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New pyrrolidin-, piperidin- and azepin-2-oxocarboxylic acid esters are preferential M_1, M_3 muscarinic antagonists. Synthesis and bronchospasmolytic activity

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Summary — A series of new 3-tropanol and 3-quinuclidinol esters of phenyl-substituted pyrrolidin-, piperidin- and azepin-2-oxocarboxylic acid were synthesized and tested for antimuscarinic activity. The compounds showed a preferential *in vitro* activity at M_1 and M_3 receptor subtypes and an interesting activity profile *in vivo*. A potential use as selective bronchospasmolytic agents has been suggested for selected compounds.

quinuclidinol ester / tropanol ester / antimuscarinic activity / bronchospasmolytic activity

Introduction

The interest in muscarinic antagonists, despite their long history, is far from fading. The intense research carried out in the last decade allowed the discovery of at least 5 muscarinic receptor subtypes (m_1–m_5), 3 of which are pharmacologically defined (M_1–M_3) [1]. This has provided a new impetus in the search for selective ligands assuming that a specific interference with one of the receptor subtypes results in an improved therapeutic treatment of disease. The M_1, M_2 and M_3 muscarinic receptors are involved differently in secretory and cardiovascular functions, as well as in smooth muscle control and in central nervous system transmission. As a consequence, different disorders have been targeted with several compounds according to their own peculiar affinity profile.

Pirenzepine, the M_1 antagonist that first produced evidence of the heterogeneity of muscarinic receptors [2], is marketed for the treatment of duodenal ulcer and gastritis [3]. M_2 antagonists like otenzepad [4] and M_3 antagonists like zamifenacin [5] are being investigated for the treatment of cardiac disorders and spasms of the gastrointestinal tract, respectively. More recently, centrally acting lipophylic M_2 antagonists have been suggested as a new strategy to improve learning and memory in neurodegenerative disorders [6] and a potential role as selective bronchodilators has been proposed for M_3 or mixed M_1 and M_3 antagonists [7].

The aim of our research was the identification of structurally new muscarinic antagonists possessing a selectivity profile suitable to use in the therapeutic treatment of the various disorders.

Medicinal chemistry

Study design

In the medicinal chemistry approach, we focused on a phenyl-substituted cycloalkyl ring to serve as a hydrophobic bulky moiety. To balance the overall lipophilicity of the compounds, we thought it worthwhile to introduce an endocyclic carboxamido group, which, owing to its high intrinsic hydrophilicity, may contribute to favourable physico-chemical properties, as has been suggested for pirenzepine [8]. The traditional ester group, properly placed to give a geminal substitution pattern with the phenyl ring, was selected to

connect the bulky moiety to a cationic head which is locked into a cyclic structure. A similar geminal substitution is not unusual since it is present in the antimuscarinic drug dicyclomine [9]. All the substitution positions were investigated so as not to exclude any effect of the change in the distance between the key atoms or the overall shape of the compound. The general structure of the resulting compounds is represented in figure 1.

According to this study design, we first selected the 6-membered ring phenyl-2-oxopiperidine carboxylic acid, esterified with 2 alcoholic moieties. The latter are very common in the chemistry of muscarinic ligands, *ie* 3-tropanol and 3-quinuclidinol. The pure *R* form of the latter was used as this is known to be responsible for good muscarinic receptor recognition [10]. The corresponding 5- and 7-membered rings were considered as bulky moieties but only their 3-quinuclidinol esters were prepared as the observed antimuscarinic activity was better in quinuclidine than in tropane-containing compounds.

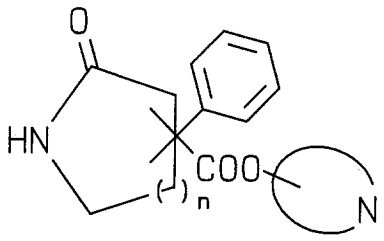


Fig 1. General structure of the compounds.

Chemistry

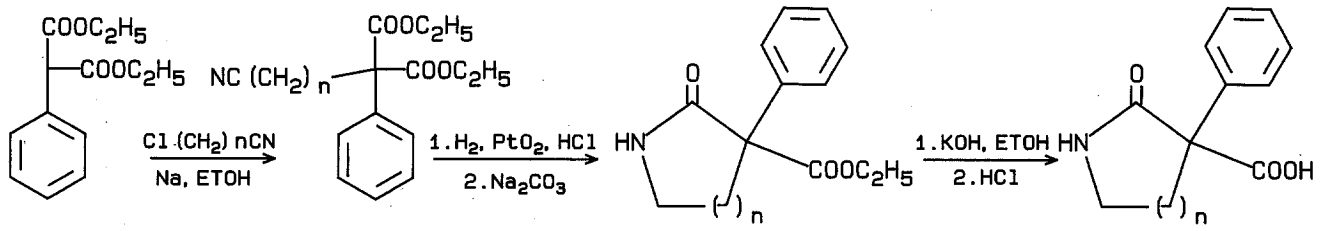
The ethyl esters **1a**, **2a** and **5a–7a** were prepared following a general strategy in which a cyano group was envisaged as a precursor of a primary amine which spontaneously cyclizes when an ester functionality is properly placed to give a 5-, 6- or 7-membered carboxamido ring.

The synthetic pathway to the known 3-ethoxycarbonyl-3-phenyl-2-oxopiperidine **2a** and the new 3-ethoxycarbonyl-3-phenyl-2-oxopyrrolidine **1a** is outlined in scheme 1 according to the method of Hill *et al* [11]. Alkylation of **9** with 2-chloroacetonitrile or propionitrile afforded **10** and **11**, respectively. These were hydrogenated in the presence of PtO_2 and HCl and, after neutralisation to generate the free amine, the intermediates **1a** and **2a** were easily obtained.

