The synthesis, conformation and antimuscarinic properties of ketone analogues of tropane esters

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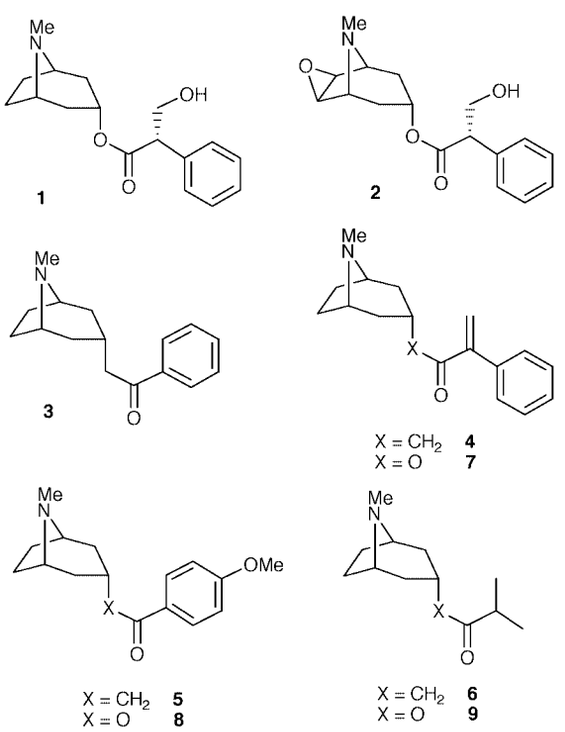
A synthesis of the ketone analogues of three naturally occurring tropane esters has been developed and one of the ketones has been evaluated for its efficacy as a muscarinic acetylcholine receptor (mAChR) antagonist in guinea-pig ileum. The ketone analogue of apoatropine displayed an IC_{50} value in the micromolar range when compared to atropine, which has antagonist activity in the nanomolar range. The conformational change in replacing the bridging oxygen of the ester moiety by CH_2 has been assessed by X-ray structure analysis of one of the compounds and has been evaluated more widely in a Cambridge Crystallographic Database survey. The structural data reveals that both the tropane esters and tropane ketones adopt similar conformations.

Introduction

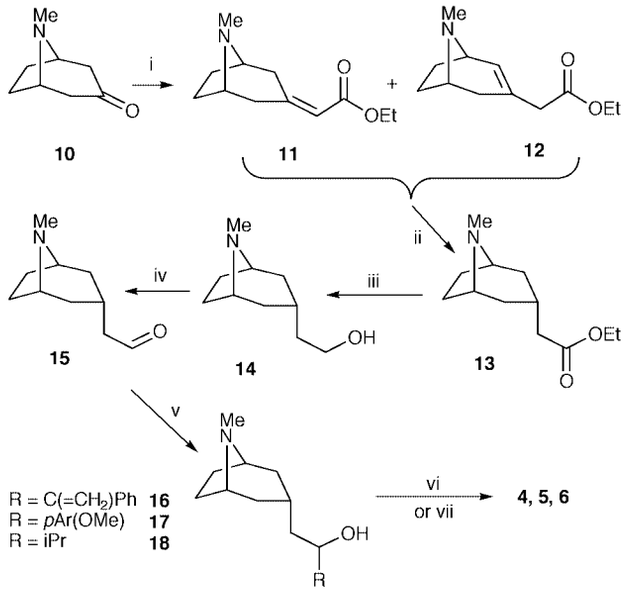
Carboxylic acid esters of tropine (8-methyl-8-azabicyclo[3.2.1]-octan-3-α-ol) form a major plant alkaloid class[1] and have been identified in plants largely of the *Solanaceae* family, and notably in *Datura, Atropa* and *Hyoscyamus*.[2] Perhaps the two most significant tropane alkaloids are hyoscyamine **1** and scopolamine **2** as they are natural products which have found roles in medicinal chemistry. Solutions of hyoscyamine **1** (and its racemate, atropine) have long been recognised for their ability to dilate the pupils of eyes and have been used both cosmetically and in opthalmics for that reason.[3] Scopolamine **2** is used widely as a pre-medication for arresting salival secretions prior to general anaesthetic.[4]



The biological activity of the tropane alkaloids lies in their antagonistic properties at various receptors and many analogues have been generated in synthesis programmes in medicinal chemistry research to explore and refine these properties.[2] In particular tropanes are found to display antagonist behaviour towards 5-hydroxytryptamine (5-HT)[5,6] receptors, and muscarinic[7–9] and nicotinic[7–9] acetylcholine receptors. In this paper we discuss a synthetic route to ketone analogues of the tropane esters, where the bridging ester oxygen is replaced by CH_2. Perhaps surprisingly such ketone analogues have hardly been explored and we are aware only of the benzyl ketone **3**,[10] which emerged to have similar antagonist potency to benzoyl tropane against the 5-HT receptors in bradycardia. Thus the replacement of the CH_2 for O in that case did not have a significantly deleterious effect. In this paper we report the synthesis of tropanes **4**–**6** which are the corresponding ketone analogues of the naturally occurring tropane esters **7**–**9**,[11–13] and we evaluate the ability of ketone **4**, the analogue of apoatropine, as an antagonist of the muscarinic acetylcholine receptors of guinea-pig ileum. A Cambridge Crystallographic Database survey has revealed that the conformation of tropane esters is matched closely by the ketone analogues.

Results and discussion

In the first instance we envisaged that selective Grignard reactions of ester **13** would provide the desired ketones in a straightforward manner and therefore we prepared ester **13** following a previously reported synthesis.[14] This involved a Horner–Wadsworth–Emmons reaction between tropinone **10** and triethyl phosphonoacetate to generate the α,β-unsaturated ester **11** (Scheme 1). The product was contaminated (11%) with ester **12** which contained an endocyclic double bond. It proved unnecessary to separate this mixture as direct hydrogenation converted both to the saturated tropane ester **13** in a 5:1 ratio of *endo*:*exo* isomers. Grignard reactions of this ester with isopropylmagnesium chloride and *p*-methoxyphenylmagnesium bromide were investigated at some length both by us and previously by Zirkle *et al.*[15] These reactions proved sluggish and did not proceed satisfactorily, giving mixtures of the required ketone with starting material and other addition products. These mixtures proved very difficult to purify by chromato-



Scheme 1 i. $(EtO)_2PCH_2CO_2Et$, NaH, reflux 35 h, 77%; ii. Pd–C, H_2, 4–5 atm, 87%; iii. $LiAlH_4$, ether, reflux, 24 h, 86%; iv. DMSO, oxalyl chloride, Et_3N, −78 °C, 93%; v. RMgBr, THF–ether, 64–79%; vi. MnO_2, CH_2Cl_2, 51–55%; vii. PCC, CH_2Cl_2, 44%.

graphy and pursuing this reaction as a means of direct access to the desired ketones was judged both by us and the previous investigators[15] to be unsatisfactory.

