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Short communication

1-Substituted-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-O-alkyloximes as novel orally active and long-lasting muscarinic cholinergic agonists

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Summary — Our previous attempts to design muscarinic agonists related to arecoline with the prerequisites for clinical use were successful with the discovery of 1,2,5,6-tetrahydropyridine-3-carboxaldehyde-O-methyloxime hydrochloride, RU 35963, and structurally related compounds, in which the metabolically labile ester group of arecoline was replaced with the bioisosteric and stable aldoxime group. With the aim of obtaining compounds with improved cholinomimetic properties, several aryl- and alkyl-carbamates of RU 35963, as well as O-alkyl-, and O-aryl-carbamates of the 1-hydroxy-1,2,5,6-tetrahydropyridine-3- carboxaldehyde-O-methyloxime hydrochloride, **24**, have been synthesized and evaluated biologically. The most interesting molecules to emerge from the primary screening have been evaluated more extensively and their cholinomimetic profiles compared with those of the parent molecules. *In vitro* studies indicate that none of these prodrugs have affinity for muscarinic receptor sites and some of them (aryl-carbamates) have cholinesterase inhibiting properties. Results from *in vivo* experiments in mice and rats showed that these new substances, 1-[4-chlorophenyl] oxycarbonyl-1,2,5,6-tetrahydropyridine-3- carboxaldehyde-O-methyloxime (**16** = RU 47213) in particular, have cholinomimetic properties that compare favourably with those of the parent compounds. After oral administration **16** was clearly superior to RU 35963 in terms of central selectivity, duration of action and therapeutic indexes.

tetrahydropyridine aldoxime / prodrug / RU 47213 / muscarinic receptor agonist / cholinomimetic profile / Alzheimer's disease

Introduction

Cholinergic mechanisms are known to play important roles in memory and other aspects of behaviour, and impairment of central cholinergic neurotransmission appears to be responsible for loss of memory and other cognitive dysfunctions in Alzheimer's disease (AD). This cholinergic hypothesis has led to the belief that enhancement of central cholinergic transmission by muscarinic agonists should be beneficial for treatment of the intellectual decline associated with AD [1–5]. Obviously, the major requirements for such therapy are long-lasting, steady–state drug effects and low levels of side-effects.

Our program to search for arecoline derivatives with the prerequisites for clinical use has led to the development of several new promising muscarinic agonists with potent oral activity and with a duration of action longer than that of the parent molecule [6–8]. One of the most promising compounds, 1,2,5,6-tetrahydropyridine-3-carboxaldehyde-O-methyloxime hydrochloride, RU 35963, whose cholinomimetic profile compares favourably with those of other muscarinic drugs, such as RS-86, *O-N*-dimethyl-THPO, isoarecoline, aceclidine and oxotremorine, is a potential candidate for clinical studies in AD patients [9].

In the search for new compounds with even better pharmacological properties we synthesized and biologically evaluated several prodrugs of RU 35963 and of its 1-hydroxy derivative [10, 11]. The most promising molecules to emerge from the primary screening were evaluated more extensively and their cholinomimetic profiles compared with those of the parent molecules.

Chemistry

Carbamates **1–23** (table I) were prepared in high yield by acylation of RU 35963 and RU 47029 with various chloroformates and triethylamine in dichloromethane or with 4-nitrophenylcarbonates (**11** and **12**) or with di-tert-butyl dicarbonate (**7**) *(Method A)*.

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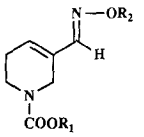
Table I. Physical properties and *in vivo* activity of 1-alkyl (or 1-aryl) oxycarbonyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-alkyloximes.



