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Methyllycaconitine analogues have mixed antagonist effects at nicotinic acetylcholine receptors

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Abstract—Bicyclic analogues of methyllycaconitine (MLA), such as **12**, have been synthesised that incorporate the C1–OMe substituent present in the natural product. Electrophysiology experiments using *Xenopus* oocytes expressing nicotinic acetylcholine receptors (nAChRs) were conducted on these analogues and a related tricyclic analogue **2**. The most potent compound, **2**, was an antagonist at all receptors studied but displayed different antagonist effects at each receptor subtype. This study more clearly defines the biological effects of MLA analogues at nAChRs and demonstrates that these analogues are not selective ligands for the α7 nAChR subtype.

1. Introduction

Neuronal nicotinic acetylcholine receptors (nAChRs) are a class of ligand gated ion channels and a rapidly emerging field of medicinal chemistry and biology, with potential therapeutic applications for the treatment of neurological conditions including schizophrenia, Alzheimer's disease and epilepsy.[1] However, this task is greatly complicated by the fact that nAChRs are pentameric proteins with numerous subtypes and these are poorly characterised in terms of subunit composition, localisation and neuronal function.[1] Despite recent progress there is still a great need for subtype selective agonists and antagonists to elucidate the biological roles of these receptors and to provide candidates for drug discovery.[2–4] We have been engaged in the design and synthesis of a class of nAChR ligands based on the potent and α7 selective nAChR antagonist methyllycaconitine (MLA, **1**, Fig. 1). This program has resulted in the synthesis of a range of analogues, such as compound **2**,[5–8] containing the tertiary amine and benzoate ester side chain that form the putative acylated homocholine pharmacophore of MLA (**1**).[9]

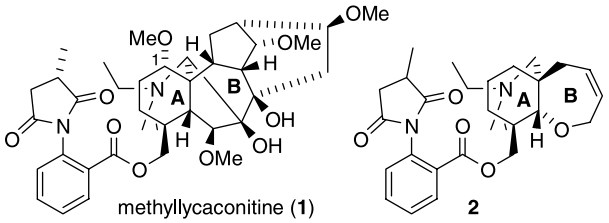

Figure 1. Methyllycaconitine (MLA, **1**) and analogue **2**.

It is known from structure–activity relationship studies of MLA and related norditerpenoid alkaloids that variation of the C1–OMe of MLA (**1**) has a significant influence on biological activity.[10] The object of this study was thus to examine the influence of the C1–OMe group on the activity of MLA analogues related to **2**, through the synthesis of structures incorporating this functionality, for example derivative **3** (Scheme 1). In this paper we report the methodologically distinct synthetic route to AE bicyclic analogues of MLA (**1**) that, in contrast to our previous compounds,[5–8] contain the C1-methoxy substituent as a part of the A-ring. We also report the biological evaluation of these new analogues and compound **2** by electrophysiology against a number of nAChR subtypes expressed in *Xenopus* oocytes. These

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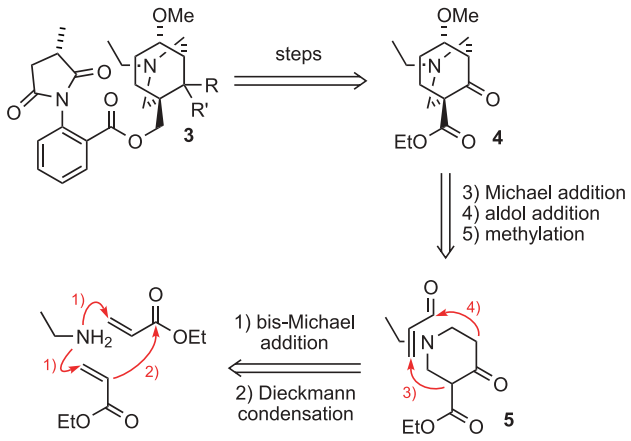


Scheme 1. Synthetic strategy.

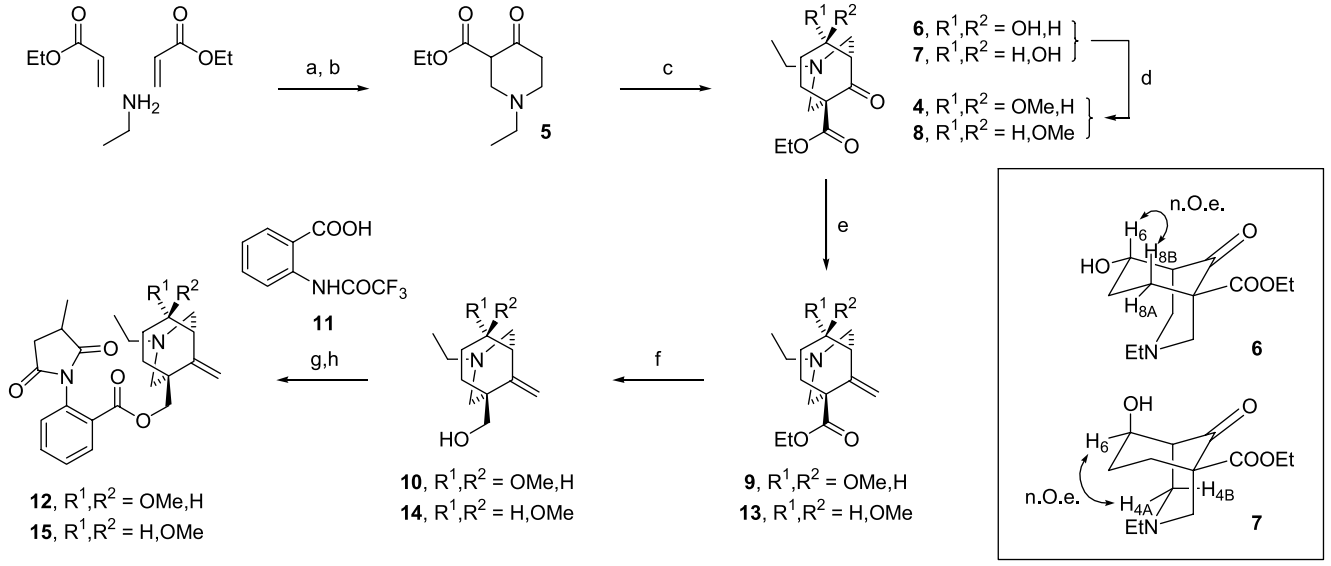
results more clearly define the biological effects of this class of ligand and show that methyllycaconitine analogues function as antagonists across a range of nAChR subtypes.

