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Short communication

Synthesis and pharmacological investigation of cholinergic ligands structurally related to muscarone*

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Summary — Five new analogs of muscarone were synthesized in order to evaluate the influence of the carbonyl group on muscarinic activity. We chose to introduce structural variations at the C-2 and C-3 positions of the tetrahydrofuran ring. The muscarinic activity was evaluated *in vitro* on guinea pig atria and ileum as well as on rat jejunum and urinary bladder. All the new derivatives are less potent than muscarone and three of them displayed a potency very close to that previously reported for muscarine. The tissue selectivity observed for the 3-methylene derivative which is eight times more potent on guinea pig ileum than atria is worth noting. The present data show the lack of a simple relationship between the polarity of the group located in the 3-position of the ring and the muscarinic activity.

Résumé — **Synthèse et investigation pharmacologique de ligands cholinergiques structuralement reliés à la muscarone.** *Cinq nouveaux analogues de la muscarone ont été synthétisés dans le but d'évaluer l'influence de son groupement carbonyle sur l'activité muscarinique. Nous avons choisi d'effectuer des modifications structurales au niveau des carbones 2 et 3 du noyau tétrahydrofuranne. L'activité muscarinique a été évaluée* in vitro *sur l'atrium et l'iléon du Cobaye ainsi que sur le jejunum et la vessie de Rat. Tous les nouveaux dérivés se sont avérés moins efficaces que la muscarone et trois d'entre eux ont fait preuve d'une activité tout à fait voisine de celle décrite antérieurement pour la muscarine. Il convient de noter la sélectivité tissulaire observée pour les dérivés à méthylène en 3 qui est huit fois plus actif sur l'iléon que sur l'atrium de Cobaye.*

Ces résultats montrent l'absence d'une relation simple entre la polarité du groupe situé en position 3 sur le noyau et l'activité muscarinique.

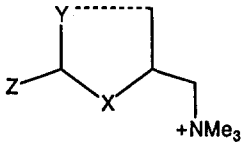
cholinergic ligands / muscarone analogs / tissue selectivity

Introduction

In the course of our studies on quantitative structure—activity relationships of muscarinic ligands, we have examined compounds possessing both rigid (*i.e.*, benzene, pyridine and furan derivatives) and flexible (*i.e.*, ethers and esters of choline) structures (see **1**, Scheme 1). We were able to demonstrate that all of the tested compounds bind in essentially one mode [1]. The picture derived from such an analysis is in agreement with the hypothesis that a maximum of activity is reached when the molecular component parts involved in the interaction with the receptor sites are: a trimethylammonium head, a terminal methyl group (Z) and an oxygen atom at X as depicted in Scheme 1.

One point which has been the subject of detailed study concerns the nature of the interaction involving substructure Y of **1**, *i.e.*, the carbonyl group of acetylcholine as well as the carbonyl group of muscarone **2**, the hydroxy group of muscarine **3** or the second heteroatom of dioxolane **4** and oxathiolane **5** in comparison to the methylene group of **6**.

The existence of a hydrogen bond between this part of



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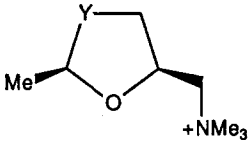

2 : Y = C=O
3 : Y = CHOH (*trans*)
4 : Y = O
5 : Y = S
6 : Y = CH_2

Scheme 1.

**We dedicate this paper to Professor Pietro Pratesi on the occasion of his 80th birthday.*
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the molecule and a donor group on the receptor site has been proposed by several authors [2—6] and was thought to explain the high potency observed in some of the above mentioned compounds.

Later on, this hypothesis was questioned by Elferink [7], who observed that oxathiolane **5**, despite the lower propensity of sulfur in comparison to oxygen to form a hydrogen bond, displayed a potency very close to that reported for a dioxolane **4**. In addition, a recent study [8] of the relationship between stereochemistry and enantioselectivity *versus* muscarinic activity shows for oxathiolane **5** a pharmacological profile similar to that of dioxolane **4** and of muscarine **3**.

