Central Cholinergic Agents. III. Synthesis of 2-Alkoxy-2,8-diazaspiro[4.5]decane-1,3-diones as Muscarinic Agonists

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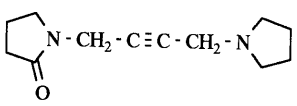
A series of 2-alkoxy-2,8-diazaspiro[4.5]decane-1,3-diones and related compounds were synthesized and tested for muscarinic receptor binding affinity using [^{3}H]pirenzepine and [^{3}H]oxotremorine M as ligands. They were also evaluated for agonistic activities in the guinea pig ileum assay. 2-Methoxy-2,8-diazaspiro[4.5]decane-1,3-dione (1i) was found to be a relatively M1 selective agonist. It reversed CO_2-induced impairment of passive avoidance response with long duration of action, but also displayed peripheral effects at low doses. To minimize these side effects, we proposed the idea of conjugation of 1i with a muscarinic antagonist. The carbamate linked conjugate (1u) of 1i with methylatropine was therefore examined.

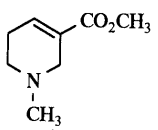
Keywords 2,8-diazaspiro[4.5]decane; muscarinic agonist; [^{3}H]pirenzepine binding; [^{3}H]oxotremorine M binding; CO_2-induced memory impairment; conjugation; methylatropine

Senile dementia of the Alzheimer type (SDAT) has been shown to be associated with central cholinergic dysfunction.[1] Therefore, the use of agents capable of stimulating central cholinergic transmisson has been regarded as a viable strategy for the treatment of SDAT. We have started studies on both inhibitors of acetylcholinesterase (AChE) and muscarinic agonists, and recently reported studies on AChE inhibitors based on a new working hypothesis.[2] Following an alternative approach, this paper describes the synthesis and biological activities of novel muscarinic agonists: 2-alkoxy-2,8-diazaspiro[4.5]decane-1,3-diones (**1**) and structurally related compounds.[3] In order to minimize the peripheral effects of agonists, chemical modification into labile derivatives (prodrugs)[4] or conjugates with muscarinic antagonists was also examined.

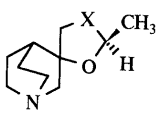
Design and Chemical Modification The therapeutic benefit of muscarinic agonists such as arecoline, oxotremorine, or RS-86 (Chart 1) is said to be limited by peripheral effects and short duration of action.[5] The use of M1 selective (pirenzepine sensitive) agonists has been suggested as one of the more promising approaches to overcome these disadvantages.[6] Some rigid analogues of acetylcholine (*cis*-AF30 and AF102B in Chart 1) have been synthesized based on the idea that rigidity of the molecule would produce selectivity towards M1 receptors.[7] We assumed that introduction of hetero atoms into these rigid compounds would improve their M1 selectivity and/or physicochemical properties through changes in steric, hydrophobic, and electronic characteristics. This might provide us with a muscarinic agonists with longer duration of action without showing peripheral effects. We therefore designed and synthesized a variety of spiro compounds which are shown in Chart 2. Among those synthesized (**1a**—**l**, **2**—**7**), 2-methoxy-2,8-diazaspiro[4.5]decane-1,3-dione (**1i**) was found to be a relatively M1 selective agonist and effective at reversing CO_2-induced memory impairment with long duration of action (see Biological Results and Discussion). The compound, however, showed peripheral effects at fairly low doses.



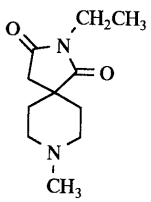
oxotremorine



arecoline



X=O : *cis*-AF30
X=S : AF102B



RS-86

Chart 1

To minimize the adverse side effects of **1i** by increasing its penetration into and hence concentration in the brain, chemical modification into labile derivatives (prodrugs)[4] with improved physicochemical properties was carried out. Verbiscar and Abood have suggested that "latentiation of centrally active amine appears feasible *via* carbamoylation."[8a] It has also been indicated that hydrolysis of carbamates might be facilitated through chemical manipulation such as activation of the leaving group by electron-withdrawing substituents.[8] This idea prompted us to prepare a variety of carbamate derivatives (**1m**—**t**) as prodrugs of **1i**.

As an alternative way to minimize the peripheral side effects of **1i**, carbamate linked conjugation with methylatropine, a well-known muscarinic antagonist, seemed attractive for the following two reasons: (1) because methylatropine is a quarternary salt, it was expected that the antagonist part would not penetrate the blood–brain barrier, thus antagonizing only the peripheral muscarinic effects of the mother compound **1i**; (2) the conjugation may improve absorption and/or distribution. Thus the carbamate linked conjugate (**1u**) of **1i** with methylatropine was prepared.

Synthesis Synthesis of the 2,8-diazaspiro[4.5]decane-1,3-diones (**1**) was carried out starting from the known diesters (**8**)[9] as outlined in Chart 3. Acid hydrolysis of the esters followed by treatment with dicyclohexylcarbodiimide (DCC) gave the anhydrides (**9**). Reaction of **9** with alkoxylamines or *N*,*N*′-dimethylhydrazine and subsequent cyclization yielded 2,8-diazaspiro[4.5]decane-1,3-diones (**1b**, **f**—**h**, **l**) and 2,3,9-triazaspiro[5.5]undecane-1,4-dione

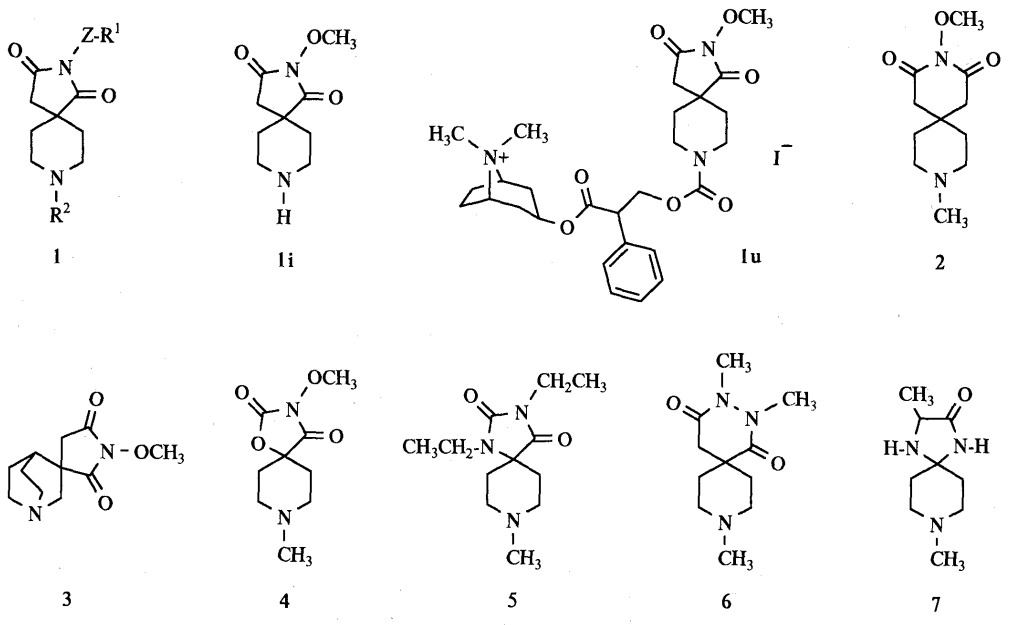
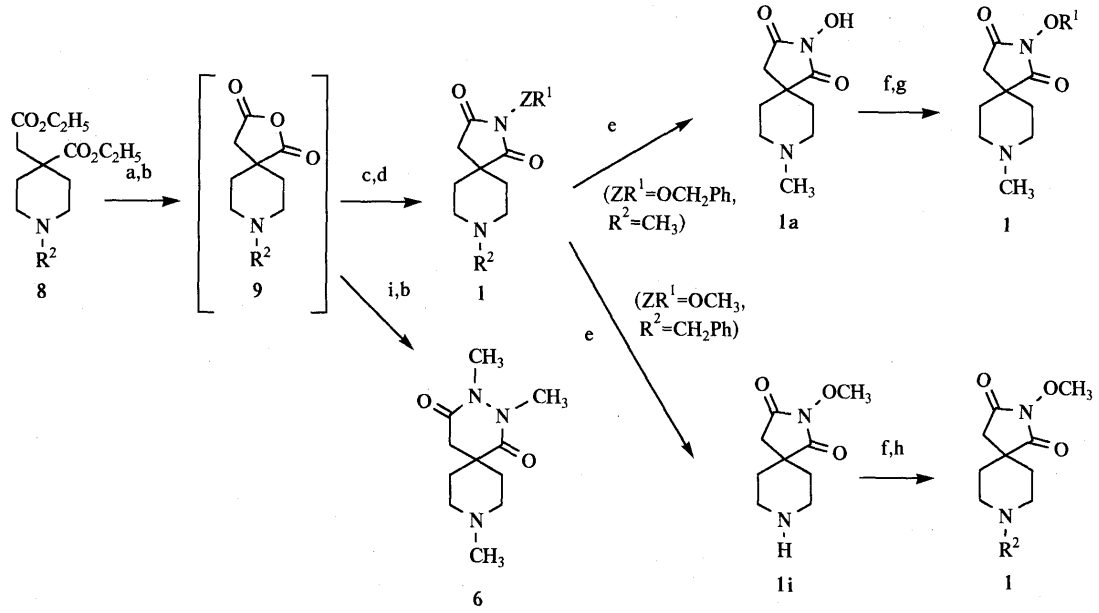


