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Muscarinic Thioligands with Cyclopentane Nucleus†

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Abstract—Some thio- and the benzoyl-derivatives of deoxamuscarine were synthesized and tested as muscarinic agonists using radioligand binding assays and functional tests. In comparison with deoxamuscarine, used as reference compound, no dimension/distance modification is tolerated for correct lipophilic pocket recognition. The substitution of the ammonium group with a sulphonium group significantly decreased muscarinic potency. The so-called 'muscarinic sub-site' accepts relatively bulky functions as long as it is bound to the cyclopentane carrier by an oxygen bridge. Esterification of this moiety increases the M_2 subtype selectivity, while etherification heightens that of M_3. Copyright © 1996 Elsevier Science Ltd

Introduction

A large number of natural and synthetic drugs contain a sulphur atom in all oxidation states and organic combinations.[1] The ability of this atom to modify physicochemical properties of reference molecules (steric hindrance, length and angle of bond and, particularly, electronic distribution) has inspired the preparation of several sulphur bioisosters of cholinergic compounds. The effect of substituting the oxygen atoms of acetylcholine (ACh) has been studied. The thiono analogue preserves the natural model properties almost intact, while acetylthiocholine, because of the change of the conformation of the X-C-C-N fragment from *gauche* to *trans*, shows a drop in activity.[2] A similar situation occurs with thiomuscarine: the larger size and lower hydrogen bonding of the sulphur atom in comparison with the oxygen atom of muscarine markedly hinder interaction with the corresponding receptor site.[3] However, in many cases the presence of thio-carriers such as 1,2,5-thiadiazole[4] or 1,3-oxathiolane[5] nuclei improves the muscarinic activity. Interesting results were found with the replacement of the lactone moiety of pilocarpine with a thiolactone ring. In fact, the resultant thiopilocarpine shows M_1 agonist and M_2 antagonist activities at the same time,[6] making it an interesting lead for potential drugs in the treatment of Alzheimer's disease.

In view of this, we decided to study the effect of the introduction of sulphur in a biologically accredited carrier such as deoxamuscarine (**1**),[7] in positions of the structure critical for interaction with the corresponding active sites, i.e. the lipophilic pocket (a), the polar area (b) and the muscarinic site (c) (Fig. 1; compounds **2–6**). The muscarinic M_1–M_4 subtype profile of these compounds was evaluated to investigate potential discrimination for the development of new therapeutic agents.

Finally, benzoyl derivative **7** was prepared for bioisosteric comparison. For the same reason, the previously described ester **8**[8] and ether derivatives **9** and **10**[9] are reported in Figure 1 and in Tables 1 and 2.

Chemistry

Methiodide **2** was prepared from *trans*-1,2-epoxy-4-cyclopentanecarboxylic acid dimethylamide (**11**)[10] by opening with sodium thiomethoxide, followed by reduction with $LiAlH_4$ and quaternization with iodomethane (Scheme 1). The stereochemistry of **2** is exactly definable on the basis of the course of the reaction which, as well noted,[11] orientates the incoming group (in this case $-SCH_3$) from the opposite part of the oxygen bridge, namely *cis* in terms of the amidic function. Accordingly, the spatial disposition of the different active groups is optimal for correct interaction with the corresponding receptorial sites.

Compound **3** was in turn synthesized from *c*-4-methyl-*c*-3-hydroxy-*r*-1-cyclopentanecarboxylic acid dimethylamide (**13**)[12] according to the sequence of reactions reported in Scheme 2, which involves a configuration inversion in the replacement of the methylsulfonyl group with a nucleophilic group (in our case the thiobenzyl group).[13]

The methiodide **4** was prepared from the corresponding tosylderivative (**16**)[14] first by treating with sodium thiomethoxide and then with MeI.

The two sulphones **5** and **6** were obtained through esterification of the -OH function of *c*-4-methyl-

Key words: muscarinic binding affinity; M_2/M_3 selectivity; muscarinic thioligands; deoxamuscarine thioderivatives.
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t-3-hydroxy-*r*-1-*N*,*N*-dimethylaminomethylcyclopentane (**17**)[14] with methyl- and benzenesulphonyl chloride, respectively, followed by quaternization with iodomethane (Scheme 3).

Finally, the benzoyl derivative **7** was obtained by simple reaction of the same amine **17** with benzoyl chloride and subsequent quaternization with iodomethane (Scheme 3).

