV. Compound I has the same absolute configuration as muscarine and muscarone, and its muscarinic activity is stereospecific, that is, the (+)-isomer of I is not active.[3] The optical resolution of racemic I, however, was difficult and resulted in low yield, probably due to the long distance between the stereogenic center and the basic nitrogen atom. A possible approach to skirt this problem is to search for an achiral compound with similar activities. Substitution of the chiral carbon atom with a nitrogen atom seemed advantageous for this purpose, because II and IV have *N*-alkyl structure. The substitution, followed by alteration of an apparently labile enamine structure to an amide, yielded a 1-oxa-2,8-diazaspiro[4.5]decan-3-one skeleton. Herein we report on the synthesis and biological activity of the compounds obtained. In addition, 2,8-dimethyl-1,2,8-triazaspiro[4.5]-decan-3-one (13) is included in this report as a 2-aza analogue of V. As previously described,[1g,3e] compounds were sought which had high M_1 receptor binding affinity *in vitro*, receptor selectivity *versus* M_2 receptors, M_1 agonistic activity, and antiamnesic effect separated from muscarinic side effects.

Chemistry

A simple method for the synthesis of isoxazolidin-3-one

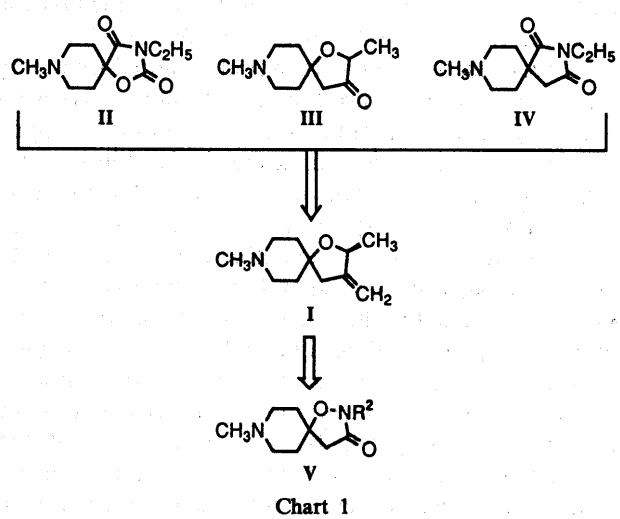

Chart 1

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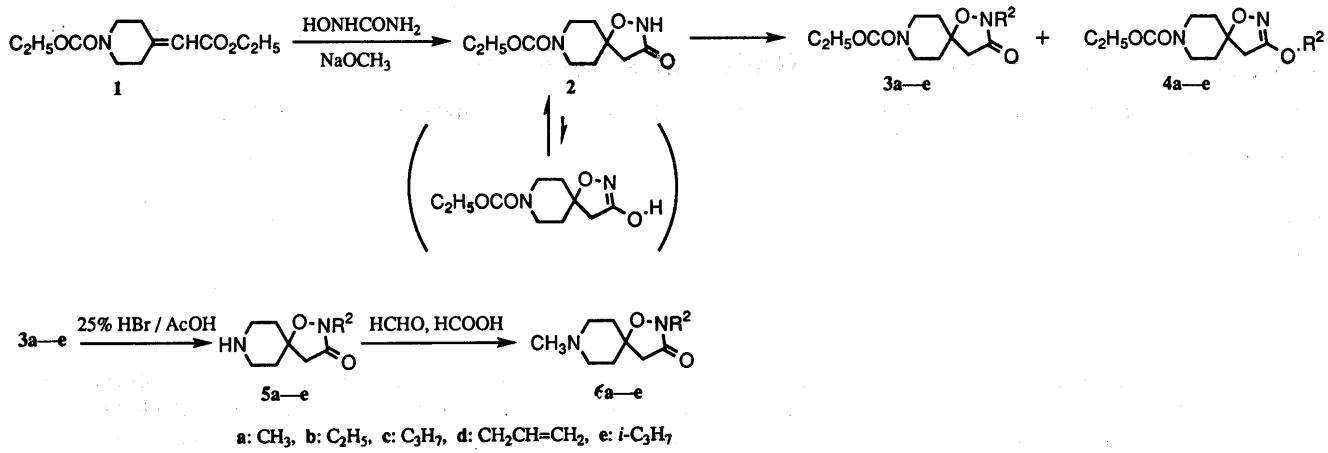


Chart 2

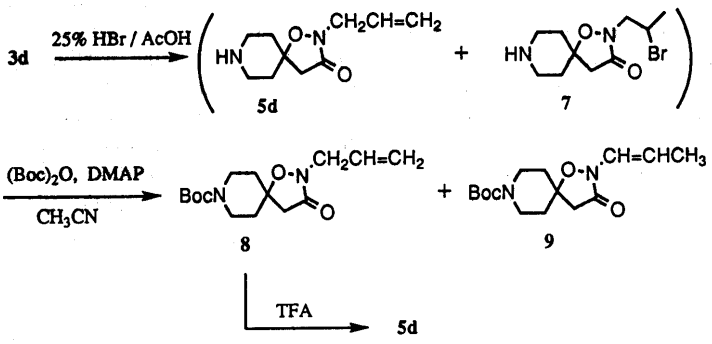


Chart 3

was reported by Olive *et al.*,[4] based on the reaction of an α,β-unsaturated ester with hydroxyurea. We adapted this method to construct the designed spiro-cyclic isoxazolidin-3-one. Treatment of ethyl (1-ethoxycarbonyl-4-piperidinylidene)acetate (**1**) with hydroxyurea in the presence of sodium methoxide gave 8-ethoxycarbonyl-1-oxa-2,8-diazaspiro[4.5]decane-3-one (**2**) in 64% yield (Chart 2). The structure was determined from the ^{1}H-NMR and MS spectra, and was supported by the IR spectra of the deprotected derivatives as described later. The product obtained by column chromatography exhibited two singlet peaks (δ 2.59 ppm, 0.6H; 2.78 ppm, 1.6H) in its ^{1}H-NMR spectrum, indicating the presence of tautomers. The former peak was assigned to H-4 protons of the NH form and the latter peak was assigned to those of the OH form based on the ^{1}H-NMR spectra of *N*- or *O*-alkylated derivatives described later. It should be noted that recrystallization of the crude product from hexane–ethyl acetate (2:1, v/v) gave a mixture of these tautomers (approximately 1:1) as crystals and, interestingly, dilution of the mother liquid with hexane gave pure NH form, which exhibited only one two-proton singlet peak at δ 2.59 ppm.

