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Synthesis, Affinity Profile and Functional Activity of Potent Chiral Muscarinic Antagonists with a Pyrrolidinylfuran Structure†

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Starting from the structure of previously studied muscarinic agonists, characterized by a pyrrolidinylfuran scaffold, a new series of muscarinic antagonists was synthesized by substituting the 5-position of the furane cycle with bulky hydrophobic groups. Both tertiary amines and the corresponding iodomethyl derivatives were obtained and studied. All the new compounds show high affinity toward cloned human muscarinic M_1-M_5 receptors expressed in Chinese hamster ovary (CHO) cells and behave as competitive antagonists on classical models of muscarinic receptors. The diastereoisomeric mixture of the highest affinity compound of the series was resolved into the four optical isomers by chiral HPLC. The relative and absolute configuration of the obtained compounds was established by means of a combined strategy based on X-ray crystallography and chiroptical techniques. Although generally fairly potent, the compounds showed only modest subtype selectivity, with the exception of **2a** and **6a**, which in functional assays presented clear-cut selectivity for the muscarinic receptors present in rabbit vas deferens.

Introduction

Five muscarinic acetylcholine receptor subtypes (M_1-M_5) are found in the central and peripheral nervous system where they mediate a variety of vital functions.[1] Four of these subtypes (M_1-M_4) have been characterized from a pharmacological point of view.[2] Due to the physiological role of such receptors, muscarinic drugs have the potential for therapeutic use in several pathological states.[3,4] The high sequence conservation within the orthosteric domain across all five muscarinic subtypes[5] has made it difficult to identify either natural or synthetic selective ligands.[6] As a consequence, despite the ubiquitous presence of muscarinic receptors in the human body, the number of muscarinic drugs that have been introduced into therapy is limited, particularly when one considers the importance of this class of receptors.[3] The recent identification of allosteric sites,[7−9] which could be different in the five subtypes, and the discovery of allosteric agonists such as AC-42[10,11] that can interact with them may open a new promising approach toward selective modulation of muscarinic subtypes. In the meantime, classical research into orthosteric-site-directed ligands both for agonists and antagonists is still active. It aims to identify muscarinic ligands that can be used as therapeutic drugs or as pharmacological tools for studying muscarinic receptors.[4] Muscarinic subtype characterization is far from being satisfactory, and more potent and selective ligands are still needed. There is intense research to identify selective M_3 subtype antagonists for use in smooth muscle disorders such as urinary incontinence (UI), chronic obstructive pulmonary disease (COPD), and irritable bowel syndrome (IBS).[12−15]

For several years, we have been working on cholinergic ligands, both agonists and antagonists.[16−20] Recently, we reported the muscarinic agonist activity of a series of pyrrolidinylfuran derivatives. Of these, *S*-(−)-2-(5-methyl-2-furyl)-1-methylpyrrolidine metiodide, whose structure is shown in Chart 1, was the most interesting, because it was a potent and selective M_2 receptor partial agonist.[21]

In the present paper, we report the synthesis of a new series of pyrrolidinylfuran derivatives (**1**−**8**) that were transformed into potent antagonists by the insertion of bulky hydrophobic groups in position 5 of the furan ring (Chart 1) as successfully done in previous studies on pentatomic cyclic compounds.[17,20,22] We reasoned that by extending the length of the molecules it would be possible to explore the properties of less conserved proximal regions of the orthosteric binding site of the receptor, as also suggested by the chemical structure of some recently reported subtype-selective muscarinic antagonists.[23−26]

Chemistry

Initially, to save time and to avoid labor-intensive experimental work, we decided to synthesize the designed compounds, disregarding the problem of stereoisomerism. Once the pharmacological activity of the obtained mixtures was evaluated, we would then resolve the complex mixture of the

†Dedicated to Prof. Fulvio Gualtieri on the occasion of his retirement.

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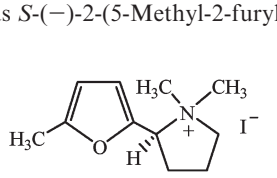
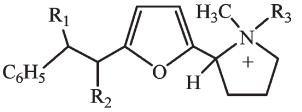
Chart 1. Structure of Compounds *S*-(−)-2-(5-Methyl-2-furyl)-1-methylpyrrolidine Methiodide and **1**−**8**



