Brief Articles

Discovery of Diaryl Imidazolidin-2-one Derivatives, a Novel Class of Muscarinic M3 Selective Antagonists (Part 2)

Ilaria Peretto,*,† Claudia Fossati,† Giuseppe A. M. Giardina,† Alessandra Giardini,† Matilde Guala,† Elena La Porta,† Paola Petrillo,† Stefano Radaelli,† Luigi Radice,† Luca F. Raveglia,† Enza Santoro,† Roberta Scudellaro,† Francesca Scarpitta,† Alberto Cerri,† Sergio Menegon,† Giulio M. Dondio,† Andrea Rizzi,‡ Elisabetta Armani,‡ Gabriele Amari,‡ Maurizio Civelli,‡ Gino Villetti,‡ Riccardo Patacchini,‡ Marco Bergamaschi,‡ Franco Bassani,‡ Maurizio Delcanale,‡ and Bruno P. Imbimbo*,‡

NiKem Research, Via Zambeletti 25, 20021 Baranzate, Milan, Italy, and Research and Development, Chiesi Farmaceutici S.p.A., Via Palermo 26/A, 43100 Parma, Italy

Received October 6, 2006

Synthesis and biological activity of a novel class of quaternary ammonium salt muscarinic M3 receptor antagonists, showing high selectivity versus the M2 receptor, are described. Selected compounds exhibited potent anticholinergic properties, in isolated guinea-pig trachea, and good functional selectivity for trachea over atria. In vivo, the same compounds potently inhibited acetylcholine-induced bronchoconstriction after intratracheal administration in the guinea pig.

Introduction

Muscarinic receptors modulate the activity of an extraordinarily large number of physiological functions. Individual muscarinic receptor subtypes are expressed in a complex, overlapping fashion in most tissues and cell types.[1] In human airways, in particular, M2 and M3 receptor subtypes are expressed and differentially distributed. M2 receptors are localized on the postganglionic cholinergic nerve terminals and provide a functional negative feedback of acetylcholine (Ach) release. M3 receptors are localized on effector cells, smooth muscle, mucosal glands, and blood vessels and mediate bronchoconstriction, mucus secretion, and vasodilatation.[2] In asthma and chronic obstructive pulmonary disease (COPD), bronchoconstriction and mucus secretion are increased and the airways are hyperresponsive to contractile agents as a result of increased parasympathetic nerve activity. The number and function of post-junctional M3 muscarinic receptors in the airways are unchanged; rather, it is the supply of Ach to the post-junctional effector cells (smooth muscle and submucosal gland) that is increased. The increase in Ach release occurs because prejunctional, inhibitory M2 muscarinic receptors on the parasympathetic nerves are dysfunctional.[3] Therefore, while inhibition of M3 receptors is useful to limit bronchoconstriction and mucus secretion, inhibition of M2 muscarinic receptors at the airways level may potentiate bronchoconstriction.

Quaternary ammonium muscarinic M3 antagonists have been successfully used by inhalation to antagonize bronchoconstriction in asthma and COPD, because their fixed positive charge assures a poor absorption in the systemic circulation, thus limiting the side effects. The clinically most effective quaternary ammonium muscarinic antagonist is tiotropium bromide, a compound with subnanomolar M3 affinity and long-lasting bronchodilating activity. Unfortunately, tiotropium bromide binds with similar subnanomolar affinity to the human muscarinic M2 receptor subtype (although it displays kinetic selectivity toward the M2 receptor).[4] Thus, new quaternary ammonium muscarinic antagonists with selectivity for M3 over M2 receptors may provide more ideal anticholinergic therapy for both asthma[5] and COPD.[6]

Chemistry

Following the discovery of a class of tertiary amine derivatives as selective M3 antagonists,[1] we considered the possibility of derivatization of these tertiary amines as quaternary ammonium salts to obtain a class of compounds more suitable for the topical treatment of pulmonary diseases. Starting from the imidazolidinone core structure previously identified (Scheme 1), several possible structural modifications were considered and implemented.

The general synthetic pathway employed for the synthesis of all the derivatives described in this paper is outlined in Scheme 2.