2. Results

2.1. Chemistry

The strategy devised for the synthesis of C1–OMe AE bicyclic MLA analogues, typified by compound **3**, is shown in Scheme 1. We anticipated that analogue **3** could be derived in a short sequence of steps from the AE-bicyclic core structure **4**. This keto-ester **4** could in turn be derived from piperidone **5** and acrolein by a three-step sequence involving Michael addition and aldol addition to effect ring formation with regioselective introduction of the desired C1 secondary hydroxyl group, followed by methylation. In this respect we were inspired by related sequences for the annulation of 4-piperidones.[11] The piperidone **5** required for this synthesis could itself be obtained by twofold Michael addition of ethyl amine to ethyl acrylate, followed by base mediated Dieckmann condensation.[12]

The first key intermediate, piperidone **5** was readily synthesised in two steps and 76% overall yield by the twofold Michael addition of ethylamine to excess ethyl acrylate followed by treatment of the resulting diester with KO^{t}Bu in THF to facilitate Dieckmann condensation.[12] With piperidone **5** in hand we were now in a position to investigate the pivotal Michael addition/aldol addition sequence for the introduction of the A-ring. A number of bases were investigated for this conversion, including triethylamine,[11] NaOMe,[13] K_2CO_3[14] and 1,1,3,3-tetramethylguanidine (TMG)[15] with the finding that only TMG cleanly converted piperidone **5** to a 1:1 diastereomeric mixture of secondary alcohol products **6** and **7** in 73% yield. These were separated by flash chromatography and the stereochemistry assigned by 2D NMR analysis. In each case, reciprocal NOESY cross-peaks were observed, consistent with the assigned structures of **6** and **7** (see box, Scheme 2). Methylation of **6** under standard conditions, using sodium hydride and methyl iodide, led to rapid decomposition, presumably due to a sequence initiated by base promoted retro-aldol reaction. Using silver(I) oxide as base was found to methylate **6** in moderate yield, however in this instance epimerisation of the hydroxy-bearing stereocentre occurred, as a result of a retro-aldol/aldol addition sequence, to give a mixture of diastereomeric methyl ethers **4** and **8**. Once again these diastereoisomers could be separated and the stereochemistry of each was assigned in an analogous manner to that reported for alcohols **6** and **7** above. For preparative purposes, the initially formed mixture of alcohols **6** and **7** were



Scheme 2. (a) EtOH; (b) KO^{t}Bu, THF; (c) acrolein, 1,1,3,3-tetramethylguanidine, CH_2Cl_2; (d) Ag_2O, MeI, CH_2Cl_2; (e) Ph_3PCH_3Br, ^{n}BuLi, THF; (f) $LiAlH_4$, THF; (g) **12**, DCC, DMAP, CH_2Cl_2; then $NaBH_4$, EtOH; (h) methylsuccinic anhydride.

subjected to methylation to give a 1.2:1 mixture of methyl ethers **4** and **8** in 55% combined yield. Thus, both relative configurations at the methoxy bearing stereocentre were prepared, with the major product of this sequence corresponding to the stereochemistry observed for the C1–OMe of MLA (**1**).

Elaboration of the AE-bicyclic structure **4** proceeded in four steps. Wittig olefination of the ketone gave alkene **9** in 61% yield and the ester was then reduced to give the primary alcohol **10** (90%). The side chain of analogue **12** was introduced by a two-step procedure, firstly forming the anthranilate ester (76%) with *N*-(trifluoroacetyl)anthranilic acid (**11**), followed by fusion of the resulting primary amine with methylsuccinic anhydride to form the succinimide ring (84%).[7] The diastereomeric AE-bicyclic core structure **8** was elaborated by the same sequence. Wittig olefination of ketone **8** gave alkene **13** (67%), which was reduced to give primary alcohol **14** (94%) and the side chain added to give analogue **15** in 61% over two steps. Thus we had prepared two new AE bicyclic analogues of MLA, which differed in the stereochemistry of the methoxy substituent.

2.2. Pharmacology

2.2.1. Inhibition of ACh-current by compounds 2, 12 and 15. Expression of rat α7 (a), α3β4 (b) and α4β2 (c) cRNA in *Xenopus* oocytes generated respective nicotinic receptors, which showed dose-dependent ACh-activated inward current when the cell was voltage clamped at −60 mV (Fig. 2). The corresponding expression levels ranged from 75 to 200, 90 to 7000 and 70 to 1200 nA, respectively. This could be blocked by compounds such as bicyclic analogues **12** and **15** or tricyclic analogue **2** as shown in Figure 2 and Table 1. Each oocyte was first screened with a submaximal dose of ACh (α7, 300 μM; α3β4, 150 μM; α4β2, 60 μM) to determine expression level. Cells expressing currents >50 nA were used in screening. Analogues **2**, **12** and **15** were then tested to determine agonist activity, followed by antagonist activity by application with a submaximal dose of ACh. On their own, these compounds trigger no response, but when co-applied with ACh they act as antagonists, blocking or reducing the response of ACh.

2.2.2. Inhibition of ACh-current by compound 2. Acetylcholine dose response curves for rat neuronal α7, α3β4 and α4β2 receptors expressed in oocytes were constructed as described in data analysis using GraphPad 'Prism'(3.02) and are shown in Figure 3a–c, respectively. The resulting EC_{50} values for ACh under these conditions were 277 ± 1 μM for α7, 151 ± 2 μM for α3β4 and 97 ± 1 μM for α4β2 nAChRs (Table 2). The corresponding Hill coefficients (n_H) were 1.3 ± 0.1, 1.5 ± 0.3 and 1.1 ± 0.2, respectively.

The dose response curves for ACh in the presence of 30 μM analogue **2** are also shown in Figure 3 and the resulting EC_{50} values were 277 ± 1 μM, for the homomeric α7 and 151 ± 2 and 97 ± 1 μM for heteromeric α3β4 and α4β2 nAChRs, respectively (Table 2). The corresponding Hill coefficients (n_H) were 1.3 ± 0.1, 1.5 ± 0.3 and 1.1 ± 0.2, respectively. The dose response curve for ACh in the presence of 1 nM MLA (**1**) at the α7 receptor (not shown) was also completed and comparative data is reported in Table 2. As indicated by Figure 3a and b, higher concentrations of ACh decrease the amplitude of the current obtained during application. This is due to receptor desensitisation, which is a well known characteristic of nAChRs.