In order to circumvent this difficulty, aldehyde **15** was identified as an alternative intermediate as it was anticipated that it would be amenable to Grignard reactions to generate secondary alcohols **16**–**18**. Subsequent oxidation of these alcohols would then afford the desired ketones **4**–**6**. Direct DIBAL-H reduction[16] of ester **13** to afford aldehyde **15** was only partially successful. The conversion was high (71%), but purification was troublesome as the product decomposed both on alumina and silica gel. Thus ester **13** was reduced under more forcing conditions with $LiAlH_4$ to generate alcohol **14**, with complete conversion. Oxidation of **14** to aldehyde **15** was initially unsuccessful using PDC[17] or PCC,[18] however oxidation using Swern's conditions[19] proved straightforward and the required aldehyde **15** was isolated in excellent yield, and with an *endo*:*exo* ratio of 5:1 reflecting that of ester **13**. Aldehyde **15** offered an excellent intermediate by which to approach the target ketone analogues. Accordingly aldehyde **15** was reacted with the different Grignard reagents and generated the resultant alcohols **16**–**18** in moderate to good yields. The *endo*:*exo* ratios of these product alcohols again reflected that of the starting materials. Finally, oxidation of the alcohols to the target ketones was accomplished with manganese dioxide[20] for alcohols **16** and **17**. Alcohol **18** on the other hand was oxidised with PCC.

It was important to unambiguously determine the configuration of the major stereoisomer in these products. When the Grignard reactions were run for short reaction times at low temperature (−78 °C) the predominant isomer was also the faster reacting isomer. Thus a reaction of aldehyde **15** with *p*-methoxyphenylmagnesium bromide at low temperature generated a sample of alcohol **17** (13% yield), as a single stereoisomer as determined by ^{1}H- and ^{13}C-NMR. This material was then oxidised with MnO_2 to generate a sample of ketone **5** (also as a single stereoisomer) which was then converted to its hydrochloride salt. Slow crystallisation from diethyl ether–ethanol yielded single crystals of **5**·HCl suitable for an X-ray diffraction study, which confirmed this (major) isomer to be *endo*. The structure comprises ionic pairs (Fig. 1), held together by strong N–H · · · Cl hydrogen bonds.

For tropane ketones to be convincing analogues of tropane

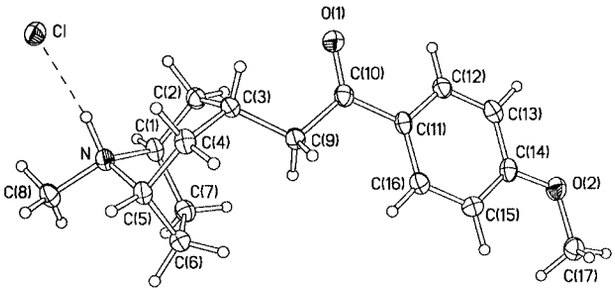


Fig. 1 X-Ray structure of **5**·HCl (50% displacement ellipsoids). Distances: N · · · Cl 3.048(2), H(N) · · · Cl 2.05(3) Å.

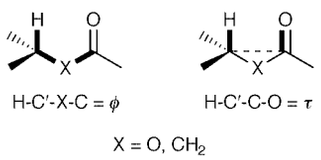


Fig. 2 Description of torsion angles φ and τ used in the X-ray structural survey.

esters for biological studies, clearly it is desirable that the carbonyl (C=O) and the C–H bond at C-3 of the tropane ring, orientate similarly to each other in the esters and the ketones. To compare the orientation of the C(10)=O(1) carbonyl group relative to the C(3)–H bond in **5** with that in tropane esters and other related compounds in the solid state, we have performed a survey of the current (April 1999) release of the Cambridge Crystallographic Database (CSD).[21] This orientation can be described by torsion angles H–C′–O–C (φ) or H–C′ · · · C=O (τ) as shown in Fig. 2.

If the C=O bond adopts a *syn* (rather than *anti*, which is very rare) orientation relative to the C–H bond, then (due to near-planarity of the ester or keto-group) the two angles φ and τ coincide within a few degrees and show the same statistical trends. The following analysis considers only (the absolute value of) τ. It has previously been noted[22] that esters of secondary alcohols usually have synperiplanar C′–H and C=O bonds ($\tau = 0$). A CSD-based study by Schweizer and Dunitz[23] covered 77 such compounds and showed that φ always lies in the range 0–60° and mostly in the range 0–30°. Our present survey showed the same tendency on a much wider base of over 4300 structures, nearly all of which have τ between 0 and 56°, with the majority (88%) in the 0–40° range. However, if we restrict the scope to structures where C′ is bonded to two methylene groups (fragment i, Fig. 3), as found in the tropanes, then a very convincing picture emerges. In all 221 such cases, the C′–H and C=O bonds also adopt *syn* orientations, τ varying from 1° to 56°. However, 82% of observations are in the range 18–42°. The distribution maximum, mean and median values nearly coincide at 30–32°, indicating a substantially skewed conformation (see Fig. 3). So it seems that the ideal synperiplanar conformation, which is widely promulgated, is not intrinsically favourable (*e.g.* due to attractive H · · · O interactions) in this situation, but is rather forced upon the ester group by substituents next to [in α-positions to] C′. 12 structures of tropane ester derivatives (containing fragment ii) were found; they have τ from 20 to 48° (mean 36°), as shown by the crosses in Fig. 3.

Compounds containing fragment iii (where CH_2 replaces O) are few; only 13 structures with 15 independent fragments were found as shown by the circles in Fig. 3. Of these, 14 adopt *syn* (and one *anti*) conformation, τ ranging from 34 to 49° (mean 41°), *i.e.* from the centre to the upper limit of the τ distribution for esters. Thus the conformation of **5** is common for both i and iii fragments. The torsion angle φ H(3)C(3)C(9)C(10) = −39(2)° and torsion angle τ H(3)C(3) · · · C(10)O(1) = −34(2)° for the refined position of H(3) [or −41° and −35° respectively for the

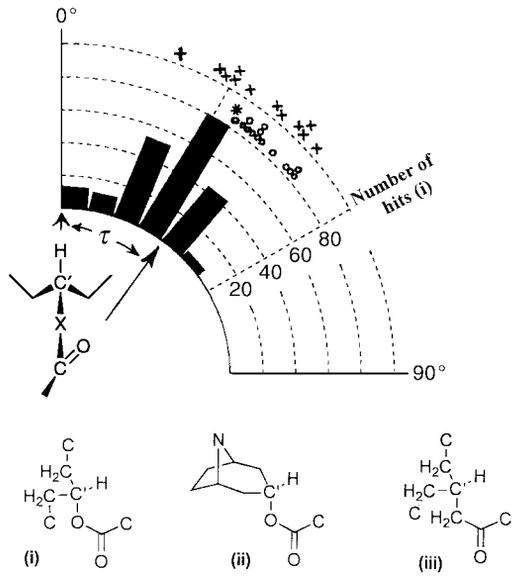


Fig. 3 Torsion angles H–C′···C=O (τ) in esters of secondary alcohols (fragment i, histogramm), tropane esters (fragment ii, crosses), ketones (fragment iii, circles) and in **5**·HCl (star).

idealised position of this atom in compound **5** is indicated by the asterisk in Fig. 3.