Treatment of **2** with diazomethane gave the *N*-methyl compound (**3a**) in 42% yield and the *O*-methyl compound (**4a**) in 30% yield. On the other hand, alkylation of **2** with ethyl iodide, propyl iodide, and allyl iodide in the presence of potassium carbonate gave *N*-alkylated compounds (**3b—d**) as major products and *O*-alkylated compounds (**4b—d**) as minor products. The purification of these products was accomplished easily by column chromatography and their structures were determined from the ^{1}H-NMR spectra; for example, the *N*-methyl protons of **3a** resonated at δ 3.18 ppm, whereas the *O*-methyl protons of **4a** resonated at δ 3.84 ppm. The signals of the H-4 protons of **3a—d** appeared at approximately δ 2.6 ppm and those of **4a** and **4b** appeared at approximately δ 2.7 ppm, which corresponded to the two singlet peaks in the ^{1}H-NMR spectrum of the mixture of **2** and its tautomer described above. The alkylation reaction with isopropyl iodide proved to be sluggish. Thus we tried the Mitsunobu reaction. Reaction of **2** with isopropanol in the presence of triphenylphosphine and diethyl azodicarbonate afforded the *N*-isopropyl derivative (**3e**) and no contamination with the *O*-isopropyl derivative (**4e**) was detected. This finding prompted us to examine the methylation by means of the Mitsunobu reaction. Interestingly, in contrast to the result of methylation with diazomethane, this reaction with methanol gave exclusively the *N*-methyl derivative (**3a**) in 74% yield. The ^{1}H-NMR spectrum and the TLC analysis of the crude product did not indicate any contamination with *O*-methylated product (**4a**). Deprotection of **3a—e** was accomplished with 25% HBr–acetic acid. In the case of the *N*-allyl compound (**3d**), treatment with 25% HBr–acetic acid gave a mixture of the desired deprotected compound (**5d**) and another product, the mass spectrum of which exhibited peaks at *m/z* 278, 276, and 196, corresponding to the molecular ion peak of the *N*-(2-bromopropyl) derivative (**7**) and that of **5d** (Chart 3). Since the separation of **5d** and **7** by column chromatography was difficult, they were treated with di-*tert*-butyldicarbonate to give two *N*-*tert*-butoxycarbonyl de-

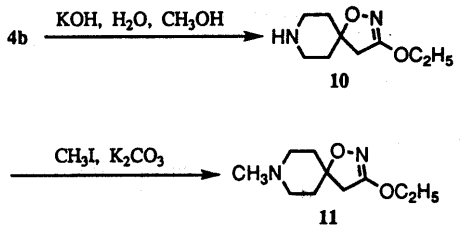


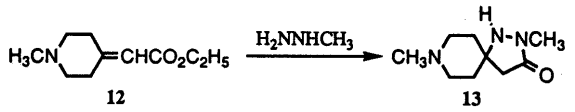
Chart 4

rivatives. Compound **8** was purified successfully by column chromatography and deprotected to give **5e**. Another product exhibited a peak at *m/z* 297, corresponding to the quasimolecular ion of **9**, a dehydrobrominated derivative of **7**. The deprotected products **5a**—**e** exhibited C=O stretching bands (1690—1726 cm^{-1}) in their IR spectra, confirming the isoxazolidin-3-one structure. Reductive methylation of **5a**—**e** with formalin-formic acid afforded the *N*-methyl derivatives (**6a**—**e**).

Deprotection of **4b** with 25% HBr–acetic acid gave complicated highly polar products, suggesting decomposition of the isoxazoline ring (Chart 4). Thus, deprotection under basic conditions was attempted and proved successful. Treatment of **4b** with potassium hydroxide in aqueous MeOH gave the deprotected product **10** in 46% yield. Compound **10** was methylated under basic conditions to yield **11** (Chart 4). Methylation of **10** in formalin–formic acid again gave complex products. The IR spectra of **10** and **11** (**10**: 1632 cm^{-1}, **11**: 1586 cm^{-1}) supported their isoxazoline structures.

After the disclosure of our patent relating to these synthetic studies,[5] synthesis of 3-hydroxy-1-oxa-2,8-diazaspiro[4.5]dec-2-ene as an analogue of 3-hydroxy-5-(4-piperidyl)isoxazole, a $GABA_A$ agonist, was reported by De Amici and his coworkers.[6] Their synthesis was based on a 1,3-dipolar cycloaddition reaction between *exo*-olefin and bromonitrile oxide. The synthesis of 2,8-dialkyl-1-oxa-2,8-diazaspiro[4.5]decan-3-ones, however, has not been reported as far as we know. Our results indicate that the Michael addition-cyclization reaction of hydroxyurea and α,β-unsaturated esters is useful for the synthesis of these compounds.

The synthesis of 2,8-dimethyl-1,2,8-triazaspiro[4.5]-decan-3-one (**13**) was accomplished by heating the α,β-unsaturated ester (**12**) and methylhydrazine in toluene in 94% yield (Chart 5). The structure was determined from the IR, ^{1}H-NMR, and MS data. The dihydrochloride of **13** exhibited a C=O stretching band at 1744 cm^{-1} in the IR spectrum and the free base exhibited a three-proton singlet peak at δ 3.02 ppm assigned to the methyl protons at N2 and a two-proton singlet at δ 2.38 ppm assigned to the H-4 protons in the ^{1}H-NMR spectrum. Similar treatment with *N,N'*-dimethylhydrazine failed to give spiropyrazolidin-3-one, probably due to the steric congestion in the β-position caused by the *N*-methyl group. It was reported by Bohrisch and coworkers that the reaction of a butenolide derivative, α,β-unsaturated lactone, with methylhydrazine gave regioselectively a 1-methylpyrazolidin-3-one derivative.[7] Altogether, these results suggest that sterically less hindered substrates such as a β-monosubstituted α,β-unsaturated ester are attacked



12 13

Chart 5

first by the more nucleophilic methyl-substituted nitrogen and sterically more hindered substrates such as **12** are attacked by the less bulky unsubstituted nitrogen, leading to inverse regioselectivity. Following this successful construction of spiropyrazolidinone, we examined the reaction of **1** and *N*-methylhydroxylamine in the presence of sodium hydride but the desired spirocyclic compound (**2**) was not obtained.

Pharmacology

The desired activity profile of compounds was as follows: (1) high affinity for M_1 receptors with high selectivity over M_2 receptors; (2) antiamnesic effect in the scopolamine-induced amnesia model with high selectivity over muscarinic side effects; and (3) efficacy as an M_1 agonist on the second messenger system, as demonstrated by stimulation of phosphoinositide (PI) hydrolysis. The affinities of compounds for M_1 and M_2 receptors were evaluated in terms of the abilities of the compounds to displace [^{3}H]pirenzepine (PZ), an M_1-selective ligand, from rat cerebral cortex membrane[8] and [^{3}H]quinuclidinyl benzilate (QNB) from rat cerebellum membrane,[9] respectively. These affinities are summarized as K_i values in comparison with those of carbachol, I, III, and IV in Table 1. The ratio of affinities, [K_i (QNB)/K_i (PZ)], is a measure of M_1 selectivity. Antiamnesic effects were evaluated in terms of the ability to ameliorate scopolamine-induced impairments in one-trial passive avoidance tasks of rats.