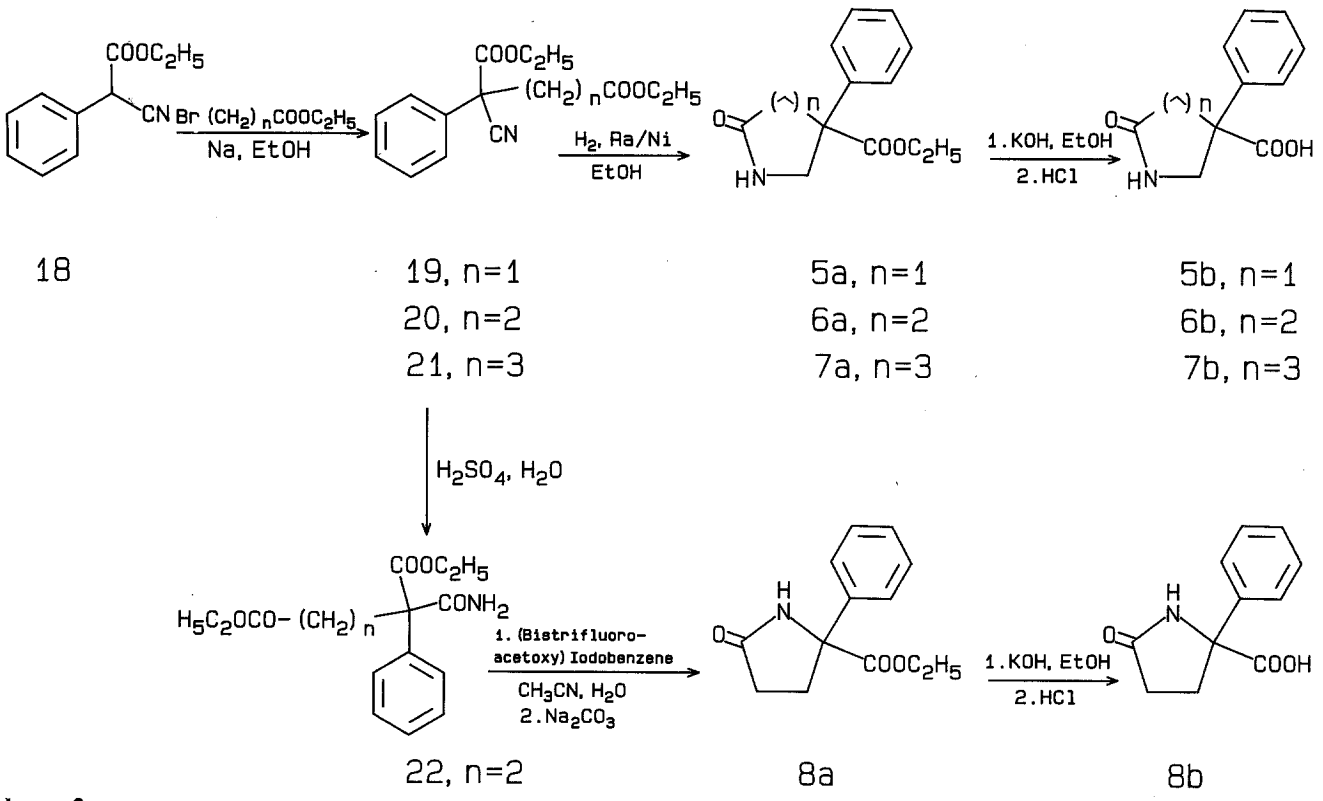
The method of Bishop *et al* [12], used in the preparation of 5-ethoxycarbonyl-5-phenyl-2-oxopiperidine **6a**, served as a base for the synthesis of the new 4-ethoxy-4-phenyl-2-oxopyrroline **5a** and 6-ethoxy-6-phenyl-2-oxoazepine **7a** (scheme 2). In this case, the cyano derivatives **19**, **20**, and **21**, which come from the alkylation of **18** with the suitable bromo esters in EtONa solution, were hydrogenated in the presence of Raney nickel as a catalyst.

The synthesis of 6-ethoxycarbonyl-6-phenyl-2-oxopiperidine **4a** is described in scheme 3. The cyclizing precursor amine comes from a smooth acidic hydrolysis of the properly alkylated Schiff base **16**. The benzylidene groupment proved very useful both in the protection of the basic function of 2-phenyl glycine ethyl ester and in the activation of the carbon atom to be alkylated. A similar approach was attempted in the synthesis of **8a** but an instant intramolecular self-condensation of the intermediate Schiff base took place and only the diphenyldiethoxycarbonyl pyrrolidine **17** was obtained, thus preventing the achievement of the desired 5-ethoxycarbonyl-5-phenyl-2-oxopyrrolidine **8a**. A Hoffman-type rearrangement carried out under neutral conditions with bis-(trifluoroacetoxy)iodobenzene [13] on the carboxamido intermediate **22** afforded the precursor amino derivative to be cyclized to **8a** (scheme 2) in a satisfactory yield.

The synthetic pathway to 4-ethoxycarbonyl-4-phenyl-2-oxopiperidine **3a** is outlined in scheme 4. The 4-ethoxycarbonyl-4-phenyl piperidine **12** [14] was first acylated to **13**, then selectively oxidated by a



Scheme 1.



Scheme 2.

mixture of RuO and $NaIO_4$ according to the method described by Yoshifuji *et al* in the oxidation of proline [15].

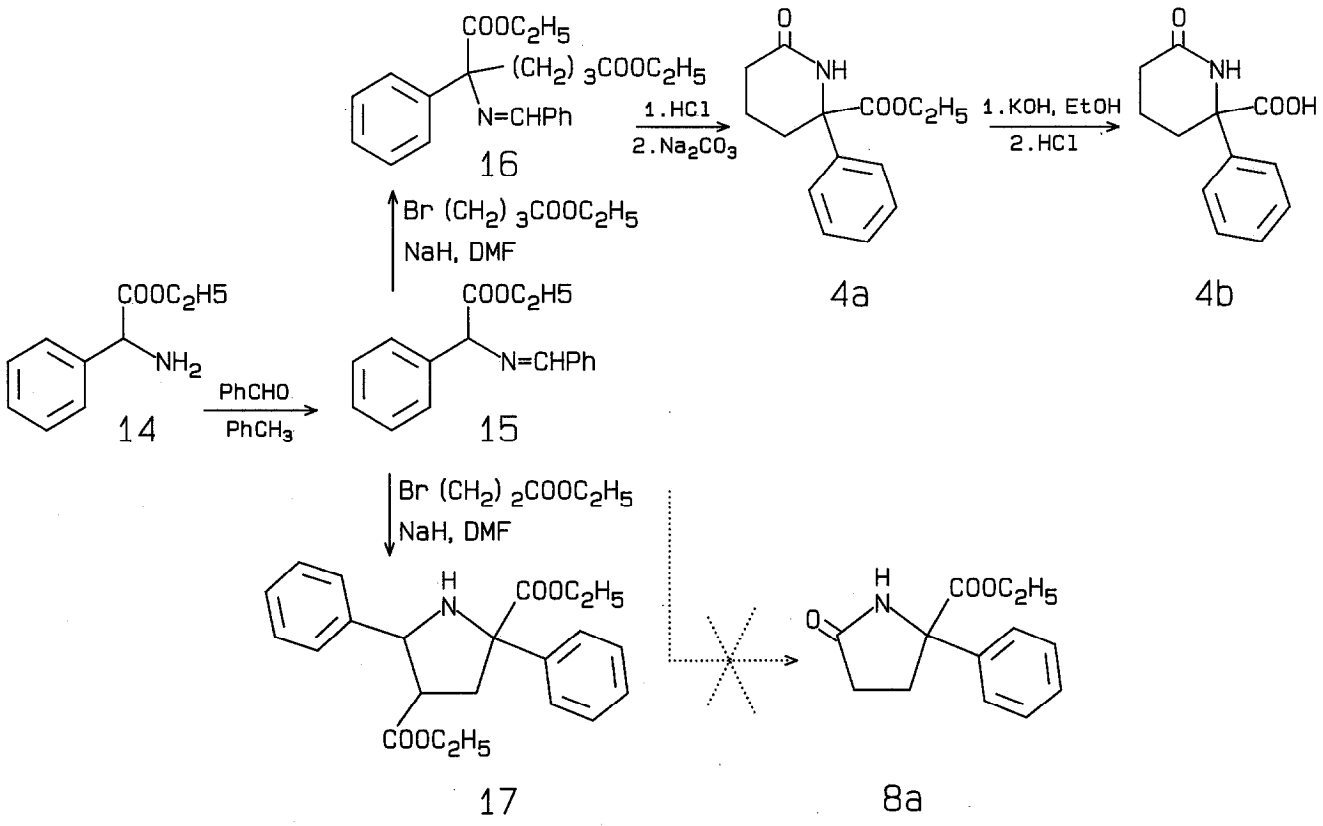
The carboxylic acids **1b**, **3b–8b** were obtained by acidification of aqueous solutions of their potassium salts, which were prepared by the saponification of the corresponding ethyl esters in 95% EtOH.

