Highly Chiral Muscarinic Ligands: The Discovery of (2*S*,2′*R*,3′*S*,5′*R*)-1-Methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine 3-sulfoxide Methyl Iodide, a Potent, Functionally Selective, M_2 Partial Agonist

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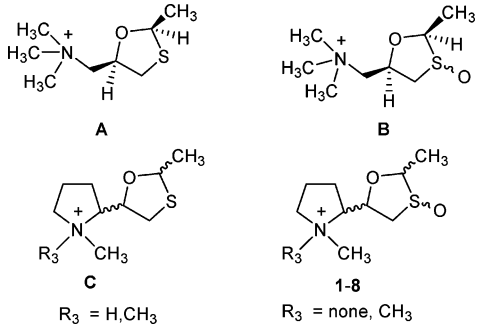
By further steric complication of previously studied highly chiral muscarinic agonists, we have obtained a small chiral library of enantiomeric and diasteromeric 1-methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine 3-sulfoxides. Binding studies on cloned human muscarinic receptors expressed in CHO cells show that the introduction of a fourth stereogenic center gives undetectable affinity for hm1, hm3, hm4 and hm5 subtypes while leaving a quite modest affinity only for hm2 subtypes. However, functional studies on model M_1-M_4 muscarinic tissues have shown that three compounds of the series [(−)-**5**, (−)-**7**, (+)-**8**] are endowed with functional activity and behave as M_2 selective partial agonists. Among them, compound (2*S*,2′*R*,3′*S*,5′*R*)-1-methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine 3-sulfoxide methyl iodide [(+)-**8**] is particularly interesting, as it is a potent partial agonist on guinea pig atrium (force) (M_2; $pD_2 = 7.65$, $\alpha = 0.41$) while being a poor antagonist on M_1, M_3, and M_4 model tissues ($pK_b < 5$).

Introduction

Cholinergic compounds have been intensively studied over the past years to find agents to treat cholinergic receptor dysfunctions and to identify molecules useful to characterize muscarinic[1] and nicotinic[2] receptor subtypes. A few compounds of this class have found use as medicines, while many others have been used as pharmacological tools for cholinergic receptor characterization. However, while antagonists have been immensely useful for both purposes, agonists have hardly found any use in receptor subtypes classification, in particular as regards muscarinic receptors.[3] Thus, new agonists, selective for one of the several muscarinic 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 such as Alzheimer's disease.[6,7] For several years we have been working on cholinergic agonists characterized by a pentatomic cycle, such as that of 1,3-oxathiolanes (compounds **A** and **B**, Chart 1),[8] and recently we have reported the synthesis and the pharmacological profile of a new series of 1,3-oxathiolane derivatives (compounds **C**, Chart 1)[9] whose structure had been sterically complicated, with respect to the parent compound (+)-(*R*,*R*)-2-methyl-5-[(dimethylamino)methyl]-1,3-oxathiolane methyl iodide, by introducing a third stereogenic center. We reasoned that exalting the molecular complexity of **A**, through stereochemical complication in the proximity of the critical cationic head of the molecule, would result in agonists able to detect the subtle structural differences between muscarinic receptor subtypes, whose recognition sites are highly conserved.[10,11] Indeed, binding studies on the five cloned human muscarinic receptors have shown that these compounds (**C**, Chart 1) possess some selectivity toward hm2 subtypes, a property confirmed in functional assays, where they show clear-cut functional selectivity on guinea pig atrium.[12]

Chart 1



Building on this hypothesis, we have now designed and synthesized, by oxidation of the sulfur atom of these 1,3-oxathiolanes derivatives, the corresponding sulfoxides **1**−**8** reported in Chart 1. The new compounds present a fourth stereogenic center, and we anticipated that further increase of stereochemical complexity of our molecules would improve further M_2 selectivity.

Chemistry. The starting material for the synthesis of sulfoxides **1**−**8** were oxathiolanes **18**−**21** that, in turn, were obtained from commercially available (*R*)- and (*S*)-prolinol, as previously reported.[9]

However, in the present work, we have definitely improved the synthetic procedure by isolating and characterizing all intermediates and obtaining all possible isomers of **18**−**21**, as described in Scheme 1 for the (*R*)-prolinol series. The same holds true for the series of compounds derived from (*S*)-prolinol.

The yield of (*R*)-2-vinylpyrrolidine-1-carboxylic acid benzyl ester (**9**) was increased from 47 to 85% by using the base KN-

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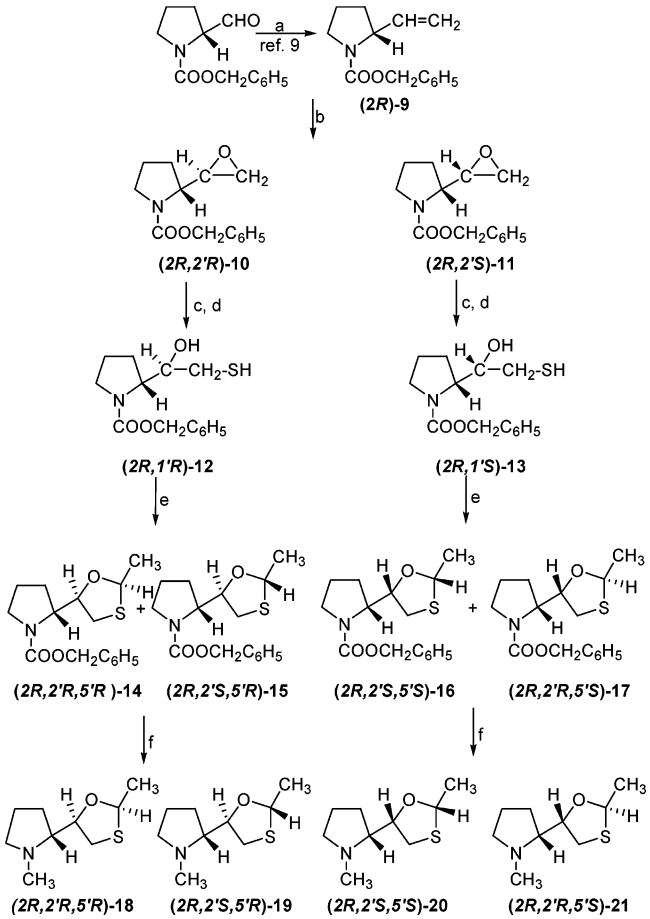
Scheme 1[a]