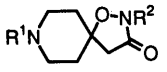
Since muscarinic agonists are known to induce hypothermia and tremor, effects suggested to be associated with central M_2 receptors,[10] and to increase salivary secretion *via* peripheral muscarinic receptors, the effects of test compounds on rectal temperature, behavior, and salivary secretion of mice were observed.[1g,3e] These three effects were regarded not only as indices of muscarinic activity but also as indices of cholinergic side effects in anticipation of future clinical use. In Table 1, only the hypothermia-inducing activity of test compounds is shown for simplicity.

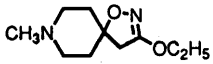
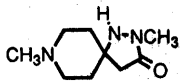
Activation of M_1 receptors in the hippocampus has been suggested to stimulate PI hydrolysis as a second messenger system.[11] The ability of some compounds to stimulate PI metabolism was examined in rat hippocampal slices in comparison with carbacol as a full agonist.

Results and Discussion

The designed compound **6a** exhibited high affinity for M_1 receptors and ameliorated scopolamine induced impairment in passive avoidance tasks at 0.1 mg/kg as shown in Table 1. In addition, **6a** stimulated PI hydrolysis with an efficacy about half of those of III and carbachol, as shown in Table 2. These findings indicate that **6a** is a novel M_1 agonist capable of penetrating the blood–brain barrier. On the other hand, **6a** also exhibited high affinity

Table 1. Muscarinic Activities of 1-Oxa-2,8-diazaspiro[4.5]decan-3-one Derivatives and Related Compounds



Compd.	Structure		Binding data: K_i,[a] μM			Antiamnesic effect,[e] MED mg/kg, s.c.	Hypothermia $ED_{\Delta 2°C}$ mg/kg, s.c.
	R^1	R^2	[^{3}H]PZ[b]	[^{3}H]QNB[c]	M_2/M_1 index[d]		
5a·HCl	H	CH_3	1.2 (1.1—1.3)	0.53 (0.47—0.59)	0.44	>1	>30
5b·HCl	H	C_2H_5	0.12 (0.11—0.13)	0.11 (0.10—0.13)	0.91	>3	3
5c·HCl	H	n-C_3H_7	0.15 (0.15—0.15)	0.32 (0.31—0.33)	2.1	>3	>30
5d·HCl	H	$CH_2CH=CH_2$	0.18 (0.18—0.19)	0.54 (0.51—0.57)	3.0	NT[f]	>30
6a·HCl	CH_3	CH_3	1.6 (1.5—1.8)	0.74 (0.68—0.80)	0.46	0.1	3
6b·HCl	CH_3	C_2H_5	0.28 (0.24—0.32)	0.34 (0.31—0.37)	1.2	>1	1
6c·HCl	CH_3	n-C_3H_7	0.33 (0.30—0.36)	1.2 (1.1—1.3)	3.6	>3	>30
6d·HCl	CH_3	$CH_2CH=CH_2$	0.42 (0.40—0.44)	NT[f]		NT[f]	>30
6e·HCl	CH_3	iso-C_3H_7	0.43 (0.40—0.47)	2.9 (2.8—3.0)	6.7	0.01	>30
9·HCl			6.7 (6.5—7.0)				
13·2HCl			25 (20—32)				
I			1.2 (1.1—1.2)	2.1 (1.9—2.3)	1.8	0.03[g]	23[g]
III			0.37 (0.36—0.38)	0.12 (0.11—0.12)	0.3	0.3	0.3
IV			0.46 (0.45—0.46)	0.97 (0.95—1.00)	2.1	0.1	1
Carbachol			1.5 (1.4—1.6)	0.11 (0.11—0.12)	0.073		

a) K_i values were determined by experiments performed in triplicate. Values in parentheses are 95% confidence intervals. *b*) Displacement of [^{3}H]pirenzepine from rat cortex membrane preparations. *c*) Displacement of [^{3}H]quinuclidinyl benzilate from rat cerebellum membrane preparations. *d*) The ratio of QNB/PZ, K_i's. *e*) Ameliorating effect on scopolamine-induced impairment of rat passive avoidance tasks. *f*) Not tested. *g*) *p.o.*

Table 2. Effects of Selected Compounds on Phosphoinositide Turnover in Rat Hippocampal Slices[a]

Compd.	Control	Test compd.			Carbachol (10^{-4} M)[b]
		10^{-5} M	10^{-4} M	10^{-3} M	
6a	0.162±0.003	0.185±0.005** (9%)[c]	0.280±0.005*** (48%)	0.306±0.003*** (59%)	0.406±0.007***
6b	0.162±0.003	0.228±0.006*** (27%)	0.257±0.001*** (39%)	0.261±0.002*** (41%)	0.406±0.007***
6c	0.164±0.010	0.174±0.005	0.170±0.004	0.189±0.013	0.622±0.010***[d]
6e	0.162±0.003	0.166±0.007	0.147±0.008	0.154±0.001	0.406±0.007***
III	0.172±0.003	0.361±0.016*** (62%)	0.452±0.006*** (92%)	0.460±0.012*** (94%)	0.478±0.019***
IV	0.180±0.005	0.229±0.006*** (15%)	0.251±0.011*** (21%)	0.268±0.007*** (27%)	0.512±0.015***

a) Results are expressed as [^{3}H]IPs/([^{3}H]lipids+[^{3}H]IPs)±S.E. of triplicate experiments. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ *vs.* control. *b*) Effects of carbachol (10^{-4} M) determined in the same experiment with the test compound are shown as an index of a full agonist. *c*) The increase of [^{3}H]IPs/([^{3}H]lipids+[^{3}H]IPs) is expressed as a percentage of that elicited by carbachol (10^{-4} M). *d*) Carbachol 10^{-3} M.

for M_2 receptors and induced a decrease of rectal temperature at the relatively low dose of 3 mg/kg. The separation of the doses for inducing antiamnesic activity and hypothermia is somewhat similar to that in the case of IV. Consequently, the activity profile *in vitro* of **6a** is similar to that of III, a nonselective M_1 agonist, and that *in vivo* is similar to that of IV.