Intermediates **3a**−**i** were synthesized utilizing procedures previously described for analogue compounds.[1] Reduction of the hydantoin moiety with Red-Al (sodium bis-(2-methoxyethoxy)aluminum hydride), followed by reaction with the appropriate alkylating agent, allowed obtaining the imidazolidin-2-one final compounds **4**−**34**. Substitution at the basic nitrogen with two different groups (R3 and R4) resulted in the formation of isomers that were not separated. If another stereogenic center was already present in the molecule, the final compounds were obtained as a mixture of diastereoisomers that could not be easily separated by chromatography and were tested as such.

Biology

The binding affinity of the compounds toward the muscarinic M3 and M2 receptors was evaluated in membranes of CHO-K1 clone cells expressing the human M2 or M3 receptors. The nonselective muscarinic radioligand [^{3}H]-*N*-methyl scopo-

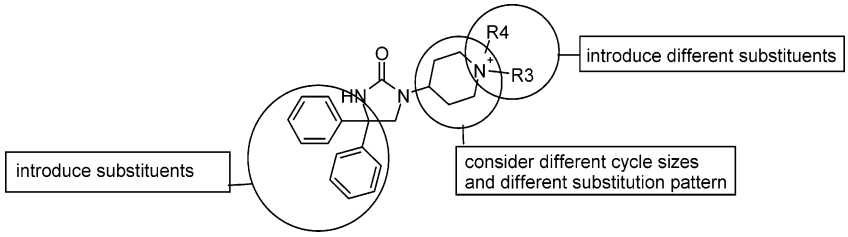
* To whom correspondence should be addressed. Bruno P. Imbimbo for editorial communication and pharmacology and Ilaria Peretto for medicinal chemistry. Phone: +39 0521 279278 (B.P.I.); +39 02 356947587 (I.P.). Fax: +39 0521 279579 (B.P.I.); +39 02 356947606 (I.P.). E-mail: b.imbimbo@chiesigroup.com (B.P.I.); ilaria.peretto@nikemresearch.com (I.P.).

† NiKem Research.

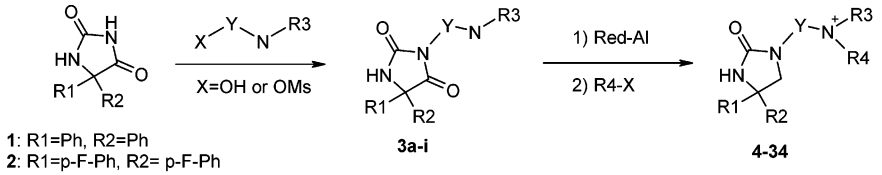
‡ Chiesi Farmaceutici S.p.A.

10.1021/jm061160+ CCC: $37.00
Published on Web 03/13/2007

Scheme 1. Planned Chemical Variations on Imidazolidinone Scaffold



Scheme 2. General Synthetic Pathway for Imidazolidinone Derivatives



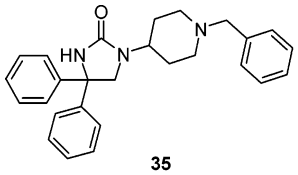
1: R1=Ph, R2=Ph
2: R1=p-F-Ph, R2= p-F-Ph

3a-i

4-34

3a: R1=R2=Ph, YNR3=N-methylpiperidin-4-yl
3b: R1=R2=Ph, YNR3=tropin-4-yl
3c: R1=R2=Ph, YNR3=(R,S)-N-methylpyrrolidin-3-yl
3d: R1=R2=Ph, YNR3=(S)-N-methylpyrrolidin-3-yl
3e: R1=R2=Ph, YNR3=(R)-N-methylpyrrolidin-3-yl
3f: R1=R2=Ph, YNR3=N,N-dimethylaminoethyl
3g: R1=R2=Ph, YNR3=N,N-dimethylaminopropyl
3h: R1=R2=Ph, YNR3=(R,S)-N-phenoxyethylpyrrolidin-3-yl
3i: R1=R2=p-F-Ph, YNR3=(R)-N-methylpyrrolidin-3-yl

Scheme 3. Compound **35** Selected as Initial Hit



35
M3 binding: Ki = 1.9 nM

lamine was used to label the M2 and M3 binding sites, while the nonspecific binding was determined in the presence of cold *N*-methyl scopolamine.

In vitro functional activity of selected test compounds against M3 and M2 receptors was investigated in isolated guinea-pig trachea and atria, respectively. Ach-induced bronchoconstriction in anaesthetized guinea pig was used to investigate in vivo potency of the most interesting compounds.