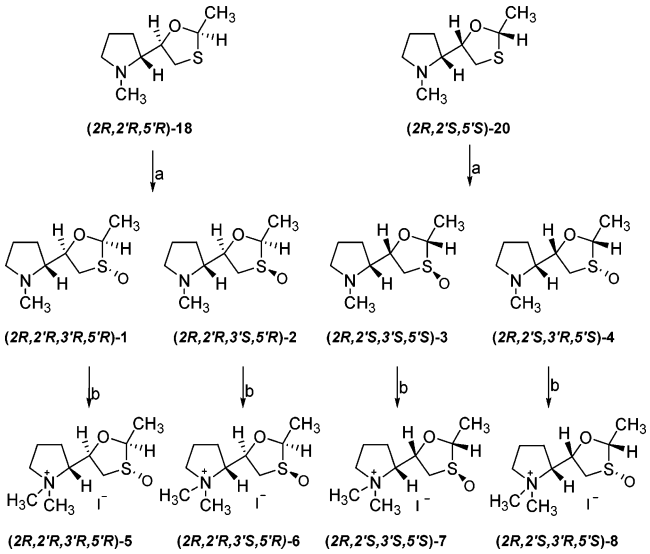


[a] (a) $KN(TMS)_2$, Ph_3PCH_3Br; (b) *m*-CPBA, chromatographic separation; (c) CH_3COSH; (d) HCl, MeOH; (e) *p*-toluensulfonic acid, $CH_3CH(OMe)_2$; (f) $LiAlH_4$, chromatographic separation. For the synthesis of the compounds of the *S*-series, starting from (**2*S***)-**9**, the same procedure was followed.

$(TMS)_2$ in the Wittig reaction.[13] Epoxides **10** and **11** were obtained by reaction with *m*-CPBA as previously described,[9] but now the two diastereoisomers (2*R*,2′*R*)-**10** and (2*R*,2′*S*)-**11** were isolated by flash chromatographic separation on silica gel and characterized; their absolute configuration at C2′ was attributed on the basis of the C2′ configuration[9] of the compounds **18**−**21** obtained from each isomer. In this respect, according to previous reports,[14] we found that each epoxide was a mixture of rotamers (^1H and ^{13}C NMR evidence).

The opening of epoxides **10** and **11** with thioacetic acid and subsequent hydrolysis of the thioesters under acidic conditions, gave (2*R*,1′*R*)-**12** and (2*R*,1′*S*)-**13**, which could thus be completely characterized. Cyclization of each mercapto alcohol (**12**, **13**) afforded a diastereomeric mixture of cis and trans isomers—**14** and **15** from **12**, **16** and **17** from **13**—that were not separated but reduced as such with $LiAlH_4$ to give compounds **18**−**21**. Column chromatography on Al_2O_3 allowed separation of the two mixtures to obtain two cis isomers, **18** and **20**, and two trans isomers, **19** and **21**, in a 5:1 ratio (NMR). The inefficient chromatographic separation that in our hands resisted any improvement, allowed isolation of only a small amount of trans isomers. As a consequence, the following sulfoxidation reaction was performed only on the cis isomers **18** and **20** as shown in Scheme 2 for the 2*R* series. Each isomer was oxidized with H_2O_2 to give the expected mixture of diastereomeric sulfoxides **1**−**4** that were separated by column chromatography and then transformed into the corresponding methyl iodides **5**−**8** with

Scheme 2[a]



[a] (a) H_2O_2, CH_3COOH, chromatographic separation; (b) CH_3I. For the synthesis of the compounds of the 2*S*-series the same procedure was followed

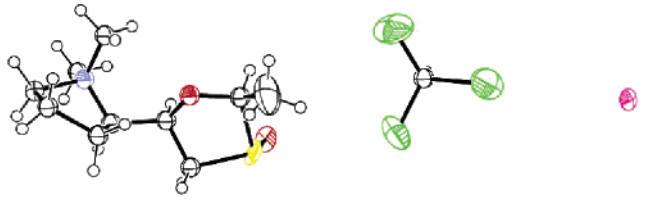


Figure 1. Thermal ellipsoid plot (30% ellipsoids) of compound (−)-**5**.

CH_3I. As expected, the same reaction sequence carried out on the (2*S*)-derivatives yielded comparable results.

The absolute configuration of 1,3-oxathiolane 3-sulfoxide compounds was established on the basis of the X-ray crystallography of the methiodide (−)-**5** (whose crystallographic structure is shown in Figure 1) and of the 1D and 2D ^1H NMR spectra, exploiting the fact that all *cis*-sulfoxides (where cis and trans is referred to the relationship with the substituent on C5′) show fairly similar spectra, as do *trans*-sulfoxides. NMR experiments were performed on the tertiary amines, but their results can obviously be extended to the corresponding methiodide.

