Synthesis and Pharmacological Characterization of Chiral Pyrrolidinylfuran Derivatives: The Discovery of New Functionally Selective Muscarinic Agonists

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Received February 11, 2008

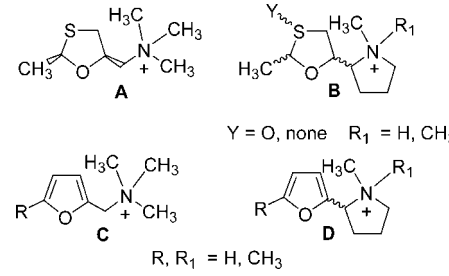
Building on the previously and successfully applied hypothesis that stereochemical complication in the proximity of the critical cationic head of a cholinergic agonist would result in subtype selective compounds, we synthesized a series of chiral derivatives of furmethide and 5-methylfurmethide, with the aim of obtaining compounds that are useful for treating diseases derived from cholinergic receptor dysfunctions and/or useful for further characterizing subtypes of cholinergic receptors. Unlike their parent compounds, the new molecules lack nicotinic activity, being pure muscarinic ligands. While binding studies on the five cloned human muscarinic receptors showed no subtype selectivity, functional assays revealed that some of the molecules of the series are potent M_2 selective partial agonists with interesting pharmacological profiles.

Introduction

For a long time, cholinergic compounds have been the object of intense research to find molecules that are useful for characterizing muscarinic[1] and nicotinic[2] receptor subtypes and for identifying medicines for cholinergic receptor dysfunctions. Only a few compounds of this class have been introduced in therapy, while many have been used as pharmacological tools for cholinergic receptor characterization. However, while antagonists have been immensely useful for both purposes, agonists have found little use in either therapy or receptor subtypes classification, particularly regarding muscarinic receptors.[3] Thus, new agonists, selective for one of the several muscarinic and nicotinic receptor subtypes, would be extremely useful not only to further characterize the receptors but also for their therapeutic potential in pathological states such as pain,[4] schizophrenia,[5] and neurodegenerative pathologies like Alzheimer's disease.[6–8] In particular, it has been documented that muscarinic agonists are able to inhibit β-amyloid protein production, possibly stimulating nonamyloidogenic α-secretase.[9–11] This observation has renewed interest in muscarinic agonists, and a new M_1 selective compound, AF267B, is reported to enter soon in clinical trials.[12,13]

For several years we have been working with cholinergic agonists that are characterized by a pentatomic cycle such as that of 1,3-oxathiolanes.[14] Recently, we reported the synthesis and the pharmacological profile of a new series of 1,3-oxathiolane derivatives whose structure had been sterically complicated with respect to the parent compound (*R*,*R*)-(+)-2-methyl-5-dimethylaminomethyl-1,3-oxathiolane methyl iodide **A** (Chart 1), introducing more stereogenic centers.[15–17] We reasoned that enhancing the molecular complexity of **A**, through stereochemical complication in the proximity of the critical cationic head of the molecule, would result in agonists that are able to detect the subtle structural differences between muscarinic receptor subtypes, whose recognition sites are highly conserved.[18,19] At the same time, we expected that the introduction of a pyrrolidinyl moiety would augment the modest nicotinic activity that was present in **A**.[20] As a matter of fact, we were successful in identifying some M_2 selective compounds, but the new derivatives **B** (Chart 1) lacked any nicotinic activity.

Chart 1. Structures of Compounds **A**−**D**



Compounds that show both muscarinic and nicotinic activity with proper subtype selectivity are considered to be beneficial for the cognitive deficits present in Alzheimer's disease.[21,22] We therefore decided to explore the consequences of a similar pyrrolidinyl substitution in cholinergic ligands like furmethide and 5-methylfurmethide **C** (Chart 1), which show comparable nicotinic and muscarinic activities[23] but do not present subtype selectivity. We thus designed the series of compounds **D** (Chart 1).

Chemistry

The synthetic pathway for obtaining the racemic compounds (**D**, Chart 1) is reported in Scheme 1. Compounds (±)-**1** and (±)-**2** were obtained as reported in the literature.[24–28] **1** and **2** were alkylated, as shown in Scheme 1, by reductive methylation with HCOOH/HCHO to give the tertiary amines (±)-**3**, and (±)-**4**, respectively, which in turn were transformed into the corresponding methyl iodides (±)-**5** and (±)-**6**, with an excess of CH_3I.

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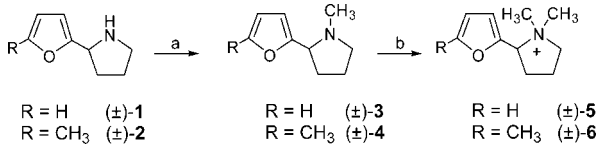
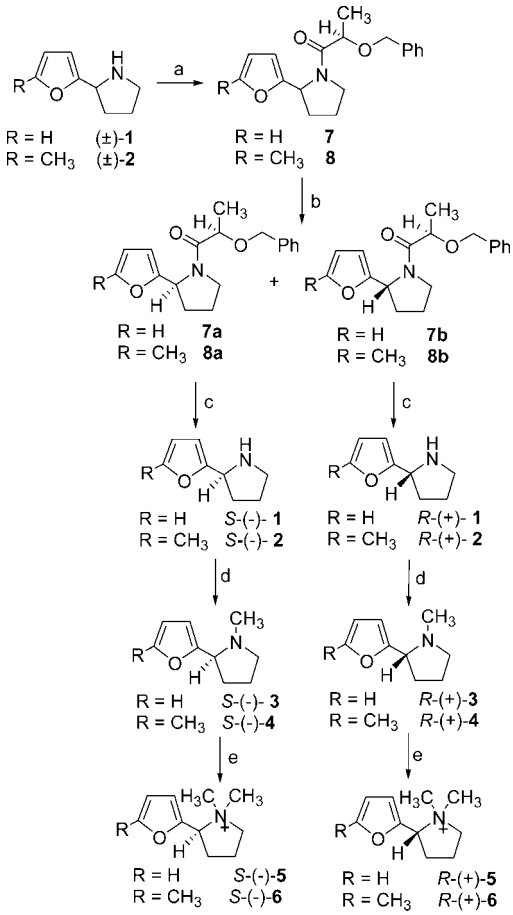
10.1021/jm800145d CCC: $40.75
Published on Web 06/11/2008

Scheme 1. Synthesis of Racemic Compounds **1**−**6**[a]



[a] a) HCOOH/HCHO; b) CH_3I.

Scheme 2. Synthesis of the Enantiomers of Compounds **1**−**6**[a]



[a] a) R(+)-benzyloxypropionyl chloride; b) chromatographic separation; c) H_2O, *t*-BuOK; d) HCOOH/HCHO; e) CH_3I.

In an attempt to obtain the enantiomers of the designed compounds, the racemic mixture of (±)-**3** was resolved by fractional crystallization of the diastereomeric salts with D-(+) and L-(−)-dibenzoyltartaric acid, obtaining the two enantiomers (+)-**3** and (−)-**3** with an optical rotation of +84.7 and −85.2, respectively. However, this approach failed to resolve the racemic mixtures of both **4** and the secondary amines **1** and **2**. For that reason we decided to shift to the synthetic pathway shown in Scheme 2, using an optically active resolving agent.