Three methods, outlined in scheme 5, were used in the preparation of the target esters. *Method A* describes the transesterification process between an ethyl ester and (*R*)-3-quinuclidinol [16] in the presence of catalytic amount of Na in toluene which afforded compounds **24–26** and **31–35** without any racemisation in good to moderate yields. Stronger conditions were needed to react the hindered 3-tropanol. According to *Method B*, esters **28–30** were thus obtained in only moderate yields after activation the carboxyl and alcoholic functions with carbonyl diimidazole and stoichiometric sodium, respectively. DMF was the solvent of choice. Finally, esters **23** and **27** were obtained in satisfactory yields, under their own reaction conditions, as shown in *Method C*. The ester **2a** did not survive prolonged heating at 80°C (*Method A*) and the use of the corresponding acid (*Method B*) was prevented by instant decarboxylation. Therefore, the carbonyl chloride derivative of **2a**, which is easily obtained from the stable potassium salt with $SOCl_2$, was chosen as a suitable form of the carboxylic acid, and was activated enough to react under smooth conditions. Tables I and II collect the physical properties and the spectroscopic data, respectively, of the new esters **23–35**.

Pharmacology

The binding affinities (K_D) of the compounds for the muscarinic receptor subtypes were evaluated on different rat tissue homogenates (cerebral cortex, M_1; heart, M_2; and submandibular gland, M_3). The results are summarized in table III. The most interesting quinuclidine compounds were further tested for their ability to antagonize a functional muscarinic response on guinea-pig ileum and guinea-pig left atrium, models of M_3 and M_2 mediated responses. The results are expressed as K_B values and are shown in table IV.

Finally, the compounds were tested in *in vivo* preparations related to the suggested application as selective spasmolytics which comes from their *in vitro* profile. As models, we chose the inhibition of the acetylcholine-induced bronchoconstriction (M_1, M_3 mediated) and bradycardia (M_2 mediated) in the

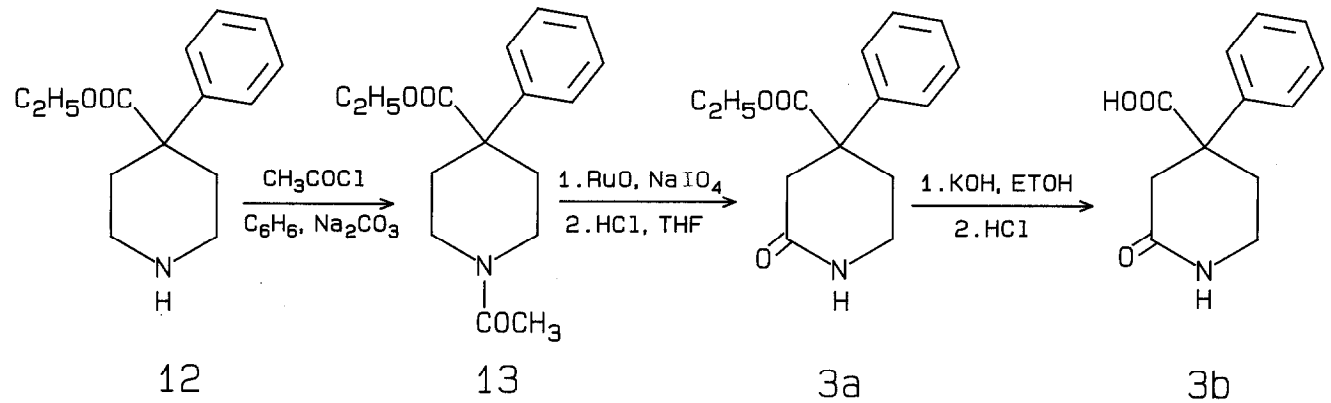


Scheme 3.

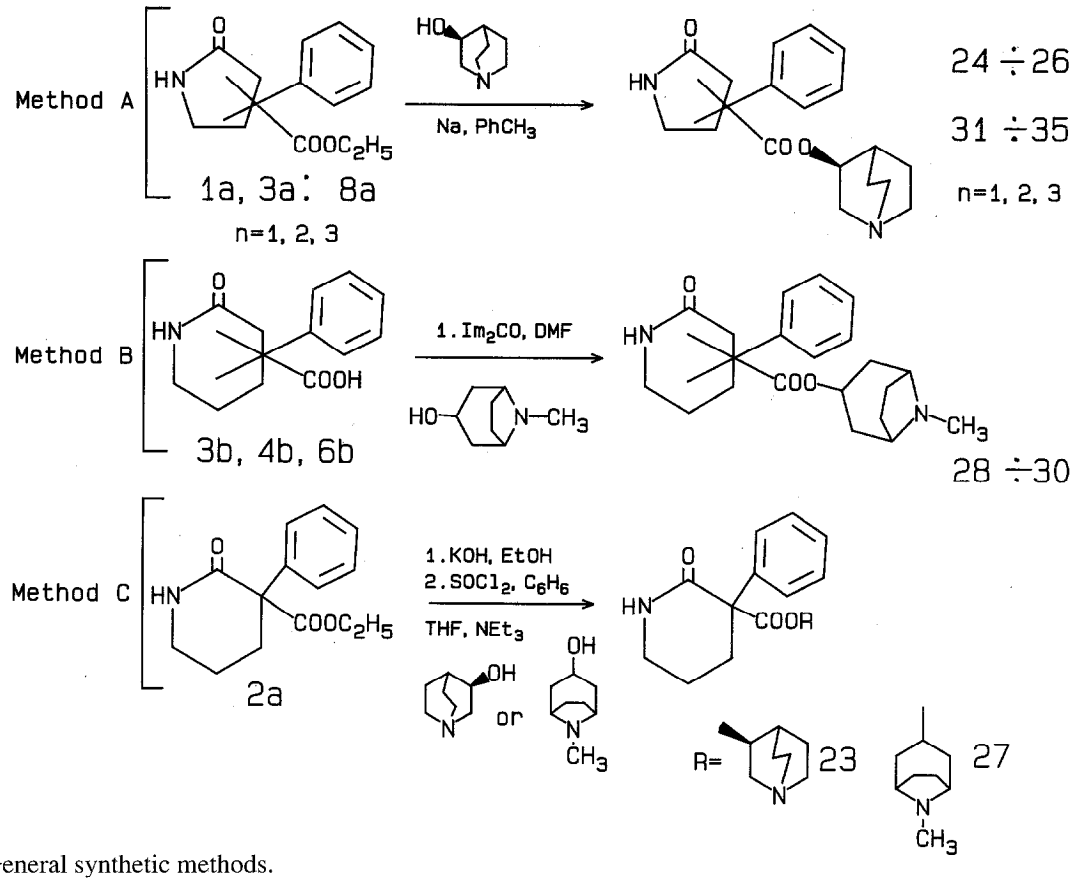
guinea-pig bronchi and heart. A comparison of the effective doses, expressed as $-\log ID_{50}$ and listed in table V, gives an idea of the selectivity ratio of the different compounds.