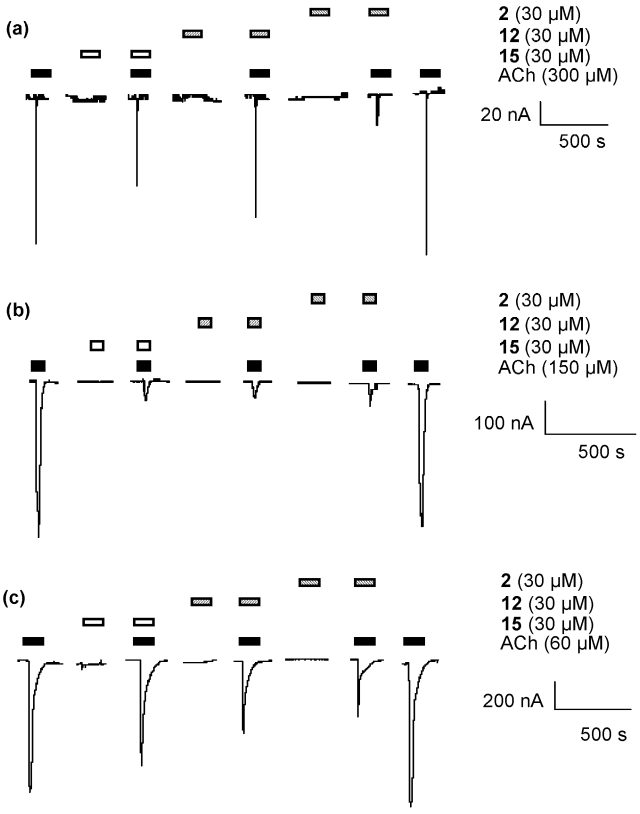


Figure 2. A comparison of the activity of compounds **2**, **12** and **15**. (a) α7; compounds **2**, **12** and **15** (30 μM; duration indicated by backward hatched bar, forward hatched and open bar, respectively), produced no effect alone but when applied with ACh (300 μM, submaximal dose), compounds **2**, **12** and **15** inhibited ACh response by 95 ± 1%; 37 ± 4% and 37 ± 2%, respectively. (b) α3β4; the same concentration of **2**, **12** and **15** (30 μM) were applied in the same manner. In the presence of ACh (150 μM, submaximal dose), compound **2**, **12** and **15** inhibited ACh response by 90 ± 1%; 78 ± 5% and 78 ± 6%, respectively. (c) α4β2; procedure described was repeated with ACh (60 μM, submaximal dose). Compound **2**, **12** and **15** inhibited ACh response by 53 ± 3%; 42 ± 4% and 30 ± 6%, respectively. For (a), (b) and (c) a second maximal current was obtained using submaximal concentration of ACh to confirm receptor recovery. Data are the mean ± SEM (n = 3–4 oocytes).

Table 1. Percentage inhibition by compounds **2**, **12** and **15** (30 μM) of acetylcholine at nicotinic acetylcholine receptors—α7 (300 μM), α3β4 (150 μM) and α4β2 (60 μM)

Compounds	α7	α3β4	α4β2
2	95 ± 1	90 ± 1	53 ± 3
12	37 ± 4	78 ± 5	42 ± 4
15	37 ± 2	78 ± 6	30 ± 6

Data are mean ± SEM (n = 3–4).

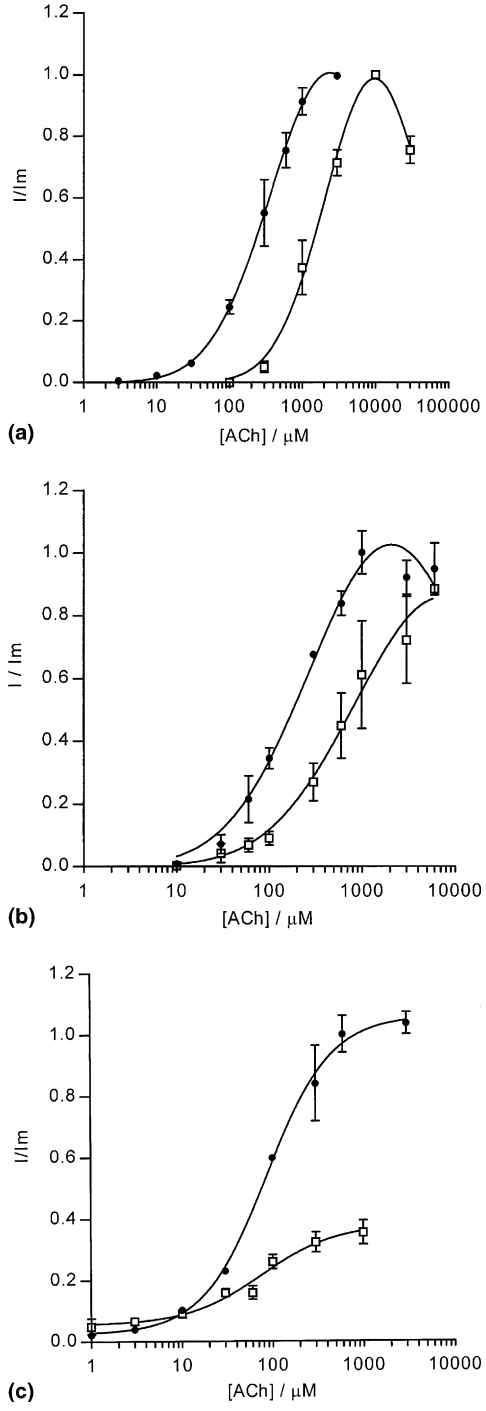


Figure 3. ACh dose response curve (●) and increasing concentrations of ACh in the presence of 30 μM **2** (□) for recombinant (a) α7, (b) α3β4 and (c) α4β2 receptors expressed in *Xenopus* oocytes. The amplitude of the current recorded in response to each dose applied was normalised to the amplitude of the current response to α7 (3 mM); α3β4 (300 μM) and α4β2 (30 μM). Data are the mean ± SEM (n = 3–15 oocytes).

The application of tricyclic analogue **2** in combination with ACh reduced the response generated by ACh (300 μM, α3β4; 100 μM, α4β2). The corresponding IC_{50} values were 14.7 ± 1.1 and 15.4 ± 1.2 μM, respectively (Fig. 4).

