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Synthesis of a new series of 2,7-diazaspiro[3.5]nonan-1-ones and study of their cholinergic properties

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Summary — A new series of substituted 2,7-diazaspiro[3.5]nonan-1-ones (**1a–l**), structurally related to the muscarinic agonist RS–86, has been synthesized and tested for cholinergic properties both *in vitro* and *in vivo*. None of the compounds showed significant cholinergic properties at either the central or peripheral level. A possible explanation for the lack of activity is given.

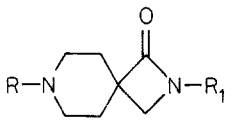
Alzheimer's disease / cholinergic system / RS–86 / muscarinic agonists

Introduction

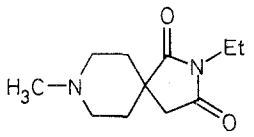
Alzheimer's disease (AD) is a progressive degenerative encephalopathy associated with behavioral disorders, loss of memory, physical debility and ultimately death.

A deficit in the central cholinergic function has been recognized to be at least partially responsible for the symptoms of AD and therefore many attempts to synthesize cholinomimetic drugs have been undertaken. In particular, acetylcholinesterase inhibitors, precursors of acetylcholine and muscarinic agonists have been considered [1, 2].

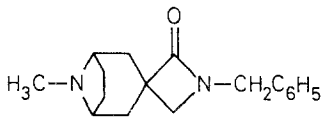
We describe in this paper the synthesis and pharmacological profile of a new series of 2,7-diazaspiro[3.5]nonan-1-ones (**1a–l**) structurally related to the muscarinic agonist RS–86 [2], which has been used in the treatment of AD patients since 1984 [3] (see scheme 1 for symbols).