Results and discussion

All 4 quinuclidine esters of phenyl-2-oxopiperidin carboxylic acid bind the muscarinic receptors but behave in different ways at each subtype. Their affinity is high at M_1 (ranging from 13 to 51 nM), intermediate at M_3 (from 86 to 417 nM) and quite low at M_2 subtype (from 413 to 1865 nM). Thus the compounds discriminate better M_1 and M_2 (from about 20 times for compound **25** and **26** up to more than 30 times for **23** and **24**) than M_1 and M_3 (3–13 times). The different relative positions of key substituents affect differently, but not substantially, the



Scheme 4.



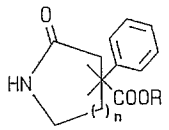
Scheme 5. General synthetic methods.

binding affinities for the different subtypes; therefore the net result of the distance change and shape modification is a finer regulation in the drug–receptor interaction. The tropanol esters **27–30** are less active than the corresponding quinuclidinol esters; in addition, since the range of affinities within the former class is wider than in the latter, particularly in the case of M_1 and M_3 receptors, either weak (**28–30**) or totally inactive compounds (**27**) are produced. Clearly, in this class of compounds the presence of the quinuclidinol affects receptor interaction more favourably than tropanol. Decreasing the size of the phenyl-substituted azacyclic ring results in increased affinity for the M_1 subtype, compounds **32** and **33** possessing the highest M_1 affinity (2.9 and 4.5 nM, respectively). The observed increase in affinity is even higher at the M_2 receptor; the net selectivity ratio between the 2 subtypes is therefore decreased and the discrimination ability for **31–33** never exceeds 20–25 fold higher. Both the high M_1 affinity and the desired selectivity profile are retained in the bulkier 7-membered rings, at least for compound **34** (M_1 affinity 2.1 nM and M_2 affinity 86 nM). A deeper inspection of the binding affinities of **34** and **35**, which are pure diastereoisomers, suggests that a further optimisation could be achieved by preparing the pure diastereoisomers of 5- and 6-membered rings **23–26**, and **31–33**.

In *in vitro* functional models, all the compounds, except **35**, preferentially antagonized the effects of bethanechol on guinea-pig ileum (M_3 activity) over those on heart (M_2 activity). The resulting selectivity ratio M_3 *versus* M_2 is poor, as shown by the differences in K_B values which are rather small and never exceed 6–7 fold, but well parallels the binding study findings. As M_3 affinity is intermediate between M_1 and M_2, the selectivity ratio M_3 *versus* M_2 is lower than M_1 *versus* M_2.

It is interesting to consider the compounds' ability to inhibit the bronchoconstriction and the bradycardia induced by acetylcholine in the guinea pig *in vivo*. The activities observed on the respiratory model were quite remarkable (in particular for compounds **25**, **32**, and **34**) and, most interestingly, they were clearly separated from those observed on the cardiac model. Selectivity ratios between smooth muscle and cardiac M_2 muscarinic receptors *in vivo* were raised up to 60

Table I. Preparation and physical properties of compounds **23–35**.



Compound	*n*	*Position of gem-substituent*	*R*	*Method of preparation*[a]	*Crystallisation solvent*[b]	*Yield (%)*[c]	*Mp (°C)*	*Formula*
23	2	3	quinuclidin-3-yl	C	diethylether	45	153–156	$C_{19}H_{24}N_2O_3$
24	2	4	quinuclidin-3-yl	A	diethylether	48	177–179	$C_{19}H_{24}N_2O_3$
25	2	5	quinuclidin-3-yl	A	petroleum ether	60	184–186	$C_{19}H_{24}N_2O_3$
26	2	6	quinuclidin-3-yl	A	diethylether[e]	35	145–149 dec[e]	$C_{19}H_{25}ClN_2O_3$
27	2	3	tropan-3-yl	C	diethylether	18	121–122	$C_{20}H_{26}N_2O_3$
28	2	4	tropan-3-yl	B	diethylether	21	156–158	$C_{20}H_{26}N_2O_3$
29	2	5	tropan-3-yl	B	ethylacetate[f]	12	94–100 dec[f]	$C_{24}H_{32}N_2O_9$
30	2	6	tropan-3-yl	B	ethylacetate[f]	10	80–85 dec[f]	$C_{24}H_{32}N_2O_9$
31	1	3	quinuclidin-3-yl	A	diethylether	42	142–143	$C_{18}H_{22}N_2O_3$
32	1	4	quinuclidin-3-yl	A	diisopropylether	40	147–148	$C_{18}H_{22}N_2O_3$
33	1	5	quinuclidin-3-yl	A	diethylether[e]	43	125–128 dec[e]	$C_{18}H_{23}ClN_2O_3$
34[d]	3	6	quinuclidin-3-yl	A	diethylether	20	156–158	$C_{20}H_{26}N_2O_3$
35[d]	3	6	quinuclidin-3-yl	A	diethylether	26	172–175	$C_{20}H_{26}N_2O_3$

[a]See *Experimental protocols*; [b]all compounds, except **31**, were first purified by flash chromatography on silica gel (eluent mixture: CH_2Cl_2/MetOH 90:10/conc NH_4OH) then crystallised from the indicated solvent. Compound **31** was purified directly from diethylether; [c]yields were not optimised; [d]compounds **34** and **35** are 2 pure diastereoisomers separated by flash chromatography on silica gel; **34** is the lower component (R_f 0.25), **35** is the higher component (R_f 0.3); [e]hydrochloride salt; [f]tartrate salt.

times (**34** and **32**, respectively). A tentative explanation can be put forward to account for the different selectivity *versus* M_2 receptors observed in *in vitro* and *in vivo* models. First, it must be considered that guinea-pig heart, which contains almost exclusively M_2 subtypes [1], was used as a cardiac tissue in both cases. In contrast, 2 contracting tissues were used in *in vitro* and *in vivo* spasmolytic models: the guinea-pig ileum and bronchi, respectively. These tissues have been shown to contain a mixed muscarinic receptor population, that is $M_2 + M_3$ for the former [1] and $M_1 + M_3$ for the latter [17]. The better spasmolytic effect, which appears particularly favourable if the activity ratio of bronchi *versus* heart rather than ileum *versus* heart is considered, could be ascribed to those differences in receptor subtypes population. An additional or alternative explanation may be inferred from the physico-chemical properties of the compounds which could affect pharmacokinetic parameters relevant to *in vivo* models. As a matter of fact, compounds **25** and **33** possess experimental log P values of 0.7 and 1.01, respectively. The rather high basicity of the quinuclidine ring present in both compounds (pK_a 8.5 and 8.8) lowers these values even further to 0.37 and 0.40, respectively, when measured at physiological pH 7.4, reflecting a proper lipophilicity–hydrophilicity balance as anticipated in the study design.

