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Design, Synthesis and Binding at Cloned Muscarinic Receptors of *N*-[5-(1′-Substituted-Acetoxymethyl)-3-Oxadiazolyl] and *N*-[4-(1′-Substituted-Acetoxymethyl)-2-Dioxolanyl] Dialkyl Amines

Stefano Manfredini,[a,*] Ilaria Lampronti,[a] Silvia Vertuani,[a] Nicola Solaroli,[a] Maurizio Recanatini,[b] David Bryan[c] and Michael McKinney[c,†]

[a]*Dipartimento di Scienze Farmaceutiche, Università di Ferrara, Via Fossato di Mortara 17-19, I-44100 Ferrara, Italy*
[b]*Dipartimento Scienze Farmaceutiche, Università di Bologna, Via Belmeloro 6, Bologna, Italy*
[c]*Mayo Clinic Jacksonville, 4500 San Pablo Road, Jacksonville, FL 32224, USA*

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Abstract—Few muscarinic antagonists differentiate between the M_4 and M_2 muscarinic receptors. In a structure–activity study, aimed at discovering leads for the development of a M_4 muscarinic receptor-selective antagonist, we have synthesized and tested at cloned muscarinic receptors the binding of a group of dioxolane- or oxadiazole-dialkyl amines, and compared them to our compound **1**, which contains the furan nucleus. Although none of these agents were particularly potent at M_4 receptors (K_d values were typically 30–70 nM), furan derivatives **(−)1** and **(+)1** were significantly more potent at M_4 receptors than at M_2 receptors (~3- and 4-fold, respectively). The dioxolane derivatives **12b** and **12c** were more than 10-fold selective for the M_4 versus the M_2 receptors, while the dioxolane derivative **12e** was 15-fold more potent at M_4 receptors than for M_2 receptors. However, these agents bound to M_3 receptors with potencies like that for the M_4 receptor, so they are not M_4-selective. The M_4/M_2 relative selectivities of some of our compounds are similar to the better hexahydrosiladifenidol derivatives, and may provide some important structural clues for the development of potent and selective M_4 antagonists. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

The five muscarinic receptors can be classified into two biochemical classes based upon structural similarity and second messenger coupling.[1] The M_2 and M_4 muscarinic receptors are members of the subclass that couple to the inhibition of adenylate cyclase and the activation of potassium channels. These two receptors are difficult to distinguish pharmacologically, in that they bind most agonists and antagonists with similar affinities.[2] For example, the potent antagonists AF-DX 384 and himbacine bind to M_2 and M_4 receptors with essentially the same affinities.[3,4] Truly M_4-selective agents would be particularly useful studies of receptors in the central nervous system, because most brain regions express mixtures of several muscarinic receptor subtypes and the M_4 receptor is one of the more abundant ones, and is the most abundant subtype in the striatum, a region important in pharmacotherapy for Parkinson's and Huntington's diseases.[1]

Most muscarinic antagonists are non-selective; a few are marginally subtype-selective, but most of these are M_1-selective.[5] Secoverine and tropicamide are two agents reported to be 3–11-fold selective for the M_4 subtype, relative to the other subtypes.[6] Recently, preparations of cloned muscarinic receptors have become commercially available; thus, materials for clearly distinguishing the affinities of antagonists at all five subtypes are available for rapid screening with binding assays.[7,8] Most recently, using cloned muscarinic receptor preparations for binding screening, a 38-fold M_4/M_2-selective benzoxazine isoquinoline antagonist was discovered.[9]

In our recent work, we developed muscarinic antagonists for differentiating between the ileal and bladder receptors.[10] A series of compounds with various substitutions on the furan ring were synthesized, of which **1** was the most selective in tissue screening assays. In view of this unexpected tissue selectivity, both are believed to express the M_3 receptor; we started the present study to determine the true receptor subtype selectivity of these furan compounds in assays with cloned muscarinic receptors. Additionally, we became interested in the possible M_4-selectivity of our agents. In

*Corresponding author. Tel.: +39-532-291-292; fax: +39-532-291-296.
†Second corresponding author.

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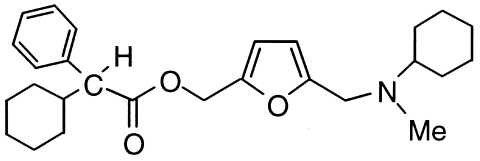

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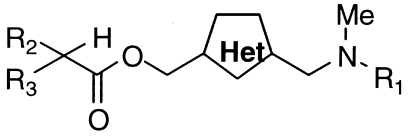


12a-h; 13a,d,e,h

Formulae 1 and general. **a**: R_1 = methyl; R_2 = phenyl; R_3 = cyclohexyl; **d**: R_1 = cyclohexyl; R_2 = phenyl; R_3 = cyclohexyl; **e**: R_1 = methyl; R_2 = cyclohexyl; R_3 = cyclohexyl; **h**: R_1 = cyclohexyl; R_2 = cyclohexyl; R_3 = cyclohexyl; Het = furan; 1,2,4-oxadiazole; 1,3-dioxolane; R_1, R_2 R_3 = methyl, cyclohexyl, phenyl.

order to explore the potential to develop M_4-selective antagonists, we prepared a new series of compounds containing the dioxolane moiety.[11] The dioxolane moiety, originally synthesized as an analogue to muscarone,[12] was subsequently shown to be suitable for development of a potent muscarinic agonist, *cis*-dioxolane.[13,14] Addition of progressively larger lipophillic groups to the dioxolane nucleus produces antagonist.[14,15] Our dioxolane series is represented by compounds **12a–g** and several analogous agents were prepared with the oxadiazole moiety (**13a,d,e,h**). This latter moiety has been used in the field of muscarinic receptors, but only as an ester replacement in the modification of arecoline or azabicyclic muscarinic receptor ligands and not as a scaffold to achieve subtype selectivity.[16–20] The two stereo-isomers of compound **1**, compounds **12a–g**, and compounds **13a,d,e,h** were screened in binding assays at cloned M_1–M_4 receptors, all expressed in Chinese hamster ovary (CHO) cell membranes.