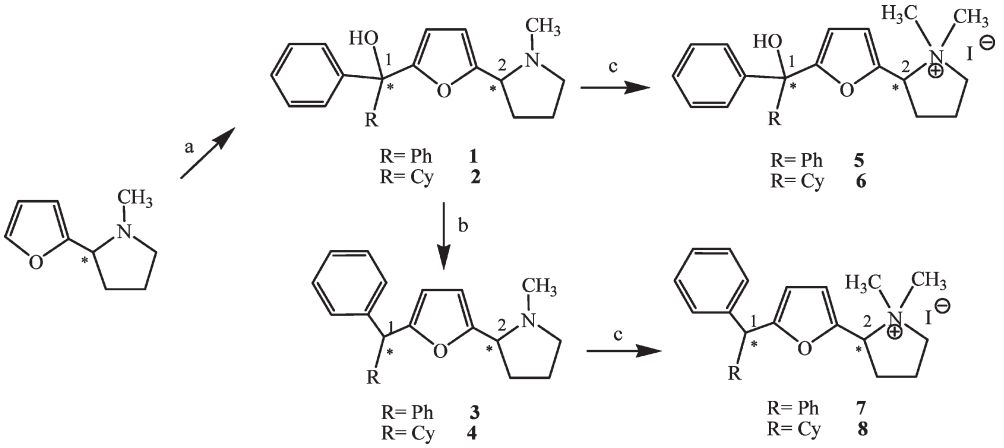
S-(-)-2-(5-methyl-2-furyl)-1-methylpyrrolidine methiodide



1-8

$R_1 = C_6H_5, C_6H_{11}$
$R_2 = H, OH$
$R_3 = none, CH_3$

Scheme 1. Synthesis of Compounds **1**−**8**[a]



[a] (a) BuLi, PhCOCy or PhCOPh; (b) $NaBH_4$, CF_3COOH; (c) CH_3I.

Table 1. Binding Affinity[a] of Compounds **1**−**8**

compound	hM_1	hM_2	hM_3	hM_4	hM_5
1	7.45 ± 0.06	7.53 ± 0.12	7.02 ± 0.08	6.98 ± 0.06	7.01 ± 0.06
2	8.91 ± 0.08	8.17 ± 0.09	8.11 ± 0.11	7.85 ± 0.06	8.15 ± 0.07
(1*R*,2*R*)-**2a**	8.91 ± 0.09	8.34 ± 0.10	8.78 ± 0.11	8.40 ± 0.09	8.48 ± 0.08
(1*R*,2S)-**2b**	7.64 ± 0.07	7.22 ± 0.11	7.63 ± 0.12	7.26 ± 0.08	7.56 ± 0.04
(1*S*,2*R*)-**2c**	6.72 ± 0.07	6.44 ± 0.11	6.49 ± 0.11	6.26 ± 0.07	6.33 ± 0.08
(1*S*,2*S*)-**2d**	5.91 ± 0.08	5.49 ± 0.11	5.78 ± 0.11	5.51 ± 0.10	5.81 ± 0.07
3	7.37 ± 0.10	7.19 ± 0.18	7.07 ± 0.08	6.80 ± 0.05	6.98 ± 0.05
4	7.87 ± 0.07	6.85 ± 0.09	7.47 ± 0.12	7.11 ± 0.07	7.26 ± 0.08
5	7.63 ± 0.07	7.50 ± 0.10	7.44 ± 0.09	7.19 ± 0.07	7.43 ± 0.07
6	8.68 ± 0.06	7.99 ± 0.09	8.14 ± 0.12	7.81 ± 0.08	7.94 ± 0.08
(1*R*,2*R*)-**6a**	9.16 ± 0.08	8.63 ± 0.10	8.82 ± 0.12	8.37 ± 0.10	8.81 ± 0.07
(1*R*,2S)-**6b**	8.04 ± 0.07	7.70 ± 0.09	8.03 ± 0.12	7.48 ± 0.08	7.92 ± 0.07
(1*S*,2*R*)-**6c**	7.32 ± 0.07	7.06 ± 0.09	6.81 ± 0.12	6.43 ± 0.08	6.64 ± 0.06
(1*S*,2*S*)-**6d**	6.48 ± 0.07	6.12 ± 0.10	6.29 ± 0.12	5.63 ± 0.10	6.06 ± 0.06
7	7.94 ± 0.11	7.87 ± 0.2	7.63 ± 0.08	7.42 ± 0.08	7.50 ± 0.07
8	8.18 ± 0.07	7.76 ± 0.09	8.05 ± 0.11	7.71 ± 0.08	7.81 ± 0.09
NMS[b]	9.49 ± 0.06	9.75 ± 0.10	9.87 ± 0.10	9.85 ± 0.07	9.68 ± 0.05

[a] Binding affinity at cloned human muscarinic receptor subtypes expressed in CHO cells. 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. The affinity is estimated as pK_i ($-\log K_i$), where the unit of measure for K_i is M. [b] *N*-Methylscopolamine.

most interesting ones. Scheme 1 illustrates the synthetic pathway that was followed.