SAR Discussion

In a previous paper[1] we described the discovery of a class of tertiary amines as selective M3 antagonists. The hit compound **35** displayed good M3 binding affinity (K_i = 1.9 nM) and also quite good selectivity toward the M2 receptor (40-fold; Scheme 3).

We considered the possibility of derivatization of these tertiary amines as quaternary ammonium salts. Compound **4** (Table 1), unfortunately, proved to be only weakly active (M3 K_i = 612 nM), and the dimethyl quaternary ammonium salt **5** was only slightly more active (M3 K_i = 249 nM). We then considered different cycle sizes and different substitution patterns on the basic moiety. Introduction of tropine did not significantly improve binding affinity: derivative **6**, bearing the methyl-benzyl substitution, was almost inactive, while the corresponding dimethyl derivative **7** displayed a better affinity for the M3 receptor (K_i = 153 nM). Pyrrolidinyl derivative **8**, despite being a mixture of four diastereoisomers, was almost as active as compound **4** (K_i = 727 nM). Fixing the stereochemistry at the pyrrolidine stereogenic center resulted in a significant separation of activity: compound **10** (*R* stereochemistry at this center) showed M3 K_i = 282 nM, while compound **11** (*S* stereochemistry) was almost inactive. Linear basic moieties were also considered, such as in compounds **12**−**15**; none of these compounds displayed a significant improvement of activity if compared to the cyclic derivatives.

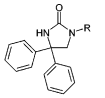
We then investigated in more detail the substitution patterns at the quaternary nitrogen atom, selecting the pyrrolidine scaffold as template. In fact, among the compounds reported in Table 1, pyrrolidine derivatives maintained an appreciable affinity both in the presence of the dimethyl- and the methyl-benzyl substitution (compounds **9** and **10**); thus, these derivatives might better tolerate diverse substituents on the quaternary nitrogen atom.

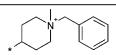
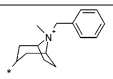
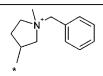
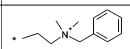
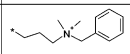
As a first attempt, we considered the phenethyl-methyl derivative **16** (Table 2), which surprisingly displayed a significant improvement of activity (M3 K_i = 0.67 nM) if compared to the corresponding benzyl-methyl derivative **8** (M3 K_i = 727 nM). We further investigated the phenethyl moiety by insertion of additional carbon or oxygen atoms in the side chain (compounds **17**−**20**), but neither affinity nor selectivity were improved. The importance of the aromatic ring in the side chain was assessed with compound **21**, featuring the 3,3-dimethyl-butan-2-one moiety, which proved to be completely inactive. On the other hand, the corresponding acetophenone derivative **22**, maintaining the phenyl moiety, displayed a good affinity for the M3 receptor (K_i = 1.89 nM) and the best selectivity observed so far in this class of quaternary ammonium salts (16.7-fold). Other compounds (**23** and **24**) featuring the phenyl moiety and a hydrogen-bond acceptor group in a different substitution pattern within the side chain did not result in significant improvement in the activity or the selectivity.

Replacement of the methyl group on the quaternary nitrogen atom with cyanomethyl or methoxyethyl moieties was detrimental for affinity, as shown by compounds **25** and **26** (M3 K_i = 541 nM and 356 nM, respectively).

To confirm the importance of the stereochemistry at the pyrrolidine C3 stereogenic center, compounds **27** and **28**, consisting of (*R*)- and (*S*)-isomers, were synthesized and tested. The (*S*)-isomer **28** displayed M3 K_i = 7.60 nM and 20-fold selectivity toward the M2 receptor, while the (*R*)-isomer **27** was the more active (M3 K_i = 1.04 nM) although poorly selective (only 2.3-fold); the (*R*)-isomer was then considered as privileged scaffold due to its higher binding affinity.

Table 1. Modifications of the Basic Moiety and of the Substituents on the Basic Nitrogen



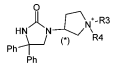
Cmpd	R	Number of stereoisomers[a]	M3 affinity (Ki, nM)	M2 affinity (Ki, nM)[b]	M2/M3 selectivity ratio
4		2	612	inactive	-
5		1	249	1810	7.3
6		2	2535	inactive	-
7		1	153	498	3.2
8		4	727	2010	2.8
9		1	310	862	2.8
10		2	282	884	3.0
11		2	3158	inactive	-
12		1	507	inactive	-
13		1	335	inactive	-
14		1	619	inactive	-
15		1	2065	inactive	-

[a] When the final compounds were obtained as a mixture of stereoisomers, they were not separated and were tested as such. [b] Inactive: Percent of control binding <50% at the concentration of 10 μM.