Amines (±)-**1** and (±)-**2** were reacted with (*R*)-(+)-benzyloxypropionyl chloride to give the diastereomeric amides **7** and **8**. Amides **7** were separated by flash chromatography on silica gel using 6/4 hexane/ethyl acetate as the eluting system. After a sequence of four chromatographic separations, compounds **7a** and **7b** (where **7a** is the first eluted amide and **7b** the second one) were obtained. The same procedure was applied to the diastereomeric amides **8** that were separated, using 7/3 cyclohexane/ethyl acetate as an eluting system, to obtain **8a** and **8b**. As observed before for 1,3-oxathiolane sulfoxides[17] and quinoline[29] derivatives, ^{1}H NMR spectra showed that each amide was a mixture of rotamers.

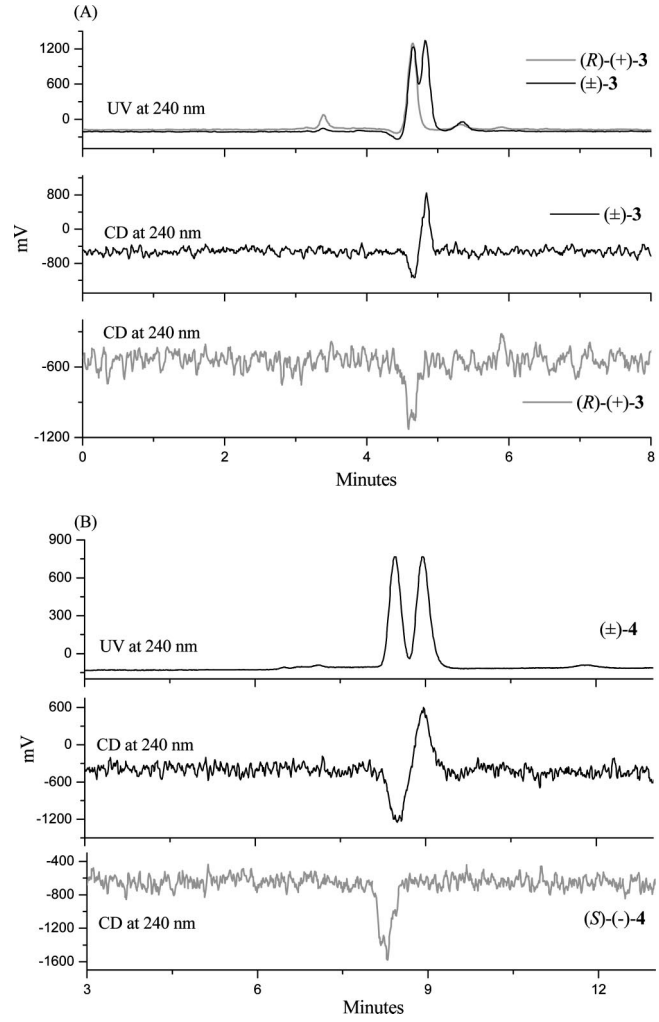


Figure 1. UV and CD chromatograms of (±)-**3**, (±)-**4**, (*R*)-(+)-**3**, and (*S*)-(−)-**4**. (A) CSP, Chiralcel OD (250 mm × 4.6 mm i.d.); eluent, *n*-hexane−2-propanol−DEA 100:1:0.1 (v/v/v); flow-rate, 1 mL/min; temperature, 25 °C; detection, UV and CD at 240 nm. (B) CSP: Chiralcel OB-H (250 mm × 4.6 mm i.d.); eluent, *n*-hexane−2-propanol−DEA 100:0.5:0.1; flow-rate, 0.5 mL/min; temperature, 25 °C; detection, UV and CD at 240 nm.

Compounds **7a** and **8a** were hydrolyzed according to Gassman and co-workers,[30] affording (−)-**1** and (−)-**2**, respectively. In the same way, compounds (+)-**1** and (+)-**2** were obtained from the amides **7b** and **8b**, respectively.

The secondary amines (+)-**1**, (−)-**1**, (+)-**2**, and (−)-**2** were alkylated (Scheme 2) by reductive methylation with HCOOH/HCHO to give the tertiary amines (+)-**3**, (−)-**3** (their optical activity was identical to that of the compounds obtained by fractional crystallization), (+)-**4**, and (−)-**4**, which in turn were transformed into the corresponding methyl iodides (+)-**5**, (−)-**5**, (+)-**6**, and (−)-**6** with an excess of CH_3I.

The enantiomeric excess (ee) of the enantiomeric forms of **3** and **4** was evaluated by enantioselective HPLC (see Experimental Section) on the polysaccharide-based Chiralcel OD and Chiralcel OB-H chiral stationary phases (CSPs) (Figure 1). The chromatographically determined ee values were higher than 98.5%.

The absolute configuration of compounds (*R*)-(+)-**3** and (*S*)-(−)-**3** was established on the basis of the X-ray crystallography of the D-(+)-dibenzoyltartaric salt of (+)-**3** and the L-(−)-

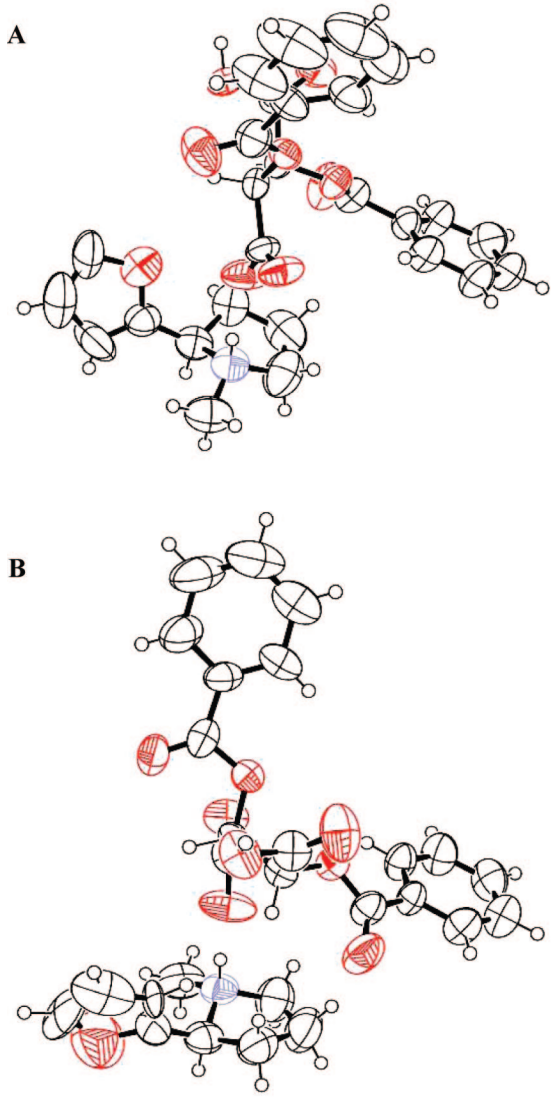


Figure 2. (A) ORTEP drawing of D-(+)-dibenzoyltartaric salt of compound *R*-(+)-**3**. (B) ORTEP drawing of L-(−)-dibenzoyltartaric salt of compound *S*-(−)-**3**.

dibenzoyltartaric salt of (−)-**3** (whose crystallographic structures are shown in Figure 2). The absolute configuration of the 5-methyl derivative (*S*)-(−)-**4** was assigned by comparison of its CD (circular dichroism) spectra with that of the unsubstituted analogue (*S*)-(−)-**3** (Figure 3). The absolute configurations of the enantiomers of compounds (±)-**1**, (±)-**2**, (±)-**5**, and (±)-**6** come straightaway, as the reactions d and e (Scheme 2) occur without racemization. The absolute configuration and the optical rotation of all compounds are reported in Table 1.

