Brief Articles

Synthesis, Affinity Profile, and Functional Activity of Muscarinic Antagonists with a 1-Methyl-2-(2,2-alkylaryl-1,3-oxathiolan-5-yl)pyrrolidine Structure†

Silvia Dei,*,‡ Cristina Bellucci,‡ Michela Buccioni,§ Marta Ferraroni,|| Luca Guandalini,‡ Dina Manetti,‡ Elisabetta Martini,‡ Gabriella Marucci,§ Rosanna Matucci,⊥ Marta Nesi,⊥ Maria Novella Romanelli,‡ Serena Scapecchi,‡ and Elisabetta Teodori‡

Dipartimento di Scienze Farmaceutiche, Università di Firenze, Via U. Schiff 6, 50019 Sesto Fiorentino (FI), Italy, Dipartimento di Farmacologia Preclinica e Clinica, Università di Firenze, Viale G. Pieraccini 6, 50139 Firenze, Italy, Dipartimento di Scienze Chimiche, Università di Camerino, Via S. Agostino 1, 62032 Camerino (MC), Italy, Dipartimento di Chimica, Università di Firenze, Via della Lastruccia 5, 50019 Sesto Fiorentino (FI), Italy

Received November 29, 2006

Starting from a previously studied muscarinic ligand, characterized by a 1,3-oxathiolane nucleus, a new series of muscarinic antagonists were designed by increasing the stereochemical complexity of the molecules. A small library of enantiomeric and diastereomeric 2,2-diphenyl- and 2-cyclohexyl-2-phenyl substituted compounds was thus obtained. All the tested compounds show a high affinity toward cloned human muscarinic hm1−hm5 receptors expressed in CHO cells and a good antagonistic activity on functional assays, with a modest selectivity on rabbit vas deferens.

Introduction

Muscarinic receptors play an important role in several vital functions such as movement control, cognition, smooth muscle tone, and glandular secretion.[1] As a consequence of this vast array of actions, muscarinic drugs have the potential for therapeutic use in several pathological states.[2,3]

Five distinct subtypes of muscarinic receptors have been cloned (hm1−hm5), but only four have been satisfactorily characterized from a pharmacological point of view (M_1-M_4).[4] As a matter of fact, several ligands (mainly antagonists) do show some subtype selectivity,[2,5] but none displays high selectivity for one subtype to the relative exclusion of all others, possibly because of the high sequence conservation within the orthosteric domain across all five muscarinic subtypes.[6] As a consequence, the number of muscarinic drugs that have been introduced in therapy is relatively small[2] compared to the importance of this receptor in mammals. Currently, the development of extremely selective muscarinic drugs, both agonists and antagonists,[2,5,7] remains the main instrument for studying this class of receptors, as the detection of the molecular structure of protein G-coupled receptors remains a difficult task and homology models present obvious limits.

In the past few years we have been engaged in the synthesis and preliminary pharmacological characterization of muscarinic agonists characterized by a 1,3-oxathiolane scaffold.[8] More recently we have designed and studied new compounds of the same class characterized by several stereogenic centers.[9−11] We reasoned that highly chiral compounds would have the chance to selectively interact with the fairly conserved recognition sites of muscarinic receptor subtypes. Indeed, among the small library of agonists synthesized, we found some interesting M_2-selective compounds.

In the present paper, we report an extension of this approach to 1,3-oxathiolane derivatives carrying bulky substituents in position 2, such as compound (2*R*,5*R*)-**A** (Chart 1), which behave as potent, although not selective, muscarinic antagonists.[12,13] Accordingly, applying the frozen analog strategy,[14] we designed and synthesized the series of pyrrolidine derivatives **1**, **2**, and **3** and the corresponding dimethyl pirrolidinium iodides **4**, **5**, and **6** that carry two or three stereogenic centers; they are reported in Chart 1.

Chemistry

The key intermediates for the synthesis of the 1,3-oxathiolane derivatives **1**−**6** (shown in Scheme 1) were the thiol-alcohols (−)-**7**, (+)-**7**, (−)-**8**, and (+)-**8** obtained from commercially available (*S*)- and (*R*)-prolinol, according to the previously reported procedure.[9,11] The thiol derivatives were reacted with the suitable ketone to give the cyclic 1,3-oxathiolane compounds.