Results

Chemistry

The synthetic procedure for derivatives **12a–h** and **13a,d,e,h** are outlined in Schemes 1–3. In the case of the dioxolanes **12a–h** two different routes have been envisaged. Route (a) was based on our previous studies, the methodology now being improved by the use of $BF_3{\cdot}Et_2O$ as the catalyst instead of toluensulfonic acid (TSOH). Indeed, taking advantage of our other previous findings on the transacetalization of THP-derivatives[21,22] we have evaluated some Lewis acids (i.e. trimethylsilyltriflate, $SnCl_4$ and BF_32O) as possible catalysts for the dioxolane ring formation. Among these catalysts, $BF_3{\cdot}Et_2O$ gave the better results both in terms of yields and stereospecificity: while *cis*:*trans* mixtures (4:1 ratios) were obtained with TSOH, $BF_3{\cdot}Et_2O$ gave only the *cis* compound without traces of the other isomer. Moreover, the reaction could be accomplished at lower temperature, about 35 °C, without any traces of decomposition products. *Cis*:*trans* ratios were inferred by ^{1}H NMR analysis, according to our previous studies.[11] In order to deprotect the hydroxy function at position 4, removal of the benzyl group was performed in reductive conditions, with Pd/C as the catalyst, to give compound **4** in almost quantitative yield. Next substitution of the chlorine atom at position 2 was initially attempted in sealed vial, as described previously by us.[11] Whereas compounds **5a,d** were obtained in appreciable yields (60–80%), only traces of compounds **5b,c** could be recovered in these conditions (Scheme 1). After several attempts, in order to overcome the above described synthetic difficulties, we envisaged route (b) as a possible alternative pathway. Instead of reacting the appropriate amine on the functionalized dioxolane ring, as for route (a), chloroacetaldehyde dimethyl acetal was directly converted into the corresponding amino-derivatives (**6b,c**), and then transacetalized with isopropylidenglycerine-1-benzyl ether in the presence of TSOH, to give compounds **7b,c** in 57–59% overall yields. Next deprotection by hydrogenolysis, as for **4**, gave the expected **5b,c** in 47–68% yields.

3,5-Substituted-1,2,4-oxadiazoles (**10a,d**) were obtained, adapting the procedure described by Street et al.,[23] via cyclization of the amidoxime precursor **9** with the appropriate *N*-substituted-amino acid ethyl ester (Scheme 2).[24] Compound **9** was in turn obtained by the reaction of benzyloxyacetonitrile (**8**)[25] with hydroxylamine.

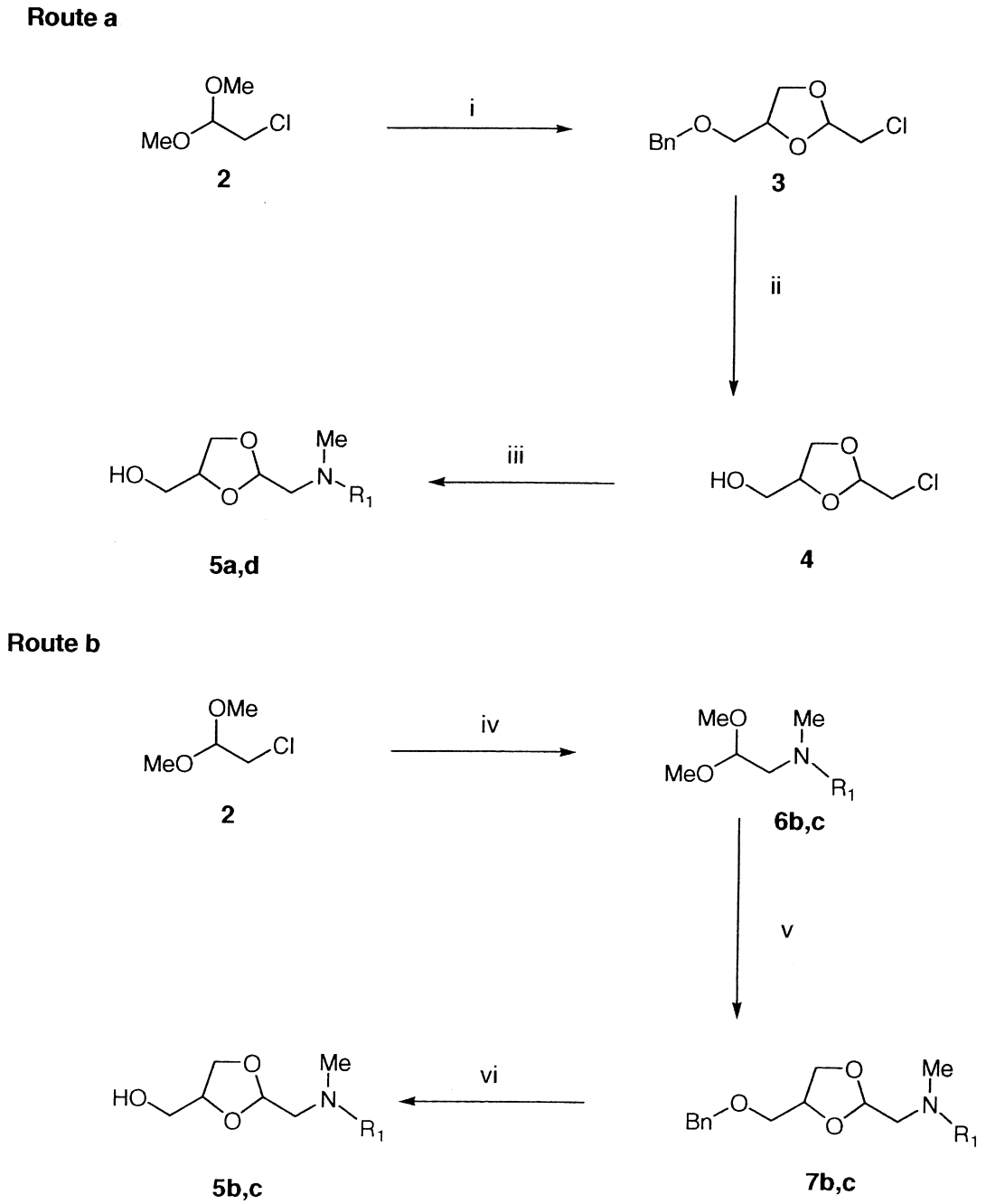
Due to the presence of the oxadiazole ring, it was impossible to use the standard catalytic hydrogenation, as for **3** and **7b,c**, for the next removal of the benzyl protective group. An alternative procedure, based on the use of BCl_3 at −73 °C, allowed us to obtain the intermediate **11a,d** in good yields and short reaction times. Finally, the amino-alcohols **5a-d** and **11a,d** were condensed on the appropriate substituted acid chlorides, prepared in situ from the corresponding carboxylic acid and oxalyl chloride, in benzene (Scheme 3).

