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1-Alkyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-alkyl-oximes: a new class of potent orally active muscarinic agonists related to arecoline

E Toja[1]*, C Bonetti[1], A Butti[1], P Hunt[3], M Fortin[3], F Barzaghi[2], ML Formento[2], A Maggioni[2], A Nencioni[2], G Galliani[2]**

[1]*Chemistry Department, Roussel-Pharma SpA, Milan;*
[2]*Pharmacology Department, Roussel-Pharma SpA, Viale Gran Sasso 18, 20131 Milan, Italy;*
[3]*Centre de Recherche Roussel-Uclaf, Romainville, France*

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Summary — On the basis of the knowledge acquired from basic studies of several known arecoline derivatives about the structural requirements for potent agonistic activity and oral efficacy, a series of 1-alkyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-alkyl-oximes was synthesized and biologically evaluated in a battery of *in vitro* and *in vivo* assays. The most interesting molecules to emerge from the primary screening, 1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-methyloxime hydrochloride (**11**, RU 35963), 1-methyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-methyloxime hydrochloride (**12**, RU 35926), and 1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-propargyloxime hydrochloride (**30**, RU 47029) and 1-methyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-propargyloxime hydrochloride (**33**, RU 35986), were evaluated more extensively, and their cholinomimetic profile was compared with that of the parent molecule, arecoline. The pharmacological results after oral administration to mice and rats revealed that their efficacy is 2–3 orders of magnitude higher than that of arecoline, and, in addition, they show a longer duration of action. The 4 aldoximes showed anti-amnesic properties in many respects superior to those of arecoline. Their anti-amnesic doses were 2 orders of magnitude lower than those inducing obvious cholinergic symptoms, and 3–5 orders of magnitude lower than the lethal doses. These new compounds can be regarded as potential candidates for clinical studies in AD (Alzheimer's disease) patients.

Résumé — 1-Alkyl-1,2,5,6-tétrahydropyridine-3-carboxaldéhyde-*O*-alkyloximes : une nouvelle classe de puissants agonistes muscariniques actifs par voie orale, apparentés à l'arécoline. *Une série de 1-alkyl-1,2,5,6-tétrahydropyridine-3-carboxaldéhyde-O-alkyloximes a été synthétisée en fonction des critères structuraux qui s'étaient avérés nécessaires, dans une étude de quelques dérivés de l'arécoline, pour obtenir une forte activité agoniste cholinergique ainsi qu'une efficacité par voie orale. Les produits les plus intéressants retenus à partir d'un premier triage pharmacologique* in vitro *et* in vivo, *1,2,5,6-tétrahydropyridine-3-carboxaldéhyde-O-méthyloxime (chlorhydrate **11**, RU 35963), 1-méthyl-1,2,5,6-tétrahydropyridine-3-carboxaldéhyde-O-méthyloxime (chlorhydrate **12**, RU 35926), et 1,2,5,6-tétrahydropyridine-3-carboxaldéhyde-O-propargyloxime (chlorhydrate **30**, RU 47029) et 1-méthyl-1,2,5,6-tétrahydropyridine-3-carboxaldéhyde-O-propargyloxime (chlorhydrate **33**, RU 35986) ont été essayés de façon plus approfondie et leur profil cholinomimétique a été comparé à celui du produit de départ, l'arécoline. Les résultats pharmacologiques, après administration chez la souris ou le rat, ont montré une efficacité supérieure, de 2–3 ordre de grandeur à celle de l'arécoline et une durée d'action plus longue. Les quatre aldoximes montrèrent des propriétés anti-amnésiques supérieures, sous différents aspects, à celles de l'arécoline. Leurs doses anti-amnésiques étaient de 2 ordres de grandeur inférieures à celles induisant des symptômes cholinergiques extérieurs, et de 3–5 ordres de grandeur inférieures aux doses létales. Ces nouveaux produits peuvent être considérés comme des candidats potentiels dans le traitement de la maladie d'Alzheimer.*

tetrahydropyridine aldoximes RU 35926, RU 35963 / muscarinic receptor agonists / cholinomimetic profiles / Alzheimer's disease

Introduction

Senile dementia of the Alzheimer type is a complex, degenerative brain disease that affects ≈ 6% of people over the age of 65 years; it is characterized clinically by progressive and inexorable loss of memory and intellectual function [1, 2]. Because of the severe disability that accompanies this disorder, it represents a major public health problem [3, 4].

Although most of the major neurotransmitter systems have been implicated in the etiology of Alzheimer's disease (AD), the most dramatic and

*Present address: Malesci-50144, Florence, Italy
**Correspondence and reprints

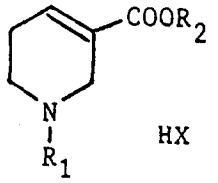
consistent findings are related to a deficit in the cholinergic systems [5, 6]. Cholinergic neurons in the basal forebrain are markedly and selectively damaged in AD [7, 8]. This neuronal loss is associated with a substantial reduction in presynaptic markers [9, 10], particularly in the hippocampus and in the cerebral cortex, regions concerned with memory and cognition [11, 12]. Presynaptic muscarinic receptor sites of the M_2 subtype are reduced, whereas postsynaptic M_1 receptors seem to be relatively spared in AD patients, suggesting that selective M_1 agonists could be of particular therapeutic interest [13, 14]. Other evidence supporting the 'cholinergic hypothesis' of AD is the similarity of the AD memory deficit to the deficit induced in both young and elderly normal volunteers by the anticholinergic drug scopolamine [15, 16], and to the small but reliable memory improvement observed in AD patients after administration of muscarinic agonists, cholinesterase inhibitors and combined therapies [17, 18]. Until recently, however, because of the serious pharmacokinetic limitations of the available cholinomimetic agents, it seemed likely that the development of therapeutically useful new drugs would have to await the discovery and development of a new generation of cholinergic agents [19].

Most of the known cholinergic agonists, including acetylcholine itself, contain a quaternary ammonium group [20, 21] and, consequently, penetrate the blood–brain barrier poorly. They can therefore be expected to induce undesirable peripheral side-effects at doses lower than those needed to stimulate central cholinergic receptors. Arecoline, a naturally occurring alkaloid with cholinergic properties, is a tertiary amine. This muscarinic agonist has strong lipophilic properties and can easily cross the blood–brain barrier after systemic administration [22]. Many experimental studies in animals [23, 24] as well as a few clinical studies in demented patients and normal volunteers [25, 26] have demonstrated the ability of arecoline to prevent or to reduce memory deficit. Due to the rapid *in vivo* hydrolysis of the ester function, however, arecoline has an extremely short half-life and negligible activity after oral administration [27, 28], and is thus therapeutically useless.

Since we have been involved in the search for arecoline analogues with the pre-requisites for clinical use, we aimed to find compounds with both potent oral activity and long duration of action. A large number of arecoline derivatives, already known in the literature for their muscarinic activity *in vitro* were synthesized and evaluated pharmacologically both *in vivo* and *in vitro*. A list of the most representative compounds **1–8** is reported in table I with the appropriate references [29–34], and the pharmacological results are given in table V.

These basic studies gave us interesting information that has been useful in the search for the desired

Table I. Muscarinic agents derived from 1,2,5,6-tetrahydro-3-carboxypyridine (guvacine).



No	R_1	R_2	*HX*	*Ref*[a]
1	H	CH_3	HCl	29
2	H	C_2H_5	HCl	30
3	H	CH_2-C≡CH	HCl	29
4	CH_3	H	HBr	31
5	CH_3	CH_3	HBr	Arecoline[b]
6	CH_3	C_2H_5	HBr	32
7	CH_3	CH_2-C≡CH	HCl	33
8	C_2H_5	CH_3	HCl	34

[a]The first practical method of preparation is cited in the references. [b]Fluka commercial product.