3. Discussion

The α7 nAChR subtype is the second most prevalent in the brain and has been implicated as playing a key role in conditions such as schizophrenia, Alzheimer's disease and epilepsy.[1] Despite recent progress in the synthesis of competitive agonists selective for the α7 nAChR subtype,[3] very few antagonists are known that bind with high affinity and selectivity at this receptor.[4] These include the peptide toxins α-bungarotoxin,[16] α-conotoxin ImI[17] and the norditerpenoid alkaloid methyllycaconitine (MLA, **1**, Fig. 1).[18] MLA is the major toxic component of *Delphinium brownii*[19] and is a potent antagonist of the α7 nAChR in mammalian neuronal membranes that exhibits high selectivity for this subtype over other neuronal nAChRs. The high affinity binding, functional potency and subtype selectivity renders MLA a prime lead for the development of new therapeutic agents or pharmacological tools targeting the α7 nAChR.

It has been proposed that the tertiary amine and ester side chain of MLA (**1**) form an acylated homocholine pharmacophore at physiological pH that gives rise to the high affinity nAChR binding.[9] In support of this hypothesis, structure–activity studies on MLA have shown the *N*-substituted anthranilate ester moiety as an essential structural feature for pharmacological activity. Removal of the side-chain methyl group, succinimide ring or ester side chain of MLA results in a 20-, 210- or 1300-fold decrease, respectively, in binding affinity at rat brain α7 nAChR.[9,20,21]

A number of approaches to the synthesis of small molecule analogues of MLA incorporating the putative pharmacophore have been disclosed,[5–8,22–35] however there have been fewer reports of significant biological activity.[22,28,31,32,35,36] In this study we address two areas. Firstly we sought synthetic access to analogues that incorporated the C1–OMe substituent present in the natural product, which is situated in close proximity to the acylated homocholine pharmacophore of this ligand. It is known that the intravenous toxicity of MLA (**1**) and the related norditerpenoid alkaloids in mice are strongly influenced by variation in the C1 substituent (Fig. 5). Specifically, the toxicity of MLA (**1**) is of a similar magnitude to grandiflorine (**20**) bearing a C1 hydroxy group but grandiflorine acetate (**21**) bearing a C1-acetoxy substituent is an order of magnitude less toxic.[10] Despite this correlation, there have been few reports describing the synthesis[28–30] and biological evaluation[28] of MLA analogues incorporating C1 substitution to date. The synthetic work developed in this paper provides a succinct route to AE-bicyclic analogues **12** and **15** (Scheme 2) that incorporate the methoxy substituent with the same regiochemistry to that of MLA (**1**) but differing relative stereochemistry.

The second aim was to test the analogues derived from our synthetic endeavours in functional assays across a range of nAChR receptor subtypes to evaluate the potency and function of these ligands. This would allow us to obtain a more complete understanding of how ligand architecture influences biological activity at

Table 2. EC_{50}, and n_H values for ACh alone and in the presence of analogue **2** and MLA (**1**) tested on rat neuronal nicotinic AChRs (α7, α3β4 and α4β2)

Compounds	α7		α3β4		α4β2	
	EC_{50}[a] (μM)	n_H[b]	EC_{50} (μM)	n_H	EC_{50} (μM)	n_H
ACh	277 ± 1	1.3 ± 0.1	151 ± 2	1.5 ± 0.3	97 ± 1	1.1 ± 0.2
ACh + **2** (30 μM)	1176 ± 1	1.9 ± 1.3	618 ± 1	1.1 ± 0.2	108 ± 1	1.2 ± 1.0
ACh + **1** (1 nM)	1447 ± 15	0.8 ± 0.3	—	—	—	—

[a] EC_{50} is the concentration that activates 50% of receptors. Data are the mean ± SEM (n = 3–15 oocytes).
[b] n_H is the Hill coefficient. Data are the mean ± SEM (n = 3–15 oocytes).

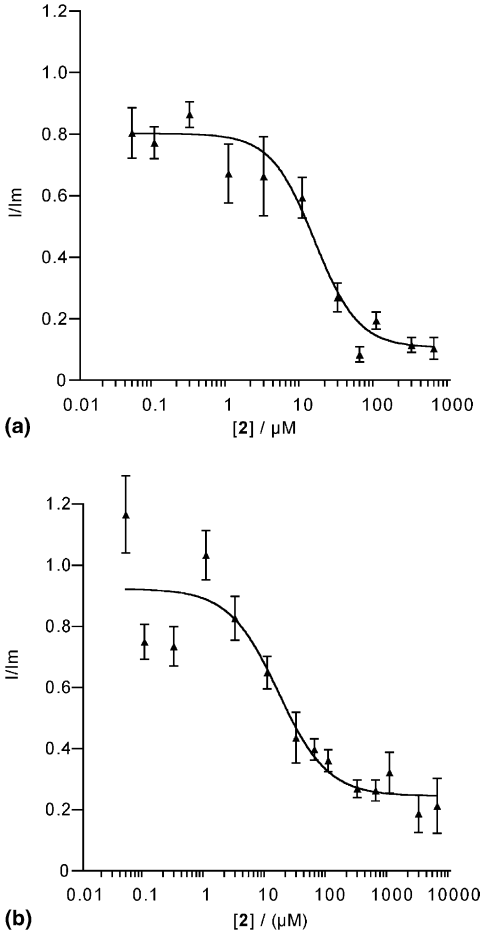
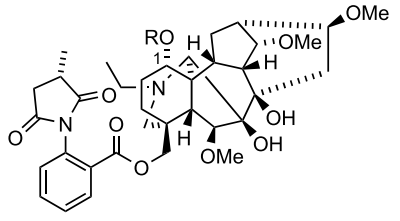


Figure 4. ACh dose response curve in the presence of increasing concentrations of **2** for recombinant (a) α3β4 and (b) α4β2 receptors expressed in *Xenopus* oocytes. The amplitude of the current recorded in response to each dose applied was normalised to the amplitude of the current of ACh alone at α3β4 (300 μM) and α4β2 (100 μM). Data are the mean ± SEM (n = 3–15 oocytes).

nAChRs. Attempts to extend the approach described in this report for the synthesis of ABE tricyclic analogues incorporating methoxy substitution have been unsuccessful. However, the availability of related ABE-tricyclic analogues, such as the previously prepared compound **2**,[5] allows an assessment of the significance of the B-ring to biological activity.

