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Synthesis and Antagonistic Activity at Muscarinic Receptor Subtypes of Some 2-Carbonyl Derivatives of Diphenidol

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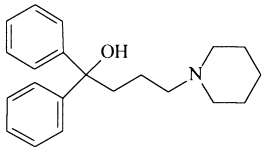
Abstract—A series of 2-carbonyl analogues of the muscarinic antagonist diphenidol bearing 1-substituents of different lipophilic, electronic, and steric properties was synthesized and their affinity for the M_2 and M_3 muscarinic receptor subtypes was evaluated by functional tests. Two derivatives (**2g** and **2d**) showed an M_2-selective profile which was confirmed by functional tests on the M_1 and M_4 receptors. A possible relationship between M_2 selectivity and lipophilicity of the 1-substituent was suggested by structure–activity analysis. This work showed that appropriate structural modification of diphenidol can lead to M_2-selective muscarinic antagonists of possible interest in the field of Alzheimer's disease.

Introduction

The heterogeneity among muscarinic receptor has been widely demonstrated, and five unique gene sequences coding for muscarinic receptors (m_1–m_5) have been cloned;[1] four of them (M_1–M_4) have been pharmacologically defined.[2] The search for potent and selective ligands is still in progress, mostly because of the need for reliable pharmacological standards which would make for a more complete classification and represent potential therapeutic agents. In fact, muscarinic receptor subtypes are variously involved in secretory and cardiovascular functions, smooth muscle control and in central nervous system transmission.

As a part of our studies on diphenidol (1,1-diphenyl-4-piperidin-1-yl-butan-1-ol, **1**), we initially turned our attention to the part of the molecule interacting with the hydrophobic receptor pocket introducing a substituent in either position *para* or *meta* or *ortho* to one phenyl ring of the lead compound.[3] To extend our research, we then modified the intermediate chain connecting the lipophilic head of the molecule to the cationic center rendering it more polar or less flexible.[4] The introduction of a carbonyl group in position 2 of the butyl chain of diphenidol led to compound **2** with enhanced affinity for both the M_2 and the M_3 receptor subtypes and a selectivity ratio M_3/M_2 better than that of diphenidol (**1**, Table 1).



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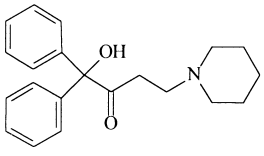

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Considering the important role of the benzilic OH in position 1 of diphenidol (**1**), we designed and synthesized a number of 1-substituted-2-carbonyl derivatives (**2a**–**i**, Table 1) with the aim to evaluate the contribution of 1-substituents to the affinity for the muscarinic receptor subtypes. Substituents were chosen in such a way as to provide orthogonality and as much variability as possible with respect to the classical lipophilic (π), electronic (σ) and steric (MR) parameters.

In this paper, we report the synthesis and the evaluation by functional studies of the affinity and selectivity for muscarinic receptor subtypes of the title compounds, and discuss their structure–activity and structure–selectivity relationships.

Key words: Receptors; cholinergic activity; antagonists; substituent effects.
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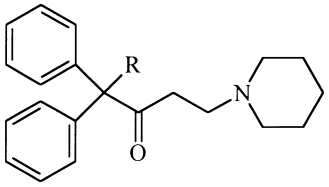
Table 1. Affinity values at the muscarinic M_2 and M_3 receptor subtypes, selectivity ratios, and physicochemical parameters of the studied compounds



No.	R	$pK_b \pm SEM$ M_2[a]	M_3[a]	M_2/M_3[b]	Sel[c]	π	σ*	MR
2[d]	OH	7.48 ± 0.05	8.12 ± 0.03	0.2	−0.64	−0.67	1.37	0.285
2a	H	6.64 ± 0.06	6.54 ± 0.13	1.3	0.10	0.00	0.49	0.103
2b	CH_3	7.13 ± 0.21	6.75 ± 0.06	2.4	0.38	0.56	0.00	0.565
2c	C_2H_5	6.69 ± 0.01	6.10 ± 0.03	3.9	0.59	1.02	−0.10	1.030
2d	C_6H_5	7.06 ± 0.14[e]	5.85 ± 0.14[f]	16	1.21	1.96	0.60	2.536
2e	OCH_3	7.60 ± 0.21	6.94 ± 0.01	4.6	0.66	−0.02	1.77	0.787
2f	OC_2H_5	6.97 ± 0.07	6.21 ± 0.14	5.7	0.76	0.38	1.68	1.247
2g	SC_2H_5	7.19 ± 0.10[e]	5.40 ± 0.02[f]	62	1.79	1.07	1.44	1.842
2h	$SO_2C_2H_5$	5.20 ± 0.10	6.02 ± 0.07[f]	0.2	−0.82	−1.07	3.74	1.810
2i	Cl	7.89 ± 0.02	7.40 ± 0.16	3.1	0.49	0.71	2.94	0.603
Diphenidol (**1**)[d]		[6.72 ± 0.02]	7.02 ± 0.04	[0.5]	[—]	[—]	[—]	[—]

[a] Affinity constants calculated from the equation $\log(DR-1) = \log[ant] - \log K_b$ for a single concentration of the antagonist, according to van Rossum,[23] are reported.
[b] Antilog of the difference between the pK_b values for M_2 and M_3 muscarinic receptor subtypes.
[c] Log (M_2/M_3).
[d] Ref 4.
[e] pA_2 values.
[f] A decrease of the maximum effect of the reference agonist was present at 3×10^{-5} M.

