Eur J Med Chem (1994) 29, 153–161
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Short communication

Cholinergic agents structurally related to furtrethonium. 1

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(Received 8 March 1993; accepted 25 October 1993)

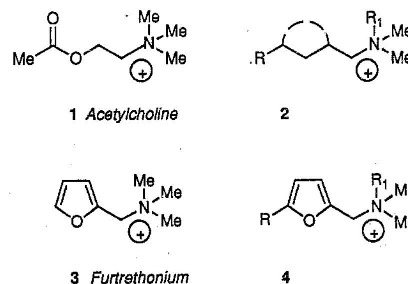
Summary — A series of 5-substituted-2-(dimethylaminomethyl)-furyl derivatives **4** was prepared, with the aim of discovering novel antimuscarinic agents which are selective for smooth muscle as opposed to cardiac tissue. Both non-quaternary and quaternary ammonium compounds were synthesised. The agonist starting point, furtrethonium **3**, was gradually transformed into antagonist by introduction of lipophilic and bulky groups in position 5 of this molecule. In particular, the introduction of α-hydroxy-α-cyclohexylbenzyl moiety (compound **9b**), a lipophilic group characteristic of antimuscarinic agents, caused an appreciable increase of the antagonist's potency, and the lengthening of the distance between this lipophilic group and the furan ring, obtained by introduction of an ester, ether or amide group, led to some selectivity towards smooth muscle (compounds **19**, **21**, **25**). Interestingly, compound **19**, with an ester moiety as a spacer group, proved to be at least 20 times more potent in rat ileum (pK_B = 7.3) and rat bladder (pK_B = 7.2) than guinea-pig atria (pK_B = 5.9).

furtrethonium derivatives / cholinergic agents / antimuscarinic activity

Introduction

A large number of studies have led to muscarinic receptors being divided into receptor subclasses in the various peripheral tissues [1–6]. The classification into subclasses was made possible by the use of suitable selective antagonists: thus cardiac muscarinic receptors are called M_2-receptors, those in the glands are M_3-receptors and smooth muscle receptors are a mixture of M_2 and M_3 [7–9], but only the M_3 are the functional receptors responsible for contraction. This differentiation suggests that it might be possible to discover tissue-selective antimuscarinic agents.

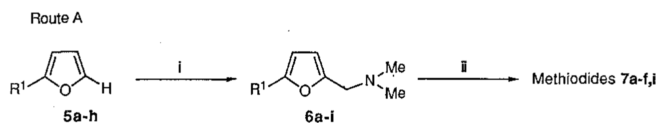
Structure–activity relationships in the field of muscarinic agents have been the object of many investigations and the structural features required for the interaction with the receptor (cationic ending and lipophilic tail at a suitable distance) have been extensively discussed [10–14]. It is generally accepted that the capacity of acetylcholine to interact with different cholinergic receptor types and subtypes is related to its conformational flexibility and, consequently, selective antagonists should be obtainable from rigid isosters **2** of acetylcholine **1**. Continuing our studies [15] on cholinergic agents featured by conformational restrictions we report here the synthesis and biological evaluation of some partially rigid analogues of acetylcholine structurally related to furtrethonium **3**. Substitutions at position 5 of the furan ring **4** were performed with groups of widely varying bulk and lipophilicity with the aim of discovering antimuscarinic agents which are selective for smooth muscle as opposed to cardiac tissue. In most cases, both quaternary ammonium and non-quaternary compounds were prepared. The influence of optical isomerism was also investigated.



Chemistry

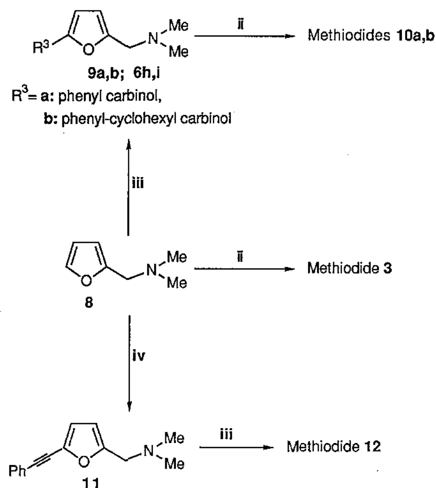
The synthetic strategies (schemes 1–3) used for the preparation of the furtrethonium derivatives with general formula **4** can be divided as follows: a) *route A* [16–19] is based on the Mannich reaction between a starting 2-alkyl or aryl, alkyl (**5a–f**) or hydroxyalkyl (**5g** and **h**) furane, formaldehyde and dimethylamine (scheme 1); b) *route B* [20, 21] is based on the lithiation and subsequent reaction of 2-dimethylfurfurylamine **8** [16] with the appropriate aldehyde or ketone (scheme 1); and c) *route C* is based on 2-furoic acid derivatives as key starting materials (scheme 2). Thus, appropriately prepared or commercially available derivatives **5a–h** were reacted (*route A*) with 40% aqueous dimethylamine and 40% aqueous formaldehyde in glacial acetic acid to give the target compounds **6a–i** in 40–75% yield. Attempts to prepare **6h** resulted in the formation of **6i** with only traces of the expected compound; the carbinols **5h** and **6h** are not stable under Mannich conditions and readily become **6i**. Another approach (*route B*) involved the reaction of 2-furfuryldimethylamine **8** [16], which was lithiated with *n*-BuLi and quenched with the appropriate derivative to give **9a**, **b** and **6h**, **i**. This approach was undertaken in view of the importance of a hydroxy-cyclopentyl moiety at position 5; this modification gives a cyclic moiety, and includes a hydrophobic chain and a hydroxy group, both of which are important for the binding in the area of the receptor reserved for the lipophilic tail [12]. Here again the compound **6h** initially formed in 80% yield was not stable even if stored at –17°C, undergoing slow spontaneous dehydration to **6i**. Preparation of compound **11** (*route B*), first attempted with the classic Stephens–Castro method [22], was finally achieved by a complementary approach [23], the reaction of 5-furyl-copper-*N*,*N*-dimethylfurfurylamine (generated *in situ* from 5-furyl-lithium-2-*N*,*N*-dimethylfurfurylamine) with iodoethynylbenzene at –20°C gave the ethynyl derivative **11** in 45% yield (scheme 1). The

Route A



R^1= **a:** Me, **b:** Et, **c:** Ph-CH_2-, **d:** Ph-$(CH_2)_3$-, **e:** Ph-CH=CH-, **f:** MeO-CH_2- , **g:** HO-CH_2-, **h:** 1'hydroxy-cyclopentyl, **i:** 1'cyclopentenyl.