Introduction of 4-fluoro substituents on the phenyl rings of the imidazolidinone scaffold as in compound **29** (Table 3), featuring the methyl-phenethyl substitution on the basic nitrogen, maintained good binding affinity (M3 K_i = 0.75 nM) and significantly improved the selectivity toward the M2 receptor (almost 30-fold selective). This finding suggested combining the same difluoro substitution pattern with the best substituent on the quaternary nitrogen atom in terms of selectivity, that is, the acetophenone side chain; the resulting compound **30** displayed M3 K_i = 1.84 nM and 51-fold selectivity toward the M2 receptor.

Introduction of 3-fluoro substitution on the acetophenone moiety (compound **31**) did not improve activity or selectivity. Replacement of the phenyl ring of acetophenone with the heterocycles was then considered. Thienyl derivative **32** was as active and selective as the corresponding acetophenone derivative **30**. Conversely, pyridyl derivatives proved to be more interesting, and the position of the basic pyridine nitrogen proved to be crucial for affinity and selectivity. In fact, 2-pyridyl derivative **34** maintained good activity (M3 K_i = 2.61 nM) and displayed excellent selectivity toward the M2 receptor (100-

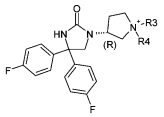
Table 2. Investigation of Substitution on the Basic Nitrogen: Pyrrolidine Derivatives



Cmpd	N. of stereo-isomers[a]	Configura-tion of (*)	R3	R4	M3 affinity (Ki, nM)	M2 affinity (Ki, nM)[b]	M2/M3 selectivity ratio
8	4	(R,S)	benzyl	Me	727	2010	2.8
10	2	(R)	benzyl	Me	282	884	3.1
11	2	(S)	benzyl	Me	3158	inactive	-
16	4	(R,S)	phenethyl	Me	0.67	5.4	8.1
17	4	(R,S)	phenylpropyl	Me	0.70	0.73	1.0
18	4	(R,S)	phenoxyethyl	Me	1.89	1.66	0.9
19	4	(R,S)	benzyloxyethyl	Me	6.70	47.8	7.1
20	4	(R,S)	phenoxypropyl	Me	1.76	8.84	5.0
21	4	(R,S)	-CH_2CO-tBu	Me	inactive	inactive	-
22	4	(R,S)	-CH_2CO-Ph	Me	1.89	31.5	16.7
23	4	(R,S)	-CH_2CONHPh	Me	9.11	9.92	1.1
24	4	(R,S)		Me	6.66	27.4	4.1
25	4	(R,S)	phenoxyethyl	CH_2CN	541	1152	2.1
26	4	(R,S)	phenoxyethyl	CH_2CH_2OMe	356	552	1.6
27	2	(R)	phenoxyethyl	Me	1.04	2.39	2.3
28	2	(S)	phenoxyethyl	Me	7.60	157	20.7

[a] When the final compounds were obtained as a mixture of stereoisomers, they were not separated and were tested as such. [b] Inactive: Percent of control binding <50% at the concentration of 10 μM.

Table 3. Difluorophenyl Imidazolidin-2-one Derivatives: Investigation of the Substituents on the Basic Nitrogen



Cmpd	N. of stereoisomers[a]	R3	R4	M3 affinity (Ki, nM)	M2 affinity (Ki, nM)	M2/M3 selectivity ratio
29	2	phenethyl	Me	0.75	21.9	29.2
30	2	-CH_2CO-Ph	Me	1.84	94.5	51.4
31	2		Me	3.01	108	35.9
32	2		Me	1.62	88.5	54.6
33	2		Me	22.9	1014	44.3
34	2		Me	2.61	268	102.7

[a] When the final compounds were obtained as a mixture of stereoisomers, they were not separated and were tested as such.