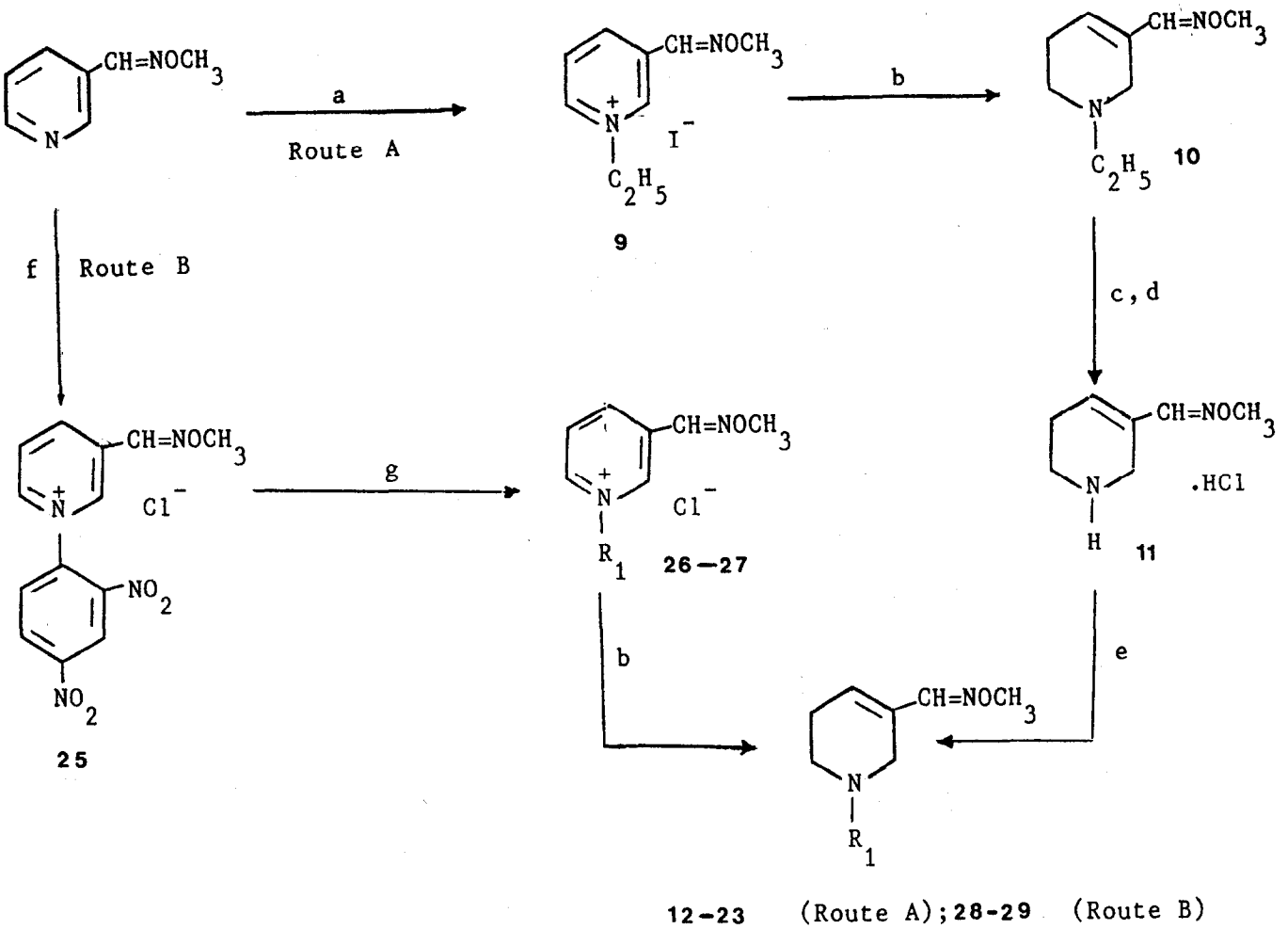
arecoline derivatives. It is worth mentioning, for example, that guvacoline (**1**), the *N*-desmethyl analogue of arecoline, showed good oral activity, 14–60-fold greater than the parent molecule (**5**). Since guvacoline has a weaker affinity than arecoline for muscarinic receptors [35], it would seem that *N*-demethylation can protect the tetrahydropyridine moiety from first-pass metabolic deactivation. This assumption was also supported by the lower ratio between oral and subcutaneous activity (1/6 for guvacoline; 1/60 for arecoline). In agreement with the literature, the propargyl analogues of guvacoline (**3**) and of arecoline (**7**) showed the highest affinity for muscarinic receptors in the isolated guinea-pig ileum [29, 33], but had very weak oral activity. Data on their anti-amnesic activity showed a good correlation between cholinergic potency and the potency for reversing a scopolamine-induced memory deficit. Interestingly, the oral anti-amnesic doses were 1/10–1/100 of those inducing overt peripheral and central cholinergic effects [23]. The acute toxicity data demonstrated that an increase in oral cholinomimetic activity is not necessarily accompanied by a parallel increase in general toxicity. The toxic doses were up to 2 orders of magnitude greater than those able to reverse a scopolamine-induced memory deficit.

In the light of these encouraging findings we have continued our research, with the aim of finding a hydrolytically stable bioisostere of the ester function of arecoline. In this context, the oxime derivative of acetylcholine, *O*-(β-dimethylamino) ethylacetaldoxime methiodide has been described in the literature as having cholinergic properties similar to those of carbamoylcholine [36]. Moreover, an aldoxime analogue of arecoline, 1-methyl-1,2,5,6-tetrahydropyridine-3-aldoxime, was reported by Wells *et al* in 1967 [37] to be a parasympathomimetic agent and 1-methyl-3-acetyl-1,2,5,6-tetrahydropyridine oxime was described by Druey *et al* in 1961 [38]. In our primary screening test, the first of these 2 arecoline derivatives (compound **37** in tables III and VI) demonstrated an affinity for muscarinic receptors comparable to that of the structurally related acid **4** (arecaidine) and ≈ 2 orders of magnitude lower than that of **5** (arecoline). In spite of its relatively poor fulfillment of the structural requirements for muscarinic activity, **37** showed good oral activity and, more interestingly, the activity was the same after oral or parenteral (sc) administration, with a longer duration of action than arecoline. Thus **37** could be regarded as a lead compound for the design of proper cholinomimetic agents less vulnerable to metabolic attack.

In this paper we report the synthesis and the pharmacological evaluation of a new series of 1-alkyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-alkyloximes which fit into the above-mentioned strategy. The results of more extensive studies of the most interesting molecules to emerge from the primary screening are also reported.

Chemistry

The synthetic route utilized for the preparation of the majority of the compounds is shown in scheme 1.

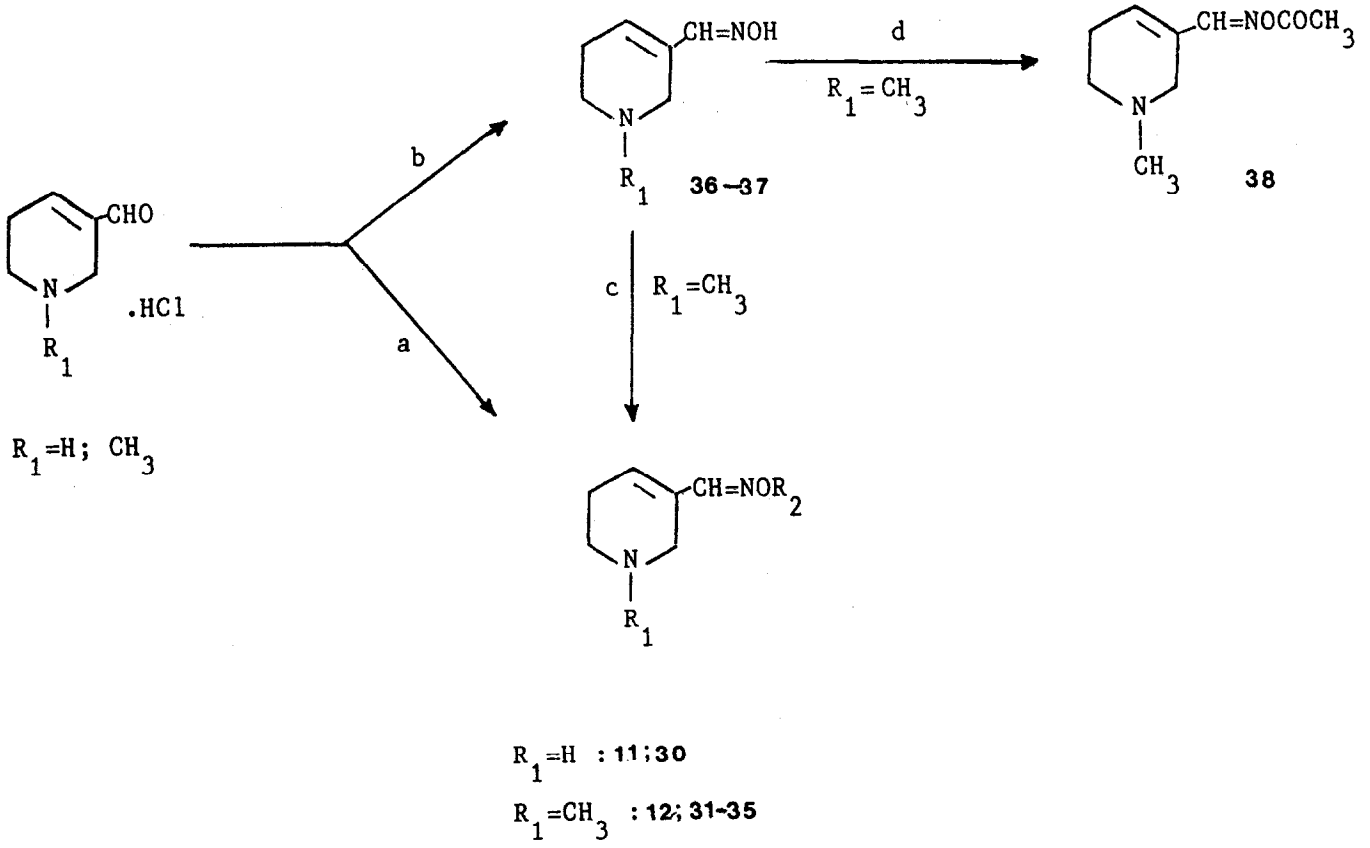


Scheme 1. Reagents: a) EtI-EtOH; b) $NaBH_4$-MeOH; c) $CH_3CHClOCOCl$-CH_2ClCH_2Cl; d) MeOH; e) R_1 (halogen)-TEA-DMF; f) 1-chloro-2,4-dinitrobenzene; g) R_1NH_2-MeOH.

Quaternization of pyridine-3-carboxaldehyde-*O*-methyloxime [39] with ethyl iodide followed by reduction with sodium borohydride in methanol of the pyridinium iodide **9** yielded 1-ethyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-methyloxime **10**. Reaction of **10** with 1-chloroethyl chloroformate [40] in 1,2-dichloroethane and methanolysis of the intermediate carbamate directly gave the 1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-methyloxime hydrochloride **11** (route A).

We had previously described [41] the use of 3-methoxyiminomethyl-1-benzylpyridinium bromide instead of **9** as the starting material for the preparation of **11** in an overall yield comparable to that of the procedure reported here. When an amount up to 100 g of **11** was required, this last procedure was more advantageous because **10** could be purified by distillation, unlike the 1-benzyl congener, which required a chromatographic purification. *N*-Alkylation of **11** was achieved by reaction of various primary and secondary alkyl halides and triethylamine in dimethylformamide to give **12–23**. The 1-carboxymethyl derivative **24** was obtained by cleavage of the *tert*-butyl ester **23** under acidic conditions. Cyclopropyl and *tert*-butyl derivatives **28**, **29** could not be prepared by alkylation of **11** and were obtained by reduction with sodium borohydride in methanol of the corresponding 1-alkylpyridinium chlorides **26**, **27**. These quaternary salts were in turn obtained from 3-methoxyiminomethyl-1-(2,4-dinitrophenyl) pyridinium chloride **25** and cyclopropylamine or *tert*-butylamine in methanol according to the method of Zincke [42] (route B).