In order to improve the selectivity, several modifications were carried out as shown in Table 1. 1-Oxa-2,8-diazaspiro[4.5]decan-3-ones (**5a—d**, **6a—e**) showed similarly high affinity for both M_1 and M_2 receptors. On the other hand, the affinities for M_1 receptors of 1-oxa-2,8-diazaspiro[4.5]dec-2-ene (**9**) and 1,2,8-triazaspiro[4.5]decan-3-one (**13**) were low. Thus, substitution with a nitrogen atom at the 2-position, but not that at the 1-position, is tolerated for binding to the M_1 receptors. These findings suggest that the oxygen atom or carbonyl group at the 1-position directly interacts with the recognition site in the receptor and the atom at the 2-position plays a role as the juncture of the oxygen atom, the carbonyl group, and the alkyl group. In addition, the presence of an alkyl group on the 2-position seems important, as found in the 1-oxa-8-azaspiro[4.5]decan-3-ones.[3e] Among these compounds, the 2-isopropyl derivative **6e** was the most M_1-selective and had the most potent antiamnesic activity. But, unfortunately, **6e** did not enhance PI hydrolysis.

Elongation of the alkyl group at N2 from a methyl group to a propyl group increased the M_1 selectivity in terms of binding affinity. But M_1 agonistic activity (indicated by the ability to potentiate PI hydrolysis) was decreased by this modification (Table 2). The ethyl derivative **6b** exhibited moderate stimulation and the

propyl derivative **6c** did not show any activity. Similar relations between selectivity and agonistic activity were also observed for 1-oxa-8-azaspiro[4.5]decan-3-ones[3e] and other derivatives.[1d] Compound **6b** did not show antiamnesic activity at doses from 0.03 mg/kg to 1 mg/kg, though it induced hypothermia at 1 mg/kg. Although demethylation at N8 resulted in slightly increased affinity for M_1 and M_2 receptors, no antiamnesic activity of the demethylated derivatives was detected. The results *in vivo* of **5a** may be partly attributed to its low lipophilicity in comparison to **6a**.

Conclusions

A series of novel 2,8-dialkyl-1-oxa-2,8-diazaspiro[4.5]-decan-3-ones and 2,8-dimethyl-1,2,8-triazaspiro[4.5]decan-3-one (**13**) were synthesized by using Michael addition reaction of hydroxyurea or methylhydrazine to α,β-unsaturated esters followed by cyclization reaction. The Mitsunobu reaction was proved to be useful for selective *N*-alkylation of isoxazolidin-3-one.

The aza substitution of the stereogenic carbon of III and the oxa substitution of the carbonyl group at the 1-position of IV gave a novel potent, though nonselective, M_1 partial agonist **6a** and **6b**. The alteration of the alkyl group at N2 increased the binding selectivity for M_1 over M_2 receptors. Such compounds as **6c** and **6e**, however, lacked M_1 agonistic activity or antiamnesic activity. The investigations of **13** suggest that a basic nitrogen atom is not tolerated as a substitute for an oxygen atom or a carbonyl group at the 1-position of **6a** or IV in M_1 receptor binding.

Experimental

All melting points were determined with a Yanaco MP-3 melting point apparatus and are uncorrected. ^{1}H-NMR spectra were measured with either a JEOL FX90Q or a FX100 spectrometer; chemical shifts are recorded in δ units from tetramethylsilane as an internal standard and the following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, and dd = double doublet. Mass spectra were recorded with a Hitachi M-80 or JEOL JMS-DX300 spectrometer. Infrared spectra were recorded on a Hitachi 270-30 infrared spectrophotometer. All solutions were dried over anhydrous magnesium sulfate.

8-Ethoxycarbonyl-1-oxa-2,8-diazaspiro[4.5]decan-3-one (2) Sodium (5.2 g, 226 mmol) was stirred in MeOH (88 ml) at room temperature until it had all dissolved. Hydroxyurea (13.2 g, 174 mmol) was added at once and the solution was stirred for 3 h. Ethyl (1-ethoxycarbonyl-4-piperidinylidene)acetate (**1**, 40 g, 166 mmol) was added and the reaction mixture was stirred for 7 d, then poured into ice-water (500 ml). Concentrated HCl was added to adjust the pH to 1, then the mixture was extracted with $CHCl_3$. The extract was washed with saturated aqueous NaCl, dried and evaporated *in vacuo*. The residue was purified through a silica gel column (ethyl acetate–*n*-hexane, 4:1, v/v) to give **2** (24.3 g, 64%); mp 125 °C. IR (KBr): 1696 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (t, 3H, J = 7 Hz), 1.34—2.13 (m, 4H), 2.59 (s, 2H), 3.17—3.47 (m, 2H), 3.67—3.94 (m, 2H), 4.15 (q, 2H, J = 7 Hz). MS *m/z*: 228 (M^+), 156, 42. *Anal.* Calcd for $C_{10}H_{16}N_2O_4$: C, 52.62; H, 7.07; N, 12.27. Found: C, 52.67; H, 7.05; N, 12.05.

8-Ethoxycarbonyl-2-methyl-1-oxa-2,8-diazaspiro[4.5]decan-3-one (3a) and 8-Ethoxycarbonyl-3-methoxy-1-oxa-2,8-diazaspiro[4.5]dec-2-ene (4a) *N*-Nitrosomethylurea (5.08 g, 49 mmol) was added to a mixture of 40% aqueous NaOH (90 ml) and ether (120 ml) at 0 °C and stirred at the same temperature. The resulting ether solution of diazomethane was added dropwise to an ether solution of **2** (2.15 g, 9.42 mmol) at 0 °C and the mixture was stirred for 19 h at the same temperature. Formic acid was added until the yellow color was discharged. The solution was evaporated *in vacuo* and the residue was purified through a silica gel column ($CHCl_3$–MeOH–27% aqueous NH_3, 50:1:0.1, v/v) to give **3a** (0.96 g, 42%) and **4a** (0.68 g, 30%), each as a colorless oil. **3a**: IR (neat): 1704 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (t, 3H, J = 7 Hz), 1.45—2.08 (m, 4H), 2.60 (s, 2H), 3.18 (s, 3H), 3.22—3.51 (m, 2H), 3.64—3.92 (m, 2H), 4.16 (q, 2H, J = 7 Hz). MS *m/z*: 242 (M^+), 196, 168. **4a**: IR (neat): 1700 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.24 (t, 3H, J = 7 Hz), 1.48—2.05 (m, 4H), 2.71 (s, 2H), 3.23—3.54 (m, 2H), 3.63—3.88 (m, 2H), 3.84 (s, 3H), 4.12 (q, 2H, J = 7 Hz). MS *m/z*: 242 (M^+), 197, 154.