No	R_1	R_2	*Formula*[a]	*mp°C recrystn solvent*[b]	*bp °C/mbar*[c]	*Method*	*Yield*[d] *%*	LD_{50} ≈ *mg/kg po*	*Hypothermia*[e] *mg/kg po*	*sc*	*Diarrhoea*[f] *mg/kg po*
1	CH_3	CH_3	$C_9H_{14}N_2O_3$	37–38	140/0.05	A	90	200	20	20	> 50
2	C_2H_5	CH_3	$C_{10}H_{16}N_2O_3$		150/0.05	A; B	94; 45	175	3.9	3.6	25
3	C_2H_5	H_2C-C≡CH	$C_{12}H_{16}N_2O_3$	55–56 A	–	A	82	125	5.0	4.8	13
4	$(CH_2)_2CH_3$	CH_3	$C_{11}H_{18}N_2O_3$		170/0.07	A	95	175	4.4	4.5	30
5	$CH(CH_3)_2$	CH_3	$C_{11}H_{18}N_2O_3$	40–41	180/0.08	A	60	250	11	≈ 12	> 50
6	$(CH_2)_3CH_3$	CH_3	$C_{12}H_{20}N_2O_3$		220/0.08	A	72	125	8	8	30
7	$C(CH_3)_3$	CH_3	$C_{12}H_{20}N_2O_3$		150/0.07	A	86	> 500	> 50	> 50	> 50
8	$CH_2CH{=}CH_2$	CH_3	$C_{11}H_{16}N_2O_3$		200/0.08	A	94	175	1.6	1.8	3
9	$CH_2C_6H_5$	CH_3	$C_{15}H_{18}N_2O_3$		230/0.06	A; B	72; 43	400	30	25	25
10	$(CH_2)_2C_6H_5$	CH_3	$C_{16}H_{20}N_2O_3$		250/0.07	A	76	250	17	16	> 50
11	$(CH_2)_2SO_2CH_3$	CH_3	$C_{11}H_{18}N_2O_5S$	93–94 A	–	A	51	> 500	21	25	100
12	$(CH_2)_2SiMe_3$	CH_3	$C_{13}H_{24}N_2O_3Si$		220/0.08	A	76	> 500	≈ 25	≈ 50	> 50
13	adamantyl	CH_3	$C_{18}H_{26}N_2O_3$	105–106 B	–	A	76	> 500	> 50	> 50	> 50
14	C_6H_5	CH_3	$C_{14}H_{16}N_2O_3$		220/0.06	B	47	> 500	4.6	2.3	100
15	4-FC_6H_4	CH_3	$C_{14}H_{15}FN_2O_3$	100–101 A	–	A	96	500	3.2	1.5	> 50
16	4-ClC_6H_4	CH_3	$C_{14}H_{15}ClN_2O_3$	106–108 C	–	A; B	84; 82	> 1000	2.2	2.9	> 50
17	4-ClC_6H_4	H_2C-C≡CH	$C_{16}H_{15}ClN_2O_3$	91–93 C	–	A	84	> 500	> 50	> 50	> 50
18	4-BrC_6H_4	CH_3	$C_{14}H_{15}BrN_2O_3$	93–94 A	–	A	92	> 500	1.7	1.3	> 50
19	4-$NO_2C_6H_4$	CH_3	$C_{14}H_{15}N_3O_5$	109–110 A	–	A	95	500	4.0	5.0	> 50
20	4-$CH_3C_6H_4$	CH_3	$C_{15}H_{18}N_2O_3$	70–71 A	–	A	91	250	1.5	1.2	> 50
21	4-$CH(CH_3)_2C_6H_4$	CH_3	$C_{17}H_{22}N_2O_3$	103–105 C	–	A	77	> 1000	3.4	8.8	> 50
22	4-$C(CH_3)_2CH_2C(CH_3)_3C_6H_4$	CH_3	$C_{22}H_{32}N_2O_3$	91–93 D	–	A	94	> 500	6.9	15	> 50
23	3,4-$(OCH_3)_2C_6H_3$	CH_3	$C_{16}H_{20}N_2O_5$	143–145 A	–	A	68	175	0.73	0.74	> 50

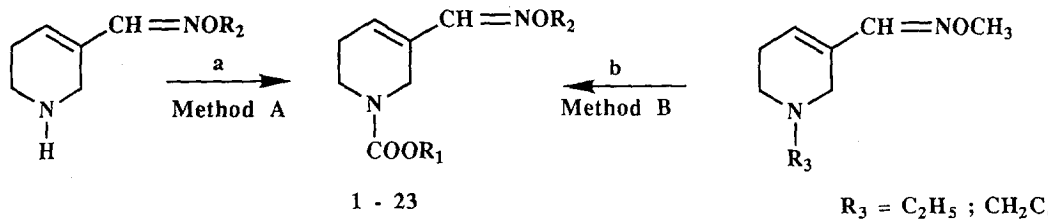
[a]C, H, N analyses were within ± 0.4% of the calculated values except for 22: C calculated 70.94; found 71.62; [b]A: benzene-cyclohexane; B: hexane; C: cyclohexane; D: ethanol-water; when the solvent is not shown, the oily substance obtained crystallized on standing; [c]distillations were carried out in a Kugelrohr apparatus; [d]yields are referred to distilled or recrystallized compounds and were not optimized except for **16**; [e]dose lowering rectal temperature by 1°C after *po* or sc administration to mice; [f]dose causing diarrhoea in 50% of the animals (mice).

RU 35963, however, was best prepared by N-dealkylation of the corresponding 1-ethyl or 1-benzyl derivatives by means of 1-chloroethyl chloroformate [8]. Thus, in some cases it was more expedient to directly prepare final carbamates by cleavage of ethyl or benzyl groups with the required chloroformates in dichloroethane at reflux *(Method B)*, although the overall yields were generally lower than with *Method A*.