Chart 2



a: conc. HCl b: DCC c: R^1ZNH_2 d: Ac_2O, NaOAc e: H_2/Pd f: NaH g: R^1I h: R^2I i: $CH_3NHNHCH_3$

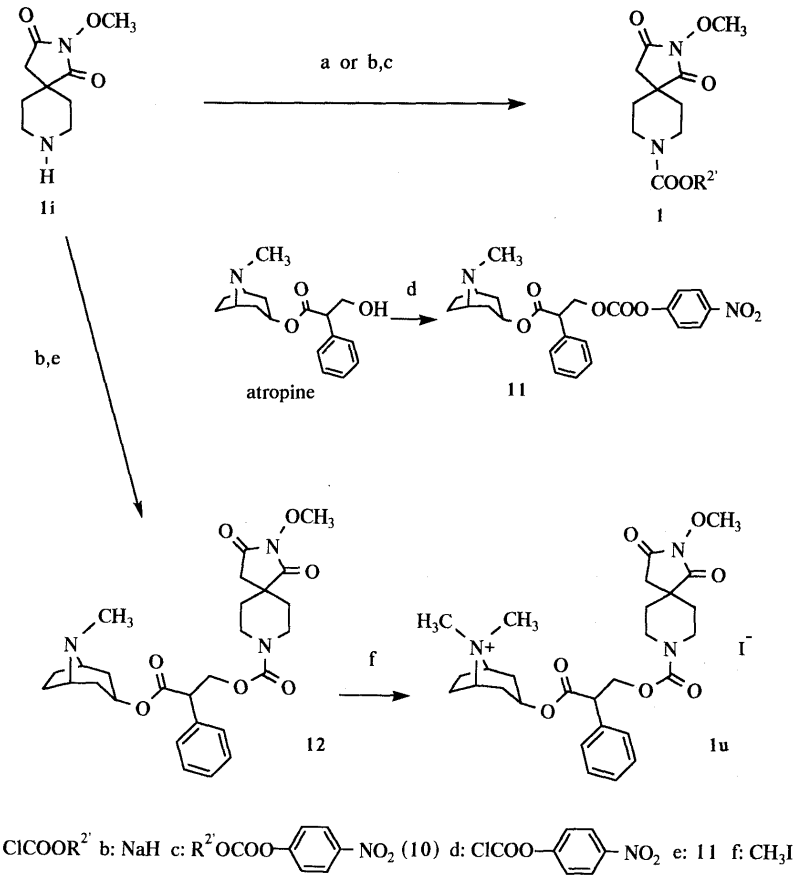
Chart 3

(**6**). The 2-alkoxy derivatives (**1c—e**) were prepared by alkylation of the 2-hydroxy derivative (**1a**), which in turn was obtained by hydrogenation of the 2-benzyloxy derivative (**1f**) over Pd. Catalytic hydrogenation of the 8-benzyl derivative (**1l**) gave 2-methoxy-2,8-diazaspiro-[4.5]decane-1,3-dione (**1i**), which was alkylated to yield the 8-alkyl derivatives (**1j** and **1k**).

Preparation of the carbamate derivatives of **1i** (**1m, n, q, t**) was made by the Shotten–Baumann procedure as shown in Chart 4. The other carbamates (**1o, p, r, s**) were obtained by successive treatment of **1i** with NaH followed by *p*-nitrophenyl alkoxycarbonate (**10**), which was prepared by reaction of the corresponding alcohol and chloro *p*-nitrophenylcarbonate.

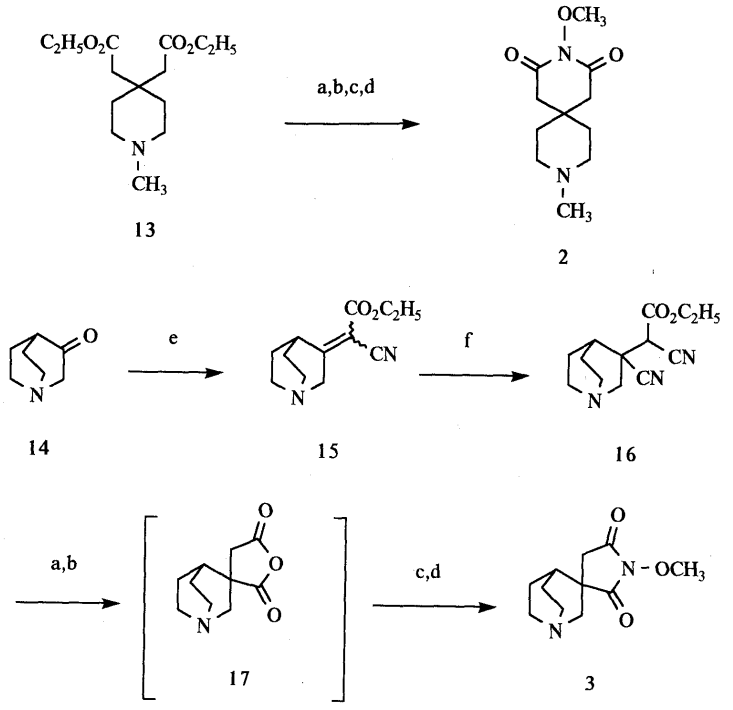
Atropine was allowed to react with chloro *p*-nitrophenylcarbonate to give the *p*-nitrophenylcarbonate (**11**). Successive treatment of **1i** with NaH followed by **11** yielded the carbamate (**12**), which led to the desired conjugate (**1u**) upon methylation with iodomethane.

The ring expanded analogue (**2**) and quinuclidine analogue (**3**) of **1b** were obtained as shown in Chart 5. In the same manner as described for **1b**, 3-methoxy-3,9-diazaspiro[5.5]undecane-2,4-dione (**2**) was prepared starting from the diester (**13**).[9b] Knoevenagel condensation of quinuclidin-3-one (**14**) with ethyl cyanoacetate gave the α,β-unsaturated ester (**15**). Michael addition of **15** with KCN yielded the ester (**16**), which was hydrolyzed, decarboxylated, and treated with DCC to give the acid anhydride



a: $ClCOOR^{2'}$ b: NaH c: $R^{2'}OCOO$–C_6H_4–NO_2 (10) d: $ClCOO$–C_6H_4–NO_2 e: 11 f: CH_3I