1 a-j



RS-86

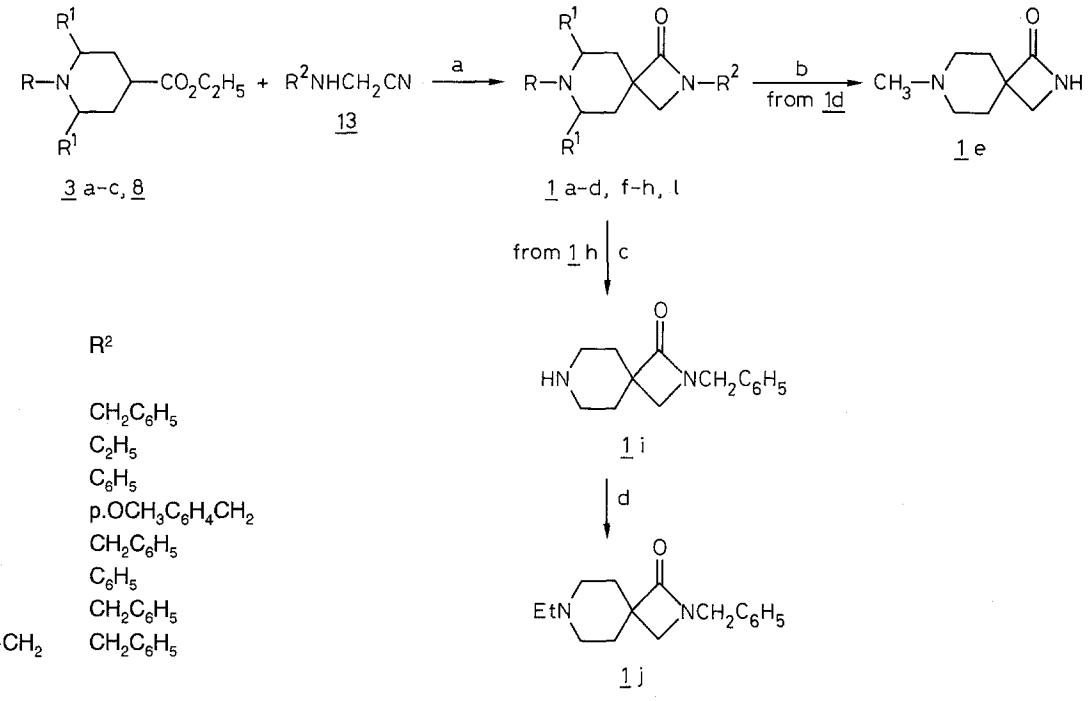


1l

Chemistry

All compounds were prepared as shown in scheme 1, starting from the appropriate esters **3a–c**, **8**, which were condensed with the required *N*-cyanomethylamines (**13a–d**) at –70°C in the presence of lithium diisopropylamide (LDA), generated *in situ* from butyllithium and diisopropylamine. The so-obtained **1a–d**, **f–h**, **l** were then purified by silica-gel chromatography followed by distillation under vacuum or crystallization. Compound **1e** was prepared from **1d** by deprotecting the azetidinonic nitrogen with ceric ammonium nitrate while **1j** was synthesized from **1h** by deprotection with trifluoroacetic acid (**1i**) and subsequent condensation with iodoethane in acetone.

The *N*-substituted piperidin-4-carboxylic esters **3a–c** were easily obtained from ethyl isonipecotate by standard methods. However, the synthesis of the tropine ester **8** required a more laborious procedure involving reduction of tropinone (**4**) with sodium in isobutanol and toluene to pseudotropine (**5**), which reacted with thionyl chloride to give **6**. Conversion of



Compd	R	R^1	R^2
1a	CH_3	H	$CH_2C_6H_5$
1b	CH_3	H	C_2H_5
1c	CH_3	H	C_6H_5
1d	CH_3	H	p.$OCH_3C_6H_4CH_2$
1f	$CH_2C_6H_5$	H	$CH_2C_6H_5$
1g	$CH_2C_6H_5$	H	C_6H_5
1h	BOC	H	$CH_2C_6H_5$
1l	CH_3	CH_2–CH_2	$CH_2C_6H_5$

Scheme 1.

the latter with potassium cyanide to **7**, followed by acid hydrolysis led to **8** (see scheme 2). It is to be noted that treatment of the beta-epimer of **6** with potassium cyanide affords 2-allyl-4-cyan-1-methyl-pyrrolidine [4].

Finally, the required *N*-cyanomethylamines were prepared from the corresponding amines by treatment with potassium cyanide and aqueous formaldehyde (**13a–c**) [5] or by condensing aniline with chloro-acetonitrile in the presence of triethylamine (**13d**).

Pharmacology

Compounds **1a–l** were tested *in vitro* and *in vivo* for cholinergic activity both at the central and peripheral levels. The muscarinic agonist RS–86 was used as a reference drug (see also pharmacological section in *Experimental protocols*).

Results and discussion

Preliminary *in vitro* and *in vivo* results show that compounds **1a–l**, are devoid of significant cholinergic properties both at the central and peripheral levels.

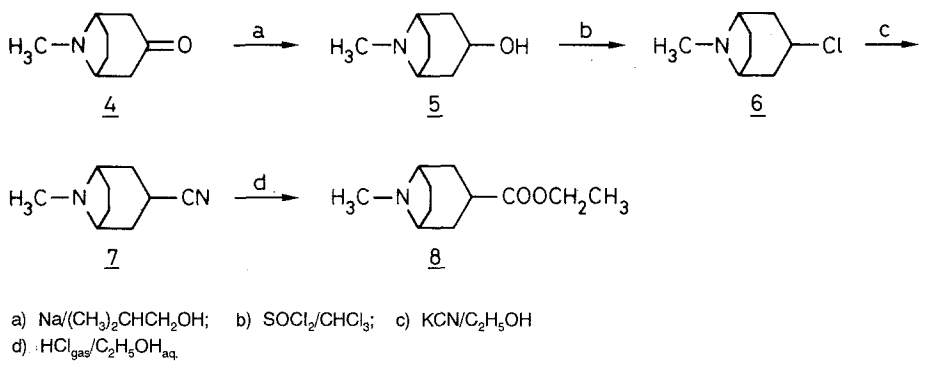
Molecular modelling studies (Wermuth and Pinto, personal communication) showed that by superimposing structures **1** and **2** on their common piperidine ring, the resulting distance between the cationic nitrogen and the negative end of the C=O dipole (4 Å) is significantly lower than the reported distance for optimum activity in muscarinic agents (5 Å) [6] (fig 1). Although some examples of longer distances are reported to maintain good affinity for muscarinic receptors [7], shortening of this distance does not appear to be tolerated.