2-Methyl-1-oxa-2,8-diazaspiro[4.5]decan-3-one (5a) A mixture of **3a** (400 mg, 1.65 mmol) and 25% HBr–acetic acid (5 ml) was stirred for 1 h at 100 °C. The mixture was concentrated *in vacuo* and the resulting residue was dissolved in water. The solution was basified with potassium carbonate and extracted with $CHCl_3$. The extract was dried and concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$–MeOH–27% aqueous NH_3 (10:1:0.1, v/v) to yield **5a** (180 mg, 64%) as a colorless oil. The oil was treated with 4 N HCl in ethyl acetate to afford the hydrochloride; mp 280 °C. IR (KBr): 1726 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.91—2.03 (m, 4H), 2.71 (s, 2H), 3.04 (s, 3H), 2.98—3.12 (m, 4H). MS *m/z*: 170 (M^+), 95, 42. *Anal.* Calcd for $C_8H_{14}N_2O_2 \cdot HCl$: C, 46.49; H, 7.32; N, 13.55; Cl, 17.25. Found: C, 46.36; H, 7.15; N, 13.54; Cl, 17.42.

2,8-Dimethyl-1-oxa-2,8-diazaspiro[4.5]decan-3-one (6a) Formic acid (2 ml) and 37% aqueous formaldehyde (2 ml) were added to **5a** (260 mg, 1.53 mmol), then the mixture was stirred for 1 h at 100 °C and concentrated *in vacuo*. The residue was dissolved in water, and the solution was basified with potassium carbonate and extracted with $CHCl_3$. The extract was dried and concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$–MeOH–27% aqueous NH_3 (20:1:0.1, v/v) to yield **6a** (240 mg, 85% yield) as a colorless oil. The oil was treated with 4 N HCl in ethyl acetate to afford the hydrochloride; mp 228 °C (MeOH–ether). IR (KBr): 1734 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.04—2.18 (m, 4H), 2.71—2.85 (m, 5H), 2.93—3.60 (m, 4H), 3.39 (s, 3H). MS *m/z*: 184 (M^+), 110. *Anal.* Calcd for $C_9H_{18}N_2O_2 \cdot HCl \cdot 0.1H_2O$: C, 48.58; H, 7.79; N, 12.59; Cl, 15.93. Found: C, 48.33; H, 7.57; N, 12.42; Cl, 15.82.

8-Ethoxycarbonyl-2-ethyl-1-oxa-2,8-diazaspiro[4.5]decan-3-one (3b) and 8-Ethoxycarbonyl-3-ethoxy-1-oxa-2,8-diazaspiro[4.5]dec-2-ene (4b) A mixture of **2** (2.0 g, 8.8 mmol) and potassium carbonate (1.82 g, 13 mmol), acetone (50 ml), and ethyl iodide (4.1 g, 26.3 mmol) was refluxed for 3 h. After having been cooled to room temperature, the mixture was filtered and the filtrate was concentrated *in vacuo*. Water was added and the mixture was extracted with $CHCl_3$. The extract was dried and evaporated *in vacuo* and the residue was purified through a silica gel column ($CHCl_3$–MeOH–27% aqueous NH_3, 50:1:0.1, v/v) to give **3b** (1.92 g, 85% yield) and **4b** (290 mg, 13% yield) as oils. **3b**: IR (neat): 1704 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.22 (t, 3H, J = 7 Hz), 1.27 (t, 3H, J = 7 Hz), 1.51—2.07 (m, 4H), 2.59 (s, 2H), 3.19—3.52 (m, 6H), 4.16 (q, 2H, J = 7 Hz). MS *m/z*: 256 (M^+), 168. **4b**: IR (neat): 1704 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.26 (t, 3H, J = 7 Hz), 1.34 (t, 3H, J = 7 Hz), 1.51—2.07 (m, 4H), 2.73 (s, 2H), 3.25—3.91 (m, 4H), 4.16 (q, 2H, J = 7 Hz), 4.21 (q, 2H, J = 7 Hz). MS *m/z*: 256 (M^+), 154.

2-Ethyl-1-oxa-2,8-diazaspiro[4.5]decan-3-one Prepared by the same method as described for **5a** in 71% yield. Hydrochloride: mp 159 °C. IR (KBr): 1690 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.11 (t, 3H, J = 7 Hz), 1.95—2.08 (m, 4H), 2.73 (s, 2H), 2.95—3.26 (m, 4H), 3.50 (q, 2H, J = 7 Hz). MS *m/z*: 184 (M^+), 95, 42. *Anal.* Calcd for $C_9H_{16}N_2O_2 \cdot HCl \cdot 0.2H_2O$: C, 48.19; H, 7.82; N, 12.49; Cl, 15.81. Found: C, 48.25; H, 8.12; N, 12.55; Cl, 15.84.

2-Ethyl-8-methyl-1-oxa-2,8-diazaspiro[4.5]decan-3-one (6b) Compound **6b** was prepared by the same method as described for **6a** in 95% yield. Hydrochloride; mp 186 °C. IR (KBr): 1704 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.13 (t, 3H, J = 7 Hz), 2.04—2.18 (m, 4H), 2.74 (s, 3H), 2,74 (s, 2H), 2.92—3.63 (m, 6H). MS *m/z*: 198 (M^+), 110, 42. *Anal.* Calcd for $C_{10}H_{18}N_2O_2 \cdot HCl$: C, 51.17; H, 8.16; N, 11.93; Cl, 15.10. Found: C, 51.05; H, 8.06; N, 12.02; Cl, 15.26.

8-Ethoxycarbonyl-2-propyl-1-oxa-2,8-diazaspiro[4.5]decan-3-one (3c) Compound **3c** was prepared from **2** and propyl iodide in a manner similar to that described for **3b** in 92% yield. IR (neat): 1702 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.96 (t, 3H, J = 7 Hz), 1.30 (t, 3H, J = 7 Hz), 1.48—2.05 (m, 6H), 2.62 (s, 2H), 3.20—3.90 (m, 6H), 4.16 (q, 2H, J = 7 Hz). MS *m/z*: 270 (M^+), 168.

2-Propyl-1-oxa-2,8-diazaspiro[4.5]decan-3-one (5c) Compound **5c** was prepared from **3c** by the same method as described for **5a** in 83% yield. Hydrochloride; mp 171 °C. IR (KBr): 1694 cm^{-1}. ^{1}H-NMR

(DMSO-d_6) δ: 0.87 (t, 3H, J=7 Hz), 1.37—1.77 (m, 2H), 1.95—2.08 (m, 4H), 2.75 (s, 2H), 2.92—3.24 (m, 4H), 3.43 (t, 2H, J=7 Hz). MS m/z: 198 (M^+), 124, 95, 42. *Anal.* Calcd for $C_{10}H_{18}N_2O_2 \cdot HCl$: C, 51.17; H, 8.16; N, 11.93; Cl, 15.10, Found: C, 51.04; H, 8.04;, N, 11.88; Cl, 15.16.