A second approach utilized to obtain this series of compounds is shown in scheme 2. Compound **11**, for example, was prepared by oximation of 1,2,5,6-tetrahydropyridine-3-carboxaldehyde hydrochloride [43] with methoxylamine hydrochloride in water. Similarly, the *O*-propargyloxime **30** was obtained by oximation with propargyloxyamine. *O*-Alkyloximes **12**, **31–35** of 1-methyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde [43] were prepared either by direct oximation with the corresponding alkyloxyamine hydrochlorides in water or by selective *O*-alkylation with alkyl bromides of 1-methyl-1,2,5,6-tetrahydropyridine-3-aldoxime **37** [44] in the presence of sodium ethanolate, according to a general procedure [45]. This second route is preferred when alkyloxyamines are not commercially available because of



Scheme 2. Reagents: a) R_2ONH_2·HCl-H_2O; b) $HONH_2$·HCl-H_2O; c) R_2Br-EtONa-EtOH; d) CH_3COCl-TEA-THF.

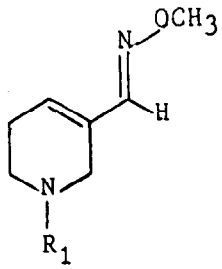
their troublesome preparation. 1,2,5,6-Tetrahydropyridine-3-carboxaldehydes were obtained in ≈ 50% yield from 3-chloropropionaldehyde diethyl acetal and methylamine or ammonia and ring closure of the resulting bis (3,3-diethoxyethyl) amine with HCl, without modification of the original procedure [43, 44]. The corresponding aldoximes **36**, **37** were prepared by reaction with hydroxylamine hydrochloride in water [43, 44]. Finally, *O*-acetylation of **37** with acetyl chloride and triethylamine gave **38** which was tested as a free base because of the fast hydrolytic cleavage of the acetyl group observed for the corresponding hydrochloride.

All the oximes presented in tables II and III were obtained as pure, sterically favored E isomers as shown by the appearance in the NMR spectrum of one singlet attributable to the proton of the oxime group. The signal appeared in the range 7.60–7.80 ppm for the hydrochlorides **10–37** dissolved in DMSO–d_6 and in the range 7.43–7.50 ppm for the corresponding free bases dissolved in $CDCl_3$. Specific examples are found in the *Experimental protocols*. NMR spectra of **11** and **12** obtained by the 2 different sequences (schemes 1 and 2) were identical. It is worth mentioning in this context that NMR studies of oxime configurations have demonstrated that pyridine-3-aldoximes exist in the E-form exclusively [46, 47].

Pharmacological results and discussion

Following our primary screening program all the compounds listed in tables I–III were submitted to a battery of tests to assess their affinity and selectivity for muscarinic receptors, their anti-cholinesterase activity, their acute toxicity and their effects on the

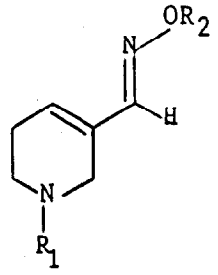
Table II. 1-Alkyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-methyloxime hydrochlorides.



No	R_1	*Formula*[a]	*Decomp temp (°C) recrystn solvent*[b]		*Yield*[c] *(%)*
10	C_2H_5	$C_9H_{17}ClN_2O$	220	A	69
11	H	$C_7H_{13}ClN_2O$	208	A	81
12	CH_3	$C_8H_{15}ClN_2O$	223	B	41
13	$(CH_2)_2CH_3$	$C_{10}H_{19}ClN_2O$	220	B	48
14	$CH(CH_3)_2$	$C_{10}H_{19}ClN_2O$	210	A	45
15	$(CH_2)_3CH_3$	$C_{11}H_{21}ClN_2O$	195	A	59
16	$(CH_2)_4CH_3$	$C_{12}H_{23}ClN_2O$	191	A	52
17	Cyclopentyl	$C_{12}H_{21}ClN_2O$	213	A	70
18	CH_2-CH=CH_2	$C_{10}H_{17}ClN_2O$	221	B	81
19	CH_2-CH=CH-CH_3[d]	$C_{11}H_{19}ClN_2O$	215	A	63
20	CH_2-C≡CH	$C_{10}H_{15}ClN_2O$	229	B	76
21	CH_2-cyclopropyl	$C_{11}H_{19}ClN_2O$	233	A	36
22	CH_2-C_6H_5	$C_{14}H_{19}ClN_2O$	261	B	87
23	$CH_2COOC(CH_3)_3$	$C_{13}H_{23}ClN_2O_3$	182	A	74
24	CH_2COOH	$C_9H_{15}ClN_2O_3$	213	B	65
28	Cyclopropyl	$C_{10}H_{17}ClN_2O$	231	A	26
29	$C(CH_3)_3$	$C_{11}H_{21}ClN_2O$	215	A	20

[a]C, H, N analyses were within ± 0.4% of the calculated values; [b]A: EtOH-Et_2O; B: EtOH; [c]yields are referred to recrystallized hydrochlorides and were not optimized; [d]E-configuration.

Table III. 1-Alkyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-alkyloxime hydrochlorides and *O*-acetyloxime.



No	R_1	R_2	*Formula*[a]	*Decomp temp (°C) recrystn solvent*[b]		*Yield*[c] *(%)*
30	H	CH_2-C≡CH	$C_9H_{13}ClN_2O$	202	B	85
31	CH_3	C_2H_5	$C_9H_{17}ClN_2O$	197	B	61
32	CH_3	$CH(CH_3)_2$	$C_{10}H_{19}ClN_2O$	234	B	59
33	CH_3	CH_2-C≡CH	$C_{10}H_{15}ClN_2O$	157	B	90
34	CH_3	CH_2-CH=CH_2	$C_{10}H_{17}ClN_2O$	168	A	29
35	CH_3	CH_2-CH=CH-CH_3[d]	$C_{11}H_{19}ClN_2O$	164	A	34
36	H	H	$C_6H_{11}ClN_2O$	252[e]	C	80
37	CH_3	H	$C_7H_{13}ClN_2O$	249[f]	B	91
38	CH_3	$COCH_3$	$C_9H_{14}N_2O_2$	[g]		81

[a,c,d]See corresponding footnotes in table II; [b]A: EtOH-Et_2O; B: EtOH; C: EtOH-H_2O; [e]see ref [43]; [f]see ref [44]; [g]used as a free base

central (hypothermia) and peripheral (diarrhoea) cholinergic systems. Comparative studies of sc and *po* administration were also carried out to obtain some information about the influence of metabolic first pass and oral absorption factors on the biological activity. Since all the tested compounds are tertiary amines with good oral absorption expected, the ratio of *po* to sc activity can be considered as an index of the degree of metabolic first pass. Moreover, the central duration of action of the compounds was estimated biologically from the time-course of the hypothermic effect, by comparing the responses elicited by approximately equiactive doses.

The compounds were initially screened for their ability to interact with a number of neurotransmitter receptors, including muscarinic receptors, and drug binding sites. The ratio of the IC_{50} value for the displacement of the M_1-selective antagonist [^{3}H]PZ to that for the displacement of the non-selective antagonist [^{3}H]QNB in the presence of pirenzepine to block M_1 sites was used as an index of M_1 selectivity (lower values of this index indicate higher degrees of M_1 selectivity), and contractile responses in the isolated guinea-pig ileum indicate agonistic properties.

The results concerning the interaction of compounds **12**, **33** and reference drugs with the various receptors labelled with specific radioligands are reported in table IV. The 2 compounds showed significant affinity only for muscarinic receptors labelled either with [^{3}H]PZ or [^{3}H]QNB. Similarly, all the other compounds tested had little or no affinity ($IC_{50} \geq$ 5000 nM) for receptors other than the muscarinic receptors (results shown only for [^{3}H]PZ and [^{3}H]QNB binding: tables V and VI).

In agreement with our *in vitro* experimental findings (table V and unpublished data) and those of others [33, 48] for tertiary arecoline ester derivatives, the methyl oxime derivatives **11**, **12** and propargyl oxime derivatives **30**, **33** showed potent agonistic properties in the isolated guinea-pig ileum. The compounds had pD_2 values from 6.26 to 6.58, the corresponding value for arecoline being 6.48. As in the arecoline series [29, 48 and table V], propargyl oximes had greater potency than the corresponding methyl oxime analogues. Dose–response curves for **11**, **12**, **30** and **33** in the isolated guinea pig ileum were similar in shape and maximal response to those of acetylcholine and arecoline, indicating full agon-

Table IV. Affinity (IC_{50}, nM) of compounds **12**, **33** and reference drugs for neurotransmitter receptor and drug binding sites. Data are means of at least 3 separate experiments carried out in triplicate. In = inactive at the concentration (nM) shown.

Receptor or binding site	*Radioligand*	*Tissue/organ*	*Inhibition of specific ^{3}H-Ligand binding by:* *Compd* **12**	*Compd* **33**	*ref compounds*	
m_1-Muscarinic	^{3}H-Pirenzepine	Rat cortex	2100	430	Pirenzepine	9.7
'm_2'-Muscarinic	^{3}H-QNB	Rat brain	13000	2200	Benztropine	6.3
Dopamine	^{3}H-Spiperone	Rat striatum	In 5000	In 5000	Haloperidol	8.5
α_1-Adrenergic	^{3}H-Prazosin	Rat brain	In 5000	In 5000	Phentolamine	15
α_2-Adrenergic	^{3}H-Rauwolscine	Rat cortex	In 5000	21000	Phentolamine	49
β_1-Adrenergic	^{3}H-Dihydroalprenolol	Rat cortex	In 5000	In 5000	Propranolol	9.1
β_2-Adrenergic	^{3}H-Dihydroalprenolol	Rat cerebellum	In 5000	In 5000	Propranolol	6.0
5HT-Serotonin	^{3}H-5HT	Rat brain	In 5000	In 5000	Serotonin	2.5
$5HT_2$-Serotonin	^{3}H-Spiperone	Rat frontal cortex	In 5000	In 5000	Mianserin	7.0
H_1-Histamine	^{3}H-Pyrilamine	Guinea pig cerebellum	In 5000	In 5000	Triprolidine	1.3
Benzodiazepine	^{3}H-Diazepam	Rat cortex	In 5000	In 5000	Diazepam	12
GABA	^{3}H-Muscimol	Rat brain	In 50 000	In 50 000	GABA	61
μ-Opioid	^{3}H-Dihydromorphine	Rat brain	In 5000	In 5000	Morphine	3.7
K-Opioid	^{3}H-Ethylketocyclazocine	Guinea pig cerebellum	In 5000	In 5000	U 50488	3.0

Table V. Pharmacological profile of 1-alkyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-methyloximes and some known structurally related esters. nd = not determined; In = inactive.