Conclusions

A new class of quinuclidine esters of 2-oxopyrrolidine, piperidine and azepine carboxylic acids as original bulky moieties has been made available. Their antimuscarinic activity is preferentially expressed at M_1 and M_3 receptor subtypes and a particularly meaningful selectivity is observed *in vivo*. According to their profile, some compounds (**25**, **32** and **34**) will be further evaluated as selective bronchodilators.

Experimental protocols

Chemistry

Melting points were determined in capillary tubes on a Büchi apparatus and are uncorrected. The IR spectra were recorded on a Perkin–Elmer Model 218 spectrometer. Mass spectra were

Table II. Spectroscopic data of compounds **23–35**.

Compound	*NMR (δ, ppm)**	*MS (CI) (M + H)*
23	1.25 (m, 1H); 1.4–1.9 (ov, 5H); 2.01 (m, 1H); 2.4 (m, 1H); 2.6–2.9 (ov, 6H); 3.1–3.6 (ov, 3H); 4.88 (m, 1H); 6.15 (d, 1H); 7.3–7.5 (ov, 5H)	329
24	1.1–3.4 (ov, 17H); 4.75 (m, 1H); 6.03 (b, 1H); 7.25 (s, 5H)	329
25	1.28 (m, 1H); 1.3–1.7 (ov, 3H); 1.89 (m, 1H); 2.3–2.8 (ov, 9H); 3.12 (m, 1H); 3.65 (m, 1H); 4.04 (m, 1H); 4.77 (m, 1H); 6.7 (b, 1H); 7.3–7.4 (ov, 5H)	329
26	1.2–1.8 (ov, 11H); 3–3.08 (ov, 6H); 5.15 (m, 1H); 7.41 (s, 5H); 8.45 (s, 1H); 12.25 (b, 1H)	329
27	1.2–2.3 (ov, 10H); 2.21 (s, 3H); 2.4–2.8 (m, 2H); 2.97 (b, 2H); 3.3–3.5 (m, 2H); 5.08 (t, 1H); 6.03 (b, 1H); 7.34 (s, 5H)	343
28	1.2–3.1 (ov, 16H); 2.12 (s, 3H); 4.88 (t, 1H); 7.33 (s, 5H); 7.49 (b, 1H)	343
29	1.1–2.6 (ov, 12H); 2.44 (s, 3H); 3.44 (b, 2H); 3.4–4 (ov, 2H); 4.04 (s, 2H); 4.17 (b, 4H); 4.93 (t, 1H); 7.37 (s, 5H); 8.19 (b, 1H)	343
30	1.4–2.6 (ov, 14H); 2.52 (s, 3H); 3.53 (b, 2H); 4.09 (s, 2H); 4.33 (b, 4H); 4.94 (t, 1H); 7.2–7.5 (ov, 5H); 8.09 (s, 1H)	343
31	1.1–3.6 (ov, 15H); 4.84 (m, 1H); 6.34 (b, 1H); 7.2–7.6 (ov, 5H)	315
32	1.1–1.7 (ov, 4H); 1.87 (m, 1H); 2.2–3.2 (ov, 7H); 3.31 (g, 1H); 3.62 (g, 1H); 4.37 (g, 1H); 4.75 (m, 1H); 6.31 (b, 1H); 7.25 (s, 5H)	315
33	1.5–3.7 (ov, 15H); 5.09 (m, 1H); 7.1–7.5 (ov, 5H); 9.29–8.66 (ds, 1H); 11.81 (b, 1H)	315
34	1.2–2.9 (ov, 16H); 3.19 (m, 1H); 3.70 (g, 1H); 3.98 (g, 1H); 4.84 (m, 1H); 5.91 (t, 3H); 7.2–7.5 (ov, 5H)	343
35	1.1–3.0 (ov, 16H); 3.19 (m, 1H); 3.6–4.1 (g, 2H); 4.82 (m, 1H); 6.27 (t, 1H); 7.27 (s, 5H)	343

*ov: overlapped; g: geminal.

recorded on a Finnigan 1020 spectrometer. ^{1}H-NMR spectra were recorded on a Varian CFT-20 (80 MHz) or a Varian VXR 200 (200 MHz) with TMS as internal standard in the indicated solvent. Chemical shifts are given in ppm (δ). The reactions were followed by analytical TLC in Kieselgel 60 F 254 or in neutral alumina with the appropriate eluents; spots were visualised by UV, iodine vapour or Dragendorff. The analytical purity of the compounds was checked on HPLC apparatus equipped with a 1040 A diode array detector and a MP 2225 recorder. Elemental analyses were performed on a C Erba elemental analyzer Model 1106 and the data for C, H, N, and Cl were within ± 0.4% of the theoretical values.

*2-Cyanomethyl-2-phenyldiethyl malonate **10***
A mixture of 2-phenyldiethyl malonate (83.5 g, 0.35 mol), diethylcarbonate (350 ml), and freshly prepared sodium ethylate (24 g, 0.35 mol) was heated at 60°C until about 200 ml of a mixture of ethyl alcohol and diethylcarbonate was distilled off by means of a Liebig apparatus. At this point, chloroacetonitrile (26.4 g, 0.35 mol) was added and the reaction mixture was heated overnight at 100°C, cooled and poured into water. The pH was adjusted to 7 with 10% aqueous HCl and the oil that separated was extracted into diethylether. The organic layer was washed with water, desiccated over $MgSO_4$ and evaporated to dryness. From the residue after distillation the title compound was obtained in a sufficiently pure form to be used in the following step; 46.5 g (48% yield); bp 140–142°C, 0.8 mmHg. ^{1}H-NMR ($CDCl_3$) δ 1.19 (t, 6H); 3.20 (s, 2H); 4.35 (q, 4H); 7.41 (s, 5H). IR (film) peaks at 2250, 1740 cm^{-1}.

*3-Ethoxycarbonyl-3-phenylpyrrolidin-2-one **1a***
A solution of the intermediate **10** (19 g, 60 mmol) in anhydrous ethanol (180 ml) was hydrogenated in the presence of 30% alcoholic HCl solution (0.18 mol) and 17% Na_2CO_3 (6.2 g). When the theoretical amount of hydrogen was taken up, the filtered solution was evaporated to dryness and the residue dissolved in water and washed with light petroleum ether. The cooled aqueous acid solution was basified with 17% Na_2CO_3 solution and the separated oil was extracted into ethyl acetate. The organic solution was washed with water, desiccated and evaporated to dryness to give the crude title compound which was purified by crystallisation from a mixture of diethylether and light petroleum ether; 10.7 g (69% yield); mp 91–93°C. ^{1}H-NMR ($CDCl_3$) δ 1.23 (t, 3H); 2.52 (m, 1H); 2.9–3.5 (ov, 3H); 4.23 (q, 2H); 6.84 (b, 1H); 7.2–7.5 (ov, 5H). IR (nujol) peaks at 3200, 1725, 1675 cm^{-1}. Anal $C_{13}H_{15}NO_3$ (C, H, N).

Table III. Receptor binding affinity for compounds **23–35** (K_D[a] x 10^{-9} M ± SE[a]).