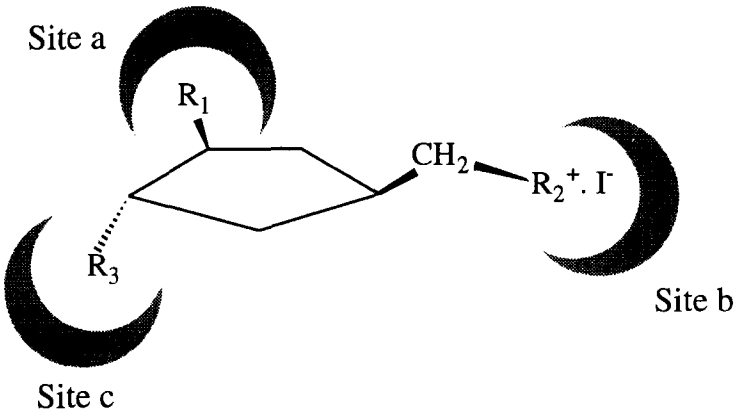
Biological Studies

Molecules **2**–**7**, reference compound **1** (deoxamuscarine) and its derivative **10** were preliminarily tested on M_1-M_4 preparations with radioligand binding assay techniques. This screening was done in frozen sections[15] of rat frontal cortex (M_1), heart (M_2), submaxillary glands (M_3), and striatum (M_4), using as a ligand the non-selective muscarinic receptor antagonist [^{3}H]-*N*-methylscopolamine (NMS). These tissues were chosen since they represent the source of different subtypes of muscarinic cholinergic receptors indicated above in parentheses[16]. The binding of [^{3}H]-NMS to the above tissues was time-, temperature- and concentration-dependent.

Scatchard analysis data of [^{3}H]-NMS binding to sections of the different tissues investigated are summarized in Table 3. As can be seen, the dissociation constant (K_d) and the maximum density of binding sites (B_{max}) of these tissues are consistent with data reported in literature.[17]

Competitor dissociation constant (K_i)[18] of the compounds tested indicates their affinity for the subtypes of muscarinic cholinergic receptors expressed by the tissues investigated. To allow an easier comparison with functional data (see below), K_i will be expressed in the text as pK_i values (Table 1). Analysis of K_i values allowed the receptor profiles of the compounds tested to be evaluated.

In addition, molecules **2**–**7** were tested in functional studies performed in preparations of guinea pig left atrium and ileum. These preparations allow evaluation of M_2 and M_3 receptor activity, respectively.[19] Results



1.	R_1=CH_3,	R_2=NMe_3,	R_3=OH
2.	R_1=SCH_3,	R_2=NMe_3,	R_3=OH
3.	R_1=CH_3,	R_2=NMe_3,	R_3=$SCH_2C_6H_5$
4.	R_1=CH_3,	R_2=SMe_2,	R_3=OH
5.	R_1=CH_3,	R_2=NMe_3,	R_3=OSO_2CH_3
6.	R_1=CH_3,	R_2=NMe_3,	R_3=$OSO_2C_6H_5$
7.	R_1=CH_3,	R_2=NMe_3,	R_3=$OCOC_6H_5$
8.	R_1=CH_3,	R_2=NMe_3,	R_3=$OCOCH_3$
9.	R_1=CH_3,	R_2=NMe_3,	R_3=OCH_2CH_3
10.	R_1=CH_3,	R_2=NMe_3,	R_3=$OCH_2C_6H_5$

Figure 1.

for these newly synthesized compounds as well as for the reference molecule deoxamuscarine (compound **1**) and for derivatives **8–10** are shown in Table 2.

Discussion

Cyclopentane derivatives **1–7** and **10** (Table 1) show low binding affinity values consistent with the labelling of muscarinic agonists and/or weak antagonists. The presence of a phenyl group in compounds **3**, **6**, **7**, and **10** increases the affinity for M_1–M_4 subtypes in comparison with the reference deoxamuscarine (**1**). Compounds **3**, **6**, and **7** display a slight preference for the M_2 subtype, compound **10** for the M_3 subtype. Compound **4** shows a good selectivity for the M_2 receptor. Molecule **5** has a pharmacological profile similar to the lead compound deoxamuscarine (1), with higher affinity for M_1 and M_2 receptor subtypes rather than for M_3 and M_4 sites. The low affinity of deoxamuscarine (**1**) at the M_3 subtype is not in good agreement with its potency shown in functional assays even though measured on a different preparation (Table 2).

The modification at the methyl group level (compound **2**) of the reference molecule deoxamuscarine (**1**) causes a drop in affinity and in potency, confirming the critical role of the receptorial lipophilic pocket for muscarinic agonism. The requirement of this area (site a in Fig. 1) probably does not tolerate dimensional or distance changes of the corresponding complement in the interacting molecule.