	In vitro studies						*In vivo studies*				
	Receptor binding[a]			*Guinea pig ileum*[b]		*Anti-ChE*[c] *activity*	*Approx* LD_{50}	*Hypothermia*[d]			*Diarrhoea*[e]
Compd	*[^{3}H] PZ* IC_{50} *(nM)*	*[^{3}H] QNB* IC_{50} *(nM)*	*M1/Non* M_1 *index*	*Agonism* pD_2	*Antagonism* IC_{50} *(M)*	IC_{50} *(μg/ml)*	*mg/kg po*	*mg/kg po*	*mg/kg sc*	*Ratio po/sc*	*mg/kg po*
1	20000	In 5000	< 4	5.85	–	> 100	750	3.3	0.6	6	2.5
2	2300	> 5000	< 0.46	5.99	–	> 100	750	12	0.9	13	4.5
3	1600	4200	0.38	6.67	–	> 100	500	> 100	1.0	> 100	50
4	nd	nd	–	4.44	–	> 100	> 1000	> 200	> 100	–	> 200
5	9500	42000	0.23	6.48	–	> 100	550	194	2.9	67	35
6	990	4100	0.24	6.45	–	> 100	1000	200	2.2	90	35
7	940	3200	0.29	6.62	–	> 100	750	> 100	2.5	> 40	50
8	17000	In 5000	< 3	4.53	–	nd	750	81	nd	–	> 100
10	4200	In 5000	< 1	< 3	2.0×10^{-4}	> 20	60	0.97	1.16	0.8	10
11	4300	28000	0.15	6.58	–	> 100	70	0.11	0.12	0.9	0.15
12	2100	13000	0.16	6.28	–	> 100	450	0.85	0.22	4	1.5
13	16000	In 5000	< 3	4.60	–	> 20	100	0.34	0.83	0.4	1.7
14	5400	In 5000	< 1	< 3	1.2×10^{-4}	> 20	60	0.77	0.98	0.8	2
15	18000	In 5000	< 4	< 3	2.3×10^{-4}	> 20	30	0.79	0.85	0.9	3.5
16	14000	In 5000	< 3	< 3	1.6×10^{-4}	> 20	175	3.2	4.2	0.8	5.0
17	32000	In 5000	< 6	< 3	9.1×10^{-5}	> 20	60	1.5	2.1	0.7	3
18	3300	In 5000	< 7	4.80	–	> 20	175	0.34	0.86	0.4	1.2
19	17000	In 5000	< 3	< 3	2.0×10^{-3}	> 20	60	0.92	1.8	0.5	2.5
20	26000	In 5000	< 5	≈ 3	1.3×10^{-3}	> 20	50	0.43	0.43	1	1
21	8100	In 5000	< 2	< 3	3.4×10^{-4}	< 20	40	0.81	0.88	0.9	2.8
22	53000	In 5000	< 11	< 4	$> 1.0 \times 10^{-4}$	> 20	125	6.6	15	0.4	27
23	> 10000	In 5000	–	< 3	3.0×10^{-4}	< 20	400	30	30	1	100
24	> 10000	In 5000	–	< 3	$> 1.0 \times 10^{-3}$	> 20	> 1000	> 50	> 50	–	> 50
28	3200	In 5000	< 0.6	< 4	7.9×10^{-5}	> 20	50	1.8	2	0.9	20
29	> 1000	In 5000	–	< 3	$> 1.0 \times 10^{-3}$	> 20	500	50	50	1	> 50

[a–e]See corresponding footnotes in table VI.

Table VI. Pharmacological profile of 1-alkyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-alkyloximes and *O*-acetyloxime. nd = not determined. In = inactive.

	In vitro studies						*In vivo studies*				
	Receptor binding[a]			*Guinea pig ileum*[b]		*Anti-ChE*[c] *activity*	*Approx* LD_{50}	*Hypothermia*[d]			*Diarrhoea*[e]
Compd	*[³H] PZ* IC_{50} *(nM)*	*[³H] QNB* IC_{50} *(nM)*	*M1/Non* M_1 *index*	*Agonism* pD_2	*Antagonism* IC_{50} *(M)*	IC_{50} *(μg/ml)*	*mg/kg po*	*mg/kg po*	*mg/kg sc*	*Ratio po/sc*	*mg/kg po*
30	1500	> 5000	< 0.30	6.58	–	> 100	90	0.70	0.49	1.4	0.8
31	840	9300	0.09	< 3	1.8×10^{-4}	> 20	250	13	11	1.2	50
32	510	11000	0.05	< 3	1.1×10^{-5}	> 20	350	> 100	nd	–	> 100
33	430	2200	0.20	6.32	–	> 100	60	2.1	0.63	3.3	5.5
34	450	5000	0.09	< 4	1.0×10^{-5}	> 20	250	38	30	1.2	> 100
35	240	6500	0.04	< 3	1.1×10^{-5}	> 20	250	80	40	2.0	100
36	> 10000	In 5000	–	3.78	–	> 20	> 1000	53	50	1.1	75
37	39000	In 5000	< 8	4.42	–	> 20	750	35	28	1.3	200
38	> 10000	In 5000	–	4.29	–	> 20	500	39	nd	–	> 200

[a]Affinity for muscarinic receptors binding sites in the rat cortex or brain labeled with [³H] pirenzepine ([³H] PZ) or [³H] quinuclidinyl benzylate ([³H] QNB) in the presence of 100 nM pirenzepine; IC_{50} values are the concentrations that cause 50% inhibition of the specific ³H-ligand binding; M_1/non M_1 index is the ratio between the IC_{50} value obtained in [³H] PZ and [³H] QNB binding assays (a low ratio indicates high selectivity for M_1 binding sites). [b]Agonist or antagonist effects are expressed as pD_2, negative logarithm of the concentration inducing 50% of the maximal contractile response or as IC_{50}, concentration causing 50% inhibition of acetylcholine induced contractions. [c]Concentration required to inhibit cholinesterase activity *in vitro* by 50%; the IC_{50} value for the reference drug physostigmine was 0.028 ± 0.001 μg/ml (mean ± SEM of 4 experiments made in duplicate). [d]Dose lowering rectal temperature by 1°C after *po* or sc administration to mice; the ratio *po* /sc was used as an index of minor (values near to 1) or major (values > than 1) metabolic first pass. [e]Dose causing diarrhoea in 50% of the animals (mice).

istic activity. The muscarinic nature of the contractile response was confirmed by the results obtained in the presence of atropine or hexamethonium; that is, atropine but not hexamethonium inhibited the response produced by maximally effective concentrations of the compounds. In our binding assays compounds **11** and **12** had better selectivity than arecoline for the M_1 receptor with respect to 'non M_1' (M_2, M_3 etc) receptors labelled by ³H-QNB in the presence of pirenzepine (table V). Although further studies are needed to better define the selectivity of these new aldoximes for muscarinic subtypes, this experimental finding is interesting; M_1-selective agonists may be of particular value for the treatment of AD [13, 14]. In analogy with the data for arecoline esters ([33], table V) indicating a stringent structural requirement for potent agonistic activity, replacement of the 1-methyl group by larger substituents (*ie*, ethyl, *n*-propyl, *n*-butyl, propargyl, etc) or of the methyl or propargyl groups of the oxime by a hydrogen (**36** and **37**), ethyl (**31**), isopropyl (**32**), allyl (**34**) or butenyl (**35**) gave compounds with low agonistic or antagonistic properties or to inactive compounds. In the aldoxime series, a more dramatic loss of muscarinic receptor affinity was observed when the optimal substituents at the nitrogen atom (hydrogen and methyl) or at the oxygen atom of the oxime group (methyl and propargyl) were replaced by other substituents. This indicates a more rigid electronic or steric requirement for the interaction with the receptor protein in the case of the oxime moiety.

Our *in vitro* studies also revealed that over the concentration range of 20–100 μg/ml, compounds **1–38** have no anticholinesterase activity (tables V, VI); in the same assay, the ID_{50} value for the anticholinesterase reference drug physostigmine was 0.028 μg/ml.

Time course studies of central and peripheral effects in mice of different subcutaneous and/or oral doses of the compounds showed clear dose-dependency for both peak action and duration of action. The doses that lowered body temperature by 1°C or induced diarrhoea in 50% of the animals are reported in tables V and VI.

Moreover a good correlation between *in vivo* and *in vitro* cholinergic potency for compounds with hydrogen or methyl in position 1 was observed. Thus, in agreement with the *in vitro* data, *O*-methyloximes **11** and **12** and the corresponding *O*-propargyloximes **30** and **33** were the most potent compounds of this series. The pharmacological results after oral administration showed that their central efficacy was 2–3 orders of magnitude higher than that of arecoline, and that their duration of action was longer. The most potent compound was **11**, followed by **30**, **12** and **33**; **11** and **30** showed longer-lasting effects than the corresponding 1-methyl derivatives **12** and **33**. The 4 aldoximes had *po*/sc ratios much lower than that of

arecoline. In accordance with our introductory remarks, the 2 *N*-demethylated compounds **11** and **30** had the lowest ratios; compound **11** showed the same activity after oral and subcutaneous treatment, suggesting that this molecule is completely protected from metabolic first pass. As suggested by the data obtained in the hypothermia and diarrhoea tests, the compounds had better ratios of central to peripheral effects than arecoline (see also below).

Contrary to the effect observed with the aldoxime moiety, alkylation of the ring nitrogen with groups longer than methyl determined a dramatic difference between *in vivo* and *in vitro* results. Except for compounds bearing CH_2-C_6H_5 (**22**), $CH_2COOC(CH_3)_2$ (**23**), CH_2COOH (**24**) or $C(CH_3)_3$ (**29**) groups, all the other 1-alkyl derivatives had potent hypothermic activity. Since these compounds had little or no agonistic activity *in vitro*, it is logical to assume that they are *N*-dealkylated in the body to the biologically active parent molecule **11**. The greater activity of compounds **13**, **18** and **20** *vs* the shorter homologue **10**, longer chain compounds **15**, **19** and **16** and compounds with branched alkyl chains (**14**, **17**, **21** and **28**) indicates that there is an optimal 3-carbon linear alkyl chain for this well-known enzymatic *N*-dealkylation [49]. These 'prodrugs' were more effective when given orally than parenterally, and their duration of action was long. It is also worth noting that, unlike the parent compound **11**, some of these 1-alkyl derivatives, **10** in particular, induced hypothermia at doses considerably lower than those necessary to induce diarrhoea.