Ketones carrying bulky groups are known to be poorly reactive in ketalization reactions, so it was necessary to modify the procedures used for the synthesis of 2-monosubstituted 1,3-oxathiolanes.[9,11] After a few attempts with different catalysts and solvent and reaction conditions,[15,16] acceptable yields (around 50% in the case of diphenyl derivatives and 60% in the case of cyclohexylphenyl derivatives) were obtained by reacting the thiol-alcohols with the dimethyl ketals of benzophenone or cyclohexylphenyl ketone[17] in a 1:2 ratio in an anhydrous solvent with *p*-toluenesulfonic acid as the catalyst and using a Dean−Stark trap. As expected, cyclization of thiol-alcohols gave a single derivative in the case of benzophenone and a mixture of two diastereoisomers in the case of cyclohexylphenyl ketone. However, the diastereomeric thiol-alcohols showed quite different reactivity. While (−)-**7** and (+)-**7** gave

† Dedicated to Professor Fulvio Gualtieri on the occasion of his 70th birthday.

* To whom correspondence should be addressed. Phone: +39-055-4573689. Fax: +39-055-4573780. E-mail silvia.dei@unifi.it.

‡ Dipartimento di Scienze Farmaceutiche, Università di Firenze.

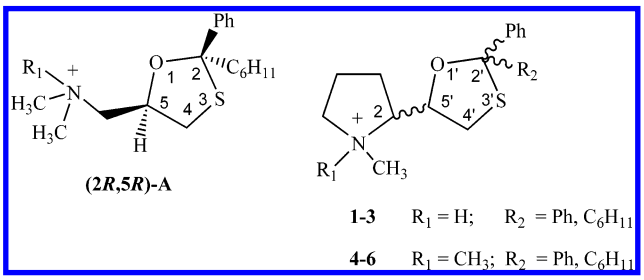
§ Dipartimento di Scienze Chimiche, Università di Camerino.

|| Dipartimento di Chimica, Università di Firenze.

⊥ Dipartimento di Farmacologia Preclinica e Clinica, Università di Firenze.

10.1021/jm061374r CCC: $37.00
Published on Web 02/17/2007

Chart 1. [a]



[a] Lead and newly designed compounds.

the cyclized compounds with acceptable yields, their diastereoisomers (−)-**8** and (+)-**8** afforded only very poor yields of the thioketals (around 5% yield) under a variety of reaction conditions. As a consequence, the following reactions were performed only on (−)-**7** and (+)-**7**.

Cyclization with benzophenone dimethyl ketal afforded compounds (−)-**9** and (+)-**9**, which were then reduced with $LiAlH_4$, yielding the methylated compounds (−)-**1** and (+)-**1**, respectively.

When the cyclohexylphenyl dimethyl ketal was used, each thiol-alcohol afforded a diastereoisomeric mixture of two isomers, both from (−)-**7** and from (+)-**7**. The mixtures were not separated at this step, but rather reduced with $LiAlH_4$ to give compounds **2** and **3**, which were conveniently separated by column chromatography to give (+)-**2** and (−)-**3** from (−)-**7** and the corresponding enantiomers (−)-**2** and (+)-**3** from (+)-**7**, in both cases, in a 88:12 ratio. The *N*-methyl derivatives **1**, **2**, and **3** were then transformed into the corresponding dimethyl pirrolidinium iodides **4**−**6** with CH_3I.

The absolute configuration of diphenyl derivatives (−)-**1** and (+)-**1**, which presented only two stereocenters, is the same as the parent compounds (−)-**7** and (+)-**7**, whose structure was already known.

In the case of 2-cyclohexylphenyl compounds, the relative configuration of the new stereocenter in position 2 was suggested by comparison of the properties of the two diastereomeric couples **2** and **3** with those of the parent diastereomeric couple **A**, whose stereochemistry was already known.[13] As found for (2*R**,5*R**)-**A** (Chart 1), the less-abundant diastereoisomer **3** was eluted, in this case too, as second in the chromatographic separation and represented only 15% of the diastereomeric mixture; moreover, analysis of its 1D and 2D ^1H and ^{13}C NMR spectra showed that the peaks relative to H5 (0.3 ppm) and C5 (3 ppm) were deshielded with respect to the corresponding signals of the **2** diastereoisomer, suggesting that, as happens in (2*R**,5*R**)-**A**, the phenyl ring of **3** is trans to the proton in **5**. On these bases, knowing the absolute configuration of the other stereocenters, it was possible to hypothesize the absolute configuration of the four optical isomers, as shown in Scheme 1. This attribution was eventually confirmed by the X-ray crystallography of (−)-**2** oxalate, which, as shown in Figure 1, is indeed 2*R*,2′*S*,5′*R*.

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, rabbit stimulated vas deferens (putative M_1), guinea pig stimulated left atria (M_2), guinea pig ileum (M_3), and guinea pig lung strips (putative M_4), following the protocols reported previously.[18] In this respect, it is necessary to point out 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.[4,19] Analogously, the validity of guinea pig lung strips as a M_4 model[20] has been questioned.[21] 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 and 4-Cl-McN-A-343 were used as agonists. Results are expressed as pK_i values (affinity) and as pK_b values calculated from the equation $pK_b = \log(DR-1) - \log[B]$, where DR is the ratio of ED_{50} values of agonist after and before treatment with one or two antagonist concentrations [*B*].[22] In selected cases, antagonist potency is expressed in terms of pA_2, estimated by Schild plots constrained to slope −1.0, as required by the theory.[23] Results are shown in Table 1, where the data of *N*-methylscopolamine and compound (2*R*,5*R*)-**A** (Chart 1) are reported for comparison.