In conclusion, substitution of the imidic pentatomic ring of the model RS–86 with an azetidinonic moiety to give compounds **1** was found to be detrimental to cholinergic activity.

Experimental protocols

Chemistry

Melting points were determined on a Büchi 510 capillary melting point apparatus and are uncorrected. Analyses indicated by the symbols were within ± 0.4% of theoretical values. IR spectra were recorded on a Perkin–Elmer 1310 infrared spectrophotometer. ^{1}H-NMR spectra were recorded on a Hitachi–Perkin–Elmer R 600 FR spectrometer; chemical shifts are reported as δ (ppm), relative to tetramethylsilane as an internal standard. $CDCl_3$ was used as a solvent, unless other-



a) $Na/(CH_3)_2CHCH_2OH$; b) $SOCl_2/CHCl_3$; c) KCN/C_2H_5OH
d) $HCl_{gas}/C_2H_5OH_{aq.}$

Scheme 2.

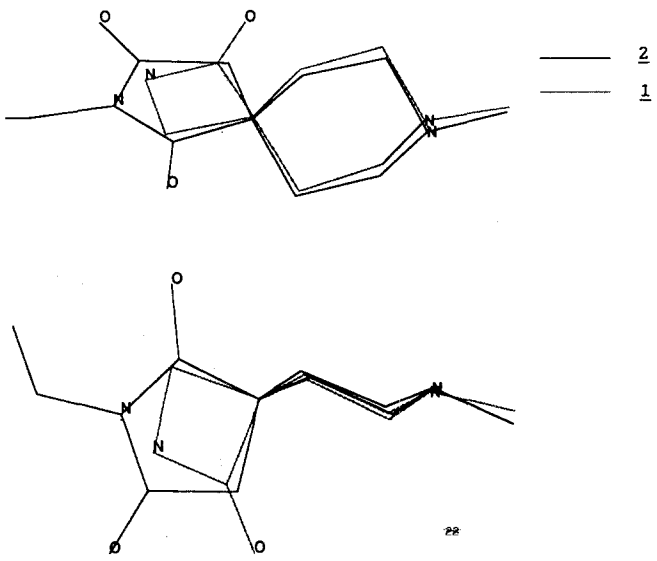


Fig 1. Superposition of structures **1** and **2** (front view and orthogonal view).

wise noted. TLC on silica-gel plates was used to check product purity. Silica gel 60 (Merck; 70–230 mesh) was used for column chromatography. The structures of all compounds were consistent with their analytical and spectroscopic data.

Ethyl-N-methyl-4-piperidine carboxylate ***3a***
To a solution of ethyl isonipecotate (15.7 g; 0.1 mol) in 99% formic acid (150 ml), 40% formaldehyde (7.2 ml; 0.1 mol) was added and the mixture heated at 110°C for 2.5 h. After cooling, the solvent was evaporated under vacuum, the residue treated with a saturated solution of $NaHCO_3$ and then extracted with ethyl ether. After evaporation of the solvent, distillation under vacuum gave 10.2 g (60%) of **3a**; bp: 100–102°C/25 mmHg (lit [8]: 94–95°C/12 mmHg).

Ethyl-N-benzyl-4-piperidine carboxylate ***3b***
To a solution of benzyl chloride (15.2 g; 0.12 mol) in acetone (60 ml) and sodium hydroxide (12 g; 0.3 mol), a solution of ethyl isonipecotate (18.2 g; 0.12 mol) in acetone (50 ml) was added dropwise. The mixture was then stirred at rt for 0.5 h, the precipitate filtered off and the solvent evaporated under vacuum. The residue was purified by column chromatography (eluent cyclohexane/ethyl acetate, 7:3) to give 19.0 g (64%) of **3b**; bp: 150°C/0.1 mmHg; IR (oil): 1730 (CO) cm^{-1}; ^{1}H-NMR δ: 1.2 (t, 3H); 1.5–3.2 (m, 9H); 3.5 (s, 2H); 4.2 (q, 2H); 7.3 (s, 5H). Anal $C_{15}H_{21}NO_2$ (C, H, N).