It is clear from this analysis of solid state structures that the replacement of O by CH_2 in going from tropane esters to ketones does not introduce a dramatic conformational change.

Biological evaluation of ketone analogue 4

To assess the potential biological effects of the structural changes imposed by the CH_2 replacement, the muscarinic acetylcholine receptor (mAChR) antagonist activity of the ketone analogue **4** was determined using an *in vitro* guinea-pig ileum preparation.[24] The antagonist activity was compared to the tropane alkaloid, atropine (±)-**1** as a reference standard. The isolated guinea-pig ileum contains heterogeneous populations of muscarinic receptors, and is a well established assay for the investigation of mAChR acting drugs.[25] Addition of ACh (3 nM–10 μM) evoked concentration-dependent contraction of the guinea-pig ileum, as depicted in Fig. 4. The ACh EC_{50} was determined to be 0.1 ± 0.09 μM. After the concentration response curve was completed, addition of 0.1 μM ACh yielded reproducible responses. Atropine (0.1 nM–0.1 μM) inhibited the control ACh (0.1 μM) response in a concentration dependent manner, as illustrated in Fig. 5(a). The IC_{50} value was determined to be 3 ± 0.08 nM, which was very close to those of the IC_{50} values (2 nM) reported by other research groups.[26,27] Ketone **4** similarly inhibited the control ACh (0.1 μM) response in a concentration dependent manner, as shown in Fig. 5(b), where the IC_{50} value was determined to be 1.5 ± 0.2 μM. The inhibitory effects of both atropine and ketone **4** were reversible upon washing, but the reversibility of ketone **4** was much quicker than that observed for atropine.

In conclusion, a synthetic route to the ketone analogues **4**–**6** of certain naturally occurring tropane esters has been developed and the route is clearly amenable to the preparation of other such tropane derivatives. The X-ray data suggest that the CH_2 for O replacement does not dramatically change the conformation of the ketone relative to the ester. This is reinforced to some extent after examination of the biological activity of **4**. Ketone **4** is a mAChR antagonist displaying 500-fold less potency than atropine. It should be noted however that this level of activity is more potent (33-fold in guinea-pig ileum)[26,27] than pirenzepine, a mAChR antagonist, which has been used to reduce gastric acid and pepsin secretion. We have not compared **4** in gastric muscarinic receptors and the specificity there may improve. Ketone **4** was selected for biological studies as it was structurally most similar to atropine, and was a direct analogue of apoatropine, the dehydrated product of atropine. The antagonist activity of these tropane ketones remains to be evaluated more fully in this system and with other receptors, before their clinical potential can be fully assessed.

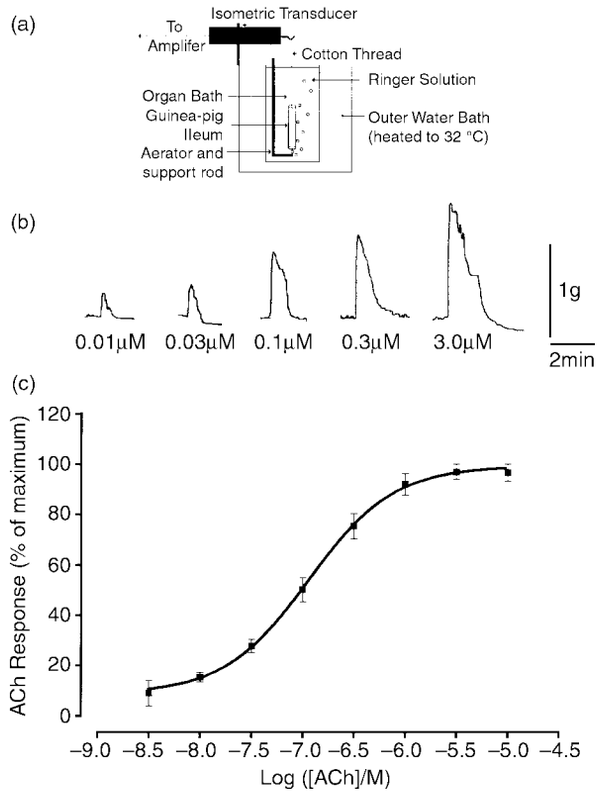


Fig. 4 Acetylcholine (ACh) evokes concentration-dependent contractions of the guinea-pig ileum *in vitro*. Insert (a) shows a simple scheme of the experimental setup. For further details see the Experimental section. Insert (b) shows the actual chart recorder traces of ACh-evoked responses from one of the six experiments. The concentrations applied to the preparation are given beneath the responses. Insert (c) presents the concentration-response data plotted from an average of 6 experiments. The ACh response is given on the *y*-axis, as a percentage of the maximal response, against the final bath concentration of the drug, given on the *x*-axis. The EC_{50} for ACh from these experiments was found to be 0.1 ± 0.09 μM.

Experimental

General methods

IR spectra were recorded on a Perkin-Elmer 257 spectrometer and mass spectra on a VG-7070E instrument. NMR spectra were obtained on Varian 200, 400 and 500 instruments in $CDCl_3$. Chemical shifts are quoted relative to TMS for ^{1}H- and ^{13}C-NMR spectra. Solvents were dried and distilled prior to use. Reactions requiring anhydrous conditions were conducted under an atmosphere of nitrogen and column chromatography was carried out over silica gel (Merck, Kieselgel 60, 230–400 mesh). All reagents, commercially available materials, were used without purification. Tetrahydrofuran and diethyl ether were dried over Na metal, dichloromethane was dried over calcium hydride.

Ethyl (tropan-3-ylidene)acetate 11 †

Neat triethyl phosphonoacetate (27.1 g, 0.121 mol) was added

† The IUPAC systematic name for tropane is 8-methyl-8-azabicyclo-[3.2.1]octane.