Methods

Series design

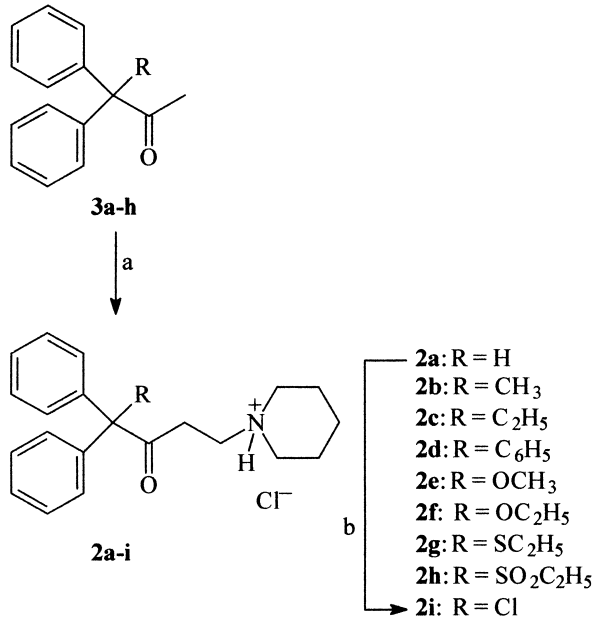
The substituents to be introduced in the benzilic position of **2** (R in Table 1) were selected following, as far as possible, the criteria of minimum redundancy and maximum variance in physicochemical properties. Synthetic restraints limited the choice to a small number of groups. The selection was based on the consideration of the lipophilic, electronic and steric properties which were parameterized by means of the substituent constants[5] π, σ* and MR, respectively. The R groups and the physicochemical descriptors are shown in Table 1. The squared correlation matrix[6] of the parameters is reported in Table 2, and it shows that a reasonable orthogonality among the properties was obtained with the selected series of substituents. This means that the variation of each parameter within the series is independent from the variation of the others as indicated by the low values of the squared correlation coefficients. It is remarkable that almost no collinearity exists between the lipophilic (π) and steric (MR) parameters.

Table 2. Squared correlation matrix for the physicochemical parameters of the substituents of Table 1

	π	σ*	MR
π	—	0.269	0.207
σ*		—	0.030
MR			—

Chemistry

The compounds reported in this study were synthesized by means of the Mannich reaction on the appropriate 1-substituted-1,1-diphenylpropan-2-one (**3a**–**h**) and piperidine hydrochloride in 2-methoxyethanol as high-boiling solvent (**2a**–**h**, Scheme 1). Compound **2i** was prepared



Scheme 1. (a) CH_2O, piperidine hydrochloride, HCl, CH_3OCH_2–CH_2OH, reflux, (b) Cl_2, THF, rt.

by reaction of **2a** with chlorine in THF. Compounds **2a–i** were obtained as hydrochloride salts, because of the better characterizability and stability. Starting ketones were commercially available (**3a**) or synthesized according to literature methods (**3b–f**). Compound **3g** was obtained from 1-chloro-1,1-diphenylpropan-2-one and ethylmercaptan, in presence of anhydrous $CaCO_3$. Oxidation of the ethylthio-derivative **3g** with *m*-chloroperbenzoic acid (MCPBA) in CH_2Cl_2 led to the sulfone **3h** (Scheme 2).

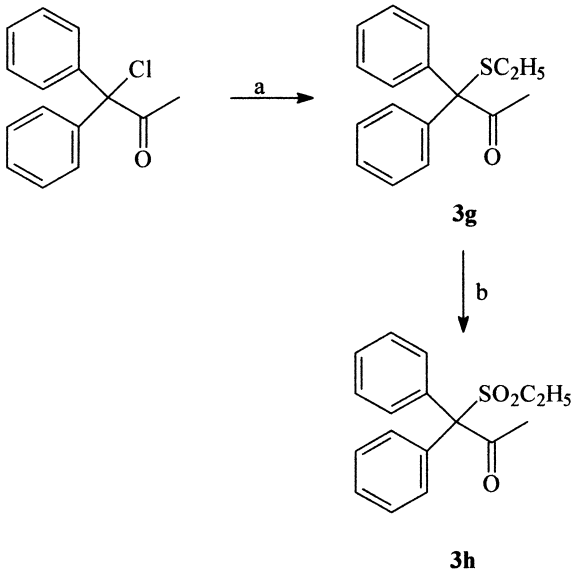
Pharmacology

All the compounds were tested on M_2 (guinea pig heart) and M_3 (guinea pig ileum) muscarinic receptor subtypes for their antimuscarinic activity using arecaidine propargyl ester (APE) as agonist. Compounds **2d** and **2g** were also tested on M_1 (rabbit vas deferens) and M_4 (guinea pig lung) subtypes using *p*-Cl-McN-A-343 and APE as agonists, respectively. The antagonist potency was expressed as dissociation constant (pK_b), and in some cases pA_2 values were also determined.