Ethyl-N-t-butyloxycarbonyl-4-piperidine carboxylate ***3c***
To a solution of ethyl isonipecotate (2.3 g; 0.015 mol) in $CHCl_3$ (33 ml) a solution of di-*tert*-butyldicarbonate (3.5 g; 0.016 mol) in $CHCl_3$ (8.5 ml) was added dropwise and the mixture stirred under nitrogen overnight. After evaporation of the solvent, the residue was purified by silica-gel chromatography (eluent *n*-hexane/ethyl acetate, 8:2) to give 2.7 g (86%) of **3c**; bp: 130°C/0.5 mmHg; IR (oil): 1820, 1730 (CO) cm^{-1}; ^{1}H-NMR δ: 1.2 (t, 3H); 1.5 (s, 9H); 1.4–3.2 (m, 9H); 4.2 (q, 2H). Anal $C_{13}H_{23}NO_4$ (C, H, N).

Ethyl-tropane-3-carboxylate ***8***
To a boiling mixture of sodium (8.8 g; 0.38 g atom) and toluene (54 ml), a solution of tropinone (**4**; 27.0 g; 0.19 mol) in toluene (54 ml) and isobutanol (21.6 ml) was added dropwise and the mixture refluxed for 24 h. After cooling, water (100 ml) was cautiously added, the organic layer separated and the aqueous layer extracted with benzene. After evaporation of the solvent, the residue was dissolved with benzene and the pseudotropine **5** precipitated by adding petroleum ether (17.5 g; 65%); mp: 106–107°C; ^{1}H-NMR δ: 1.2–2.6 (m, 9H); 2.3 (s, 3H); 2.9–3.5 (m, 2H); 3.6–4.3 (m, 1H).

To a cooled solution of **5** (15.3 g; 0.11 mol) in $CHCl_3$ (72.5 ml), thionyl chloride (33 ml) was added dropwise, the mixture was refluxed for 3 h and then stirred at rt overnight. After evaporation of the solvent, the residue was made alkaline with 4 N NaOH and extracted with chloroform. The desired **6** was finally distilled under vacuum; yield 64%; bp: 90°C/1 mmHg (lit [4] 56°C/0.01 mmHg).

To a solution of KCN (6.0 g; 0.09 mol) in water (9 ml), a solution of **6** (14.0 g; 0.09 mol) in ethanol (30 ml) was added and the mixture refluxed for 18 h. After evaporation of the ethanol, the mixture was made alkaline by 35% NaOH and extracted with ether. The cyanoderivative **7** was finally crystallized from *n*-pentane; mp: 61–62°C; Yield 63%.

Gaseous HCl was bubbled for 1 h into a solution of **7** (7.5 g; 0.05 mol) in ethanol (100 ml) and water (1 ml). The mixture

was then refluxed overnight and, after cooling, made alkaline with 30% NaOH. After evaporation of the solvent, the residue was distilled under vacuum to give **8** (43%); bp: 100°C/0.5 mmHg; IR (oil): 1730 (CO) cm^{-1}; ^{1}H-NMR δ: 1.2 (t, 3H); 1.4–2.9 (m, 9H); 2.3 (s, 3H); 3.0–3.4 (m, 2H); 4.1 (q, 2H).

N-(Cyanomethyl)-ethylamine ***13a***
To a solution of $NaHSO_3$ (40 g) in water (60 ml), 37% formaldehyde (17.0 g; 0.22 mol) and ethylamine (10.0 g; 0.22 mol) were added followed by a solution of KCN (14.4 g; 0.22 mol) in water (20 ml). The mixture was stirred for 15 min, treated with a saturated solution of NaCl (20 ml) and then extracted with ether. After evaporation of the solvent, **13a** was obtained by distillation (49%); bp: 90°C/25 mmHg; IR (oil): 3340 (NH); 2240 (C≡N) cm^{-1}; ^{1}H-NMR δ: 1.1 (t, 3H); 1.4 (s, 1H); 2.8 (q, 2H); 3.6 (s, 2H) [9].