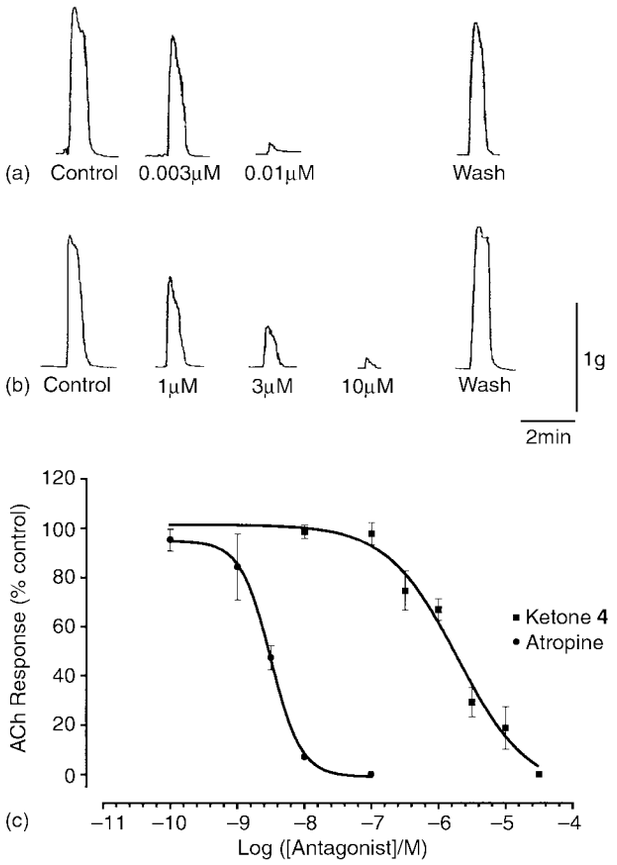


Fig. 5 Ketone **4** inhibits the ACh-evoked response in a concentration dependent manner in the isolated guinea-pig ileum. Inserts (a) and (b) show actual chart traces of (0.1 μM) ACh-evoked responses in the absence (control) and then presence of atropine (a) or ketone **4** (b). Also shown is the response following wash in each case. Insert (c) shows the plotted concentration–inhibition data. The ACh (0.1 μM)-evoked response is given on the *y*-axis, as percent of control, plotted against the final bath concentration of atropine (●) and ketone **4** (■), given on the *x*-axis. The IC_{50} values for atropine and ketone **4** were 0.003 ± 0.0008 μM ($n = 3$) and 1.5 ± 0.2 μM ($n = 4$), respectively.

dropwise to a slurry of NaH (3.0 g, 0.125 mol) in THF (150 ml) at −30 °C. The mixture was stirred for 1 h and then a solution of tropinone (15.0 g, 0.108 mol) in THF (100 ml) was added. The entire mixture was allowed to warm to room temperature for 2 h and was then heated under reflux for 35 h. The reaction mixture was quenched with water (50 ml) and concentrated to give a residue. The residue was partitioned between water (300 ml) and Et_2O (100 ml) and the organics were extracted into Et_2O (4 × 100 ml). The combined organic layers were treated with 2 M HCl (150 ml) and then washed with Et_2O (2 × 300 ml). After basification with 2.5 M NaOH (130 ml), the title compound was extracted into Et_2O (4 × 100 ml), dried over $MgSO_4$ and concentrated to give the title compound (17.4 g, 77%) as a pale-yellow oil. The product was contaminated with 11% of *endo*-cyclic ester **12**. This material was used directly for the next step without purification. δ_H (400 MHz) 1.23 (3H, t, $J = 7.2$ Hz, CH_3CH_2O), 1.46 (2H, broad dd, $J = 8.8$ and 2.4 Hz, *endo*-C6,7), 1.88–1.98 (3H, m, *exo*-C6,7 and *ax*-C4), 2.33 (3H, s, NCH_3), 2.30–2.40 (1H, broad d, *eq*-C2), 2.65 (1H, broad d, $J = 14.0$ Hz, *eq*-C4), 3.21 (2H, broad s, C1,5), 3.46 (1H, broad d, $J = 14.8$ Hz, *ax*-C2), 4.10 (2H, q, $J = 7.2$ Hz, CH_3CH_2O), 5.65 (1H, s, C=CH). δ_C (100 MHz) 14.2 (CH_3CH_2O), 26.7 and 26.9 (C6,7), 38.8 (NCH_3), 35.0 and 41.6 (C2,4), 59.5 (CH_3CH_2O), 61.0 and 61.4 (C1,5), 117.7 (C=CH), 157.9 (C3), 166.6 (CO). ν/cm^{-1} (thin film): 2940, 2874, 2846, 2795 (N–Me), 1714 (C=O), 1643 (C=C), 1199, 1153, 1041. *m/z* (CI) 210 ($M + H^+$).

Ethyl (tropan-3-yl)acetate 13

A solution of ethyl (tropan-3-ylidene)acetate **11** (16.4 g, 78.4 mmol) in MeOH (400 ml) and 10% palladium (834 mg, 7.8 mmol) on activated carbon was shaken for 51 h under 4–5 atm pressure of hydrogen at room temperature. The catalyst was removed by filtration through Hyflo Super Cell and the filtrate concentrated to give a crude product. Purification by distillation (66 °C; 0.1–0.01 mmHg) gave the title compound (15.3 g, 87%) (5:1 *endo–exo*). *endo*-Isomer: δ_H (300 MHz) 1.12 (3H, t, $J = 6.9$ Hz, CH_3CH_2O), 1.17 (2H, broad d, $J = 14.7$ Hz, *eq*-C2,4), 1.50 (2H, broad d, $J = 8.4$ Hz, *endo*-C6,7), 1.88–1.96 (2H, m, *exo*-C6,7), 1.97–2.09 (2H, m, *ax*-C2,4), 2.10–2.20 (1H, m, C3), 2.12 (3H, s, N-CH_3), 2.32 (2H, d, $J = 8.1$ Hz, CH_2CO), 2.96 (2H, broad s, C1,5), 3.99 (2H, q, $J = 6.9$ Hz, CH_3CH_2O). *endo*-Isomer: δ_C (75 MHz) 14.4 (CH_3), 24.7 (C3), 26.5 (C6,7), 35.3 (C2,4), 40.2 (N-CH_3), 43.2 (C9 and O-CH_2), 60.4 (C1,5), 173.2 (C=O). *exo*-Isomer: δ_H (300 MHz) 1.11 (3H, t, $J = 6.9$ Hz, CH_3CH_2O), 1.08–1.56 (4H), 1.84–2.20 (5H), 2.12 (3H, s, N-CH_3), 2.34 (2H, d, $J = 8.1$ Hz, CH_2CO), 2.92–3.02 (2H, broad s, C1,5), 3.97 (2H, q, $J = 6.9$ Hz, CH_3CH_2O). *exo*-Isomer: δ_C (75 MHz) 14.4 (CH_3CH_2O), 24.7 (C3), 25.6 and 26.2 (C6,7), 37.9 and 41.5 (C2,4), 40.4 (N-CH_3), 42.9 (CH_2CO and CH_3CH_2O), 60.3 and 61.3 (C1,5), 172.8 (CO). ν/cm^{-1} (thin film): 2932, 2796 (N–Me), 1735 (C=O), 1478, 1450, 1368, 1342, 1307, 1169, 1149, 1063, 1033. *m/z* (EI^+) 211 (M^+, 28%), 124 (55%), 96 (79%), 82 (100%), 42 (65%).