Results and Discussion

The antagonistic activities at the M_2 and M_3 muscarinic receptors and the subtype selectivity of the new compounds studied are reported in Table 1. The corresponding data of the previously published[4] diphenidol analogue **2** are also shown. Compounds **2a–i** behave as competitive (causing a parallel shift to the right of the agonist dose–response curves) and noncompetitive (a decrease of the maximum effect of the reference agonist was present at 1×10^{-5} M) antagonists at M_2 and M_3 subtypes, respectively (Figs 1 and 2).

Examination of the pK_b values of Table 1 reveals that the introduction in position 1 of substituents different from the hydroxylic group, always decreases the affinity for the M_3 receptor subtype in a range reaching 100-fold differences (5–525, for compounds **2i** and **2g**, respectively). The effects of the substitution are different in the case of the M_2 receptor subtype. In fact, while the Cl and OCH_3 substituents slightly enhance the affinity of the corresponding compounds (**2i** and **2e**, respectively) for this subtype, all the others cause a moderate decrease (up to sevenfold with compound **2a**) in this parameter, the only exception being the $SO_2C_2H_5$ substituent which causes a remarkable fall (190 times) in the affinity of



Scheme 2. (a) C_2H_5SH, $CaCO_3$, 80°C, (b) MCPBA, CH_2Cl_2, rt.

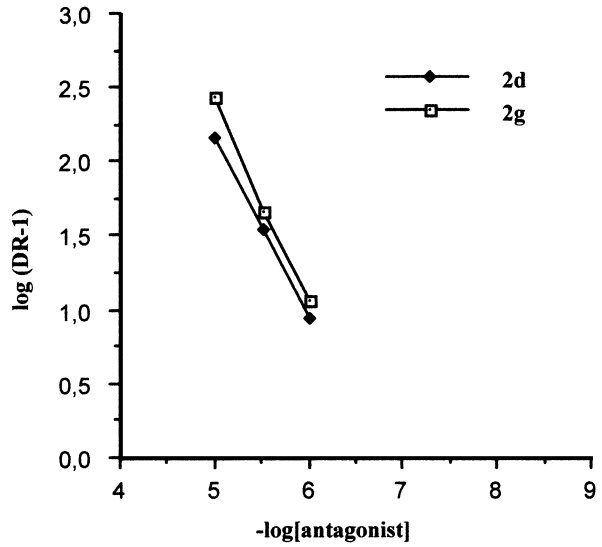


Figure 1. Schild plots obtained for the antagonists **2d** and **2g** on guinea-pig atria force (M_2). The points are the means of five to seven experiments. S.E.M.s are not reported for clarity and are less than 10%. Ordinate: log(DR-1) where $DR = EC_{50}$ ratio measured from the displacement of the agonist concentration-response curves by the antagonist. Abscissa: −log[antagonist]. Schild correlations, based on three points, do not deviate from linearity, suggesting a competitive behavior of the two antagonists.

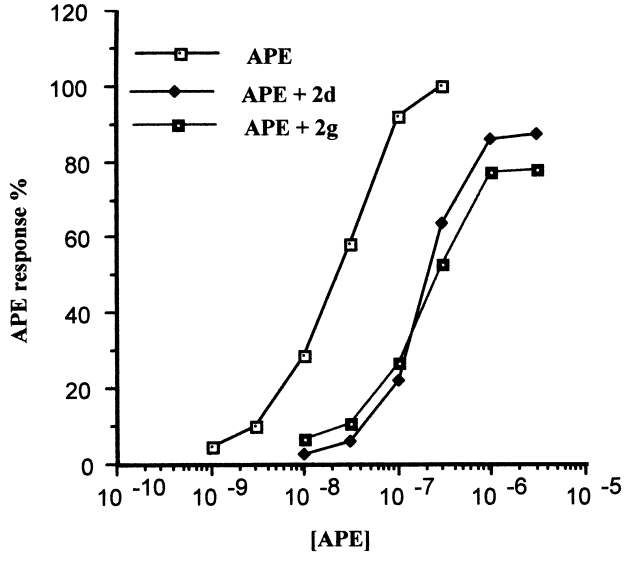


Figure 2. Experimental dose–response curves of APE on guinea-pig ileum (M_3) in the absence (□) and in the presence of 10 μM of **2d** (◆) and **2g** (■). The decrease of the maximum effect of APE suggests a non-competitive behavior of the two antagonists.

compound **2h**. As a consequence, the M_2/M_3 selectivity profile of the antagonists **2a–g** and **2i** results reversed compared with that of the reference compound **2**, which shows higher affinity for the M_3 subtype.