Pharmacology. Muscarinic receptor affinity was evaluated in CHO cells expressing the five human muscarinic subtypes (hm1−hm5). Functional activity was evaluated in vitro on classical preparations, following previously reported methods:[15,16] rabbit stimulated vas deferens (M_1), guinea pig stimulated left atria (M_2), guinea pig ileum (M_3), and guinea pig lung strips (M_4). In this respect, it is appropriate to mention that, for a long time, the contraction of rabbit vas deferens was considered as an effect mediated by M_1-receptor subtypes, whereas more recent studies attribute the same effect to an M_4-activation.[17,18] Therefore, since the pharmacological characterization of the receptor subtype involved does not appear to be clearly established, in this work the rabbit vas deferens will be considered a putative M_1 subtype. Carbachol, arecaidine propargyl ester (APE), and 4-Cl-McN-A-343 were used as reference compounds. Results are expressed as pK_i values (affinity), as pD_2 (pED_{50}; agonist potency), or as pK_b (antagonist affinity) and are reported in Table 1. The affinity constants (K_a) of (−)-**5**, (−)-**7**, (+)-**8**, which showed functional activity as agonists, were evaluated using the method of Waud for partial agonists,[19]

Table 1. Binding Affinity and Functional Selectivity of Compounds **1**−**8**

	binding affinity[a] pK_i±SEM)					functional activity[b]					
							guinea pig				
						rabbit vas deferens	atrium (force) (M_2)		ileum (M_3)		lung (M_4)
compd	hm1	hm2	hm3	hm4	hm5	(M_1)[c] pD_2 [pK_b]	α	pD_2 [pK_b]	α	pD_2 [pK_b]	pD_2 [pK_b]
(−)-(2*R*,2′*R*,3′*R*,5′*R*)-**1**	<4	<4	<4	<4	<4	–	–	–	–	–	–
(+)-(2*R*,2′*R*,3′*S*,5′*R*)-**2**	<4	<4	<4	<4	<4	–	–	–	–	–	–
(+)-(2*R*,2′*S*,3′*S*,5′*S*)-**3**	<4	<4	<4	<4	<4	–	–	–	–	–	–
(+)-(2*R*,2′*S*,3′*R*,5′*S*)-**4**	<4	<4	<4	<4	<4	–	–	–	–	–	–
(−)-(2*R*,2′*R*,3′*R*,5′*R*)-**5**	<4	5.03(0.14)	<4	<4	<4	[<5]	0.58(0.03)	6.11(0.15)	1	5.58(0.08)	[<5]
(+)-(2*R*,2′*R*,3′*S*,5′*R*)-**6**	<4	<4	<4	<4	<4	–	–	–	–	–	–
(+)-(2*R*,2′*S*,3′*S*,5′*S*)-**7**	<4	4.09(0.11)	<4	<4	4.24(0.13)	[<5]	0	[<5]	0	[<5]	[<5]
(−)-(2*R*,2′*S*,3′*R*,5′*S*)-**8**	<4	<4	<4	<4	<4	[<5]	0	[<5]	0	[<5]	[<5]
(+)-(2*S*,2′*S*,3′*S*,5′*S*)-**1**	<4	4.06(0.13)	<4	<4	<4	[<5]	0	[<5]	0	[<5]	[<5]
(−)-(2*S*,2′*S*,3′*R*,5′*S*)-**2**	<4	<4	<4	<4	<4	-	-	-	-	-	-
(−)-(2*S*,2′*R*,3′*R*,5′*R*)-**3**	<4	<4	<4	<4	<4	[<5]	0	[<5]	0	[<5]	[<5]
(−)-(2*S*,2′*R*,3′*S*,5′*R*)-**4**	<4	4.05(0.12)	<4	<4	<4	[<5]	0	[<5]	0	[<5]	[<5]
(+)-(2*S*,2′*S*,3′*S*,5′*S*)-**5**	<4	4.11(0.16)	<4	<4	<4	[<5]	0	[<5]	0	[<5]	[<5]
(−)-(2*S*,2′*S*,3′*R*,5′*S*)-**6**	<4	4.54(0.17)	<4	<4	<4	[<5]	0	[<5]	0	[<5]	[<5]
(−)-(2*S*,2′*R*,3′*R*,5′*R*)-**7**	4.03(0.08)	4.81(0.11)	4.47(0.06)	4.33(0.06)	4.47(0.13)	[<5]	0.56(0.06)	6.37(0.10)	0.54(0.05)	4.62(0.15)	[<5]
(+)-(2*S*,2′*R*,3′*S*,5′*R*)-**8**	<4	4.29(0.12)	<4	<4	<4	[<5]	0.41(0.03)	7.65(0.15)	0	[<5]	[<5]
carbachol	4.42(0.09)	5.91(0.06)	4.36(0.10)	5.20(0.07)	4.16(0.07)	–	1	7.33(0.08)	1	6.68(0.01)	5.43[d](0.03)
APE	5.91(0.05)	7.06(0.09)	6.07(0.04)	6.01(0.05)	6.03(0.04)	7.14[d](0.18)	1	8.67(0.04)	1	7.64(0.09)	5.56[d](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)	6.33[d](0.06)	–	–	–	–	–

[a] Binding affinity on cloned human muscarinic receptors expressed in CHO cells. The values represent the mean (SEM) of at least three experiments. [b] Evaluated as reported in the experimental part 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. [c] The presence of the M_1 subtype in rabbit vas deferens has been questioned in favor of the M_4 subtype; this attribution must then be considered putative. [d] The intrinsic activity of the compounds is 1.

Table 2. Potency and Affinity of Compounds **5**, **7**, and **8**

	guinea pig atrium (force)[a] (M_2)				guinea pig ileum (M_3)			
compd	α[b]	pD_2 ± SEM	pK_a ± SEM	K_a/ED_{50}[c]	α[b]	pD_2 [pK_b] ± SEM	pK_a ± SEM	K_a/ED_{50}[c]
(−)-**5**	0.58 ± 0.03	6.11 ± 0.15	5.11 ± 0.18	12	1.00 ± 0.08	5.58 ± 0.08	4.87 ± 0.20	7
(−)-**7**	0.56 ± 0.06	6.37 ± 0.10	4.64 ± 0.18	58	0.54 ± 0.05	4.62 ± 0.15	4.59 ± 0.16	1
(+)-**8**	0.41 ± 0.03	7.65 ± 0.15	6.13 ± 0.20	84	0	[<5]	–	–
(−)-(2*R*,3*R*,5*R*)-**B**[d]	0.40 ± 0.03[e]	6.66 ± 0.05	5.22 ± 0.08	28	1.20 ± 0.09[e]	6.95 ± 0.05	5.38 ± 0.05	37
carbachol	1	7.33 ± 0.08	6.02 ± 0.18	9	1	6.68 ± 0.01	4.79 ± 0.03	51

[a] The compounds do not show any effect on frequency. [b] α represents Ariens's intrinsic activity of the agonist. [c] K_a/ED_{50} represents an estimate of Furchgott's efficacy of the agonist. [d] See ref 36. [e] Relative efficacy (see ref 37).

and the results are reported in Table 2, together with that of carbachol, evaluated with the method of Furchgott[20] for full agonists and of the parent compound (−)-(2*R*,3*R*,5*R*)-**B**.