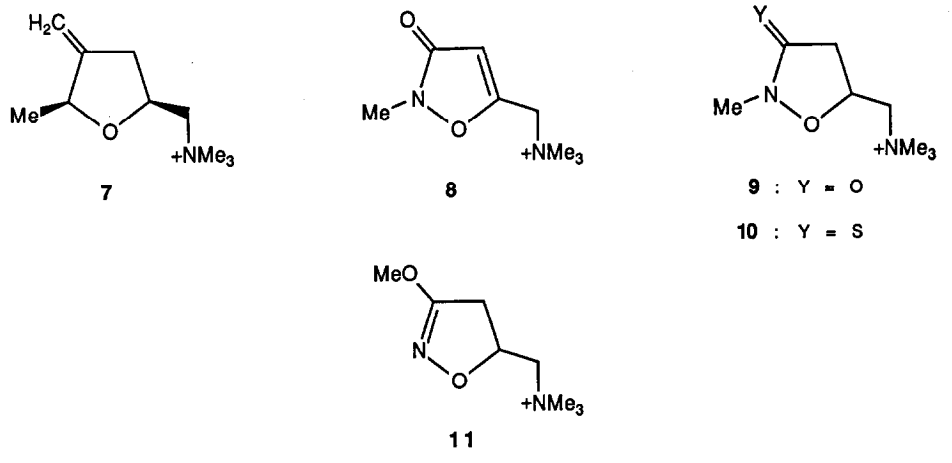
An alternative hypothesis [9, 10] considers a strong dipole or a polarizable moiety at the crucial position as the feature necessary to provide high activity.

Since muscarone is one of the most attracting muscarinic ligands due to its peculiar pharmacological profile [2—4, 11—14], we tested the above reported hypotheses by reducing and increasing the polarity of substructure Y (Scheme 1). As a matter of fact, we prepared derivative **7** (Scheme 2) which contains the low polar and bulky methylene group which, in addition, is unable to form a hydrogen bond. We also attempted the synthesis of the thio-carbonyl analogue, which, unfortunately, could not be isolated due to its high instability.

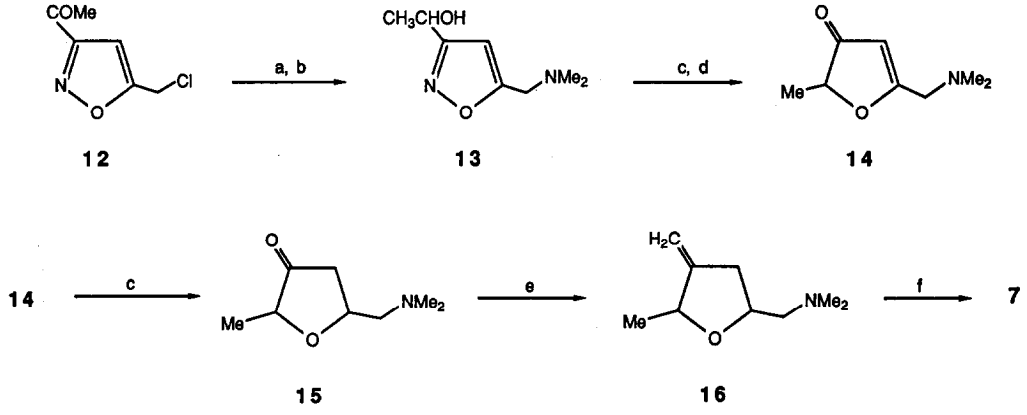
Compounds **8** and **9** were prepared in order to increase the polarity of the carbonyl group of muscarone; in the case of **9**, the polarity of this moiety was reduced by exchanging sulfur for oxygen (**10**). Finally compound **11** was prepared with the aim of testing the influence of the *N*-methyl group on the biological activity.

Chemistry

Compounds **7**, **8**, **9** and **11** were obtained *via* 1,3-dipolar cycloaddition of a nitrile oxide to the appropriate dipolarophile, whereas **10** was prepared from **21a**. Thus 3-acetyl-5-chloromethylisoxazole **12** was prepared following the methodology used in the synthesis of muscarone [15]: minor modifications increased the overall yield. The pyruvonitrile oxide was generated *in situ* and was reacted with commercially available propargyl chloride. The reduction of the carbonyl group of **12** prior to reaction with excess dimethylamine then gave **13** in 72% yield (Scheme 3).



Scheme 2.



Scheme 3. Reagents: a: $NaBH_4/EtOH$; b: $NHMe_2$; c: H_2-Pd/C; d: H^+; e: $CH_2{=}PPh_3$; f: CH_3I.

β-Furanone **14** was obtained by catalytic reduction (H_2, Pd/C) of **13** and subsequent cyclization under acidic conditions. A further catalytic hydrogenation (H_2, Pd/C) of **14** yielded crude nor-muscarone **15** which was contaminated by 23% of the *trans*-isomer; the two isomers could not be separated by column chromatography. The ratio between the two isomers was determined by ^1H NMR and was confirmed by capillary gas—liquid chromatography (GLC). The NMR spectrum of crude **15** showed the major differences between the two isomers to be the chemical shifts of protons H-2, H-5 and the methyl group. As a matter of fact, the *trans*-isomer showed H-2 and H-5 at lower fields (H-2: δ 4.06 *vs.* 3.82; H-5: δ 4.49 *vs.* 4.27), whereas the opposite was observed for the methyl group (δ 1.25 *vs.* 1.29).

Crude **15** was then subjected to a Wittig olefination under standard conditions and gave **16** in 50% yield. ^1H NMR spectrum of the reaction mixture showed the presence of two isomers in a ratio (78:22) very close to that observed in the starting material.