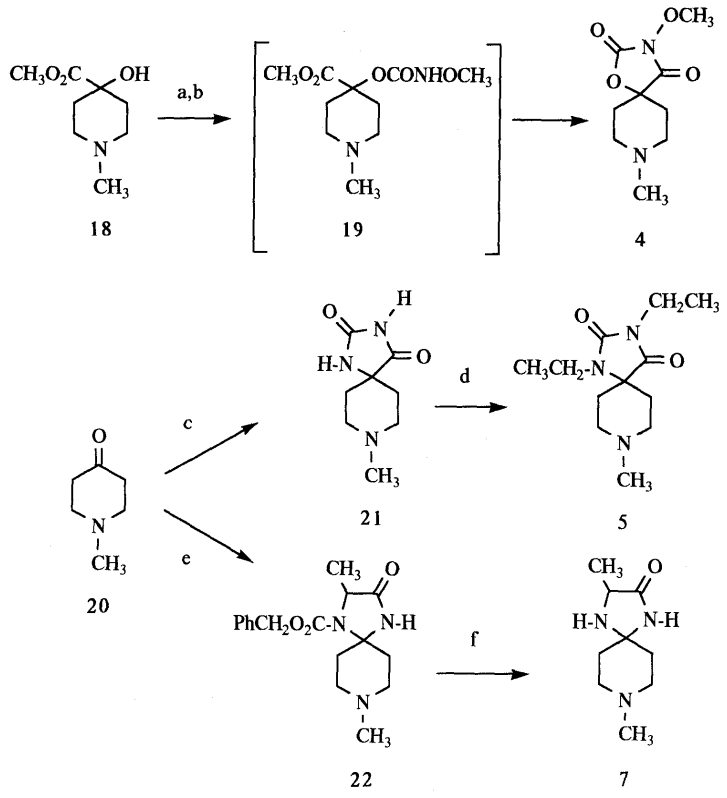
Chart 4



a: conc. HCl b: DCC c: CH_3ONH_2·HCl d: Ac_2O, NaOAc

e: $NCCH_2CO_2C_2H_5$, NH_4OAc, AcOH f: KCN

Chart 5



a: ClCOO-⟨⟩-NO_2 b: CH_3ONH_2·HCl c: $(NH_4)_2CO_3$, KCN d: NaH, C_2H_5I

e: $PhCH_2OCONHCH(CH_3)CONH_2$, *p*-toluenesulfonic acid f: H_2/Pd-C

Chart 6

(**17**). The desired quinuclidine derivative (**3**) was obtained from **17** in the same manner as described for **1b**.

Chart 6 illustrates the preparation of other spiro compounds (**4**, **5**, and **7**). 3-Methoxy-1-oxa-3,8-diazaspiro-[4.5]decane-2,4-dione (**4**) was obtained by cyclization of the carbamate intermediate (**19**), which was prepared by successive treatment of 4-hydroxy-1-methylpiperidine-4-carboxylic acid methyl ester (**18**)[10] with chloro *p*-nitrophenylcarbonate and methoxylamine. A mixture of 1-methyl-4-piperidone (**20**), ammonium carbonate, and KCN was heated to give 8-methyl-1,3,8-triazaspiro[4.5]-decane-2,4-dione (**21**), which was alkylated to yield the 1,3-diethyl derivative (**5**). Treatment of **20** with (2-amino-1-methyl-2-oxoethyl)carbamic acid phenylmethyl ester followed by catalytic hydrogenation led to 1,4,8-triaza-spiro[4.5]decan-2-one (**7**).

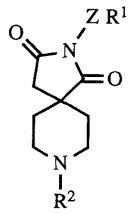
Biological Results and Discussion

In vitro receptor binding studies were carried out to assess the affinity of compounds for muscarinic receptors in rat brain.[11] [^{3}H]Pirenzepine (Pir, M1 selective antagonist) and [^{3}H]oxotremorine M (Oxo-M, M2 agonist)[12] were used as ligands and the ratio of the affinity, [IC_{50}(Oxo-M)/IC_{50}(Pir)] × 100,[13] gave a measure of the M1 selectivity. The compounds were also tested for their agonistic or antagonistic activities in the isolated guinea pig ileum. In these assays, oxotremorine, arecoline, and RS-86 were used as reference compounds.

Table I shows that 2-alkoxy-2,8-diazaspiro[4.5]decane-1,3-diones (**1b**—**f**) exhibit medium to strong receptor binding affinity whereas the 2-hydroxy derivative (**1a**) has no affinity. With bulkier alkoxy groups the Oxo-M/Pir ratio increased and **1d**—**f** displayed larger Oxo-M/Pir ratio than RS-86. Among the 2-alkoxy derivatives (**1b**—**f**), 2-methoxy derivative (**1b**) acted as a strong agonist in the ileum assay; **1c** and **1d** were weak muscarinic agonists. The 2-benzyloxy derivative (**1f**), which showed large M1 selectivity, did not display agonistic activity. Replacement of the OMe group at the 2-position by NHMe or NMe_2 groups reduced the binding affinity for Oxo-M (**1g**, **h** *vs.* **1b**), thus increasing the Oxo-M/Pir ratio. These compounds maintained agonistic potency. Among compounds (**1b**, **i**—**l**) bearing various alkyl substituents on nitrogen atom at 8-position, the unsubstituted derivative (**1i**: R^2=H) showed strong affinity for muscarinic receptors and acted as an agonist in the ileum assay.

The ring expanded analogue of the strong agonist **1b**, 3-methoxy-3,9-diazaspiro[5.5]undecane-2,4-dione (**2**), exhibited neither receptor binding affinity nor agonistic activity. Surprisingly, the quinuclidine analogue (**3**) acted as an antagonist in the ileum assay. 3-Methoxy-1-oxa-3,8-diazaspiro[4.5]decane-2,4-dione (**4**), which is an oxa analogue of **1b**, maintained agonistic character but was less potent than **1b**. 1,3,8-Triazaspiro[4.5]decane-2,4-dione (**5**) displayed weak receptor binding affinity for Pir and was inactive in the ileum assay. Both 2,3,9-triazaspiro[5.5]un-decane-1,4-dione (**6**) and 1,4,8-triazaspiro[4.5]decan-2-one (**7**) showed weak binding affinity for Oxo-M and were weak

TABLE I. Muscarinic Receptor Binding Affinity and Agonistic Activities of 2,8-Diazaspiro[4.5]decane-1,3-diones (**1a**—**l**) and Their Related Compounds (**2**—**7**)[a]



1a-l

Compound No.	R^2	ZR^1	Binding data: IC_{50} (μM)			Agonism[e]
			Oxo-M[b]	Pir[c]	Ratio[d]	pD_2
Oxotremorine			0.0021	0.20	1.05	6.8
Arecoline			0.036	3.0	1.20	6.5
RS-86			0.33	0.26	127	6.0
1a	CH_3	OH	>100	>100	—	Inactive
1b	CH_3	OCH_3	0.27	4.7	5.74	5.9
1c	CH_3	OCH_2Cl	1.6	4.2	38.1	3.5
1d	CH_3	OCH_2CH_3	44	11	400	<3.5
1e	CH_3	$OCH_2CH_2CH_3$	33	6.6	500	Inactive
1f	CH_3	OCH_2Ph	3.2	0.14	2290	Inactive
1g	CH_3	$NHCH_3$	1.4	4.5	31.1	5.5
1h	CH_3	$N(CH_3)_2$	6.2	5.4	115	4.5
1i	H	OCH_3	0.3	4.6	6.52	5.3
1j	CH_2CH_3	OCH_3	4.3	45	9.56	Inactive
1k	$CH_2CH_2CH_3$	OCH_3	>100	>100	—	Inactive
1l	CH_2Ph	OCH_3	39	35	111	Inactive
2[a]	—	—	50	>100	<50	Inactive
3[a]	—	—	1.2	0.22	545	(5.5)[f]
4[a]	—	—	3.8	18	21.1	4.4
5[a]	—	—	>100	21	>476	Inactive
6[a]	—	—	69	>100	<69	<3.5
7[a]	—	—	4.8	>100	<4.8	4.2

a) See Chart 2 for the structure. *b*) Displacement of [^{3}H]oxotremorine M. *c*) Displacement of [^{3}H]pirenzepine. *d*) The ratio of [IC_{50}(Oxo-M)/IC_{50}(Pir)] × 100. *e*) Effect on guinea pig ileum. *f*) pA_2 value as an antagonist.