N-(Cyanomethyl)-benzylamine ***13b***
Compound **13b** was obtained following the above-reported method for **13a**, starting from benzylamine; yield 83%; bp: 100°C/0.7 mmHg; IR (oil): 3340 (NH); 2240 (C≡N) cm^{-1}; ^{1}H-NMR δ: 1.8 (s, 1H); 3.6 (s, 2H); 4.0 (s, 2H); 7.5 (s, 5H) [10].

N-(Cyanomethyl)-p-methoxybenzylamine ***13c***
Compound **13c** was obtained following the above-reported method for **13a**, starting from *p*-methoxybenzylamine. However, **13c** was purified by silica-gel chromatography (eluent cyclohexane/ethyl acetate, 7:3); yield 58%; IR (oil): 3340 (NH); 2240 (C≡N) cm^{-1}; ^{1}H-NMR δ: 1.6 (s, 1H); 3.5 (s, 2H); 3.7 (s, 3H); 3.8 (s, 2H); 6.8–7.3 (d, d, 4H, J = 7 Hz).

N-(Cyanomethyl)-phenylamine ***13d***
A mixture of aniline (15.7 g; 0.17 mol), triethylamine (23.4 ml; 0.17 mol) and chloroacetonitrile (10.6 ml; 0.17 mol) in ethanol (100 ml) was refluxed for 4 h. After evaporation of the ethanol, the residue was treated with ethylacetate and the precipitate which formed was filtered off. The organic layer was washed with water, the solvent evaporated and the residue distilled under vacuum to give the unreacted aniline and **3d** in that order: yield 22%; bp: 150°C/0.6 mmHg; IR (oil): 3400 (NH); 2250 (C≡N) cm^{-1}; ^{1}H-NMR δ: 3.6 (s, 1H); 3.7 (s, 2H); 6.2–7.1 (m, 5H) [11].

2,7-Disubstituted-2,7-diazaspiro[3.5]nonan-1-ones ***1a–d, f–h, l*** *(general method)*
To a solution of diisopropylamine (7.1 ml; 0.05 mol) in anhydrous THF (190 ml) cooled at –70°C and under nitrogen, a 2.5 M solution of *n*-butyllithium (20 ml; 0.05 mol) was added dropwise. After stirring for 30 min, a solution of the appropriate ester (**3** or **8**; 0.047 mol) in anhydrous THF (50 ml) was added. Stirring was continued for 1 h at –70°C and then a solution of the required *N*-cyanomethylamine (**13**; 0.024 mol) in THF was added. After 1 h cooling was interrupted and the mixture stirred for further 20 h. A solution of NH_4Cl (18.0 g; 0.36 mol) in water (120 ml) was then added and the mixture extracted with chloroform. After evaporation of the solvent the residue was purified by silica-gel chromatography ($CHCl_3$/MeOH, 9:1) to give the desired **1** (see table I for data).

7-Methyl-2,7-diazaspiro[3.5]nonan-1-one ***1e***
To a cooled solution of **1d** (10.0 g; 0.036 mol) in acetonitrile (340 ml), a solution of ceric ammonium nitrate (CAN; 60 g; 0.1 mol) in water (360 ml) was added and the mixture stirred for 20 min at 0°C. The solution was then diluted with water (≈ 500 ml) and made alkaline with 2 N NaOH. Inorganic salts were filtered off and the solution extracted with chloroform. The residue was purified by silica-gel chromatography eluting with $CHCl_3$/MeOH, 9:1; yield 25%; mp: 110–113°C; ^{1}H-NMR δ: 1.5–2.2 (m, 6H); 2.3 (s, 3H); 2.5–3.0 (m, 2H); 3.3 (s, 2H); 6.0 (s, 1H). Anal $C_8H_{14}N_2O$ (C, H, N).