Reduction of the two pyridine-3-aldoxime-*N*-oxides with sodium borohydride in methanol gave the corresponding 1-hydroxy-1,2,5,6-tetrahydropyridines **24** and **25** (scheme 2). To our knowledge, the preparation of 1-hydroxy-1,2,5,6-tetrahydropyridines by reduction of pyridine-*N*-oxides with sodium borohydride has never been reported previously.

O-acylation was easily effected with various acyl chlorides and triethylamine in tetrahydrofuran to yield **26–29** (table II). Aminocarbonyloxy derivatives (**30–43**) (table II) were finally obtained by condensation with alkyl and aryl isocianates in toluene.

As for the determination of oxime configuration, we have previously reported [8] that RU 35963 and RU 47029 were obtained as pure E isomers. Similarly, **24** and **25**, in the NMR spectrum run in DMSO-d_6, showed one singlet attributable to the proton of the

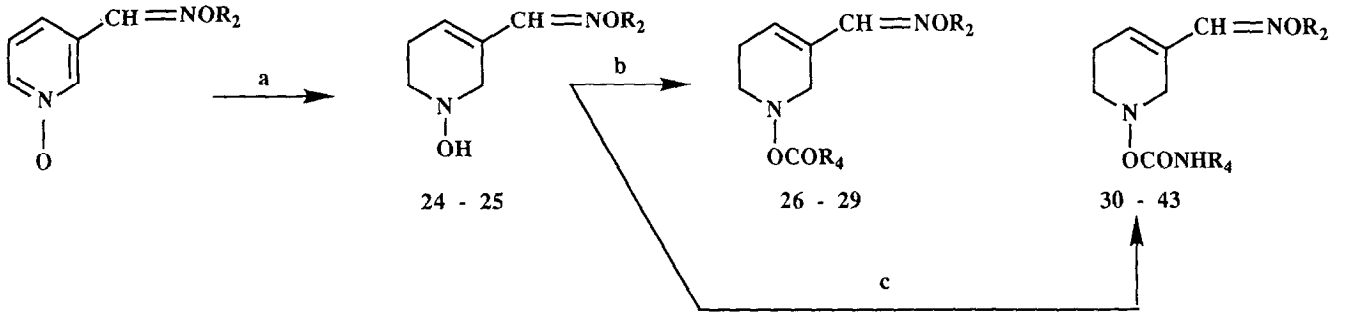


RU 35 963 ($R_2 = CH_3$) ;

RU 47 029 ($R_2 = CH_2—C\equiv CH$)

Reagents a) R_1OCOX - TEA - CH_2Cl_2 ; X = Cl ; F ; $OC_6H_4NO_2$; $OCOOR_1$

b) R_1OCOCl - CH_2ClCH_2Cl -∇



$R_2 = CH_3$; $CH_2—C\equiv CH$

R_4 = Alkyl ; Aryl ; Alkoxy

Reagents a) $NaBH_4$-MeOH ; b) R_4COCl-TEA-THF ; c) R_4NCO-toluene

Schemes 1 and **2.**

oxime group at 7.76 ppm in accordance with previous findings. As a consequence, all carbamates **1–23** and all *N*-hydroxy derivatives **26–43** are to be considered as pure E isomers.

Pharmacological results and discussion

The compounds listed in tables I and II were submitted to a battery of primary screening tests to assess their affinity and selectivity for muscarinic receptors, their anticholinesterase activity and their acute toxicity in mice. Moreover, their effects on the central (hypothermia) and peripheral (diarrhoea) cholinergic systems were investigated after oral and parenteral administration to mice.

All the tested compounds had no or negligible affinity for muscarinic receptors labelled either with [^{3}H]PZ or [^{3}H]QNB, and showed little (compounds **2**, **26**, **27** and **29**) or no agonistic activity in the isolated guinea-pig ileum. In this latter assay the 1-hydroxy derivatives **24** ($pD_2 = 5.38$) and **25** ($pD_2 = 5.24$) were found to be respectively 1/16 and 1/22 as potent as the corresponding 1-unsubstituted derivatives ($pD_2 = 6.58$ for both compounds) [8]. This can be imputed either to the electronic disturbance around the ring nitrogen or to the steric hindrance of the attached groups that hamper the ligand-receptor interaction. Actually, as shown by us and by others, only hydrogen or methyl on the nitrogen of the tetrahydropyridine ring confer potent agonistic properties to the molecule [8, 12].