Metalation with BuLi at 0 °C of racemic 2-furan-2-yl-1-methylpyrrolidine[21] and subsequent treatment with the appropriate electrophile (diphenyl ketone for **1** and phenylcyclohexyl ketone for **2**) gave compounds **1** and **2**; their reduction with $NaBH_4$ in CF_3COOH[27] afforded compounds **3** and **4**. Diastereomeric mixtures **1**−**4** were transformed into the corresponding methiodides (**5**−**8**) with an excess of CH_3I in the dark at room temperature. The binding affinity of these mixtures of four (**2**, **4**, **6**, **8**) or two (**1**, **3**, **5**, **7**) stereoisomers was evaluated. Based on the obtained results (Table 1), we decided to resolve mixture **2**, which would in turn allow the preparation of each of the four stereoisomers of methiodide **6** separately.

Therefore, our first attempt was to separate the two diastereoisomers of compound **2** by flash chromatography, but we obtained only enriched mixtures of each diastereoisomer. The diastereo- and enantioselective HPLC performed on the Chiracel OD chiral stationary phase (CSP) using the *n*-hexane/2-propanol/DEA 100:1:0.1 (v/v/v) mixture as mobile phase (Figure 1) was more useful. These conditions, scaled-up to semipreparative level, permitted isolation of milligram amounts of each stereoisomer for a single chromatographic run within 12 min (Figure 2a). The CD[a] spectra (Figure 3) and the specific rotations (Figure 2b) of the four isomers indicated

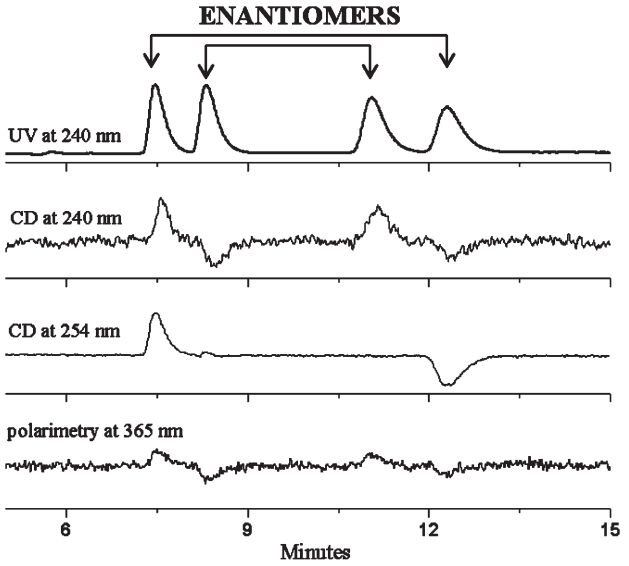


Figure 1. Chromatograms of **2** obtained by simultaneous UV and chiroptical (polarimetric or CD) detection. Column, Chiralcel OD (250 mm × 4.6 mm I.D); eluent, *n*-hexane/2-propanol/DEA 100:1:0.1 (v/v/v); flow-rate, 1 mL min^{-1}; temperature, 25 °C.