Biology

All compounds were then tested for their capability to displace [^{3}H]QNB binding on cloned M_1–M_4 human muscarinic receptors. Affinities, determined by competition experiments, are reported in Table 1.

Discussion

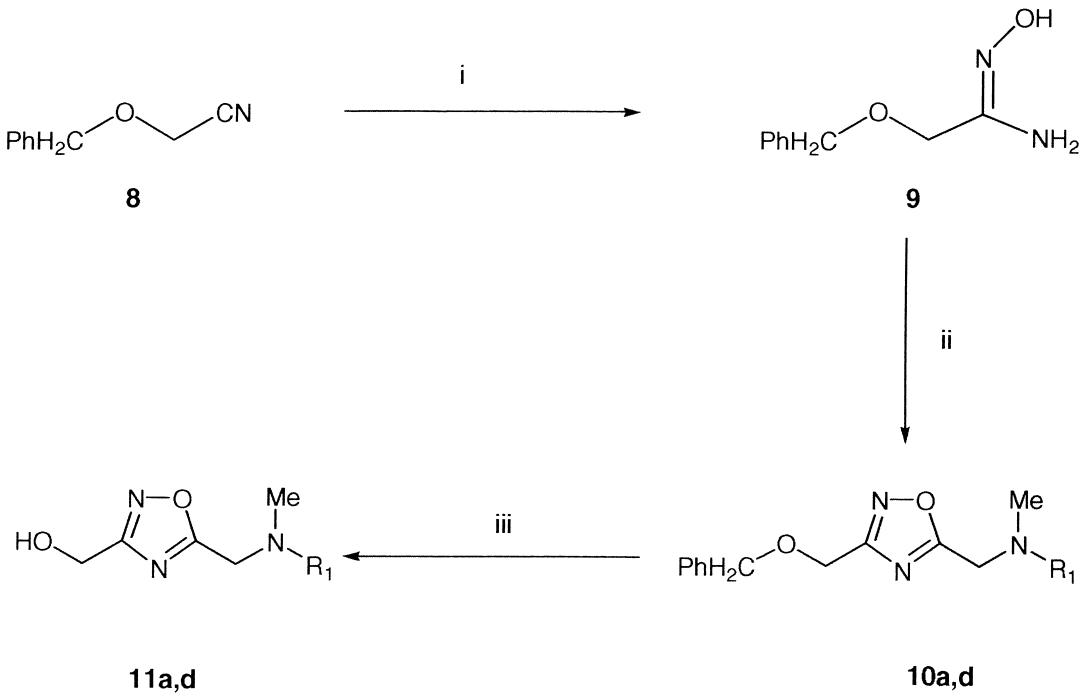
Compound **1**, which was selected as a lead at the beginning of this study, showed modest potency (69 nM) and significant selectivity (4.46-fold; $p < 0.05$) for the M_4 subtype (Table 1). Compound (−)**1** was also significantly selective (3.54-fold; $p < 0.05$) for the M_4 receptors versus the M_1 receptors. Most M_1-selective antagonists do not differentiate the M_1 and M_4 receptors,[1] so compound (−)**1** seems to be an exception to this generality. Compound (−)**1** also displayed a tendency



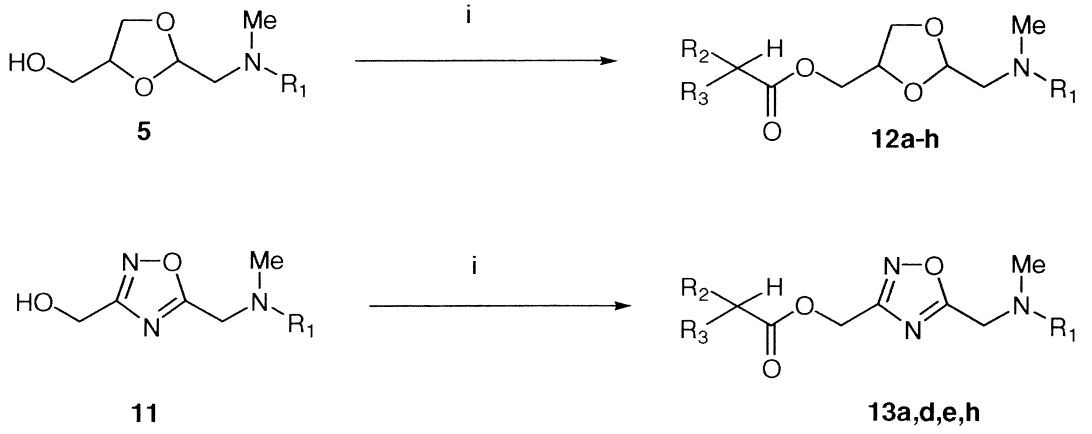
Scheme 1. **a**: R_1 = methyl; **b**: R_1 = isopropyl; **c**: R_1 = butyl; **d** R_1 = cyclohexyl. Reagents and conditions: (i) isopropylidenglycerine-1-benzylether, $BF_3{\cdot}Et_2O$, 40 °C; (ii) H_2, Pd/C; (iii) $MeNHR_1$; (iv) $MeNHR_1$, KI, K_2CO_3, 60 °C; (v) 2,2-dimethyl-4-benzyl-dioxolane, TSOH; (vi) H_2, Pd/C.