Compound	*Cerebral rat cortex*[b]	*Rat heart*[c]	*Submandibular rat glands*[c]
23	13.7 ± 0.7	413 ± 19	180 ± 5.0
24	51 ± 4.7	1865 ± 49	417 ± 44
25	13 ± 1.2	483 ± 20	113 ± 9.3
26	26 ± 2.1	541 ± 32	86 ± 4.1
27	5083 ± 109	33 300 ± 1650	10 765 ± 650
28	543 ± 20	10 500 ± 765	2230 ± 145
29	203 ± 15	4930 ± 234	1650 ± 87
30	123 ± 9	1600 ± 58	433 ± 14
31	48 ± 3.8	817 ± 61	152 ± 10
32	2.9 ± 0.2	57 ± 4.3	10.3 ± 0.9
33	4.5 ± 0.3	136 ± 7.4	25 ± 2.6
34	2.1 ± 0.2	86 ± 5.8	8.2 ± 0.9
35	11.3 ± 0.9	123 ± 8.8	52 ± 4.4

[a]See the *Experimental protocols* for the method of determination and calculation. Data are means ± SE from 3–5 replicates. Hill coefficients were not significantly different from 1; [b]radioligand was [^{3}H]pirenzepine; [c]radioligand was [^{3}H]NMS.

3-Ethoxycarbonyl-3-phenylpiperidin-2-one ***2a***
This compound was prepared as described by Hill *et al* [11].

1-Acetyl-4-ethoxycarbonyl-4-phenylpiperidine ***13***
A solution of acetylchloride (6.29 g, 80.1 mmol) in anhydrous toluene (40 ml) was dropped into a well-stirred suspension of **12** [14] and sodium carbonate (7.73 g, 72.8 mmol) in toluene (190 ml) and water (115 ml). The reaction was stirred for 2 h at room temperature. The organic layer was separated, washed with dilute 10% hydrochloric acid, then with water, desiccated and evaporated to dryness. The title compound was obtained as a white solid, after crystallisation from petroleum ether; 18.5 g (92 % yield); mp 84–85°C. ^{1}H-NMR ($CDCl_3$) δ 1.20 (t, 3H); 1.90 (m, 2H); 2.13 (s, 3H); 2.62 (m, 2H); 2.8–3.6 (ov, 2H); 3.6 (m, 1H); 4.20 (q, 2H); 4.50 (m, 1H); 7.2–7.6 (ov, 5H). IR (nujol) peaks at 1720, 1630 cm^{-1}.

Table IV. *In vitro* functional studies (–log K_B ± SE, M)[a].

Compound	*Guinea-pig ileum*[b]	*Guinea-pig left atrium*[b]
23	7.65 ± 0.11	7.08 ± 0.01
24	7.13 ± 0.01	6.37 ± 0.12
25	7.83 ± 0.10	7.20 ± 0.12
26	7.76 ± 0.13	6.96 ± 0.06
31	7.38 ± 0.01	7.11 ± 0.10
32	8.01 ± 0.18	7.83 ± 0.03
33	7.92 ± 0.05	7.47 ± 0.06
34	8.42 ± 0.04	7.57 ± 0.06
35	6.90 ± 0.04	7.37 ± 0.03

[a]See the *Experimental protocols* for the method of determination and calculation; data are means ± SE from at least 3 replicates; [b]agonist bethanechol.

Table V. *In vivo* functional studies (–log ID_{50} ± SE, mol/kg)[a].

Compound	*Guinea-pig bronchi*[b]	*Guinea-pig heart*[b]	*Selectivity ratio*
24	6.62 ± 0.09	5.57 ± 0.11	11
25	7.25 ± 0.06	5.99 ± 0.09	18
26	6.96 ± 0.08	5.57 ± 0.07	25
31	6.44 ± 0.10	5.25 ± 0.08	15
32	8.4 ± 0.07	6.6 ± 0.08	62
33	7.14 ± 0.11	6.19 ± 0.09	9
34	7.98 ± 0.11	6.19 ± 0.09	64
35	7.38 ± 0.07	6.28 ± 0.03	12

[a]See the *Experimental protocols* for the methodology of determination; [b]agonist: acetylcholine.

4-Ethoxycarbonyl-4-phenylpiperidin-2-one ***3a***
A 10% aqueous solution of $NaIO_4$ (585 ml) was added to a well-stirred suspension of **13** (16.5 g, 59.9 mmol) and ruthenium (IV) oxide hydrate (260 mg). The 2-phase suspension was stirred for 3 d at room temperature, then the organic layer was separated and washed with an aqueous solution of sodium bisulfite and water. After drying over $MgSO_4$ the solution was evaporated *in vacuo* to give 10.8 g of the intermediate 1-acetyl-4-ethoxycarbonyl-4-phenylpiperidin-2-one (mp 45–46°C, from light petroleum ether). This compound was dissolved in tetrahydrofuran (90 ml) and 5 drops of 10% aqueous hydrochloric acid was added. The resulting solution was slowly stirred at room temperature for 5 d, then evaporated to dryness. The crude residue was partitioned between ethyl acetate and a 17% aqueous solution of Na_2CO_3. The organic layer was separated, washed with water, desiccated and evaporated to dryness. The pure title compound was obtained after crystallisation from diethylether; 7.13 g (48% overall yield); mp 137–138°C. ^{1}H-NMR ($CDCl_3$) δ 1.17 (t, 3H); 2.4 (m, 2H); 2.7–3.1 (g, 2H); 3.27 (m, 2H); 4.20 (q, 2H); 7.22 (b, 1H); 7.40 (s, 5H). IR (nujol) peaks at 3280, 3220, 1720, 1680. Anal $C_{14}H_{17}NO_3$ (C, H, N).

N-Benzyliden-2-phenylglycine ethyl ester ***15***
A suspension of α-phenylglycine ethyl ester [18] (6.3 g, 40 mmol), benzaldehyde (4.05 g, 40 mmol) and $MgSO_4$ (20 g) in toluene (80 ml) was stirred for 48 h at room temperature. The reaction mixture was filtered and evaporated to dryness under vacuum. From the crude residue, 6.8 g of the pure **15** was obtained after distillation (67% yield); bp 164–166°C, 0.05 mmHg. ^{1}H-NMR ($CDCl_3$) δ 1.23 (t, 3H); 4.20 (q, 2H); 5.18 (s, 1H); 7.2–7.6 (ov, 8H); 7.83 (m, 2H); 8.34 (s, 1H).

N-Benzyliden-2-phenyl-2-(3-ethoxycarbonyl propyl)glycine ethyl ester ***16***
4-Bromoethyl butyrate (6.1 g, 30 mmol) was slowly dropped into a well-stirred solution of the Schiff base **15** (7.2 g, 30 mmol) and 80% NaH (0.59 g, 33 mmol) in anhydrous DMF. The temperature was kept below 30°C during stirring overnight.

The reaction mixture was poured into ice-water. The oily phase was extracted several times into diethylether, the combined organic extracts were washed with water, dried over $MgSO_4$ and evaporated to dryness. This crude compound (9.9 g) was not characterized due to its instability, but was used directly in the following step.