2-Benzyl-2,7-diazaspiro[3.5]nonan-1-one ***1i***
To a solution of **1h** (1.0 g; 0.003 mol) in CH_2Cl_2 (10 ml) under nitrogen, a mixture of CF_3COOH (10 ml) and anisole (2 ml) was added. After stirring for 15 min, the solvent was evaporated, the solution made alkaline by 6 N NaOH and extracted with CH_2Cl_2. After evaporation of the dichloromethane and distillation under vacuum of anisole, the residue was purified by silica-gel chromatography (eluent $CHCl_3$/MeOH, 8:2); yield 80%; IR (oil): 3320 (NH); 1740 (CO) cm^{-1}; ^{1}H-NMR δ: 1.1–2.3 (m, 5H); 2.4–3.6 (m, 4H); 3.0 (s, 2H); 4.4 (s, 2H); 7.3 (s, 5H). Anal $C_{14}H_{18}N_2O$ (C, H, N).

2-Benzyl-7-ethyl-2,7-diazaspiro[3.5]nonan-1-one ***1j***
To a solution of **1i** (0.46 g; 0.002 mol) in acetone (10 ml), a solution of iodoethane (0.37 g; 0.002 mol) in acetone (5 ml) was added under nitrogen. The mixture was then stirred for 1 h, the solvent evaporated and the residue crystallized from ether to give 0.30 g (40%) of **1j** as the iodidrate; mp: 177–178°C; ^{1}H-NMR δ: 1.05 (t, 3H); 1.6–2.9 (m, 11H); 2.95 (s, 2H); 4.4 (s, 2H); 7.3 (s, 5H). Anal $C_{16}H_{22}N_2O$•HI (C, H, I, N).

Pharmacology

Receptor binding
Binding to muscarinic receptors was carried out according to the method described by JR Farrar *et al* [12]. Binding was determined by the ability of compounds to compete with ^{3}H-(*R*)quinuclidinyl benzylate (^{3}H-QNB) in a suspension of brain membranes for 2 h (≈ 900 μg proteins, 0.4 nM ^{3}H-QNB). Non-specific binding was evaluated by adding 0.01 μM atropine to a separate set of samples. Compounds were tested at doses ranging from 10^{-9} to 10^{-4}. K_i are reported as means of 3 experiments (for RS–86 K_i = 1.44 μM; for atropine K_i = 0.0003 μM).

Guinea-pig bioassay
A distal portion of guinea-pig ileum was cut and a segment (5–6 cm) was tied at both ends. One end was connected to a force displacement transducer (Brush) and the other to a muscle holder in an organ bath. The tissue was suspended in Krebs solution (KCl 0.35; NaCl 6.6; $CaCl_2$ 0.28; KH_2PO_4 0.16; $MgSO_4$•$7H_2O$ 0.29; $NaHCO_3$ 2.1; and glucose 2.05 g/l) aerated with 95% O_2 and 5% CO_2 and maintained at 37°C. After the tissue was allowed to equilibrate for 45–60 min, single doses of agonists were administered into the bath and isotonic contractions were recorded on a Brush Mark 220 polygraph.

Oxotremorine interaction in mice
Male albino Swiss mice (22–24 g; Charles River) were used. The test compounds were administered intraperitoneally at doses of 50 and 100 mg/kg 15 min prior to oxotremorine (0.5 mg/kg ip in 0.9% NaCl solution) to groups of 5 animals, while controls received the vehicle only. Mice were allocated into individual cages and their rectal temperatures read with a calibrated electric thermocouple thermometer. The measurement was repeated 30, 60, 90 and 120 min after medication. Tremors were recorded over a 30-s observation time at 15, 30, 45, 60, 90 and 120 min after treatment.