Results and Discussion

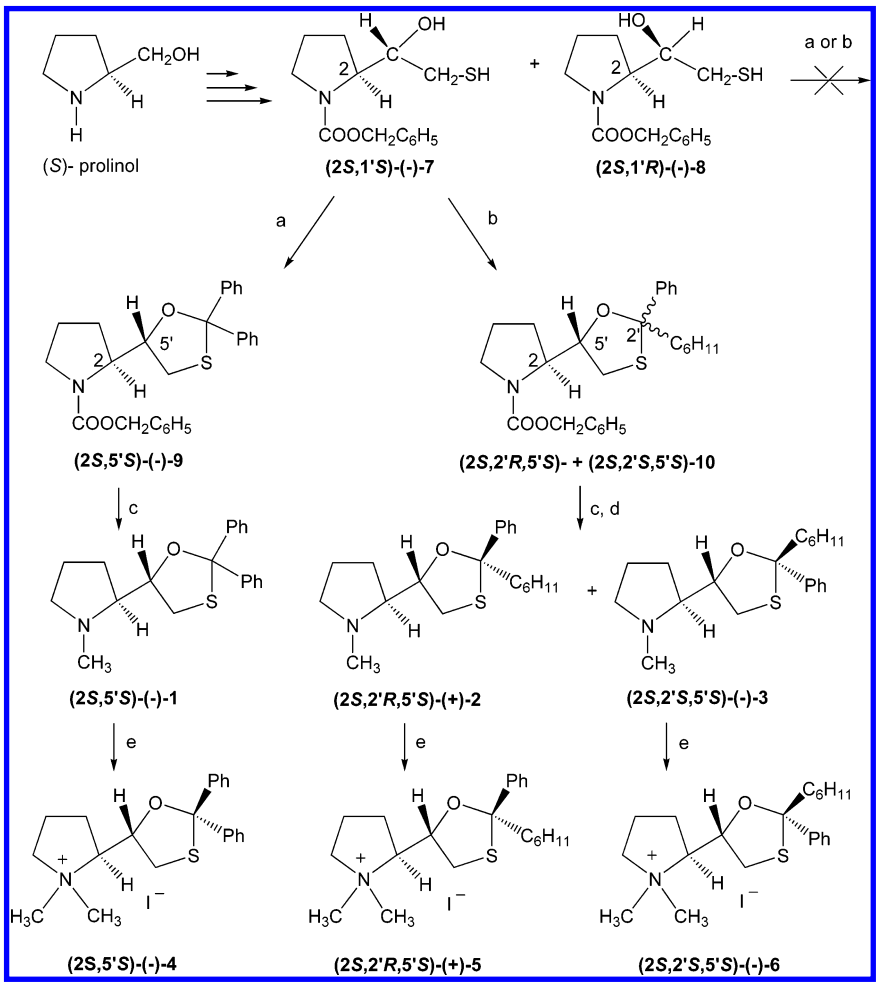
The results of binding studies and the functional activities of compounds **1**−**6** are reported in Table 1 and compared with *N*-methylscopolamine as a general reference and with compound (2*R*,5*R*)-**A**, which was the most interesting among the previously studied parent compounds.[12,13] Its pharmacological profile has been evaluated anew, using the same protocols of the new series.

From Table 1, it can be observed that all compounds bind with very high affinity to the five human muscarinic receptors. Affinity is consistently highest for the quaternary salts (**4**−**6**), but is rather good also for the tertiary bases (**1**−**3**). Accordingly, the amines are potent antagonists also in functional models, even if their potency is lower than that of the corresponding methyl iodides. From the same table it can be seen that the agreement between affinity and functional activity is good for the well characterized M_2 (guinea pig atrium) and M_3 (guinea pig ileum) models. Functional activity on rabbit vas deferens preparation (a putative M_1 model) agrees with both hm1 and hm4 binding, which leaves open the problem of the subtype present in this model. On the contrary, there is no correlation between functional activity on guinea pig lung preparation (a putative M_4 model)[20] and the binding data on hm4 as well as on the other human cloned subtypes; as shown in Table 1, this is also for the reference compound *N*-methylscopolamine and the parent compound (2*R*,5*R*)-**A**. The great difference between activity on guinea pig lung preparation and affinity on the five cloned human muscarinic subtypes (up to 3 orders of magnitude) is difficult to explain and casts doubts on the validity of the model, supporting the point of view of Roffel et al.[21]

Contrary to our expectations, the new compounds lack any appreciable selectivity in binding to human cloned muscarinic subtypes. In functional studies, however, some selectivity is observed, although less than 1 order of magnitude, concerning the rabbit vas deferens receptor model for 2,2-cyclohexylphenyl compounds (**2**, **3**, **5**, and **6**).