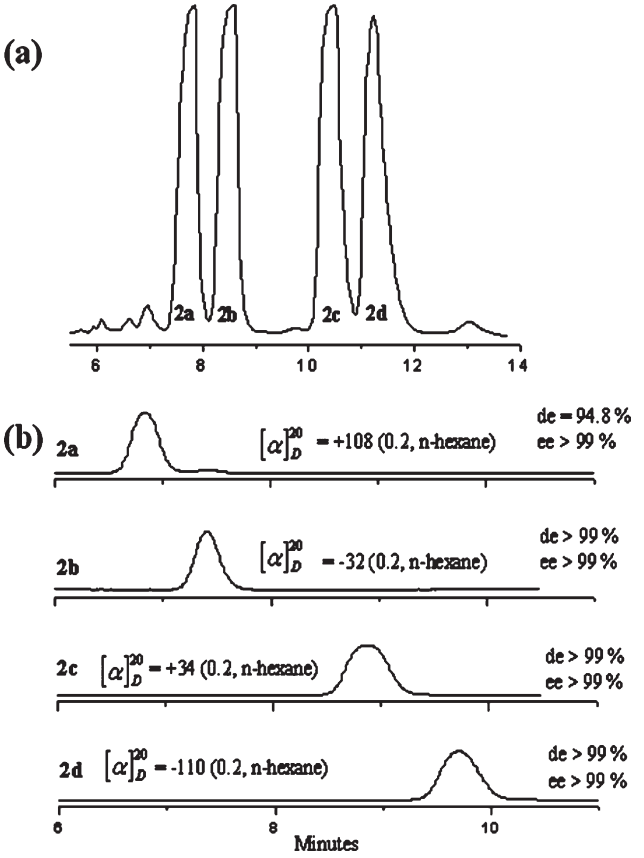


Figure 2. (a) Simultaneous diastereo- and enantioseparation of 10 mg of **2**; (b) chromatographic and polarimetric analysis of the isolated stereoisomers. Column, (a) Chiralcel OD (250 mm × 10 mm I.D.), (b) Chiralcel OD (250 mm × 4.6 mm I.D.); eluent, *n*-hexane/2-propanol/DEA 100:1:0.1 (v/v/v); flow-rate, (a) 5.0 mL min^{-1}, (b) 1 mL min^{-1}; detector, UV at 254 nm; temperature, 25 °C.

that the enantiomeric couples are **2a**/**2d** and **2b**/**2c** where **a**−**d** indicates the stereomeric elution order. To establish the absolute configuration of the four isomers, the first reaction

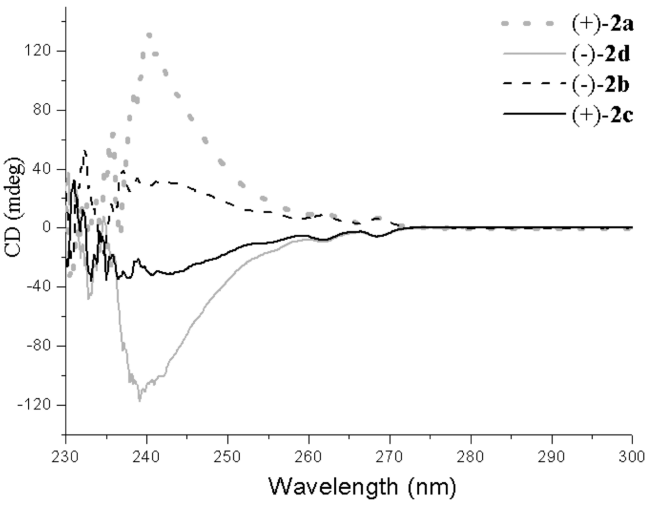


Figure 3. CD spectra of the **2a**−**2d** stereoisomers in *n*-hexane.

of Scheme 1 was performed on the (2*R*)-(+)-(2-furyl)-1-methylpyrrolidine isomer.[21] In this case, only two diastereoisomers of compound **2** with known stereochemistry on the pyrrolidine center were obtained. Diastereoselective HPLC showed that the isomers obtained were **2a** and **2c** (Figure 4). The structure of (+)-**2a** (first eluted on the OD CSP) was readily secured by X-ray crystallography. As illustrated in Figure 5, the absolute configuration of (+)-**2a** was determined as (1*R*,2*R*); thus the (1*S*,2*S*)-configuration was assigned [(1*S*,2*S*)-(−)-**2d**] to the (−)-enantiomer of **2a** (**2d**, fourth eluted on the OD CSP). On the basis of combined chromatographic, chiroptical, and NMR studies, the absolute configuration assignment was unambiguously completed, and configurations were assigned to the second and third eluting enantiomers: (1*R*,2*S*)-(−)-**2b** and (1*S*,2*R*)-(+)-**2c**.

Reaction of **2a**−**d** with methyl iodide, according to Scheme 1, afforded the corresponding methiodides **6a**−**d**.

Pharmacology

Muscarinic receptor affinity was evaluated in CHO cells expressing the five human muscarinic subtypes (hM_1−hM_5). 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 previously reported methods.[28] It should be remembered that the contraction of rabbit vas deferens has long been considered an effect mediated by M_1-receptor subtypes, whereas more recent studies attribute the same effect to an M_4 activation.[2,29] Analogously, the validity of the guinea pig lung strips as an M_4 model[30] has been questioned.[31] For this reason, these two preparations are indicated as putative M_1 and M_4 receptor models in the present work. Carbachol, arecaidine propargyl ester (APE), 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 $pK_b = \log(DR\text{-}1) - \log[B]$, where DR is the ratio of ED_{50} values of agonist after and before treatment with one or two antagonist concentrations, [B].[32] Results are shown in Tables 1 and 2, where the *N*-methylscopolamine data are reported for comparison.