The substitution of the ammonium group of deoxamuscarine (**1**) with a sulphonium group (compound **4**) weakens the potency of the molecule. Modest antagonist properties on the M_2 subtype are also shown in functional tests. The cationic groups of **4** and of the reference compound **1** have a similar volume. Hence the drop in activity could be attributed to conformational reasons or to increased lipophilicity. In fact, the value of the hydrophobicity constant, π, of the $-S^+Me_2$ function is 0.74 times more positive than the corresponding $-N^+Me_3$ one, i.e. −0.50 and −1.24, respectively.[20]

Of particular interest are the results obtained with compound **3**, which shows about the same affinity on M_1–M_4 preparations in binding experiments and only a weak antagonist activity on both M_2 and M_3 subtypes in functional tests. This does not occur for the oxygenated analogue **10**[9], which displays an agonist activity exceeding that of deoxamuscarine (**1**) at the M_3 site.

The sulphones **5** and **6**, as well as the benzoylderivative **7**, display substantial agonist activity. The same is true for other compounds, such as acetoxy- and ethoxy-derivatives, **8**[8] and **9**,[9] respectively, which, independently of the substituent, maintain the presence of an oxygen bridge at the same molecular position.

The above data collectively suggest that the 'muscarinic sub-site' (area c in Fig. 1) is capable of accepting

Table 1. Affinity constants (pK_i) in rat frontal cortex (M_1), heart (M_2), submaxillary glands (M_3) and striatum (M_4) muscarinic subtypes

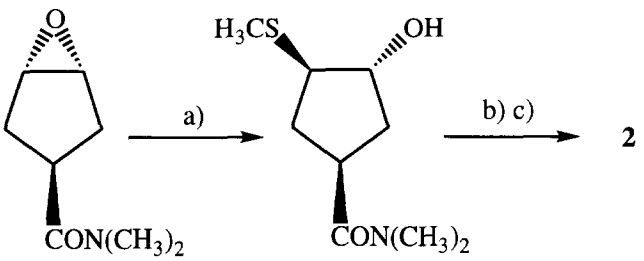
Compound	pK_i[a]			
	M_1	M_2	M_3	M_4
1	4.43 ± 0.17	4.82 ± 0.15	[b]	[b]
2	[b]	[b]	[b]	[b]
3	5.72 ± 0.29	6.25 ± 0.25	5.65 ± 0.21	5.58 ± 0.19
4	[b]	5.52 ± 0.27	[b]	[b]
5	4.41 ± 0.19	5.44 ± 0.23	[b]	[b]
6	4.74 ± 0.22	5.58 ± 0.15	5.01 ± 0.21	4.39 ± 0.17
7	5.12 ± 0.18	6.00 ± 0.24	5.47 ± 0.30	5.56 ± 0.23
10	4.48 ± 0.21	5.71 ± 0.33	6.23 ± 0.25	4.61 ± 0.15

[a]Values are the mean ± SE of at least three separate experiments performed in triplicate. All Hill numbers (nH) were not significantly different from unit ($p > 0.05$).
[b]$IC_{50} > 100\ \mu M$.

Table 2. Maximal response (E_{max}),[a] potency expressed as -log ED_{50} (pD_2) and dissociation constant (pK_b)[b] on isolated guinea pig left atrium and ileum (M_2 and M_3 muscarinic subtypes, respectively[c])

Compound	Isolated G.P. left atrium		Isolated G.P. ileum		Ref.
	E_{max}[a]	pD_2 (pK_b)[b]	E_{max}[a]	pD_2 (pK_b)[b]	
1	1.00 ± 0.02	5.93 ± 0.05	1.03 ± 0.02	6.13 ± 0.07	9
2		(< 4)	0.28 ± 0.13	4.5 ± 0.25	
3		(4.33 ± 0.01)		(≃ 5)	
4		(4.38 ± 0.01)	0.97 ± 0.05	3.87 ± 0.07	
5	1.00 ± 0.03	6.26 ± 0.35	1.00 ± 0.02	5.40 ± 0.09	
6	0.70 ± 0.09	6.57 ± 0.46	1.00 ± 0.03	5.36 ± 0.30	
7	0.96 ± 0.02	6.56 ± 0.15	0.86 ± 0.01	5.41 ± 0.11	
8	1.00 ± 0.03	6.09 ± 0.08[d]	0.97 ± 0.02	5.31 ± 0.12	8
9	0.60 ± 0.19	5.27 ± 0.06	0.95 ± 0.02	5.56 ± 0.01	9
10	0.92 ± 0.05	5.59 ± 0.08	1.00 ± 0.05	6.81 ± 0.08	9

[a]E_{max} is given relative to that of carbachol.
[b]Dissociation constants are calculated from the equation: $\log(DR-1) = \log[ant] - \log K_b$.
[c]The results are the means (± SE) of four to six independent experiments.
[d]Not published.