8-Methyl-2-propyl-1-oxa-2,8-diazaspiro[4.5]decan-3-one (6c) Compound **6c** was prepared by the same method as described for **6a** in 97% yield. Hydrochloride; mp 187—188 °C. IR (KBr): 1698 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.88 (t, 3H, J=7 Hz), 1.37—1.72 (m, 2H), 2.72 (s, 3H), 2.04—2.20 (m, 4H), 2.66—3.60 (m, 8H). MS m/z: 212 (M^+), 110, 42. *Anal.* Calcd for $C_{11}H_{20}N_2O_2 \cdot HCl \cdot 0.3H_2O$: C, 51.98; H, 8.57; N, 11.02; Cl, 13.95. Found: C, 52.04; H, 8.66; N, 11.03; Cl, 14.08.

8-Ethoxycarbonyl-2-(2-propenyl)-1-oxa-2,8-diazaspiro[4.5]decan-3-one (3d) Compound **3d** was prepared from **2** and allyl iodide in a manner similar to that described for **3b** in 73% yield. IR (neat): 1704 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (t, 3H, J=7 Hz), 1.52—2.08 (m, 4H), 2.66 (s, 2H), 3.21—3.50 (m, 2H), 3.68—3.82 (m, 2H), 4.07—4.39 (m, 4H), 5.20—5.45 (m, 2H), 5.66—6.04 (m, 1H). MS m/z: 268 (M^+), 168.

2-(2-Propenyl)-1-oxa-2,8-diazaspiro[4.5]decan-3-one (5d) The same treatment as described for **5a** using **3e** (5.66 g, 21 mmol) gave a mixture of **5d** and 2-(2-bromopropyl)-1-oxa-2,8-diazaspiro[4.5]decan-3-one (**7**, 2.20 g). This mixture (1.7 g) was dissolved in acetonitrile (30 ml), and di-*tert*-butyldicarbonate (2.10 g, 9.6 mmol) and a catalytic amount of 4-dimethylaminopyridine were added at 0 °C. The mixture was stirred for 4 h at room temperature and for 1 h at 50 °C, then concentrated *in vacuo*. The residue was chromatographed on silica gel with hexane–ethyl acetate (2:1) to yield 8-*tert*-butoxycarbonyl-2-(2-propenyl)-1-oxa-2,8-diazaspiro[4.5]decan-3-one (**8**, 1.67 g). Trifluoroacetic acid (14 ml) was added to the product. The mixture was stirred for 1 h at room temperature, then concentrated *in vacuo* and the residue was mixed with water, basified with potassium carbonate and extracted with $CHCl_3$. The $CHCl_3$ solution was dried and concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$–MeOH–27% aqueous NH_3 (5:1:0.1) to yield **5d** (1.08 g, 26%) as a colorless oil. The oil was treated with 4 N HCl in ethyl acetate to afford the hydrochloride: mp 168 °C. IR (KBr): 1704 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.94—2.08 (m, 4H), 2.76 (s, 2H), 2.82—3.20 (m, 4H). 4.04—4.14 (m, 2H), 5.12—5.38 (m, 2H), 5.61—6.03 (m, 1H). MS m/z: 196 (M^+), 124, 42. *Anal.* Calcd for $C_{10}H_{16}N_2O_2 \cdot HCl$: C, 51.61; H, 7.36; N, 12.04; Cl, 15.23. Found: C, 51.37; H, 7.25; N, 11.78; Cl, 15.10.

8-Methyl-2-(2-propenyl)-1-oxa-2,8-diazaspiro[4.5]decan-3-one (6d) Compound **6d** was prepared by the same method as described for **6a** in 90% yield. Hydrochloride: mp 188 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.04—2.28 (m, 4H), 2.67—3.56 (m, 6H), 2.72 (s, 3H), 4.04—4.16 (m, 2H), 5.13—5.40 (m, 2H), 5.63—6.06 (m, 1H). MS m/z: 210 (M^+), 110, 42. *Anal.* Calcd for $C_{11}H_{18}N_2O_2 \cdot HCl$: C, 53.55; H, 7.76; N, 11.35; Cl, 14.37. Found: C, 53.25; H, 7.64; N, 11.43; Cl, 14.54.

8-Ethoxycarbonyl-2-(2-propyl)-1-oxa-2,8-diazaspiro[4.5]decan-3-one (3e) Triphenylphosphine (1.76 g, 6.71 mmol), **2** (1.53 g, 6.70 mmol), and isopropanol (0.51 ml, 6.70 mmol) were dissolved in tetrahydrofuran (39 ml) and the solution was cooled to 0 °C. Diethyl azodicarboxylate (1.06 mg, 6.73 mmol) was added dropwise at 0 °C and the mixture was stirred for 12 h at room temperature. The solution was concentrated *in vacuo* and the residue was chromatographed on silica gel with $CHCl_3$–MeOH–27% aqueous NH_3 (10:1:0.1) to yield a mixture of **3e** and triphenylphosphine oxide (3.84 g). ^{1}H-NMR ($CDCl_3$) δ: 1.20—1.36 (m, 9H), 1.51—2.07 (m, 4H), 2.58 (s, 2H), 3.19—3.94 (m, 4H), 4.04—4.58 (m, 4H),4.04—4.58 (m, 3H). MS m/z: 270 (M^+), 168.

2-(2-Propyl)-1-oxa-2,8-diazaspiro[4.5]decan-3-one (5e) Compound **5e** was prepared from a mixture of **3e** and triphenylphosphine oxide by the same method as described for **3a** in 42% yield based on **2**. Hydrochloride; mp 203 °C. IR (KBr): 1692 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.18 (d, 6H, J=6 Hz), 1.93—2.06 (m, 4H), 2.73 (s, 2H), 3.00—3.10 (m, 4H), 4.25 (q, 1H, J=6 Hz).

8-Methyl-2-(2-propyl)-1-oxa-2,8-diazaspiro[4.5]decan-3-one (6e) Coumpound **6e** was prepared by the same method as described for **6a** in 77% yield. Hydrochloride; mp 209 °C. IR (KBr): 1678 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.20 (t, 6H, J=6 Hz), 2.01—2.15 (m, 4H), 2.60—3.54 (m, 9H), 4.25 (m, 1H). MS m/z: 212 (M^+), 110, 42. *Anal.* Calcd for $C_{11}H_{20}N_2O_2 \cdot HCl \cdot 0.3H_2O$: C, 51.98; H, 8.57; N, 11.02; Cl, 13.95. Found: C, 51.74; H, 8.50; N, 11.05; Cl, 14.10.