Results and Discussion

It is immediately apparent from the binding data, in the left part of Table 1 that sulfoxides **1**−**8** possess undetectable affinity for cloned human muscarinic subtypes. Compared to the parent 1,3-oxathiolanes **C** (Chart 1),[12] sulfoxides show a sharp drop in affinity: their pK_is are below 4 and only in the case of the hm2 subtype do a few of them showed a modest affinity, i.e., the case of (−)-**5**, (−)-**6**, (−)-**7**, (+)-**8** (pK_i = 5.03, 4.54, 4.81, 4.29, respectively). This was a frustrating result indeed, but we knew, from the literature and from our previous research,[12] that binding affinities for cloned receptor subtypes do not always accurately predict the functional behavior of muscarinic ligands.

As a matter of fact, it is well-documented that partial muscarinic agonists, such as talsaclidine and sabcomeline,[21,22] do not show subtype selectivity in binding studies, while they do show M_1 selectivity in functional assays. In particular, it has been reported that talsaclidine shows only minor differences in affinity among hm1−hm5 receptor subtypes, while in functional assays it is more potent and efficacious in cells expressing M_1 receptors, slightly less for M_3, and inactive for M_2.[22] There are several factors that might explain the discrepancies between binding and functional studies,[23] in particular when cloned human receptors expressed in CHO cells, which represent an artificial system loosely related to the tissues used for functional assays, are used for binding studies. The agonist-induced receptor trafficking concept proposed by Kenakin[24] seems to be one of the most convincing. Here, different agonists are considered able to stabilize different conformations of the same receptor, which have the ability to selectively activate one G protein over another. Moreover, tissue variation in receptor density and signaling efficiencies may influence the expression of selective agonism over and above the inherent affinity differences; as a consequence, receptor agonists may exhibit "functional" rather than absolute subtype selectivity.[25] Another finding that can be at the origin of such discrepancies has recently been individuated in the presence in tissues of receptor homo-oligomers and/or hetero-oligomers that may possess different affinity for some ligands with respect to the monomers mainly present in binding experiments.[26,27]

Therefore, we tested some selected compounds on functional models of muscarinic receptors, namely M_1−M_4 subtypes; the results are reported in the right side of Table 1. It can be seen that, in general, the poor affinity of sulfoxides for muscarinic receptors is confirmed and the compounds are inactive as agonists while being, in several cases, poor antagonists (pK_b < 5). However, three out of four compounds that showed some affinity for the hm2 subtype were endowed with functional activity [(−)-**5**, (−)-**7**, (+)-**8**]; the fourth compound [(−)-**6**] did not confirm its modest hm2 selectivity and lacks any functional activity on M_1−M_4 subtypes. Compound (−)-**5**, which in the binding test appeared to be the most promising in terms of affinity and selectivity, is, in functional assays, a partial agonist

with modest selectivity for M_2 versus M_3 receptors ($pD_2 = 6.11$ and 5.58, respectively) where it behaves as a full agonist. On the contrary, compound (−)-**7**, which showed some affinity for all five cloned receptors resulted to be fairly selective, as it is a poor antagonist on M_1 and M_4 subtypes and is an agonist 2 orders of magnitude more potent on M_2 with respect to M_3 receptors ($pD_2 = 6.37$ and 4.62, respectively). Compound (+)-**8** emerged as the most interesting and intriguing one; indeed, even if in binding assays it showed a modest affinity for the hm2 subtype, much to our surprise, in functional assays compound (+)-**8** resulted to be a fairly potent partial agonist on the M_2 subtype ($pD_2 = 7.65$) while being a poor antagonist on the other subtypes.

Trying to find an explanation for these results, we have evaluated the affinity constants of these three compounds using the method of Waud for partial agonists;[19] the results are reported in Table 2.

It can be seen that, in the case of compounds (−)-**5** and (−)-**7**, affinities evaluated in functional assays are in agreement with those obtained in binding studies. This suggests that differences between binding and functional data can be explained on the basis of efficacy and, from molecular point of view, by one of the mechanisms discussed above.

This is not the case for compound (+)-**8** that, being inactive on the other subtypes, shows an affinity on guinea pig atrium (force) that is some 2 orders of magnitude larger than that found in binding studies (hm2, $pK_i = 4.29$; M_2, $pK_a = 6.13$). At the moment, we do not have an explanation for this fact, and more studies, which are under way, will be necessary to understand the situation. However, one can speculate that this difference could be related to the presence in the guinea pig atrium tissue of oligomeric receptors showing for compound (+)-**8** an affinity different from that of the monomer usually present in binding experiments. Whatever the case, compound (+)-**8** is a new, potent, partial agonist showing remarkable functional selectivity for M_2 muscarinic receptor subtypes that may represent a useful pharmacological tool.

Any attempt to relate stereochemistry to binding and functional activity is frustrated by the lack of activity of most of the compounds studied. More information will hopefully be collected by an eudismic analysis, which is under way, on the set of compounds described in this and in previous papers on the subject[9,12,28−30] (shown in Chart 1). Only a few qualitative considerations can be made. Two of the most affinitive compounds on hm2 receptors [(−)-**5** and (−)-**7**], as far as the 1,3-oxathiolane moiety is concerned, show the same stereochemistry (2′*R*,3′*R*,5′*R*) of the most potent compounds of the 1,3-oxathiolane (2*R*,5*R*)[30] and 1,3-oxathiolane 3-oxide (2*R*,3*R*,5*R*)[29] series (**A** and **B**, Chart 1). These two compounds are also among the few showing functional activity on the M_2 receptor model. The situation appears to be more complicated when the comparison is made with the 1,3-oxathiolanylpyrrolidines that we have recently described (**C**, Chart 1).[12] In this case, even if the most affinitive compounds for the hm2 subtype belong to the 2′*R*,5′*R* series, compounds with fairly good affinity were found also in the 2′*S*,5′*S* series. The position of compound (+)-**8** is, once again, peculiar, since the absolute stereochemistry of its 1,3-oxathiolane ring is 2′*R*,3′*S*,5′*R*. It is hoped that eudismic analysis[28] will cast some light on the whole matter. At the moment, it can be said that the introduction of more sterogenic centers into 1,3-oxathiolanes disturbs the interaction with muscarinic receptor subtypes, resulting in hm2 subtype selectivity, but the centers complicate the interpretation of the results in terms of stereochemistry.