Table 4. In Vitro Functional Selectivity and In Vivo Potency of Selected Compounds in Guinea Pig

cmpd	isolated trachea (IC_{50}, nM)	left atria (IC_{50}, nM)	selectivity (M_2/M_3)	in vivo potency[a] (ID_{50} nmol/kg)
29	4.5	140	31.1	1.04
31	7.8	1000	128.2	3.86
32	7.6	880	115.8	5.76

[a] The ID_{50} is the dose producing 50% inhibition of Ach-induced bronchoconstriction.

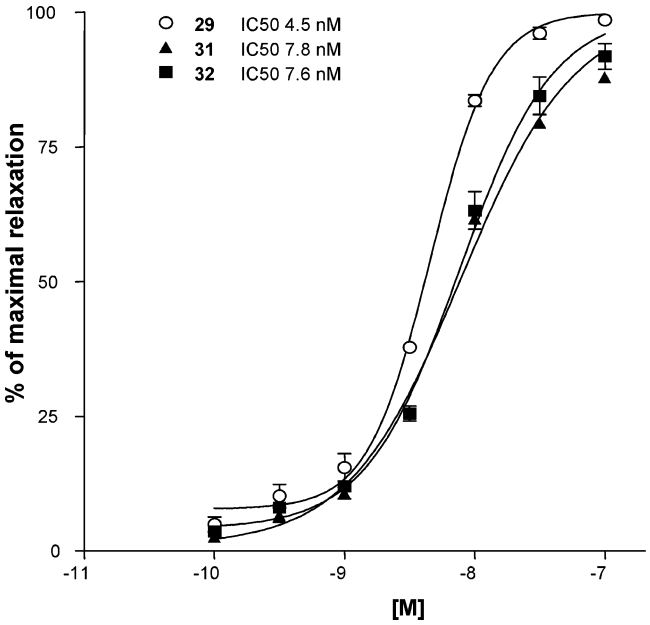


Figure 1. Effect of compounds **29**, **31**, and **32** in the isolated guinea-pig trachea assay. Isoprenaline-induced relaxation was used as the maximal inhibitory reference effect.

fold selectivity), while 3-pyridyl derivative **33** showed a significant loss of activity (M3 K_i = 22.9 nM).

The functional selectivity of selected compounds was investigated in vitro by studying their ability to inhibit carbachol-induced contractions in guinea-pig trachea and methacholine-induced bradycardia in the guinea-pig left atria (Table 4).

Compounds **29**, **31**, and **32** showed concentration-dependent inhibition of carbachol-induced contraction in guinea-pig trachea, with IC_{50} values of 4.5, 7.8, and 7.6 nM, respectively (Figure 1). Furthermore, the same compounds showed good to high functional selectivity toward the M2 receptor, determined in the in vitro assay on guinea-pig isolated atria, thus confirming that these quaternary ammonium salts act as potent and selective functional antagonists at the M3 receptor.

Finally, compounds **29**, **31**, and **32** were tested in vivo in anesthetized guinea pig for their ability to inhibit bronchoconstriction produced by i.v. injection of Ach after intratracheal administration (Table 4). All the compounds showed a potent bronchodilator activity, with ID_{50} values of 1.04, 3.86, and 5.76 nmol/kg, respectively.

Conclusions

A novel class of M3 receptor antagonists was discovered,[7] characterized by the moiety of a quaternary ammonium salt, suitable for the topical treatment of pulmonary diseases. Compounds with binding affinity in the low nanomolar range were obtained, with up to 100-fold selectivity toward the M2 receptor. Functional in vitro studies on selected compounds demonstrated high subtype selectivity for tracheal M3 over cardiac M2 receptors in the isolated guinea-pig tissues. Furthermore, compounds **29**, **31**, and **32**, after intratracheal administration, potently inhibited Ach-induced bronchoconstriction in guinea pigs, suggesting that these compounds could provide efficient bronchodilator activity with minimal side effects for the treatment of obstructive airway diseases.