to be selective for the M_4 over the M_3 receptors, but with the small number of assays performed did not quite reach the $p = 0.05$ level of significance. Compound (+)**1** was also significantly M_4-selective (4.28-fold; $p < 0.05$) relative to the M_2 subtype. However, this compound was significantly ($p < 0.05$) more potent at the M_3 receptors ($K_d = 65$ nM) than was compound (−)**1**. Compounds (−)**1** and (+)**1** were about equipotent at the M_1 receptors. The substitution of the furan ring, in the lead compound **1**, with the dioxolane (**12a–h**) and oxadiazole one (**13a,d,e,h**), induced an overall lipophilicity variation of −0.6 and −1.2 log *P* units in the case of furan–dioxolane and of dioxolane–oxadiazole, respectively (CLOGP values were calculated with the C-QSAR program).[26] As a result of this modification, a consistent decrement of activity at muscarinic receptors was observed, in particular for the oxadiazole derivatives.

However, compounds **12b**, **12c** and **12e** were more than 10-fold selective for the M_4 versus the M_2 receptors. Of these, **12b** and **12c** also distinguished between the M_4 and M_1 receptors. Compound **12e** bound to the M_1 and M_4 receptors with similar potency, but was notable for having the highest (15-fold; $p < 0.2$) selectivity for the M_4 versus the M_2 receptors. Compound **12** was reminiscent of drugs like 4-DAMP, which bind with similar potencies at M_1, M_3, and M_4 receptors, but with low affinity to the M_2 subtype.



Scheme 2. **a**: R_1 = methyl; R_2 = phenyl; R_3 = cyclohexyl; **d**: R_1 = cyclohexyl; R_2 = phenyl; R_3 = cyclohexyl. Reagents and conditions: (i) 60 °C, NH_2OH; (ii) NaH, **a**: *N,N*-dimethylamino- or **d**: *N*-cyclohexyl-*N*-methyl-glycine ethyl ester; (iii) BCl_3.



Scheme. 3. **a**: R_1 = methyl; R_2 = phenyl; R_3 = cyclohexyl; **b**: R_1 = isopropyl; R_2 = phenyl; R_3 = cyclohexyl; **c**: R_1 = butyl; R_2 = phenyl; R_3 = cyclohexyl; **e**: R_1 = methyl; R_2 = cyclohexyl; R_3 = cyclohexyl; **f**: R_1 = isopropyl; R_2 = cyclohexyl; R_3 = cyclohexyl; **g**: R_1 = butyl; R_2 = cyclohexyl; R_3 = cyclohexyl; **h**: R_1 = cyclohexyl; R_2 = cyclohexyl; R_3 = cyclohexyl. Reagents and conditions: **i**: $R_2R_3CHCOOH$, $(COCl)_2$.

Table 1. Affinities (K_d) for muscarinic receptors

Compound	M_1 K_d (nM)	M_2 K_d (nM)	M_3 K_d (nM)	M_4 K_d (nM)
(−)1	244±6[a]	308±18[a]	199±17.2[a]	69±11.9[a]
(+)1	161±3[a]	214±29[a]	65±10.5[a]	50±6[a]
12a	80±8[a]	192±24[a]	34±5[a]	36±7[a]
12b	189	327	28	30
12c	202	392	27	33
12d	316	302	32	39
12e	73±8[a]	796±55[a]	26±3[a]	53±10[a]
12f	634	> 10,000	98	65
12g	588	1280	29	49
13a	> 10,000	> 10,000	3430	6710
13d	> 10,000	> 10,000	> 10,000	6810

[a]Data are averages of three to five experiments with each receptor.

Conclusion

Compounds **13a** and **13d** were two or more orders of magnitude less potent at the muscarinic receptors than the other drugs listed in Table 1, indicating that substitution of the oxadiazole ring for the dioxolane structure is detrimental to muscarinic activity.

Since this substitution does not affect the size of the molecule and the distance between the polar head and the lipophilic tail, the affinity may be reduced due to the increased hydrophilicity and/or different electronic properties. In our opinion, having maintained the same substitution pattern (R_1 to R_3) of furan and dioxolane

series, these results confirm the importance of the overall lipophilicity in the affinity profile of compound **1**.

The dioxolane derivatives **12b** and **12c** were more than 10-fold selective for the M_4 versus the M_2 receptors, while the dioxolane derivative **12e** was 15-fold more potent at M_4 receptors than for M_2 receptors. However, these agents bound to M_3 receptors with potencies like that for the M_4 receptor, so they are not M_4-selective.

The potencies observed in the present assays, for the isolated enantiomers of **1**, are higher than those observed for the racemate in isolated tissues;[10] however, it is not unusual for receptors in tissues to bind less efficiently than receptors in homogenates, due to diffusional and other mechanical constraints.[27] Further studies will address this question also with respect to compounds **12a**–**h**.

However, it is noteworthy that in the current research we have been able to demonstrate the true enantioselectivity of **1** in its binding to the M_4 muscarinic receptors; this illustrates the advantages of screening with cloned receptors.

In conclusion, we believe these data of interest for structure–activity relationship (SAR) studies in the field of muscarinic agents and, in view of the demand for subtype-specific ligands, particularly in the case of M_4 receptors. Indeed, the reinvestigation of compound **1** resulted in the discovery of a new M_4-selective ligand, that might be useful as a lead compound in the design of more potent and selective ligands.