TABLE II. Ameliorating Effects,[a] Side Effects, and the Resulting Therapeutic Indices of 2-Methoxy-2,8-diazaspiro[4.5]decane-1,3-diones (**1b, i, q, u**)

Compound	Ameliorating effects MED (mg/kg, *p.o.*)	Side effects ED_{50} (mg/kg, *p.o.*)			Therapeutic indices		
		sal.[b]	lac.[c]	dia.[d]	Ratio (sal.)[e]	Ratio (lac.)[f]	Ratio (dia.)[g]
Oxotremorine	0.01	0.12	0.20	0.19	12	20	19
Arecoline	0.3	>200	>3	>200	>666	>10	>666
RS-86	0.3	0.44	0.66	2.8	1.5	2.2	9.3
1b	0.3	1.7	3.3	10.0	5.7	11	33
1i	0.1	3.8	9.2	26.0	38	92	260
1q	1.0	5.2	14.2	65.6	5.2	14.2	65.6
1u	0.1	>100	>100	>100	>1,000	>1,000	>1,000

a) Ameliorating effects on CO_2-induced impairment of passive avoidance response. *b*) Salivation. *c*) Lacrimation. *d*) Diarrhea. *e*) ED_{50}(sal.)/MED(ameliorating effect). *f*) ED_{50}(lac.)/MED(ameliorating effect). *g*) ED_{50}(dia.)/MED(ameliorating effect).

agonists.

The compounds (**1b**—**d**, **g**—**i**, **4**, **6**, and **7**) with agonistic character were evaluated for their ameliorating effects on CO_2-induced impairment of passive avoidance response. Oxotremorine, arecoline, and RS-86 showed significant improvement of the memory impairment at doses of 0.01, 0.3, and 0.3 mg/kg (*p.o.*), respectively. Among the compounds synthesized, **1b** and **1i** significantly improved the impairment at doses of 0.3 and 0.1 mg/kg (*p.o.*), respectively (Table II). Table III illustrates that **1i** has a duration of action longer than four hours.[14] Unfortunately, the compounds with greater M1 selectivity than **1b** or **1i** (**1c**, **d**, **g**, **h**, **4**, **6**, and **7**) were not effective at reversing memory impairment. Table II also shows that **1b** and **1i** exhibit peripheral effects at fairly low doses and that their therapeutic indices[15] are lower than those of arecoline. Furthermore, RS-86 with large M1 selectivity displayed the lowest therapeutic indices. These may suggest that the approach using M1 selective agonists is not sufficient to overcome these side effects.

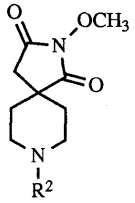
We hoped to minimize the adverse side effects of **1i** by chemical modification to carbamates or by conjugation with

TABLE III. Duration of Ameliorating Effects of **1i** and **1u** on CO_2-Induced Impairment of Passive Avoidance Response

Compound (dose)	Mean latency (% of control) Time after administration			
	30 min	1 h	2 h	4 h
1i (0.3 mg/kg, *p.o.*)	321[a]	189[b]	241[b]	199[b]
1u (1.0 mg/kg, *p.o.*)	243[b]	157	153	122

Statistically significant at *a*) $p=0.01$, *b*) $p=0.05$.

TABLE IV. Muscarinic Receptor Binding Affinity and Agonistic Activities of 2-Methoxy-2,8-diazaspiro[4.5]decane-1,3-diones (**1m—u**)



1m-u

Compd. No.	R^2	Binding data: IC_{50} (μM)			Agonism[d]
		Oxo-M[a]	Pir[b]	Ratio[c]	pD_2
1m	CO_2CH_3	>100	>100	—	Inactive
1n	$CO_2CH_2CH_3$	>100	>100	—	Inactive
1o	$CO_2CH_2CH_2F$	>100	>100	—	Inactive
1p	$CO_2CH_2CH_2Cl$	76	>100	<76	Inactive
1q	$CO_2CH_2CH_2Br$	0.93	17	5.5	4.9
1r	$CO_2CH_2CH_2I$	0.15	65	0.23	4.7
1s	$CO_2(CH_2)_3Br$	2.6	>100	<2.6	Inactive
1t	CO_2-C$_6$H$_4$-NO_2	>100	3.8	>2630	Inactive
1u[e]	—	0.21	0.048	438	(7.1)[f]

a) Displacement of [^{3}H]oxotremorine M. *b*) Displacement of [^{3}H]pirenzepine. *c*) The ratio of $[IC_{50}(\text{Oxo-M})/IC_{50}(\text{Pir})]\times 100$. *d*) Effect on guinea pig ileum. *e*) See Chart 2 for the structure. *f*) pA_2 value as an antagonist.

methylatropine (see Design and Chemical Modification).

Among the carbamate derivatives (**1m—t**) of **1i**, 2-halogenoethyl carbamates (**1q** and **1r**) exhibited strong affinities for muscarinic receptors as well as agonistic activity (Table IV). The stronger affinity of 2-iodoethyl carbamate (**1r**) for Oxo-M compared with **1i** suggests that these carbamates might be acting not only as prodrugs of **1i** but also as agonists themselves. The 3-bromopropyl carbamate (**1s**) showed weaker binding affinities than **1q**. The *p*-nitrophenyl carbamate (**1t**), which has been shown by Verbiscar and Abood to be the most effective,[8a] displayed binding affinity only for Pir, giving the highest Oxo-M/Pir ratio. Among the carbamates (**1m—t**), **1q** significantly reversed CO_2-induced memory impairment at a dose of 1.0 mg/kg (*p.o.*). Unexpectedly, therapeutic indices of **1q** were less than those of **1i**. Other derivatives including **1t** did not reverse the memory impairment at doses of up to 30 mg/kg (*p.o.*).[16]

The conjugate (**1u**) of **1i** with methylatropine displayed strong affinities for muscarinic receptors and acted as an antagonist in the ileum assay (Table IV). As we expected (see Design and Chemical Modification), **1u** showed significant effect on reversing CO_2-induced memory impairment at a dose of 0.1 mg/kg (*p.o.*) and adverse peripheral effects were not detected at doses of up to 100 mg/kg (*p.o.*). Unfortunately, the duration of action of **1u** was less than one hour, which was shorter than **1i** (Table III). This could be due to the difference of absorption and/or metabolism. Further investigation is necessary to assess the idea of conjugation of muscarinic agonists with muscarinic antagonists, nevertheless, this preliminary study showed that the idea might be an attractive approach for the treatment of SDAT.[17]

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were measured on a Jasco IR-810 infrared spectrophotometer in KBr disks for solids and liquid films for oils. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian EM-390 NMR spectrometer with tetramethylsilane for $CDCl_3$ and dimethylsulfoxide-d_6 (DMSO-d_6) or with 3-(trimethylsilyl)propionic acid sodium salt for D_2O as internal standard. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad.

2-Methoxy-8-methyl-2,8-diazaspiro[4.5]decane-1,3-dione Hydrochloride (1b) A mixture of [1-methyl-4-(ethoxycarbonyl)piperidin-4-yl]acetic acid ethyl ester[9a] (6.00 g) and concentrated HCl (40 ml) was gently refluxed for 16 h. Excess concentrated HCl was evaporated *in vacuo*. Toluene was added to the residue and the remaining water in the mixture was removed completely by azeotropic distillation with a Dean–Stark water separator to give (1-methyl-4-carboxypiperidin-4-yl)acetic acid hydrochloride (5.30 g, 96%) as an amorphous powder.

DCC (4.50 g) was added to a solution of the acid (4.70 g) in *N*,*N*-dimethylformamide (DMF, 100 ml) and the solution was stirred at room temperature for 1 h. Methoxylamine hydrochloride (1.65 g) and Et_3N (2.8 ml) were added to the solution and the resulting mixture was stirred at room temperature for 30 min. The precipitate was removed by filtration and the filtrate was concentrated to give an oil. A mixture of the oil, acetic anhydride (80 ml), and sodium acetate (4.20 g) was heated at 100 °C for 30 min. The precipitate was removed by filtration and the filtrate was concentrated to give a residue. Treatment of the residue with ethanolic HCl (1 eq) afforded a crude solid, which was recrystallized from ethanol–ethyl acetate to give colorless prisms (2.30 g, 42%), mp 259 °C. IR (KBr): 1715 cm^{-1}. ^{1}H-NMR (D_2O) δ: 2.00—2.40 (4H, m), 2.90 (3H, s), 2.93 (2H, s), 3.03—3.80 (4H, m), 3.90 (3H, s). *Anal.* Calcd for $C_{10}H_{16}N_2O_3\cdot HCl$: C, 48.29; H, 6.89; N, 11.26. Found: C, 48.18; H, 6.90; N, 10.97.