In analogy to **15**, the *cis*-structure of the major isomer of the reaction mixture was attributed by taking into account the differences in the chemical shift of protons H-2, H-5 and 2-Me. Also, in this case, the minor isomer of **16** (the *trans* isomer) showed protons H-2 and H-5 at lower field (H-2: δ 4.47 *vs.* 4.27; H-5: δ 4.17 *vs.* 3.96), whereas the opposite holds true for the methyl group (δ 1.21 *vs.* δ 1.25). The chemical shifts of the protons were assigned through decoupling experiments; in particular, by irradiating the multiplet at δ 4.27, we observed, in addition to the expected coupling with the 2-methyl group, long-range couplings with H-4 and H-4′. These hydrogens are also coupled with the two vinylic protons. It is worth pointing out that the relative chemical shifts of H-2 and H-5 in **15** and **16** are reversed (δ 3.82 and 4.27 *vs.* δ 4.27 and 3.96). A different conformation of the ring in the two compounds can account, at least in part, for the experimental observation.

By treating **16** with an excess of methyl iodide, compound **7** was obtained in quantitative yield and was purified from the *trans*-isomer by crsytallization.

The synthesis of derivatives **8**, **9** and **11** was achieved by using bromonitrile oxide [16, 17]. Thus, bromonitrile oxide reacted with propargyl chloride to produce **17** in 93% yield (Scheme 4). In turn **17** was reacted with an excess of trimethyloxonium tetrafluoroborate and the isoxazolium salt directly transformed, under basic conditions, into intermediate **18a**; the final derivative **8** was then prepared by standard reactions.

The same strategy was applied to the synthesis of **9**. Compound **20a** was prepared in good yield following the reaction sequence reported in Scheme 5, whereas **20b—d** were prepared from **20a** by standard reactions. When we tried to react the iodo-derivative **20c** or the corresponding mesylate **20b** with dimethylamine, we failed to obtain the desired product, possibly due to the lability of the heterocyclic ring under basic conditions. The same result was obtained by reacting **20c** with trimethylamine. The required tertiary amine **21a** was finally prepared by reacting trifluoromethanesulfonate **20d** with a stoichiometric amount of dimethylamine at low temperature (— 15°C). Treatment of **21a** with an excess of methyl iodide gave **9** as a slightly hygroscopic salt.

Tertiary amine **21a** was also reacted with the Lawesson's reagent and gave the corresponding thio-analogue **21b** in 62% yield which was then transformed into the ammonium salt **10**.

Finally, derivative **11** was prepared in 40.5% yield starting from **22** *via* the reaction sequence reported in Scheme 5.

Results and Discussion

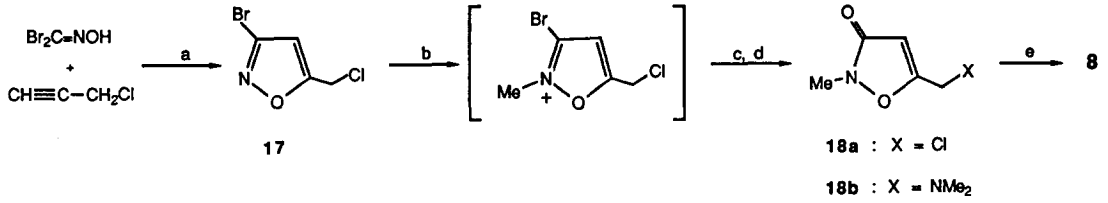
All the newly synthesized compounds (**7—11**) behaved as muscarinic agonists in the *in vitro* experiments.

The potencies for compounds **6** [18] and **7—11** were compared (Table I) with those of the previously reported compounds **2—5**. Compounds **6—11** show a slope of the dose—response curve as well as a maximum response similar to that of the reference compound muscarine **3**.