The role played by stereochemistry in affinity appears to be complex and may possibly be clarified using molecular modeling and eudismic analysis[24] approaches, which will be performed soon after the present series of 1,3-oxathiolane agonists and antagonists are completed with the synthesis of diphenyl and cyclohexylphenyl 3-sulfoxides. In the meantime, some interesting preliminary observations can be made. The first regards enantioselectivity, which is modest overall (in general eudismic ratio values, ER ≤ 10), as was the case for the parent compounds;[13] the highest enantioselectivity being that presented by compound **5**, both in binding (ER = 8 for hm1; ER = 14 for hm3; and ER = 30 for hm5) and functional studies (ER =

Scheme 1[a]



[a] Reagents: (a) benzophenone dimethyl ketal, anhydrous toluene, *p*-toluenesulfonic acid; (b) cyclohexylphenyl ketone dimethyl ketal, anhydrous toluene, *p*-toluenesulfonic acid; (c) anhydrous Et_2O, $LiAlH_4$; (d) chromatographic separation; (e) anhydrous Et_2O, CH_3I. The corresponding 2*R*-enantiomers were obtained from (*R*)-prolinol using the same pathways.

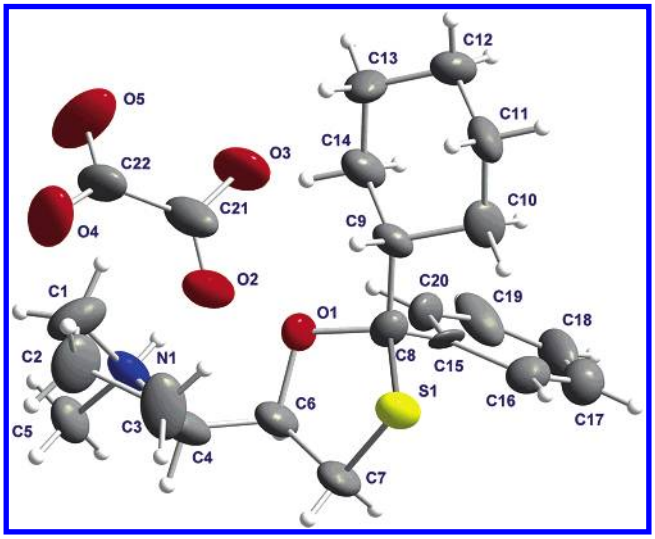


Figure 1. Thermal ellipsoid plot (50% ellipsoids) of compound (−)-**2** oxalate.

30 for rabbit vas deferens; ER = 7 for M_3; and ER = 28 for guinea pig lung). In general, in functional tests, enantioselectivity is greater on the rabbit vas deferens (**1**, ER = 5; **2**, ER = 56; **4**, ER = 12). In this respect, a very interesting observation is that, both in binding hm1 and in rabbit vas deferens functional studies, the *N*-methyl compound (+)-**1**, having a 2*R*,5′*R* stereochemistry, is the eutomer, whereas, in the case of the corresponding dimethyl pirrolidinium iodide, the eutomer (−)-**4** shows the opposite 2*S*,5′*S* stereochemistry. The same situation can be observed in the enantiomeric couple **2** with respect to the methyl iodide **5** and, as far as binding is concerned, for **3** with respect to **6**. Identification of the eutomers in the functional activity of **6** is not straightforward, suggesting that the free bases (**1**, **2**, and **3**) and the corresponding dimethyl pirrolidinium derivatives (**4**, **5**, and **6**) have different binding modes in the recognition site of the receptors, a fact that deserves further study.

In conclusion, the new series of synthesized antagonists shows very high affinity for all human cloned muscarinic receptors but, despite the presence of two or three stereogenic centers, subtype selectivity remains poor, with no major improvement with respect to the parent compound. It will be interesting to see what happens after the introduction of an additional stereogenic center (studies which are currently in progress).