Experimental

Chemistry

Material and methods. Reaction courses and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated F254 Merck plates, with detection under 254 nm UV lamp and/or by spraying with a diluted potassium permanganate solution. Nuclear magnetic resonance (^{1}H NMR) spectra were determined for solution in $CDCl_3$–DMSO-d_6 on a Bruker AC-200 spectrometer, and peak positions are given in parts per million (δ) downfield from tetramethylsilane as internal standard, whereas coupling constants (*J*) are in hertz. Melting points were obtained in open capillary tubes and are uncorrected. Column chromatographies were performed with Merck 60–200 mesh silica gel. Ambient temperature was 22–25 °C. All drying operations were performed over anhydrous magnesium or sodium sulfate. Microanalyses, unless indicated, were in agreement with calculated values within ±0.4%.

Synthesis of 2,4-substitued-dioxolanes. Route a.

Synthesis of 2-chloromethyl-4-benzyloxymethyl-1,3-dioxolane (3). To a stirred and refluxed solution of isopropylidenglycerine-1-benzylether (11 mL, 50 mmol) and chloroacetaldehydedimethylacetal (**2**) (12 mL, 100 mmol) in Et_2O (800 mL) was added BF_3 in Et_2O (48 g, 43 mL, 338 mmol). The reaction mixture was heated at reflux conditions for 2 h, cooled and washed with a saturated solution of $NaHCO_3$ (20 mL×2). This mixture was then washed with a saturated solution of NaCl and water. The organic layer was dried (Na_2SO_4), evaporated under vacuum, and the residue was purified by column chromatography on silica gel (eluent hexane:Et_2O 4:1) to give the compound **3** (yield 98%). ^{1}H NMR ($CDCl_3$) δ 3.52 (d, 2H, J = 3.6 Hz), 3.86 (m, 2H), 4.15 (m, 1H), 4.31 (m, 2H), 4.69 (m, 2H), 5.16 (t, 1H, J = 3.6 Hz, H-2 *cis*), 5.37 (t, 1H, J = 3.6 Hz, H-2 traces of *trans*), 7.20–7.50 (m, 5H).

Synthesis of 2-chloromethyl-4-hydroxymethyl-1,3-dioxolane (4). The compound **3** (6.9 g, 28 mmol) was dissolved in methyl alcohol, hydrogenated at 40 psi over 10% Pd/C for 18 h. The catalyst was removed by filtration on a Celite pad, and the solvent was then evaporated under vacuum. The residue oil was purified by column chromatography on silica gel (eluent hexane:Et_2O 4:1) to give the compound **4** (40%). ^{1}H NMR ($CDCl_3$) δ 2.50–4.15 (br, 1H), 3.22 (d, 2H, J = 3.9 Hz), 3.51 (m, 2H), 3.70–3.90 (m, 2H), 4.12 (m, 1H), 5.03 (t, 1H, J = 3.9 Hz, H-2 *cis*).

Synthesis of 2-dimethylaminomethyl- and 2-(*N*-methyl-*N*-cyclohexylaminomethyl)-4-hydroxymethyl-1,3-dioxolanes (5a,d). The compound **4** (0.7 g, 4.5 mmol) was dissolved in ethyl alcohol (10 mL) and mixed with the appropriate amine (15 mmol) in a sealed vial for 12 h at 120 °C. The solvent was removed on a rotary evaporator and the residue was purified by column chromatography on silica gel (eluent CH_2Cl_2:MeOH 8:2) to give the compounds **5a**,**d** as oils.

5a: yield 82%; ^{1}H NMR ($CDCl_3$) δ 2.43 (s, 6H), 2.79 (d, 2H, J = 2.6 Hz), 3.44 (br, 1H), 3.51 (m, 2H), 3.65–4.20 (m, 2H), 4.25 (m, 1H), 5.10 (t, 1H, J = 2.6 Hz, H-2 *cis*).

5d: yield 67%; ^{1}H NMR ($CDCl_3$) δ 1.29–1.35 (m, 10H), 2.43 (s, 3H), 2.61 (m, 1H), 2.79 (d, 2H, J = 2.6 Hz), 3.44 (br, 1H), 3.51 (m, 2H), 3.65–4.20 (m, 2H), 4.25 (m, 1H), 5.10 (t, 1H, J = 2.6 Hz, H-2 *cis*).

Route b.

Synthesis of (2,2-dimethoxy-ethyl)-isopropyl-methyl-amine and (2,2-dimethoxy-ethyl)-butyl-methyl-amine (6b,c). A mixture of chloroacetaldehydedimethylacetal (**2**) (0.7 g, 4.6 mmol), the appropriate *N*-methylamine-derivative (4.6 mmol), KI (catalytic amount) and K_2CO_3 (1.27 g, 9.2 mmol) in acetonitrile was heated under argon atmosphere and at reflux conditions for 18 h. The solvent was then removed under vacuum and the resulting oil was purified by column chromatography on silica gel (eluent CH_2Cl_2:MeOH 9.5:0.5) to give the compounds **6b** and **6c**.

6b: yield 35%; ^{1}H NMR ($CDCl_3$) δ 1.05 (m, 6H), 2.27 (s, 3H), 2.65 (m, 2H), 2.97 (m, 1H), 3.24 (s, 6H), 4.43 (m, 1H).

6c: yield 43%; ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3H, J = 7 Hz), 1.33–1.39 (m, 4H), 2.36 (m, 2H), 2.66 (m, 2H), 3.24 (s, 6H), 4.43 (m, 1H).

Synthesis of 2-(*N*,*N*-methylisopropylaminomethyl)- and 2-(*N*,*N*-methylbutylaminomethyl)-4-benzyloxymethyl-1,3-dioxolanes (7b,c). To a stirred and refluxed solution of **6b**,**c** (100 mmol) and isopropylidenglycerine-1-benzylether (11 mL, 50 mmol) in benzene (200 mL) were added water (9 mL) and TSOH (1 g, 5 mmol). The reaction mixture was heated at reflux conditions for 24 h, cooled and washed with a saturated solution of $NaHCO_3/H_2O$ (2×100). The organic phase was then washed with a saturated solution of $NaCl/H_2O$ and water. The organic layer was finally dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by column chromatography on silica gel (eluent hexane:Et_2O 4:1).