Compounds **11**, **12**, **30** and **33**, which had potent agonistic properties, potent oral efficacy and longer duration of action than arecoline in the primary screening, were further evaluated in a battery of secondary assays. The parent molecule arecoline was used as the reference drug.

Studies of typical overt central (hypothermia, tremors and analgesia) and peripheral (diarrhoea, lacrimation, salivation and mydriasis) cholinergic symptoms, imputable to stimulation of muscarinic receptors [50–52], were carried out in mice and/or rats. Moreover, the ability of the compounds to increase local cerebral blood flow in the frontal cortex of conscious rats and to induce synchronization of the hippocampal EEG under halothane anesthesia in rats were evaluated. Finally, the pro-mnemonic properties of the compounds were evaluated by investigating their effects on scopolamine-induced amnesia in mice. The results obtained in these secondary assays are summarized in table VII.

Table VII. *In vivo* pharmacological profile of selected compounds in comparison with arecoline.

			Effective equipotent doses (mg/kg)				
Test	*Species route*		*Compd 11*	*Compd 12*	*Compd 30*	*Compd 33*	*Arecoline*
Central effects							
Hypothermia[a]	Mouse	*po*	0.11	0.85	0.70	2.1	194
	Mouse	sc	0.12	0.22	0.49	0.6	2.9
	Rat	*po*	0.11	1.80	0.56	4.6	> 200
	Rat	sc	0.09	0.38	0.36	1.5	5.2
Tremors[b]	Mouse	*po*	0.25	2.0	0.9	6.0	200
Analgesia[c]	Mouse	*po*	0.25	2.0	1.0	6.0	220
CCBF[d]	Rat	*po*	0.25	1.0	1.0	> 5	> 50
Hippocampal RSA[e]	Rat	sc	0.02	0.10	0.05	< 0.5	0.5
Anti-amnesic[f]	Mouse	*po*	0.001	0.007	0.002	0.020	1
Peripheral effects							
Diarrhoea[b]	Mouse	*po*	0.15	1.5	0.8	5.5	35
	Rat	*po*	0.23	2.5	1.0	6.0	200
Salivation[b]	Mouse	*po*	0.25	1.7	0.9	3.5	200
Lacrimation[b]	Mouse	*po*	0.30	2.0	1.0	4.0	250
Mydriasis[g]	Mouse	*po*	0.41	2.6	1.2	4.3	> 200
Acute toxicity							
LD_{50}	Mouse	*po*	70	600	90	60	550
LD_{50}	Rat	*po*	12	300	80	> 200	2500

[a]Dose that lowers rectal temperature by 1°C; [b]dose that causes symptoms in 50% of animals; [c]dose that increases the time to avoidance response to the hot plate by 100%; [d]dose that significantly increases cortical cerebral blood flow (CCBF) in conscious rats; [e]dose that induces 30% synchronization of the rhythmical slow wave activity (RSA) in the hippocampus of the halothane-anesthetized rat; [f]dose that increases retention performance by 50%; [g]dose that increases pupillar diameter by 50%.

After oral administration to mice and rats, **11** was the most potent in inducing overt central cholinergic effects, followed by **30**, **12** and **33**. The central actions of these novel muscarinic agonists were 700–1400, 200–400, 100–200 and 30–190 times that of arecoline, respectively. The doses needed to induce overt central symptoms were not substantially different from those needed to induce overt peripheral ones, implying that their penetration into the CNS is good. From this point of view, arecoline had similar ratios of central to peripheral effects; the only exception was the diarrheogenic response. The marked diarrheogenic effect observed after oral treatment with arecoline is probably due to a direct action of the drug on the intestine. These experimental findings indicate that the compounds behave similarly in the central and peripheral cholinergically innervated structures involved in the expression of hypothermia, tremors, analgesia, diarrhoea, salivation, lacrimation and mydriasis. All these biological effects were completely blocked by pre-treatment with the muscarinic receptor antagonist atropine, confirming that the overt symptoms induced by the compounds are manifestations of their muscarinic activity. Compounds **11** and **30** had similar central activity in mice and rats, whereas **12**, **33** and arecoline were somewhat less effective in rats. Their central duration of action, as estimated from the time-course of the hypothermic effect after oral or subcutaneous administration, was longer than that of arecoline; **11** and **30** were the longest-lasting (table VIII). After oral administration to conscious rats, the compounds dose-dependently increased local cerebral blood flow in the frontal cortex. In this test, too, **11** was the most potent, and was more than 200 times as active as arecoline. The very potent central muscarinic action of the compounds was confirmed by measuring the scopolamine-sensitive hippocampal slow-wave activity (RSA) in the halothane-anesthetized rat. In this electrophysiological assay the order of potency of the compounds correlates well with the order for other central cholinergic effects, and the effective doses were lower than those required to induce overt central and peripheral symptoms.

The validity of using scopolamine, a centrally active anticholinergic agent, as a model for dementia, is widely accepted. Scopolamine produces amnesia in both experimental and clinical situations [53, 54] and scopolamine-induced memory impairment in animals qualitatively resembles that seen in patients with AD and in elderly persons [55]. In animal studies, scopolamine inhibits the learning in several situations, particularly inhibitory avoidance [56, 57]. We investigated the anti-amnesic profiles of the 4 selected compounds and of arecoline on amnesia induced by scopolamine in mice in a 1-trial inhibitory avoidance task. All compounds reversed scopolamine-induced memory deficit. Dose-retention curves had the inverted-U shape typical of agents believed to improve cognitive functions. The effective doses were much lower than those inducing other central cholinergic symptoms, and there is good correlation between anti-amnesic and central cholinergic potency. The most potent compound was **11**, which was 2, 7, 20 and 1000 times as effective as **30**, **12**, **33** and arecoline. The 4 aldoximes showed anti-amnesic properties in many respects superior to those of arecoline. In fact, in addition to being much more potent than the parent reference drug, they significantly reversed scopolamine-induced amnesia over a wide range of doses and caused greater reversal of amnesia. These experimental data, although having limitations, are of

Table VIII. Time course of the hypothermic response in the rat after equipotent *po* or sc doses of selected compounds and arecoline. nd = not determined.

Compound	*Dose (mg/kg)*	*Route*	*Changes in body temperature (°C) at*				
			0'	*30'*	*60'*	*120'*	*180'*	*240'*
11	0.2	*po*	± 0	− 1.7**	− 1.8**	− 1.4**	− 0.8**	− 0.2
11	0.2	sc	+ 0.2	− 2.0**	− 2.0**	− 1.5**	− 0.5**	+ 0.1
12	4	*po*	− 0.1	− 1.7**	− 2.0**	− 0.6**	± 0	nd
12	1	sc	+ 0.1	− 2.0**	− 1.1**	± 0	− 0.1	nd
30	1	*po*	+ 0.1	− 1.1**	− 1.7**	− 1.3**	− 0.4**	± 0
30	0.75	sc	± 0	− 2.0**	− 1.8**	− 0.8**	− 0.2	± 0
33	8	*po*	± 0	− 1.5**	− 0.9**	− 0.3*	− 0.1	nd
33	4	sc	+ 0.1	− 2.0**	− 1.3**	− 0.1	± 0	nd
Arecoline	10	sc	+ 0	− 1.7**	− 0.3	+ 0.1	+ 0.1	nd

6–12 animals per group were used; *$P < 0.05$; **$P < 0.01$ compared with controls (Student's *t*-test).

considerable practical interest in view of the clinical use of the compounds.

Finally, the acute toxicity data in mice and rats indicate that the lethal effect of the compounds is not a direct result of their cholinomimetic activity. In agreement with our previous findings for arecoline ester derivatives (see *Introduction*) and with the results with the other oxime derivatives in mice (see tables V, VI), the lethality of the selected compounds seems to be primarily due to the characteristics of the compounds themselves. In fact, we found no correlation between cholinergic doses and LD_{50} values. The LD_{50} of the four selected compounds were 2–4 orders of magnitude higher than those inducing overt central effects, and their therapeutic indices were much more favourable than that of the parent molecule, arecoline.

In conclusion, present knowledge strongly advocates giving muscarinic agonists as therapeutic agents to prevent or reverse cognitive deficits in elderly subjects with cholinergic deficit. In a search for new cholinergic agents for treatment of AD we have synthesized and tested a large number of compounds structurally related to arecoline. Amongst these, **11** (RU 35963), **12** (RU 35926), **30** (RU 47029) and **33** (RU 35986) had spectra of pharmacological properties that fit the aims of our research well. Because of their characteristic cholinomimetic profile, as potent orally active muscarinic agents with several advantages over the natural parent compound, arecoline, they are presently undergoing further preclinical and toxicological evaluation.

Experimental protocols

Chemistry

Melting points were determined on a Buchi 510 melting point apparatus and are uncorrected. IR spectra were recorded with a Perkin–Elmer model 881 spectrophotometer. NMR spectra were determined on Varian T-60 or Varian VXR-200/s spectrometers; chemical shifts are reported in units downfield from Me_4Si; the coupling constants are expressed in hertz; standard abbreviations are used. Thin layer chromatography was carried out on silica gel 60 F 254 precoated glass plates (Merck, 0.25 mm). Column chromatography was performed on silica gel 60 (Merck, 230–400 mesh) or alumina (Merck, 70–230 mesh). Microanalyses were performed by the Analytical Laboratory Service of the Department of Industrial Chemistry of the University of Milan, and agreed with theoretical values to within ± 0.4% except where indicated.

3-Methoxyiminomethyl-1-ethylpyridinium iodide ***9***

A solution of 3-pyridinecarboxaldehyde-*O*-methyloxime [39] (22.7 g, 167 mmol) and ethyl iodide (20 ml, 250 mmol) in ethanol (220 ml) was heated at reflux for 10 h. The reaction mixture was concentrated *in vacuo* to half volume, diethyl ether (100 ml) was added and the yellow solid was collected by filtration, washed with diethyl ether and dried *in vacuo* to give 45 g (92%) of **9**. mp = 107–108°C with dec. This material was used in the next step without purification. A sample recrystallized from acetone melted at 118°C with dec. Anal $C_9H_{13}IN_2O$ (C, H, N).