Experimental Section

Chemistry. All melting points were taken on a Büchi apparatus and are uncorrected. NMR spectra were recorded on a Gemini 200 spectrometer (200 MHz for ^{1}H NMR, 50.3 MHz for ^{13}C), and on Brucker 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). When necessary, chromatographic separations were performed on an Al_2O_3 column by gravity chromatography (aluminum oxide 90 standardized, Merck). Yields are given after purification, unless differently stated. Where analyses 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 ±0.002°). When reactions were performed under anhydrous conditions, the mixtures were maintained under nitrogen. Compounds were named following IUPAC rules as applied by Beilstein-Institute AutoNom (version 2.1) software for systematic names in organic chemistry.

(*R*)-2-Vinylpyrrolidine-1-carboxylic Acid Benzyl Ester (*R*)-9. Potassium bis(trimethylsilyl)amide (1.01 g, 5.06 mmol) was added to a solution of methyltriphenylphosphonium bromide (1.81 g, 5.06 mmol) in 30 mL of anhydrous THF at room temperature and the mixture was stirred at room temperature for 1 h and at −78 °C for 10 min. A solution of (*R*)-phenylmethyl 2-formylpyrrolidine-1-carboxylate (0.59 g, 2.53 mmol)[9] in THF (5 mL) was then added and the whole was stirred at −78 °C for 10 min and for 2 h at room temperature. Then methanol (9 mL) and aqueous, saturated Rochelle salt were added, and stirring was continued at room temperature for 30 min. The organic compounds were extracted with ether and the organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. Then Ph_3PO was eliminated with hexane and the desired alkene obtained in 85% yield.[9]

The same procedure was applied to the synthesis of the (*S*)-enantiomer (*S*)-**9** that was obtained in 85% yield.

(2*R*,2′*R*)-Phenylmethyl 2-Oxiran-2-ylpyrrolidine-1-carboxylate (2*R*,2′*R*)-10 and (2*R*,2′*S*)-Phenylmethyl 2-Oxiran-2-ylpyrrolidine-1-carboxylate (2*R*,2′*S*)-11. To a solution of 0.5 g (2.16 mmol) of olefin (*R*)-**9** in dry CH_2Cl_2 at 0 °C was added a solution of 0.75 g (4.32 mmol) of *m*-chloroperbenzoic acid in dry CH_2Cl_2. The mixture was stirred for 20 h at room temperature, ethyl acetate was added, and the solution washed consecutively with saturated $NaHCO_3$, 10% $NaHSO_3$, saturated $NaHCO_3$, water, and brine. The organic phase was then dried over Na_2SO_4, filtered, and evaporated to dryness, yielding 0.46 g of product as a mixture of two diastereoisomers. Flash chromatography (hexane:ether 5:2) afforded 0.2 g (0.81 mmol, 38% yield) of **(2*R*,2′*R*)-10** and 0.11 g (0.45 mmol, 21% yield) of (**2*R*,2′*S*)-11**.

(2*R*,2′*R*)-10. ^{1}H NMR ($CDCl_3$) 400 MHz δ (ppm): 1.80−1.97 (m, 4H, CH_2CH_2), 2.51 and 2.81 (2 bs, 1H, CH*H*O), 2.67 and 2.86 (2 bs, 1H, C*H*HO), 2.91 and 3.05 (2 bs, 1H, CHO), 3.41−3.57 (m, 2H, CH_2N), 3.61 and 3.74 (2 bs, 1H, CHN), 5.08−5.21 (m, 2H, CH_2Ph), 7.30−7.41 (m, 5H, aromatics). ^{13} C NMR ($CDCl_3$) 100 MHz δ (ppm): 23.20 and 24.09 (CH_2), 27.32 and 28.65 (CH_2), 46.78 and 47.21 (CH_2−N), 47.45 and 47.63 (CH_2O), 52.54 and 52.95 (CHO), 58.52 and 59.12 (CHN), 66.78 and 67.17 (CH_2Ph), 127.82, 127.97, 128.18, 128.25, and 128.51 (CH aromatics), 136.53 and 136.81 (C aromatics), 155.22 and 155.38 (C=O). $[\alpha]^{20}_D = +15.8$ (c 1.0, $CHCl_3$).

(2*R*,2′*S*)-11. ^{1}H NMR ($CDCl_3$) 400 MHz δ (ppm): 1.80−2.08 (m, 4H, CH_2CH_2), 2.42−2.53 (m, 1H, C*H*HO), 2.59−2.72 (m, 1H, CH*H*O), 2.97−3.10 (m, 1H, CHO), 3.36−3.54 (m, 2H, CH_2N), 4.12 and 4.24 (2 bs, 1H, CHN), 5.08−5.23 (m, 2H, CH_2Ph), 7.26−7.42 (m, 5H, aromatics). ^{13} C NMR ($CDCl_3$) 100 MHz δ (ppm): 23.52 and 24.31 (CH_2), 28.40 and 28.89 (CH_2), 44.36 (CH_2N), 46.96 and 47.21 (CH_2O), 53.87 (CHO), 56.37 (CHN), 66.84 (CH_2Ph), 127.82, 127.95 and 128.46 (CH aromatics), 136.82 (C aromatic), 154.65 and 155.0 (C=O). $[\alpha]^{20}_D = +68.9$ (c 1.0, $CHCl_3$).