7b, *cis* isomer: yield 45%; ^{1}H NMR ($CDCl_3$) δ 1.16 (d, 3H, J = 6.6 Hz), 1.19 (d, 3H, J = 6.6 Hz), 2.65 (s, 3H), 2.96 (d, 2H, J = 2.6 Hz), 3.29 (m, 1H), 3.48–4.35 (m, 5H), 4.63 (m, 2H), 5.18 (t, 1H, J = 2.6 Hz, H-2 *cis*), 7.20–7.50 (m, 5H).

Trans isomer: yield 12%; ^{1}H NMR ($CDCl_3$) δ 5.32 (t, 1H, J = 2.6 Hz, H-2 *trans*).

7c, *cis* isomer: yield 52%; ^{1}H NMR ($CDCl_3$) δ 0.91 (t, 3H, J = 7.2 Hz), 1.28 (m, 2H), 1.47 (m, 2H), 2.43 (s, 3H), 2.52 (m, 2H), 2.70 (d, 2H, J = 2.2 Hz), 3.45–4.15 (m, 4H), 4.25 (m, 1H), 4.63 (m, 2H), 5.06 (t, 1H, J = 2.2 Hz, H-2 *cis*), 7.20–7.50 (m, 5H).

Trans isomer: yield 7%; ^{1}H NMR ($CDCl_3$) δ 5.16 (t, 1H, J = 2.2 Hz, H-2 *trans*).

Synthesis of 2-(*N*-methyl-*N*-isopropylaminomethyl)- and 2-(*N*-methyl-*N*-butylaminomethyl)-4-hydroxymethyl-1,3-dioxolanes (5b,c). The compounds **7b**,**c** (28 mmol) were dissolved in methyl alcohol, hydrogenated at 40 psi over 10% Pd/C for 18 h. The catalyst was removed by filtration on a Celite pad, and the solvent was then evaporated under vacuum. The obtained oil was purified by column chromatography on silica gel (eluent CH_2Cl_2: MeOH 4:1).

5b: yield 68%; ^{1}H NMR ($CDCl_3$) δ 1.16 (d, 3H, J = 6.6 Hz), 1.19 (d, 3H, J = 6.6 Hz), 2.65 (s, 3H), 2.96 (d, 2H, J = 2.6 Hz), 3.29 (m, 1H), 3.48–4.35 (m, 5H), 5.18 (t, 1H, J = 2.6 Hz, H-2 *cis*), 5.80–7.50 (br, 1H).

5c: yield 47%; ^{1}H NMR ($CDCl_3$) δ 0.91 (t, 3H, J = 7.2 Hz), 1.28 (m, 2H), 1.47 (m, 2H), 2.43 (s, 3H), 2.52 (m, 2H), 2.70 (d, 2H, J = 2.2 Hz), 3.45–4.15 (m, 4H), 4.25 (m, 1H), 4.97 (s. br. 1H), 5.06 (t, 1H, J = 2.2 Hz, H-2 *cis*).

Synthesis of 3,5-substituted-1,2,4-oxadiazoles.

Synthesis of 2-benzyloxyacetamidoxime (9). A sodium butoxide solution was prepared by dissolving sodium (0.34 g, 16 mmol) in butanol (10 mL) under argon atmosphere and heating at reflux conditions for 1 h. The above obtained sodium butoxide solution (kept warmed) was added to a well-stirred *n*-butanol solution (10 mL) of hydroxylamine hydrochloride (1.2 g, 15 mmol) and phenolphthaleine (catalytic amount). The rate of the addition should be slow enough to maintain the pink color of the solution. When the addition was complete, the mixture was heated at reflux conditions for an additional 2 h, then filtered and 2-benzyloxyacetonitrile (**8**) (2 g, 14 mmol) was added to the above prepared hydroxylamine free base solution. The mixture was heated at reflux conditions for 48 h. The reaction mixture was then cooled to room temperature, dried over Na_2SO_4 and evaporated to dryness. The residue was dissolved in Et_2O (20 mL) and crystallized from hexane to give **9**.

Yield 63%; mp 73–74 °C; ^{1}H NMR (DMSO-d_6) δ 3.86 (s, 2H), 4.44 (s, 2H), 5.56 (br, 2H), 7.20–7.40 (m, 5H), 8.80–9.50 (br, 1H).

Synthesis of 3-benzyloxymethyl-5-dimethylaminomethyl- and 3-benzyloxymethyl-5-methylcyclohexylaminomethyl-1,2,4-oxadiazoles (10a,d). Compound **9** (0.36 g, 2 mmol) was dissolved in THF (anhydrous, 10 mL). NaH 80% (0.05 g, 2.1 mmol) was added to the solution, and the mixture was heated at reflux for 1 h followed by the addition of the appropriate amino acid ethyl ester (4 mmol). Reflux was continued for additional 90 min. The mixture was then cooled and filtered through a Celite pad, and the residue was purified by column chromatography on silica gel (eluent Et_2O:CH_2Cl_2 1:4) to give **10a,d** as oils.

10a: yield 60%; ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 6H), 3.79 (s, 2H), 4.63 (s, 2H), 4.65 (s, 2H), 7.20–7.45 (m, 5H).

10d: yield 62%; ^{1}H NMR ($CDCl_3$) δ 1.10–1.90 (m, 10H), 2.42 (s, 3H), 2.46 (m, 1H), 3.96 (s, 2H), 4.67 (s, 2H), 4.49 (s, 2H), 7.25–7.45 (m, 5H).

Synthesis of 3-hydroxymethyl-5-dimethylaminomethyl- and of 3-hydroxymethyl-5-methylcycloesylaminomethyl-1,2,4-oxadiazoles (11a,d). Compounds **10a**,**d** were dissolved in CH_2Cl_2 (anhydrous, 30 mL) under argon atmosphere. The solution was cooled to −73 °C and boron trichloride solution, 1 M in dichloromethane (4 mL, 4 mmol), was slowly added. The reaction was then treated, after 10 min, by very slow addition of a mixture of CH_2Cl_2 and MeOH (1:1). After 10 min, trimethylamine (2.6 mL) was added and the reaction mixture warmed to room temperature, dried over Na_2SO_4 and concentrated in vacuo. The crude oil obtained was purified by column chromatography on silica gel (eluent CH_2Cl_2:MeOH 5:0.25) to give **11a**,**d** as oils.