1-Ethyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-O-methyloxime hydrochloride ***10***

Sodium borohydride (8 g, 0.21 mol) was added in portions to a stirred solution of **9** (32 g, 0.11 mol) in methanol (320 ml), with external cooling to maintain the temperature at 0°C. At the end of the addition the reaction mixture was stirred for an additional hour at room temperature. The solvent was distilled under reduced pressure, excess reducing agent was decomposed with water (40 ml) and after saturation with sodium chloride, the mixture was thoroughly extracted with ethyl acetate. The combined organic extracts were dried (Na_2SO_4) and evaporated. The residual oil was distilled at 80°C, 1.3 mbar to give 12.7 g (69%) of **10** as a yellow oil. The hydrochloride was prepared in Et_2O with HCl gas. The white solid after recrystallization from EtOH–Et_2O melted at 220°C with dec. Anal $C_9H_{16}N_2O$•HCl (C, H, N). ^{1}H-NMR ($CDCl_3$) of the base: 1.13 (3H, t, J = 7, CH_3CH_2), 2.13–2.73 (6H, m, 5-CH_2, 6-CH_2, CH_2CH_3), 3.10–3.23 (2H, m, 2-CH_2), 3.8 (3H, s, OCH_3), 5.76–6.03 (1H, m, 4-CH), 7.46 (1H, s, CH=N).

1,2,5,6-Tetrahydropyridine-3-carboxaldehyde-O-methyloxime hydrochloride ***11***

Method A (scheme 1). 1-Chloroethyl chloroformate (98%, 4 g, 27.4 mmol) was cautiously added at 0°C under nitrogen to a stirred solution of the free base of **10** (4.5 g, 26.7 mmol) in dichloroethane (60 ml). The reaction mixture was warmed to room temperature and heated at reflux for 2 h. Dichloroethane was evaporated under reduced pressure, and the residue was taken up in diethyl ether to remove by filtration a small amount (0.4 g) of unreacted **10**. Diethyl ether was evaporated and the crude carbamate so obtained was dissolved in methanol (40 ml) and heated at reflux for 30 min. Methanol was evaporated under reduced pressure, and the residue was recrystallized from EtOH + Et_2O to yield 3.8 g (81%) of **11** as a white solid. mp = 208°C with dec. Anal $C_7H_{12}N_2O$•HCl (C, H, N).

To obtain the free base, **11** was dissolved in the minimum amount of water which was saturated with K_2CO_3 and extracted with Et_2O. Distillation of the base was effected in a Kugelrohr apparatus at 150–160°C, 0.2 mbar. ^{1}H-NMR ($CDCl_3$) of the base: 1.8 (1H, s, NH), 2.0–2.43 (2H, m, 5-CH_2), 2.93 (2H, t, J = 5, 6-CH_2), 3.53 (2H, d, J = 2, 2-CH_2), 3.78 (3H, s, OCH_3), 5.83–6.06 (1H, m, 4-CH), 7.43 (1H, s, CH=N).

The intermediate carbamate is a yellow oil which slowly decomposes at room temperature. An analytical sample was obtained by Kugelrohr distillation at 200°C, 0.08 mbar. Anal $C_{10}H_{15}ClN_2O_3$ (C, H, N). IR (film): 1727 cm^{-1} (NCOO). ^{1}H-NMR ($CDCl_3$): 1.8 (3H, d, J = 6, CH_3), 2.10–2.50 (2H, m, 5-CH_2), 3.56 (2H, t, J = 6, 6-CH_2), 3.80 (3H, s, OCH_3), 4.16 (2H, d, J = 2, 2-CH_2), 5.86–6.10 (1H, m, 4-CH), 6.53 (1H, q, J = 6, CHCl), 7.46 (1H, s, CH=N).

This dealkylation procedure when applied to 1-benzyl ***22*** *and 1-methyl* ***12*** *derivatives, gave* ***11*** *in 87 and 60% yield respectively.*

Method B (scheme 2). 1,2,5,6-Tetrahydropyridine-3-carboxaldehyde hydrochloride [43] (5 g, 34 mmol) was dissolved in water (30 ml), methoxylamine hydrochloride (2.85 g, 34 mmol) was added and the solution was stirred at room temperature for 1 h. Water was distilled at reduced pressure and the residue was recrystallized from ethanol to give 4.8 g (80%) of **11**, mp = 208°C with dec.

*1-Alkyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-O-methyloximes hydrochlorides **12–23**. General procedure*

A solution of the alkyl halide (10 mmol) in dry dimethylformamide (5 ml) was added to a stirred dispersion of **11** (1.76 g, 10 mmol) and triethylamine (2.8 ml, 20 mmol) in dry DMF (20 ml). The rate of addition was controlled in order to keep the temperature of the reaction below 30°C without external cooling. At the end of the addition, stirring was continued at room temperature for additional 3 h, or at 60°C for 8 h in the preparation of **17**. The solvent was removed under reduced pressure, the residue was dissolved in water (10 ml) which was saturated with solid K_2CO_3 and extracted with ethyl acetate (3 x 60 ml). The combined organic extracts were dried (Na_2SO_4) and evaporated. The yellow liquid so obtained was chromatographed on a silica gel column eluted with $CHCl_3$–CH_3OH/4:1 to yield pure 1-alkyl derivatives 12–23 as the free bases. The hydrochlorides were prepared in Et_2O with HCl gas and recrystallized from the solvent indicated in table II.

Iodomethane was used in the preparation of **12** and chloromethyl cyclopropane in the preparation of **21**. In all other cases, alkyl bromides were used.

Variation of the general procedure. It was more expedient to prepare **14** and **21** by dissolving the free base of **11** (2.8 g, 20 mmol) in excess alkyl halide (2.5 ml) and heating the stirred reaction at gentle reflux for 4 h when 2-bromopropane was used (**14**) and at 75–80°C for 12 h when chloromethyl cyclopropane was used (**21**). Excess alkyl halide was then distilled, saturated K_2CO_3 solution added and the aqueous phase was extracted with ethyl acetate (3 x 80 ml). The oil obtained after evaporation of the solvent was chromatographed on a silica gel column eluted with $CHCl_3$-CH_3OH/4:1 and unreacted material (~ 40%) was recovered. The hydrochlorides **14** and **21** were prepared as described under *General procedure*.

*1-Carboxymethyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-O-methyloxime hydrochloride **24***

A solution of the free base of **23** (3.05 g, 12 mmol) and *p*-toluensulfonic acid monohydrate (2.28 g, 12 mmol) in toluene (30 ml) was heated at reflux for 1 h. The oily residue obtained after evaporation of the solvent was dissolved in 1,2-dichloroethane (30 ml) and saturated with HCl gas. Dilution with Et_2O gave a solid which was collected by filtration and recrystallized from ethanol to yield 1.8 g (65%) of **24**. mp = 213°C with dec. Anal $C_9H_{14}N_2O_3$•HCl (C, H, N). IR (nujol): 1750 cm^{-1} (COOH). ^{1}H-NMR (DMSO–d_6): 2.40–2.80 (2H, m, 5-CH_2), 3.26–3.56 (2H, m, 6-CH_2), 3.76 (3H, s, CH_3), 3.96 (2H, br s, 2-CH_2), 4.16 (2H, s, CH_2-COOH), 6.16–6.40 (1H, m, 4-CH), 7.76 (1H, s, CH=N).

*3-Methoxyiminomethyl-1-(2,4-dinitrophenyl) pyridinium chloride **25***

3-Pyridinecarboxaldehyde-*O*-methyloxime [39] (20 g, 147 mmol) and 1-chloro-2,4-dinitrobenzene (89 g, 439 mmol) were reacted at 60–70°C for 2 h with occasional shaking. The reaction was cooled to room temperature, water was added (300 ml) and unreacted material was extracted with diethyl ether (3 x 100 ml). The aqueous solution was treated with charcoal, filtered and evaporated under vacuum. The residue was triturated with acetone and the yellow solid collected by filtration to give 31 g (62%) of **25**. mp = 161°C with dec. Anal $C_{13}H_{11}ClN_4O_5$ (C, H, N).

*3-Methoxyiminomethyl-1-cyclopropylpyridinium chloride **26***

Cyclopropylamine (6.2 ml, 89 mmol) was added at 0°C to a stirred solution of **25** (10 g, 29.5 mmol) in methanol (100 ml) and the brown reaction was heated at 45–50°C for 15 min. The solvent was evaporated, water (100 ml) was added and the aqueous phase was repeatedly extracted with diethyl ether, decolorized with charcoal and evaporated under vacuum to yield 6 g (95%) of crude **26**. A sample recrystallized from i-PrOH-Et_2O melted at 198°C with dec. Anal $C_{10}H_{13}ClN_2O$ (C, H, N).

*3-Methoxyiminomethyl-1-(1,1 dimethyl)ethyl pyridinium chloride **27***

Tert-butylamine (11.2 ml, 107 mmol) was added at 0°C to a stirred solution of **25** (12 g, 35.4 mmol) in methanol (120 ml) and the brown reaction was heated at 45–50°C for 1 h. After cooling to room temperature, the insoluble material was discarded and the solvent was evaporated to a final volume of ≈ 20 ml. Dilution with diethyl ether (200 ml) afforded a tarry brown solid which was used without purification in the reduction step. Attempts to obtain a crystalline substance were unsuccessful.

*1-Cyclopropyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-O-methyloxime hydrochloride **28***

The compound was obtained in a 26% yield from **26** using the same procedure described for compound **10**. The base was purified by chromatography on a silica gel column eluted with ethyl acetate–toluene 1:1. Anal $C_{10}H_{16}N_2O$•HCl (C, H, N). ^{1}H-NMR ($CDCl_3$) of the free base: 0.40–0.56 (4H, m, CH_2-cyclopropyl), 1.53–1.90 (1H, m, CH-cyclopropyl), 2.06–2.45 (2H, m, 5-CH_2), 2.73 (2H, t, J = 5, 6-CH_2), 3.23–3.4 (2H, m, 2-CH_2), 3.80 (3H, s, CH_3), 5.73–6.0 (1H, m, 4-CH), 7.46 (1H, s, CH=N).