The following compounds (**1f—h**, **1**, **2**, and **6**) were prepared in the same manner as described for **1b**.

8-Methyl-2-(phenylmethoxy)-2,8-diazaspiro[4.5]decane-1,3-dione Hydrochloride (**1f**): Colorless prisms, mp 261—263 °C. Yield: 76%. IR (KBr): 1715 cm^{-1}. ^{1}H-NMR (D_2O) δ: 2.00—2.30 (4H, m), 2.91 (3H, s), 2.93 (2H, s), 3.05—3.80 (4H, m), 4.37 (2H, s), 7.53 (5H, s). *Anal.* Calcd for $C_{16}H_{20}N_2O_3\cdot HCl$: C, 59.17; H, 6.52; N, 8.62. Found: C, 59.21; H, 6.62; N, 8.50.

8-Methyl-2-(methylamino)-2,8-diazaspiro[4.5]decane-1,3-dione Hydrochloride (**1g**): Colorless powder, mp 235—260 °C. Yield: 13%. IR (KBr): 1710 cm^{-1}. ^{1}H-NMR (D_2O) δ: 2.00—2.40 (4H, m), 2.62 (3H, s), 2.87 (3H, s), 2.91 (2H, s), 3.00—3.70 (4H, m). *Anal.* Calcd for $C_{10}H_{17}N_3O_2\cdot HCl$: C, 48.49; H, 7.32; N, 16.96. Found: C, 48.25; H, 7.47; N, 16.83.

2-(Dimethylamino)-8-methyl-2,8-diazaspiro[4.5]decane-1,3-dione Hydrochloride (**1h**): Colorless cubes, mp 229—230 °C. Yield: 42%. IR (KBr): 1710 cm^{-1}. ^{1}H-NMR (D_2O) δ: 2.00—2.40 (4H, m), 2.87 (6H, s), 2.93 (3H, s), 2.97 (2H, s), 3.08—3.80 (4H, m). *Anal.* Calcd for $C_{11}H_{19}N_3O_2\cdot HCl$: C, 50.48; H, 7.70; N, 16.05. Found: C, 50.30; H, 7.81; N, 16.01.

2-Methoxy-8-(phenylmethyl)-2,8-diazaspiro[4.5]decane-1,3-dione Hydrochloride (**1l**): Colorless prisms, mp 227—229 °C. Yield: 37% (from [4-ethoxycarbonyl-1-(phenylmethyl)piperidin-4-yl]acetic acid ethyl ester).[9a] IR (KBr): 1725 cm^{-1}. ^{1}H-NMR (D_2O) δ: 2.00—2.40 (4H, m), 2.90 (2H, s), 3.05—3.70 (4H, m), 3.92 (3H, s), 4.35 (2H, s), 7.45 (5H, s). *Anal.* Calcd for $C_{16}H_{20}N_2O_3\cdot HCl$: C, 59.17; H, 6.52; N, 8.62. Found: C, 59.21; H, 6.62; N, 8.50.

3-Methoxy-9-methyl-3,9-diazaspiro[5.5]undecane-2,4-dione Hydrochloride (**2**): Colorless cubes, mp 275—284 °C. Yield: 44% (from [4-(ethoxycarbonylmethyl)-1-methylpiperidin-4-yl]acetic acid ethyl ester hydrochloride).[9b] IR (KBr): 1690 cm^{-1}. ^{1}H-NMR (D_2O) δ: 1.72—1.93

(4H, m), 2.83 (2H, s), 2.87 (3H, s), 3.00—3.67 (6H, m), 3.80 (3H, s). *Anal.* Calcd for $C_{11}H_{18}N_2O_3 \cdot HCl$: C, 50.29; H, 7.29; N, 10.66. Found: C, 50.35; H, 7.17; N, 10.57.

2,3,9-Trimethyl-2,3,9-triazaspiro[5.5]undecane-1,4-dione Hydrochloride (**6**): Colorless cubes, mp 246—248 °C. Yield: 25%. IR (KBr): 1670 cm^{-1}. ^1H-NMR (D_2O) δ: 2.00—2.30 (4H, m), 2.64 (2H, s), 2.88 (3H, s), 3.10—3.60 (10H, m). *Anal.* Calcd for $C_{11}H_{19}N_3O_2 \cdot HCl$: C, 50.48; H, 7.70; N, 16.05. Found: C, 50.35; H, 7.77; N, 15.87.

2-Methoxy-2,8-diazaspiro[4.5]decane-1,3-dione Hydrochloride (1i) A solution of **1l** (3.24 g) in water (20 ml) was hydrogenated over Pd catalyst at room temperature and atmospheric pressure for 10 h. The catalyst was removed by filtration and the filtrate was concentrated to give a solid, which was recrystallized from ethanol to afford colorless prisms (2.10 g, 89%), mp 273 °C. IR (KBr): 1710 cm^{-1}. ^1H-NMR (D_2O) δ: 2.00—2.40 (4H, m), 2.87 (2H, s), 3.00—3.80 (4H, m), 3.93 (3H, s). *Anal.* Calcd for $C_9H_{14}N_2O_3 \cdot HCl$: C, 46.06; H, 6.44; N, 11.94. Found: C, 45.81; H, 6.46; N, 12.03.

2-Hydroxy-8-methyl-2,8-diazaspiro[4.5]decane-1,3-dione hydrochloride (**1a**) was prepared by catalytic hydrogenation of **1f** in the same manner as described for **1i**. Recrystallization of crude product from ethanol gave colorless prisms, mp 284—186 °C. Yield: 99%. IR (KBr): 2700, 1710 cm^{-1}. ^1H-NMR (D_2O) δ: 2.00—2.50 (4H, m), 2.92 (3H, s), 2.95 (2H, s), 3.00—3.80 (4H, m). *Anal.* Calcd for $C_9H_{14}N_2O_3 \cdot HCl$: C, 46.06; H, 6.44; N, 11.94. Found: C, 46.13; H, 6.65; N, 12.11.

8-Ethyl-2-methoxy-2,8-diazaspiro[4.5]decane-1,3-dione Hydrochloride (1j) Sodium hydride (60% in oil, 0.88 g) was added portionwise to a suspension of **1i** (2.37 g) in DMF (50 ml) and the mixture was stirred at room temperature for 1 h. Bromoethane (1.09 g) was then added and, after stirring for 2 h at room temperature, the solvent was removed *in vacuo* to give a residue. The residue was chromatographed on silica gel eluting with CH_2Cl_2–methanol–water (14 : 6 : 1) to afford an oil. Treatment of the oil with ethanolic HCl (1 eq) gave colorless prisms (1.50 g, 57%) after recrystallization from ethanol, mp 232—235 °C. IR (KBr): 1720 cm^{-1}. ^1H-NMR (D_2O) δ: 1.22 (3H, t, J=7 Hz), 2.00—2.40 (4H, m), 2.83 (2H, s), 3.03 (2H, q, J=7 Hz), 3.00—3.80 (4H, m), 3.94 (3H, s). *Anal.* Calcd for $C_{11}H_{18}N_2O_3 \cdot HCl$: C, 50.29; H, 7.29; N, 10.66. Found: C, 50.21; H, 7.32; N, 10.71.

The following compounds (**1c—e, k**) were prepared in the same manner as described above.

2-(Chloromethoxy)-8-methyl-2,8-diazaspiro[4.5]decane-1,3-dione Hydrochloride (**1c**): Colorless prisms, mp 195—198 °C. Yield: 35%. IR (KBr): 1713 cm^{-1}. ^1H-NMR (D_2O) δ: 2.05—2.35 (4H, m), 2.90 (3H, s), 2.92 (2H, s), 3.05—3.80 (4H, m), 5.90 (2H, s). *Anal.* Calcd for $C_{10}H_{15}ClN_2O_3 \cdot HCl$: C, 42.42; H, 5.70; N, 9.89. Found: C, 42.34; H, 5.93; N, 9.69.