Experimental Section

Chemistry. ^{1}H NMR spectra were recorded on a Bruker ARX 300 (300 MHz); chemical shifts are reported downfield in parts per million (ppm) relative to TMS, utilizing the solvent peaks as the reference. EI mass spectra were recorded on a Thermo Finnigan TSQ700 spectrometer; ESI-LCMS analysis was performed on a Phenomex Luna C-18, 3 μm, 4.6 × 50 mm column, with an AQA Thermo Finnigan single quadrupole instrument or with a Waters Micromass ZQ2000 instrument; high performance liquid chromatography (HPLC) analysis was performed on a Shimadzu SCL-10A equipped with an SIL-10AD injector and an SPD-M10A detector normally operating in a 200−360 nm range, with a Waters Symmetry C-18, 3.5 μm, 4.6 × 75 mm column, using a 10 min gradient of 0−100% solvent B, where solvent A is 90:10:0.05 CH_3CN-H_2O-TFA and solvent B is 90:10:0.05 H_2O-CH_3CN-TFA. Reactions were monitored by TLC using 0.25 mm Merck silica gel plates (60 F254); column chromatography was performed on Merck silica gel 60 (particle size 0.063−0.2 mm); and flash chromatography was conducted using a Biotage-Quad3 apparatus and prepacked silica gel columns (KP-SIL, particle size 32−60 μm). Anhydrous solvents were purchased from Aldrich and used as received. "Brine" refers to a saturated aqueous solution of NaCl. Unless otherwise specified, solutions of common inorganic salts used in workups are aqueous solutions.

Synthetic Procedures. General Procedure 1. Mitsunobu Reaction. 3-(1-Methyl-piperidin-4-yl)-5,5-diphenyl-imidazolidin-2,4-dione (3a). Commercially available 5,5-diphenyl hydantoin (phenytoin, 0.50 g, 1.98 mmoles) is dissolved in dry THF[a] (20 mL); triphenyl phosphine (0.78 g, 2.97 mmoles) and 4-hydroxy-*N*-methyl piperidine (0.342 g, 2.97 mmoles) are added to the reaction mixture. The resulting solution is cooled to 0 °C and DEAD (0.47 mL, 2.97 mmoles) is added dropwise. The reaction is then stirred at room temperature for 24 h. The solvent is evaporated under vacuum, and the product is purified by chromatography on silica gel (500 g, eluent: AcOEt/hexane = 2:8 to AcOEt) to yield 0.8 g of pure product as a white solid. LC-MS (ESI pos): 350.52 (MH^+).

Compounds **3c**−**i** were synthesized, following the same procedure, starting from the corresponding hydantoin derivatives.

3-(1-Methyl-tropin-4-yl)-5,5-diphenyl-imidazolidine-2,4-dione (3b). (a) Synthesis of Methanesulfonic Acid 8-Methyl-8-aza-bicyclo[3.2.1]oct-3-yl Ester (Tropine Mesilate). Commercially available tropine (0.150 g, 1.063 mmoles) is added to a solution of triethyl amine (0.207 mL, 1.5 mmoles) in dry DCM (10 mL); the resulting mixture is cooled to 0 °C and mesyl chloride (0.099 mL, 1.276 mmoles) is added. The reaction is stirred at 0 °C for 1 h, then the solvent is evaporated in vacuum and the product obtained is employed in the next step without purification.

(b) Synthesis of 3-(8-Methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-5,5-diphenyl-imidazolidin-2,4-dione (3b). Commercially available 5,5-diphenyl hydantoin (phenytoin, 0.20 g, 0.793 mmoles) is dissolved in dry DMF (10 mL), and K_2CO_3 (0.33 g, 2.38 mmoles) and *N*-methyl-tropine mesilate (prepared in the previous step) dissolved in dry DMF (3 mL) are added. The reaction is then stirred at 80 °C for 18 h. The reaction mixture is diluted with water and extracted with ethyl acetate; the organic phase is washed with water, then with brine, and finally dried and concentrated under vacuum to give a yellowish solid that is crystallized from ethyl ether to give the desired product as a white solid (0.18 g). LC-MS (ESI pos): 376.10 (MH^+). ^{1}H NMR ($CDCl_3$): 7.41−7.29 (m, 10H), 5.89 (s

[a] Abbreviations: DCM, dichloromethane; DEAD, diethyl azodicarboxylate; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; THF, tetrahydrofuran.

br, 1H), 4.45 (m, 2H), 3.53 (m, 2H), 2.72 (s, 3H), 2.33 (m, 2H), 1.82 (m, 2H), 1.27 (m, 2H).