*1-(1,1-Dimethyl)ethyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-O-methyloxime hydrochloride **29***

By employing the procedure described for compound **10** but limiting the reduction time to 15 min at 0°C, **29** was obtained from crude **27** in 20% yield. The base was purified by filtration on a short alumina column eluted with methylene chloride and then by chromatography on a silica gel column eluted with ethyl acetate. Anal $C_{11}H_{20}N_2O$•HCl (C, H, N). ^{1}H-NMR ($CDCl_3$) of the free base: 1.13 (9H, s, CH_3), 2.06–2.43 (2H, m, 5-CH_2), 2.61 (2H, t, J = 5, 6-CH_2), 3.20–3.40 (2H, m, 2-CH_2), 3.76 (3H, s, OCH_3), 5.75–6.0 (1H, m, 4-CH), 7.46 (1H, s, CH=N).

*1,2,5,6-Tetrahydropyridine-3-carboxaldehyde-O-propargyloxime hydrochloride **30***

The compound was obtained in an 85% yield according to the Method B described for compound **11** by using propargyloxylamine hydrochloride [58]. Anal $C_9H_{12}N_2O$•HCl (C, H, N). ^{1}H-NMR (DMSO–d_6): 2.33–2.63 (2H, m, 5-CH_2), 3.0–3.5 (3H, 2m, 6-CH_2 and HC≡C)), 3.63 (2H, br s, 2-CH_2), 4.6 (2H, d, J = 3, CH_2O), 6.2–6.4 (1H, m, 4-CH), 7.76 (1H, s, CH=N), 9.5 (2H, br s, NH_2^+).

*1-Methyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-O-alkyloximes hydrochlorides **31–35***

Method A. Compounds **31–33** were obtained according to method B described for compound **11** from 1-methyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde hydrochloride [44] and ethoxylamine hydrochloride (**31**), iso-propyloxylamine hydrochloride [59] (**32**) and propargyloxylamine hydrochloride [58] (**33**). Yields are reported in table III. Compound **12** could also be obtained by this method in 54% yield by using methoxylamine hydrochloride

^{1}H-NMR (DMSO-d_6) of **31**: 1.16 (3H, t, J = 7, CH_3), 2.36–2.66 (2H, m, 5-CH_2), 2.76 (3H, s, N-CH_3), 3.06–3.40 (2H, m, 6-CH_2), 3.75 (2H, br s, 2-CH_2), 4.0 (2H, q, J = 7, CH_2O), 6.10–6.36 (1H, m, 4-CH), 7.73 (1H, s, CH=N), 10.9–11.6 (1H, br b, HCl).

^{1}H-NMR (DMSO-d_6) of **32**: 1.16 (6H, d, J = 6, CH_3), 2.38–2.70 (2H, m, 5-CH_2), 2.76 (3H, s, N-CH_3), 3.06–3.43 (2H, m, 6-CH_2), 3.76 (2H, br s, 2-CH_2), 4.23 (1H, q, J = 6, CHO), 6.10–6.35 (1H, m, 4-CH), 7.70 (1H, s, CH=N), 11.1–11.5 (1H, br b, HCl).

^{1}H-NMR (DMSO-d_6) of **33**: 2.36–2.66 (2H, m, 5-CH_2), 2.76 (3H, s, N-CH_3), 3.03–3.46 (3H, 2m, 6-CH_2 and HC≡C), 3.73 (2H, br s, 2-CH_2), 4.60 (2H, d, J = 2, CH_2O), 6.16–6.43 (1H, m, 4-CH), 7.80 (1H, s, CH=N), 11.30 (1H, br s, HCl).

Method B. O-Alkylation of 1-methyl-1,2,5,6-tetrahydropyridine-3-aldoxime **37**

1-Methyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-O-allyloxime hydrochloride ***34***

Sodium (0.5 g, 21.7 mmol) was added under nitrogen to absolute ethanol (20 ml) and, after dissolution, 1-methyl-1,2,5,6-tetrahydropyridine-3-aldoxime [44] (2.7 g, 19.2 mmol) was added under stirring. Allylbromide (1.7 ml, 19.6 mmol) was dropped and the reaction heated at reflux for 3 h. The solvent was evaporated, the residue partitioned between water (60 ml) and methylene chloride (120 ml). Evaporation of the organic phase gave a brown oil which was chromatographed on a silica gel column eluted with ethyl acetate–methanol 95:5. The free base of **34** thus obtained was distilled in a Kugelrohr apparatus at 125–130°C, 5 mbar, then dissolved in diethyl ether and treated with HCl gas. The hydrochloride was recrystallized from CH_3OH/Et_2O to give 1.2 g (29%) of **34** as a white crystalline powder. mp = 168–169°C with dec. Anal $C_{10}H_{16}N_2O$•HCl (C, H, N). ^{1}H-NMR ($CDCl_3$) of the free base: 2.11–2.63 (4H, m, 5- and 6-CH_2), 2.36 (3H, s, NCH_3), 3.0–3.20 (2H, m, 2-CH_2), 4.46 (2H, dd, J = 6 and 1, CH_2O), 4.96–5.36 (2H, m, CH_2=C), 5.56–6.23 (2H, m, 4-CH and CH=C), 7.50 (1H, s, CH=N).

1-Methyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-O-2-butenyloxime hydrochloride ***35***

35 was similarly obtained in a 34% yield by using *trans*-crotylbromide. The free base was distilled in a Kugelrohr apparatus at 160–165°C, 3 mbar. Anal $C_{11}H_{18}N_2O$•HCl (C, H, N). ^{1}H-NMR ($CDCl_3$) of the free base: 1.60–1.80 (3H, m, CH_3-C), 2.10–2.66 (4H, m, 5- and 6-CH_2), 2.36 (3H, s, NCH_3), 3.03–3.20 (2H, m, 2-CH_2), 4.33–4.50 (2H, m, CH_2O), 4.50–5.76 (2H, 2m, CH=CH), 5.76–5.96 (1H, m, 4-CH), 7.50 (1H, s, CH=N). The NMR spectrum evidenced the presence of about 18% of the isomeric *O*-1-methyl-2-propenyl oxime derived from 3-bromo-1-butene contained in the commercial crotyl bromide.

1-Methyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-O-acetyloxime ***38***

Acetyl chloride (1 ml, 14 mmol) was slowly dropped into a stirred solution of 1-methyl-1,2,5,6-tetrahydropyridine-3-aldoxime [43] (2 g, 14.2 mmol) and triethylamine (2 ml, 14.3 mmol) in dry tetrahydrofuran and stirring was continued for 1 h at room temperature. The solvent was removed and the residue taken up in 5% aqueous $NaHCO_3$ and extracted with ethyl acetate (2 x 50 ml). Evaporation of the solvent gave an oil which was distilled in a Kugelrohr apparatus at 170–175°C, 0.05 mbar to finally yield 2.1 g (81%) of a pale yellow oil. Anal $C_9H_{14}N_2O_2$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 2.10 (3H, s, CH_3-CO), 2.26–2.66 (4H, m, 5- and 6-CH_2), 2.36 (3H, s, NCH_3), 3.13–3.30 (2H, m, 2-CH_2), 6.03–6.26 (1H, m, 4-CH), 7.70 (1H, s, CH=N).

Pharmacology

Male CD1 mice (25–30 g), male Sprague–Dawley rats (150–300 g), male Wistar rats (300–400 g) (Charles River, Calco, Italy) and male albino guinea pigs weighing 250–400 g (Bettinardi, Momo, Italy) were used. The animals were housed in separate air-conditioned rooms with food and water *ad libitum* and maintained on a 12 h light/dark illumination cycle.

Test compound and reference drugs were dissolved in double-distilled water (*in vitro* assays) or in 0.9% saline (parenteral injections), while for oral administration they were dissolved in 0.25% carboxymethylcellulose. Vehicle alone was administered as the control for each experiment, and the volumes were: 10 ml/kg (sc) or 20 ml/kg (*po*) for mice and 2 ml/kg (sc) or 5–10 ml/kg (*po*) for rats. Doses refer to the salt form.

Binding studies

[^{3}H]Pirenzepine (70–87 Ci/mmol) and [^{3}H] quinuclidinyl benzilate (QNB) (30–60 Ci/mmol) were purchased from New England Nuclear (Paris).

[^{3}H] Pirenzepine binding to the M_1 receptor was carried out according to the method of Watson *et al* [60]. A rat brain was rapidly dissected over ice and the cortex homogenized (Polytron, setting 3.3 x 10 s) in 100 vol cold $NaKHPO_4$ buffer (10 mM, pH 7.4). Aliquots (0.5 ml) of the homogenate were incubated for 60 min at 25°C with [^{3}H] pirenzepine (0.25 nM) and various concentrations of the test compound. Bound ligand was separated from the incubation medium by rapid filtration through Whatman GF/C filters pre-soaked for 30 min in 0.05% polyethyleneimine. The filters were washed with 3 x 5 ml ice-cold buffer before radioactivity measurement. Non-specific binding was determined by an identical incubation in the presence of unlabelled pirenzepine (10 μM).

[^{3}H] QNB binding to 'non-M_1' (M_2, M_3, M_4...) muscarinic receptors in a crude synaptic membrane preparation from rat brain was carried out by a method similar to that described by Yamamura and Snyder [61] but in the presence of pirenzepine (100 nM) to eliminate binding to the M_1 receptor (15–20% of total specific binding). After removal of the cerebellum, the remaining brain tissue was homegenised (Potter–Elvehjem glass homogeniser with a Teflon pestle) in 20 vol ice-cold 0.32 M sucrose. The homogenate was centrifuged for 10 min at 1000 *g* and the resulting supernatant recentrifuged for 15 min at 30 000 *g*. The membrane pellets were resuspended in 25 vol Tris–HCl buffer (50 mM, pH 7.5) and centrifuged for 15 min at 30 000 *g*. The pellets were stored at – 30°C and then resuspended in the same buffer for incubation. The membrane suspension was pre-incubated for 15 min at 25°C with pirenzepine (100 nM) and re-cooled to 4°C. Aliquots (2 ml) of the homogenate were incubated for 60 min at 25°C in the presence of [^{3}H] QNB (0.3 nM) and various concentrations of the test compound. The filtration and washing steps were as described for [^{3}H] pirenzepine. Non-specific binding was determined in the presence of benztropine (30 μM).