11a: yield 62%; ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 6H), 3.78 (s, 2H), 3.80–4.20 (br, 1H), 4.76 (s, 2H).

11d: yield 77%; ^{1}H NMR ($CDCl_3$) δ 0.90–2.00 (m, 10H), 2.34 (s, 3H), 2.39 (m, 1H), 3.60–4.60 (br, 1H), 3.88 (s, 2H), 4.75 (s, 2H).

General procedure for the preparation of *N*-[4-(1′-substituted-acetoxymethyl)-2-dioxolanyl] and *N*-[5-(1′-substituted-acetoxymethyl)-3-oxadiazolyl] dialkyl amines 12a–h and 13a,d,e,h. Phenylcyclohexyl acetic acid or dicyclohexyl acetic acid (0.87 mmol) were dissolved in benzene (anhydrous, 5 mL) under argon atmosphere; to the

solution was then added oxalyl chloride (110 μL, 1.26 mmol). After 1 h under stirring at room temperature, the solvent was evaporated and the residue was dissolved in anhydrous benzene (3 mL). This solution was added dropwise, under argon, to a solution of the appropriate amino-alcohol **5a–d** or **11a,d** (0.76 mmol) in benzene (anhydrous, 10 mL) containing triethylamine (0.4 mL) and DMAP (catalytic amount). The mixture was stirred at room temperature for 24 h, then washed with a saturated solution of $NaHCO_3$, the aqueous layer was dried ($MgSO_4$), concentrated, and the residue was purified by column chromatography on silica gel (eluent CH_2Cl_2: MeOH 5:0.25) to give the expected compound.

4-(1′-Cyclohexyl-1′-phenyl-acetoxymethyl)-2-(*N,N*-dimethyl-aminomethyl)-1,3-dioxolane (12a). Yield 60%; ^{1}H NMR ($CDCl_3$) δ 1.00–1.40 (m, 6H), 1.45–1.80 (m, 5H), 2.27 (s, 6H), 2.47 (m, 2H), 3.23 (d, 1H, J = 10.6 Hz), 3.65–3.90 (m, 2H), 4.00–4.30 (m, 3H), 4.95 (t, 1H, J = 2.60 Hz, H-2 *cis*), 7.20–7.40 (m, 5H). Anal. C, H, N ($C_{21}H_{31}NO_4$).

4-(1′-Cyclohexyl-1′-phenyl-acetoxymethyl)-2-(*N*-isopropyl-*N*-methyl-aminomethyl)-1,3-dioxolane (12b). Yield 51%; ^{1}H NMR ($CDCl_3$) δ 0.91 (d, 6H, J = 6.6 Hz), 1.00–1.40 (m, 6H), 1.40–2.05 (m, 5H), 2.21 (s, 3H), 2.47 (m, 2H), 2.79 (m, 1H), 3.18 (d, 1H, J = 10.6 Hz), 3.30–4.15 (m, 5H), 4.86 (t, 1H, J = 4.5 Hz, H-2 *cis*), 7.05–7.30 (m, 5H). Anal. C, H, N ($C_{23}H_{35}NO_4$).

4-(1′-Cyclohexyl-1′-phenyl-acetoxymethyl)-2-(*N*-butyl-*N*-methyl-aminomethyl)-1,3-dioxolane (12c). Yield 72%; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 7.2 Hz), 1.00–1.80 (m, 15H), 2.23 (s, 3H), 2.34 (m, 2H), 2.49 (d, 2H, J = 4.4 Hz), 3.17 (d, 1H, J = 10.8 Hz), 3.50–3.80 (m, 2H), 3.80–4.25 (m, 3H), 4.90 (t, 1H, J = 4.4 Hz, H-2 *cis*), 7.10–7.40 (m, 5H). Anal. C, H, N ($C_{24}H_{37}NO_4$).

4-(1′-Cyclohexyl-1′-phenyl-acetoxymethyl)-2-(*N*-methyl-*N*-cyclohexyl-aminomethyl)-1,3-dioxolane (12d). Yield 75%; ^{1}H NMR ($CDCl_3$) δ 0.90–1.35 (m, 13H), 1.35–1.75 (m, 8H), 2.20 (m, 1H), 2.26 (s, 3H), 2.53 (d, 2H, J = 4.6 Hz), 3.17 (d, 1H, J = 10.6 Hz), 3.55–3.85 (m, 2H), 3.85–4.20 (m, 3H), 4.85 (t, 1H, J = 4.6 Hz, H-2 *cis*), 7.10–7.40 (m, 5H). Anal. C, H, N ($C_{26}H_{39}NO_4$).

4-(1′-Dicyclohexyl-acetoxymethyl)-2-(*N,N*-dimethyl-aminomethyl)-1,3-dioxolane (12e). Yield 85%; ^{1}H NMR ($CDCl_3$) δ 0.90–1.35 (m, 10H) 1.35–1.80 (m, 12H), 2.02 (t, 1H, J = 7.4 Hz), 2.29 (s, 6H), 2.56 (d, 2H, J = 4.4 Hz), 3.65–3.80 (m, 2H), 3.95–4.10 (m, 2H), 4.21 (m, 1H), 5.01 (t, 1H, J = 4.4 Hz, H-2 *cis*). Anal. C, H, N ($C_{21}H_{37}NO_4$).