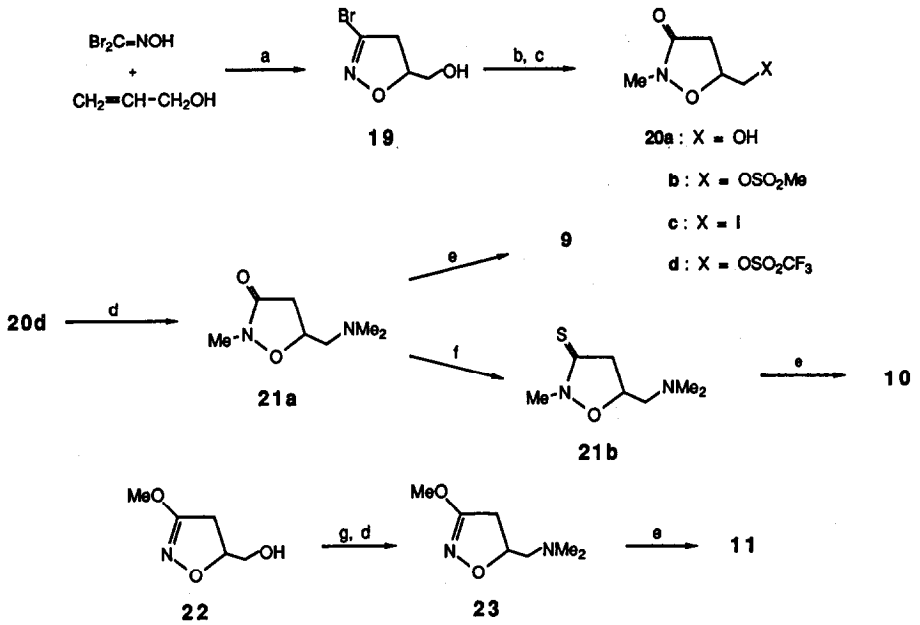
A better comparison between the activities of the various compounds can be made by considering the equipotent molar ratios *(EPMR)* relative to muscarone **2** (Table II).

By combining the horizontal and vertical reading of the data presented in Table II, it is possible to deduce the relative potencies of compounds **3—11** in the various tissues. In addition, the table reports the ileum/atria selectivities iu guinea pig are reported.

Due to the organ selectivity observed with some of the ligands reported in Table II, the pharmacological results can be better discussed by separating the data on smooth muscle organs from those on the cardiac tissue. In all smooth muscle preparations, muscarone is always the most active compound, followed by dioxolane **4** and oxathiolane **5** which, depending upon the organ considered, are 2—6 times less potent. The aza-derivatives **8** and **9** (3.5—12 times less potent than **2**) possess a potency in-between oxathiolane **5** and muscarine **3**. In addition, the potency of **9** is decreased 5—20 times when the oxygen of its carbonyl group is replaced by sulfur. The low activity displayed by **11** can be, at least



Scheme 4. Reagents: a: $NaHCO_3/CH_3COOEt$; b: $Me_3O^+BF^-_4/CH_3NO_2$; c: $NaHCO_3/H_2O$; d: $NHMe_2$; e: CH_3I.



Scheme 5. Reagents: a: $NaHCO_3/CH_3COOEt$; b: $Me_3O^+BF^-_4/MeNO_2$; c: $NaHCO_3/H_2O$; d: $NHMe_2$/MeOH at —15°C; e: MeI/acetone; f: Lawesson's reagent at reflux in toluene; g: triflic anhydride/Py from —78°C to RT.

Table I. Potencies of muscarinic agonists in guinea pig and rat tissues.[a]

Compd.	Guinea pig[b]		Rat[b]	
	atria (force)	ileum	jejunum	urinary bladder
2	8.62±0.06	9.00±0.07	7.89±0.03	7.28±0.05
3	7.23±0.07	7.50±0.05	6.81±0.04	6.24±0.05
4	8.02±0.04	8.38±0.06	7.58±0.11	6.91±0.05
5	7.37±0.03	8.23±0.06	7.55±0.03	6.64±0.04
6	5.88±0.06	6.87±0.08	6.19±0.05	5.62±0.03
7	6.10±0.08	7.38±0.08	6.78±0.03	6.13±0.03
8	7.87±0.12	7.92±0.08	7.06±0.06	6.74±0.04
9	7.62±0.05	7.95±0.03	7.13±0.03	6.44±0.03
10	6.36±0.05	6.74±0.06	5.80±0.09[c]	5.77±0.05[d]
11	4.81±0.04	5.49±0.09	4.36±0.03	4.03±0.03[e]

[a]The values for compounds **2**—**5** were taken from [11].
[b]Arithmetic means of —logs of the molar concentrations which give 50% of the maximum response (—log EC_{50})±SEM are reported; if not otherwise stated, the compounds possess intrinsic activity *(α)* equal to 1.
[c]$\alpha = 0.81$.
[d]$\alpha = 0.71$.
[e]$\alpha = 0.76$.

Table II. Equipotent molar ratios *(EPMR)* relative to muscarone **2.**

Compd.	Guinea pig		Selectivity ileum:atria	Rat	
	atria (force)	ileum		jejunum	urinary bladder
3	24.5	31.6	1:1	12.0	11.0
4	4.0	4.2	1:1	2.0	2.3
5	17.8	5.9	3:1	2.2	4.4
6	549.5	134.9	4:1	50.1	45.7
7	331.1	41.7	8:1	12.9	14.1
8	5.6	12.0	1:2	6.8	3.5
9	10.0	11.2	1:1	5.8	6.9
10	181.9	181.9	1:1	123.0	32.3
11	6456.5	3235.9	2:1	3388.4	1698.2

in part, related to the absence of the terminal methyl group in the appropriate position.