Pharmacology

The compounds, tested on rat brain homogenates, did not show any nicotinic affinity up to 10 μM dose. Muscarinic receptor affinity was evaluated in CHO[a] cells expressing the five human muscarinic subtypes (hm1−hm5). Functional activity was evaluated in vitro on classical preparations: rabbit stimulated vas deferens, guinea pig stimulated left atria (M_2), guinea pig ileum (M_3), and guinea pig lung strips, following the methods

[a] Abbreviations: CD, circular dichroism spectra; CHO, Chinese hamster ovary cells; APE, arecaidine propargyl ester; DEA, diethylamine; dec, decomposition; equiv, equivalent; abs EtOH, absolute ethanol; PSS, physiological salt solution.

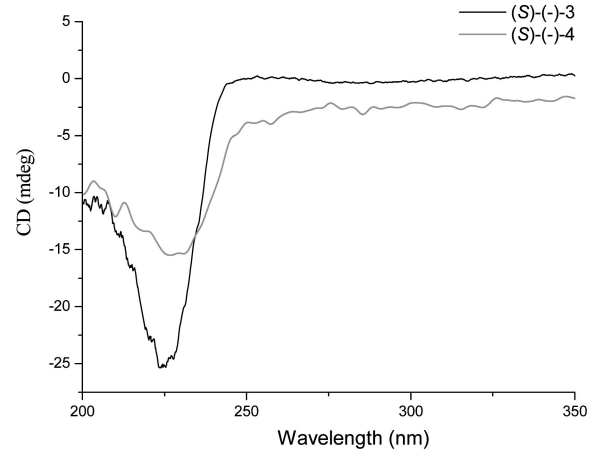


Figure 3. CD spectra of (*S*)-(−)-**3** (black line) and (*S*)-(−)-**4** (gray line) in *n*-hexane.

Table 1. Absolute Configuration and the Optical Rotation of the Enantiomers of Compounds **1**−**6**

	$[\alpha]_D^{20}$		$[\alpha]_D^{20}$
S-(−)-**1**	−13.33	*R*-(+)-**1**	+16.66
S-(−)-**2**	−18.00	*R*-(+)-**2**	+19.00
S-(−)-**3**	−85.22	*R*-(+)-**3**	+84.7
S-(−)-**4**	−81.48	*R*-(+)-**4**	+80.00
S-(−)-**5**	−5.13	*R*-(+)-**5**	+5.15
S-(−)-**6**	−4.67	*R*-(+)-**6**	+4.79

reported previously.[31] In this respect, it is fair to recall that, for a long time, the contraction of rabbit vas deferens was considered an effect mediated by M_1-receptor subtypes, whereas more recent studies attribute the same effect to an M_4-activation.[32,33] Analogously, the validity of the guinea pig lung strips as an M_4 model[34] has been questioned.[35] For this reason, in the present work, these two preparations are indicated as putative M_1 and M_4 receptor models. Carbachol, arecaidine propargyl ester (APE), 5-methylfurmethide, and McN-A-343 were used as reference agonists. Results are expressed as pK_i values (binding affinity), as pD_2 (pED_{50}, agonist potency), or as pK_b (antagonist affinity) and are reported in Tables 2 and 3.

Results and Discussion

Contrary to our expectations, the synthesized compounds ((±)-**1** to (±)-**6**) do not show any nicotinic activity up to 10 μM; they appear to be pure muscarinic agonists. The results of their binding and functional activity on muscarinic receptors are reported in Tables 2 and 3, together with those of carbachol, McN-A-343, 5-methylfurmethide (Table 2), and APE (Table 3), used as reference compounds. While in general the studied compounds show lower affinity compared to the reference compounds, some of them, such as (±)-**5** and *S*-(−)-**6**, show comparable or higher affinity with respect to the standards (Table 2). Much like the reference compounds, none of them showed subtype selectivity, although there is a modest increase of affinity for hm2 subtype with respect to the other subtypes, and in the case of the racemate (±)-**2** and its enantiomers, hm2 selectivity is quite considerable (1 order of magnitude). Enantioselectivity is very modest or nearly nonexistent except in the case of (±)-**2** where it is close to the value of 10 at all five receptor subtypes. All the binding affinities of the enantiomers lay within the range of the standard errors. In this respect, it is fair to emphasize that the binding profile of (±)-**5** is quite unexpected and difficult to rationalize. In this case, the racemate presents consistently higher affinity than the single enantiomers. At present, we do

Table 2. Binding Affinity[a] of Compounds **1**−**6** and of Their Enantiomers

	pK_i ± SEM				
compd	hm1	hm2	hm3	hm4	hm5
(±)-**1**	3.75 ± 0.09	4.53 ± 0.20	4.94 ± 0.16	4.06 ± 0.19	4.78 ± 0.10
R-(+)-**1**	4.33 ± 0.10	4.11 ± 0.21	4.60 ± 0.12	4.05 ± 0.20	4.50 ± 0.10
S-(−)-**1**	4.20 ± 0.11	3.97 ± 0.21	4.36 ± 0.13	3.99 ± 0.18	4.32 ± 0.09
(±)-**2**	4.42 ± 0.06	5.37 ± 0.09	4.74 ± 0.11	4.29 ± 0.08	4.72 ± 0.07
R-(+)-**2**	4.84 ± 0.09	5.49 ± 0.13	4.92 ± 0.10	4.53 ± 0.07	4.80 ± 0.07
S-(−)-**2**	3.81 ± 0.08	4.54 ± 0.11	3.83 ± 0.12	3.68 ± 0.09	3.86 ± 0.08
(±)-**3**	4.48 ± 0.07	4.38 ± 0.11	4.35 ± 0.12	4.16 ± 0.07	4.35 ± 0.07
R-(+)-**3**	4.11 ± 0.10	4.32 ± 0.12	4.09 ± 0.10	4.10 ± 0.07	4.12 ± 0.07
S-(−)-**3**	4.36 ± 0.09	4.51 ± 0.12	4.28 ± 0.09	4.25 ± 0.07	4.20 ± 0.05
(±)-**4**	4.57 ± 0.07	4.84 ± 0.11	4.64 ± 0.10	4.61 ± 0.07	4.66 ± 0.06
R-(+)-**4**	4.35 ± 0.08	4.71 ± 0.11	4.54 ± 0.10	4.23 ± 0.07	4.50 ± 0.07
S-(−)-**4**	4.44 ± 0.07	4.80 ± 0.09	4.48 ± 0.11	4.32 ± 0.07	4.40 ± 0.07
(±)-**5**	5.28 ± 0.06	5.43 ± 0.11	5.56 ± 0.10	5.19 ± 0.08	5.50 ± 0.07
R-(+)-**5**	4.29 ± 0.08	4.85 ± 0.09	4.75 ± 0.13	4.55 ± 0.09	4.63 ± 0.07
S-(−)-**5**	4.51 ± 0.07	5.13 ± 0.09	4.67 ± 0.09	4.67 ± 0.09	4.68 ± 0.07
(±)-**6**	5.13 ± 0.07	5.72 ± 0.09	5.16 ± 0.08	5.36 ± 0.09	5.33 ± 0.08
R-(+)-**6**	4.86 ± 0.08	5.57 ± 0.10	5.11 ± 0.10	4.86 ± 0.07	5.02 ± 0.07
S-(−)-**6**	5.25 ± 0.08	5.78 ± 0.10	5.57 ± 0.10	5.29 ± 0.08	5.44 ± 0.08
carbachol	4.42 ± 0.09	5.91 ± 0.06	4.36 ± 0.10	5.20 ± 0.07	4.16 ± 0.07
McN-A-343	5.71 ± 0.09	5.49 ± 0.07	5.62 ± 0.09	5.04 ± 0.07	5.42 ± 0.06
5-methylfurmethide	5.10 ± 0.08	6.02 ± 0.09	5.21 ± 0.09	5.20 ± 0.07	5.16 ± 0.10

[a] Binding affinity on cloned human muscarinic receptors expressed in CHO cells. The values represent the mean (±SEM) of at least three experiments.