2-(Tropan-3-yl)ethanol 14

A solution of ethyl (tropan-3-yl)acetate **13** (12.4 g, 58.9 mmol) in Et_2O (50 ml) was added dropwise to a suspension of $LiAlH_4$ (4.5 g, 0.118 mol) in Et_2O (250 ml) at −60 °C. The reaction mixture was allowed to warm to room temperature and was then heated under reflux for 24 h. The reaction mixture was sequentially quenched with water (4.8 ml), 15% NaOH (4.8 ml) solution and water (15 ml) to leave a granular precipitate. The precipitate was filtered through a sintered funnel and the clear solution was washed with Et_2O (2 × 50 ml). The combined filtrate was dried over $MgSO_4$ and concentrated to give the title compound (8.5 g, 86%) as a clear oil and as a 5:1 *endo–exo* mixture. δ_H (300 MHz) 1.29 (2H, broad d, $J = 14.1$ Hz, *eq*-C2,4), 1.34–1.86 (7H), 1.61 (2H, broad d, $J = 8.1$ Hz, *endo*-C6,7), 1.72 (2H, t, $J = 6.6$ Hz, CH_2CH_2OH), 1.66–1.86 (1H, m, C3), 1.90–2.04 (2H, m, *exo*-C6,7), 2.04–2.16 (2H, m, *ax*-C2,4), 2.21 (3H, s, N-CH_3), 2.78 (1H, broad s, OH), 3.06 (2H, broad s, C1,5), 3.59 (2H, t, $J = 6.6$ Hz, CH_2OH). *endo*-Isomer: δ_C 24.1 (C3), 26.6 (C6,7), 35.9 (C2,4), 40.4 (N-CH_3), 41.7 (CH_2CH_2-OH), 60.7 (C1,5), 61.7 (CH_2OH). *exo*-Isomer: δ_C 24.5 (C3), 26.3 (C6,7), 38.5 (C2,4), 40.0 (N-CH_3), 40.7 (CH_2CH_2OH), 60.5 (CH_2OH), 61.5 (C1,5). ν/cm^{-1} (thin film): 3201, 3161 (OH), 2967, 2927, 2886, 2851, 2797 (N-CH_3), 1473, 1342, 1026, 1011. Accurate mass (EI^+): Found 169.1470; Calculated for (M^+) $C_{10}H_{19}NO$ 169.1467 (−1.8 ppm). *m/z* (EI) 169 (M^+, 21%), 96 (83%), 82 (100%).

(Tropan-3-yl)acetaldehyde 15

A solution of dimethyl sulfoxide (3.2 g, 41.3 mmol) in CH_2Cl_2 was added dropwise to a solution of oxalyl chloride (2.7 g, 21.2 mmol) in CH_2Cl_2 at −78 °C. After stirring for 15 min, a solution of 2-(tropan-3-yl)ethanol **14** (2.0 g, 11.8 mmol) in CH_2Cl_2 was added. The mixture was stirred for 2 h at −78 °C and was then quenched with triethylamine (6.6 g, 64.9 mmol). After stirring for 15 min at room temperature, 10% NaOH solution was added to make the reaction mixture strongly basic. The organics were then extracted into CH_2Cl_2 (4 × 80 ml) and were combined, dried over $MgSO_4$ and concentrated to give the title compound (1.84 g, 93%) as a light-brown liquid and as a 5:1 *endo–exo* mixture. *endo*-Isomer: δ_H (300 MHz) 1.17 (2H, broad

d, J = 14.4 Hz, *eq*-C2,4), 1.52 (2H, broad d, J = 8.4 Hz, *endo*-C6,7), 1.90–2.02 (2H, m, *exo*-C6,7), 2.08–2.16 (2H, m, *ax*-C2,4), 2.18 (3H, s, N-CH_3), 2.24–2.38 (1H, m, C3), 2.48 (2H, dd, J = 7.8 and 2.0 Hz, CH_2CHO), 3.06 (2H, broad s, C1,5), 9.54 (1H, t, J = 1.8 Hz, CHO). *endo*-Isomer: δ_C (75 MHz) 20.5 (C3), 25.2 (C6,7), 34.2 (C2,4), 38.9 (N–CH_3), 51.4 (CH_2CHO), 59.4 (C1,5), 201.6 (CHO). *exo*-Isomer: δ_H (300 MHz) 1.08–1.62 (4H), 1.90–2.38 (5H), 2.22 (3H, s, N–CH_3), 2.45–2.52 (2H, CH_2CHO), 3.14 (2H, broad s, C1,5), 9.60 (1H, t, J = 1.8 Hz, CHO). *exo*-Isomer: δ_C (75 MHz) 21.7 (C3), 24.8 (C6,7), 36.4 (C2,4), 39.1 (N–CH_3), 49.3 (CH_2CHO), 60.6 (C1,5), 200.4 (CHO). ν/cm^{-1} (thin film): 2930, 2795 (N–CH_3), 1723 (C=O), 1450, 1339, 1231, 1129. *m*/*z* (EI) 167 (M^+, 9%), 124 (58%), 96 (49%), 83 (67%), 82 (100%), 42 (59%). Accurate mass (EI^+): Found 167.1311; Calculated for (M^+) $C_{10}H_{17}NO$ 167.1310.