Compounds **2**, **12** and **15** were tested against α7, α3β4 and α4β2 nicotinic acetylcholine receptors expressed in *Xenopus* oocytes and were found to be antagonists at these receptors (Fig. 2 and Table 1). The potency of each



methyllycaconitine (**1**), R = Me (LD_{50} = 7.5 mg kg^{-1})
grandiflorine (**16**) R = H (LD_{50} = 8.3 mg kg^{-1})
grandiflorine acetate (**17**) R = Ac (LD_{50} ~ 100 mg kg^{-1})

Figure 5. Toxicity of selected norditerpenoid alkaloids.

compound was dependent on the nAChR receptor subtype tested with ABE tricyclic analogue **2** being the most potent compound at each receptor subtype. Compounds **12** and **15** were equipotent at α7 and α3β4 receptors with compound **12** having potency between that of compounds **2** and **15** at the α4β2 receptor. The lower and near equal potency of the diastereomeric methoxy bearing analogues **12** and **15** at each receptor subtype suggests that the stereochemistry methoxy group has little influence over binding or selectivity of these compounds.

To gain further insight, the most potent analogue, compound **2**, was further evaluated for activity and the type of inhibition at the different receptor subtypes and was found to exert different antagonistic effects at α7, α3β4, α4β2 nicotinic acetylcholine receptors (Fig. 3 and Table 2). At the α7 nAChR, analogue **2** (K_B = 6.0 ± 1.5 μM), like MLA (**1**, K_B = 237 ± 79 pM), appeared to be a competitive antagonist over the concentration tested albeit 25,000-times weaker (Fig. 3a). In contrast, **2** was a non-competitive antagonist at α4β2, (Fig. 3c) having little to no effect at lower doses but inhibiting the maximum ACh response (*Im*) without changing the affinity of ACh for the receptor as evidenced by the little change in the EC_{50}. At α3β4 receptors (Fig. 3b), compound **2** affected the maximum ACh response *and* the affinity of ACh in the presence of the antagonist. Thus compound **2** has mixed competitive and non-competitive antagonist actions at this receptor.

The binding affinity of AE bicyclic and ABE tricyclic analogues of MLA have also been investigated by Blagbrough and co-workers[35] in competitive ligand binding assays against [^{125}I]α-bungarotoxin on rat brain membranes. Similar trends were observed, with one tricyclic analogue of undefined stereochemistry (IC_{50} = 478 nM) displaying greater potency than a

diastereomer or related bicyclic analogue (IC_{50} = 107–110 μM) but much lower than that of MLA itself (IC_{50} = 2.18 nM). The competitive nature of binding for bicyclic and tricyclic analogues of MLA contrasts with results obtained for monocyclic E-ring analogues, which are not effective competitive inhibitors.[36] Studies on rat brain α7 nAChR showed little inhibition of [^{125}I]α-bungarotoxin binding by these simple analogues ($IC_{50} \geqslant 177$ μM) under similar conditions.[36] Taken together these results suggest that the incorporation of a tricyclic ring system increases the potency of competitive inhibitors at the α7 nAChR.

In contrast to competitive antagonism of the α7 nAChR, compound **2** displayed non-competitive antagonism at the α4β2 receptor (Figs. 3c and 4b, IC_{50} = 15.4 μM). Previous studies on rat brain α4β2 nAChR showed little inhibition of [^{3}H]nicotine binding by simple bicyclic and tricyclic analogues and from this data it was concluded that these compounds retained selectivity for the α7 subtype.[35] The results obtained from functional assays performed on analogue **2** show that this analogue has non-competitive antagonist effects at the α4β2 receptor, which would not be apparent in a competitive binding assay.[35]

Bergmeier and co-workers[31,32,36] have synthesised simple E ring analogues of MLA that act as antagonists ($IC_{50} \geqslant 11.4$ μM) of bovine adrenal α3β4* nAChRs. However, competition binding assays against [^{3}H]-epibatidine at the α3β4* nAChR suggested a non-competitive mode of inhibition in contrast to the competitive binding observed for MLA at this receptor.[36] In this study, compound **2** showed intermediate behaviour acting as a mixed competitive and non-competitive antagonist at the α3β4 receptor expressed in oocytes (Figs. 3b and 4a, IC_{50} = 14.7 μM).

A number of alkaloids have been shown to have mixed inhibitory effects on nACh receptors including bicuculline.[37] Bicuculline was found to be a competitive antagonist at human α7 nACh receptors but displays non-competitive antagonism at rat α4β2 nAChRs expressed in oocytes. The two distinct antagonistic effects by **2** and bicuculline is presumably due to its binding at different binding sites, where one site is located within the ion channel of the receptors and the other is in the close proximity of, or at the same binding site as, acetylcholine.[37]

4. Conclusion

In conclusion we have reported the synthesis and biological evaluation of a number of MLA analogues. The results from functional assays indicate the tricyclic analogue **2** was more potent than bicyclic analogues **12** and **15** at all the receptors investigated. The stereochemistry of methoxy group in analogues **12** and **15** corresponding to the C1–OMe of MLA has little influence on the activity or selectivity of the analogues. Analogue **2** was an antagonist at all receptors studied but displayed different antagonist effects at each receptor subtype. The compound was a competitive inhibitor at the α7 subtype with a lower potency than the natural product MLA (**1**) and showed non-competitive inhibition of α3β4 and α4β2 receptors. This synthetic and functional study more clearly defines the biological effects of MLA analogues across a number of nAChR receptor subtypes and demonstrates that these analogues are not selective ligands for the α7 nAChR subtype.

5. Experimental

5.1. Chemistry

General experimental details have been reported elsewhere.[8]

5.1.1. Ethyl 3-[*N*-ethyl-*N*-(2-ethoxycarbonylethyl)amino]-propionate.[12] To a freshly prepared solution of ethylamine (22.5 g, 32.7 ml, 0.5 mol) in absolute ethanol (100 ml) at 0 °C, was added ethyl acrylate (148.7 g, 160.9 ml, 1.5 mol) and the mixture heated under reflux for 20 h. The mixture was then cooled to room temperature and concentrated in vacuo to give the crude product, which was purified by high vacuum distillation to give the title compound (110.4 g, 90%). Bp 105–107 °C/0.5 mmHg. (lit.[12] bp 126–128 °C/2 mmHg). δ_H (200 MHz; $CDCl_3$) 1.01 (3H, t, *J* 7.2, NCH_2CH_3), 1.23 (3H, t, *J* 7.2, OCH_2CH_3), 2.40–2.57 (6H, m, $3 \times NCH_2$), 2.78 (4H, t, *J* 7.2, $2 \times COCH_2$) and 4.10 (4H, q, *J* 7.2, $2 \times OCH_2CH_3$).