Table 3. Functional Activity and Selectivity of Compounds **2**−**6** and of Their Enantiomers[a]

	rabbit vas deferens[b]		guinea pig atrium (M_2)		guinea pig ileum (M_3)		guinea pig lung[b]	
compd	α	pD_2	α	pD_2	α	pD_2	α	pD_2
(±)-**2**	nd		1.00 ± 0.01	6.66 ± 0.04	0.97 ± 0.04	5.57 ± 0.21	pK_b = 5.51 ± 0.22	
R-(+)-**2**	1.00 ± 0.01	5.75 ± 0.2	1.00 ± 0.01	6.71 ± 0.12	1.00 ± 0.01	6.16 ± 0.01	0.32 ± 0.11	5.45 ± 0.20
S-(−)-**2**	pK_b < 5		0.60 ± 0.04	6.10 ± 0.18	1.00 ± 0.01	5.00 ± 0.19	pK_b = 5.11 ± 0.07	
(±)-**3**	0.22 ± 0.02	5.59 ± 0.12	0.40 ± 0.11	7.25 ± 0.20	0.35 ± 0.04	5.70 ± 0.04	0.25 ± 0.03	6.87 ± 0.16
R-(+)-**3**	0.47 ± 0.11	5.26 ± 0.20	0.45 ± 0.09	7.38 ± 0.17	0.31 ± 0.09	5.65 ± 0.27	pK_b < 5	
S-(−)-**3**	0.30 ± 0.04	5.27 ± 0.20	0.65 ± 0.13	6.82 ± 0.09	0.37 ± 0.09	5.83 ± 0.20	0.33 ± 0.09	6.72 ± 0.24
(±)-**4**	nd		0.37 ± 0.04	6.31 ± 0.20	pK_b < 5		pK_b < 5	
R-(+)-**4**	pK_b < 5		0.32 ± 0.04	6.40 ± 0.14	0.22 ± 0.02	4.93 ± 0.22	pK_b < 5	
S-(−)-**4**	pK_b < 5		0.47 ± 0.05	6.60 ± 0.09	pK_b < 5		pK_b < 5	
(±)-**5**	0.26 ± 0.01	5.84 ± 0.07	0.38 ± 0.12	6.65 ± 0.03	0.19 ± 0.03	5.65 ± 0.18	pK_b < 5	
R-(+)-**5**	0.28 ± 0.02	5.57 ± 0.14	0.40 ± 0.05	6.49 ± 0.20	0.25 ± 0.04	5.37 ± 0.18	pK_b < 5	
S-(−)-**5**	0.21 ± 0.01	5.70 ± 0.08	0.30 ± 0.11	7.19 ± 0.12	0.41 ± 0.08	5.77 ± 0.13	pK_b < 5	
(±)-**6**	nd		0.33 ± 0.05	7.55 ± 0.26	0.28 ± 0.02	7.25 ± 0.10	pK_b < 5	
R-(+)-**6**	pK_b = 5.67 ± 0.22		0.44 ± 0.03	7.66 ± 0.20	0.26 ± 0.04	7.10 ± 0.20	pK_b < 5	
S-(−)-**6**	pK_b = 5.90 ± 0.12		0.42 ± 0.02	7.49 ± 0.08	pK_b 7.82 ± 0.08		pK_b = 5.96 ± 0.19	
carbachol			1	7.33 ± 0.08	1	6.68 ± 0.01	5.43[c] ± 0.03	
APE	7.14[c] ± 0.18		1	8.67 ± 0.04	1	7.64 ± 0.09	5.56[c] ± 0.07	
MCN-A-343	6.33[c] ± 0.06							

[a] Evaluated as reported in the Experimental Section and expressed as pD_2 (−log ED_{50}) for agonism and as pK_b (−log K_b) for antagonism. For agonists, α represents intrinsic activity. [b] In the present work, these two preparations are indicated as putative M_1 and M_4 receptor models. [c] The intrinsic activity of the compounds is 1.

not know the reasons for such behavior. Apparently, the enantiomers seem to influence each other's binding, resulting in a higher affinity of the racemate. This would be consistent with one or both of the enantiomers being positive allosteric modulators of the human muscarinic receptors expressed in CHO cells, but of course, more experiments will be needed to clarify this point.

On the basis of our previous experience, which showed that poor binding would not prevent good functional activity,[16,17] we tested the compounds on functional models of muscarinic receptors. In our experience, discrepancies between binding studies and functional activity of agonists are quite common[16,17] and can be explained on the basis of Furchgott's efficacy and other arguments,[17] including the theory of receptor trafficking.[36,37] The results are reported in Table 3, and apparently, with these models, the compounds show a much more interesting pharmacological profile. With the exception of *R*-(+)-**2**, which is a full agonist, the other compounds are all partial agonists and present selectivity for the M_2 receptor. This subtype selectivity is generally modest except in compounds *S*-(−)-**2**, *R*-(+)-**3**, *S*-(−)-**4**, *S*-(−)-**5**, *S*-(−)-**6**. Compound *S*-(−)-**2** is inactive on rabbit vas deferens and guinea pig lung (pK_b < 5 and pK_b = 5.11, respectively) and presents 1 order of magnitude of selectivity for the M_2 vs M_3 receptors pD_2 = 6.10 and pD_2 = 5.00, respectively).

Compound *S*-(−)-**6** is a potent partial agonist on M_2 receptors (pD_2 = 7.49) while being an antagonist on M_3, rabbit vas deferens, and guinea pig lung preparations (pK_b = 7.82, 5.90, 5.96, respectively). Very similarly, compound *S*-(−)-**4** is active as a partial agonist on M_2 receptors (pD_2 = 6.60) and is inactive on the other functional models (pK_b < 5 for all). Compound *R*-(+)-**3** is one of the most interesting because it presents a selectivity of nearly 2 orders of magnitude on M_2 vs M_3 and rabbit vas deferens (pD_2 = 7.38, 5.65, 5.26, respectively) while being inactive on guinea pig lung preparation (pK_b < 5). A fairly similar profile is that of compound *S*-(−)-**5** (pD_2 = 7.19, 5.77, 5.70 on M_2, M_3, and rabbit vas deferens respectively; pK_b < 5 on guinea pig lung preparation). As predicted by binding studies, enantioselectivity is nearly absent, with the exception of the enantiomers of compound **2**, which present a modest enanti-

oselectivty (eudismic index EI = 0.61 on M_2 and EI = 1.16 on M_3 models). Two properties deserve a final comment: (i) 5-methyl substitution seems to produce compounds that behave generally as antagonists, especially on rabbit vas deferens preparation (see compounds *S*-(−)-**2**, *R*-(+)-**4**, *S*-(−)-**4**, *R*-(+)-**6**, *S*-(−)-**6**); (ii) with the exception of *R*-(+)-**3**, all of the most interesting compounds belong to the *S*-series.