Another interesting point is the difference in the selective profile observed between compounds **2g** and **2h** carrying sulfide and sulfone substituents, respectively. The 310-fold difference in selectivity (Table 1) between these two antagonists suggests that the two oxygens in the substituent of compound **2h** could play a role in binding with the muscarinic receptor subsites. In particular, it seems possible to suppose that the presence of two oxygen atoms improves the binding of compound **2h** at M_3 subtype, while it hinders the binding of this compound at the M_2 one. Moreover, as already pointed out,[7] it seems that oxygen and sulfur can differentiate the behaviour of the muscarinic antagonists: in fact, compounds **2f** and **2g**, which present substituents differing for oxygen (**2f**) and sulfur (**2g**), display a tenfold difference in selectivity (Table 1).

Since the M_2/M_3 selectivity ratios for compounds **2g** and **2d** showed interesting values (62 and 16, respectively), we decided to complete the study of these two antagonists testing them at M_1 (rabbit vas deferens) and M_4 (guinea pig lung) muscarinic receptor subtypes as well. In Table 3, the whole results for these two ligands are reported, from which their M_2-selective profile clearly emerges. Actually, these compounds show M_2 selectivity ratios ranging from 62 to >155 (**2g**) and from 16 to >115 (**2d**), and therefore they can be considered as candidates for further development in an area of great therapeutic interest as that of the treatment of Alzheimer's disease.

It is well known that the cholinergic system is involved in memory and learning.[8] Although acetylcholine is not the only neurotransmitter involved in Alzheimer's disease, an illness presenting degenerative alterations which occur in certain brain regions involved in the cognitive processes, nevertheless the 'cholinergic hypothesis' suggests the restoration of central cholinergic tone in order to alleviate and slow down the symptoms and the progress of this disease.[9] The presence of M_1 and M_2 muscarinic receptors has been investigated in cortex and hippocampus with selective radioligands. M_1 receptors control excitatory processes and are postsynaptically located, whereas M_2 receptors are mostly located presynaptically and modulate the release of acetylcholine.[10] Since M_1 receptors are apparently untouched in these areas of the brain of Alzheimer's disease patients, a possible mean of restoring central cholinergic tone is the substitution of the reduced amount of acetylcholine with exogenous M_1 selective agonists.

On the other hand, since stimulation of M_2 presynaptic autoreceptors will reduce, and blockade will enhance the acetylcholine release, the use of M_2 selective antagonists could improve memory and learning, amplifying the physiological cholinergic transmission. The increase of acetylcholine release will be so possible by blocking the presynaptic M_2 receptors with selective M_2 antagonists possessing the ability to penetrate the blood–brain barrier but leaving unaffected the postsynaptic M_1 receptors.[10]

Attempts to quantitatively correlate the observed affinity data at both the M_2 and M_3 muscarinic receptor subtypes with the substituents' physico-chemical properties were carried out following the traditional Hansch method, but no significant QSAR equation could be obtained. As an alternative approach, a factor analysis[6] was performed on the matrix formed by the biological and physico-chemical data of Table 1. This method allows one to extract the relevant information from a data matrix and to calculate linearly independent factors from the original variables. Each factor corresponds to one of the eigenvalues of the matrix and, generally, only a few of them are needed to account for most of the variance of the data. In the present case, as shown in Table 4, the first three factors are able to explain 94% of the variance, indicating that some intercorrelation exists within the six (biological and physicochemical) variables. Looking at the loadings of each original variable on to the relevant factors, it is possible to see which variables are correlated. Considering Factor1, it appears that the selectivity ratio (Sel) and π are correlated to some extent, although other variables contribute to that factor. Factor2 is mainly composed of $pK_b(M_2)$ correlated with $pK_b(M_3)$ and MR; Factor3 is mostly loaded by σ^*. Factor1 also shows a negative correlation between $pK_b(M_3)$ and π, which might explain the favorable effect of the latter parameter on the M_2 versus M_3 selectivity. As regards $pK_b(M_2)$, it is not easy to say which physicochemical variable (if any) has a quantitative effect on it, even if MR (negative association in Factor2) might play a role.

The important outcome of this analysis is the relationship between selectivity and lipophilicity, which can also

Table 3. Affinity values, pK_b[a] or $(pA_2) \pm$ SEM, and selectivity ratios[b] for the muscarinic antagonists **2d** and **2g** on rabbit vas deferens (M_1), guinea-pig atria force (M_2), ileum (M_3) and lung (M_4)

	pK_b or $(pA_2) \pm$ SEM									
No.	M_1	M_2	M_3	M_4	M_2/M_1	M_2/M_3	M_2/M_4	M_3/M_1	M_3/M_4	M_4/M_1
2d	<5	$(7.06) \pm 0.14$	$5.85^c \pm 0.14$	$5.32^c \pm 0.15$	>115	16	55	>7.1	3.4	>2.1
2g	<5	$(7.19) \pm 0.10$	$5.40^c \pm 0.02$	$5.35^c \pm 0.10$	>155	62	69	>2.5	1.1	>2.1

[a] See note a of Table 1.
[b] See note b of Table 1.
[c] A decrease of the maximum effect of the reference agonist was present at 1×10^{-5} M.