Interesting enough is the result obtained with the methylene derivative **7** which is 13—40 times less potent than **2** but is equipotent with muscarine **3** and definitely more active than desoxymuscarine **6**.

The structure—activity relationship among the homogeneous set of compounds **2—10** is not easily accounted for by current hypotheses. By comparing the potency of **7** with that of muscarine **3** and desoxymuscarine **6**, we must infer a productive contribution of the methylene group for muscarinic receptor interaction which cannot be explained by the formation of a hydrogen bond as proposed for muscarone, muscarine and dioxolane [2—6].

On the other hand, the hypothesis of a dipole—dipole interaction [9, 10] between the Y moiety (see **1**) and the corresponding receptor site can explain, at least in part, the decline in activity noted on passing from muscarone **2** to the methylene derivative **7** and from the aza-derivative **9** to its thio-analogue **10**. The remarkable activity of **7** which, in spite of the presence of a low polar and bulky substituent in such a crucial position, possesses a potency very close to that of muscarine and not far from that displayed by the more polar **8** and **9** remains rather inexplicable. The role played by the methylene moiety in **7** is not fully explained by the existence of a dipole—dipole interaction between this group and the complementary receptor site. Similar considerations can be derived from the data reported by Pigini *et al.* [19] for oxathiolane **5** and its more polar S-oxide *(trans)*.

These considerations are derived from the interaction ligand—muscarinic receptors in smooth muscle organs. Now, if we turn our attention to the ileum/atria potency ratios (Table II) and assume as a criterion for the existence of an organ selectivity a ratio higher than 4 [11], it becomes evident that **7** is the only selective compound. This finding is consistent with the proposed heterogeneity [20, 21] between atrial and smooth muscle muscarinic receptors. In any case, the significance of such an organ selectivity needs to be further explored by studying more appropriate pharmacodynamic parameters, such as dissociation constants and relative efficacies; these data will be reported in the near future.

In conclusion, the results presented in this paper indicate the lack of a simple relationship between the polar character of substructure Y (see **1**) and the muscarinic activity displayed by the corresponding ligand. In our opinion, more data on analogs of muscarone are necessary to solve the puzzling nature of its high activity.

In addition, the synthesis of the corresponding chiral derivatives has been undertaken in order to define the difference in the stereochemical requirements between muscarinic and nicotinic receptors as well as among the muscarinic M_2-subtypes.

Experimental protocols

Chemistry

Melting points were determined in capillary tubes and are uncorrected. Elemental analyses were performed on a Carlo Erba Elemental Analyzer model 1106 and agreed with theoretical values $\pm 0.4\%$. ^{1}H NMR spectra were recorded on Perkin—Elmer R-600FT and Varian XL-200 instruments. Chemical shifts are expressed in ppm (δ) relative to internal Me_4Si and the coupling constant in Hz. All NMR spectra were recorded in $CDCl_3$ unless otherwise stated. IR spectra were recorded with a Perkin—Elmer 1310 spectrometer as a film or in a KBr disc. Gas chromatographic analyses were carried out with a Carlo Erba HRGC 5160 Mega Series equipped with an SP-2140 (30 mt) capillary column. Merck silica gel 60 F_{254} analytical thin—layer chromatography plates were used throughout this work.

3-Acetyl-5-chloromethylisoxazole ***12***
A suspension of pyruvohydroximoyl chloride (12 g, 0.1 mol), propargyl chloride (18 ml, 0.25 mol) and $NaHCO_3$ (25 g, 0.3 mol) in ethyl acetate (100 ml) was stirred at room temperature (RT) overnight. The solids were filtered off and washed with ethyl acetate. The organic solvent was removed under vacuum and the residue distilled at reduced pressure with a Kugelrohr apparatus. bp: 150—152°C (furnace temp.)/18 mm Hg; yield 90%; NMR: 2.65 (s, 3, Me), 4.78 (s, 2, CH_2), 6.75 (s, 1, H-4).

3-(1-Hydroxyethyl)-5-dimethylaminomethylisoxazole ***13***
The methodologies previously reported [15] were used but we found it more convenient to reverse the reaction sequence: the carbonyl was reduced before the reaction with dimethylamine (overall yield: 72%).

cis-*2-Methyl-5-dimethylaminomethyl-tetrahydrofuran-3-one* ***15***
Compound **14** [15] (3.1 g, 0.02 mol) was hydrogenated at atmospheric pressure in the presence of 5% Pd/C; crude **15** was purified by column chromatography (eluent: chloroform—MeOH, 4:1) to give material (45% yield) which contained some of the *trans*-isomer (23%) as a by-product (200 MHz NMR) [22]. ^{1}H NMR: 1.29 (d, 3, Me; $J = 6.1$); 2.23 (m, 1, H-4a); 2.33 (s, 6, NMe_2); 2.35—2.70 (m, 3, H-4b and CH_2N); 3.82 (q, 1, H-2; $J = 6.1$); 4.27 (m, 1, H-5).