In conclusion, although our primary goal of synthesizing cholinergic agonists endowed with both muscarinic and nicotinic activity was not attained, we have identified a series of potent and functionally selective agonists of the muscarinic M_2 subtype, which could be useful as pharmacological tools for muscarinic receptor characterization. Compounds *S*-(−)-**6** and *R*-(+)-**3** seem the most promising. In particular, the latter does not bear a permanent charge and might be able to cross the blood−brain barrier.

Experimental Section

Chemistry. All melting points were taken on a Büchi apparatus and are uncorrected. Unless otherwise stated, NMR spectra were recorded on Bruker Avance 400 spectrometer (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C). Chromatographic separations were performed on a silica gel column by gravity chromatography (Kieselgel 40, 0.063−0.200 mm; Merck) or flash chromatography (Kieselgel 40, 0.040−0.063 mm; Merck). Yields are given after purification, unless otherwise stated. Where analysis results are indicated by symbols, the analytical results are within ±0.4% of the theoretical values. Optical rotation was measured at a concentration of 1 g/100 mL (c = 1), unless otherwise stated, with a Perkin-Elmer polarimeter (accuracy of ±0.002°). When reactions were performed in anhydrous conditions, the mixtures were maintained under nitrogen. Compounds were named following IUPAC rules as applied by Beilstein-Institute AutoNom (version 2.2), a software for systematic names in organic chemistry.

HPLC-grade solvents were supplied by Carlo Erba (Milan, Italy). Diethylamine (DEA) was obtained from Fluka Chemie (Buchs, Switzerland). HPLC enantioseparations were performed by using stainless steel Chiralcel OD (250 mm × 4.6 mm i.d.) and Chiralcel OB-H (250 mm × 4.6 mm i.d.) (Daicel, Chemical Industries, Tokyo, Japan) columns. HPLC apparatus consisted of a Perkin-Elmer (Norwalk, CT) 200 lc pump equipped with a Rheodyne (Cotati, CA) injector (20 μL sample loop), a HPLC Dionex (CA) model TCC-100 oven, and a Jasco (Jasco, Ishikawa-cho, Hachioji City, Tokyo, Japan) model 2095 Plus UV/CD detector. The (*S*)-(−)-**3** and (*S*)-(−)-**4** enantiomers were dissolved in *n*-hexane (concentrations of about 4 mg/mL), and their circular dichroism (CD) spectra were measured in a quartz cell (0.1 cm path length) using a Jasco J-710 spectropolarimeter (Japan Spectroscopic, Tokyo, Japan), maintained thermostatically at 25 °C. The mean spectra from three instrumental scans were calculated as ellipticity values (mdeg).

2-(2-Furyl)pyrrolidine ((±)-1) and 2-(5-Methyl-2-furyl)-pyrrolidine ((±)-2). (±)-**1** and (±)-**2** were obtained according to literature.[28]

2-(2-Furyl)-1-methylpyrrolidine ((±)-3). An amount of 0.5 g (0.036 mol) of **1** was dissolved in 10 mL of absolute ethanol, and 17 equiv of HCOOH and 12 equiv of HCHO (40% solution in H_2O) were added. The mixture was refluxed for 2 h. The solvent was then removed and the residue basified with 10% NaOH aqueous solution and extracted with diethyl ether. The organic phase was dried on Na_2SO_4 and then the solvent removed under reduced pressure to give 0.505 g of **3** (94% yield) as an oily product. Anal. ($C_9H_{13}NO$) C, H, N. ^{1}H NMR ($CDCl_3$): δ = 1.60−1.63 (m, 1H, pyrrolidine), 1.78−1.92 (m, 3H, pyrrolidine), 2.07 (s, 3H, NCH_3), 2.11 (t, 1 H, J = 9.2 Hz, 2′-H), 2.96 −3.00 (m, 1 H, 5′-H), 3.00−3.07 (m, 1 H, 5′-H), 6.01 (dd, 1 H, J = 3.2, 2.0 Hz, 4-H), 6.11 (d, 1 H, J = 3.2 Hz, 3-H), 7.17 (d, 1 H, J = 2.0 Hz, 5-H). ^{13}C NMR ($CDCl_3$) δ (ppm): 22.3 (CH_2, t), 30.7 (CH_2, t), 39.9 (NCH_3, q), 56.1 (C-5′, t), 64.5 (C-2′, d), 106 (furan, d), 110 (furan, d), 141.1 (C-5, d), 155 (C-2, s).

Following the same procedure and starting from *S*-(−)-**1** and *R*-(+)-**1**, compounds *S*-(−)-**3** and *R*-(+)-**3** (Anal. $C_9H_{13}NO$) were obtained as oily products. Their optical rotations are reported in Table 1.

2-(5-Methyl-2-furyl)-1-methylpyrrolidine ((±)-4). Following the same procedure described for (±)-**3**, starting from 0.145 g (0. 96 mmol) of (±)-**2**, 0.120 g (80% yield) of compound (±)-**4** was obtained as an oil. Anal. ($C_{10}H_{15}NO$) C, H, N. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.76−1. 96 (m, 1H, pyrrolidine), 1.98−2.10 (m, 3H, pyrrolidine), 2.28 (t, 1H, J = 8.8 Hz), 2.26 (s, 3H, CH_3), 2.28 (s, 3H, NCH_3), 3.00−3.17 (m, 1H, 5′-H), 3.17−3.22 (m, 1H, 5′-H), 5.87 (d, 1H, J = 3.2 Hz, 4-H), 6.07 (d, 1H, J = 3.2 Hz, 3-H). ^{13}C NMR ($CDCl_3$) δ (ppm): 14.0 (CH_3, q), 22.0 (CH_2, t), 31.0 (CH_2, t), 40.5 (NCH_3, q), 57.0 (C-5′, t), 64.0 (C2′, d), 105.0 (CH, d, furan), 107.5 (CH, d, furan), 152.0 (C-5, d), 153.0 (C-2, s).

Following the same procedure and starting from *S*-(−)-**2** and *R*-(+)-**2**, compounds *S*-(−)-**4** and *R*-(+)-**4** (Anal. $C_{10}H_{15}NO$) were obtained as oily products. Their optical rotations are reported in Table 1.

2-(2-Furyl)-1-methylpyrrolidine Methiodide ((±)-5). An anhydrous diethyl ether solution of (±)-**3** was treated with an excess of methyl iodide and kept overnight at room temperature in the dark. The solid obtained was filtered, dried under vacuum, and recrystallized from absolute ethanol, mp 175−178 °C. Anal. ($C_{10}H_{16}NOI$) C, H, N. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.22−2.62 (m, 4H, pyrrolidine), 2.86 (s, 3H, NCH_3), 3.48 (s, 3H, NCH_3), 4.07−4.16 (m, 1H, 5′-H), 4.16−4.24 (m, 1H, 5′-H), 5.50 (t, 1H, J = 9.2 Hz, 2′-H), 6.46−6.47 (m, 1H, 4-H), 6.88 (d, 1H, J = 3.2 Hz, 3-H), 7.53−7.54 (m, 1H, 5-H). ^{13}C NMR ($CDCl_3$) δ (ppm): 19.98 (CH_2, t), 25.14 (CH_2, t), 47.23 (NCH_3, q), 51.30 (NCH_3, q), 65.61(C-5′, t), 72.91 (C-2′, d), 111.48 (furan, d), 115.8 (furan, d), 143.67 (C-5, d), 145.46 (C-2, s).