5.1.2. Ethyl 1-ethyl-4-oxo-piperidine-3-carboxylate 5.[12] To a solution of potassium *tert*-butoxide (3.43 g, 0.031 mol) in dry THF (150 ml) at 0 °C, under an atmosphere of nitrogen, was slowly added a solution of ethyl 3-[*N*-ethyl-*N*-(2-ethoxycarbonylethyl)amino]propionate (5.0 g, 0.020 mol) in dry THF (100 ml) over 10 min. The mixture was then allowed to warm to room temperature and stirred for a further 15 min, after which the reaction mixture was quenched by the addition of water (20 ml). The mixture was then concentrated to dryness in vacuo and the resulting crude solid was dissolved in 2 M HCl (100 ml) and washed with ethyl acetate (2×100 ml). The combined organic layers were then back-extracted with 2 M HCl (100 ml) and the combined acidic aqueous layers neutralised with 2.5 M NaOH then extracted with ethyl acetate (3×100 ml). The combined organic layers were then dried (Na_2SO_4) and concentrated in vacuo to give the title compound **5** (3.39 g, 84%), which was used without further purification. An analytical sample was prepared by treating a small sample of the piperidone **5** (250 mg) in dry ether (10 ml) with excess gaseous dry hydrogen chloride in order to precipitate the hydrochloride salt. The hydrochloride salt was recrystallised from ethanol-ether to give ethyl 1-ethyl-4-oxo-piperidine-3-carboxylate hydrochloride, mp 143–145 °C (lit.[12] mp 143–145 °C).

5.1.3. Ethyl (1*R,5*S**,6*S**)-3-ethyl-6-hydroxy-9-oxo-3-azabicyclo[3.3.1]nonane-1-carboxylate 6 and ethyl (1*R*,*, 5*S**,6*S**)-3-ethyl-6-hydroxy-9-oxo-3-azabicyclo[3.3.1]nonane-1-carboxylate 7.** To a solution of **5** (2.00 g, 10 mmol)

in dichloromethane (50 ml) at 0 °C was added 1,1,3,3-tetramethyl guanidine (1.39 g, 1.51 ml, 12.1 mmol) and the mixture stirred, under an atmosphere of nitrogen, for 5 min. Acrolein (0.84 g, 15 mmol) was then added dropwise over 5 min and the mixture stirred for 24 h. After this time the mixture was concentrated in vacuo and the resultant residue dissolved in dichloromethane (20 ml), washed with water (2 × 50 ml) and satd sodium bicarbonate solution (50 ml) and brine (50 ml) then dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by flash chromatography (1:1 hexane–ethyl acetate) to give **6** (0.94 g, 37 %) (R_f 0.55) as a yellow oil. ν_{max} (NaCl)/cm^{-1} 3423 (OH), 2968 (CH), 1731 (ester, C=O), 1715 (ketone, C=O), 1456 and 1260; δ_H (400 MHz; $CDCl_3$) 1.07 (3H, t, *J* 7.2, NCH_2CH_3), 1.24 (3H, t, *J* 7.1, OCH_2CH_3), 1.85–1.90 (1H, m, 7B-H), 2.07–2.13 (1H, m, 8A-H), 2.26–2.34 (1H, m, 8B-H), 2.35–2.42 (4H, m, 4B-H, NCH_2CH_3 and OH), 2.62 (1H, m, 5-H), 2.73–2.79 (1H, m, 7A-H), 2.90 (1H, dd, $J_{2B,8B}$ 1.7, J_{gem} 11.4, 2B-H), 3.14 (1H, dd, $J_{2A,4A}$ 2.5, J_{gem} 11.4, 2A-H), 3.49 (1H, dt, $J_{4A,2A}$ 2.5, $J_{4A,5}$ 2.5, J_{gem} 11.4, 4A-H), 4.11–4.15 (1H, m, 6-H) and 4.16 (2H, q, *J* 7.1, OCH_2CH_3); δ_C (100 MHz; $CDCl_3$) 12.5 (CH_3, NCH_2CH_3), 14.0 (CH_3, OCH_2CH_3), 29.9 (CH_2, C-7), 30.4 (CH_2, C-8), 50.8 (CH_2, NCH_2CH_3), 53.7 (CH, C-5), 53.8 (CH_2, C-4), 58.0 (quat., C-1), 61.2 (CH_2, C-2), 61.3 (CH_2, OCH_2CH_3), 72.2 (CH, C-6) 170.8 (quat., OC=O) and 209.7 (quat., C-9); *m/z* (EI) 255 (M^+, 29%), 238 (M−OH, 100), 226 (M−CH_2CH_3, 10) and 210 (M−OCH_2CH_3, 36); found: 255.1467. $C_{13}H_{21}NO_4(M^+)$ requires 255.1471.

A second fraction afforded **7** (0.93 g, 36%) (R_f 0.5) as a yellow oil. ν_{max} (NaCl)/cm^{-1} 3424 (OH), 2933 (CH), 1733 (ester, C=O), 1717 (ketone, C=O), 1452 and 1259; δ_H (400 MHz; $CDCl_3$) 1.01 (3H, t, *J* 7.2, NCH_2CH_3), 1.21 (3H, t, *J* 7.1, OCH_2CH_3), 1.64–1.69 (1H, m, 7B-H), 2.06 (1H, dd, $J_{8A,7A}$ 5.1, J_{gem} 14.0, 8A-H), 2.40 (2H, q, *J* 7.2, NCH_2CH_3), 2.46 (1H, dd, $J_{4B,5}$ 3.7, J_{gem} 11.4, 4B-H), 2.52–2.52 (1H, m, 5H), 2.75 (1H, ddd, $J_{8B,7B}$ 6.2, $J_{8B,7A}$ 14.0, J_{gem} 14.0, 8B-H), 2.88 (1H, d, J_{gem} 11.4, 2B-H), 3.02–3.11 (2H, m, 7A-H and OH), 3.15 (1H, d, J_{gem} 11.4, 4A-H), 3.21 (1H, d, J_{gem} 11.4, 2A-H), 4.18 (2H, q, *J* 7.1, OCH_2CH_3) and 4.40 (1H, d, *J* 3.0, 6-H); δ_C (100 MHz; $CDCl_3$) 12.4 (CH_3, NCH_2CH_3), 13.9 (CH_3, OCH_2CH_3), 28.7 (CH_2, C-7), 31.7 (CH_2, C-8), 50.6 (CH_2, NCH_2CH_3), 54.8 (CH, C-5), 56.1 (CH_2, C-4), 58.4 (quat., C-1), 61.0 (CH_2, OCH_2CH_3), 61.6 (CH_2, C-2), 76.2 (CH, C-6) 170.8 (quat., OC=O) and 210.8 (quat., C-9); *m/z* (EI) 255 (M^+, 24%), 238 (M−OH, 100), 226 (M−CH_2CH_3, 16) and 210 (M−OCH_2CH_3, 29); found: 255.1466. $C_{13}H_{21}NO_4$ (M^+) requires 255.1471.