Table 4. Factor analysis

	Factor1	Factor2	Factor3	Factor4	Factor5	Factor6
Eigenvalues:	2.87	1.96	0.82	0.26	0.09	0.00
Variance explained:	0.48	0.81	0.94	0.98	1.00	1.00
Variables			Loadings			
$pK_b(M_2)$	0.15	0.90	0.41	0.00	−0.08	0.00
$pK_b(M_3)$	−0.75	0.59	0.18	0.24	−0.01	0.00
Sel	0.91	0.24	0.20	−0.25	−0.06	0.00
π	0.91	0.30	0.03	0.19	0.22	0.00
σ*	−0.43	−0.53	0.72	−0.10	0.11	0.00
MR	0.67	−0.62	0.25	0.30	−0.14	0.00

be shown by the plot of Figure 3. The correlation between the two variables is not sharp ($r^2 = 0.718$), but the trend is evident: increasing the lipophilicity of the benzilic substituent (R) favors the affinity for the M_2 muscarinic receptor subtype rather than that for the M_3 subtype. From inspection of the plot, it appears that the effect of the SC_2H_5 substituent on selectivity is not only strong, but also much stronger than its lipophilicity would predict. The same seems to hold, even if at a lesser extent, also for the OC_2H_5 and OCH_3 substituents.

The effect of the SC_2H_5 substituent on the M_2 (and M_1) versus M_3 selectivity was reported for a series of 2,2-diphenyl-2-ethylthioacetic acid esters (**4**, R=SC_2H_5) developed as muscarinic antagonists structurally correlated to adiphenine (**4**, R=H).[11] In an attempt to understand the chemical reasons at the basis of such an effect, Romanelli et al.[12] performed an accurate theoretical study comparing the electronic and steric properties of the ethoxy- and ethylthio-groups. Based on some ligand–receptor models, and postulating that these substituents bind to a receptor pocket made by several aromatic residues, the authors reached the conclusion that sulfur derivatives are favored over oxygen analogues, because in the overall balance of the contributions to the binding energy, repulsion forces favor oxygen (due to its smaller size), but dispersion and electrostatic forces favor sulfur (due to its higher polarizability and lower electron density).

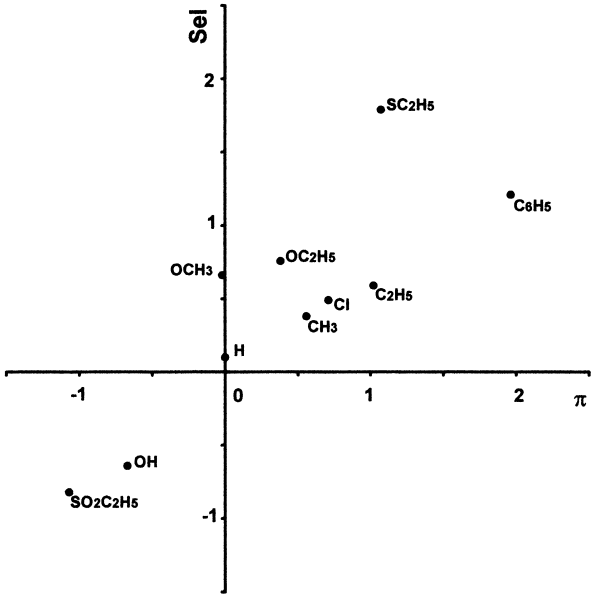
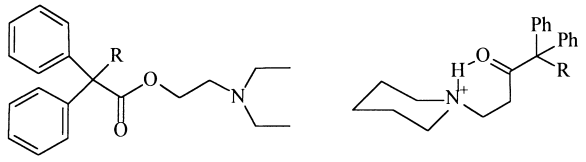


Figure 3. Plot of the M_2/M_3 selectivity (Sel) versus lipophilicity (π).

The similarity of the molecular skeletons on which the substituent groups are introduced (the carbonyl analogue of diphenidol, **2**, and adiphenine, **4**, R=H) leads us to believe that the results reported in the present paper, as regards the effect of the SC_2H_5 group on the M_2/M_3 selectivity, can be ascribed to the same reasons outlined above. In such a context, it is reasonable that lipophilicity exerts a generalized influence on selectivity (perhaps limiting the M_3 affinity), while more specific interactions determine the binding affinity of each single compound to the different receptor subtypes.



4 **5**

Finally, in a previous work,[4] we hypothesized that the bioactive conformation of these carbonyl-derivatives of diphenidol might be one that exposes the pharmacophoric groups to the receptor in a favorable arrangement (**5**). At the light of the present results, namely the relevant effect of the R substituents on both affinity and selectivity towards the M_2 and M_3 receptor, we can confirm that hypothesis and point out the critical role of R in recognizing and binding the different muscarinic receptor subtypes.