The *in vitro* data also show that some of the compounds have cholinesterase-inhibiting properties. These properties, already described in the literature for other carbamates [15, 16], can be ascribed to this moiety, since the parent molecule, bearing either hydrogen (RU 35963) or hydroxy (**24**) in position 1, are devoid of anticholinesterase activity [8]. All the alkyl carbamates tested had no cholinesterase-inhibiting properties, whereas most of the aryl-carbamates were endowed with this property. Compound **14** was 1/10 as effective as the corresponding 1-phenylamino-carbonyloxy derivative, **36**. In both series the potency was unchanged or reduced by substitutions at the phenyl ring. The most potent compounds were **36**, **37**, **38**, **40** and **42**, which as *in vitro* cholinesterase inhibitors were 1/3–1/5 as active as physostigmine. However, the physiological significance of this activity is at present unknown. Preliminary results in mice treated orally with a highly effective cholinergic dose of **38** and **16** (10 mg/kg) or intravenously with 10 mg/kg of **16** revealed no effect on serum or brain cholinesterase activity. In the same assay, dose- and time- related inhibition of serum and brain cholinesterase activity was obtained with 0.25–0.50 mg/kg, sc, of physostigmine salicylate (Roussel Pharma files).

The *in vivo* results in tables I and II show that, when given *po* or sc, most of the tested prodrugs induce a central muscarinic effect such as hypothermia; depending on the substituent in position 1 the effective doses vary from a few hundred μg/kg to more than 50 mg/kg. The *N*-hydroxy *O*-methyloxime **24** and the *N*-hydroxy *O*-propargyloxime **25** have a cholinergic potency comparable to that of the corresponding N-H derivatives [8]. When the doses inducing hypothermia are compared with those that induce diarrhoea, it appears that several of the new compounds have relatively low diarrheogenic properties. It is interesting to note that the separation between central and peripheral effect is mostly apparent in the first series of carbamates (table I). The acute toxicity data in mice confirm our previous findings indicating that there is no correlation between cholinergic doses and LD_{50} values [8]. Among the tested compounds the para-chloro phenyl derivatives **16** and **38** had the highest therapeutic indices.

Compounds **2**, **4**, **14**, **16**, **21**, **23**, **33** and **38** were further evaluated in our battery of secondary assays. Studies of typical central (hypothermia, tremors and analgesia) and peripheral (diarrhoea, lacrimation and salivation) cholinergic symptoms, imputable to stimulation of muscarinic receptors [17, 18] were carried out in rats and mice. Additional evidence concerning their central muscarinic action was obtained by measuring the scopolamine-sensitive hippocampal rhythmical slow-wave activity in the halothane-anesthetized rat, and by evaluating their ability to reverse scopolamine-induced memory deficit in a one-trial passive avoidance response in mice.

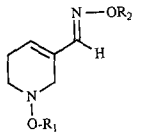
The results obtained with the selected compounds in the primary and secondary assays are summarized in table III, and compared with those of the parent molecules RU 35963 and **24**.

When given orally or parenterally all the selected carbamates exhibited the same cholinergic effects induced by RU 35963 and **24**, indicating that in the body they are converted to the biologically active parent compound(s). However, some important differences were found. All of them were somewhat less potent than the parent molecules, suggesting that only a fraction of the given dose underwent biotransformation; incomplete absorption seems unlikely, since there were no substantial differences of activity after oral or parenteral administration. The prodrugs showed less pronounced peripheral effects than those of the parent compounds, especially the diarrhoeagenic effect. In fact, as opposed to RU 35963 and **24**, all carbamates induced diarrhoea at doses higher or much higher than those required to induce other parasympathomimetic symptoms. The duration of their central action, estimated by the time-course of equipotent hypothermic doses [8], was comparable to or longer than those of the parent compounds. Compounds **14**, **16**, **21**, **23** and **38** had the longest-lasting effects. Dramatic differences in acute toxicity were found, with resulting therapeutic indexes sometimes more, sometimes less favourable than those of RU 35963 and **24**.

The different activity profiles observed *in vivo* can be ascribed to variations of the physicochemical properties induced by the chemical modifications of the parent molecule, and presumably reflect changes in the rate of absorption or biotransformation, and/or changes in distribution or enzymatic metabolism in target tissues. Obviously, full metabolic and pharmacokinetic studies will be necessary in order to clarify the mechanism(s) involved.

Among the selected products, **16** seems to be one of the most promising. Although 1/20–1/40 (1/10–1/20 as active principle) as potent as RU 35963 in producing central muscarinic effects, **16** showed some superiority over the parent drug in terms of central selectivity and duration of action. Thus, **16** induced overt central effects such as hypothermia, tremors and analgesia at doses lower than those required to induce peripheral cholinergic signs such as salivation, lacrimation and diarrhoea (2–10 mg/kg, *po vs* 12–> 50 mg/kg, *po*). The corresponding values for RU 35963 were 0.11–0.25 *vs* 0.15–0.30 mg/kg, *po*. The central action of **16** was longer (mice) or considerably longer (rat) than that of the parent compound. After oral administration of a dose able to

Table II. Physical properties and *in vivo* activity of 1-hydroxy-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-alkyloximes and derivatives: 1-acyloxy; 1-alkoxycarbonyloxy; 1-alkyl (or 1-aryl)aminocarbonyloxy.