4-(Tropan-3-yl)-2-phenylbut-2-en-3-ol 16

A solution of α-bromostyrene (657 mg, 3.59 mmol) in Et_2O (10 ml) was added dropwise to a suspension of magnesium turnings (96 mg, 3.95 mmol) and iodine crystals in Et_2O (5 ml) at room temperature. After addition was complete, the mixture was heated under reflux for 15 min and was then cooled to ambient temperature. This Grignard reagent was added to a solution of (tropan-3-yl)acetaldehyde **15** (400 mg, 2.39 mmol) in THF (30 ml) at −78 °C. The reaction mixture was diluted with Et_2O (45 ml) and was then stirred for 4.5 h between −78 °C and −10 °C. The reaction mixture was concentrated and the residue diluted with saturated NH_4Cl (40 ml) and organics were extracted into AcOEt (3 × 40 ml). After the aqueous layer was made basic with NaOH pellets the organics were extracted into CH_2Cl_2 (4 × 50 ml). The combined extracts were washed once with 2.5 M NaOH solution (40 ml), dried over $MgSO_4$ and concentrated to give the title compound (514 mg, 79%) as a pale-yellow viscous oil and as a 5:1 *endo–exo* mixture. δ_H (300 MHz) 1.10–1.50 (4H), 1.60–2.20 (7H), 2.09 (3H, s, NCH_3), 2.93 (2H, broad s, C1 and C5), 3.95 (1H, broad s, OH), 4.46–4.58 (1H, m, CH–OH), 5.18 (1H, s, =CH_2), 5.30 (1H, s, =CH_2), 7.14–7.34 (5H, m, Ar). *endo*-Isomer: δ_C (75 MHz) 24.3 (C3), 26.3 and 26.4 (C6,7), 34.3 and 37.0 (C2,4), 40.2 (N–CH_3), 45.3 (C9), 60.6 (C1,5), 72.3 (CH–OH), 112.3 (*β*-C), 127.1, 127.8, 128.6, 140.3 (*ipso*-C), 153.0 (*α*-C). *exo*-Isomer: δ_C (75 MHz) 24.5 (C3), 26.2 (C6,7), 28.0 and 30.3 (C2,4), 40.6 (N–CH_3), 43.9 (C9), 61.5 and 61.6 (C1,5), 71.1 (CH–OH), 112.3 (*β*-C), 127.0, 127.8, 128.6, 140.2 (*ipso*-C), 153.2 (*α*-C). *m*/*z* (EI) 271 (M^+, 13%), 254 (61%), 138 (41%), 96 (93%), 82 (100%). Accurate mass (EI^+): Found 271.1936; Calculated for (M^+) $C_{18}H_{25}NO$ 271.1936 (0.05 ppm). ν/cm^{-1} (thin film): 3343 (O–H), 3079, 3055, 3028, 2923, 2855, 2800 (N–CH_3), 1627, 1597, 1492, 1450, 1338, 1122, 1071.

2-(Tropan-3-yl)-1-(*p*-methoxyphenyl)ethan-1-ol 17

A solution of 4-bromoanisole (673 mg, 3.60 mmol) in Et_2O (15 ml) was added dropwise to a suspension of magnesium turnings (96 mg, 3.96 mmol) and iodine crystals in Et_2O (5 ml) at room temperature. After addition was complete, the mixture was heated under reflux for 15 min and then cooled to ambient temperature. This Grignard reagent was added to a solution of (tropan-3-yl)acetaldehyde **15** (200 mg, 1.20 mmol) in THF (20 ml) at −78 °C. The reaction mixture was allowed to warm to room temperature for 21 h and then concentrated. The residue was diluted with saturated NH_4Cl and impurities were extracted into Et_2O (3 × 30 ml). After the aqueous layer was made basic (pH 10–11) with NaOH pellets, organics were then extracted into CH_2Cl_2 (5 × 40 ml). The combined extracts were washed once with 2.5 M NaOH solution, dried over $MgSO_4$ and concentrated to give the title compound (210 mg, 64%) as a pale-yellow viscous oil and as a 5:1 *endo–exo* mixture. δ_H ($CDCl_3$, 300 MHz) 1.20–1.50, 1.60–2.30, 2.18 (3H, s, NCH_3), 3.10 (2H, broad s, C1,5), 3.74 (3H, s, OCH_3), 4.50 (1H, t, CH–OH), 6.80 (2H, d, J = 8.7 Hz, Ar), 7.18 (2H, d, J = 8.7 Hz, Ar). *endo*-Isomer: δ_C ($CDCl_3$, 50 MHz) 24.1 (C3), 26.5 and 26.6 (C6,7), 34.7 and 36.0 (C2,4), 39.6 (N–CH_3), 48.4 (C9), 55.4 (OCH_3), 60.5 (C1,5), 72.3 (CH–OH), 113.9 (C3′), 127.3, 138.2, 159.0 (C4′). *exo*-Isomer: δ_C ($CDCl_3$, 50 MHz) 24.5 (C3), 26.3 (C6,7), 37.7 and 38.5 (C2,4), 39.6 (N–CH_3), 46.7 (C9), 53.6 (OCH_3), 61.4 and 61.5 (C1,5), 71.0 (CH–OH), 113.9 (C3′), 127.1, 138.3, 159.0 (C4′). *m*/*z* (EI) 275 (M^+, 23%), 257 (8%), 138 (78%), 96 (73%), 82 (100%). Accurate mass (EI^+): Found 275.1882; Calculated for (M^+) $C_{17}H_{25}NO_2$ 275.1885 (1.2 ppm). ν/cm^{-1} (thin film): 3355 (O–H), 2931, 2836, 2801 (N–CH_3), 1610, 1585, 1510, 1451, 1338, 1245, 1075, 1037.

1-(Tropan-3-yl)-3-methylbutan-2-ol 18

A 2 M solution of isopropylmagnesium chloride (492 mg, 4.78 mmol) was added to a solution of (tropan-3-yl)acetaldehyde **15** (400 mg, 2.39 mmol) in THF (30 ml) at −78 °C. The reaction mixture was allowed to warm to room temperature for 14 h and was then concentrated under reduced pressure. The residue was diluted with saturated NH_4Cl and impurities were extracted into Et_2O (3 × 30 ml). After the aqueous layer was made basic (pH 10–11) with NaOH pellets. The organics were then extracted into CH_2Cl_2 (5 × 40 ml). The combined extracts were washed once with 2.5 M NaOH solution, dried over $MgSO_4$ and concentrated to give the title compound (337 mg, 67%) as a clear oil and as a 5:1 *endo–exo* mixture. δ_H (300 MHz) 0.86 (3H, d, J = 6.6 Hz, CH_3), 0.88 (3H, d, J = 6.6 Hz, CH_3), 1.20–1.40 (3H, m), 1.50–1.70 (4H, m), 1.80–2.30 (6H, m), 2.22 (3H, s, NCH_3), 3.10 (2H, broad s, C1,5), 3.40 (1H, m, CH–OH). *endo*-Isomer: δ_C (75 MHz) 17.5 and 19.0 (CH_3), 24.1 (C3), 26.5 and 26.7 (C6,7), 34.2 and 37.4 (C2,4), 40.3 (N–CH_3), 42.8 (C9), 60.8 (C1,5), 74.9 (CH–OH). *exo*-Isomer: δ_C (75 MHz) 17.3 and 18.9 (CH_3), 24.3 (C6,7), 26.2 and 26.3 (C2,4), 34.3, 37.4, 41.3 (N–CH_3), 42.8, 61.7 and 61.9 (C1,5), 73.7 (CH–OH). *m*/*z* (EI) 211 (M^+, 3%), 168 (3%), 96 (64%), 82 (100%), 42 (74%). Accurate mass (EI^+): Found 211.1936; Calculated for (M^+) $C_{13}H_{25}NO$ 211.1936 (0.07 ppm). ν/cm^{-1} (thin film): 3373 (O–H), 2958, 2928, 2797 (N–CH_3), 1470, 1451, 1338, 1230, 1131, 1063.