Following the same procedure and starting from *S*-(−)-**3**, compound *S*-(−)-**5** was obtained (Anal. $C_{10}H_{16}NOI$; mp 160 °C dec). Starting from *R*-(+)-**3**, compound *R*-(+)-**5** was obtained. Their optical rotations are reported in Table 1.

2-(5-Methyl-2-furyl)-1-methylpyrrolidine Methiodide ((±)-6). Following the same procedure described for **5**, starting from 0.045 g (0.3 mmol) of (±)-**4**, 0.050 g (76.7% yield) of compound (±)-**6** was obtained, mp 184−187 °C. Anal. ($C_{11}H_{18}NOI$) C, H, N. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.31−2.60 (m, 4H, pyrrolidine), 2,31 (s, 3H, CH_3), 2.88 (s, 3H, NCH_3), 3.49 (s, 3H, NCH_3), 4.10−4.14 (m, 1H, 5′-H), 4.20−4.28 (m, 1H, 5′-H), 5.34−5.39 (m, 1H, 2′-H), 6.12 (d, 1H, J = 3.2 Hz, 4-H), 6.76 (d, 1H, J = 3.2 Hz, 3-H). ^{13}C NMR ($CDCl_3$) δ (ppm): 13.5 (CH_3, q), 20 (CH_2, t), 25.10 (CH_2, t), 46.23 (NCH_3, q), 51.30 (NCH_3, q), 65 (C5′, t), 73 (C2′, d), 109.8 (CH, d, furan), 117 (CH, d, furan), 141.5 (C5, d), 145.46 (C-2, s).

Following the same procedure and starting from *S*-(-)-**4**, compound *S*-(−)-**6** was obtained (Anal. $C_{11}H_{18}NOI$; mp 142 °C). Starting from *R*-(+)-**4**, compound *R*-(+)-**6** was obtained. Their optical rotations are reported in Table 1.

2-Benzyloxy-1-(2-furan-2-ylpyrrolidin-1-yl)propan-1-one (7). An amount of 2 g of (±)-**1** was dissolved in 30 mL of $CHCl_3$ stabilized with amylene. To the resulting mixture, 1.77 g of Et_3N and 3.48 g of 2-benzyloxypropionyl chloride solubilized in 10 mL of $CHCl_3$ stabilized with amylene were added. The mixture was refluxed for 2 h. The solvent was then removed and the residue basified with 10% NaOH solution and extracted with ether. The organic phase was dried on Na_2SO_4 and then the solvent removed under reduced pressure to give 4.2 g (96.2% yield) of a 1:1 mixture of the two diastereoisomers **7a** and **7b**. A cycle of four chromatographic separations, using hexane/ethyl acetate = 6:4 as eluting system, afforded 0.46 g of the first eluted isomer **7a** (as oily compound) and 0.36 g of the second eluted **7b** (as oily compound). Anal. ($C_{18}H_{21}NO_3$) C, H, N.

As observed before for 1,3-oxathiolane sulfoxides,[17] ^{1}H NMR spectra showed that each amide was a mixture of rotamers. ^{1}H NMR (CDCl3) δ (ppm) **7a**, 1.17 and 1.42 (d, 3H, J = 6.4 Hz, CH_3), 1.89−2.13 (m, 4H, 3′-CH_2 and 4′-CH_2), 3.52−3.67 (m, 2H, 5′-H), 4.17 and 4.22 (q, 1H, J = 6.4 Hz, CHOBz), 4.33−4.59 (m, 2H,

CH_2-benzylic), 4.86−4.88 and 5.29−5.31 (m, 1H, 2′-H), 5.95 and 6.18 (d, 1H, J = 1.6 Hz, 3-H), 6.26−6.27 and 6.30−6.31 (m, 1H, 4-H), 7.30−7.37 (m, 6H: 1H, 5-H + 5H aromatics). ^{1}H NMR ($CDCl_3$) δ (ppm) **7b**, 1.36 and 1.43 (d, 3H, J = 6.4 Hz, CH_3), 1.93−2.18 (m, 4H, 3′-CH_2 and 4′-CH_2), 3.59−3.66 (m, 2H, 5′-H), 4.08−4.11 and 4.41−4.62 (m, 2H, CH_2-benzylic), 4.21 and 4.23 (q, 1H, J = 7.2 Hz, CHOBz), 5.04−5.06 and 5.31−5.35 (m, 1H, 2′-H), 6.10 and 6.16 (d, 1H, J = 1.6 Hz, 3-H), 6.26−6.28 and 6.29−6.30 (m, 1H, 4-H), 7.24−7.36 (m, 6H: 1H, 5-H + 5H aromatics).

2-Benzyloxy-1-[2-(5-methylfuran-2-yl)pyrrolidin-1-yl]propan-1-one (8). Following the same procedure described for **1**, starting from 1.6 g of (±)-**2** dissolved in 25 mL of $CHCl_3$ stabilized with amylene, 1.28 g of Et_3N, and 2.52 g of 2-benzyloxypropionyl chloride solubilized in 10 mL of $CHCl_3$ stabilized with amylene, 3.2 g (96.5% yield) of a 1:1 mixture of the two diastereoisomers **8a** and **8b** was obtained.

Four subsequent chromatographic separations, using cyclohexane/ethyl acetate = 7:3 as eluting system, afforded 0.56 g of the first eluted isomer **8a** (as oily compound) and 0.18 g of the second eluted **8b** (as oily compound). Anal. ($C_{19}H_{23}NO_3$) C, H, N. Also in this case, ^{1}H NMR spectra showed that each amide was a mixture of rotamers. ^{1}H NMR ($CDCl_3$) δ (ppm) **8a**: 1.19 and 1.41 (d, 3H, J = 6.4 Hz, CH_3), 1.80−2.12 (m, 4H, 3′-CH_2 and 4′-CH_2), 2.22 and 2.23 (s, 3H, CH_3), 3.52−3.70 (m, 2H, 5′-H), 4.20 (q, 1H, J = 6.4 Hz, CHOBz), 4.33−4.56 (m,2H, CH_2-benzylic), 4.79−4.80 and 5.23−5.25 (m, 1H, 2′-H), 5.80−5.82 (m, 1H, 3-H and 4-H), 5.86 (d, 0.5H, J = 2.4 Hz, 3-H), 6.02 (d, 0.5H, J = 2.4 Hz 4-H), 7.26−7.34 (m, 5H, aromatics). ^{1}H NMR ($CDCl_3$) δ (ppm) **8b**: 1.36 and 1.42 (d, 3H, J = 6.4 Hz, CH_3), 1.69−2.13 (m, 4H, 3′-CH_2 and 4′-CH_2), 2.22 and 2.23 (s, 3H, CH_3), 3.50−3.76 (m, 2H, 5′-H), 4.21 (q, 1H, J = 6.4 Hz, CHOBz), 4.07−4.66 (m,2H, CH_2-benzylic), 4.94−4.96 and 5.25−5.30 (m, 1H, 2′-H), 5.84−5.85 (m, 1H, 3-H and 4-H), 5.95 (d, 0.5H, J = 2.4 Hz, 3-H), 6.00 (d, 0.5H, J = 2.4 Hz, 4-H), 7.23−7.35 (m, 5H, aromatics).