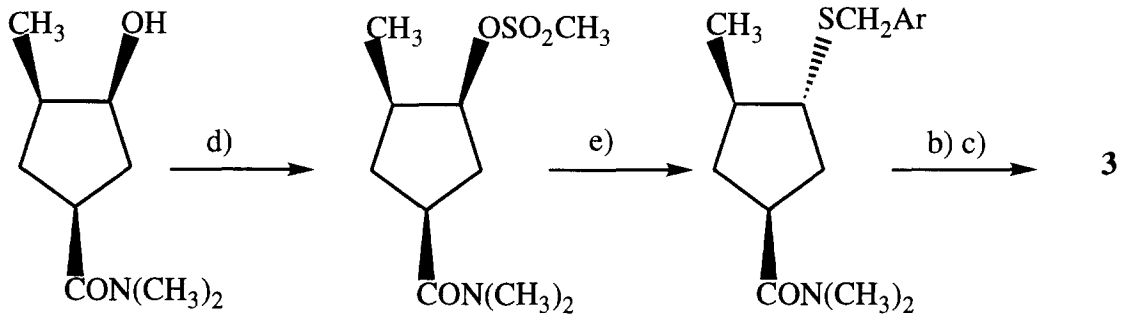
11 **12**

Scheme 1. (a) CH_3SNa; (b) $LiAlH_4$; (c) MeI.

relatively bulky functions and of recognizing bioisosteric groups such as -$CH_2C_6H_5$, -COC_6H_5, -$SO_2C_6H_5$ (compounds **10**, **7**, and **6**, respectively), as long as they are bound to the pentatomic carrier by an oxygen bridge. The critical role of this atom could be better clarified by its replacement with CH_2, NH or SO functions.

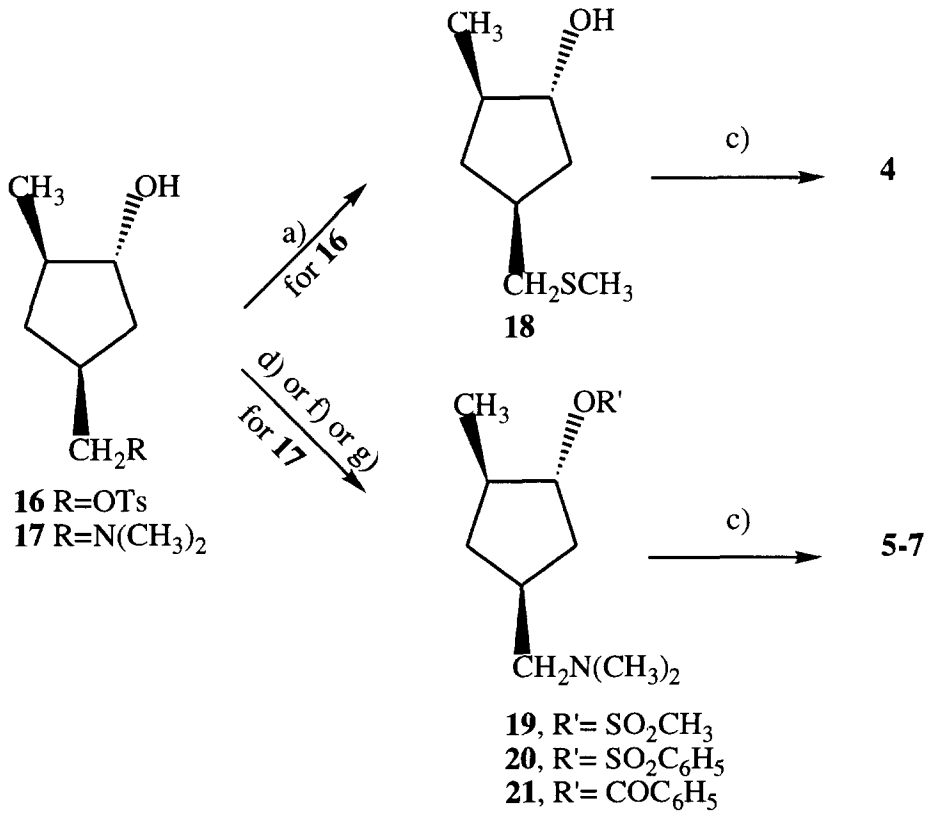
It is worth noting, as well, that esterification of the deoxamuscarine hydroxy moiety (compounds **5–7**) causes a significative increase of selectivity toward the M_2 subtype, while etherification (compounds **9** and **10**) improves M_3 selectivity.