Results and Discussion

The results reported in Table 1 show that the compounds studied are endowed with high affinity for the five human

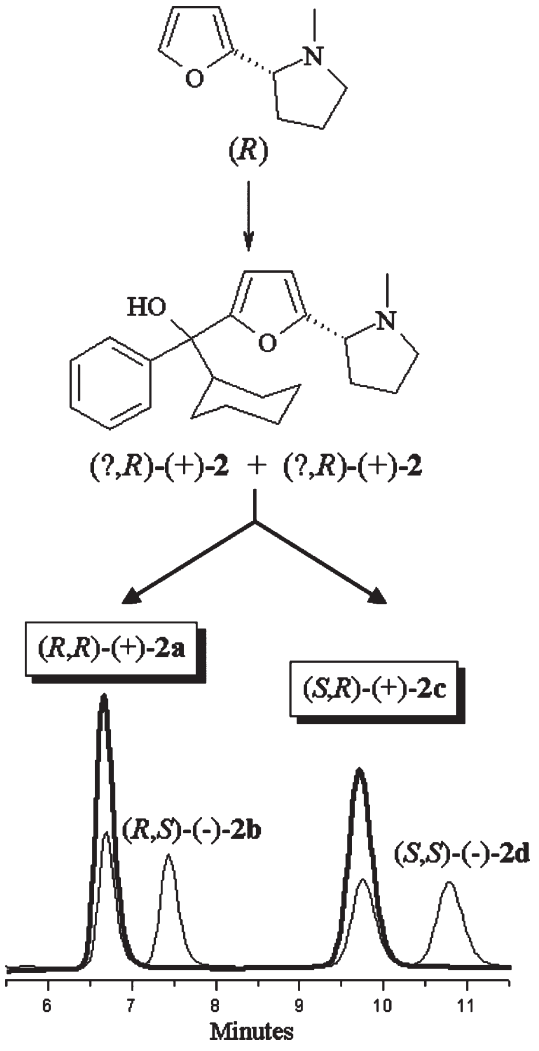


Figure 4. Identification of the diastereomeric pairs of **2**. Column, Chiralcel OD (250 mm × 4.6 mm I.D.); eluent, *n*-hexane/2-propanol/DEA 100:1:0.1 (v/v/v); flow-rate, 1 mL min^{-1}; detector, UV at 240 nm; temperature, 25 °C.

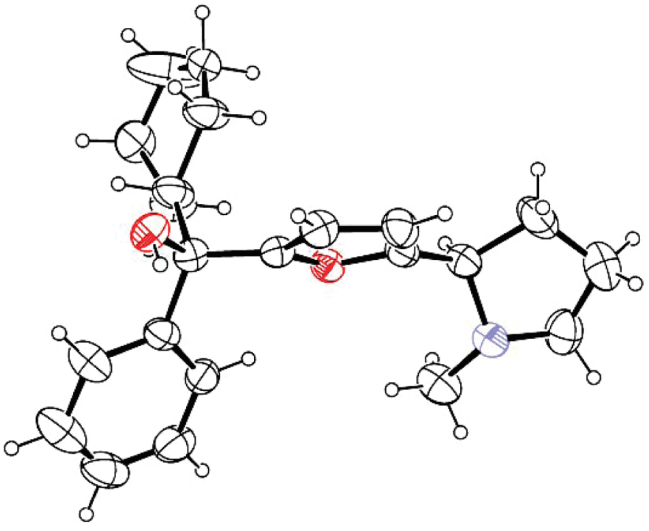


Figure 5. Ortep drawing of compound of (+)-(1*R*,2*R*)-**2a**.

muscarinic receptor subtypes expressed in CHO cells but show only low or modest subtype selectivity. The most potent was the mixture of the four stereoisomers **2**, which was therefore chosen for the resolution procedure. Compound **2a** had the highest affinity for the muscarinic receptors, while its enantiomer **2d** had the lowest. As a consequence, the enantioselectivity, expressed by the eudismic ratio (ER) between the affinity of two enantiomers, is very high for all receptor subtypes varying from 468 for hM_5 to 708 for hM_2, 776 for hM_4, and 1000 for hM_1 and hM_3. In contrast, the other enantiomeric couple (**2b**, **2c**) shows only modest enantioselectivity, about 2 orders of magnitude lower (ER = 6 for hM_2; 8 for hM_1; 10 for hM_4; 14 for hM_3, 17 for hM_5). Apparently, the configuration of **2a** is optimal to interact with muscarinic receptors. Unfortunately, once again, this is true for all subtypes. As a matter of fact, subtype selectivity also remains very low for the pure stereoisomers and this behavior is maintained in the corresponding methiodides (**6a**−**6d**). As expected, **6a**−**6d** show a slightly higher affinity, but they maintain similar eudismic ratios and subtype selectivity compared with the corresponding tertiary amines. This confirms that the presence of a quaternary nitrogen is not critical for muscarinic antagonists.[33]