2-Ethoxy-8-methyl-2,8-diazaspiro[4.5]decane-1,3-dione Hydrochloride (**1d**): Colorless prisms, mp 222—225 °C. Yield: 51%. IR (KBr): 1715 cm^{-1}. ^1H-NMR (D_2O) δ: 1.28 (3H, t, J=7 Hz), 2.00—2.50 (4H, m), 2.92 (3H, s), 2.95 (2H, s), 3.00—3.80 (4H, m), 4.18 (2H, q, J=7 Hz). *Anal.* Calcd for $C_{11}H_{18}N_2O_3 \cdot HCl$: C, 50.29; H, 7.29; N, 10.66. Found: C, 50.25; H, 7.25; N, 10.76.

8-Methyl-2-propoxy-2,8-diazaspiro[4.5]decane-1,3-dione Hydrochloride (**1e**): Colorless prisms, mp 231—234 °C. Yield: 53%. IR (KBr): 1710 cm^{-1}. ^1H-NMR (D_2O) δ: 0.93 (3H, t, J=7 Hz), 1.53—1.85 (2H, m), 2.00—2.30 (4H, m), 2.91 (3H, s), 2.93 (2H, s), 3.03—3.80 (4H, m), 4.06 (2H, t, J=7 Hz). *Anal.* Calcd for $C_{12}H_{20}N_2O_3 \cdot HCl$: C, 52.08; H, 7.65; N, 10.12. Found: C, 51.84; H, 7.89; N, 10.10.

2-Methoxy-8-propyl-2,8-diazaspiro[4.5]decane-1,3-dione Hydrochloride (**1k**): Colorless prisms, mp 192 °C. Yield: 64%. IR (KBr): 1710 cm^{-1}. ^1H-NMR (D_2O) δ: 0.97 (3H, t, J=7 Hz), 1.60—1.90 (2H, m), 2.00—2.40 (4H, m), 2.90 (2H, s), 3.00—3.80 (6H, m), 3.94 (3H, s). *Anal.* Calcd for $C_{12}H_{20}N_2O_3 \cdot HCl$: C, 52.08; H, 7.65; N, 10.12. Found: C, 52.01; H, 7.53; N, 10.18.

2-Methoxy-1,3-dioxo-2,8-diazaspiro[4.5]decane-8-carboxylic Acid 2-Bromoethyl Ester (1q) A mixture of $NaHCO_3$ (4.50 g) and **1i** (5.00 g) in dioxane–water (100/100 ml) was stirred at room temperature for 20 min. 2-Bromoethyl chlorocarbonate (6.00 g) was added and the mixture was stirred at room temperature for 1 h and extracted with CH_2Cl_2. The extracts were washed with water, dried over Na_2SO_4, and the solvent was removed *in vacuo* to give a residue. The residue was chromatographed on silica gel eluting with CH_2Cl_2–ethyl acetate (20 : 1) to afford colorless crystals. Recrystallization from CH_2Cl_2–ether gave colorless prisms (6.50 g, 87%), mp 134 °C. IR (KBr): 1780, 1730, 1705, 1695 cm^{-1}. ^1H-NMR ($CDCl_3$) δ: 1.40—1.70 (2H, m), 1.85—2.20 (2H, m), 2.55 (2H, s), 3.00—3.40 (2H, m), 3.52 (2H, t, J=6 Hz), 3.80—4.20 (5H, m), 4.40 (2H, t, J=6 Hz). *Anal.* Calcd for $C_{12}H_{17}BrN_2O_5$: C, 41.28; H, 4.91; N, 8.02. Found: C, 41.51; H, 4.95; N, 7.98.

The following carbamates (**1m, n, t**) were prepared in the same manner as described for **1q**.

2-Methoxy-1,3-dioxo-2,8-diazaspiro[4.5]decane-8-carboxylic Acid Methyl Ester (**1m**): Colorless prisms, mp 128 °C. Yield: 90%. IR (KBr): 1725, 1685 cm^{-1}. ^1H-NMR ($CDCl_3$) δ: 1.30—1.70 (2H, m), 1.80—2.20 (2H, m), 2.53 (2H, s), 2.95—3.30 (2H, m), 3.70 (3H, s), 3.93 (3H, s), 3.94—4.20 (2H, m). *Anal.* Calcd for $C_{11}H_{16}N_2O_5$: C, 51.56; H, 6.29; N, 10.93. Found: C, 51.56; H, 6.37; N, 10.74.

2-Methoxy-1,3-dioxo-2,8-diazaspiro[4.5]decane-8-carboxylic Acid Ethyl Ester (**1n**): Colorless prisms, mp 88—90 °C. Yield: 88%. IR (KBr): 1725, 1680 cm^{-1}. ^1H-NMR ($CDCl_3$) δ: 0.95 (3H, t, J=7 Hz), 1.35—1.72 (2H, m), 1.83—2.18 (2H, m), 2.53 (2H, s), 2.96—3.35 (2H, m), 3.95 (3H, s), 3.80—4.22 (4H, m). *Anal.* calcd for $C_{12}H_{18}N_2O_5$: C, 53.33; H, 6.71; N, 10.36. Found: C, 53.05; H, 6.71; N, 10.30.

2-Methoxy-1,3-dioxo-2,8-diazaspiro[4.5]decane-8-carboxylic Acid 4-Nitrophenyl Ester (**1t**): Colorless prisms, mp 193—194 °C. Yield: 78%. IR (KBr): 1785, 1730, 1710 cm^{-1}. ^1H-NMR ($CDCl_3$) δ: 1.50—1.84 (2H, m), 2.14 (2H, ddd, J=4, 10, 14 Hz), 2.62 (2H, s), 3.20—3.65 (2H, m), 3.85—4.33 (2H, m), 3.96 (3H, s), 7.29 (2H, d, J=9 Hz), 8.25 (2H, d, J=9 Hz). *Anal.* Calcd for $C_{16}H_{17}N_3O_7$: C, 52.89; H, 4.72; N, 11.57. Found: C, 52.75; H, 4.72; N, 11.52.

2-Methoxy-1,3-dioxo-2,8-diazaspiro[4.5]decane-8-carboxylic Acid 2-Chloroethyl Ester (1p) Pyridine (0.29 ml) was added dropwise to a solution of 2-chloroethanol (0.24 ml) and *p*-nitrophenyl chlorocarbonate (0.80 g) in ether (10 ml) at 0—5 °C. The mixture was stirred at 0—5 °C for 1 h and then at room temperature for 2 h. Resulting precipitate was removed by filtration. The filtrate was washed successively with 10% HCl and water, dried over Na_2SO_4, and concentrated to give 2-chloroethyl *p*-nitrophenylcarbonate as a colorless oil.

A mixture of **1i** (0.40 g) and NaH (oil free, 90 mg) in DMF (5 ml) was heated at 50—55 °C for 30 min and cooled to 0—5 °C. A solution of the above oil in DMF (2 ml) was added to the mixture at 0—5 °C. The mixture was stirred at room temperature for 2 h, quenched with methanol, and the solvent was evaporated to give a residue. The residue was chromatographed on silica gel eluting with CH_2Cl_2–ethyl acetate (20 : 1) to afford colorless crystals. Recrystallization from CH_2Cl_2–ether gave colorless cubes (0.40 g, 77%), mp 87—90 °C. IR (KBr): 1790, 1730, 1695 cm^{-1}. ^1H-NMR ($CDCl_3$) δ: 1.37—2.20 (4H, m), 2.56 (2H, s), 3.03—3.37 (2H, m), 3.67 (2H, t, J=5.5 Hz), 3.83—4.20 (5H, m), 4.35 (2H, t, J=5.5 Hz). *Anal.* Calcd for $C_{12}H_{17}ClN_2O_5$: C, 47.30; H, 5.62; N, 9.19. Found: C, 47.45; H, 5.32; N, 9.37.

The following compounds (**1o, r, s**) were prepared in the same manner as described for **1p**.