General Procedure 2. Reduction with Red-Al and Alkylation. Synthesis of 1-Benzyl-1-methyl-4-(2-oxo-4,4-diphenyl-imidazolidin-1-yl)-piperidinium (4). (1) 3-(1-Methyl-piperidin-4-yl)-5,5-diphenyl-imidazolidin-2-one. A 3.5 M solution of sodium bis-(2-methoxyethoxy)aluminum hydride (Red-Al) in toluene (5.37 mL, 18.4 mmoles) is dissolved in dry THF (15 mL) under nitrogen atmosphere and cooled to 0 °C. A solution of compound **3a** (0.803 g, 2.3 mmoles) in dry THF (20 mL) is added dropwise. The mixture is then heated to 85 °C for 4 h. The reaction is cooled again to 0 °C and quenched with water (5 mL); then 2 M sodium hydroxide is added (10 mL) and the mixture is extracted with ethyl acetate; and the organic phase is washed with water, then with brine, and finally dried and concentrated under vacuum to give a yellowish solid that is crystallized from acetone to give the desired product as a white solid (0.65 g). LC-MS (ESI POS): 336.20 (MH^+). ^{1}H NMR (DMSO; 343 K): 7.80 (s br, 1H), 7.40−7.21 (m, 10H), 3.91 (s, 2H), 3.80 (m, 1H), 3.41 (m, 2H), 3.09 (m, 2H), 2.72 (s, 3H), 2.01 (m, 2H), 1.80 (m, 2H).

(2) 1-Benzyl-1-methyl-4-(2-oxo-4,4-diphenyl-imidazolidin-1-yl)-piperidinium (4). 3-(1-Methyl-piperidin-4-yl)-5,5-diphenyl-imidazolidin-2-one (0.08 g, 0.24 mmoles) is dissolved in CH_3CN (4 mL) in a microwave tube. Benzyl bromide (0.164 g, 0.96 mmol) is added, the tube is sealed, and the solution is heated at 120 °C in a microwave oven for 2 h. The solvent is evaporated and the crude compound is triturated with *i*-Pr_2O/DCM. The desired product **4** is obtained as a pale yellow solid (0.08 g). LC-MS (ESI pos): 426.18 (MH^+). ^{1}H NMR ($CDCl_3$): 7.66−7.21 (m, 15H), 5.00 (s br, 1H), 4.13 (m, 2H), 4.09 (s, 2H), 3.92 (m, 2H), 3.56 (m, 2H), 3.23 (s, 3H), 2.59 (m, 2H), 2.00 (m, 2H).

All the quaternary ammonium salts reported in Tables 1−3 were synthesized following the same procedure starting from the corresponding tertiary amine and the appropriate alkylating agent.

Biology. Cell lines and membrane preparations and radioligand binding conditions are as described previously.[1]

Isolated Guinea-Pig Trachea. Tracheal zigzag preparations were obtained from tracheal segments excised from male albino Dunkin−Hartley guinea pigs (450−550 g, Charles River Laboratories Italia, Calco, Italy) and set up as described previously.[8] Test compounds were assayed for their ability to relax a smooth muscle tone raised by a submaximally effective concentration (0.3 μM) of carbachol. The antagonist concentration producing a 50% reversal of carbachol-induced tonic contraction (IC_{50}) was taken as a measure of antagonist potency. Isoprenaline-induced relaxation was used as the maximal inhibitory reference effect.

Isolated Guinea-Pig Left Atrium. The hearts were rapidly removed from male albino Dunkin−Hartley guinea pigs (325 ± 25 g), and the left and right atria were separately excised. The left atria were mounted with a preload of 0.5 g in McEwens, pH 7.4. The solution was maintained at 32 °C, and tissues were stimulated by the application of methacholine 1 μM. Negative ionotropic responses to the addition of methacholine were recorded as changes in isometric tension, as previously reported.[9] Dose−response curves were obtained for selected test compounds.

In Vivo Bronchoconstriction in Guinea Pig. All in vivo experiments were performed on male albino Dunkin−Hartley guinea pigs (450−550 g, Charles River Laboratories Italia, Calco, Italy), as described previously.[8] Test compounds (given intratracheally to anesthetized animals) were assayed for their ability to counteract bronchoconstriction produced by i.v. injection of Ach, 20 μg kg^{-1}. Bronchoconstriction, quantified as a reduction of tidal volume, was evaluated according to the method of Konzett and Roessler.[9] Ach challenge was applied at 5, 15, and 30 min and then every 30 min up to 180 min from the intratracheal administration of test compounds. The effect of test compounds was expressed as percentage of inhibition of Ach-evoked bronchoconstriction, as compared to basal response obtained in control animals. Ethical approval of the experimental protocols with animals (in vitro and in vivo) was obtained from the local Ethics Committee.