In conclusion, appropriate modifications at the hydroxy group level of deoxamuscarine (**1**) seem to be



13 **14** **15**

Scheme 2. (b) (c) see Scheme 1; (d) CH_3SO_2Cl; (e) $C_6H_5CH_2SH$/Na.



Scheme 3. (a), (c) and (d) see Schemes 1 and 2, respectively; (f) $C_6H_5SO_2Cl$; (g) C_6H_5COCl.

Table 3. Scatchard analysis of [^{3}H]NMS binding to muscarinic cholinergic receptor subtypes expressed in the different tissues investigated

Tissue	K_d[a] (nM)	B_{max}[b] (fmol/mg tissue)	Receptor subtype
Frontal cortex	0.17 ± 0.015	2814.25 ± 112.3	M_1
Heart	0.46 ± 0.04	121 ± 4	M_2
Submaxillary glands	0.18 ± 0.019	378.7 ± 15.41	M_3
Striatum	0.12 ± 0.02	2239.2 ± 89.6	M_4

[a]Dissociation constant.
[b]Maximum density of binding sites.

promising in modulating M_2/M_3 selectivity; in contrast, modifications of the functions interacting with the lipophilic pocket or polar area (sites a or b in Fig. 1) are detrimental to muscarinic cholinergic activity.

Experimental

Chemistry

Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Gemini-200 (200 MHz) spectrometer. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS). The microanalyses were performed by the microanalytical laboratory of our department, and the elemental compositions of the compounds agreed to within ± 0.4% with the calculated values. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040–0.063, Merck) by flash chromatography. Compounds were named following IUPAC rules as applied by · AUTONOM, a PC software for systematic names in organic chemistry, Beilstein-Institut and Springer-Verlag.

***t*-3-Hydroxy-*c*-4-methylsulphanyl-*r*-1-cyclopentanecarboxylic acid dimethylamide (12).** The epoxide **11**[10] (0.82 g, 5.28 mmol) was added to a soln of $NaHCO_3$ (1.17 g, 13.93 mmol) in water (20 mL) at 0 °C. $NaSCH_3$ (0.53 g, 7.56 mmol) was added in portions over 2 min, and the capped mixture was stirred at room temperature for 72 h. The reaction was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, evapd in vacuo and the crude product was chromatographed using EtOAc:EtOH (97:3) as the eluent to give **12** (0.52 g, 2.25 mmol, 48%). ^{1}H NMR ($CDCl_3$): δ (ppm): 1.84 (2H, m, cyclo), 2.11 (3H, s, S-CH_3), 2.30 (2H. m, cyclo), 2.85 (1H, m, C_1-H), 2.94–3.03 (6H, ds, NMe_2), 3.20 (1H, m, C_4-H), 4.19 (1H, m, C_3-H), 5.20 (1H, d, OH).

***t*-3-Hydroxy-*c*-4-methylsulphanyl-*r*-1-*N*,*N*-dimethylaminomethylcyclopentane methiodide (2).** A soln of the amide **12** (0.52 g, 2.25 mmol) in dry Et_2O (10 mL) was added dropwise to a stirred mixture of $LiAlH_4$ (0.4 g, 10.52 mmol) in dry Et_2O (15 mL) at 0 °C over a period of 20 min. The mixture was refluxed for 4 h, then decomposed with H_2O (0.4 mL), a diluted solution of NaOH (0.4 mL) and H_2O (2 mL). After stirring for 1 h, the solid was filtered off and the filtrate was dried over anhydrous Na_2SO_4. The solvent was evapd in vacuo and the residue chromatographed using $CHCl_3$:MeOH:conc NH_4OH (9:1:0.01) as the eluent. The resulting amine (95% yield), dissolved in dry acetone, was treated with CH_3I (0.15 mL); after 2 h, the solid was collected by filtration and recrystallized from *i*-PrOH to afford **2** (85%, mp 122–123 °C). ^{1}H NMR (DMSO): δ (ppm) 1.15 (1H, m, cyclo), 1.63 (1H, m, cyclo), 1.82 (1H, m, cyclo), 2.10 (3H, s, S-CH_3), 2.48 (1H, m, cyclo), 2.61 (1H, m, cyclo), 2.85 (1H, m, C_1-H), 3.09 (9H, ds, NMe_3), 3.36 (1H, m, CH_2), 3.95 (1H, m, C_4-H), 5.02 (1H, d, OH). Anal. $C_{10}H_{22}INOS$: C 36.26; H 6.69; N 4.23; S 9.68. Found: C 36.65; H 6.59; N 4.11; S 9.68.