Table 2. Functional Activity of Compounds **1**−**8**[a]

compound	rabbit vas deferens	guinea pig atrium (M_2)	guinea pig ileum (M_3)	guinea pig lung
1	6.91 ± 0.19	6.92 ± 0.11	6.65 ± 0.09	< 5
2	*c*	6.98 ± 0.21	7.80 ± 0.02	< 5
(1*R*,2*R*)-**2a**	9.58 ± 0.20	7.91 ± 0.20	8.76 ± 0.02	5.92 ± 0.13
(1*R*,2S)-**2b**	*c*	6.65 ± 0.03	7.66 ± 0.18	5.84 ± 0.09
(1*S*,2*R*)-**2c**	*c*	6.23 ± 0.20	6.77 ± 0.18	< 5
(1*S*,2*S*)-**2d**	*c*	5.95 ± 0.02	6.41 ± 0.11	< 5
3	6.64 ± 0.15	6.52 ± 0.19	6.81 ± 0.08	5.19 ± 0.23
4	*c*	5.36 ± 0.15	7.05 ± 0.20	< 5
5	8.46 ± 0.23	7.08 ± 0.20	7.60 ± 0.15	6.20 ± 0.04
6	*c*	7.88 ± 0.17	8.35 ± 0.04	5.90 ± 0.04
(1*R*,2*R*)-**6a**	10.15 ± 0.16	8.49 ± 0.15	8.95 ± 0.09	7.75 ± 0.03
(1*R*,2S)-**6b**	*c*	7.32 ± 0.21	7.95 ± 0.18	6.16 ± 0.12
(1*S*,2*R*)-**6c**	*c*	6.85 ± 0.03	6.95 ± 0.09	6.33 ± 0.10
(1*S*,2*S*)-**6d**	*c*	5.92 ± 0.02	6.32 ± 0.02	< 5
7	7.91 ± 0.05	7.09 ± 0.07	7.59 ± 0.10	< 5
8	*c*	6.21 ± 0.25	7.88 ± 0.15	5.69 ± 0.20
NMS[b]	*c*	9.33 ± 0.03[d]	9.21 ± 0.07[d]	8.19 ± 0.06[d]

[a] pK_b values from functional tests ± SEM; $n = 3$. [b] *N*-Methylscopolamine. [c] Not tested [d] pA_2 values.

The results of functional activity on the classic models of M_2 (guinea pig atrium) and M_3 (guinea pig ileum) muscarinic receptors are reported in Table 2, together with the results obtained in two other tissues (rabbit vas deferens and guinea pig lung) for which the nature of the muscarinic subtypes involved, as mentioned above, is currently controversial; to save animals and money, only selected compounds were assayed on rabbit vas deferens. As far as M_2 and M_3 receptors are concerned, the results of functional tests are in fairly good agreement with binding assays. This was expected, since antagonists usually do not present the discrepancies between binding and functional assays often found with agonists.[18,19,21] There are only small variations in potency and the enantioselectivity of the couples **2a/2d**, **2b/2c**, **6a/6d**, and **6b/6c** is of the same order of magnitude as that found in binding. The same occurs for subtype selectivity, which unfortunately remains low. A different situation is found in the other two tissues: the functional data on guinea pig lung do not correlate with the binding of any of the human muscarinic subtypes expressed in CHO cells, confirming the doubts[31] on the validity of this tissue as a model for M_4, as proposed previously,[30] or for any other subtype. It is interesting that the functional data on rabbit vas deferens, performed on isomer **2**,

shows that **2a** and **6a**, which were the highest affinity isomers on hM_1 receptors (pK_i = 8.91 and 9.16, respectively), are also the most potent on rabbit vas deferens (pK_b = 9.58 and 10.15 respectively).

In conclusion, we have synthesized and studied a new series of muscarinic antagonists where the length of the molecule has been extended compared with similar classes of compounds studied previously.[17,20,22] Some members of the series are fairly potent and show higher enantioselectivity but still fail to present subtype selectivity. More work, such as a further extension of the molecule length, will be necessary to explore the viability of this approach.