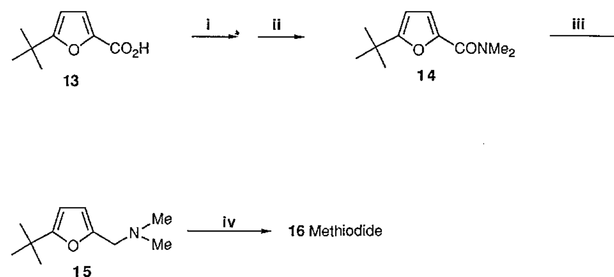
Route B



i = Me_2NH, HCO_2H, HCHO; ii = MeI, MeOH; iii = nBuLi, THF, -30°C, benzaldehyde or ketones; iv = nBuLi, THF, -30°C, CuI, iodoethynyl-benzene.

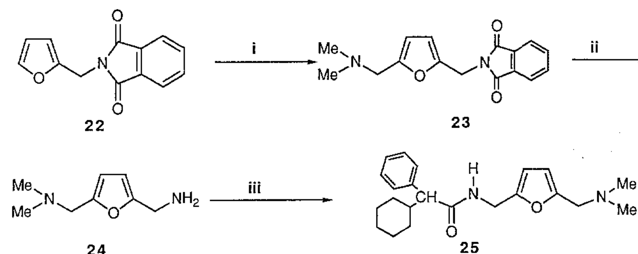
Scheme 1.

Route C



i = $SOCl_2$; ii = DMF; iii = $LiAlH_4$; iv = MeI, MeOH

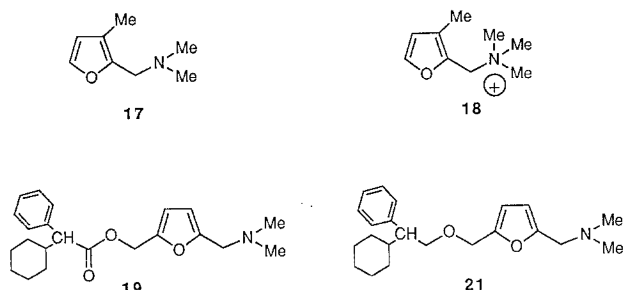
Scheme 2.



i = dimethylamine, formaldehyde, acetic acid,Δ; ii = hydrazine hydrate, EtOH, Δ; iii = dimethoxyethane, 0°C, phenylcyclohexylacetic acid-hydroxysuccinimidester.

Scheme 3.

preparation of compounds **15** and **17** required an alternative access to the dimethylamino-methylene function (*route C*). This was accomplished for **15**, as depicted in scheme 2, starting from **13** [24] and following and adapting the procedure reported for **17** [25, 26]. Compound **19** was prepared by esterification of **6g** with phenylcyclohexyl acetic acid and dicyclohexylcarbodiimide in dichloromethane, in the presence of a catalytic amount of 4-dimethylaminopyridine [27] (scheme 3). The direct condensation of **6g** with 2-phenylcyclohexyl ethanol (see *Experimental protocols*), in the presence of methanesulfonic acid, produced **21** in satisfactory yield. Finally, the amide analogue **25** was prepared by condensation between diamine **24** and phenylcyclohexyl acetic acid *via* activation of the acid as hydroxysuccinimidester. The unknown diamine **24** was prepared from furfurylamine, which was in turn protected, **22**, reacted to **23** and deprotected with hydrazine-hydrate to give the expected compound in 45% overall yield.



The title compounds were submitted to the biological tests as methiodides and/or oxalate salts, in order to evaluate them in both the permanent and non-permanent charged form. The oxalic acid was chosen in view of the salts formed, which were quite stable and generally not as hygroscopic as the hydrochloride salts. To further investigate structure–activity relationships of compound **19**, the 2 optical isomers were prepared. This required optical resolution of the racemic phenylcyclohexyl acetic acid and condensation with **6g** under the same conditions as used for the racemic product. The optical purity of enantiomers of **19** was determined by the observation of the ^{1}H-NMR shift of the -CH_2N- singlet, centered at 3.50 ppm, induced by the addition of the chiral shift reagent Eu(Tfc)$_3$.

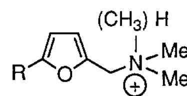
Pharmacology

Rat ileum and bladder were used as target tissues to evaluate the muscarinic or antimuscarinic properties of synthesized compounds at M_3 receptors. Guinea-pig atria, endowed with M_2 receptors, were used to check their selectivity. For compounds exhibiting muscarinic agonist properties (contraction in the ileum and bladder, negative inotropic effect in the atria), potencies were expressed as $-\log EC_{50}$ (negative logarithm of the molar concentration of agonist causing 50% of maximum response), while abilities to produce responses were quantified as α (ratio between maximal response to test agonists and that to furtrethonium in ileum and bladder or that to carbachol in atria). The potencies of compounds exhibiting antimuscarinic properties were expressed as pK_B.