***c*-3-Methanesulphonyl-*c*-4-methyl-*r*-1-cyclopentanecarboxylic acid dimethylamide (14).** To a stirred solution of **13**[12] (0.84 g, 4.91 mmol) and triethylamine (1.36 mL) in dry CH_2Cl_2 (20 mL) was added a solution of methanesulphonyl chloride (1 mL) in dry CH_2Cl_2 (15 mL). The mixture was allowed to warm to room temperature and stirred for 3 h. The reaction was washed with ice and H_2O (5 mL) and the organic phase was dried over anhydrous Na_2SO_4, filtered and concd in vacuo, to obtain a residue which was chromatographed using $CHCl_3$:EtOH (97:3) as the eluent. Mesylate **14** (1 g, 81%) was obtained as an oil. ^{1}H NMR ($CDCl_3$): δ (ppm): 1.10 (3H, d, CH_3), 2.00–2.32 (4H, m, cyclo), 2.49 (1H, m, cyclo), 2.98–3.09 (6H, ds, NMe_2), 3.00 (1H, m, C_1-H), 3.04 (3H, s, SO_2CH_3), 5.01 (1H, m, C_3-H).

***t*-3-Benzylsulphanyl-*c*-4-methyl-*r*-1-cyclopentanecarboxylic acid dimethylamide (15).** A soln of Na (0.075 g) in *i*-PrOH (25 mL) was heated to reflux under N_2. After cooling, benzyl mercaptane (0.4 mL, 3.45 mmol) was added, followed after 0.5 h by a soln of mesylate **14** (0.86 g, 3.45 mmol) in *i*-PrOH (10 mL). The reaction mixture was allowed to warm to room temperature and was stirred for 5 h. A solution of 0.1 N iodine (20 mL) was added to the reaction and the mixture was evapd. Water was added to the residue and extracted with $CHCl_3$ (3 × 40 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and concd in vacuo. Chromatography with $CHCl_3$:MeOH (10:0.01) as the eluent gave thioether **15** (0.47 g, 49%). ^{1}H NMR ($CDCl_3$): δ (ppm) 1.05 (3H, d, CH_3), 1.50 (1H, m, cyclo), 1.83 (2H, m, cyclo), 2.12 (1H, m, cyclo), 2.35 (1H, m, cyclo), 2.71 (1H, m, cyclo), 2.92–3.00 (6H, ds, NMe_2), 3.11 (1H, m, C_1-H), 3.76 (2H, s, CH_2Ar), 7.18–7.32 (5H, m, Ar).

***t*-3-Benzylsulphanyl-*c*-4-methyl-*r*-1-*N*,*N*-dimethylaminomethylcyclopentane methiodide (3).** In the same way as compound **2**, amide **15** was converted to **3** in 70% yield. Mp 154–155 °C (from EtOH:Et_2O). ^{1}H NMR (DMSO): δ (ppm) 1.00 (3H, d, CH_3), 1.02 (1H, m, cyclo), 1.70–2.19 (4H, m, cyclo), 2.59 (2H, m, cyclo), 3.05 (9H, s, NMe_3), 3.32 (2H, d, CH_2N), 3.78 (2H, s,

CH_2Ar), 7.19–7.32 (5H, m, Ar). Anal. $C_{17}H_{28}INS$: C 50.37; H 6.96; N 3.46; S 7.91. Found: C 50.73; H 6.88; N 3.35; S 7.89.

***t*-3-Hydroxy-*c*-4-methyl-*r*-1-methylsulphanylmethylcyclopentane methiodide (4).** $NaSCH_3$ (0.12, 1.7 mmol) was added to a soln of tosyl derivative **16**[14] (0.50 g, 1.7 mmol) in DMSO (10 mL), under N_2. The mixture was refluxed for 4 h, then ice (15 g) was added and the mixture extracted with cyclohexane (5 × 20 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and concd in vacuo. Chromatography with cyclohexane:EtOAc (70:30) as the eluent gave compound **18** (0.20 g, 74%). ^1H NMR ($CDCl_3$): δ (ppm) 0.90 (1H, m, cyclo), 1.05 (3H, d, CH_3), 1.52 (1H, d, OH), 1.62–1.91 (3H, m, cyclo), 2.10 (3H, s, SMe), 2.13 (1H, m, cyclo), 2.40 (1H, m, cyclo), 2.49 (2H, m, CH_2S), 3.78 (1H, m, C_3-H).