Experimental Section

Chemistry. All melting points were taken on a Büchi apparatus and are uncorrected. Unless otherwise stated, NMR spectra were recorded on a 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 analyses are indicated by symbols, the analytical results are within ±0.4% of the theoretical values. The purity of the tested compounds not subjected to combustion analysis (**2a**−**d**) was determined by HPLC (see details below). All reported compounds had a purity of at least 95%. 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 4.01.305), a software for systematic names in organic chemistry. Diethylamine (DEA) was obtained from Fluka Chemie (Buchs, Switzerland).

HPLC enantioseparations were performed using stainless-steel Chiralcel OD (250 mm × 4.6 mm I.D. and 250 mm × 10 mm I.D.) columns (Chiral Technologies Europe, Illkirch, France). HPLC-grade solvents were supplied by Carlo Erba (Milan, Italy). The HPLC apparatus consisted of a Perkin-Elmer (Norwalk, CT) 200 lc pump equipped with a Rheodyne (Cotati, CA) injector, a 100-μL sample loop, and a HPLC Dionex TCC-100 oven (Sunnyvale, CA). A Jasco (Jasco, Tokyo, Japan) model CD 2095 Plus UV/CD and a Perkin-Elmer polarimeter model 241 equipped with Hg/Na lamps and a 40 μL flow cell were used as detectors for HPLC. The signal was acquired and processed by Clarity software (DataApex, Prague, Czech Republic).

The mobile phases were filtered and degassed by sonication just before use. In semipreparative enantioseparations, a standard solution was prepared by dissolving 1 g of **2** into 10 mL of mobile phase. The injection volume was 100 μL. After semipreparative separation, the collected fractions were analyzed by chiral analytical columns to determine their enantiomeric excess (ee) and diastereomeric excess (de).

Specific rotations of stereoisomers **2a**−**2d**, dissolved in *n*-hexane, were measured at 589 nm with a Perkin-Elmer polarimeter model 241 equipped with a Na lamp. The volume of the cell was 1 mL, and the optical path was 10 cm. The system was set at a temperature of 20 °C using a Neslab RTE 740 cryostat.

The circular dichroism (CD) spectra of stereoisomers **2a**−**2d**, dissolved in *n*-hexane (concentration about 0.15 mg/mL) in a quartz cell (0.1 cm-path length) at 20 °C were measured using a Jasco model J-700 spectropolarimeter. The spectra were averaged over three instrumental scans, and the intensities are presented in terms of ellipticity values (mdeg).

[5-(1-Methylpyrrolidin-2-yl)furan-2-yl]diphenylmethanol (1). 2-Furan-2-yl-1-methylpyrrolidine[21] (0.50 g, 3.3 mmol) was dissolved in anhydrous THF (36 mL) and cooled to 0 °C. Butyllithium, 1.6 M solution in hexane (4.55 mL, 7.3 mmol), was then added, and the mixture stirred for 2 h at 0 °C. Diphenylmethanone (7.28 mmol) was added, and the mixture maintained at 0 °C for 10 min and then warmed to 25 °C. The solution was acidified with 2 N HCl aqueous solution (10 mL) and extracted with diethyl ether (3 × 20 mL). Then the aqueous phase was made alkaline with NaOH 10% solution and extracted with diethyl ether (3 × 20 mL), and the organic phase was dried (Na_2SO_4). The mixture obtained was purified by column chromatography, using CH_2Cl_2/abs. EtOH/Et_2O/petroleum ether/NH_4OH = 180:45:450:180:25 as eluting system. Compound **1** was obtained as an oil in 80% yield. Anal. ($C_{22}H_{23}NO_2$) C, H, N. ^{1}H NMR and ^{13}C NMR spectra are reported in Table 3 (Supporting Information).

Cyclohexyl-[5-(1-methylpyrrolidin-2-yl)furan-2-yl]phenylmethanol (2). By the same procedure as that for **1** with cyclohexylphenylmethanone as the electrophile, **2** was obtained in 98% yield as an oil that was the expected mixture of four stereoisomers (two racemates).

When the reaction was performed on the (+)-2*R*-furan-2-yl-1-methylpyrrolidine isomer,[21] only two diastereoisomers were obtained: (1*R*,2*R*)- and (1*S*,2*R*)-[5-(1-methylpyrrolidin-2-yl)-furan-2-yl]diphenylmethanol. Anal. ($C_{22}H_{29}NO_2$) C, H, N. ^{1}H NMR and ^{13}C NMR spectra of the isolated enantiomers (1*R*,2*R*)-**2a** and (1*S*,2*R*)-**2c** are reported in Table 3 (Supporting Information).