Results and discussion

The pharmacological results are summarized in table I. The increment in potency obtained by the transformation of furtrethonium **3** into methyl-furtrethonium **7a** was consistent with Ing's rule [28, 29] and can also be observed in the non-quaternary analogues **8** and **6a**, while the introduction of a methyl group in position 3 of the furane ring gave a compound **18** that was equiactive to **3**. The introduction of the furan ring of more lipophilic and sterically hindering group in position 5 determined a variety of consequences. 1) Only quaternary ethyl **7b** and phenylethynyl **12** derivatives maintained muscarinic-agonist properties, being partial agonists in the ileum, bladder and atria, with a potency from 30 to > 100 times lower than that of methyl-furtrethonium **7a**. The non-quaternary ethyl derivative **6b** proved to be practically inactive. This result is consistent with that for the non-quaternary furtrethonium **8** and methyl-furtrethonium **6a** derivatives, which showed a marked loss in activity in all tissues. 2) Introduction of the methoxy-methyl group led to total loss of activity for both the quaternary and the tertiary amino derivatives **7f**, **6f**. 3) Quaternarized compounds having the α-hydroxybenzyl **10a**, styryl **7e**, *t*-butyl **16**, benzyl **7c** or 1-cyclopentenyl **7i** groups in position 5 proved to be weak antagonists, without any selectivity between the 3 tissues examined. In their synthesised non-quaternary counterparts, as well as in the 3-phenylpropyl derivative **6d**, a weak antagonist activity was maintained **6i** or further reduced **15**, **6c** to the point of disappearing **9a**, **6e**. 4) Introduction of the α-hydroxy-α-cyclohexyl benzyl moiety, a bulky lipophilic group characteristic of antimuscarinic agents [12, 13], greatly increased antagonist potency. In contrast with the previous results, the non-quaternary derivative **9b** was slightly more potent than its corresponding quaternary analogue **10b**. However, no selectivity was observed between tissues. Following these results, attempts to induce tissue selectivity were made, introducing a spacer group between the furan ring and some of the bulky lipophilic moieties, known

Table I. *In vitro* muscarinic agonist/antagonist potencies of tested compounds[a].



COMPD.	R	Rat ileum AGONISM -logEC50	Rat ileum AGONISM α	Rat ileum ANTAGONISM pKB	Rat bladder AGONISM -logEC50	Rat bladder AGONISM α	Rat bladder ANTAGONISM pKB	Guinea-pig atria AGONISM -logEC50	Guinea-pig atria AGONISM α	Guinea-pig atria ANTAGONISM pKB
3*	H	6.1±0.1(6)	1.03±0.04(6)[b]		5.5±0.1(6)	1.02±0.03(6)[b]		6.5±0.1(5)	0.96±0.04(5)[b]	
8		3.9±0.2(3)	0.95±0.10(3)[b]		3.3±0.4(3)	0.96±0.05(3)[b]		NT		
7a*	Me-	7.2±0.2(4)	1.04±0.07(4)[b]		6.4±0.1(4)	1.06±0.04(4)[b]		7.2±0.2(4)	1.05±0.06(4)[b]	
6a		4.5±0.2(4)	0.71±0.06(4)[b]		3.7±0.1(3)	0.73±0.08(3)[b]		NT		NT
7b*	Et-	5.4±0.1(4)	0.56±0.14(4)[b]		4.5±0.2(4)	0.51±0.05(4)[b]		NT		NT
6b		NSE			NSE			NT		NT
7f*	Me–O–	NSE			NSE			NT		NT
6f		NSE			NSE			NT		NT
10a*	OH			4.5±0.2(3)			4.6±0.1(3)	NT		NT
9a		NSE			NSE			NT		NT
7e*				5.1±0.2(3)[c]			5.2±0.1(4)[c]			5.5±0.2(3)
6e		NSE			NSE			NT		NT
12*		5.2±0.2(4)	0.62±0.09(4)[b]		4.8±0.1(4)	0.52±0.05(4)[b]		5.2±0.1(4)	0.77±0.06(4)[b]	
16*	tBut-			5.0±0.2(4)[c]			5.1±0.1(4)			5.3±0.1(3)
15				4.6±0.1(4)[c]			4.9±0.2(4)[c]			5.0±0.2(3)[d]
7c*				5.1±0.2(4)[c]			5.0±0.1(4)[c]			5.4±0.2(3)
6c				4.7±0.2(4)			4.4±0.2(4)[c]			5.0±0.1(3)
6d				5.2±0.2(3)[c]			5.0±0.1(3)[c]			NT
7i*				5.2±0.2(3)			5.3±0.2(3)			5.1±0.2(3)
6i				5.1±0.1(4)			5.0±0.2(4)	NT		NT
10b*	OH			7.7±0.1(6)			7.5±0.1(6)			7.5±0.2(4)
9b				8.0±0.1(4)			7.9±0.1(4)			7.8±0.1(4)
20*	O, O			6.4±0.1(4)[c]			6.2±0.2(4)[c]			5.5±0.2(3)[d]
19				7.3(7.2-7.5)[c,e]			7.2(7.0-7.4)[c,e]			5.9±0.1(4)[d]
21	O			6.0±0.1(4)[c]			5.9±0.1(4)[c]			5.7±0.2(3)[d]
25	H, N, O			5.8±0.1(4)[c]			5.9±0.2(4)[c]			5.1±0.2(3)[d]
Atropine[f]				9.5			9.4			9.4
4-DAMP[f]				9.05			NT			8.3
Hexahydro siladifenidol[g]				7.96			7.76			6.53

NT = not tested; NSE = no significant muscarinic agonist/antagonist effects at 100 μM; *methiodides; [a]values represent mean ± standard error with number of experiments in brackets; [b]agonist effects inhibited by atropine; [c]depression of the maximum at concentrations of 10–100 μM; [d]appreciable negative inotropic and/or chronotropic effects (atropine-insensitive) at the concentrations of 10–30 μM; [e]95% confidence limits; [f]data from [15]; [g]data from [1].