Binding experiments with other neurotransmitter receptors were carried out using similar procedures with tritiated ligands and sources of tissue as shown in table IV.

Isolated guinea pig ileum

Segments of the ileum (2.5–3 cm long) were excised, washed and immediately suspended in a 10 ml organ bath containing

Tyrode's solution at 37°C and continuously aerated with a mixture of 95% O_2, 5% CO_2. Contractions were recorded isometrically at 1 g tension using an electromechanical displacement transducer and a potentiometric recorder (Basile).

After equilibration the test compounds were added to the bath. Each dose was allowed to exert its maximal contractile effect before being washed out. The tissue was to return to spontaneous baseline activity prior to each dose. The interval between 2 doses of agonist was usually 2 min. The dose–response curve for each compound was obtained from 4–6 different concentrations and 3–5 independent observations. The maximal contractile response was set at 100 and the responses to other doses are reported as percentages of the maximal response. Agonistic potencies were expressed as pD_2 values (negative logarithm of the concentration inducing 50% of the maximal effect). The intrinsic activity [62] was expressed as the maximal contraction amplitude elicited by the compounds in comparison with the maximal effect of acetylcholine. We also investigated to what extent the agonistic action of the compounds could be antagonized by the antimuscarinic agent atropine (1×10^{-7} M) or by hexamethonium (3×10^{-4} M) which blocks the nicotinic receptors in the ileal smooth muscle [63]. The antagonists were added to the bath 1 min before a dose of the agonist able to induce the maximal contraction.

In antagonist assays contractions were induced by acetylcholine (1×10^{-6} M). Comparisons were made of the acetylcholine-induced contractions before and 1 min after addition of the compound in 3–5 different concentrations. The ED_{50}, *ie*, the concentration inhibiting the acetylcholine-induced contraction by 50%, was expressed as a molar concentration, with the activity threshold set at 10^{-3} M.

Anticholinesterase activity

Anticholinesterase activity was determined *in vitro* by the method of Ellman *et al* [64] slightly modified in our laboratory, using the test-combination cholinesterase kit (Boehringer Mannheim Diagnostica). Briefly, the products were left in contact with the enzyme (Control Sera, Boehringer Mannheim) for 5 min at room temperature. The substrate was then added and mixed in, and the time in seconds to an adsorbance increase of 0.100 was measured. Physostigmine was used as a reference drug.

Hypothermia

The rectal temperatures of mice and rats (6–18 per group) were measured with a calibrated electric thermometer (LRI Milano). The thermistor probe was carefully inserted ≈ 2.5 cm (mice) or 4 cm (rats) into the rectum of manually lightly restrained animals. Groups of animals with similar mean body temperatures (within a range of ± 0.2°C) were used. 3–6 doses of each compound were administered *po* or sc, and body temperature was measured at time 0, 30, 60, 120 and 180 min, or longer when necessary, after treatment. Results are expressed as differences between mean body temperature of the treated group and that of the control group, measured at the same time. The dose that lowered rectal temperature by 1°C at the most effective time was determined. The duration of action was estimated by comparing the time courses of the hypothermic responses produced by approximately equipotent doses of the compounds.

Diarrhoea

Mice and rats (5–15 per group) were placed singly in cages with the bottoms covered with paper towels. The paper towel was replaced at each observation. The compounds were administered orally at 3–4 doses. The diarrhoea, if any, was assessed on an all or nothing basis 30, 60, 120 and 180 min after treatment. The dose inducing diarrhoea in 50% of the animals was determined.

Tremors, lacrimation and salivation

Mice and rats (5–18 per group) were treated orally with various doses of test compounds and checked for the presence or absence of other typical central (tremors) or peripheral (lacrimation and salivation) cholinergic symptoms. Tremor, lacrimation and salivation were observed and scored at 30, 60, 120 and 180 min after treatment. The dose inducing these symptoms in 50% of the animals was determined.

Atropine antagonism

Mice (6 per group) were treated with vehicle or atropine sulphate (1 mg/kg sc) 30 min before oral administration of highly effective doses of the compounds. Changes in body temperature, tremors, diarrhoea lacrimation and salivation were recorded 30, 60, 120 and 180 min after treatment.

Mydriasis

The pupil diameter was measured with a graded scale in the eyepiece of a dissecting microscope (American Optical 570, magnification 4 x, low constant illumination). After a basal reading the test compounds were administered *po* to 6–18 mice per dose. Subsequent measurements were made 15, 30, 60, 120 and 180 min after drug administration. Mydriatic activity was expressed as % ratio of the mean pupillar diameter at different times *vs* the basal value. On the basis of the mydriatic effects at 3–5 doses the ED_{50} value was calculated.

Antinociceptive effect

The antinociceptive effect of the compounds was measured by the method of Eddy and Leinbach [65], recording the reaction time of mice placed on a hot plate (Basile) maintained at 50 ± 0.1°C. The time to the first avoidance response (jumping, paw-licking or rapid stamping) was measured. The animals were immediately removed after the first response to the noxious stimulus; a 60-s cut-off time was arbitrarily selected as a maximal antinociceptive response. The animals (6–12 per group) were treated with various *po*, doses of the compounds or vehicle and the response time to the hot plate was measured at time 0, 15, 30, 60 and 120 min after treatment. The effect of the compounds was calculated as percent increase in the latency time compared with the controls. The dose that increases the time to avoidance response by 100% was determined.

Cerebral cortical blood flow (CCBF)

CCBF was measured by the hydrogen clearance method of Auckland *et al* [66], as modified by Haining *et al* [67]. Three days before the experiment the animals were anesthetized with 5% chloral hydrate and 2 electrodes for measuring hydrogen clearance were inserted 1 mm into the right and left frontal cortical areas ($A = 3$; $L = 3$). These electrodes were 90% platinum-10% iridium wire, 125 μm in diameter, insulated in glass with a bare tip ≈ 500 μm in length platinized in a 5% chloroplatinic acid solution. The common reference electrode, a silver-silver

chloride ball, was placed epidurally on the left frontal cortical area. All the electrodes were fixed with acrylic dental cement. On the day of the experiment, the animals were placed in individual boxes (20 x 20 x 24 cm). The electrodes were connected to a rotatory connector (Air Precision) and polarized at + 0.2 V. Current generated by oxidation of H_2 (5% in air) was recorded on a nanoamperometer in a circuit designed to maintain a constant polarization current. Hydrogen was administered by inhalation. Before treatment the CCBF was measured 3 times at 15-min intervals and the third value was taken as the basal value. The products were administered after the third recording. The compounds were administered *po* at 2–4 doses to 5–8 animals per group. CCBF was measured 0.5, 1, 2, 4 and 6 h after treatment. At each time readings from the 2 hemispheres were averaged to give a single CCBF value. Values are expressed in ml/min per g. On the basis of the differences between basal values and those obtained at different times after treatment, the minimal dose inducing a statistically significant increase in CCBF was determined.

Hippocampal EEG recording in the halothane-anesthetized rat

Male Wistar rats were used. Three days before the experiment the animals were anesthetized with 5% chloral hydrate and a bipolar recording electrode was inserted in the hippocampus. Stereotaxic coordinates were $A = -2.4$ $L = 2$ $V = -3.5$ [68]. The electrode was made according to a technique described by Cooley and Vanderwolf [69]. In short it consisted of 2 parallel insulated nichrome wires 160 μm in diameter with bevelled bare tips ≈ 500 μm in length. The distance between the wires was ≈ 1 mm and one wire was 1 mm longer than the other. Once lowered into position the shorter tip was in the CA1 nucleus and the longer one in nucleus CA3. (Position of the electrodes was confirmed histologically at autopsy.) The reference electrode a silver-silver chloride ball was placed epidurally on the frontal cortical area and a retaining screw was inserted on the skull bone controlaterally to the recording electrode. All the electrodes were soldered to a micro connector (Souriau) and the assembly was fixed on the skull with acrylic dental cement. On the day of the experiment the animals were lightly anaesthetized with halothane in a glass jar and were then put in a plastic box connected to a powerful aspirator. Air was bubbled through a halothane-filled Deschsel bottle and mixed with room air on a 1/10 ratio. The mixture was continuously delivered to the animal through a mask at the rate of 10 l/min. The micro-connector on the head of the animal was connected to a polygraph (Battaglia Rangoni) and to an IBM PS/2 computer through an A/D converter (Cambridge Electronic Design). Body temperature was kept close to 37°C with a heating lamp and an automatic temperature controller (YSI Co). The compounds were administered sc at various doses (5–10 animals per dose) 10 min after induction of anesthesia. Ten 5–s epochs each time were analyzed by fast Fourier transform (sampling frequency of 100 Hz). The power spectrum was evaluated between 0 and 25 Hz with a resolution of 0.2 Hz. The quantitative analyses of the EEG are expressed as % of power associated with the 3.8–5.8 Hz band (RSA) over the total power. The dose that induced about 30% synchronization of RSA was determined.

Scopolamine-induced amnesia

The effects of the compounds on scopolamine-induced amnesia for a passive avoidance response in mice was assessed by the previously described method [23]. Briefly, mice were trained to avoid a dark chamber in which they were punished by a single footshock. To produce amnesia for the avoidance task, mice were treated with scopolamine (0.7 mg/kg, ip) 15 min before training. Latency to reenter the shock box 24 h later was used as a measure of memory retention (cut-off time = 180 s). The animals (20–50 per group) were treated orally with 5–6 doses of the compounds, or with vehicle immediately after training. Results were expressed as percent increases in the retention latency in comparison to the corresponding controls, and the dose increasing retention performance by 50% was determined.

Acute toxicity

Mice and rats (4–6 per group) fasted 16 h were treated orally with various doses of the compounds and observed for 1 week after treatment; deaths were recorded daily. The approximate median lethal dose (LD_{50}) was then calculated.

Statistics

LD_{50}, ED_{30-100} and pD_2 values were calculated from the dose–response curves for the effects of the different doses against the logarithm of the doses by computer programs or graphically. Statistical significance was usually calculated by Student's *t*-test. Dunnet's *t*-test was used to evaluate the results in the scopolamine test.

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