No	R_1	R_2	*Formula*[a]	*mp°C recrystn solvent*[b]	*Yield*[c] *%*	$LD_{50}\approx$ *mg/kg po*	*Hypothermia*[d] *mg/kg po*	*sc*	*Diarrhoea*[e] *mg/kg po*
24	H	CH_3	$C_7H_{12}N_2O_2HCl$	146–147 A	58	60	0.19	0.20	0.5
25	H	H_2C-C≡CH	$C_9H_{12}N_2O_2HCl$	132–135 B	48	80	0.48	0.49	0.9
26	$COCH_3$	CH_3	$C_9H_{14}N_2O_3$	69–70 D	79	100	0.22	0.20	0.5
27	$COC(CH_3)_3$	CH_3	$C_{12}H_{20}N_2O_3$	oil	52	125	0.34	0.29	1.0
28	COC_6H_5	CH_3	$C_{14}H_{16}N_2O_3$	74–76 E	61	90	0.42	0.21	1.5
29	$COOC_2H_5$	CH_3	$C_{10}H_{16}N_2O_4$	oil	73	125	0.26	0.24	0.6
30	$CONHC_2H_5$	CH_3	$C_{10}H_{17}N_3O_3$	127–128 F	52	300	2.0	1.3	15
31	$CONH(CH_2)_2CH_3$	CH_3	$C_{11}H_{19}N_3O_3$	110–111 E	88	125	1.4	1.3	20
32	$CONHCH(CH_3)_2$	CH_3	$C_{11}H_{19}N_3O_3$	96–98 E	70	350	2.3	2.5	25
33	$CONH(CH_2)_3CH_3$	CH_3	$C_{12}H_{21}N_3O_3$	117–118 G	58	700	2.0	1.2	25
34	$CONHCH(CH_3)C_6H_5$[f]	CH_3	$C_{16}H_{21}N_3O_3$	115–116 g	87	500	5.3	17	30
35	$CONHCH(CH_3)C_6H_5$[h]	CH_3	$C_{16}H_{21}N_3O_3$	115–116 g	81	350	6.0	12	25
36	$CONHC_6H_5$	CH_3	$C_{14}H_{17}N_3O_3$	76–78 H	80	250	0.74	0.43	5
37	CONH-3-ClC_6H_4	CH_3	$C_{14}H_{16}ClN_3O_3$	99–100 H	90	250	2.0	0.95	5
38	CONH-4-ClC_6H_4	CH_3	$C_{14}H_{16}ClN_3O_3$	142–144 H	96	> 1000	1.8	1.8	10
39	CONH-4-ClC_6H_4	H_2C-C≡CH	$C_{16}H_{16}ClN_3O_3$	81–83 H	71	150	4.8	2.5	15
40	CONH-3,4-$Cl_2C_6H_3$	CH_3	$C_{14}H_{15}Cl_2N_3O_3$	117–118 H	91	500	4.3	3.1	16
41	CONH-4-$CH(CH_3)_2C_6H_4$	CH_3	$C_{17}H_{23}N_3O_3$	115–117 E	88	350	1.9	2.4	20
42	CONH-3-$OCH_3C_6H_4$	CH_3	$C_{15}H_{19}N_3O_4$	92–93 E	92	350	1.4	0.49	5
43	CONH-4-$OCH_3C_6H_4$	CH_3	$C_{15}H_{19}N_3O_4$	115–116 I	72	60	0.88	0.78	6

[a]C, H, N analyses were within ± 0.4% of the calculated values; [b]A: i-PrOH-Et_2O; B: MeOH-Et_2O; C: i-PrOH; D: hexane; E: cyclohexane; F: benzene; G: EtOAc; H: benzene-cyclohexane; I: Me_2CO-H_2O; [c]yields are referred to recrystallized compounds and were not optimized; [d]dose lowering rectal temperature by 1°C after *po* or sc administration to mice; [e]dose causing diarrhoea in 50% of the animals (mice); [f]R-configuration; [g]the two compounds were triturated with diethyl ether; [h]S-configuration.

decrease body temperature by 1.3–1.5°C its hypothermic effect peaked after 1 or 3 h, and lasted up to 2 or 5 h in mice and rats; the corresponding figures for RU 35963 were 0.5 or 1 h and 1 or 2 h.