5.1.4. Ethyl (1*R,5*S**,6*S**)-3-ethyl-6-methoxy-9-oxo-3-azabicyclo[3.3.1]nonane-1-carboxylate 4 and ethyl (1*R**,5*S**,6*R**)-3-ethyl-6-methoxy-9-oxo-3-azabicyclo[3.3.1]nonane-1-carboxylate 8.** To a solution of **6** and **7** (3.0 g, 11.8 mmol) and suspended silver(I) oxide (10.9 g, 47.0 mmol) in dichloromethane (60 ml) was added iodomethane (16.68 g, 7.32 ml, 0.118 mol) and the mixture stirred in a foil-covered sealed flask for 48 h. After this time the mixture was filtered through Celite and the solvents removed in vacuo to give the crude product mixture, which was purified by flash chromatography (4:1 hexane–ethyl acetate) to give **4** (R_f 0.35) (0.94 g, 30%) as a pale yellow oil. ν_{max} (NaCl)/cm^{-1} 2934 (CH), 1737 (ester, OC=O), 1720 (ketone, C=O), 1452 and 1259; δ_H (400 MHz; $CDCl_3$) 1.02 (3H, t, *J* 7.1, NCH_2CH_3), 1.24 (3H, t, *J* 7.1, OCH_2CH_3), 1.89–1.96 (1H, m, 7B-H), 2.03 (1H, ddd, $J_{8A,7A}$ 1.3, $J_{8A,7B}$ 7.0, J_{gem} 14.1, 8A-H), 2.20 (1H, m, 8B-H), 2.27–2.38 (3H, m, 4B-H and NCH_2CH_3), 2.72–2.75 (1H, m, 5-H), 2.83–2.94 (1H, m, 7A-H), 2.90 (1H, dd, $J_{2B,8B}$ 1.9, J_{gem} 11.4, 2B-H), 3.14 (1H, dd, $J_{2A,4A}$ 2.2, J_{gem} 11.4, 2A-H), 3.27 (3H, s, OCH_3), 3.35 (1H, ddd, $J_{4A,2A}$ 2.2, $J_{4A,5}$ 2.2, J_{gem} 11.2, 4A-H), 3.50–3.52 (1H, m, 6-H) and 4.13 (2H, q, *J* 7.1, OCH_2CH_3); δ_C (100 MHz; $CDCl_3$) 12.3 (CH_3, NCH_2CH_3), 13.9 (CH_3, OCH_2CH_3), 27.0 (CH_2, C-7), 30.3 (CH_2, C-8), 50.7 (CH and CH_2, C-5 and NCH_2CH_3), 53.1 (CH_2, C-4), 56.1 (CH_3, OCH_3), 58.1 (quat., C-1), 61.0 (2 × CH_2, C-2 and OCH_2CH_3), 81.4 (CH, C-6) 170.5 (quat., OC=O) and 208.9 (quat., C-9); *m/z* (EI) 269 (M^+, 46%), 254 (M−CH_3, 100), 240 (M−CH_2CH_3, 36), 238 (M−OCH_3, 69) and 224 (M−OCH_2CH_3, 42); found: 269.1625. $C_{14}H_{23}NO_4$ (M^+) requires M^+ 269.1627.

A second fraction afforded **8** (0.79 g, 25%) (R_f 0.30) as a pale yellow oil. ν_{max} (NaCl)/cm^{-1} 2933 (CH), 1735 (ester, OC=O), 1717 (ketone, C=O), 1458 and 1254; δ_H (400 MHz; $CDCl_3$) 1.03 (3H, t, *J* 7.2, NCH_2CH_3), 1.21 (3H, t, *J* 7.1, OCH_2CH_3), 1.70–1.75 (1H, m, 7B-H), 2.06 (1H, ddd, $J_{8A,7A}$ 3.1, $J_{8A,7B}$ 5.9, J_{gem} 13.7, 8A-H), 2.37 (2H, m, NCH_2CH_3), 2.46 (1H, dd, $J_{4B,5}$ 3.8, J_{gem} 11.5, 4B-H), 2.58–2.65 (2H, m, 5-H and 8B-H), 2.83 (1H, dd, $J_{2B,8B}$ 3.8, J_{gem} 11.4, 2B-H), 2.88–2.97 (1H, m, 7A-H), 3.07 (1H, d, J_{gem} 11.5, 4A-H), 3.21 (1H, dd, $J_{2A,4A}$ 2.2, J_{gem} 11.4, 2A-H), 3.26 (3H, s, OCH_3), 3.80 (1H, m, 6-H) and 4.09–4.18 (2H, m, OCH_2CH_3); δ_C (100 MHz; $CDCl_3$) 12.3 (CH_3, NCH_2CH_3), 14.0 (CH_3, OCH_2CH_3), 25.7 (CH_2, C-7), 31.5 (CH_2, C-8), 50.7 (CH_2, NCH_2CH_3), 51.5 (CH, C-5), 55.7 (CH_3, OCH_3), 56.4 (CH_2, C-4), 58.3 (quat., C-1), 60.9 (CH_2, OCH_2CH_3), 61.7 (CH_2, C-2), 84.9 (CH, C-6) 170.6 (quat., OC=O) and 209.2 (quat., C-9); *m/z* (EI) 269 (M^+, 33%), 254 (M−CH_3, 20), 240 (M−CH_2CH_3, 15), 238 (M−OCH_3, 100) and 224 (M−OCH_2CH_3, 30); found: 269.1624. $C_{14}H_{23}NO_4$ (M^+) requires 269.1627.