Thioderivative **18**, dissolved in dry Et_2O, was treated with CH_3I (0.4 mL); the solid obtained was collected by filtration and recrystallized to afford **4**. Mp 65 °C (from EtOH:Et_2O). ^1H NMR (DMSO): δ (ppm) 0.93 (1H, m, cyclo), 1.02 (3H, d, CH_3), 1.69 (3H, m, cyclo), 2.10 (1H, m, cyclo), 2.43 (1H, m, cyclo), 2.89 (6H, s, SMe_2), 3.31 (2H, m, CH_2S), 3.61 (1H, m, C_3-H), 4.71 (1H, d, OH). Anal. $C_9H_{19}IOS$: C 35.77; H 6.34; S 10.61. Found: C 35.68; H 6.25; S 10.90.

***t*-3-Methanesulphonyl-*c*-4-methyl-*r*-1-*N*,*N*-dimethylaminomethylcyclopentane methiodide (5).** The methanesulphonyl chloride (0.69 mL) was added dropwise to a stirred soln of compound **17**[14] (0.7 g, 4.5 mmol) and triethylamine (1.24 mL) in dry $CHCl_3$ (20 mL) at 0 °C. The soln was kept at 0 °C for 2 h. The reaction was washed with ice and water (5 mL), dried over anhydrous Na_2SO_4, filtered and concd in vacuo. The residue was chromatographed with $CHCl_3$:MeOH:conc. NH_4OH (9:1:0.01) as the eluent to give **19** (0.91 g, 74%).

This amine was dissolved in dry Et_2O and was treated with an excess of CH_3I. The solid was collected by filtration and recrystallized to give **5**. Mp 122–123 °C (from EtOH). ^1H NMR (DMSO): δ (ppm) 0.99 (1H, m, cyclo), 1.06 (3H, d, CH_3), 1.89 (1H, m, cyclo), 2.11 (3H, m, cyclo), 2.58 (1H, m, cyclo), 3.04 (9H, s, NMe_3), 3.31 (3H, s, SO_2CH_3), 3.35 (2H, m, CH_2N), 4.60 (1H, m, C_3-H). Anal. $C_{11}H_{24}INO_2S$: C 36.57; H 6.7; N 3.88; S 8.87. Found: C 36.88; H 6.59; N 3.79; S 8.99.

***t*-3-Benzenesulphonyl-*c*-4-methyl-*r*-1-*N*,*N*-dimethylaminomethylcyclopentane methiodide (6).** The amine **20** was obtained in the same way as compound **19**, adding benzenesulphonyl chloride to compound **17**. Treating this amine with an excess of CH_3I gave **6**. Mp 110–112 °C (from EtOH). ^1H NMR (DMSO): δ (ppm) 0.95 (1H, m, cyclo), 1.02 (3H, d, CH_3), 1.90–2.30 (4H, m, cyclo), 2.65 (1H, m, cyclo), 3.09 (9H, s, NMe_3), 3.38 (2H, m, CH_2N), 4.65 (1H, m, C_3-H), 7.30 (3H, m, Ar), 7.61 (2H, m, Ar). Anal. $C_{16}H_{26}INO_2S$: C 45.39; H 6.19; N 3.31; S 7.57. Found: C 45.71; H 6.38; N 3.50; S 7.85.

***t*-3-Benzoyl-*c*-4-methyl-*r*-1-*N*,*N*-dimethylaminomethylcyclopentane methiodide (7).** The amine **21** was obtained in the same way as compound **19**, adding benzoyl chloride to compound **17**. Treating this amine with an excess of CH_3I gave **7**. Mp 175–176 °C (from *i*-PrOH). ^1H NMR (DMSO): δ (ppm) 1.01 (1H, d, cyclo), 1.11 (3H, d, CH_3), 1.90–2.30 (4H, m, cyclo), 2.69 (1H, m, cyclo), 3.09 (9H, s, NMe_3), 3.41 (2H, m, CH_2N), 4.89 (1H, m, C_3-H), 7.52 (2H, m, Ar), 7.67 (1H, m, Ar), 8.00 (2H, m, Ar). Anal. $C_{17}H_{26}INO$: C 52.72; H 6.77; N 3.62. Found: C 53.00; H 6.79; N 3.48.