Experimental Section

Chemistry. All melting points were taken on a Büchi apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer spectrum RX I FT−IR spectrophotometer in Nujol mull for solids and neat for liquids. NMR spectra were recorded on a Bruker Avance 400 spectrometer (400 MHz for ^1H NMR, 100 MHz for ^{13}C), and chromatographic separations were performed on a silica gel column by gravity chromatography (Kieselgel 40, 0.063−0.200

Table 1. Binding Parameters and Functional Activities of 1,3-Oxathiolane Derivatives

compounds		binding affinities[a] (pK_i±SE)					functional activities[b] (pK_b±SE)			
cmpd	stereochem.	hm1	hm2	hm3	hm4	hm5	rabbit vas deferens	guinea pig atrium (M_2)	guinea pig ileum (M_3)	guinea pig lung
(−)-**1**	2*S*,5′*S*	8.46 ± 0.085	8.31 ± 0.08	8.28 ± 0.08	8.48 ± 0.08	8.25 ± 0.07	7.93 ± 0.09	7.58 ± 0.19	8.05 ± 0.05	6.41 ± 0.11
(+)-**1**	2*R*,5′*R*	9.12 ± 0.055	8.56 ± 0.08	8.61 ± 0.10	8.70 ± 0.10	8.49 ± 0.07	8.66 ± 0.06	7.97 ± 0.17	8.44 ± 0.14	6.06 ± 0.10
(+)-**2**	2*S*,2′*R*,5′*S*	8.86 ± 0.06	8.56 ± 0.09	9.05 ± 0.11	8.63 ± 0.12	8.87 ± 0.09	7.41 ± 0.02	8.82 ± 0.14	8.92 ± 0.34	6.22 ± 0.17
(−)-**2**	2*R*,2′*S*,5′*R*	9.46 ± 0.06	8.97 ± 0.10	8.66 ± 0.11	9.00 ± 0.075	8.70 ± 0.08	9.16 ± 0.04	8.07 ± 0.20	8.55 ± 0.17	6.03 ± 0.16
(−)-**3**	2*S*,2′*S*,5′*S*	8.83 ± 0.06	8.37 ± 0.10	8.77 ± 0.08	8.92 ± 0.10	8.70 ± 0.08	8.32 ± 0.10	6.91 ± 0.09	7.70 ± 0.11	5.92 ± 0.27
(+)-**3**	2*R*,2′*R*,5′*R*	9.04 ± 0.06	8.35 ± 0.085	8.84 ± 0.07	8.90 ± 0.06	8.92 ± 0.06	7.95 ± 0.14	7.13 ± 0.11	7.65 ± 0.06	5.98 ± 0.11
(−)-**4**	2*S*,5′*S*	9.53 ± 0.08	9.33 ± 0.09	9.13 ± 0.10	9.33 ± 0.10	9.39 ± 0.07	9.56 ± 0.17	8.78 ± 0.15	9.16 ± 0.11	7.79 ± 0.07
(+)-**4**	2*R*,5′*R*	8.63 ± 0.05	8.47 ± 0.08	8.30 ± 0.10	8.46 ± 0.09	8.46 ± 0.06	8.49 ± 0.11	7.76 ± 0.05	8.26 ± 0.16	6.52 ± 0.18
(+)-**5**	2*S*,2′*R*,5′*S*	9.93 ± 0.07	9.02 ± 0.11	9.47 ± 0.11	9.06 ± 0.12	9.97 ± 0.09	9.97 ± 0.05	9.00 ± 0.08*	9.66 ± 0.23	7.99 ± 0.12
(−)-**5**	2*R*,2′*S*,5′*R*	9.02 ± 0.045	8.83 ± 0.09	8.31 ± 0.07	8.74 ± 0.07	8.49 ± 0.06	8.49 ± 0.12	8.54 ± 0.17	8.84 ± 0.11	6.55 ± 0.15
(−)-**6**	2*S*,2′*S*,5′*S*	9.60 ± 0.05	9.11 ± 0.13	9.23 ± 0.09	9.06 ± 0.13	9.70 ± 0.11	9.52 ± 0.02	8.37 ± 0.03*	8.50 ± 0.02	7.47 ± 0.01
(+)-**6**	2*R*,2′*R*,5′*R*	9.30 ± 0.065	9.08 ± 0.10	9.04 ± 0.085	9.15 ± 0.08	9.11 ± 0.10	9.53 ± 0.08	8.60 ± 0.21	8.79 ± 0.11	6.47 ± 0.07
A	2*R*,5*R*	8.96 ± 0.07	8.12 ± 0.09	8.75 ± 0.10	8.21 ± 0.07	8.55 ± 0.08	8.81 ± 0.20	8.47 ± 0.13[c]	8.79 ± 0.09[c]	6.24 ± 0.21
NMS[d]		9.49 ± 0.06	9.75 ± 0.10	9.87 ± 0.10	9.85 ± 0.07	9.68 ± 0.05	n.t.[e]	9.33 ± 0.03*	9.21 ± 0.07*	8.19 ± 0.06*

[a] Binding parameters of muscarinic antagonists at five human muscarinic receptor subtypes. The affinity estimates (as pK_i) were derived from both [^{3}H]-NMS homologous and heterologous competition curves and represent the mean (± SEM) of at least three experiments. [b] pK_b values in functional tests ± SEM; $n = 3$. The results labeled with a star (*) represent pA_2 values. [c] See reference 13. [d] *N*-Methylscopolamine. [e] n.t. = not tested.