as the 'apolar tail', used in many series of potent antimuscarinic agents. The introduction of an esteric group bearing the cyclohexylphenyl-methyl moiety, selected for reasons of synthetic feasibility, led to the synthesis of derivative **20** and its non-quaternary counterpart **19**. The latter showed a promising profile, displaying fairly high antimuscarinic potency, at least 20 times greater in intestinal and bladder than in cardiac muscle. Interestingly, its quaternary analogue **20** proved to be 10 times less potent in the ileum and bladder and equally active in the atria. Attempts to improve the selectivity of compound **19** were made, replacing the ester function with the ether **21** and amide groups **25**. These modifications reduced antagonist activity while some selectivity towards intestinal and bladder smooth muscle is maintained for compound **25**. The optical isomers of **19**, *ie* **19**(+) and **19**(–), were also prepared but since the maximum optical purity obtained for **19**(–) was still unsatisfactory (*ee* = 77%) with respect to that of **19**(+) (*ee* > 98%) no conclusions can be drawn at present about the enantioselectivity of compound **19**. It is noteworthy that some compounds endowed with antimuscarinic activity, including **19**, when tested at concentrations from 10 to 100 μM, caused some non-muscarinic-related effects (table I). In the ileum and bladder, these effects consisted of a reduction of maximal contractile response to the reference agonist and a flattening of the concentration–response curve, which is not removed by the addition of even large concentrations of reference agonist. In the atria, negative inotropic and/or chronotropic effects were observed when antagonists were allowed to equilibrate for 30 min and these effects were not inhibited by atropine. When these concentrations produced significant differences in slope functions and maximal responses in comparison with control curves (see *Experimental protocols*) were not utilized for the estimate of pK_B values The nature of these non-muscarinic-related effects awaits investigation.

Conclusions

The structural manipulation of furtrethonium described here afforded a fairly potent antimuscarinic antagonist **19**, albeit with some selectivity toward smooth muscle preparations, together with other less selective and potent antagonists (**21** and **25**). The degree of selectivity of compound **19** is comparable to or greater than those of the familiar ileoselective 4-diphenylacetoxy-*N*-methyl piperidine methiodide (4-DAMP) and hexahydrosiladiphenidol, which are considered as standard selective M_3 antagonists [1–6]. These results were obtained by increasing the distance between the lipophilic anchoring moiety (α-cyclohexyl-phenyl group) and the charged nitrogen, which are the essential features in many antimuscarinic agents. In general, the whole series of our antagonists could be subdivided into 2 classes. The first class includes antimuscarinic antagonists in which the lipophilic bulky moiety, responsible for the antagonist activity, is located just beyond the furan ring in a position corresponding to that of the methyl groups in acetylcholine (**10a**, **7e**, **16**, **15**, **7c**, **6c**, **6d**, **7i**, **6i**, **10b**, **9b**). As for other classic antagonists, a marked increase in activity was found with the introduction of the characteristic hydroxycyclohexyl benzyl group and with the quaternarization of the amine moiety. However, these compounds proved to be non-selective. The second class contains antimuscarinic antagonists in which the lipophilic anchoring moiety is located further away from the charged nitrogen. For these compounds, which proved to be endowed with some selectivity toward smooth muscle preparations, the following considerations can be made: i) the -CO- moiety adjacent to the apolar tail is not essential (see compound **21**); and ii) the oxygen adjacent to the carbonyl in the ester function is not essential, but seems more important than the -CO- group for purposes of potency (compare compounds **19** and **21** with **25**). Interestingly, as stated above, quaternarization of the amine moiety in compound **20** produced a decrease in activity. In view of the potent and selective activity of compound **19**, further investigations on this lead compound are in progress.

Experimental protocols

Chemistry

Reaction courses and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica-gel precoated F_{254} Merck plates. Infrared spectra (IR) were measured on a Perkin–Elmer 257 instrument. Nuclear magnetic resonance (^{1}H-NMR) spectra were determined for the solution in $CDCl_3$ or $CDCl_3/d_6$–DMSO, with a Bruker WP 80 and AC–200 spectrometers. Peak positions are given in parts per million (δ) downfield from tetramethylsilane as an internal standard. Optical rotations were determined on a Perkin–Elmer 241 polarimeter. Melting points were obtained in open capillary tubes and are uncorrected. Purification by ball-tube distillation was performed on a Buchi GKR–50 instrument. Column chromatographies were perfomed with Merck 60–200 mesh silica gel. All final compounds were tested as mono-oxalate salts and/or methiodides. All of the starting compounds reported showed IR and ^{1}H-NMR spectra in agreement with their assigned structures. Room temperature was 22–25°C. Microanalyses were performed on salified compounds and were in agreement with calculated values within ±0.4%.

General procedure for the preparation of methiodide derivatives

Methiodide derivatives of **3**, **7a–g** and **i**, **10a** and **b**, **12**, **16**, **20** were generally prepared by adding, to a well-stirred solution of the compound in methanol at 0°C, 1.2 mol commercial methyl iodide. The mixture was then stirred at room temperature for

2 h and evaporated. The semisolid mass obtained was dissolved in methanol and crystallized from methanol/Et_2O. The crystalline mass, after filtration, was recrystallized from methanol/Et_2O or acetone.

*Preparation of 2-alkyl-furyl derivatives **5a–h**, route A*

Commercially available **5a**, **b**, **g** (reagent grade) were used without any further purification. 2-Benzylfurane (**5c**) [30], 2-(3'-phenyl-propyl)-furane (**5d**) and (±)2-(1'-hydroxy-cyclopentyl)-furane (**5h**) were prepared by adapting literature procedures [30, 31]. 2-Styrylfurane (**5e**) [32, 33] was better prepared (95% yield) as an $E + Z$ mixture (1:1 as indicated by ^{1}H-NMR analysis) by Wittig reaction between furfural and triphenylbenzylphosphonium bromide in dimethylsulfoxide in the presence of potassium *tert*-butoxide. 2-Methoxymethylfurane (**5f**) was prepared by standard procedure from 2-furfuryl alcohol and methyl iodide in the presence of sodium hydride. **5d**: bp 75°C (1 mmHg); yield 82%; **5f**: bp 50°C (768 mmHg); yield 70%; **5h**: bp 85°C (0.1 mmHg); yield 72%.