2-Methoxy-1,3-dioxo-2,8-diazaspiro[4.5]decane-8-carboxylic Acid 2-Fluoroethyl Ester (**1o**): Colorless plates, mp 122—124 °C. Yield: 17%. IR (KBr): 1785, 1725, 1695 cm^{-1}. ^1H-NMR ($CDCl_3$) δ: 1.36—1.76 (2H, m), 2.03 (2H, ddd, J=4, 10, 14 Hz), 2.56 (2H, s), 3.20 (2H, ddd, J=3, 9, 14 Hz), 3.80—4.95 (6H, m), 3.96 (3H, s). *Anal.* Calcd for $C_{12}H_{17}FN_2O_5$: C, 50.00; H, 5.94; N, 9.72. Found: C, 50.03; H, 5.98; N, 9.62.

2-Methoxy-1,3-dioxo-2,8-diazaspiro[4.5]decane-8-carboxylic Acid 2-Iodoethyl Ester (**1r**): Colorless cubes, mp 127—130 °C. Yield: 34%. IR (KBr): 1785, 1720, 1685 cm^{-1}. ^1H-NMR ($CDCl_3$) δ: 1.35—1.75 (2H, m), 2.03 (2H, ddd, J=4, 10, 14 Hz), 2.55 (2H, s), 3.00—3.47 (4H, m), 3.70—4.25 (2H, m), 3.93 (3H, s), 4.33 (2H, t, J=7 Hz). *Anal.* Calcd for $C_{12}H_{17}IN_2O_5$: C, 36.38; H, 4.33; N, 7.07. Found: C, 36.43; H, 4.35; N, 6.97.

2-Methoxy-1,3-dioxo-2,8-diazaspiro[4.5]decane-8-carboxylic Acid 3-Bromopropyl Ester (**1s**): Colorless cubes, mp 129—133 °C. IR (KBr): 1790, 1730, 1700 cm^{-1}. ^1H-NMR ($CDCl_3$) δ: 1.35—2.37 (6H, m), 2.55 (2H, s), 2.97—3.65 (4H, m), 3.80—4.37 (7H, m). *Anal.* Calcd for $C_{13}H_{19}BrN_2O_5$: C, 42.99; H, 5.27; N, 7.71. Found: C, 43.04; H, 5.33; N, 7.57.

Preparation of *endo*-(±)-α-[(2-Methoxy-1,3-dioxo-2,8-diazaspiro[4.5]-decan-8-yl)carbonyloxymethyl]benzene Acetic Acid 8,8-Dimethyl-8-azoniabicyclo[3.2.1]oct-3-yl Ester Iodide (1u) *endo*-(±)-α-[(2-Methoxy-1,3-dioxo-2,8-diazaspiro[4.5]decan-8-yl)carbonyloxymethyl]benzene acetic acid 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester (**12**) was prepared in the same manner as described for **1p**. The crude product was chromatographed on silica gel eluting with butanol–ethyl acetate–acetic acid–water (1 : 1 : 1 : 1) to give an oil, which was dissolved in ethyl acetate, washed with saturated aq $NaHCO_3$, and dried over Na_2SO_4. The solvent was removed *in vacuo* to afford a colorless oil. Yield: 40%. IR (film): 1735, 1715, 1700, 1420, 1210 cm^{-1}. ^1H-NMR (DMSO-d_6) δ: 1.23—2.40 (14H, m), 2.25 (3H, s), 2.48 (2H, s), 2.80—3.25 (6H, m), 3.52—3.90 (1H, m), 3.92 (3H, s), 4.98 (1H, t, J=5.5 Hz), 7.30 (5H, s).

Iodomethane (0.3 ml) was added to a solution of the oil (**12**, 0.82 g) in ethyl acetate (25 ml). The mixture was stirred at room temperature for 16 h. The resulting precipitate was collected by filtration, which was recrystallized from ethanol–ethyl acetate to give **1u** (0.37 g, 97%) as a colorless powder, mp 168—172 °C. IR (KBr): 1735, 1730, 1710, 1460 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.45—2.40 (12H, m), 2.57 (2H, s), 2.85—3.20 (9H, m), 3.23 (3H, s), 3.65—4.10 (6H, m), 4.98 (1H, t, J=5.5 Hz), 7.33 (5H, s). *Anal.* Calcd for $C_{28}H_{38}IN_3O_7$: C, 51.30; H, 5.84; N, 6.41. Found: C, 51.04; H, 5.94; N, 6.57.

1′-Methoxyspiro[bicyclo[2.2.2]octane-3,3′-pyrrolidine]-2′,5′-dione (3) A mixture of 3-quinuclidinone (**14**, 6.79 g), ethyl cyanoacetate (9.9 ml), ammonium acetate (0.99 g), and acetic acid (2.7 ml) in toluene (50 ml) was refluxed for 10 h and the resulting water was removed with a Dean–Stark water separator. After removal of the solvent, the residue was dissolved in ethyl acetate. The solution was washed with saturated aq $NaHCO_3$, dried over Na_2SO_4 and the solvent was removed *in vacuo* to give an oil. Treatment of the oil with ethanolic HCl (1 eq) afforded α,β-unsaturated ester **15** (11.9 g, 77%) as a yellow powder, mp 200—201 °C.

A solution of KCN (4.10 g) in water (5 ml) was added to a suspension of the α,β-unsaturated ester (**15**, 11.9 g) in ethanol (20 ml). The mixture was heated at 80 °C for 1 h and the solvent was evaporated to give a residue. A mixture of the residue and concentrated HCl (80 ml) was refluxed for 24 h. Excess concentrated HCl was removed completely *in vacuo* and the resulting residue was dissolved in 5 N ethanol HCl (100 ml). The solution was refluxed for 20 h. After the solvent was evaporated, water was added to the residue. The mixture was neutralized with $NaHCO_3$, extracted with CH_2Cl_2, dried over Na_2SO_4, and the solvent was removed under reduced pressure to give an oil. A mixture of the oil and concentrated HCl (50 ml) was refluxed for 20 h and excess concentrated HCl was completely evaporated to give (3-carboxylquinuclidin-3-yl)acetic acid (5.30 g, 46%) as an amorphous powder.

The desired product **3** was prepared from the diacid in the same manner as described for **1b** in 38% yield. Colorless cubes, mp 256—258 °C. IR (KBr): 1720 cm^{-1}. ^{1}H-NMR (D_2O) δ: 2.00—2.50 (6H, m), 2.97 (2H, s), 3.10—3.70 (5H, m), 3.95 (3H, s). *Anal.* calcd for $C_{11}H_{16}N_2O_3 \cdot HCl$: C, 50.68; H, 6.57; N, 10.74. Found: C, 50.67; H, 6.63; N, 10.49.

3-Methoxy-8-methyl-1-oxa-4,8-diazaspiro[4.5]decane-2,4-dione Hydrochloride (4) A mixture of 4-hydroxy-4-methoxycarbonyl-1-methylpiperidine (**18**, 0.87 g), *p*-nitrophenyl chlorocarbonate (1.20 g), and pyridine (10 ml) in DMF (10 ml) was stirred at room temperature for 2 h. A solution of methoxylamine hydrochloride (0.50 g) and Et_3N (0.84 ml) in DMF (5 ml) was added to the mixture, which was stirred at 60 °C for 15 h. The resulting precipitate was removed by filtration and the filtrate was concentrated to give a residue. The residue was dissolved in ethyl acetate, washed with brine, dried over Na_2SO_4, and concentrated to give an oil. The oil was treated with ethanolic HCl (1 eq) to afford colorless prisms (0.72 g, 57%) after recrystallization from ethyl acetate, mp 254—259 °C. IR (KBr): 1750 cm^{-1}. ^{1}H-NMR (D_2O) δ: 2.25—2.60 (4H, m), 2.96 (3H, s), 3.15—3.90 (4H, m), 4.03 (3H, s). *Anal.* Calcd for $C_9H_{14}N_2O_4 \cdot HCl$: C, 43.12; H, 6.03; N, 11.17. Found: C, 42.92; H, 5.98; N, 10.94.