6-Ethoxycarbonyl-6-phenylpiperidin-2-one ***4a***
The above intermediate (9.8 g) was dissolved in 10% aqueous hydrochloric acid and stirred for 1 h. The aqueous solution was then adjusted at pH 7.5 with 17% Na_2CO_3 solution and the separated oil extracted into ethyl acetate. The organic extracts were washed with water, dried over $MgSO_4$, filtered and evaporated to dryness. Crystallisation of the crude residue from light petroleum ether gave the pure title compound; 3.9 g (52% overall yield); mp 92–93°C. ^{1}H-NMR ($CDCl_3$) δ 1.17 (t, 3H); 1.6 (m, 2H); 2.1–2.3 (ov, 4H); 4.20 (q, 2H); 7.2–7.6 (ov, 6H). IR (nujol) peaks at 3280, 3225, 1720, 1680. Anal $C_{14}H_{17}NO_3$ (C, H, N).

2,5-Diphenyl-3,5-diethoxycarbonyl pyrrolidine ***17***
80% NaH (0.869 g, 28.8 mmol) was cautiously added to a cooled solution of **15** (7 g, 26 mmol) in anhydrous DMF. After 15 min stirring 3-bromoethyl propionate (5.2 g, 28.8 mmol) was added and the reaction mixture was stirred overnight at room temperature. It was then poured into ice and water, and the separated residue was extracted into diethylether. The organic solution was washed, desiccated over $MgSO_4$ and evaporated to dryness.

From the title compound was obtained the brown residue by crystallisation from diethylether; 6.8 g (64% yield). ^{1}H-NMR ($CDCl_3$) δ 0.82 (t, 3H); 1.24 (t, 3H); 2.63 (m, 1H); 3.14 (m, 1H); 3.23 (m, 1H); 3.5–3.8 (ov, 1H); 3.71–3.66 (dq, 2H); 4.20–4.21 (dq, 2H); 4.58 (d, 1H); 7.2–7.5 (ov, 8H); 7.74 (m, 2H). MS (CI) 368 m/e (M+H).

5-Ethoxycarbonyl-5-phenylpiperidin-2-one ***6a***
This compound was prepared as described by Bishop *et al* [12].

2-Cyano-2-phenyldiethyl succinate ***19***
Ethylphenylcyano acetate 825 g, (0.132 mol) was added to a cooled, stirred solution of sodium (3 g, 0.132 mol) in anhydrous ethanol (85 ml). The mixture was stirred for 1 h and then 2-bromoethyl acetate (22 g, 0.132 mol) was added dropwise. The reaction mixture was stirred overnight at room temperature, cooled, filtered and concentrated. The oily residue was purified by distillation to give the desired compound; 21.2 g (58% yield); bp 131–134°C, 0.3 mmHg. ^{1}H-NMR ($CDCl_3$) δ 1.37 (t, 6H); 3.12 (g, 1H); 3.66 (g, 1H); 4.31 (q, 2H); 4.37 (m, 2H); 7.2–7.6 (ov, 5H). IR (film) peaks at 2240, 1730 cm^{-1}.

4-Ethoxycarbonyl-4-phenylpyrrolidin-2-one ***5a***
The previously described intermediate **19** (7.6 g, 28.9 mmol) was dissolved in ethanol and hydrogenated at room temperature and atmospheric pressure in the presence of Raney nickel (0.6 g). When the theoretical amount of hydrogen had been taken up the mixture was filtered and evaporated to dryness. The oily residue was left 2 d under a 1:1 mixture of diethylether and light petroleum ether. The pure title compound was obtained as a white solid; 3.1 g (46% yield); mp 108–110°C. ^{1}H-NMR ($CDCl_3$) δ 1.18 (t, 3H); 2.76 (g, 1H); 3.33 (g, 1H); 3.64 (g, 1H); 4.13 (q, 2H); 4.37 (g, 1H); 6.56 (b, 1H); 7.31 (s, 5H). IR (nujol) peaks at 3200, 1725, 1695, 1670 cm^{-1}. Anal $C_{13}H_{15}NO_3$ (C, H, N).

2-Cyano-2-phenyldiethyl adipate ***21***
2-Phenylethylcyano acetate (56.7 g, 0.3 mol) was added dropwise into a cooled solution of Na (6.9 g, 0.3 mol) in absolute ethanol (200 ml). After 30 min stirring 4-bromoethyl butyrate (58.5 g, 0.3 mol) was added keeping the temperature between 15°C and 20°C. The reaction mixture was stirred overnight at room temperature, and then was evaporated to dryness. The residue was partitioned between diethylether and water, the organic solution was washed with diluted aqueous hydrochloric acid, then water, and was then dried. After evaporation, the compound was obtained by distillation from the crude residue and was sufficiently pure to be used in the next step; 79 g (86% yield); bp 152–155°C, 0.06 mmHg. ^{1}H-NMR ($CDCl_3$) δ 1.24 (t, 6H); 1.79 (m, 2H); 2.26 (t, 2H); 2.35 (t, 2H); 4.11 (q, 2H); 4.23 (q, 2H); 7.2–7.7 (ov, 5H). IR (nujol) peaks at 2240, 1730 cm^{-1}.

6-Ethoxycarbonyl-6-phenylazepin-2-one ***7a***
A solution of the intermediate **21** (20 g, 66 mmol) in ethanol (200 ml) and 30% hydrochloric acid/ethanol (23 ml) was hydrogenated at room temperature and pressure in the presence of Raney nickel as a catalyst (6.5 g), when the theoretical amount of hydrogen was taken up, the catalyst was filtered and the solution evaporated to dryness. The residue was dissolved in water and washed with diethylether. Neutralisation of the aqueous solution with 5% aqueous NaOH and extraction of the separated oil with ethyl acetate gave the title compound as a white solid; 7.2 g (42% yield); mp 140–142°C. ^{1}H-NMR ($CDCl_3$) δ 1.22 (t, 3H); 1.7–2.2 (ov, 3H); 2.2–2.8 (ov, 3H); 3.81 (d, 2H); 4.19 (q, 2H); 6.18 (t, 1H); 7.29 (s, 5H). IR (nujol) peaks at 3225, 3200, 3090, 1725, 1670, 1640 cm^{-1}. Anal $C_{15}H_{19}NO_3$ (C, H, N).

2-Phenyl-2-carbamyldiethyl glutarate ***22***
A solution of 2-cyano-2-phenyldiethyl glutarate (37.85 g, 0.13 mol) in conc sulphuric acid (85 ml) and water (3.5 ml) was stirred at room temperature overnight. The reaction mixture was cautiously poured into ice, and the separated oil was extracted several times into ethyl acetate. The organic solution was washed with diluted $NaHCO_3$ aqueous solution, with water, desiccated over $MgSO_4$ and evaporated to dryness. The crude residue was purified by crystallisation from light petroleum ether; 35 g (87% yield); mp 79–80°C. ^{1}H-NMR ($CDCl_3$) δ 1.23 (t, 3H); 1.26 (t, 3H); 2.31 (m, 2H); 2.69 (m, 2H); 4.09 (q, 2H); 4.25 (q, 2H); 5.78 (b, 1H); 7.1–7.4 (ov, 1H); 7.33 (s, 5H). IR (nujol) peaks at 3400, 3180, 1720, 1675 cm^{-1}.