*General procedure for the preparation of 5-alkyl-2-N,N-dimethylfurfurylamine derivatives **6a–i** [19]*

35% dimethylamine/H_2O (18.13 ml, 135 mmol) and 40% formaldehyde/H_2O (5.54 ml, 80 mmol) were dissolved at 0°C in 18 ml acetic acid. The suitable 2-alkyl-furyl derivative **5a–h** (50 mmol) was added and the mixture heated at 100°C for 3 h. After this time the mixture was allowed to stand overnight at room temperature (TLC, EtOAc/pyridine/H_2O/AcOH, 3.6:1.2:0.66:0.36). The dark solution was poured into NaOH/H_2O (25 g, 80 ml) at 0°C, the aqueous layer was extracted with Et_2O (3 x 100 ml) and the organic phases collected, dried (KOH) and evaporated. The residue oil was purified by distillation. Transformation of **6h** into **6i** was observed during work-up and purification procedures. Oxalate salts and methiodides (**7a–f**, **i**) of the final compounds were prepared.

Compound **6a**, **7a** see reference [16]. **6a** oxalate: mp 121°C. Anal $C_{10}H_{15}NO_5$ (C, H, N). Compounds **6b**, **7b**, **6c** and **7c** see reference [34]. **6b** oxalate: mp 138–140°C. Anal $C_{11}H_{17}NO_5$ (C, H, N). **6c** oxalate: mp 102–104°C. Anal $C_{16}H_{19}NO_5$ (C, H, N).

6d: bp 151°C (1 mmHg); yield 65%; IR ν cm^{-1}: 3100–2750, 1600, 1570; ^{1}H-NMR δ: 7.25 (m, 5 H, Ar); 6.12 (d, 1 H, J = 4 Hz, Het); 5.97 (d, 1 H, J = 4 Hz, Het); 3.45 (s, 2 H, CH_2N); 2.60–2.80 (m, 6 H, $(CH_2)_6$); 2.30 (s, 6 H, Me). **6d** oxalate: mp 127–126°C. Anal $C_{18}H_{22}NO_5$ (C, H, N). **7d**: mp 165–167°C. Anal $C_{17}H_{24}INO$ (C, H, N).

6e: bp 75–80°C (1 mmHg); yield 40% [32, 33]; $E + Z$ mixture, 1:1 ratio calculated on ^{1}H-NMR dimethylamino shift (δ 2.20 and 2.30). **6e** oxalate: mp 116–117°C. Anal $C_{17}H_{19}NO_5$ (C, H, N). **7e**: mp 152°C. Anal $C_{16}H_{20}INO$ (C, H, N).

6f: bp 75°C (1 mmHg); yield 67%; IR ν cm^{-1}: 3000–2790, 1550, 1450; ^{1}H-NMR δ: 6.19 (d, 1 H, J = 4 Hz, Het); 6.13 (d, 1 H, J = 4 Hz, Het); 4.30 (s, 2 H, CH_2O); 3.45 (s, 2 H, CH_2N); 3.30 (s, 3 H, OMe); 2.30 (s, 6 H, Me). **6f** oxalate: mp 119–121°C. Anal $C_{11}H_{17}NO_6$ (C, H, N). **7f**: mp 95–97°C. Anal $C_{10}H_{18}INO_2$ (C, H, N). **6g**: bp 85°C (1 mmHg); yield 74%. Compound **6g** was also prepared by reaction of **5g** with dimethylamine hydrochloride and paraformaldehyde in 95% EtOH.

6h: unstable oil. A sample of compound **6h**, purified by column chromatography (EtOAc/hexane) gave the following spectral data: IR ν cm^{-1}: 3600–3300, 2900–2780, 1550. ^{1}H-NMR δ: 6.12 (d, 1 H, J = 3 Hz, Het); 5.96 (d, 1 H, J = 3 Hz, Het); 3.45 (s, 2 H, CH_2N); 3.05 (sbr, 1 H, OH); 2.32 (s, 6 H, Me); 2.00–1.60 (m, 8 H, cyclopentyl). Compound **6h** evolved spontaneously to **6i** over a few days even if stored at –18°C.

6i: bp 96°C (0.1 mmHg); yield 64%; IR ν cm^{-1}: 2950–2750, 1550, 1450; ^{1}H-NMR δ: 6.25–6.05 (m, 3 H, Het, CH=); 3.45 (s, 2 H, CH_2N); 2.7–2.4 (m, 4 H, CH_2CH=); 2.30 (s, 6H, Me); 2.20–1.85 (m, 2 H, CH_2). **6i** oxalate: mp 137–139°C. Anal $C_{14}H_{19}NO_5$ (C, H, N). **7i**: mp 187–188°C. Anal $C_{13}H_{20}INO$ (C, H, N).

*Preparation of 2-N,N-dimethylfurfurylamine **8**, route B*

2-*N*,*N*-Dimethylfurfurylamine **8** was prepared following the procedure reported by Eliel and Peckham [16] using high-grade DMF. Compound **8** was also converted into methiodide **3** [16] and oxalate. **8** oxalate: mp 105–107°C. Anal $C_9H_{13}NO_5$ (C, H, N). Iodoethynyl-benzene was prepared from commercial ethynyl-benzene by reaction with 1.6 M *n*-BuLi and iodine and was used immediately after preparation.