3-Ethoxy-1-oxa-2,8-diazaspiro[4.5]dec-2-ene (10) Potassium hydroxide (2.6 g) was dissolved in water (3 ml) and MeOH (13 ml) was added. Compound **4b** (600 mg, 2.34 mmol) was added to the solution. The mixture was stirred at 80 °C for 7 h. The mixture was concentrated *in vacuo*, then the residue was dissolved in water, and extracted with $CHCl_3$. The $CHCl_3$ solution was dried and concentrated *in vacuo*. Chromatography of the residue on silica gel (elution with $CHCl_3$–MeOH–aqueous NH_3, 10:1:0.1, v/v) afforded **10** (200 mg, 46% yield) as an oil. IR (neat) 1632 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.34 (t, 3H, J=7 Hz), 1.56—2.03 (m, 4H), 2.63—3.23 (m, 6H), 4.19 (q, 2H, J=7 Hz). MS m/z: 184 (M^+), 138, 42.

3-Ethoxy-8-methyl-1-oxa-2,8-diazaspiro[4.5]dec-2-ene (11) Potassium carbonate (160 mg, 1.16 mmol) was added to a solution of **10** (190 mg, 1.03 mmol) in acetone (20 ml) and the mixture was stirred at 50 °C for 1 h. The mixture was cooled to room temperature and methyl iodide (0.064 ml, 1.03 mmol) was added to it. The whole was stirred at room temperature for 1 h, then filtered. The filtrate was concentrated *in vacuo*, mixed with water, and extracted with $CHCl_3$. The organic solution was dried and concentrated. Chromatography of the residue on silica gel (elution with $CHCl_3$–MeOH–aqueous NH_3, 20:1:0.1, v/v) afforded **11** (131 mg, 64% yield) as an oil. Maleate: mp 123 °C (MeOH–ether). IR (KBr) 1586 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.27 (t, 3H, J=7 Hz), 1.89—2.02 (m, 4H), 2.79 (s, 3H), 2.93 (s, 2H), 3.15—3.29 (m, 4H), 4.10 (q, 2H, J=7 Hz), 6.03 (s, 2H). MS m/z: 198 (M^+), 110, 96, 42. *Anal.* Calcd for $C_{10}H_{18}N_2O_2 \cdot C_4H_4O_4$: C, 53.49; H, 7.05; N, 8.91. Found: C, 53.16; H, 6.84; N, 8.93.

2,8-Dimethyl-1,2,8-triazaspiro[4.5]decan-3-one (13) Ethyl (1-methyl-4-piperidinylidene)acetate (**12**, 910 mg, 4.97 mmol) and methylhydrazine (1.15 g, 25 mmol) were dissolved in toluene (4 ml) and the solution was heated under reflux for 24 h. The reaction mixture was concentrated *in vacuo* and the residue was chromatographed on silica gel (elution with $CHCl_3$–MeOH–27% aqueous NH_3, 20:1:0.1, v/v) to afford **13** (860 mg, 94% yield) as an oil; ^{1}H-NMR ($CDCl_3$) δ: 1.67—1.80 (m, 4H), 2.29 (s, 3H), 2.38 (s, 2H), 2.20—2.70 (m, 4H), 3.02 (s, 3H), 4.15 (s, 1H). Dihydrochloride: mp 155—157 °C (MeOH). IR (KBr): 1744 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.80—2.30 (m, 4H), 2.66 (s, 3H), 2.71 (s, 2H), 2.94 (d, 3H, J=8 Hz), 2.90—3.60 (m, 4H). MS m/z: 184 [$(M+1)^+$]. *Anal.* Calcd for $C_9H_{17}N_3O \cdot 2HCl \cdot 0.1H_2O$: C, 41.90; H, 7.50; N, 16.29; Cl, 27.49. Found: C, 41.89; H, 7.37; N, 16.49, Cl, 27.70.

Biological Methods Doses are expressed in terms of the free base. The following chemicals were obtained commercially: scopolamine hydrochloride (Tokyo Kasei Co., Ltd.), and [^{3}H]pirenzepine and [^{3}H]quinuclidinyl benzilate (Du Pont-New England Nuclear).

Receptor Binding Assay Membrane preparation and tritium-labeled ligand receptor binding assay were described previously.[3a] The cerebral cortex and the cerebellum from male Wistar rats were homogenized separately in 0.32 M ice-cold sucrose (1:10, w/v) using a motor-driven Teflon/glass homogenizer. The homogenates were centrifuged at 900 × *g* for 10 min at 4 °C. The supernatants were then recentrifuged at 11500 × *g* for 20 min at 4 °C. The pellets thus obtained were washed twice in 5 mM Tris–HCl buffer (pH 7.4) by resuspension and recentrifugation. Membranes were stored at −80 °C until required.

[^{3}H]PZ binding assay for M_1 receptors was performed according to the method of Watson *et al.*[8] Frozen rat cerebral cortex membrane was resuspended in an assay buffer (8.3 mM Tris–HCl, 1.25 mM $MnCl_2$, pH 7.4). The membrane suspension, corresponding to 250 μg of protein determined by the method of Lowry with bovine serum albumin as the standard, was incubated with approximately 1.0 nM [^{3}H]PZ at 25 °C for 60 min. Test compounds were added in a volume of 50 μl to give a final assay volume of 1.0 ml. Nonspecific binding was determined using 10 μM atropine. Assays were terminated by rapid filtration under vacuum through a Whatman GF/B filter. The filters were washed immediately four times with 4 ml each of 50 mM Tris–HCl and 120 mM NaCl buffer (pH 7.4). Each filter was placed in a scintillation vial to which 10 ml of NEN Aquasol-2 cocktail was added. Radioactivity retained on the filter was determined by liquid scintillation spectrometry. All receptor binding assays were performed in triplicate. Competition binding data were analyzed by logit-log analysis using the RS1 package (BBN Software Products Corp.) to calculate the IC_{50}. The IC_{50} values were corrected for receptor occupancy by [^{3}H]PZ as described by Cheng and Prusoff[12] to give K_i values (concentration of nonlabeled ligand that causes half-maximal receptor occupancy in the absence of [^{3}H]PZ).