Pithed rat
Male normotensive Sprague–Dawley rats (Charles River; 300–400 g) were used. The animals were anesthetized and the right

Table I. Physical data of compounds **1a–d, f–h, l.**

Compd	*Yield %*	*mp (°C) bp/mmHg*	*Molecular formula*	*^{1}H-NMR δ (ppm)*
1a	29	180/3	$C_{15}H_{20}N_2O$	1.7–2.2 (m, 6H); 2.3 (s, 3H); 2.5–2.8 (m, 2H); 2.9 (s, 2H); 4.4 (s, 2H); 7.3 (s, 5H)
1b	30	140/0.4	$C_{10}H_{18}N_2O$	1.1 (t, 3H); 1.7–2.4 (m, 6H); 2.3 (s, 3H); 2.5–3.0 (m, 2H); 3.1 (s, 2H); 3.2 (q, 2H)
1c	20	105–106[a]	$C_{14}H_{18}N_2O$	1.8–2.8 (m, 8H); 2.3 (s, 3H); 3.4 (s, 2H); 7.1–7.4 (m, 5H)
1d	53	230/0.5	$C_{16}H_{22}N_2O_2$	1.7–2.3 (m, 6H); 2.3 (s, 3H); 2.7–2.9 (m, 2H); 3.0 (s, 2H); 3.8 (s, 3H); 4.3 (s, 2H); 6.8–7.3 (d, d, 4H, J = 7 Hz)
1f	30	230/0.4	$C_{21}H_{24}N_2O$	1.6–2.9 (m, 8H); 2.9 (s, 2H); 3.5 (s, 2H); 4.4 (s, 2H); 7.3 (s, 10H)
1g	20	143–144[a]	$C_{20}H_{22}N_2O$	1.2–3.0 (m, 8H); 3.4 (s, 2H); 3.6 (s, 2H); 7.2–7.4 (m, 10H)
1h	45	109–111[a]	$C_{19}H_{26}N_2O_3$	1.5 (s, 9H); 1.6–2.0 (m, 4H); 3.0 (s, 2H); 3.2–3.8 (m, 4H); 4.4 (s, 2H); 7.3 (s, 5H)
1l	21	oil[b]	$C_{17}H_{22}N_2O$	1.9–2.3 (m, 8H); 2.3 (s, 3H); 2.8 (s, 2H); 3.0–3.3 (m, 2H); 4.3 (s, 2H) 7.3 (s, 5H)

[a]Crystallized from cyclohexane; [b]decomposes on heating.

jugular vein cannulated for drug administration. Blood pressure was measured from the left common carotid artery through a PE 50 catheter connected to a pressure transducer. After catheterization of the trachea, heparin (150 IU/kg) was given iv to prevent blood coagulation. The temperature was maintained at approximately 37°C throughout the experiment by an overhead heating lamp. Rats were then pithed by insertion of a steel rod (1.5 mm diameter) through the skull and foramen magnum down into the spinal canal. The animals were respired artificially at a frequency of 60 cycles/min with a volume of 1 ml/100 g. The preparation was then allowed to equilibrate for at least 30 min before drug administration until the mean heart rate had stabilized. Test compounds were administered iv at doses of 1, 3 and 10 mg/kg. RS–86 was administered at 10, 30 and 100 μg/kg. Full recovery with return to preinjection values was allowed prior to successive administration.

Passive avoidance tasks with scopalamine-treated rats

Two groups of 5 Wistar–Kyoto rats (220–300 g; Charles River, Italy) were used: a scopolamine/vehicle control group and a scopolamine/test compound group. The test compound was administered ip at a dose of 100 mg/kg together with scopolamine (2 mg/kg). In the training session each rat was placed in the light box of a 2-compartment passive avoidance apparatus [13]. When the animal entered the dark compartment a footshock was applied by a Letica LI 2700 apparatus. Retention was assessed 24 h after the training session. Latency to enter the dark compartment was measured up to a maximum cut-off of 600 s [14].

Behavioral profile

Compounds were tested according to the procedure reported by Irwin [15]. Briefly, male Swiss mice (CD1 Nossan; 22–24 g)

were divided into 2 groups of 3 animals each and fasted for 2 h. Group 1 was treated orally with the test compound suspended in 0.5% methylcellulose at doses of 10, 30, 60 and 100 mg/kg. Group 2 received the vehicle only (0.9% NaCl, 0.5% carboxymethylcellulose, and 0.4% Tween 80 in distilled water). Animal behavior was observed both in the cage and in the open field 30 min, 2 h and 5 h after medication.

Acknowledgments

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