2-(5-Benzhydrylfuran-2-yl)-1-methylpyrrolidine (3). Sodium borohydride (0.1 g, 2.28 mmol) was added to stirring trifluoroacetic acid (3 mL) at 15 °C under nitrogen according to the procedure reported by Gribble.[27] Compound **1** (0.14 g; 4.20 mmol), dissolved in 1.5 mL of anhydrous CH_2Cl_2, was added in portions to this mixture at 15−20 °C over 15 min. The mixture was stirred under nitrogen for 19 h. Dilution in water, basification with NaOH 10% aqueous solution, and extraction with diethyl ether gave, after drying (Na_2SO_4) and concentration in vacuum, 0.13 g of racemic **3** in 97% yield as an oil. Anal. ($C_{22}H_{23}NO$) C, H, N. ^{1}H NMR and ^{13}C NMR spectra are reported in Table 3 (Supporting Information).

2-[5-(Cyclohexylphenylmethyl)furan-2-yl]-1-methylpyrrolidine (4). By starting from the mixture of compound **2** (0.14 g; 4.13 mmol) and following the same procedure described for compound **3**, we obtained **4** (0.13 g, mixture of diastereoisomers) as an oil in 92% yield. Anal. ($C_{22}H_{29}NO$) C, H, N. ^{1}H NMR spectrum is reported in Table 3 (Supporting Information).

Resolution of the Stereoisomeric Mixture 2. HPLC separation, performed on Chiracel OD CSP (Figure 1), afforded the four isomers of compound **2**: (1*R*,2*R*)-**2a** (^{1}H NMR and ^{13}C NMR spectra are reported in Table 3, Supporting Information), (1*S*,2*S*)-**2d** (^{1}H NMR and ^{13}C NMR spectra are identical to those of **2a**), (1*R*,2*S*)-**2b** (^{1}H NMR and ^{13}C NMR are reported in Table 3, Supporting Information), and (1*S*,2*R*)-**2c** (^{1}H NMR and ^{13}C NMR spectra are identical to those of **2b**).

When separation was performed on (1*R**,2*R*)-[5-(1-methylpyrrolidin-2-yl)furan-2-yl]diphenylmethanol only the two diastereoisomers **2a** and **2c** were obtained.

General Procedure for the Synthesis of Dimethyl Pyrrolidinium Iodides 5−8. An anhydrous diethyl ether solution of the suitable compound (**1**, **2a**, **2b**, **2c**, **2d**, **3**, and **4**) was treated with an excess of methyl iodide and kept overnight at room temperature in the dark. The obtained solid was filtered, dried under vacuum, and recrystallized from absolute ethanol/diethyl ether.

The chemical and physical characteristics, the ^{1}H NMR and ^{13}C NMR spectra, and the optical rotations of the obtained compounds are reported in Table 4 (Supporting Information).

Crystallographic Data for Compound (1*R*,2*R*)-2a. $C_{22}H_{29}NO_2$; M = 339.46; orthorhombic; space group $P2_12_12_1$; a = 8.091(1), b = 13.768(1), c = 17.671(1) Å; V = 1968.5(3)Å^3; Z = 4; D_c = 1.145 g cm^{-3}; μ = 0.564 mm^{-1}; $F(000)$ = 736.

Colorless, needle-shaped crystals suitable for collection were prepared, and RX-analysis was carried out with a Goniometer Oxford Diffraction KM4 Xcalibur2 at room temperature.

Cu Kα radiation (40 mA/−40 kV), monochromated by an Oxford Diffraction Enhance ULTRA assembly, and an Oxford Diffraction Excalibur PX Ultra CCD were used for cell parameter determination and data collection. The integrated intensities, measured using the ω scan mode, were corrected for Lorentz and polarization effects.[34]

Direct methods of SIR2004[35] were used in solving the structure, and it was refined using the full-matrix least-squares on F^2 provided by SHELXL97.[36]

Multiscan symmetry-related measurement was used as experimental absorption correction type.

A total of 7074 reflections were collected with a $4.07 < \theta < 64.71$ range with a completeness to θ 97.1%; 2983 were independent; the parameters were 264, and the final R index was 0.0538 for reflections having $I > 2\sigma I$, and 0.0981 for all data.

The non-hydrogen atoms were refined anisotropically, whereas hydrogen atoms were refined as isotropic, and all of them were assigned to calculated positions.

The cyclohexyl group is disordered, so it was treated as if its atoms were in double position, with occupancy factors 0.44 and 0.56; hydrogen atoms on this group were not assigned.

Pharmacology. Binding Studies. All equilibrium radioligand binding experiments were conducted using a protocol based on previously described procedures.[18] 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.[20] Details of the procedures are reported in the Supporting Information.

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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 (+)-(1*R*,2*R*)-**2a** and its crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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