Principal ^{1}H NMR signals of the *trans*-isomer: 1.25 (d, 3, Me; $J = 6.1$); 4.06 (q, 1, H-2); 4.49 (m, 1, H-5).

cis-*2-Methyl-3-methylene-5-dimethylaminomethyl-tetrahydrofuran* ***16***
To a stirred suspension of methylenetriphenylphosphorane, generated from methyltriphenylphophonium bromide (1.8 g, 5 mmol) and potassium *t*-butoxyde (0.6 g, 4.8 mmol) in tetrahydrofuran (THF, 50 ml), **15** (0.5 g, 3.3 mmol) was added dropwise and stirring was continued at RT overnight. After the usual work-up, **16** was purified through column chromatography on silica gel (eluent: chloroform—MeOH, 10:1). Yield: 50%; IR(neat): ν 1650 (CH_2=C) cm^{-1}; NMR: 1.25 (d, 3, Me; $J = 6.3$); 2.21 (m, 1, H-4a; $J = J = 2.2$; $J = 7.2$; $J = 16.0$); 2.24 (s, 6, NMe_2); 2.34 (dd, 1, $CH_{2a}N$; $J = 4.3$; $J = 12.8$); 2.47 (dd, 1, $CH_{2b}N$; $J = 7.2$; $J = 12.8$); 2.59 (m, 1, H-4b; $J = J = 2.2$; $J = 6.1$; $J = 16.0$); 3.96 (dddd, 1, H-5; $J = 4.3$; $J = 7.2$; $J = 6.1$; $J = 7.2$); 4.27 (m, 1, H-2); 4.75 (ddd, 1, CH_2=; $J = J = J = 2.2$); 4.87 (ddd, 1, CH_2=; $J = J = J = 2.2$).

Principal ^{1}H NMR signals of the *trans*-isomer: 1.21 (d, 3, Me; $J = 6.0$); 4.17 (m, 1, H-5); 4.47 (m, 1, H-2); 4.79 (ddd, 1, CH_2=; $J = J = J = 2.2$); 4.91 (ddd, 1, CH_2=; $J = J = J = 2.2$).

cis-*2-Methyl-3-methylene-5-dimethylaminomethyl-tetrahydrofuran methiodide salt* ***7***
Methyl iodide (0.5 ml) was added to a solution of **16** (0.3 g) in acetone (3 ml). After standing overnight, the solution was treated with anhydrous ether. The precipitate was collected and recrystallized 3 times from acetone—ether. mp: 157—158°C. A 200 MHz NMR did not show the presence of any *trans*-isomer. Anal. $C_{10}H_{20}INO$: C, H, N.

3-Bromo-5-chloromethylisoxazole ***17***
A suspension of dibromoformaldoxime (2.5 g, 12.3 mmol), propargyl chloride (4.54 g, 61 mmol) and $NaHCO_3$ in ethyl acetate (50 ml) was stirred at RT until gas evolution ceased. The slurry was then poured into water and extracted with ether. The organic layer was dried over Na_2SO_4, the solvent was removed under vacuum and the residue was distilled in a Kugelrohr apparatus: bp: 125—130°C (furnace temp.)/20 mm Hg; yield: 95%; NMR: 4.65 (s, 2, CH_2Cl); 6.48 (s, 1, H-4). In additionthe NMR showed the presence (s at 8.50 δ) of the 4-substituted regioisomer (5%).

2-Methyl-5-chloromethyl-Δ^4-isoxazolin-3-one ***18a***
To a solution of **17** (1.25 g, 6.36 mmol) in nitromethane (10 ml), 1.9 g (12.7 mmol) of trimethyloxonium tetrafluoroborate was added at RT. The solution was stirred at RT until the disappearance of the starting material, then treated with 30 ml of a saturated solution of $NaHCO_3$. Compound **18a** was extracted with chloroform (3 × 20 ml) and purified by a column chromatography (silica gel, eluent: cyclohexane/ethyl acetate/methanol, 5:5:0.1); Yield 36%; NMR: 3.54 (s, 3, NMe), 4.20 and 4.50 (dd, 2H, CH_2Cl; J_{ab} = 16), 5.86 (s, 1, H-4).

2-Methyl-5-dimethylaminomethyl-Δ^4-isoxazolin-3-one methiodide salt ***8***
An excess of anhydrous dimethylamine was added to a chloroform solution (15 ml) of **18a** (1 g, 6.8 mmol). The container was sealed and left at RT until the disappearance of the starting material. After the usual work-up, the tertiary amine **18b** was purified by column chromatography on silica gel (eluent: cyclohexane/ethyl acetate/MeOH, 5:5:0.5) followed by vacuum distillation (80% yield): bp: 108—110°C/0.4 mm Hg; NMR: 2.27 (s, 6, NMe_2); 3.30 (s, 2, CH_2N); 3.40 (s, 3, NMe); 5.51 (s, 1, H-4).