mm; Merck) or flash chromatography (Kieselgel 40, 0.040−0.063 mm; Merck). Although the IR spectra data are not included, they were obtained for all compounds reported and are consistent with the assigned structures. Yields are given after purification, unless otherwise 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 ($c = 1$), with a Perkin-Elmer polarimeter (accuracy ± 0.002°). Oxalates were obtained from the corresponding amine by treatment with oxalic acid (1 equiv) in ethyl acetate. When reactions were performed in anhydrous conditions, the mixtures were maintained under nitrogen. Compounds were named following IUPAC rules, as applied by Beilstein-Institut AutoNom (version 2.1), a software for systematic names in organic chemistry.

(2*S*,5′*S*)-1-Methyl 2-(2,2-Diphenyl-1,3-oxathiolan-5-yl)pirrolidine (2*S*,5′*S*)-(−)-1. A solution of (−)-**7**[11] (0.53 g, 1.88 mmol), benzophenone dimethyl ketal (0.86 g, 3.76 mmol), and *p*-toluenesulfonic acid monohydrate (0.10 g, 0.52 mmol) in anhydrous and degassed toluene was heated to reflux for 13 h with a Dean−Stark trap. The solvent was distilled off, and the residue was dissolved in CH_2Cl_2 (previously treated with Na_2CO_3 to eliminate traces of acidity), washed with a saturated solution of $NaHCO_3$, and dried. After evaporation of the solvent, the residue was chromatographed (eluent: $CHCl_3$/hexane 3:7), yielding 0.41 g (0.92 mmol) of compound (−)-**9** (yield 48.9%). Its characterization is reported in Table 2 (Supporting Information). To a solution of 0.30 g (0.67 mmol) of (−)-**9** dissolved in the minimum amount of anhydrous diethyl ether and maintained at −18 °C, 76.4 mg (2.01 mmol) of $LiAlH_4$ was added in portions. The mixture was allowed to reach rt and, after 4 h, was treated with brine and extracted with diethyl ether. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Purification by flash chromatography (eluent: CH_2Cl_2/MeOH 98:2) yielded 0.19 g (0.58 mmol) of (−)-**1**; yield 86.6%.

Starting from (+)-**7**,[11] in the same way, **(2*R*,5′*R*)-1-methyl 2-(2,2-diphenyl-1,3-oxathiolan-5-yl)pirrolidine (2*R*,5′*R*)-(+)-1** was obtained.

The chemical and physical characteristics of the enantiomers and their ^{1}H and ^{13}C NMR spectra are reported in Tables 3 and 4 (Supporting Information).

(2*S*,2′*R*,5′*S*)-1-Methyl-2-(2-cyclohexyl-2-phenyl-1,3-oxathiolan-5-yl)pirrolidine (2*S*,2′*R*,5′*S*)-(+)-2 and (2*S*,2′*S*,5′*S*)-1-Methyl-2-(2-cyclohexyl-2-phenyl-1,3-oxathiolan-5-yl)pirrolidine (2*S*,2′*S*,5′*S*)-(−)-3. According to the procedure described for (−)-**1** and using 0.73 g (2.59 mmol) of (−)-**7**,[11] 1.21 g (5.17 mmol) of cyclohexylphenyl dimethyl ketal,[17] and 0.15 g of *p*-toluenesulfonic acid monohydrate, **10** as a mixture of two diastereoisomers was obtained, which was purified from the other byproducts by flash chromatography (eluent: $CHCl_3$/hexane 3:7; yield 59.8%, 0.70 g, 1.55 mmol). Its characterization is reported in Table 2 (Supporting Information). By subsequent reduction of **10** with $LiAlH_4$, the title diastereoisomers were obtained. The flash chromatographic separation (eluent: $CHCl_3$/abs EtOH/NH_3 99:1:0.1) afforded 0.34 g (0.96 mmol, 61.9% yield) of isomer (+)-**2** and 46 mg (0.13 mmol, 8.4% yield) of isomer (−)-**3**.

Starting from (+)-**7**,[11] in the same way, **(2*R*,2′*S*,5′*R*)-1-methyl-2-(2-cyclohexyl-2-phenyl-1,3-oxathiolan-5-yl)pirrolidine (2*R*,2′*S*,5′*R*)-(−)-2** and **(2*R*,2′*R*,5′*R*)-1-methyl-2-(2-cyclohexyl-2-phenyl-1,3-oxathiolan-5-yl)pirrolidine (2*R*,2′*R*,5′*R*)-(+)-3** were obtained.

The chemical and physical characteristics of the four isomers and their ^{1}H and ^{13}C NMR spectra are reported in Tables 3 and 4 (Supporting Information).