4-(Tropan-3-yl)-2-phenylbut-1-en-3-one 4

Activated manganese dioxide (8.0 g, 92.0 mmol) was added to a solution of 4-(tropan-3-yl)-2-phenylbut-2-en-3-ol **16** (657 mg, 2.4 mmol) in CH_2Cl_2 (25 ml) and the mixture was stirred for 24 h at room temperature. The catalyst was filtered off through Hyflo Super Cell and the filtrate concentrated to give the title compound (360 mg, 55%) as a light-brown liquid and as a 5:1 *endo–exo* mixture. *endo*-Isomer: δ_H (300 MHz) 1.19 (2H, broad d, J = 14.4 Hz, *eq*-C2,4), 1.51 (2H, broad d, J = 8.1 Hz, *endo*-C6,7), 1.86–2.02 (2H, m, *exo*-C6,7), 2.04–2.22 (2H, m, *ax*-C2,4), 2.17 (3H, s, N–CH_3), 2.24–2.38 (1H, m, C3), 2.81 (2H, d, J = 7.8 Hz, CH_2CO), 3.02 (2H, broad s, C1,5), 5.80 (1H, s, =CH_2), 5.97 (1H, s, =CH_2), 7.16–7.34 (5H, m, Ar). *endo*-Isomer: δ_C (75 MHz) 23.7 (C3), 26.4 (C6,7), 35.3 (C2,4), 39.9 (N–CH_3), 48.1 (C9), 60.2 (C1,5), 123.8 (β-C), 128.1 (*o*- and *p*-C), 128.2 (*m*-C), 137.1 (*ipso*-C), 149.6 (α-C), 202.0 (C=O). *exo*-Isomer: δ_H (300 MHz) 1.12–1.66 (4H), 1.86–2.40 (5H), 2.16 (3H, s, N–CH_3), 2.52 (2H, d, J = 6.9 Hz, CH_2CO), 2.96–3.10 (2H, broad s, C1,5), 5.80 (1H, s, =CH_2), 5.99 (1H, s, =CH_2), 7.16–7.34 (5H, m, Ar). *exo*-Isomer: δ_C (75 MHz) 24.8 (C3), 25.9 and 26.9 (C6,7), 34.1 and 38.0 (C2,4), 40.4 (N–CH_3), 46.2 (C9), 59.7 and 61.2 (C1,5). *m*/*z* (EI) 269 (M^+, 6%), 96 (85%), 82 (100%). Accurate mass (EI^+): Found 269.1782; Calculated for (M^+) $C_{18}H_{23}NO$ 269.1780 (−1.0 ppm). ν/cm^{-1} (thin film): 2955, 2930, 2846, 2794 (N–CH_3), 1685 (C=O), 1493, 1446, 1130.

2-(Tropan-3-yl)-1-(*p*-methoxyphenyl)ethan-1-one 5

Activated manganese dioxide (4.3 g, 49.4 mmol) was added to

a solution of 2-(tropan-3-yl)-1-(*p*-methoxyphenyl)ethan-1-ol **17** (358 mg, 1.30 mmol) in CH_2Cl_2 (40 ml) and the mixture was stirred for 22 h at room temperature. The catalyst was filtered off through Hyflo Super Cell and the filtrate concentrated to give the title compound (181 mg, 51%) as a light-brown liquid and as a 5:1 *endo–exo* mixture. *endo*-Isomer: δ_H (300 MHz) 1.27 (2H, broad d, J = 14.4 Hz, *eq*-C2,4), 1.64 (2H, broad d, J = 8.4 Hz, *endo*-C6,7), 1.90–2.06 (2H, m, *exo*-C6,7), 2.08–2.18 (2H, m, *ax*-C2,4), 2.19 (3H, s, NCH_3), 2.32–2.46 (1H, m, C3), 3.00 (2H, d, J = 7.8 Hz, CH_2CO), 3.05 (2H, broad s, C1,5), 3.80 (3H, s, OCH_3), 6.86 (2H, d, J = 9.0 Hz, Ar), 7.84 (2H, d, J = 9.0 Hz, Ar). *endo*-Isomer: δ_C (50 MHz) 23.7 (C3), 26.4 (C6,7), 35.4 (C2,4), 40.0 (N–CH_3), 46.6 (C9), 55.4 (OCH_3), 60.3 (C1,5), 113.6 (C3′), 130.2 (C1′,2′), 163.3 (C4′), 198.5 (C=O). *exo*-Isomer: δ_H (300 MHz) 1.36–1.70 (4H), 1.90–2.30 (5H), 2.20 (3H, s, NCH_3), 2.71 (2H, d, J = 6.6 Hz, CH_2CO), 2.92 (2H, broad s, C1,5), 3.80 (3H, s, OCH_3), 6.80–6.90 (2H), 7.80–7.90 (2H). *exo*-Isomer: δ_C (50 MHz) 24.8 (C3), 25.8 (C6,7), 38.1 (C2,4), 40.3 (N–CH_3), 44.6 (C9), 55.3 (OCH_3), 61.4 (C1,5), 113.6 (C3′), 130.2 (C1′,2′), 163.3 (C4′), 198.3 (C=O). *m*/*z* (EI) 273 (M^+, 11%), 138 (77%), 124 (100%), 96 (70%), 82 (82%). Accurate mass (EI^+): Found 273.1732; Calculated for (M^+) $C_{17}H_{23}NO_2$ 273.1729 (−1.1 ppm). ν/cm^{-1} (thin film): 2959, 2931, 2844, 2795 (N–CH_3), 1671 (C=O), 1599, 1575, 1509, 1260, 1169, 1029.