*General procedure for the preparation of 5-carbinol-2-N,N-dimethylfurfurylamine derivatives **9a–b**, **6h***

After flame drying under nitrogen atmosphere, 2-furfuryl-dimethylamine **8** [16] (3.12 g, 25 mmol) in anhydrous THF (15 ml) was introduced into a 100 ml round-bottomed flask, the solution was cooled to –30°C and 1.6 M *n*-BuLi (15.6 ml) was injected dropwise by syringe through a serum cap. The reaction mixture was then warmed to room temperature and stirred for 3 h. After this, a solution in anhydrous THF (10 ml) of a suitable ketone or aldehyde (29 mmol) was slowly added over 10 min. The solution was left to stand overnight and treated with Et_2O/H_2O (50 ml, 1:1) at 0°C. The resulting mixture was extracted with Et_2O (2 x 30 ml), and the combined organic phases were dried (Na_2SO_4) and evaporated to give an oily residue. This was crystallized from Et_2O/hexane (**9a**, **b**) or purified by column chromatography (EtOAc/hexane) (**6h**) and quickly transformed into oxalate. Oxalate salts and methiodides (**10a**, **b**) of the final compounds were prepared.

9a: mp 70–71°C (Et_2O/hexane); yield 78%; IR ν cm^{-1}: 3600–3400, 3050–2800, 1640, 1500. ^{1}H-NMR δ: 7.50–7.30 (m, 5 H, Ar); 6.10 (d, 1 H, J = 3 Hz, Het); 5.94 (d,1 H, J = 3 Hz, Het); 5.81 (s, 1 H, CHOH); 3.50 (sbr, 1 H, OH); 3.40 (s, 2 H, CH_2N); 2.18 (s, 6 H, Me). **9a** oxalate: mp 128°C. Anal $C_{16}H_{19}NO_6$ (C, H, N). **10a**: mp 158–159°C. Anal $C_{15}H_{20}INO_2$ (C, H, N).

9b: mp 90–92°C (Et_2O/hexane); yield 87%; IR ν cm^{-1}: 3600–3400, 3050–2800, 1640, 1500. ^{1}H-NMR δ: 7.60–7.20 (m, 5 H, Ar); 6.11 (d, 1 H, J = 3 Hz, Het); 5.95 (d, 1 H, J = 3 Hz, Het); 3.45 (s, 2 H, CH_2N); 2.80 (sbr, 1 H, OH); 2.20 (s, 6 H, Me); 1.70–1.1 (m, 11 H, cyclohexyl). **9b** oxalate: mp 108–110°C. Anal $C_{22}H_{29}NO_6$ (C, H, N). **10b**: mp 90–92°C. Anal $C_{21}H_{30}INO_2$ (C, H, N).

6h: unstable oil; yield 85%; analytical and spectral data were consistent with those for the compound obtained by *route A*. **6h** oxalate was unstable.

*5-(Benzene-ethynyl)-2-N,N-dimethylfurfurylamine **11***

A solution (THF, 20 ml) of the 5-lithium-2-*N*,*N*-dimethylfurfurylamine prepared as described above **8** (1.56 g, 12.5 mmol), 1.6 M *n*-BuLi (8.7 ml) was added by syringe to a vigorously stirred suspension of CuI (2.28 g, 11.9 mmol) in THF (20 ml) at –30°C. After 5 min, a solution of iodophenylacetylene (3.0 g, 13.5 mmol/THF 5 ml) was added. The resulting mixture was stirred for 3 h at –30°C and then overnight at room temperature. The reaction mixture was then quenched at 0°C with water (20 ml), extracted with Et_2O (3 x 50 m), dried ($MgSO_4$) and evaporated. The residue oil was purified by transformation into oxalate derivative (see salification procedures) in THF. Filtration gave a white mass, which was washed with Et_2O (30 ml) and then reconverted to the free base by dissolution in

1 N NaOH (50 ml) and extraction with Et_2O (3 x 30 ml). The collected ethereal fractions were dried (Na_2SO_4) and evaporated to give a pale-yellow oil. After purification by column chromatography (EtOAc/hexane), compound **11** was converted into methiodide **12**.

11: Oil, yield 45%; IR ν cm^{-1}: 3000–2750, 2200, 1600, 1540. ^{1}H-NMR δ: 7.50–7.30 (m, 5 H, Ar); 6.56 (d, 1 H, J = 3 Hz, Het); 6.20 (d, 1 H, J = 3 Hz, Het); 3.50 (s, 2 H, CH_2N); 2.20 (s, 6 H, Me). **12**: mp 156–157°C. Anal $C_{16}H_{18}INO$ (C, H, N).

Route C. Compound **17** was prepared as reported by Paul and Tchelitcheff [26] and converted into the corresponding methiodide **18** [26] and oxalate. **17** oxalate: mp 122–124°C. Anal $C_{10}H_{21}NO_5$ (C, H, N).

*5-t-Butyl-2-N,N-dimethylfurfurylamine **16***

Compound **16** was synthesized following and adapting the above-cited procedure. Amide **14** was obtained, in a quantitative yield, heating (3 h) at reflux conditions of DMF the furoylchloride prepared by standard procedure from the corresponding acid **13** [24]. After evaporation to dryness, the solid mass was co-evaporated with ethanol and crystallized from hexane to give pale-yellow oily crystals (mp 45°C). A sample of pure material gave analytical data consistent with the assigned structure.

The starting amide **14** (1.95 g, 10 mmol) was then dissolved in anhydrous Et_2O (20 ml) and slowly added at 0°C to a suspension of $LiAlH_4$ (0.5 g, 13 mmol) in anhydrous Et_2O (30 ml). The mixture was allowed to stand overnight at room temperature and the hydride excess was decomposed at 0°C with H_2O (5 ml). The organic layer was separated and the aqueous phase extracted with Et_2O (10 ml). The collected Et_2O fractions were dried (KOH) and evaporated. The residue oil was purified by bulb-to-bulb distillation and transformed into oxalate or methiodide **16**.