General Procedure for the Synthesis of Dimethyl Pirrolidinium Iodides 4−6. An anhydrous diethyl ether solution of the suitable *N*-methyl amine **1**−**3** was treated with an excess of methyl iodide and kept overnight at rt in the dark. The obtained solid was filtered, dried under vacuum, and recrystallized from absolute ethanol/diethyl ether. Compounds (−)-**4**, (+)-**4**, (+)-**5**, (−)-**5**, (+)-**6**, and (−)-**6** were prepared by this procedure. The chemical and physical characteristics of the compounds and their ^{1}H NMR spectra are reported in Tables 5 and 6 (Supporting Information).

Crystal Structure Determination and Refinement Collection of (−)-2 Oxalate. $C_{22}H_{30}NO_5S$, M = 420.53, monoclinic, space group $P2_1$, $a = 8.341(0)$, $b = 9.535(4)$, $c = 14.037(6)$ Å, beta = 91.36(9) $V = 1116.141$ Å^3, $Z = 2$, $D_c = 1.253$ g/cm^3, $\mu = 0.177$ mm^{-1}, $F(000) = 450$. Details are reported in the Supporting Information. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre with number CCDC 297250.

Pharmacology. Binding Studies. All equilibrium radioligand binding experiments were conducted using a protocol based on previously described procedures.[10] Details of the procedures are reported in the Supporting Information.

Functional Studies. All in vitro experiments were conducted using a protocol based on previously described procedures,[18] slightly modified with regard to the guinea pig ileum preparation. Details of the procedures are reported in the Supporting Information.

Acknowledgment. This work was supported by the Italian M.U.R. (Ministero dell'Università e della Ricerca).

Supporting Information Available: Chemical and physical characteristics, spectral data, and elemental analysis for all the new compounds; experimental details for the determination of biological activities; and experimental details for crystal structure determination of (−)-**2** oxalate and its crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Eglen, R. M. Muscarinic receptor subtypes in neuronal and non-neuronal cholinergic function. *Auton. Autacoid Pharmacol.* **2006**, *26*, 219−233.

(2) Eglen, R. M.; Choppin, A.; Dillon, M. P.; Hedge, S. Muscarinic ligands and their therapeutic potential. *Curr. Opin. Chem. Biol.* **1999**, *3*, 426−432.

(3) Racké, K.; Juegens, U. R.; Matthiesen, S. Control by cholinergic mechanism. *Eur. J. Pharmacol.* **2006**, *533*, 57−68.

(4) Caulfield, M. P.; Birdsall, N. J. International union of Pharmacology XVII. Classification of muscarinic acetylcholine receptors. *Pharmacol. Rev.* **1998**, *50*, 279−290.

(5) Broadley, K. J.; Kelly, D. R. Muscarinic receptor agonists and antagonists. *Molecules* **2001**, *6*, 142−193.

(6) Hulme, E. C.; Lu, Z.-L.; Bee, M.; Curtis, C. A. M.; Saldanha, J. The conformational switch in muscarinic acetylcholine receptors. *Life Sci.* **2001**, *68*, 2495−2500.

(7) Felder, C. C.; Bymaster, F. P.; Ward, J.; DeLapp, N. Therapeutic opportunities for muscarinic receptors in the central nervous system. *J. Med. Chem.* **2000**, *43*, 4333−4353.

(8) Teodori, E.; Gualtieri, F.; Angeli, P.; Brasili, L.; Giannella, M.; Pigini, M. Resolution, absolute configuration and cholinergic enantioselectivity of (+)- and (−)-*cis*-2-methyl-5-[(dimethylamino)methyl]-1,3-oxathiolane methiodide. *J. Med. Chem.* **1986**, *29*, 1610−1615.

(9) Dei, S.; Bellucci, C.; Buccioni, M.; Ferraroni, M.; Gualtieri, F.; Guandalini, L.; Manetti, D.; Matucci, R.; Romanelli, M. N.; Scapecchi, S.; Teodori, E. Synthesis and cholinergic affinity of diastereomeric and enantiomeric isomers of 1-methyl-2-(2-methyl-1,3-dioxolan-4-yl)pyrrolidine, 1-methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine, and of their iodomethylates. *Bioorg. Med. Chem.* **2003**, *11*, 3153−3164.

(10) Dei, S.; Angeli, P.; Bellucci, C.; Buccioni, M.; Gualtieri, F.; Marucci, G.; Manetti, D.; Matucci, R.; Romanelli, M. N.; Scapecchi, S.; Teodori, E. Muscarinic subtypes affinity and functional activity profile of 1-methyl-2-(2-methyl-1,3-dioxolan-4-yl)pyrrolidine and 1-methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine derivatives. *Biochem. Pharmacol.* **2005**, *69*, 1637−1645.