Acknowledgment. This study was supported by Chiesi Farmaceutici, Parma, Italy. The authors thank Dr. Samuele Pedraglio and Dr. Chiara Bigogno (NiKem Research) for the analytical support.

Supporting Information Available: Spectroscopic data and elemental analyses for compounds **3a**−**i** and **4**−**34**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Peretto, I.; Forlani, F.; Fossati, C.; Giardina, G. A. M.; Giardini, A.; Guala, M.; La Porta, E.; Petrillo, P.; Radaelli, S.; Radice, L.; Raveglia, L. F.; Santoro, E.; Scudellaro, R.; Scarpitta, F.; Bigogno, C.; Misiano, P.; Dondio, G. M.; Rizzi, A.; Armani, E.; Amari, G.; Civelli, M.; Villetti, G.; Patacchini. R.; Bergamaschi, M.; Delcanale, M.; Salcedo, C.; Fernández, A. G.; Imbimbo, B. P. Discovery of diaryl imidazolidin-2-one derivatives, a novel class of muscarinic M3 selective antagonists (Part 1) *J. Med. Chem.* **2007**, *50*, 1571−1583 and references therein.

(2) Minette, P. A.; Barnes, P. J. Muscarinic receptor subtypes in lung. Clinical implications. *Am. Rev. Respir. Dis.* **1990**, *141*, S162−S165.

(3) Coulson, F. R.; Fryer, A. D. Muscarinic acetylcholine receptors and airway diseases. *Pharmacol. Ther.* **2003**, *98*, 59−69.

(4) Saberi, F.; O'Donnell, D. E. The role of tiotropium bromide, a long-acting anticholinergic bronchodilator, in the management of COPD. *Treat. Respir. Med.* **2005**, *4*, 275−281.

(5) Bowerfind, W. M.; Fryer, A. D.; Jacoby, D. B. Double-stranded RNA causes airway hyperreactivity and neuronal M2 muscarinic receptor dysfunction. *J. Appl. Physiol.* **2002**, *92*, 1417−1422.

(6) On, L. S.; Boonyongsunchai, P.; Webb, S.; Davies, L.; Calverley, P. M.; Costello, R. W. Function of pulmonary neuronal M2 muscarinic receptors in stable chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* **2001**, *163*, 1320−1325.

(7) Peretto, I., Scarpitta, F., La Porta, E., Raveglia, L. F., Giardina, G. A. M., Imbimbo, B. P., Rizzi, A., Villetti, G. Preparation of imidazolone derivatives and heteroanalogs with antimuscarinic activity. International patent WO 06066924, June 29, 2006.

(8) Villetti, G.; Bergamaschi, M.; Bassani, F.; Bolzoni, P. T.; Harrison, S.; Gigli, P. M.; Janni, A.; Geppetti, P.; Civelli, M.; Patacchini, R. Pharmacological assessment of the duration of action of glycopyrrolate vs tiotropium and ipratropium in guinea-pig and human airways. *Br. J. Pharmacol.* **2006**, *148*, 291−298.

(9) (a) Konzett, H.; Roessler, R. Versuchanornungzu untersuchungen an der bronchialmuskulatur. *Arch. Exp. Path. Pharmak.* **1940**, *195*, 71−74. (b) Salcedo, C.; Davalillo, S.; Catena, J.; Enrich, E.; Fernández-Serrat, A.; Miquel, I.; Sanagustín, J.; Farrerons, C.; Lagunas, C.; Balsa, D.; Fernández, A. G. Functional characterization of SVT 40776, a novel and potent M3 receptor antagonist, on mice detrusor and atria isolated preparations using in vitro and ex vivo protocols. *Br. J. Pharmacol.* **2002**, *136* (suppl), 45P. (c) Lambrecht, G.; Feiferi, R.; Wagner-Roder, M.; Strohmann, C.; Zilch, H.; Tacke, R.; Wallbroeck, M.; Christophe, J.; Boddeke, H.; Mutschler, E. Affinity profiles of hexahydro-siladefenidol analogues at muscarinic receptor subtypes. *Eur. J. Pharmacol.* **1989**, *273*, 959−966.

JM061160+