2-(2-Furyl)pyrrolidine (−)-1. An amount of 0.26 g (0.87 mmoli) of **7a** was dissolved in 13.3 mL of anhydrous THF, under nitrogen. To the obtained solution, cooled at 0 °C, first 0.0313 mL (1.74 mmol) of H_2O and then 0.196 g (1.74 mmol) of *t*-BuOK were added. After 10 min, an amount of 0.45 g (3.99 mmol) of *t*-BuOK was added. After 15 min the mixture was left to reach room temperature and maintained under stirring for 12 h. Then the solvent was removed under reduced pressure and the obtained solid was treated with H_2O and 2 M HCl aqueous solution and extracted with diethyl ether. The aqueous phase was then basified with 10% NaOH aqueous solution and extracted with diethyl ether. The solvent was dried on Na_2SO_4 and evaporated under reduced pressure. A total of 0.10 g of (−)-**1** (83.9% yield) was obtained. ^{1}H NMR ($CDCl_3$) 400 MHz and ^{13}C NMR ($CDCl_3$) 100 MHz spectra are identical to the spectra of the racemic mixture. $[\alpha]_D^{20}$ −13.33 (*c* 1.0, abs EtOH).

2-(2-Furyl)pyrrolidine (+)-1. Following the same procedure described for (−)-**1**, starting from 0.22 g (0.73 mmol) of **7b** in 11.2 mL of anhydrous THF, 0.026 mL (1.47 mmol) of H_2O, and 0.541 g (4.82 mmol) of *t*-BuOK, 0.09 g of (+)-**1** (90% yield) was obtained. ^{1}H NMR ($CDCl_3$) 400 MHz and ^{13}C NMR ($CDCl_3$) 100 MHz spectra are identical to the spectra of the racemic mixture. $[\alpha]_D^{20}$ +16.66 (*c* 1.0, abs EtOH).

2-(5-Methyl-2-furyl)pyrrolidine (−)-2. Following the same procedure described for (−)-**1**, starting from 0.23 g (0.74 mmol) of **8a** in 10 mL of anhydrous THF, 0.026 mL (1.47 mmol) of H_2O, and 0.541 mg (4.82 mmol) of *t*-BuOK, 0.092 g of (−)-**2** (81.4% yield) was obtained. ^{1}H NMR ($CDCl_3$) 400 MHz and ^{13}C NMR ($CDCl_3$) 100 MHz spectra are identical to the spectra of the racemic mixture. $[\alpha]_D^{20}$ −18.00 (*c* 1.0, abs EtOH).

2-(5-Methyl-2-furyl)pyrrolidine (+)-2. Following the same procedure described for (−)-**1**, starting from 0.23 g (0.74 mmol) of **8b** in 10 mL of anhydrous THF, 0.092 g of (+)-**2** (81.4% yield) was obtained. ^{1}H NMR ($CDCl_3$) 400 MHz and ^{13}C NMR ($CDCl_3$) 100 MHz spectra are identical to the spectra of the racemic mixture. $[\alpha]_D^{20}$ +19.00 (*c* 1.0, abs EtOH).

X-ray Structural Analysis of Diastereomeric Salts of *R*-(+)-3 and *S*-(−)-3. Data Collection. Both crystals were mounted on a glass fiber before RX analyses were carried out with a goniometer Oxford diffraction KM4 Xcalibur2 at room temperature. Graphite-monochromated Mo Kα radiation (40 mA/−40 kV) and a KM4 CCD/sapphire detector were used for cell parameter determination and data collection.

Structure Refinement. The integrated intensities, measured using the ω scan mode, were corrected for Lorentz and polarization effects.[38] In both cases the substantial redundancy in data allows empirical absorption corrections to be applied using multiple measurements of symmetry-equivalent reflections. Both structures were solved by direct methods of SIR2002[39] and refined using the full-matrix least-squares on F^2 provided by SHELXL97.

D-(+)-Dibenzoyltartaric Salt of *R*-(+)-3. $C_{27}H_{27}NO_9$, M = 509.50, orthorhombic, space group $P21\ 21\ 21$, a = 7.428(2) Å, b = 12.878(3) Å, c = 27.296(6) Å, V = 2611.1(1) Å^3, Z = 4, D_c = 1.296, μ = 0.098 mm^{-1}, $F(000)$ = 1072. The 14 365 reflections were collected in the range $4.19 < \theta < 34.56$, and 9052 were independent. R_{int} was 0.0657. Parameters were 343, and the final R index was 0.0401 for reflections having $I > 2\sigma I$ and 0.0502 for all data.

Analyzing this structure, we notice two strong hydrogen bonds. The first is intramolecular between N1 and O5 with the following parameters: N1−HN1, 0.797(0.001) Å; N1···O5, 2.635(0.001) Å; HN1···O5, 1.853(0.000) Å; N1−HN1···O5, 166.25(0.03) Å. The second is intermolecular between O2 and O4 of the molecule reported by the following symmetry operation: O2, x, y, z; O4, x − 1, y, z. The following are the intermolecular parameters: O2−HO2, 0.956(0.001) Å; O2···O4, 2.518(0.001) Å; HO2···O4, 1.576(0.001) Å; O2−HO2···O4, 167.61(0.04).

The non-hydrogen atoms were refined anisotropically. Hydrogen atoms on O2, N1, C2, C7, C8, C11, and C12 were found in the Fourier synthesis, and all other hydrogens were assigned in calculated positions; all of them were refined as isotropic.

L-(−)-Dibenzoyltartaric Salt of *S*-(−)-3. $C_{27}H_{27}NO_9$, M = 509.50, orthorhombic, space group $P21\ 21\ 21$, a = 7.442(3) Å, b = 12.882(4) Å, c = 27.35(1) Å, V = 2622 (1) Å^3, Z = 4, D_c = 1.291, μ = 0.098 mm^{-1}, $F(000)$ = 1072. The 16 727 reflections were collected in the range $4.35 < \theta < 33.28$, and 8820 were independent. R_{int} was 0.0625. Parameters were 346, and the final R index was 0.0465 for reflections having $I > 2\sigma I$ and 0.0863 for all data.

Also in this case there are two strong hydrogen bonds. One is intramolecular between N1 and O4 with the following parameters: N1−HN1, 0.890(0.032) Å; N1···O5, 2.616(0.004) Å; HN1···O5, 1.733(0.032) Å; N1−HN1···O5, 171.34(3.09) Å. The other is intermolecular between O2 and O4 of the molecule reported by the following symmetry operation: O2, x, y, z; O4, x + 1, y, z. The following are the intermolecular parameters: O2−HO2, 0.820(0.002) Å; O2···O4, 2.526(0.003) Å; HO2···O4, 1.727(0.002) Å; O2−HO2···O4, 164.39(0.17) Å.

The non-hydrogen atoms were refined anisotropically. Hydrogen atoms on N1, C11, and C12 were found in the Fourier synthesis, and all other hydrogen atoms were assigned in calculated positions; all of them were refined as isotropic.