A sample of **18b** was dissolved in anhydrous ether and treated with an excess of methyl iodide. The precipitate was recrystallized from abs. ethanol. mp: 198.5—202°C dec.; Anal. $C_8H_{15}IN_2O_2$: C, H, N.

3-Bromo-5-hydroxymethyl-Δ^2-isoxazoline ***19***
Starting from dibromoformaldoxime (3 g, 14.7 mmol), allylic alcohol (1.7 g, 29.7 mmol) and $NaHCO_3$ (6 g) and employing the methodology used for **17**, adduct **19** was prepared in 93% yield. Attempts to purify this compound through distillation resulted in extensive decomposition of the material. NMR: 3.28 (m, 2, H-4); 3.79 (t, 2, CH_2O); 3.95—4.20 (bs, 1, OH); 4.60—5.15 (m, 1, H-5).

2-Methyl-5-hydroxymethyl-isoxazolidin-3-one ***20a***
Intermediate **20a** was prepared similarly to **18a** employing 2.5 g (14 mmol) of **19** and 3.07 g (21 mmol) of trimethyloxonium tetrafluoroborate. After column chromatography (eluent: chloroform/MeOH, 9:1), **20a** was recovered as a viscous oil (81.5% yield). NMR: 2.82 (m, 2, H-4); 3.21 (s, 3, NMe); 3.80 (m, 2, CH_2O); 4.35—5.05 (m, 1, H-5).

2-Methyl-5-hydroxymethyl-isoxazolidin-3-one methanesulfonate ***20b***
Compound **20a** was reacted, under standard conditions, with methanesulfonyl chloride and pyridine to yield **20b** (92% yield). NMR: 2.30—3.0 (m, 2, H-4); 3.10 (s, 3, SO_2Me); 3.15 (s, 3, NMe); 4.40 (d, 2, CH_2O, J = 5.0); 5.05—6.10 (m, 1, H-5).

2-Methyl-5-iodomethyl-isoxazolidin-3-one ***20c***
Derivative **20b** (0.5 g) was refluxed with an excess of Na*J* (3 g) in acetone (25 ml) until the disappearance of the starting material. After the usual work-up, **20c** was obtained in quantitative yield as an oil: bp: 120—125°C/5 mm Hg. NMR: 2.50—3.05 (m, 2, H-4), 3.20 (s, 3, NMe); 3.35 (d, 2, CH_2J, J = 6.0), 4.30—4.90 (m, 1, H-5).

2-Methyl-5-hydroxymethyl-isoxazolidin-3-one trifluoromethanesulfonate ***20d***
To a cooled solution (— 78°C) of **20a** (1.49 g, 11.5 mmol) in anhydrous dichloromethane (50 ml) were added 1.86 ml (23 mmol) of dry pyridine followed by 1.9 ml (12 mmol) of trifluoromethanesulfonic anhydride. The reaction flask was removed from the cooling bath and the progress of the reaction monitored from time to time by TLC. After disappearance of the starting material, the solution was acidified with dil. HCl and the organic layer was extracted with ethyl acetate (3 × 20 ml) and dried over Na_2SO_4. **20c** was not further purified but was directly used in the following reaction. NMR: 2.35—3.05 (m, 2, H-4); 3.20 (s, 3, NMe); 4.28—5.20 (m, 3, CH_2O and H-5).

2-Methyl-5-dimethylaminomethyl-isoxazolidin-3-one ***21a***
Dimethylamine (0.88 ml, 13.3 mmol) was added to a methanolic solution (15 ml) of **20d** (3.5 g, 13.3 mmol) cooled at — 15°C. The reaction mixture was stirred at — 15°C overnight. The solvent was evaporated under vacuum and the residue was treated with water and was continuously extracted with dichloromethane. The crude product was purified by column chromatography (eluent: $CHCl_3$—MeOH, 9:1) to give **21a** (58.5% yield). NMR: 2.33 (s, 6, NMe_2); 2.47—2.90 (m, 4, H-4 and CH_2N); 3.16 (s, 3, NMe); 4.30—4.95 (m, 1, H-5).

2-Methyl-5-dimethylaminomethyl-isoxazolidin-3-one methiodide salt ***9***
A solution of **21a** (0.3 g, 1.9 mmol) in acetone was treated with excess methyl iodide. The ammonium salt was then crystallized from abs. ethanol and ethyl ether: mp: 160—161°C; Anal. $C_8H_{17}IN_2O_2$: C, H, N. Salt left in contact with the atmosphere rapidly absorbed moisture.