(2*S*,2′*S*)-Phenylmethyl 2-Oxiran-2-ylpyrrolidine-1-carboxylate (2*S*,2′*S*)-10 and (2*S*,2′*R*)-Phenylmethyl 2-Oxiran-2-ylpyrrolidine-1-carboxylate (2*S*,2′*R*)-11. Using the same procedure described above, starting from 4.71 g (20.4 mmol) of olefin (*S*)-**9** and 7.03 g (40.7 mmol) of *m*-chloroperbenzoic acid, 4.95 g of a mixture of two diastereoisomers (**10** and **11**) was obtained. Flash chromatography (hexane:ether 5:2) afforded 1.35 g (27% yield, 5.46 mmol) of (2*S*,2′*S*)-**10**, 0.44 g (8.7% yield,1.78 mmol) of (2*S*,2′*R*)-**11**.

(2*S*,2′*S*)-10. ^{1}H NMR ($CDCl_3$) 400 MHz and ^{13}C NMR ($CDCl_3$) 100 MHz spectra are identical to the spectra of the enantiomer. $[\alpha]^{20}_D = -15.8$ (*c* 1.0, $CHCl_3$).

(2*S*,2′*R*)-11. ^{1}H NMR ($CDCl_3$) 400 MHz and ^{13}C NMR ($CDCl_3$) 100 MHz spectra are identical to the spectra of the enantiomer. $[\alpha]^{20}_D = -68.9$ (*c* 1.0, $CHCl_3$).

(2*R*,1′*R*)-Phenylmethyl 2-(1-Hydroxy-2-mercaptoethyl)pyrrolidine-1-carboxylate (2*R*,1′*R*)-12. (2*R*,2′*R*)-**10** (0.75 g, 3.03 mmol) was reacted with 2 equiv of thioacetic acid for 20 h at 60 °C under nitrogen. The excess of thioacetic acid was removed under vacuum, and the residue was dissolved in $CHCl_3$ and washed twice with saturated Na_2CO_3 and twice with water. The organic phase was dried over Na_2SO_4, filtered, and evaporated to afford **(2*R*,1′*R*)-phenylmethyl 2-(2-acetyl-sulfanil-1-hydroxyethyl)pyrrolidine-1-carboxylate**, which was used as such for the next reaction. ^{1}H NMR ($CDCl_3$) 200 MHz δ (ppm): 1.83−2.10 (m, 5H, CH_2CH_2 + 1H), 2.33 (s, 3H, $COCH_3$), 2.62−2.83 (m, 1H), 3.10−3.22 (m, 1H), 3.29−3.43 (m, 1H), 3.55−3.71 (m, 1H), 3.77−4.11 (m, 1H), 4.14−4.25 (m, 0.5H), 4.33−4.43 (m, 0.5H), 5.12−5.27 (m, 2H, CH_2Ph), 7.29−7.45 (m, 5H, aromatics).

The product obtained was dissolved in 5.6 mL of CH_3OH, and 0.22 mL of concentrated HCl was added. The mixture was kept at 60 °C for 5 h and then the solvent was evaporated under vacuum to afford 0.84 g (yield 98%) of (2*R*,1′*R*)-**12**. ^{1}H NMR ($CDCl_3$) 200 MHz δ (ppm): 1.70−2.02 (m, 5H, CH_2CH_2 + 1H), 2.52−2.63 (m, 2H, CH_2S), 2.81 (bs, 1H), 3.29−3.43 (m, 1H), 3.52−3.68 (m, 1H), 3.75−3.84 (m, 1H), 3.94−4.07 (m, 1H), 5.15 (s, 2H, CH_2-Ph), 7.31 (s, 5H, aromatics). $[\alpha]^{20}_D = +48.5$ (*c* 1.0, $CHCl_3$).

(2*R*,1′*S*)-Phenylmethyl 2-(1-hydroxy-2-mercaptoethyl)pyrrolidine-1-carboxylate (2*R*,1′*S*)-13. Using the same procedure described for (2*R*,1′*R*)-**12**, starting from 0.75 g (3.03 mmol) of (2*R*,2′*S*)-**11** and 2 equiv of thioacetic acid, **(2*R*,1′*S*)-phenylmethyl 2-(2-acetyl-sulfanil-1-hydroxyethyl)pyrrolidine-1-carboxylate** was obtained, which was used as such for the next reaction. ^{1}H NMR ($CDCl_3$) 200 MHz δ (ppm): 1.74−2.10 (m, 4H, CH_2CH_2), 2.36 (s, 3H, $COCH_3$), 2.86−3.00 (m, 1H), 3.16−3.47 (m, 2H), 3.51−3.78 (m, 2H), 3.89−4.02 (m, 1H), 4.85−5.04 (m, 1H), 5.15 (s, 2H, CH_2Ph), 7.29−7.43 (m, 5H, aromatics).

After cleavage of the acetylsulfanil function as described above, 0.8 g (92% yield) of (2*R*,1′*S*)-**13** was obtained. ^{1}H NMR ($CDCl_3$) 200 MHz δ (ppm): 1.62−2.12 (m, 5H, CH_2CH_2 + 1H), 2.41−2.59 (m, 1H), 2.70−2.88 (m, 1H), 3.30−3.48 (m,1H), 3.55−3.73 (m, 2H), 3.88 (bs, 1H), 4.10−4.19 (m, 1H), 5.16 (s, 2H, CH_2Ph), 7.31 (s, 5H, aromatics). $[\alpha]^{20}_D = +83.3$ (*c* 1.0, $CHCl_3$).

(2*S*,1′*S*)-Phenylmethyl 2-(1-Hydroxy-2-mercaptoethyl)pyrrolidine-1-carboxylate (2*S*,1′*S*)-12 and (2*S*,1′*R*)-Phenylmethyl 2-(1-Hydroxy-2-mercaptoethyl)pyrrolidine-1-carboxylate (2*S*,1′*R*)-13. Using the same procedure already described, the enantiomers (2*S*,1′*S*)-**12** [yield 98%, $[\alpha]^{20}_D = -48.5$ (*c* 1.0, $CHCl_3$)].and (2*S*,1′*R*)-**13** [yield 98%, $[\alpha]^{20}_D = -83.3$ (*c* 1.0, $CHCl_3$)], were obtained. Their ^{1}H NMR spectra are identical to the spectra of the enantiomers.