X-Ray crystal structure determination of 5·HCl‡

The hydrochloride salt was prepared by dissolution of **5** (50 mg) in 2 M HCl solution (2–3 ml) and the solvent removed under reduced pressure. The residue was resuspended in ethanol (0.5 ml) and crystallisation was induced by placing the open sample tube in a sealed vial containing diethyl ether. Crystallisation was induced as the ether vapour dissolved in the ethanol. The experiment was performed with a SMART 1K CCD area detector, using graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å) and a Cryostream (Oxford Cryosystems) open-flow N_2 gas cryostat. The structure was solved by direct methods and refined (non-H atoms anisotropic, all H atoms isotropic) against F^2 of all data, using SHELXTL software.[28]

Crystal data: $C_{17}H_{24}NO_2^+$ Cl^-, M = 309.8, T = 120 K, orthorhombic, space group $P2_12_12_1$ (No. 19), a = 6.341(1), b = 11.558(1), c = 21.415(4) Å, U = 1569.5(4) Å^3, Z = 4, D_c = 1.31 g cm^{-3}, μ = 0.25 cm^{-1}, 10962 reflections (3571 unique, R_{int} = 0.067) with $2\theta \leq 55°$, 286 variables, R = 0.037 [3099 data, $I > 2\sigma(I)$], $wR(F^2)$ = 0.086, $\Delta\rho$ max/min = 0.29, −0.18 e Å^{-3}. The absolute structure was determined from anomalous X-ray scattering: the Flack parameter[29] −0.05(6).

1-(Tropan-3-yl)-3-methylbutan-2-one 6

A mixture of pyridinium chlorochromate (PCC) (604 mg, 2.80 mmol) adsorbed on alumina and 1-(tropan-3-yl)-3-methylbutan-2-ol **18** (295 mg, 1.40 mmol) in CH_2Cl_2 (30 ml) was stirred for 24 h. The reaction was then diluted with ether (30 ml) and filtered through Hyflo Super Cell. The alumina was washed twice with a mixture of ether and CH_2Cl_2 (1:1) and the solution concentrated to give a residue, which was made basic with 2.5 M NaOH solution. The organics were then extracted into EtOAc (3 × 30 ml) and the combined extracts were dried over $MgSO_4$ and concentrated to give the title compound (130 mg, 44%) as a light-brown liquid and as a 5:1 *endo–exo* mixture. *endo*-Isomer: δ_H (300 MHz) 1.07 (6H, d, J = 6.9 Hz, CH_3), 1.20 (2H, broad d, J = 14.1 Hz , *eq*-C2,4), 1.61 (2H, broad d, J = 8.1 Hz, *endo*-C6,7), 1.98–2.10 (2H, m, *exo*-C6,7), 2.10–2.24 (2H, m, *ax*-C2,4), 2.26 (3H, s, NCH_3), 2.28–2.42 (1H, m, C3), 2.56 (1H, septet, J = 6.9 Hz, CHCO), 2.62 (2H, d, J = 7.8 Hz, CH_2CO), 3.10 (2H, broad s, C1,5). *endo*-Isomer: δ_C (75 MHz) 17.2 (CH_3), 21.6 (C3), 25.3 (C6,7), 34.2 (C2,4), 39.6 (N–CH_3), 48.2 (CH_2CO and CHCO), 59.3 (C1,5), 213.1 (C=O). *exo*-Isomer: δ_H (300 MHz) 1.04 (6H, d, J = 6.9 Hz, 2 × CH_3), 1.13–1.70 (4H), 1.94–2.40 (5H), 2.26 (3H, s, NCH_3), 2.45–2.65 (2H), 3.06–3.16 (2H, broad s, C1,5). *exo*-Isomer: δ_C (75 MHz) 17.0 (CH_3), 22.8 (C3), 24.8 (C6,7), 36.6 (C2,4), 38.8 (N–CH_3), 45.9 (CH_2CO and CHCO), 60.5 (C1,5), 213.1 (C=O). *m*/*z* (EI) 209 (M^+, 15%), 138 (41%), 124 (52%), 96 (68%), 82 (100%). Accurate mass (EI^+): Found 209.1773; Calculated for (M^+) $C_{13}H_{23}NO$ 209.1780 (3.1 ppm). ν/cm^{-1} (thin film): 2960, 2930, 2795 (N–CH_3), 1710 (C=O), 1467, 1382, 1128, 1079, 1039.

Biological assay methods

Drugs and solutions. Acetylcholine (ACh) chloride and atropine sulfate (Sigma Chem. Co.) were prepared daily as 1 mM and 10 mM stock solutions, respectively. Ketone **4** was dissolved in 1.0 M HCl and then diluted with distilled water to prepare a 1 mM final stock solution. In these assays, the guinea-pig ileum[24] was maintained in four 50 ml organ baths. Each organ bath was filled with a modified Tyrode solution, continuously oxygenated and maintained at 32 °C. The composition of the modified Tyrode solution was as follows (mM): 136 NaCl, 2.62 KCl, 1.8 $MgCl_2·6H_2O$, 1.8 $CaCl_2·2H_2O$, 0.42 NaH_2PO_4, 11.9 $NaHCO_3$ and 5.5 glucose. All salts were obtained from BDH (UK).

Determination of acetylcholine potency (EC_{50}). A non-cumulative concentration-response curve (CRC) to ACh was obtained with eight different concentrations of ACh, ranging from 3 nM to 10 μM (n = 6; n = number of preparations involved in the experiment, see Fig. 2). ACh was added to the organ bath for 30 s and then the tissues were washed with fresh Tyrode solution. This was repeated in a 4 min time cycle. The tissue responses were measured as changes in isometric tension using an Isometric Transducer (FSG-01) and bridge-amplifier unit (SG-02, both Experimentria, Budapest) and displayed on a pen chart recorder (Model 202, Sycopel, see Fig. 2). The responses were then calculated as a percentage of the maximum response obtained to ACh and plotted against the logarithm of the ACh concentration. The ACh potency was expressed as the 50% effective concentration value (EC_{50}).

Determination of antagonist potency (IC_{50}). The tissues were incubated with antagonists for 3 min at increasing concentrations, ranging from 0.1 nM to 0.1 μM for atropine (see Fig. 3a) and from 10 nM to 30 μM for ketone (see Fig. 3b). Each incubation was followed by addition of 0.1 μM ACh for 30 s and then the tissues were washed with fresh Tyrode solution at least twice during the 4 min time cycle. The tissue responses to ACh in the presence of the drugs were measured and calculated as a percentage of the control response obtained to ACh in the absence of the antagonists. These data were plotted against the logarithm of the antagonist concentration (see Fig. 3c). The antagonist potency was expressed as the 50% inhibitory concentration value (IC_{50}).

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