In mice made amnesic by scopolamine, **16** showed interesting pro-mnesic properties. It significantly reversed scopolamine-induced impairment of a passive avoidance response over a wide range of doses (0.01–1 mg/kg, *po*); the minimal effective dose was 0.01 mg/kg, the optimal retention test performance was obtained with 0.1 mg/kg. As expected [8], the effective anti-amnesic doses were much lower than those inducing overt cholinergic symptoms.

Finally, **16** exhibited very low acute toxicity (LD_{50} > 2000 mg/kg, *po*, in mice, > 1000 mg/kg, *po*, in rats) and therapeutic indices even higher than those of the parent drug. For example, the ratios between LD_{50} and ED_{50} in the scopolamine test in mice were 70 000 for RU 35963 and more than 200 000 for **16**.

In conclusion, the pro-drug approach applied to 1,2,5,6-tetrahydropyridine-3-aldoximes has led to compounds with greater separation between central and peripheral effects, longer duration of action and higher therapeutic indices compared to the first series of cholinergic agents [8]. Compound **16** (RU 47213) has been the most extensively studied and is proposed for clinical studies in AD patients.

Experimental protocols

Chemistry

Melting points were determined on a Buchi 510 melting point apparatus and are uncorrected. IR spectra were recorded with a

Table III. *In vivo* pharmacological profile of selected compounds in comparison with RU 35963.

Test	*Species*	*Route*	*Effective equipotent doses (mg/kg) Compounds*									
			2	**4**	**14**	**16**	**21**	**23**	**24**	**33**	**38**	RU 35963
Central effects												
Hypothermia[a]	Mouse	*po*	3.9	4.4	4.5	2.2	3.4	0.73	0.19	2.0	1.8	0.11
	Mouse	sc	3.6	4.5	2.2	2.4	8.8	0.74	0.20	1.2	1.8	0.12
	Rat	*po*	5.0	nd[g]	nd	2.5	2.5	nd	0.22	nd	4.0	0.11
Tremors[b]	Mouse	*po*	4.0	7.0	5.0	8.0	8.0	2.5	0.20	4.0	4.0	0.25
Analgesia[c]	Mouse	*po*	5.0	nd	nd	10	10	nd	0.30	nd	3.5	0.25
Hippocampal RSA[d]	Rat	*po*	1.5	> 1	1.0	1.0	0.5	0.5	0.08	0.4	1.0	0.05
Anti-amnesic[e]	Mouse	*po*	0.030	0.200	0.100	0.010	0.020	0.015	0.0025	0.070	0.050	0.001
Peripheral effects												
Diarrhoea[b]	Mouse	*po*	25	30	100	> 50	> 50	> 50	0.50	25	10	0.15
	Rat	*po*	15	nd	nd	25	12	nd	0.30	nd	10	0.23
Salivation[b]	Mouse	*po*	5.0	8.0	7.0	12	10	2.0	0.30	5.0	4.0	0.25
Lacrimation[b]	Mouse	*po*	7.0	10	15	20	18	5.0	0.35	8.0	6.0	0.30
Acute toxicity												
LD_{50}	Mouse	*po*	175	175	> 500	> 2000	> 2000	175	60	700	1500	70
LD_{50}	Rat	*po*	200	nd	nd	> 1000	> 1000	nd	20	nd	500	12

[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 induces 30% synchronization of the rhythmical slow wave activity (RSA) in the hippocampus of the halothane-anesthetized rat; [e]dose that increases retention performance by 50%; [g]nd = not determined.

Perkin-Elmer model 881 spectrophotometer. NMR spectra were determined on Varian T-60 or Varian VXR-200/s spectrometers; chemical shifts are reported in δ 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 90 (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. The following reagents were not commercially available and were prepared according to the cited references: 2-phenylethylchloroformate [13]; 4-isopropylphenylchloroformate [14].

3,4-Dimethoxyphenylchloroformate

3,4-Dimethoxyphenol (1 g, 6 mmol) was added in portions to a stirred solution of phosgene (6 g, 60 mmol) in benzene (25 ml) at 20°C, followed by the dropwise addition of a solution of DBU: 1,8-diazabicyclo [5.4.0] undec-7-ene (1 ml, 6 mmol) in benzene (10 ml) and stirring was continued for an additional 45 min. The yellow solution was decanted, the solvent was evaporated and the oil was distilled in a Kugelrohr apparatus at 200°C, 0.08 mbar to yield 1.1 g (84%) of the chloroformate as a colourless oil. Anal $C_9H_9ClO_4$ (C, H).