15: bp 105–107°C (1 mmHg); yield 83%; IR ν cm^{-1}: 3000–2750, 1550, 1450. ^{1}H-NMR δ: 6.10 (d, 1 H, J = 4 Hz, Het); 5.90 (d, 1 H, J = 4 Hz, Het); 2.30 (s, 6 H, Me); 1.30 (s, 9 H, *t*-Bu). **15** oxalate: mp 180–181°C. Anal $C_{13}H_2NO_5$ (C, H, N). **16**: 196–198°C. Anal $C_{12}H_{22}INO$ (C, H, N).

*(±)N-[5-(1'-Phenyl-1'-cyclohexyl-acetoxymethyl)2-furfuryl] dimethylamine **19***

Phenylcyclohexyl acetic acid (1.5 g, 6.4 mmol) was added at 0°C to a CH_2Cl_2 (40 ml) solution containing **6g** (1 g, 6.4 mmol) and a catalytic amount of 4-dimethylaminopyridine (0.08 g, 0.64 mmol). After 5 min, dicyclohexylcarbodiimide (1.41 g, 7 mmol) was added and the reaction course was monitored by TLC (EtOAc/pyridine/H_2O/AcOH, 3.6:1.2:0.66:0.36). After 24 h at room temperature, the reaction mixture was filtered on a celite pad to remove precipitated dicyclohexyl urea. After evaporation of the filtrate, the residue was purified by column chromatography (EtOAc/MeOH, 9:3) to give (±)**19** as a pale-yellow oil. Oxalate and methiodide (±)**20** of the final compound were prepared. The 2 optical isomers of **19** were prepared as for the above-described racemic mixture, using the corresponding (+) or (–) phenylcyclohexyl acetic acid, and then converted into oxalates. Optically active phenylcyclohexyl acetic acids were prepared, following a reported procedure, from the racemic mixture [35].

19: oil; yield 75%; IR ν cm^{-1}: 3100–2750, 1740, 1650, 1600, 1550. ^{1}H-NMR δ: 7.35–7.20 (m, 5 H, Ar); 6.26 (d, 1 H, J = 3 Hz, Het); 6.13 (d, 1 H, J = 3 Hz, Het); 5.10 (d, 1 H, J = 13 Hz, CH_2O); 4.93 (d, 1 H, J = 13 Hz, CH_2O); 3.50 (s, 2 H, CH_2N); 3.23 (d, 1 H, J = 10 Hz, CHO); 2.30 (s, 6 H, Me); 2.00–1.00 (m, 11 H, cyclohexyl). **19** oxalate: mp 126–128°C. Anal $C_{24}H_{31}NO_7$ (C, H, N). **20**: mp 200–203°C. Anal $C_{23}H_{32}INO_3$ (C, H, N).

(–)**19** oxalate: $[\alpha]_D^{20}$ –1.53 (c 2, ethanol/chloroform, 1:1); *ee* = 77%. (+)**19** oxalate: $[\alpha]_D^{20}$ +1.95 (c 2, ethanol/chloroform, 1:1); *ee* > 98%.

*(±)N-[5-(2'-Phenyl-2'-cyclohexyl-ethoxymethyl)-2-furfuryl] dimethylamine **21***

A mixture of 2-phenylcyclohexyl ethanol [29] (2.22 g, 6.96 mmol) and 5-hydroxymethyl-2-furfuryldimethylamine (**6g**) (0.988 g, 3.48 mmol) in anhydrous THF (5 ml) was added at 0°C to a solution of methanesulfonic acid (3.60 ml, 31.3 mmol) in anhydrous THF (5 ml). The reaction mixture was heated to 90°C for 30 min and then cooled to room temperature. After 1 h, an excess of anhydrous sodium carbonate was added and the suspension was left under stirring for 18 h. The mixture was then filtered and the residue cake washed with THF (3 x 2 ml); after evaporation of the combined filtrates the residue oil was treated with H_2O (10 ml) and extracted with Et_2O (3 x 5 ml). The aqueous layer was made alkaline with 5% $NaHCO_3/H_2O$ and extracted with Et_2O (2 x 10 ml). The combined organic layers were dried (Na_2SO_4) and evaporated. The residue oil was purified by column chromatography (EtOAc/MeOH, 10:1) and converted into oxalate.

21: oil; yield 40%; IR ν cm^{-1}: 3000–2800, 1600, 1500. ^{1}H-NMR δ: 7.30–7.10 (m, 5 H, Ar); 6.16–6.20 (m, 2 H, Het); 4.36 (s, 2 H, CH_2O); 3.70 (m, 2 H, $CHCH_2O$); 3.48 (s, 2 H, CH_2N); 2.63 (m, 1 H, CH); 2.28 (s, 6 H, Me); 2.00–1.00 (m, 11 H, cyclohexyl). **21** oxalate: mp 115–117°C. Anal $C_{24}H_{33}NO_6$ (C, H, N).

*5-Aminomethyl-2-N,N-dimethylfurfurylamine **24***

The phthalimide derivative **22** (10 g, 44 mmol), obtained by standard procedure from furfurylamine and phthalic anhydride, was reacted with dimethylamine under Mannich conditions, as described for compounds **6a–i**, to give **23**. The crude oil obtained after work-up was dissolved in ethanol, treated with hydrazine-hydrate (an equimolar amount with reference to the initial **22**) and heated at reflux conditions for 5 h. This was followed by evaporation, addition of 1 N NaOH (50 ml) and extraction of the mixture with EtOAc (3 x 100 ml). The organic phase was anhydrified (KOH) and evaporated. The residue oil was purified by distillation.

24: bp 150°C (1 mmHg); yield 45%; IR ν cm^{-1}: 3500–3100, 3000–2800, 1550, 1450. ^{1}H-NMR δ: 6.21 (d, 1 H, J = 4 Hz, Het); 6.15 (d, 1 H, J = 4 Hz, Het); 3.75 (s, 2 H, CH_2NH_2); 3.45 (s, 2 H, CH_2N); 2.26 (s, 6 H, Me); 2.05 (s, 2 H, NH_2).