Pharmacology. Binding Studies. Cell Culture and Membrane Preparation. Chinese hamster ovary cells stably expressing cDNA encoding human muscarinic hm1−hm5 receptors were generously provided by Prof. R. Maggio (Department of Experimental Medicine, University of L'Aquila, Italy). Growth medium consisted of Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (Gibco, Grand Iland, NY), 50 units/mL penicillin G, 0.05 mg/mL streptomycin, 2 mM glutamine (Sigma-Aldrich, Milan, Italy), nonessential amino acids solution

100× (Sigma-Aldrich, Milan, Italy), 0.25 μg/mL amphotericin B, and 400 μg/mL Geneticin (Gibco, Grand Iland, NY) in a humidified atmosphere consisting of 5% CO_2 and 95% air. Confluent CHO cell lines were scraped, washed with buffer (25 mM sodium phosphate containing 5 mM $MgCl_2$ at pH 7.4), and homogenized for 30 s using an Ultra-Turrax (setting 3). The pellet was sedimented at 17000*g* for 15 min at 4 °C, and the membranes were resuspended in the same buffer, rehomogenized with Ultra-Turrax, and stored at −80 °C.[41] An aliquot was taken for the assessment of protein content according to the method of Bradford[42] using the Bio-Rad protein assay reagent (Bio-Rad Laboratories, Munchen, West Germany), and bovine serum albumin was used as the standard.

Binding Assay. The radioligand binding assay was run in polypropylene 96-well plates (Sarstedt, Verona, Italy) and performed for 120 min at room temperature in a final volume of 0.25 mL in 25 mM sodium phosphate buffer containing 5 mM $MgCl_2$ at pH 7.4. Final membrane protein concentrations were 30 μg/mL (hm1), 70 μg/mL (hm2), 25 μg/mL (hm3), 50 μg/mL (hm4), and 25 μg/mL (hm5). In homologous competition curves, [^{3}H]NMS was present at 0.2 nM in tubes containing increasing concentrations of unlabeled NMS (0.03−1000 nM) and at 0.075−0.200 nM in tubes without unlabeled ligand. In heterologous competition curves, fixed concentrations of the tracer (0.2 nM) were displaced by increasing concentrations of several unlabeled ligands (0.01−1000 μM); all measurements were obtained in duplicate. At the end of the binding reaction, free radioligand was separated from bound ligand by rapid filtration through UniFilter GF/C plates (Perkin-Elmer Life Science, Boston, MA) using a FilterMate cell harvester (Perkin-Elmer Life Science, Boston, MA). After filtration, the filters were washed several times with ice cold buffer and allowed to dry overnight at room temperature under air flow, 25 μL of scintillation liquid (Microscint-20, Perkin-Elmer Life Science, Boston, MA) was added, and the radioactivity was counted by TopCount NXT microplate scintillation counter (Perkin-Elmer Life Science, Boston, MA). The binding data were analyzed by the weighted least-squares iterative curve fitting program LIGAND[43] to obtain the affinity constant (K_i) and the binding capacity (Bmax).

Functional Studies. General Considerations. Male guinea pigs (200−300 g) and male New Zealand white rabbits (3.0−3.5 kg) were killed by cervical dislocation. The organs required were set up rapidly under 1 g of tension in 20 mL organ baths containing physiological salt solution (PSS) maintained at an appropriate temperature (see below) and aerated with 5% CO_2−95% O_2. Dose−response curves were constructed by cumulative addition of the reference agonist. The concentration of agonist in the organ bath was increased approximately 3-fold at each step, with each addition being made only after the response to the previous addition had attained a maximal level and remained steady; a cumulative dose−response curve to the agonist under study was constructed. Responses were expressed as a percentage of the maximal response obtained in the control curve. Contractions were recorded by means of a force displacement transducer connected to the MacLab system PowerLab/800. In all cases, parallel experiments in which tissues received only the reference agonist were run in order to check any variation in sensitivity. All animal testing was carried out according to European Community Council Directive of November 24, 1986 (86/609/EEC).

Guinea Pig Ileum. Two-centimeter-long portions of terminal ileum were taken at about 5 cm from the ileum−cecum junction and mounted in PSS, at 37 °C, of the following composition (mM): NaCl 118, $NaHCO_3$ 23.8, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.18, KH_2PO_4 1.18, $CaCl_2$ 2.52, and glucose 11.7. Tension changes were recorded isotonically. Tissues were equilibrated for 30 min, and dose−response curves to arecaidine propargyl ester (APE) were obtained at 30 min intervals, the first one being discarded and the second one being taken as the control.

Guinea Pig Stimulated Left Atria. The heart was rapidly removed, and the right and left atria were separately excised. Left atria were mounted in PSS (the same used for ileum) at 30 °C and stimulated through platinum electrodes by square-wave pulses (1 ms, 1 Hz, 5−10 V) (Tetra Stimulus, N. Zagnoni). Inotropic activity was recorded isometrically. Tissues were equilibrated for 2 h, and a cumulative dose−response curve to APE was constructed.

Guinea Pig Lung Strips. The lungs were rapidly removed, and strips of peripheral lung tissue were cut either from the body of a lower lobe with the longitudinal axis of the strip parallel to the bronchus or from the peripheral margin of the lobe. The preparations were mounted, with a preload of 0.3 g, in PSS with the following composition (mM): NaCl (118.78), KCl (4.32), $CaCl_2.2H_2O$ (2.52), $MgSO_4 \cdot 7H_2O$ (1.18), KH_2PO_4 (1.28), $NaHCO_3$ (25), glucose (5.55). Contractions were recorded isotonically at 37 °C after tissues were equilibrated for 1 h, and then two cumulative dose−response curves to APE (0.01, 0.1, 1, 10, 100 μM) were obtained at 45 min intervals, the first one being discarded and the second one being taken as the control.

Rabbit Stimulated Vas Deferens. This preparation was set up according to Eltze.[44] Vasa deferentia were carefully dissected free of surrounding tissue and were divided into four segments, two prostatic portions of 1 cm and two epididymal portions of approximately 1.5 cm length. The four segments were mounted in PSS with the following composition (mM): NaCl (118.4), KCl (4.7), $CaCl_2$ (2.52), $MgCl_2$ (0.6), KH_2PO_4 (1.18), $NaHCO_3$ (25), glucose (11.1); 10^{-6} M yohimbine and 10^{-8} M tripitramine were included to block α_2-adrenoceptors and M_2 muscarinic receptors, respectively. The solution was maintained at 30 °C, and tissues were stimulated through platinum electrodes by square-wave pulses (0.1 ms, 2 Hz, 10−15 V). Contractions were measured isometrically after tissues were equilibrated for 1 h, and then a cumulative dose−response curve to McN-A-343 was constructed.

Acknowledgment. Financial support from the M.I.U.R. (Ministero dell'Università e della Ricerca) is gratefully acknowledged. The authors thank Dr. Cristina Faggi for X-ray structural analysis of compounds *R*-(+)-**3** and *S*-(−)-**3**, Dr. Katia Varani (Ferrara University) for nicotinic receptor binding, and Prof. Fulvio Gualtieri for helpful discussions.

Supporting Information Available: Elemental analysis results for compounds **1**−**8** (Table 4), X-ray crystallographic data for *R*-(+)-**3** (Tables 5−7) and *S*-(−)-**3** (Tables 8−10). This material is available free of charge via the Internet at http://pubs.acs.org.

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JM800145D