1-Alkyl (or 1-Aryl) oxycarbonyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-O-alkyloximes ***1–23***

Method A. A solution of the appropriate chloroformate (or carbonate in a few cases indicated below) (10.3 mmol) in dichloromethane (15 ml) was added under nitrogen at 0°C to a stirred solution of 1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-alkyloxime [8] (9.3 mmol) and triethylamine (1.3 ml, 9.3 mmol) in dichloromethane (20 ml). The reaction mixture was stirred at room temperature for 30–60 min until disappearance of the starting compound on TLC (silicagel toluene-ethyl acetate 1:1). The solvent was evaporated and the residue was partitioned between water and ethyl acetate. The organic layer was dried (Na_2SO_4) and evaporated. The residue was either distilled in a Kugelrohr apparatus or recrystallized as shown in table I. Di-tert-butyl dicarbonate was used in the preparation of **7**; 2-(methylsulfonyl) ethyl-4-nitrophenyl carbonate in the preparation of **11**; 2-(trimethylsilyl) ethyl-4-nitrophenyl carbonate in the preparation of **12** and adamantyl fluoroformate in the preparation of **13**.

Method B. A solution of 1-ethyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-methyloxime [8] (15.6 mmol) in 1,2-dichloroethane (30 ml) was treated with the appropriate chloroformate (21 mmol) and heated at reflux for 90 min. The reaction was cooled, washed with 5% HCl (20 ml) to remove unreacted material, then with water (2 x 25 ml). The organic phase was dried (Na_2SO_4) and evaporated. The residue was distilled in a Kugelrohr apparatus.

The use of 1-benzyl derivative as the starting material gave comparable results.

Infrared and ^{1}H-NMR data are reported for **1** and **14** chosen as examples of *O*-alkyl and *O*-arylcarbamates respectively. **1**: IR (nujol): 1714 cm^{-1} (NCOO). ^{1}H-NMR ($CDCl_3$): 2.10–2.43 (2H, m, 5-CH_2), 3.53 (2H, t, J = 6, 6-CH_2), 3.66 (3H, s, $COOCH_3$), 3.80 (3H, s, $NOCH_3$), 4.12 (2H, d, J = 2, 2-CH_2), 5.85–6.06 (1H, m, 4-CH), 7.46 (1H, s, CH=N). **14**: IR (nujol):

1720 cm^{-1} (NCOO). ^{1}H-NMR ($CDCl_3$): 2.16–2.53 (2H, m, 5-CH_2), 3.63 (2H, t, J = 6, 6-CH_2), 3.80 (3H, s, $NOCH_3$), 4.30 (2H, br s, 2-CH_2), 5.90–6.13 (1H, m, 4-CH), 6.86–7.33 (5H, m, C_6H_5), 7.50 (1H, s, CH=N).

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

Sodium borohydride (0.9 g, 24 mmol) was added to a stirred solution of pyridine-3-carboxaldehyde-*O*-methyloxime-*N*-oxide [19] (1.2 g, 8 mmol) in methanol (40 ml) at – 10°C. The cooling bath was removed and the reaction was stirred at room temperature for 1 h. The solvent was distilled under reduced pressure and the residue was partitioned between water and diethyl ether. The residual oil obtained after evaporation of the organic phase was chromatographed on a silica gel column eluted with ethyl acetate to give 0.87 g of a pale yellow oil. The hydrochloride was prepared in Et_2O with HCl gas and recrystallized to yield 0.9 g of a white powder (table II). Anal $C_7H_{12}N_2O_2$•HCl (C,H,N). ^{1}H-NMR (DMSO–d_6): 2.40–2.76 (2H, m, 5-CH_2), 3.46 (2H, t, J = 6, 6-CH_2), 3.75 (3H, s, $NOCH_3$), 3.96 (2H, d, J = 4, 2-CH_2), 6.13–6.36 (1H, m, 4-CH), 7.76 (1H, s, CH=N), 11.2–11.90 (2H, br b, exch with D_2O).

Pyridine-3-carboxaldehyde-O-2-propynyloxime-N-oxide

3-Pyridinecarboxaldehyde (10 ml, 106 mmol) was dissolved in 2 N HCl (68 ml). 2-propynyloxylamine hydrochloride [20] (11.4 g, 106 mmol) was added in portions and the reaction was stirred at room temperature for 1 h. About 50 ml of water was distilled off at reduced pressure, the solution was saturated with solid K_2CO_3 and extracted with ethyl acetate. The organic phase was dried (Na_2SO_4) and evaporated. The reddish oil (16.6 g) was redissolved in 1,2-dichloroethane (160 ml) and oxidized at – 5°C by stirring with 3-chloroperoxybenzoic acid (85%, 21.5 g, 106 mmol) for 2 h. The solution was shaken with 5% $NaHCO_3$, and evaporated. The residue was purified by chromatography on a silica gel column eluted with ethyl acetate to give, after trituration with ethyl ether, 15.7 g (84%) of a white powder used as such in the next step. A sample recrystallized from EtOAc had mp 112–113°C. Anal $C_9H_8N_2O_2$ (C, H, N). ^{1}H-NMR (DMSO–d_6) 3.40–3.56 (1H, m, HC≡C), 4.76 (2H, d, J = 2, CH_2O), 7.13–7.60 and 8.0–8.3 (4H, 2m, aromatic), 8.13 (1H, s, CH=N).