Conclusion

A series of analogues of **2** was synthesized with the aim of probing the effects of the benzilic substituent on the affinity and selectivity towards the M_2 and M_3 muscarinic receptor subtypes. In almost all the compounds studied, replacing the OH group of **2** reduced the affinity for both M_2 and M_3 subtypes, but the introduction of some substituent caused different effects at the two receptors. All substituents except $SO_2C_2H_5$ reversed the M_3-selective profile of the parent compound (**2**) up to the point that we obtained some interesting M_2-selective

muscarinic antagonists. The increase in M_2/M_3 selectivity throughout the series roughly parallels the increase in lipophilicity of the substituents, with the remarkable exception of the SC_2H_5 group (compound **2g**), which leads to a much higher M_2-selectivity than expected on the basis of the lipophilicity alone. The most selective compounds **2g** and **2d** were further characterized with respect to the other muscarinic receptor subtypes (M_1 and M_4), and their M_2-selective antimuscarinic profile was confirmed. In conclusion, our study led us to obtain two M_2-selective muscarinic antagonists, which might be of potential interest in the field of the Alzheimer's disease treatment, when the present data will be confirmed with human cloned receptor binding studies. Further work will be devoted to the increase of the antimuscarinic potency and to the improvement of the physicochemical properties (lipophilicity) critical for the pharmacokinetics of the central nervous system drugs.

Experimental

Chemistry

Melting points were taken on Electrothermal open capillary apparatus and are uncorrected. Elemental analysis was performed for compounds **2a**–**i** and the results (not shown) were within ±0.4% of the theoretical values. Infrared spectra (IR) were recorded on a Perkin–Elmer 683 instrument for all compounds and were consistent with the assigned structures; because of the lack of unusual features, they are not included. ^{1}H NMR spectra were registered on a Varian VXR 300 spectrometer, peak positions are given in parts per million (δ) relative to the standard chemical shift of the solvent. Merck silica gel 60 (230–400 mesh) was used for column chromatography. Thin-layer chromatography (Merck silica gel 60 F_{254} analytical plates) was used to monitor reactions. The term 'dried' refers to the use of anhydrous sodium sulfate.

General procedure for the preparation of 1,1-diphenyl-4-piperidin-1-ylbutan-2-one hydrochloride (2a) and of 1-substituted derivatives 2b–h. A solution of 1,1-diphenylpropan-2-one (**3a**) (1.85 g, 8.8 mmol), paraformaldehyde (0.64 g) and piperidine hydrochloride (1.48 g, 12.2 mmol) in 2-methoxyethanol (10 mL) was refluxed with stirring at 140°C for 10 min. A suspension of paraformaldehyde (0.64 g) in 2-methoxyethanol (3 mL) was added during 20 min. Concentrated hydrochloric acid (0.5 mL) was added and refluxing continued for a further 10 min to produce a clear solution. The cooled solution was poured into brine (10 mL) and extracted with $CHCl_3$ (2×30 mL). The combined organic layers were washed with brine and dried. The $CHCl_3$ was evaporated and Et_2O was added to induce separation of **2a** as white solid, which was collected by filtration and recrystallized from abs. EtOH to give 2.06 g (yield 68%); mp 206–208°C (lit.[13] mp 204–205°C). ^{1}H NMR (DMSO-d_6) δ 1.30–1.70 (m, 6H), 2.70–2.85 (m, 2H), 3.10–3.30 (m, 6H), 5.44 (s, 1H), 7.2–7.34 (m, 10H), 9.98 (bs, 1H exch. D_2O).

1,1-Diphenyl-1-methyl-4-piperidin-1-ylbutan-2-one hydrochloride (2b). From 1,1-diphenyl-1-methyl-propan-2-one (**3b**)[14] (1.97 g): 1.20 g (yield 38%), mp 190–191°C from abs. EtOH:Et_2O (lit.[15] mp 188–189°C). ^{1}H NMR (DMSO-d_6) δ 1.33–1.70 (m, 6H), 1.93 (s, 3H), 2.70–2.80 (m, 2H), 3.01–3.28 (m, 6H), 7.11–7.14 (m, 4H), 7.29–7.39 (m, 6H), 10.15 (bs, 1H exch. D_2O).

1,1-Diphenyl-1-ethyl-4-piperidin-1-ylbutan-2-one hydrochloride (2c). From 1,1-diphenyl-1-ethyl-propan-2-one (**3c**)[16] (2.10 g): 1.18 g (yield 36%), mp 143–145°C from abs. EtOH:Et_2O. ^{1}H NMR (DMSO-d_6) δ 0.60 (t, 3H, J = 7.3 Hz), 1.27–1.64 (m, 6H), 2.39 (q, 2H, J = 7.3 Hz), 2.60–2.75 (m, 2H), 2.90–3.23 (m, 6H), 7.20–7.38 (m, 10H), 10.09 (bs, 1H exch. D_2O).