Pharmacological Methods

Binding techniques. [^{3}H]-NMS (specific activity 85 Ci/mmol), was purchased from Amersham Radiochemical Centre (Buckinghamshire, U.K.). Atropine and the other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Adult male Sprague–Dawley rats were used. Animals were anaesthetized with an iv injection of pentobarbital sodium (30 mg/kg) and killed by decapitation. The brain, the heart and the submaxillary glands were dissected out and washed in an ice-cold 0.9% NaCl solution to remove blood and cell debris. The striata and the frontal cortex were removed from the whole brain. Tissues were embedded in a cryoprotectant medium, frozen in a dry ice–acetone mixture and stored at −80 °C until used. Ten micrometre-thick sections of the above tissues were cut serially at −20 °C using a microtome cryostat and mounted on pre-weighed gelatine-coated microscope slides.

For labelling muscarinic cholinergic receptors, sections of rat frontal cortex, heart, submaxillary glands and striatum were incubated for 60 min at room temperature with increasing concentrations of the non-selective antagonist [^{3}H]NMS in 50 mM phosphate buffered saline (PBS), pH 7.4 containing 1.3 M NaCl, 0.07 M Na_2HPO_4, 0.03 M NaH_2PO_4. Non-specific binding was defined by adding to the incubation medium of alternate sections a 1 μM concentration of atropine. In a series of preliminary experiments the optimal incubation times and temperatures were assessed. At the end of incubation, slides exposed to the radioligand were washed in ice-cold incubation buffer (2 × 5 min) to remove unbound radioactivity. They were then wiped onto Whatman GF-B glass fiber filters and counted in a Beckman liquid scintillation spectrometer at an efficiency of 40%.

The results of binding experiments on sections of rat frontal cortex, heart, submaxillary glands and striatum were obtained by incubating sections with the radioligand in the presence of different concentrations of newly synthesized compounds. Sections were then washed and processed as indicated above. In these experiments a standard [^{3}H]-NMS concentration of 0.25 nM was used in sections of rat heart and submax-

illary gland, whereas a radioligand concentration of 0.5 nM was used in sections of rat frontal cortex and striatum. These concentrations were used since they allowed the development of the highest specific:non-specific binding ratio (data not shown).

The dissociation constant (K_d) and maximum density of binding sites (B_{max}) were calculated by linear regression analysis of Scatchard plots of saturation isotherms. Competition curves for assessing the pharmacological profile of test cholinergic compounds and of muscarinic thioligands newly synthesized were generated from three to six independent experiments for each compound. Curves were analysed by a computer-assisted non-linear, least-squares fit of the binding data to the Hill equation, which determines the IC_{50} values and slopes.[21] Apparent competitor dissociation constant (K_i) were then derived from IC_{50} values according to Cheng and Prusoff.[18]

Functional techniques. Male guinea pigs (200–300 g) were killed by cervical dislocation, and the organs required were set up rapidly under 1 g of tension in 20 mL organ baths containing physiological salt solution of the following composition (mmol): NaCl (118), $NaHCO_3$ (23.8), KCl (4.7), $MgSO_4 \cdot 7H_2O$ (1.18), KH_2PO_4 (1.18), $CaCl_2$ (2.52), glucose (11.7). The soln was kept at 37 °C (ileum) or 30 °C (heart) and bubbled with 5% CO_2/95% O_2. Left atria were stimulated through platinum electrodes by square-wave pulses (1 m, 1 Hz, 5–10 V). Tissues were equilibrated for 30 min (2 h in the case of heart) and dose–response curves were obtained at 30-min intervals by cumulative addition of carbachol, the first one being discarded and the second one taken as control. A third dose–response curve was constructed with the agonist under study. When the antagonist activity was studied, the compound was incubated for 30 min before the third dose–response curve to carbachol. Contractions were recorded isotonically (ileum) or isometrically (heart) by means of a force transducer connected to a two-channel Gemini polygraph.

Potency was expressed as -log $ED_{50} \pm$ S.E.M. derived from dose–response curves and represents -log of the concentration of agonist required to produce 50% of the maximum contraction.

Determination of dissociation constants. The results, reported in Table 2, are expressed as -log K_b calculated from the equation log (DR-1) = log[ant]-logK_b for a single concentration of antagonist in the 10–100 μM range.

Statistical analysis. The results are expressed as mean ± S.E.M. Student's *t*-test was used to assess the statistical significance of the difference between means.

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