1-Hydroxy-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-O-2-propynyloxime hydrochloride ***25***

Reduction of pyridine-3-carboxaldehyde-O-2-propynyloxime-N-oxide was carried out, as described under **24**, with sodium borohydride in methanol. The hydrochloride was a white, deliquescent powder. Anal $C_9H_{12}N_2O_2$•HCl (C, H, N). ^{1}H-NMR (DMSO–d_6): 2.40–2.73 (2H, m, 5-CH_2), 3.22–3.60 (3H, m, 6-CH_2 and HC≡C), 3.98 (2H, d, J = 3, 2-CH_2), 4.63 (2H, d, J = 1, CH_2O), 6.22–6.50 (1H, m, 4-CH), 7.76 (1H, s, CH=N), 11.0–12.40 (2H, br b, exch with D_2O).

1-Acetoxy-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-O-methyloxime ***26***

Acetyl chloride (1.7 ml, 24 mmol) was added dropwise into a stirred solution of the free base of **24** (3.8 g, 24 mmol) and triethylamine (3.4 ml, 24 mmol) in dry tetrahydrofuran (60 ml) at 5–10°C and stirring was continued at room temperature for 10 min. The solid precipitate was filtered off, the solvent was evaporated and the residue was chromatographed on a silica gel column eluted with ethyl acetate to give, after recrystallization, 3.8 g of **26** as a white powder. Anal $C_9H_{14}N_2O_3$ (C, H, N). IR (nujol): 1760 cm^{-1} (NOCO). ^{1}H-NMR ($CDCl_3$): 2.03 (3H, s, $OCOCH_3$), 2.23–2.70 (2H, m, 5-CH_2), 3.20 (2H, t, J = 6, 6-CH_2), 3.80 (5H, br s, $2CH_2$ and $NOCH_3$), 5.80–6.03 (1H, m, 4-CH), 7.46 (1H, s, CH=N).

1-Pivaloyloxy **27**, 1-benzoyloxy **28** and 1-ethoxycarbonyloxy-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-methyloxime **29** were similarly prepared by O-acylation with pivaloyl chloride, benzoyl chloride and ethyl chloroformate respectively.

1-Alkyl and 1-arylaminocarbonyloxy-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-O-alkyloximes ***30–43***

General procedure. A solution of the free base of **24** (2 g, 13 mmol) in toluene (20 ml) was treated at about 20°C with a double molar amount of the required alkyl or aryl isocyanate for 30–90 min and the course of the reaction was followed by means of TLC (silica gel-ethyl acetate). The solvent was evaporated at reduced pressure and the residue was recrystallized from the solvent shown in table II. Compound **39** was similarly obtained from the free base of **25**.

Infrared and ^{1}H-NMR data are reported for compounds **30** and **36** chosen as examples of *N*-alkyl and *N*-arylcarbamates respectively.

30: IR (nujol): 3310 (NH) and 1713 cm^{-1} (NCOO). ^{1}H-NMR ($CDCl_3$): 1.16 (3H, t, J = 7, CH_3C), 2.23–2.63 (2H, m, 5-CH_2), 3.13 (2H, t, J = 6, 6-CH_2), 3.16 (2H, q, CH_2N), 3.76 (5H, br s, 2-CH_2 and $NOCH_3$), 5.70–6.00 (1H, m, 4-CH), 6.26–6.60 (1H, br b, NH), 7.46 (1H, s, CH=N). **36**: IR (nujol): 3260 (NH) and 1713 cm^{-1} (NCOO). ^{1}H-NMR ($CDCl_3$): 2.23–2.66 (2H, m, 5-CH_2), 3.23 (2H, t, J = 6, 6-CH_2), 3.76 (5H, br s, 2-CH_2 and $NOCH_3$), 5.80–6.03 (1H, m, 4-CH), 6.86–7.40 (5H, m, C_6H_5), 7.46 (1H, s, CH=N), 8.26–8.60 (1H, br b, NH).

Pharmacology

Detailed descriptions of the *in vitro* and *in vivo* pharmacological methods used to assess the effects of the test compounds on central and peripheral cholinergic systems and to compare their cholinomimetic properties with those of the parent molecules can be found in a previous paper [8].

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 the Student's *t*-test. The Dunnet's *t*-test was used to evaluate the results in the scopolamine test.

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