(11) Scapecchi, S.; Matucci, R.; Bellucci, C.; Buccioni, M.; Dei, S.; Guandalini, L.; Martelli, C.; Manetti, D.; Martini, E.; Marucci, G.; Nesi, M.; Romanelli, M. N.; Teodori, E. 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. *J. Med. Chem.* **2006**, *49*, 1925−1931.

(12) Romanelli, M. N.; Teodori, E.; Gualtieri, F.; Angeli, P.; Brasili, L. Enantioselectivity of muscarinic antagonists. 2,2-Dicyclohexyl-5-[(dimethylamino)methyl]-1,3-oxathiolane methiodides and related oxides. *J. Med. Chem.* **1988**, *31*, 1698−1702.

(13) Romanelli, M. N.; Gualtieri, F.; Valle, G.; Brasili, L.; Angeli, P. Enantioselectivity of muscarinic antagonists. Isomeric 2-cyclohexyl-2-phenyl-5-((dimethylamino)methyl)-1,3-oxathiolane methiodides. *J. Med. Chem.* **1988**, *31*, 1703−1708.

(14) Gualtieri, F.; Romanelli, M. N.; Teodori, E. The "frozen analog" approach in medicinal chemistry. In *Progress in Medicinal Chemistry*; Choundhary, M. I., Ed.; Harwood Academic Publishers: Amsterdam, 1996; Vol. 1 (Studies in Medicinal Chemistry), pp 271−318.

(15) Mondal, E.; Rani Sahu, P.; Bose, G.; Khan, A. T. A useful and convenient synthetic protocol for interconversion of carbonyl compounds to the corresponding 1,3-oxathiolanes and vice versa employing organic ammonium tribromide (OATB). *Tetrahedron Lett.* **2002**, *43*, 2843−2846.

(16) Karimi, B.; Seradj, H. Zirconium tetrachloride ($ZrCl_4$) as an efficient and chemoselective catalyst for conversion of carbonyl compounds to 1,3-oxathiolanes. *Synlett* **2000**, *6*, 805−806.

(17) Altava, B.; Burguete, M. I.; García-Verdugo, E.; Luis, S. V.; Miravet, J. F.; Vicent, M. J. On the origin of changes in topicity observed in Diels−Alder reactions catalyzed by Ti−TADDOLates. *Tetrahedron: Asymmetry* **2000**, *11*, 4885−4893.

(18) Scapecchi, S.; Marucci, G.; Angeli, P.; Bellucci, C.; Dei, S.; Gualtieri, F.; Manetti, D.; Romanelli, M. N.; Teodori, E. Structure−activity relationships in 2,2-diphenyl-2-ethylthioacetic acid esters: Unexpected agonistic activity in a series of muscarinic antagonists. *Bioorg. Med. Chem.* **2001**, *9*, 1165−1174.

(19) Budriesi, R.; Cacciaguerra, S.; Di Toro, R.; Bolognesi, M. L.; Chiarini, A.; Minarini, A.; Rosini, M.; Spampinato, S.; Tumiatti, V.; Melchiorre, C. Analysis of the muscarinic receptor subtype mediating inhibition of the neurogenic contractions in rabbit isolated vas deferens by a series of polymethylene tetra-amines. *Br. J. Pharmacol.* **2001**, *132*, 1009−1016.

(20) Haddad, E. B.; Trifilieff, A.; Landry, Y.; Gies, J. P. Heterogeneity of guinea-pig lung muscarinic receptors revealed by [^{3}H]4-DAMP-binding. *Pulm. Pharmacol.* **1993**, *6*, 119−27.

(21) Roffel, A. F.; Davids, J. H.; Elzinga, C. R. S.; Wolf, D.; Zaagsma J.; Kilbinger, H. Characterization of the muscarinic receptor subtype-(s) mediating contraction of the guinea-pig lung strip and inhibition of acetylcholine release in the guinea-pig trachea with the selective muscarinic receptor antagonist tripitramine. *Br. J. Pharmacol.* **1997**, *122*, 133−141.

(22) Van Rossum, J. M. Cumulative dose-response curves. Technique for the making of dose-response curves in isolated organs and evaluation of drug parameters. *Arch. Int. Pharmacodyn. Ther.* **1963**, *143*, 299−330.

(23) Tallarida, R. J.; Cowan, A.; Adler, M. W. pA_2 and receptor differentiation: Statistical analysis of competitive antagonism. *Life Sci.* **1979**, *25*, 637−654.

(24) Gualtieri, F.; Romanelli, M. N.; Teodori, E. Eudismic analysis of a series of muscarinic ligands carrying a 1,3-oxathiolane nucleus. *Chirality* **1990**, *2*, 79−84.

JM061374R