1,1,1-Triphenyl-4-piperidin-1-ylbutan-2-one hydrochloride (2d). From 1,1,1-triphenyl-propan-2-one (**3d**)[17] (2.52 g): 1.59 g (yield 43%), mp 180–181°C from abs. EtOH:Et_2O. ^{1}H NMR (DMSO-d_6) δ 1.26–1.63 (m, 6H), 2.28–2.72 (m, 2H), 2.87–2.96 (m, 2H), 3.00–3.14 (m, 4H), 7.26–7.39 (m, 15H), 10.28 (bs, 1H exch. D_2O).

1,1-Diphenyl-1-methoxy-4-piperidin-1-ylbutan-2-one hydrochloride (2e). From 1,1-diphenyl-1-methoxy-propan-2-one (**3e**)[18] (2.11 g): 0.46 g (yield 14%), mp 164–165°C from abs. EtOH:Et_2O. ^{1}H NMR (DMSO-d_6) δ 1.24–1.68 (m, 6H), 2.65–2.80 (m, 2H), 3.00 (s, 3H), 3.08–3.28 (m, 6H), 7.30–7.40 (m, 10H), 10.41 (bs, 1H exch. D_2O).

1,1-Diphenyl-1-ethoxy-4-piperidin-1-ylbutan-2-one hydrochloride (2f). From 1,1-diphenyl-1-ethoxy-propan-2-one (**3f**)[19] (2.24 g): 0.51 g (yield 15%), mp 159–161°C from abs. EtOH:Et_2O (lit.[20] mp 160°C). ^{1}H NMR (DMSO-d_6) δ 1.16 (t, 3H, J = 7.0 Hz), 1.30–1.63 (m, 6H), 2.62–2.80 (m, 2H), 3.05 (q, 2H, J = 6.9 Hz), 3.12–3.23 (m, 6H), 7.28–7.41 (m, 10H), 10.10 (bs, 1H exch. D_2O).

1,1-Diphenyl-1-ethylthio-4-piperidin-1-ylbutan-2-one hydrochloride (2g). From 1,1-diphenyl-1-ethylthio-propan-2-one (**3g**) (2.38 g): 0.89 g (yield 25%), mp 149–150°C from $CHCl_3$:Et_2O. ^{1}H NMR (DMSO-d_6) δ 1.09 (t, 3H, J = 7.4 Hz), 1.30–1.71 (m, 6H), 2.08 (q, 2H, J = 7.4 Hz), 2.70–2.86 (m, 2H), 2.90–3.04 (m, 2H), 3.19–3.27 (m, 4H), 7.28–7.51 (m, 10H), 10.13 (bs, 1H exch. D_2O).

1,1-Diphenyl-1-ethylsulfonyl-4-piperidin-1-ylbutan-2-one hydrochloride (2h). From 1,1-diphenyl-1-ethylsulfonyl-propan-2-one (**3h**) (2.66 g): 0.38 g (yield 10%), mp 160–162°C from $CHCl_3$:Et_2O. ^{1}H NMR ($CDCl_3$) δ 1.19 (t, 3H, J = 7.5 Hz), 1.40–2.16 (m, 8H), 2.40–2.64 (m, 2H), 2.86 (q, 2H, J = 7.5 Hz), 3.04–3.25 (m, 4H), 7.44–7.58 (m, 10H), 9.45 (bs, 1H exch. D_2O).

1-Chloro-1,1-diphenyl-4-piperidin-1-ylbutan-2-one hydrochloride (2i). Chlorine was passed into a stirred suspension of **2a** (0.50 g) in THF (4 mL). Refluxing began after few min and the solution cleared. After a further 5 min the passage of chlorine was stopped and the solvent removed. The resulting residue was recrystallized from $CHCl_3$:Et_2O to give 0.25 g of **2i** (yield 45%), mp

127–129°C. ^{1}H NMR (DMSO-d_6) δ 1.34–1.73 (m, 6H), 2.70–2.86 (m, 2H), 3.20–3.35 (m, 6H), 7.26–7.49 (m, 10H), 10.45 (bs, 1H exch. D_2O).

1,1-Diphenyl-1-ethylthio-propan-2-one (3g). 1-Chloro-1,1-diphenylpropan-2-one[21] (7.34 g, 30 mmol) and anhydrous $CaCO_3$ (4.5 g, 45 mmol) were added to ethylmercaptan (25 mL) in a steel bomb and kept at 80°C for 4 days. After cooling, the excess of ethylmercaptan was carefully removed and the residue treated with a 10% solution of K_2CO_3 and extracted with Et_2O (2×50 mL). The organic phase was washed, dried and evaporated and afforded an oily residue, which was purified on a silica gel column (toluene), to give 4 g (yield 49%) of compound **3g**, mp 40–42°C from petroleum ether. ^{1}H NMR (DMSO-d_6) δ 1.06 (t, 3H, J = 7.5 Hz), 2.09 (q, 2H, J = 7.5 Hz), 2.15 (s, 3H), 7.25–7.47 (m, 10H).