1,3-Diethyl-8-methyl-1,3,8-triazaspiro[4.5]decane-2,4-dione Hydrochloride (5) Ammonium carbonate (1.85 g) and KCN (0.53 g) were added successively to a solution of 1-methyl-4-piperidone (**20**, 0.50 g) in ethanol–water (5/5 ml). The mixture was heated at 60 °C for 10 h and the solvent was removed under reduced pressure. The resulting solid was collected by filtration, washed successively with ethanol and ether, and dried *in vacuo* to give a pale yellow solid (**21**, 0.70 g). Sodium hydride (0.12 g, 60% in oil) was added to a solution of the solid (0.66 g) in DMF (20 ml) and the resulting mixture was heated at 100 °C for 2 h, then cooled to room temperature. Iodoethane (0.36 ml) was added to the mixture. After stirring for 1 h at room temperature, the solvent was evaporated to give a residue, which was chromatographed on silica gel eluting with methanol–acetone–CH_2Cl_2 (2:3:10) to afford an oil. Treatment of the oil with ethanolic HCl (1 eq) gave colorless cubes (0.39 g, 35% from 1-methyl-4-piperidone) after recrystallization from ethanol, mp 195 °C. IR (KBr): 1705 cm^{-1}. ^{1}H-NMR (D_2O) δ: 1.26 (3H, t, J=7 Hz), 1.38 (3H, t, J=7 Hz), 2.10—2.50 (4H, m), 3.13 (3H, s), 3.40—3.90 (8H, m). *Anal.* Calcd for $C_{12}H_{21}N_3O_2 \cdot HCl$: C, 52.26; H, 8.04; N, 15.24. Found: C, 52.01; H, 8.39; N, 15.53.

3,8-Dimethyl-1,4,8-triazaspiro[4.5]decan-2-one Dihydrochloride (7) A mixture of 1-methyl-4-piperidone (**20**, 3.39 g), (2-amino-1-methyl-2-oxoethyl)carbamic acid phenylmethyl ester (3.33 g), and *p*-toluenesulfonic acid hydrate (6.00 g) in benzene (100 ml) was refluxed for 4 h and the resulting water was collected in a Dean–Stark water separator. The solvent was removed *in vacuo* to give a residue, which was dissolved in ethyl acetate, washed with saturated aq. $NaHCO_3$, and dried over Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on silica gel eluting with methanol–acetone–CH_2Cl_2 (2:3:10) to afford crude crystals. Recrystallization from ether–hexane gave 4-(phenylmethoxycarbonyl)-3,8-dimethyl-1,4,8-triazaspiro[4.5]decan-2-one (**22**, 0.60 g, 6%) as colorless cubes, mp 194—196 °C. IR (KBr): 1720, 1705 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.49 (3H, d, J=7 Hz), 1.90—2.30 (4H, m), 2.32 (3H, s), 2.70—3.00 (4H, m), 4.22 (1H, q, J=7 Hz), 5.20 (2H, s), 7.37 (5H, s), 8.60 (1H, br s). *Anal.* Calcd for $C_{17}H_{23}N_3O_3$: C, 64.33; H, 7.30; N, 13.24. Found: C, 64.55; H, 7.56; N, 13.26.

A mixture of **22** (0.48 g) and methanolic HCl (2.2 eq) in methanol (10 ml) was hydrogenated over 5% Pd/C (30 mg) at room temperature and atmospheric pressure. The catalyst was removed by filtration and the filtrate was concentrated to give a solid, which was recrystallized from ethanol to afford colorless cubes (0.38 g, 99%), mp 206—207 °C. IR (KBr): 1745 cm^{-1}. ^{1}H-NMR (D_2O) δ: 1.57 (3H, d, J=7 Hz), 2.10—2.70 (4H, m), 2.98 (3H, s), 3.10—3.60 (2H, m), 3.60—4.00 (2H, m), 4.52 (1H, q, J=7 Hz). *Anal.* Calcd for $C_9H_{17}N_3O \cdot 2HCl$: C, 42.20; H, 7.48; N, 16.40. Found: C, 42.11; H, 7.49; N, 16.33.

***In Vitro* Receptor Binding Assay** Cholinergic receptor binding assays using [^{3}H]pirenzepine and [^{3}H]oxotremorine M were performed according to the methods of Wang *et al.*[11a] and Birdsall *et al.*, [11b] respectively. The cerebral cortex of male Wistar rat was homogenized in 0.32 M sucrose (10 vol) with a Potter Elvehjem homogenizer. The homogenate was spun for 10 min at 4 °C at 3000 × *g* or 1000 × *g*, for [^{3}H]pirenzepine and [^{3}H]oxotremorine M binding, respectively. The supernatant was then centrifuged at 20000 × *g* for 15 min. The pellet obtained was suspended in 10 nM sodium–potassium phosphate buffer (pH 7.4, 1 mg protein/0.9 ml) and used for the receptor binding assay.

After preincubation at 25 °C for 5 min, a 0.9 ml aliquot of crude synaptosome (P2) fraction was pipetted into tubes containing either [^{3}H]pirenzepine (2 nM) or [^{3}H]oxotremorine M (2.7 nM) together with test compound, and the tubes were icubated at 25 °C for 60 and 15 min, respectively. Reactions were terminated by rapid filtration with glass fiber filter (Whatman GF/B) and the filters were immediately rinsed four times with 3 ml aliquots of ice-cooled buffer. Non-specific binding was estimated in the presence of 10^{-6} M atropine. The tissue bound radioactivity was extracted in 4 ml of scintillation fluid and quantitated by liquid scintillation spectroscopy (Aloka LSC-900).

Muscarinic Agonism and Antagonism in Guinea Pig Ileum Segments of ileum (about 1.5 cm) taken from guinea pigs weighing 330—670 g were used. Isolated smooth muscle preparations were suspended, under 1.0 g tension, in a 20 ml organ bath containing tyrode solution aerated with 97% O_2 plus 3% CO_2, and the solutions were maintained at 26 °C. The preparations were equilibrated for about 30 min before each experiment. The tension of preparations was isotonically recorded through a transducer (MEC, ME-4012) with a penwriting oscillography (Nihondenki San-ei Sokki, Rectigraph-8K). Dose–response curves to test compounds were obtained, and pD_2 values for agonists or pA_2 values for antagonists were determined. The composition of the tyrode solution was as follows (mM): NaCl 137.0, KCl 1.5, $CaCl_2$ 1.8, NaH_2PO_4 0.4, $MgCl_2$ 1.0, $NaHCO_3$ 12.0, and glucose 5.0.

Ameliorating Effects on CO_2-Induced Impairment of Passive Avoidance Response Male ICR mice weighing 25—30 g, 5 weeks old, and a two-compartment step-through passive avoidance apparatus were used.[18] A front, illuminated chamber (9 × 9 × 25 cm) was connected to a rear, dark chamber (25 × 25 × 30 cm) equipped with a grid floor, the two chambers being separated by a guillotine door (5 × 5 cm). On the training day, each mouse was placed in the front chamber and the guillotine door was opened 30 s later. When the mouse entered the rear chamber on all 4 paws, the door was closed and an AC 0.6 mA (Grason–Stadler Shocker, Model 700) was applied to the floor grid for 3 s. The mouse was then removed, and put in a dessicator filled with CO_2 gas. The mouse was taken out when spontaneous respiration disappeared, then was revived by artificial respiration. The retention test was performed 24 h later. Each mouse was again placed in the front chamber, the guillotine door being opened 30 min later. The avoidance time from opening the door until entering the dark chamber was measured. If the mouse did not enter the dark compartment within 300 s, the test was terminated and a ceiling score of 300 s was assigned. Saline or test compounds were given orally 30 min before the retention test. For statistical analysis, Mann–Whitney *U*-test was used. Minimum effective doses (MED), which were statistically significant at $p<0.05$, are shown in Table II.

General Behavior Effects of test compounds on general behavior were studied using ICR mice, weighing 22—28 g, 4 to 5 weeks old. Six mice

were used in each group. Mice were first placed in stainless steel cages (13 × 18 × 25 cm) for about 1 h for habituation. Test compounds were then administered orally, and behavioral changes such as salivation, lacrimation, and diarrhea were recorded. ED_{50} value for each behavior caused by the test compound was calculated by Finney's probit analysis.[19]

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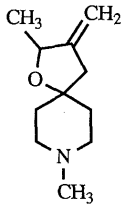
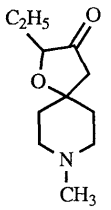


YM796



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