(2*R*,2′*R*,5′*R*)-1-Methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine (2*R*,2′*R*,5′*R*)-18 and (2*R*,2′*S*,5′*R*)-1-Methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine (2*R*,2′*S*,5′*R*)-19. To a solution of 0.79 g (2.81 mmol) of (2*R*,1′R)-**12** in 15.8 mL of 2-propanol was added 0.083 g (0.44 mmol) of *p*-toluensulfonic acid monohydrate. To this mixture, under nitrogen atmosphere, 2.4 mL (22.5 mmol) of acetaldehyde dimethylacetal was added. After 5 h at 55 °C the solvent was evaporated and the residue was dissolved in diethyl ether. The organic layer was washed with water, dried over Na_2SO_4, filtered, and evaporated to afford 0.79 g (2.59 mmol, 92% yield) of a mixture of two diastereoisomers (**14** and **15**). ^{1}H NMR ($CDCl_3$) 200 MHz δ (ppm): 1.56 (d, 3H, CHCH_3, $J_{H-Me} = 5.6$ Hz), 1.79−2.18 (m, 4H, CH_2CH_2), 2.75−3.14 (m, 2H, CH_2S), 3.39−3.54 (m, 2H), 4.00−4.12 (m, 1.5H), 4.20−4.34 (m, 0.5H), 5.05−5.23 (m, 3H, CH_2Ph + C*H*CH_3), 7.36 (s, 5H, aromatics).

A solution of 0.79 g (2.59 mmol) of the diastereomeric mixture (**14** and **15**), dissolved in the minimum amount of anhydrous THF, was added dropwise to a suspension of 0.62 g (16.3 mmol) of $LiAlH_4$ in anhydrous THF at −18 °C under nitrogen. The mixture was allowed to reach room temperature and after 4 h was treated with brine and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to afford an oily mixture of diastereoisomers (2*R*,2′*R*,5′*R*)-**18** and (2*R*,2′*S*,5′*R*)-**19**. The chromatographic separation on Al_2O_3 (eluent $CHCl_3$/petroleum ether 3:7) afforded the cis isomer (2*R*,2′*R*,5′*R*)-**18** and the trans isomer (2*R*,2′*S*,5′*R*)-**19** in a 30:1 ratio.

(2*R*,2′*S*,5′*S*)-1-Methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine (2*R*,2′*S*,5′*S*)-20 and (2*R*,2′*R*,5′*S*)-1-Methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine (2*R*,2′*R*,5′*S*)-21. Using the same procedure described for (2*R*,2′*R*,5′*R*)-**18** and *(*2*R*,2′*S*,5′*R*)-**19** starting from 1.31 g (4.66 mmol) of (2*R*,1′*S*)-**13**, 1.24 g (yield 87%) of a mixture of two diastereoisomers (**16** and **17**) was obtained. ^{1}H NMR ($CDCl_3$) 200 MHz δ (ppm): 1.55 (d, 3H, CHCH_3, $J_{H-Me} = 5.8$ Hz), 1.78−2.10 (m, 4H, CH_2CH_2), 2.80−3.00 (m, 2H, CH_2S), 3.28−3.47 (m,1H), 3.48−3.74 (m, 1H), 4.05−4.40 (m, 2H), 5.03−5.26 (m, 3H, CH_2Ph + *CH*CH_3), 7.37 (s, 5H, aromatics).

The subsequent reduction with $LiAlH_4$ and chromatographic separation afforded the cis isomer (2*R*,2′*S*,5′*S*)-**20** and the trans isomer (2*R*,2′*R*,5′*S*)-**21** in a 30:1 ratio. ^{1}H NMR and ^{13}C NMR data of the trans isomers are reported in Table 3 (Supporting Information).

(2*S*,2′*S*,5′*S*)-1-Methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine (2*S*,2′*S*,5′*S*)-(18), (2*S*,2′*R*,5′*S*)-1-Methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine (2*S*,2′*R*,5′*S*)-(19), (2*S*,2′*R*,5′*R*)-1-Methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine (2*S*,2′*R*,5′*R*)-(20), and (2*S*,2′*S*,5′*R*)-1-Methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine (2*S*,2′*S*,5′*R*)-(21). Using the same procedure described above, starting from (2*S*,1′*S*)-**12** the diastereomeric mixture of **14** and **15** (yield 93%) was obtained, and starting from (2*S*,1′*R*)-**13**, the diastereomeric mixture of **16** and **17** (yield 88%) was obtained. Their ^{1}H NMR spectra are identical to those of the diastereomeric mixtures **14** and **15**, and **16** and **17**.

The subsequent reduction with $LiAlH_4$ and chromatographic separation afforded the cis isomers (2*S*,2′*S*,5′*S*)-**18** and (2*S*,2′*R*,5′*R*)-**20** and the trans isomers (2*S*,2′*R*,5′*S*)-**19** and (2*S*,2′*S*,5′*R*)-**21** in a 30:1 ratio.

The cis isomers have already been described.[9] ^{1}H NMR and ^{13}C NMR data of the trans isomers are reported in Table 3 (Supporting Information).

(−)-(2*R*,2′*R*,3′*R*,5′*R*)-1-Methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine 3-Oxide (−)-(2*R*,2′*R*,3′*R*,5′*R*)-(1) and (+)-(2*R*,2′*R*,3′*S*,5′*R*)-1-Methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine 3-Oxide (+)-(2*R*,2′*R*,3′*S*,5′*R*)-(2). A solution of 1 equiv of (2*R*,2′*R*,5′*R*)-**18** in CH_3COOH at 0 °C was added to 3.6 equiv of H_2O_2 (30%). After 50 min at 0 °C the mixture was made alkaline with 10% NaOH, extracted with $CHCl_3$, and dried over Na_2SO_4. Filtration and evaporation afforded a mixture of two diastereomeric sulfoxides (−)-**1** and (+)-**2** in 2:1 ratio (calculated from ^{1}H NMR). The oily mixture obtained was separated by flash chromatography (eluent NH_4OH/absolute ETOH/CH_2Cl_2/petroleum ether/Et_2O = 11.2:118:460:310:99). Anal. ($C_9H_{17}NO_2S$) C, H, N.