1,1-Diphenyl-1-ethylsulfonyl-propan-2-one (3h). To a solution of **3g** (2.7 g, 10 mmol) in CH_2Cl_2 (40 mL) MCPBA (50%) (6.90 g, 20 mmol) in CH_2Cl_2 (30 mL) was added dropwise, at room temperature, and left under stirring for 2 h at room temperature. The reaction mixture was extracted with a 10% solution of Na_2CO_3 (2×20 mL) and dried. The solvent was evaporated and the residue was purified on a silica gel column (toluene: acetone, 98:2), to give 2.0 g (yield 66%) of **3h**, mp 142–144°C from MeOH. ^{1}H NMR ($CDCl_3$) δ 1.21 (t, 3H, J = 7.5 Hz), 2.09 (s, 3H), 2.89 (q, 2H, J = 7.5 Hz), 7.44–7.47 (m, 6H), 7.61–7.65 (m, 4H).

Pharmacology

General considerations. Male guinea pigs (200–300 g) and male New Zealand white rabbits (3.0–3.5 kg) were killed by cervical dislocation. The organs required were set up rapidly under 1 g of tension in 20 mL organ baths containing physiological salt solution (PSS) maintained at an appropriate temperature (see below) and aerated with 5% CO_2–95% O_2. Dose–response curves were constructed by cumulative addition of the reference agonist. The concentration of agonist in the organ bath was increased approximately threefold at each step, with each addition being made only after the response to the previous addition had attained a maximal level and remained steady. Following 30 min of washing, tissues were incubated with the antagonist for 30 min, and a new dose–response curve to the agonist was obtained. Contractions were recorded by means of a force transducer connected to a two-channel Gemini polygraph (U. Basile). In all cases, parallel experiments in which tissues received only the reference agonist were run in order to check any variation in sensitivity.

Guinea pig ileum. Two-centimeter-long portions of terminal ileum were taken at about 5 cm from the ileum–cecum junction and mounted in PSS, at 37°C, of the following composition (mM): NaCl (118), $NaHCO_3$ (23.8), KCl (4.7), $MgSO_4·7H_2O$ (1.18), KH_2PO_4 (1.18), $CaCl_2$ (2.52), and glucose (11.7). Tension changes were recorded isotonically. Tissues were equilibrated for 30 min, and dose–response curves to arecaidine propargyl ester (APE) were obtained at 30 min intervals, the first one being discarded and the second one being taken as the control.

Guinea pig stimulated left atria. The heart was rapidly removed, and the right and left atria were separately excised. Left atria were mounted in PSS (the same used for ileum) at 30°C and stimulated through platinum electrodes by square-wave pulses (1 ms, 1 Hz, 5–10 V) (Tetra Stimulus, N. Zagnoni). Inotropic activity was recorded isometrically. Tissues were equilibrated for 2 h and a cumulative dose–response curves to APE was constructed.

Guinea pig lung strips. The lungs were rapidly removed and strips of peripheral lung tissue were cut either from the body of a lower lobe with the longitudinal axis of the strip parallel to the bronchus or from the peripheral margin of the lobe. The preparations were mounted, with a preload of 0.3 g, in PSS with the following composition (mM): NaCl (118.78), KCl (4.32), $CaCl_2·2H_2O$ (2.52), $MgSO_4·7H_2O$ (1.18), KH_2PO_4 (1.28), $NaHCO_3$ (25), glucose (5.55). Contractions were recorded isotonically at 37°C after tissues were equilibrated for 1 h, then two cumulative dose–response curves to APE (0.01, 0.1, 1, 10, 100 μM) were obtained at 45 min intervals, the first one being discarded and the second one being taken as the control.

Rabbit stimulated vas deferens. This preparation was set up according to Eltze.[22] Vasa deferentia were carefully dissected free of surrounding tissue and were divided into four segments, two prostatic portions of 1 cm and two epididymal portions of approximately 1.5 cm length. The four segments were mounted in PSS with the following composition (mM): NaCl (118.4), KCl (4.7), $CaCl_2$ (2.52), $MgCl_2$ (0.6), KH_2PO_4 (1.18), $NaHCO_3$ (25), glucose (11.1); 10^{-6} M yohimbine was included to block α_2-adrenoceptors. The solution was maintained at 30°C and tissues were stimulated through platinum electrodes by square-wave pulses (0.1 ms, 2 Hz, 10–15 V). Contractions were measured isometrically after tissues were equilibrated for 1 h, then a cumulative dose–response curve to *p*-Cl-McN-A-343 was constructed.

Determination of antagonist potency. To quantify antagonist potency, pK_b values were calculated from the equation $pK_b = \log(DR\text{-}1) - \log[B]$, where DR is the ratio of ED_{50} values of agonist after and before treatment with one or two antagonist concentration [B].[23] In some cases, antagonist potency is expressed in terms of pA_2, estimated by Schild plots constrained to slope −1.0, as required by the theory.[24,25]

Statistical analysis. Values are given as mean ± standard error of four or five independent observations. Student's *t*-test was used to assess the statistical significance of the difference between two means.

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