5-Ethoxycarbonyl-5-phenylpyrrolidin-2-one ***8a***
2-Phenyl-2-carbamyldiethyl glutarate **22** (10 g, 32.5 mmol) was added portionwise to a well-stirred solution of 1,1-(bistrifluoroacetoxy)iodo benzene (20.98 g, 48.8 mmol) in acetonitrile (48 ml) and water (48 ml). The reaction mixture was further stirred for 24 h. The organic layer was separated, the aqueous phase was extracted several times with diethylether and the organic solutions were combined. To this solution conc hydrochloric acid (60 ml) and water (600 ml) were added. After 4 h stirring, the acid solution was washed with diethylether, and neutralised with solid Na_2CO_3. The separated oil was taken up into diethylether, and the title compound was obtained from this solution after usual work-up as a white solid after crystallisation from a 1:1 mixture of diethylether and light petroleum ether; 3.63 g (47% yield); mp 90–92°C. ^{1}H-NMR ($CDCl_3$) δ 1.23 (t, 3H); 2.34 (ov, 3H); 3.00 (m, 1H); 4.20 (q, 2H); 7.26 (b, 1H); 7.38 (s, 5H). IR (nujol) peaks at 3200, 1725, 1695 cm^{-1}. Anal $C_{13}H_{15}NO_3$ (C, H, N).

*General method for the preparation of pyrrolidin-, piperidin- and azepin-carboxylic acids (**1b, 3b–8b**)*
A solution of the suitable ethylester intermediate (**1a**, **3a–8a**) (25 mmol) and 85% KOH (50 mmol) in 95% aqueous ethanol (125 ml) was stirred overnight at room temperature. The potassium salt that crystallised out was filtered and dissolved in water. The desired carboxylic acid derivative was obtained as a white solid after acidification of the aqueous solution and filtration.

3-Carboxy-3-phenylpyrrolidin-2-one ***1b***. (86% yield); mp 104–106°C dec; anal $C_{11}H_{11}NO_3$ (C, H, N).

4-Carboxy-4-phenylpiperidin-2-one ***3b***. (85% yield); mp 196–197°C dec; anal $C_{12}H_{13}NO_3$ (C, H, N).

6-Carboxy-6-phenylpiperidin-2-one ***4b***. (81% yield); mp 183–185°C dec; anal $C_{12}H_{13}NO_3$ (C, H, N).

5-Carboxy-5-phenylpiperidin-2-one ***6b***. (90% yield); mp 218–220°C dec; anal $C_{12}H_{13}NO_3$ (C, H, N).

4-Carboxy-4-phenylpyrrolidin-2-one ***5b***. (88% yield); mp 228–229°C dec; anal $C_{11}H_{11}NO_3$ (C, H, N).

6-Carboxy-6-phenylazepin-2-one ***7b***. (91% yield); mp 211–214°C dec; anal $C_{13}H_{15}NO_3$ (C, H, N).

5-Carbonyl-5-phenylpyrrolidin-2-one ***8b***. (88% yield); mp 206–210°C dec; anal $C_{11}H_{11}NO_3$ (C, H, N).

*General method A for the synthesis of compounds **24–26** and **31–35***
A suspension of (*R*)-3-quinuclidinol [16] (0.042 mmol) and small pieces of Na (0.042 mmol) in dry toluene was heated at 85°C for 1 h to complete the activation process. The suitable pyrrolidin-, piperidin- and azepin-carboxylic acid ethyl ester was added dropwise (0.042 mmol) to the cooled reaction mixture. The temperature was then raised at 80°C and the reaction mixture was kept under stirring overnight. After cooling at room temperature, 10% aqueous hydrochloric acid (34 ml) was added followed by more water (100 ml). The aqueous layer was separated, washed with toluene and made alkaline by adding a 17% aqueous Na_2CO_3 solution. The separated product was taken up into CH_2Cl_2, this solution was washed with water, desiccated and evaporated to dryness to give the desired crude product. This was purified by flash chromatography on silica gel (eluent mixture, CH_2Cl_2/MeOH/conc NH_4OH 95:5:0.5).

*General method B for the synthesis of compounds **28–30***
A suspension of 3-tropanol (3 mmol) and 80% NaH (3 mmol) in anhydrous DMF was allowed to react at room temperature until the hydrogen was completely evolved. A fresh solution of the suitable imidazolide derivative prepared from the precursor piperidin-carboxylic acid **3b**, **4b** or **6b** (3 mmol) with the corresponding amount of 1,1-carbonyldiimidazole in anhydrous DMF (15 ml) was added dropwise and the reaction mixture was stirred overnight at room temperature. The crude residue obtained after evaporation of the DMF under reduced pressure was directly purified by flash chromatography on silica gel eluent mixture, CH_2Cl_2/MeOH/conc NH_4OH 95:5:0.5).

*General method C for the synthesis of compounds **23** and **27***
A solution of **2a** (5 mmol) and 85% KOH (10 mmol) in 95% EtOH (30 ml) was stirred at room temperature for 48 h. The potassium salt of the corresponding carboxylic acid which crystallised out, filtered and dried. This salt (3.8 mmol) was dissolved in a cooled solution of thionyl chloride (5 ml) and benzene (5 ml) and stirred at room temperature for 3 h. The crude chloride derivative obtained after evaporation to dryness was used immediately. A solution of this intermediate (3.4 mmol) in anhydrous THF (10 ml) was dropped into a stirred solution of 3-quinuclidinol or 3-tropanol (6.8 mmol) in anhydrous TMF (10 ml). The mixture was stirred overnight, evaporated to dryness. The corresponding 3-quinuclidinol, **23**, or 3-tropanol esters, **24**, were obtained in a pure form after flash chromatography purification on silica gel (eluent mixture, CH_2Cl_2/MeOH/conc NH_4OH 95:5:0.5).

Biochemistry

The tissue preparation for the muscarinic binding assay, the experimental procedure and the method of K_D calculation have been described previously [19] and [21].

Pharmacology

In vitro functional tests
The antagonist affinities of the compounds on guinea-pig ileal and left atrial muscarinic receptor were evaluated as described by Micheletti *et al* [20].

In vivo functional tests
Guinea pigs of either sex (DHP, 550–660 g) were anaesthetized with urethane (1.4 g/kg ip). A jugular vein and a carotid artery were cannulated for injection of drugs. A cannula was placed in the trachea and the animals were respired artificially with oxygenated room air by means of a positive pressure pump with a rate of 80 strokes/min. Except for some modifications, the effect of the compounds on bronchial tone was measured according to the method described by Konzett and Rössler (1940). Before the experiment, the trachea was clamped for a short period of time in order to obtain the maximum possible degree of bronchoconstriction for calibration. The compounds to be tested were injected *via* the jugular vein and 5 min later the increase in bronchial resistance (%) and the decrease in cardiac frequency were measured *via* an electrocardiogram (beats/min) in response to acetylcholine (50 μg/kg iv and ia). This administration method of the agonist (iv and ia) was necessary to obtain reproducible results for both parameters.

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