2-Methyl-5-dimethylaminomethylisoxazolidin-3-thione methiodide salt ***10***
To a benzene solution (8 ml) of **21a** (0.587 g, 3.715 mmol) were added 0.754 g (1.864 mmol) of Lawesson's reagent. The resulting suspension was stirred under nitrogen and heated under reflux for 1 h, then cooled at RT, treated with dil. HCl and extracted with methylene chloride. The aqueous layer was made alkaline with solid K_2CO_3 and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and the residue distilled at reduced pressure gave 0.4 g (62% yield) of **21b** as a pale yellow oil. bp: 150—155°/1 mm Hg. NMR: 2.35 (s, 6, NMe_2); 2.60 (d, 2, CH_2N); 3.34 (m, 2, H-4); 3.58 (s, 3, NMe); 4.76 (m, 1, H-5). IR (neat): ν 1510, 1450 and 1410 cm^{-1}.

The addition of an excess of methyl iodide to an ether solution of the tertiary amine **21b**, produced **10** in quantitative yield. The ammonium salt **10** crystallized from EtOH—MeOH (4:1) as colorless needles. mp: 195—200°C dec. Anal. $C_8H_{17}IN_2OS$: C, H, N.

3-Methoxy-5-dimethylaminomethyl-isoxazoline methiodide salt ***11***
Cycloadduct **22** [16] was transformed into the corresponding trifluoromethanesulfonate as previously reported for **20d**. This intermediate was not characterized but directly reacted at — 15°C with 5 ml of a solution of $NHMe_2$ in methanol (15%, v/v). The progress of the reaction was monitored by TLC. After the usual work-up, the residue was purified by column chromatography (eluent: $CHCl_3$—MeOH, 15:1) to give 0.320 g (40.5% overall yield) of the tertiary amine **23**. NMR: 2.30 (s, 6, NMe_2); 2.58 (d, 2, CH_2N; J = 6.0); 2.93 (dd, 2, H-4; J_1 = 3.0, J_2 = 9.0); 3.85 (s, 3, OMe); 4.80 (m, 1, H-5).

The methiodide salt **11** was prepared, in quantitative yield, by treating an ether solution of the tertiary amine **23** with an excess of methyl iodide. Derivative **11** crystallized from abs. EtOH as colorless prisms. mp: 204—205°C; Anal. $C_8H_{17}IN_2O_2$: C, H, N.

Pharmacology

Male Wistar Morini rats (170—220 g) and male guinea pigs from a local strain (Bettinardi, Dankin—Hartley strain, 500—600 g b. wt.) were killed by a blow to the head and exsanguinated.

Preparation of tissues
Guinea pig atria. Spontaneously beating guinea pig atria were dissected, isolated and placed in a 20 ml organ bath containing Krebs—Henseleit solution (composition in mmol/l: NaCl: 118; KCl: 5.6; $CaCl_2$: 2.5; $MgSO_4$: 1.19; NaH_2PO_4: 1.3; $NaHCO_3$: 25; glucose: 10), gassed with 5% CO_2 in O_2 (pH 7.4), thermoregulated at 29°C.
Guinea pig ileum. The ileum was cleared of connective tissue and the lumen was flushed several times with the physiological solution used above. Segments of about 2 cm in length, with one end open, were placed in a 10 ml organ bath filled with physiological solution, maintained at 37°C.
Rat jejunum. The jejunum was removed and kept viable under the same conditions used for the guinea-pig ileum.
Rat urinary bladder. Strips of the extratrigonal portion of the detrusor muscle, 8—10 mm long and 1—1.5 mm wide, were suspended in a 10 ml organ bath filled with Krebs—Henseleit solution at 37°C.

Protocols
The preparations were left to equilibrate for 40—50 min during which time the bath solution was changed every 10 min. Atrial force was recorded with a Statham force transducer connected to a Battaglia Rangoni polygraph. The resting tension was adjusted to 1 g. The responses to drugs of all the other tissues were recorded isotonically on an LNI recorder through a Basile transducer. The load was 1 g for the rat bladder and 0.5 g for all the other tissues.

Cumulative dose—response curves were obtained by increasing the concentration by 0.5 log. units except for the heart where increments of 0.25 log. units were used. Subsequent concentrations of drugs were added when the response to the previous one had reached a plateau. The concentrations were increased until the maximum response was

achieved. Muscarine was used as the reference compound and two or, occasionally three concentration—response curves were obtained in order to test their reproducibility.

Measurements

Changes in heart contraction were expressed as percentage reduction of basal values. Responses to drugs in all the other tissues were expressed in mm of shortening. — log EC_{50} concentrations producing 50% of inhibition (atrial contraction amplitude) or 50% of maximal contractile response (other tissues) values were evaluated graphically from each concentration—response curve.

Intrinsic activity (α) was determined by comparing the maximum response to muscarine with that to the other agonists.

All the data are presented as means $\pm$ SEM ($n = 8$).

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