Their spectroscopic, chemical, and physical characteristics are reported in Tables 4−6 (Supporting Information).

(+)-(2*R*,2′*S*,3′*S*,5′*S*)-1-Methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine 3-Oxide (+)-(2*R*,2′*S*,3′*S*,5′*S*)-(3) and (+)-(2*R*,2′*S*,3′*R*,5′*S*)-1-Methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine 3-Oxide (+)-(2*R*,2′*S*,3′*R*,5′*S*)-(4). Using the same procedure described above, starting from (2*R*,2′*S*,5′*S*)-**20**, compounds (+)-**3** and (+)-**4** were obtained. Anal. ($C_9H_{17}NO_2S$) C, H, N. Their spectroscopic, chemical, and physical characteristics are reported in Tables 4−6 (Supporting Information).

(+)-(2*S*,2′*S*,3′*S*,5′*S*)-1-Methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine 3-oxide (+)-(2*S*,2′*S*,3′*S*,5′*S*)-(1), (−)-(2*S*,2′*S*,3′*R*,5′*S*)-1-Methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine 3-Oxide (−)-(2*S*,2′*S*,3′*R*,5′*S*)-(2), (−)-(2*S*,2′*R*,3′*R*,5′*R*)-1-Methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine 3-Oxide (−)-(2*S*,2′*R*,3′*R*,5′*R*)-(3), and (−)-(2*S*,2′*R*,3′*S*,5′*R*)-1-Methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine 3-Oxide (−)-(2*S*,2′*R*,3′*S*,5′*R*)-(4). The same oxidative procedure applied to the *S*-series afforded the enantiomers of compounds **1**−**4**. Anal. ($C_9H_{17}NO_2S$) C, H, N. Their spectroscopic, chemical, and physical characteristics are reported in Tables 4−6 (Supporting Information).

General Procedure for the Synthesis of Methiodides 5−8. An anhydrous ether solution of the suitable sulfoxide **1**−**4** was treated with an excess of methyl iodide and kept for 1 night at room temperature in the dark. The solid obtained was filtered, dried under vacuum, and recrystallized from absolute ethanol. Anal. ($C_{10}H_{20}NO_2SI$) C, H, N. Their chemical and physical characteristics are reported in Tables 7−9 (Supporting Information).

X-ray Structural Analysis of Compound (−)-5. $C_{11}H_{21}NO_2SCl_3I$, $M = 464.60$, orthorhombic, space group *Pna*21, $a = 6.839(1)$ Å, $b = 12.968(1)$ Å, $c = 21.557(2)$ Å, $V = 1911.9(4)$ Å^3, $Z = 4$ $D_c = 1.614$, $\mu = 18.029$ mm^{-1}, $F(000) = 920$. Analysis on a prismatic transparent single crystal was carried out with a Bruker CCD X-ray diffractometer at 150 K. Cu Kα radiation monochromated by crossed Gobel mirrors was used for cell parameter determination and data collection. The intensities of some equivalents were monitored during data collection to check the stability of the crystal: no loss of intensity was recognized. The integrated intensities, measured using the ω scan mode, were corrected for Lorentz and polarization effects. The number of reflections collected was 4173 with a $3.98 < \theta < 50.97°$ range; 1808 were independent, and the final R index was 0.1483 for reflections having $I > 2\sigma I$, and 0.1797 for all data. The data acquisition, integration, data reduction, and absorption correction were performed using SMART, SAINT, and SADABS programs.[31] Structure was refined using the full-matrix least squares on F^2 provided by SHELXL97.[32] In Figure 1 a thermal ellipsoid representation of (−)-**5** is shown

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 Neuroscience, University of Pisa, Italy). Growth medium consisted in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (Gibco, Grand Iland, NY), 100 units/mL each of penicillin G and 0.1 ng/mL streptomycin, 4 mM L-glutamine (Sigma Aldrich, Milano, Italy) and nonessential amino acids (Sigma Aldrich, Milano, Italy), and 50 μg/mL of 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 30s using an Ultra-Turrax (setting 35). The pellet was sedimented 17 000*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.[33] An aliquot was taken for the assessment of protein content according to the method of Bradford[34] 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 a 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 wells containing increasing concentration of unlabeled NMS (0.03−1000 nM) and at 0.075−0.2 nM in wells 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 mM); 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 a 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[35] to obtain the affinity constant (K_i) and the binding capacity (B_{max}).[12]

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. Following 30 min of washing, tissues were incubated with the antagonist for 60 min, and a new dose−response curve to the agonist was obtained. In the case of the agonist, following 30 min of washing, 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 addition, parallel experiments in which tissues did not receive any antagonist were run in order to check any variation in sensitivity. 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 Communities Council Directive of 24 November 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), $CaCl_2 \cdot 2H_2O$ (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 mM) 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 the method of Eltze.[16] 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 p-Cl-McN-A-343 was constructed.

Determination of Affinity Constants. Affinity constants (K_a, Table 1) were determined according to the method of Furchgott and Bursztyn[20] for full agonists ($\alpha = 1$) by means of a software computation process supplied by Dr. Roberto Gagliardi. Partial agonist values were determined according to Waud[19] by comparison of its concentration−response curve with that of APE. Several equieffective concentrations of partial agonist and APE were determined graphically.

Acknowledgment. The authors are thank Dott. Cristina Faggi for the X-ray structural analysis of compound **5**.

Supporting Information Available: Tables 3−9, X-ray crystallographic data of compound (−)-**5**, and elemental analysis results of compounds **1**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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