4-(1′-Dicyclohexyl-acetoxymethyl)-2-(*N*-isopropyl-*N*-methyl-aminomethyl)-1,3-dioxolane (12f). Yield 60%; ^{1}H NMR ($CDCl_3$) δ 0.80–1.35 (m, 10H), 0.92 (d, 6H, J = 6.6 Hz), 1.35–1.80 (m, 12H), 2.02 (t, 1H, J = 7.4 Hz), 2.24 (s, 3H), 2.50 (d, 2H, J = 4.6 Hz), 2.79 (m, 1H), 3.70–3.90 (m, 2H), 4.15 (m, 2H), 4.20 (m, 2H), 4.90 (t, 1H, 4.6 Hz, H-2 *cis*). Anal. C, H, N ($C_{23}H_{41}NO_4$).

4-(1′-Dicyclohexyl-acetoxymethyl)-2-(*N*-butyl-*N*-methyl-aminomethyl)-1,3-dioxolane (12g). Yield 60%; ^{1}H NMR ($CDCl_3$) δ 0.83 (t, 3H, J = 10.8 Hz), 0.90–1.45 (m, 14H), 1.45–1.85 (m, 12H), 2.01 (t, 1H, J = 7.4 Hz), 2.24 (s, 3H), 2.32 (m, 3H), 2.52 (d, 2H, J = 4.5 Hz), 3.65–3.80 (m, 2H), 3.95–4.10 (m, 2H), 4.19 (m, 1H), 4.94 (t, 1H, J = 4.5 Hz, H-2 *cis*). Anal. C, H, N ($C_{24}H_{43}NO_4$).

4-(1′-Dicyclohexyl-acetoxymethyl)-2-(*N*-methyl-*N*-cyclohexyl-aminomethyl)-1,3-dioxolane (12h). Yield 76%; ^{1}H NMR ($CDCl_3$) δ 0.75–1.40 (m, 18H), 1.40–1.90 (m, 14H), 2.02 (t, 1H, J = 7.2 Hz), 2.25 (m, 1H), 2.29 (s, 3H), 2.59 (d, 2H, J = 4.5 Hz), 3.50–4.05 (m, 4H), 4.20 (m, 1H), 4.91 (t, 1H, J = 4.5 Hz, H-2 *cis*). Anal. C, H, N ($C_{26}H_{45}NO_4$).

3-(1′-Cyclohexyl-1′-phenylacetoxymethyl)-5-(*N,N*-dimethyl-aminomethyl)-1,2,4-oxadiazole (13a). Yield 55%; ^{1}H NMR ($CDCl_3$) δ 0.90–2.10 (m, 10 H), 2.28 (s, 6H), 2.38 (m, 1H), 3.26 (d, 1H, J = 10.6 Hz), 3.80 (s, 2H), 5.03 (d, 1H, J = 13.7 Hz), 5.21 (d, 1H, J = 13.7 Hz), 7.10–7.35 (m, 5H). Anal. C, H, N ($C_{20}H_{27}N_3O_3$).

3-(1′-Cyclohexyl-1′-phenyl-acetoxymethyl)-5-(*N*-cyclohexyl-*N*-methyl-aminomethyl)-1,2,4-oxadiazole (13d). Yield 97%; ^{1}H NMR ($CDCl_3$) δ 0.90–1.50 (m, 11H), 1.50–2.15 (m, 10 H), 2.37 (s, 3H), 2.38 (m, 1H), 3.36 (d, 1H, J = 10.6 Hz), 3.92 (s, 2H), 5.08 (d, 1H, J = 13.6 Hz), 5.26 (d, 1H, J = 13.6 Hz), 7.20–7.40 (m, 5H). Anal. C, H, N ($C_{25}H_{35}N_3O_3$).

3-(1′-Dicyclohexyl-acetoxymethyl)-5-(*N,N*-dimethyl-aminomethyl)-1,2,4-oxadiazole (13e). Yield 77%; ^{1}H NMR ($CDCl_3$) δ 0.80–1.50 (m, 10H), 1.50–1.80 (m, 12H), 2.15 (t, 1H, J = 7.2 Hz), 2.36 (s, 6H), 3.80 (s, 2H), 5.19 (s, 2H). Anal. C, H, N ($C_{20}H_{33}N_3O_3$).

3-(1′-Dicyclohexyl-acetoxymethyl)-5-(*N*-cyclohexyl-*N*-methyl-aminomethyl)-1,2,4-oxadiazole (13h). Yield 85%; ^{1}H NMR ($CDCl_3$) δ 0.80–1.50 (m, 18H), 1.50–1.90 (m, 14H), 2.10 (t, 1H, J = 7.2 Hz), 2.32 (s, 3H), 2.37 (m, 1H), 3.89 (s, 2H), 5.13 (s, 2H). Anal. C, H, N ($C_{25}H_{41}N_3O_3$).

Biology

Materials

[^{3}H]QNB (specific activity 43 Ci/mmol) was purchased from DuPont/NEN (Boston, MA). Atropine was from Research Biochemicals, Inc. (Natick, MA).

Membrane preparation

Human muscarinic receptors (hm1–hm4) transfected into CHO cells were purchased from Receptor Biology, Inc. The membranes were stored and prepared according to RBI recommendations. Each assay tube contained approximately 16–31 μg of membranes.

Radioligand binding assays

Competiton assays between [^{3}H]QNB (0.2 nM) and the unlabeled muscarinic antagonists (1 nM–10 μM) were performed in a total volume of 1 mL of 50 mM sodium–potassium phosphate buffer. At the end of the incubation

period, each suspension was aspirated onto Whatman GF/B glass fiber filters in a Brendel cell harvester. The filters were washed three times with cold 50 mM sodium–potassium phosphate buffer; radioactivity was solubilized and and counted in Redi-Safe cocktail (Beckman). Atropine (100 μM) was present in some tubes to determine the level of nonspecific binding.

Data analysis

Concentration–response data were fitted with a four parameter logistic model, ALLFIT.[28] The initial parameters were constrained using values assuming competition between [^{3}H]QNB and muscarinic antagonists. K_d was calculated by correction of IC_{50} using the Cheng and Prusoff formula[29] and K_d for [^{3}H]QNB of 50 pM.

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