[^{3}H]QNB binding assay for M_2 receptors was carried out according to the method of Yamamura and Snyder.[9] The incubation mixture consisted of 50 mM Na^+/K^+ phosphate buffer (pH 7.4), 0.06 nM [^{3}H]QNB, 150 μg protein of resuspended rat cerebellum membrane, and a test drug in a total volume of 4.0 ml. Nonspecific binding was defined with 10 μM atropine. The mixture was incubated for 60 min at 25 °C, and

the incubation was terminated by filtration through Whatman GF/B glass filters. The filters were washed three times with 3 ml of 50 mM Na^+/K^+ phosphate buffer (pH 7.4). The estimations of filter-bound radioactivity and the data analyses were similar to those in the case of [^{3}H]PZ binding.

Phosphoinositide Hydrolysis Rat hippocampal slices were prepared and prelabeled with [^{3}H]*myo*-inositol as described by Brown *et al.*[13] The slices were incubated for 60 min at 37 °C in the presence of a test compound and 10 mM LiCl in Krebs–Henseleit buffer containing 18.7 mM K^+. The [^{3}H]inositol phosphates (IPs) and [^{3}H]lipids (lipids) were separated and measured by the same method as described by Brown *et al.*[13] Results were expressed as [^{3}H]IPs/([^{3}H]lipids + [^{3}H]IPs).

Passive Avoidance Tasks with Scopolamine-Treated Rats Six male Wistar rats (Japan Slc. Inc.) were used for each dose of test compounds. A test compound or vehicle was administered subcutaneously or orally 30 min before the training session simultaneously with scopolamine (1 mg/kg i.p.). In the training session, each rat was placed in the light box of a two-compartment passive avoidance apparatus (O'Hara Co., Ltd.). When the rat entered the dark compartment, a foot-shock (70 V, AC) was applied for 1 s through the metal grid bars of the floor. Retention was assessed 24 h after the training session. Latency to enter the dark compartment was measured up to a maximum cut-off of 300 s. When the latencies of at least 4 rats were more than 60 s, the compound was evaluated as active. About 90% of vehicle-treated rats exhibited a latency of less than 60 s.

Each compound was initially tested at 0.1 and 1 mg/kg. When a compound was found to be active at one of these doses, it was retested at a three-fold lower dose, and the minimal dose required to improve the impairment of the passive avoidance tasks (MED) was thereby determined.

Hypothermia Male ICR mice (Japan Slc. Inc.) were used for these experiments. Before and 30 min after the subcutaneous or oral administration of a test compound, rectal temperature was measured with a thermoprobe (MC-111, Omron), and the dose required to reduce the rectal temperature by 2 °C ($ED_{42°C}$) was determined. Each test compound was initially tested at 30 mg/kg; the dose was then consecutively decreased to one-third of the preceding dose until hypothermia over 2 °C was not observed. Each dose group contained 3—6 mice.

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References

1) *a*) Hollander E., Davidson M., Mohs R. C., Horvath T. B., Davis B. M., Zemishlany Z., Davis K. L., *Biol. Psychiatry*, **22**, 1067 (1987); *b*) Fisher A., Brandeis R., Karton I., Pittel Z., Gurwitz D., Haring R., Sapir M., Levy A., Heldman E., *J. Pharmacol. Exp. Ther.*, **257**, 392 (1991); *c*) Kan J. P., *Eur. J. Med. Chem.*, **27**, 565 (1992); *d*) Freedman S. B., Dawson G. R., Iversen L. L., Baker R., Hargreaves R. J., *Life Sci.*, **52**, 489 (1993); *e*) Tecle H., Lauffer D. J., Mirzadegan T., Moos W. H., Moreland D. W., Pavia M. R., Schwarz R. D., Davis R. E., *ibid.*, **52**, 505 (1993); *f*) Enz A., Boddeke H., Sauter A., Rudin M., Shapiro G., *ibid.*, **52**, 513 (1993); *g*) Tsukamoto S., Ichihara M., Wanibuchi F., Usuda S., Hidaka K., Harada M., Tamura T., *J. Med. Chem.*, **36**, 2292 (1993).

2) *a*) Moos W. H., Davis R. E., Schwarz R. D., Gamzu E. R., *Med. Res. Rev.*, **8**, 353 (1988); *b*) Baker R., Saunders J., *Annu. Rep. Med. Chem.*, **24**, 31 (1989); *c*) Perry E. K., Tomlinson B. E., Blessed G., Bergmann K., Gibson P. H., Perry R. H., *Br. Med. J.*, **2**, 1457 (1978); *d*) Wilcock G. K., Esiri M. M., Bowen D. M., Smith C. C. T., *J. Neurol. Sci.*, **57**, 407 (1982).

3) *a*) Wanibuchi F., Konishi T., Harada M., Terai M., Hidaka K., Tamura T., Tsukamoto S., Usuda S., *Eur. J. Pharmacol.*, **187**, 479 (1990); *b*) Wei H., Roeske W. R., Lai J., Wanibuchi F., Hidaka K., Usuda S., Yamamura H. I., *Life Sci.*, **50**, 355 (1992); *c*) Wanibuchi F., Nichida T., Yamashita H., Hidaka K., Koshiya K., Tsukamoto S., Usuda S., *Eur. J. Pharmacol.*, **265**, 151 (1994); *d*) Tsukamoto S., Fujii M., Yasunaga T., Matsuda K., Wanibuchi F., Hidaka K., Furuya T., Tamura T., *Chem. Pharm. Bull.*, **43**, 842 (1995).

4) Olive J.-L., Petrus C., Petrus F., *Bull. Soc. Chim. Fr.*, **1976**, 1589.

5) Tsukamoto S., Nagaoka H., Usuda S., Harada M., Tamura T., Japan. Patent 02164882 (1990) [*Chem. Abstr.*, **113**, 211966 (1990)].

6) De Amici M., Frolund B., Hjeds H., Krogsgaard-Larsen P., *Eur. J. Med. Chem.*, **26**, 625 (1991).

7) Bohrisch J., Patzel M., Liebscher J., Jones P. G., *Tetrahedron Lett.*, **34**, 2749 (1993).

8) Watson M., Roeske W. R., Yamamura H. I., *Life Sci.*, **31**, 2019 (1982).

9) Yamamura H. I., Snyder S. H., *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 1725 (1974).

10) Caulfield M. P., Higgins G. A., Straughan D. W., *J. Pharm. Pharmacol.*, **35**, 131 (1983).

11) *a*) Gil D. W., Wolfe B. B., *J. Pharmacol. Exp. Ther.*, **232**, 608 (1985); *b*) Fisher S. K., Agranoff B. W., *J. Neurochem.*, **48**, 999 (1987).

12) Cheng Y., Prusoff W. H., *Biochem. Pharmacol.*, **22**, 3099 (1973).

13) Brown E., Kendall D. A., Nahorsky S. R., *J. Neurochem.*, **42**, 1379 (1984).