*(±)N-[5-(1'-Phenyl-1'-cyclohexylacetamidomethyl)-2-furfuryl]-dimethylamine **25***

Phenylcyclohexylacetic acid hydroxysuccinimidester was added to a solution of **24** in dimethoxyethane (1.5:1 molar ratio). The reaction pathway was monitored by TLC (EtOAc/MeOH, 1:1) After evaporation of the reaction mixture, the crude oil was purified by column chromatography on silica gel (EtOAc/MeOH, 1:1) and converted into oxalate.

25: oil; yield 70%; IR ν cm^{-1}: 3500–3400, 3000–2800, 1650, 1600, 1560. ^{1}H-NMR δ: 8.50 (br, 1 H, NH); 7.80–7.60 (m, 2 H, Ar); 7:40–7.20 (m, 3 H, Ar); 6.26 (d, 1 H, J = 3 Hz, Het); 6.13 (d, 1 H, J = 3 Hz, Het); 4.26 (m, 2 H, CH_2NHCO); 3.50 (s, 2 H, CH_2N); 3.23 (d, 1 H, J = 10 Hz, CH); 2.30 (s, 6 H, Me); 2.00–1.00 (m, 11 H, cyclohexyl). **25** oxalate: mp 162–164°C. Anal $C_{24}H_{32}N_2O_6$ (C, H, N).

Pharmacology

Male Wistar Morini rats (170–220 g) and male guinea pigs (500–600 g) of a local strain (Bettinardi, Dunkin–Hartley) were used. Carbachol chloride and atropine sulfate were obtained from Sigma (USA). Furtrethonium was synthesized in the Glaxo SpA Research Laboratories (Verona, Italy). All test drug solutions were freshly prepared prior to each experiment.

Tissue preparations
For the experiments, rats and guinea pigs were fasted overnight and killed by cervical dislocation. The ileum and urinary bladder were quickly removed from rats, and the atria from guinea pigs. Longitudinal segments of ileum about 2 cm in length, strips of extratrigonal portion of urinary detrusor muscle 1 cm long and 2 mm wide, and both atria were prepared for the study. These tissues were placed in a 10 or 20 ml organ bath containing Krebs–Henseleit solution (composition in mmol/l: NaCl 118; KCl 5.6; $CaCl_2$ 2.5; $MgSO_4$ 1.19; NaH_2PO_4 1.3, $NaHCO_3$ 25 and glucose 10), gassed with 5% CO_2 in O_2 (pH 7.4) and heated to 37°C (ileum and detrusor strips) or to 29°C (atria).

Experimental protocol
The preparations were left to equilibrate for 30–45 min, during which time the bathing solution was changed every 10–15 min. Contractile responses of ileum and detrusor strips were recorded isotonically on an LNI recorder through a Basile transducer, the resting tension being 0.5 g and 1 g, respectively. Atrial force was recorded with a Statham force transducer connected to a Battaglia–Rangoni polygraph, resting tension being 1 g. When testing the compound as an agonist, cumulative administration was used. Agonist concentrations were added to the organ bath in 0.5 (ileum and bladder) or 0.25 (atria) logarithmic unit multiple increments. Increasing concentrations were added after attainment of a steady-state response to the previous concentration. The concentrations were increased until a maximum response was achieved. Furtrethonium (ileum and bladder) and carbachol (atria) were used as reference agonists. To check the atropine sensitivity of the responses obtained with the study agonists, 1 or 2 concentrations of atropine ($1–3 \times 10^{-9}$ M) were used, by the procedure described below, for testing of antagonism. Atropine sensitivity was recognized when the appropriate antagonist potency was observed. When testing the compound as an antagonist, the following procedure was used. Two or, exceptionally, 3 cumulative concentration–response curves for appropriate reference agonists were constructed. Once 2 consecutive similar curves were obtained, a final cumulative concentration–response curve was repeated after the tissues had been allowed to equilibrate for at least 30 min with the compound under examination.

Quantification of agonism
Negative inotropic responses to agonists were expressed as a percentage of the basal twitch contraction, while contractile responses in ileum and bladder were expressed as a percentage of their own maximum response. The concentration–response curve data were analyzed by a 4-parameter logistic equation [36]:

$$\text{response} = a + E_{max}\,[A]^n / [A]^n + [E_{50}]^n \qquad [1]$$

where a and E_{max} are the minimal and maximal asymptotes respectively $[A]$ the agonist concentration, n the slope factor and $[E_{50}]$ the concentration of the agonist that induces 50% of the maximal effect. Potencies of agonists are expressed as $-\log EC_{50}$ (negative logarithm of the concentration inducing 50% inhibition (atrial contraction amplitude) or 50% maximal contractile response (ileum and bladder)); the values are given as mean ± standard error. The ability of a test compound to produce a response is expressed as mean ± standard error of α, which represents the ratio between the E_{max} produced by each test agonist and that of the reference agonist.

Quantification of antagonism
Because of the low potency and/or solubility of test compounds or the appearance of non-muscarinic-related effects (see *Results and discussion*), it was not possible to test a sufficient range and number of antagonist concentrations for the application of regression analysis according to Schild [37]. However, each antagonist was studied over at least 2 different concentrations. Concentration–response curves in the presence of antagonists were tested for significant differences in slope functions and maximal responses in comparison with the control curves using equation [1]. In the absence of significant difference, the analysis of antagonist effects was performed.

To quantify antagonist potency pK_B values were calculated from the equation

$$pK_B = \log(DR - 1) - \log[B]$$

where DR is the ratio of agonist E_{50} values in the presence and in absence of the antagonist concentration $[B]$. Values of pK_B were given as mean ± standard error. For compound **19**, where 3 different concentrations were tested in the ileum and bladder, the Schild regression analysis was performed [37]. Since the Schild plot slope was not significantly different from the unit, pK_B values and 95% confidence limits could be estimated.

Acknowledgments

This work was supported by Glaxo SpA, Verona, Italy. SM wishes to thank A Casolari and P Orlandini for taking the NMR spectra.

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