5.1.5. Ethyl (1*S,5*S**,6*S**)-3-ethyl-6-methoxy-9-methylidene-3-azabicyclo[3.3.1]nonane-1-carboxylate 9.** To *n*-BuLi (3.52 ml, 8.62 mmol, 2.45 M solution in hexane) was added dropwise to a suspension of methyltriphenylphosphonium bromide (3.85 g, 10.8 mmol) in dry THF (40 ml) at −78 °C. The reaction mixture was stirred at 0 °C for 15 min then cooled to −78 °C followed by the dropwise addition of a solution of **4** (1.15 g, 4.28 mmol) in dry THF (40 ml). The reaction mixture was allowed to warm to room temperature and stirred for 2 h. The reaction was quenched with distilled water (50 ml) and the solvent removed at reduced pressure. The residue was dissolved in dry ether (40 ml) and extracted with 2 M hydrochloric acid (3 × 50 ml). The aqueous extract was made basic with 10% sodium hydroxide solution then extracted with ethyl acetate (3 × 50 ml) then dried

($MgSO_4$) and concentrated in vacuo to leave the crude product, which was purified by flash chromatography (9:1 hexane–ethyl acetate) to give the **9** (0.697 g, 61%) as a pale yellow oil. ν_{max} (NaCl)/cm^{-1} 2926 (CH), 1726 (C=O), 1654 (C=C), 1451 and 1255; δ_H (200 MHz; $CDCl_3$) 0.99 (3H, t, *J* 7.2, NCH_2CH_3), 1.19 (3H, t, *J* 7.1, OCH_2CH_3), 1.55–1.76 (2H, m, 7B-H and 8A-H), 2.13–2.19 (2H, m, 4B-H and 8B-H), 2.28 (2H, q, *J* 7.2, NCH_2CH_3), 2.46 (1H, d, J_{gem} 11.2, 2B-H), 2.49–2.51 (1H, br s, 5-H), 2.57–2.72 (1H, m, 7A-H), 2.87 (1H, d, J_{gem} 11.2, 2A-H), 2.95 (1H, d, J_{gem} 11.2, 4A-H), 3.26 (3H, s, OCH_3), 3.43–3.44 (1H, m, 6-H), 4.13 (2H, q, *J* 7.1, OCH_2CH_3), 4.56 (1H, br s, 10A-H) and 4.77 (1H, br s, 10B-H); δ_C (100 MHz; $CDCl_3$) 12.3 (CH_3, NCH_2CH_3), 14.1 (CH_3, OCH_2CH_3), 25.9 (CH_2, C-7), 30.7 (CH_2, C-8), 45.1 (CH, C-5), 49.9 (quat., C-1), 51.5 (CH_2, NCH_2CH_3), 55.7 (CH_3, OCH_3), 57.6 (CH_2, C-4), 60.3 (CH_2, C-2), 61.9 (CH_2, OCH_2), 82.2 (CH, C-6), 106.3 (CH_2, C-10), 147.6 (quat., C-9) and 174.0 (quat., OC=O); *m/z* (EI) 267 (M^+, 25%), 252 (M−CH_3, 54), 238 (M−CH_2CH_3, 20), 236 (M−OCH_3, 30), 222 (M−OCH_2CH_3, 7) and 57 (100); found: 267.1834. $C_{15}H_{25}NO_3$ (M^+) requires 267.1834.

5.1.6. (1*S,5*S**,6*S**)-(3-Ethyl-6-methoxy-9-methyidene-3-azabicyclo[3.3.1]non-1-yl)methanol 10.** To a solution of **9** (545 mg, 2.04 mmol) in dry THF (50 ml) was added lithium aluminium hydride (155 mg, 4.08 mmol) and the mixture stirred, under an atmosphere of nitrogen, for 10 min. The reaction was then quenched by dropwise addition of water (20 ml), the volatiles removed in vacuo. The remaining aqueous solution was extracted with ethyl acetate (2 × 30 ml) and the combined organic layers washed with brine (50 ml) then dried ($MgSO_4$) and concentrated in vacuo to give the crude product which was purified by flash chromatography (1:1 hexane–ethyl acetate) to give **10** (412 mg, 90%) as a clear oil. ν_{max} (NaCl)/cm^{-1} 3444 (OH), 2918 (CH), 1649 (C=C), 1451 and 1241; δ_H (200 MHz; $CDCl_3$) 0.94 (3H, t, *J* 7.1, NCH_2CH_3), 1.21 (1H, dddd, $J_{7B,8A}$ 2.3, $J_{7B,8B}$ 6.9, $J_{7B,6}$ 6.9, J_{gem} 13.2, 7B-H), 1.69–1.83 (2H, m, 4B-H and 8A-H), 1.86–1.97 (2H, m, 2B-H and 8B-H), 2.17 (2H, m, NCH_2CH_3), 2.52–2.53 (1H, m, 5-H), 2.57–2.76 (2H, m, 7A-H and OH), 2.90 (1H, d, J_{gem} 10.4, 2A-H), 3.12 (1H, d, J_{gem} 10.6, 4A-H), 3.26 (3H, s, OCH_3), 3.27–3.32 (1H, m, 6′H), 3.43 (2H, m, CH_2OH), 4.48 (1H, br s, 10A-H) and 4.70 (1H, br s, 10B-H); δ_C (50 MHz; $CDCl_3$) 12.3 (CH_3, NCH_2CH_3), 27.4 (CH_2, C-7), 33.0 (CH_2, C8), 41.5 (quat., C-1), 44.4 (CH, C-5), 51.8 (CH_2, NCH_2CH_3), 53.6 (CH_2, C-4), 55.7 (CH_3, OCH_3), 61.7 (CH_2, C-2), 68.1 (CH_2, CH_2OH), 82.9 (CH, C-6), 103.6 (CH_2, C-10) and 152.0 (quat., C-9); *m/z* (EI) 225 (M^+, 52%), 210 (M−CH_3, 70), 208 (M−OH, 51), 194 (M−CH_2OH, 48) and 58 (100); found: 225.1722. $C_{13}H_{23}NO_2$ (M^+) requires 225.1729.

5.1.7. (1′*S,5′*S**,6′*S**)-(3′-Ethyl-6′-methoxy-9′-methylidene-3′-azabicyclo[3.3.1]non-1′-yl)methyl 2-aminobenzoate.** The reaction was carried out according to the standard procedure[7] using **10** (412 mg, 1.83 mmol), *N*-(trifluoroacetyl)anthranilic acid (**11**) (1.29 g, 5.53 mmol), 4(dimethylamino)pyridine (113 mg, 0.925 mmol), 1,3-dicyclohexylcarbodiimide (1.14 g, 5.53 mmol) and sodium borohydride (140 mg, 3.69 mmol) using 3:2 hexane–ethyl acetate as solvent for flash chromatography to afford the title compound (481 mg, 76%) as a clear oil. ν_{max} (NaCl)/cm^{-1} 3475 and 3370 (NH_2), 2924 (CH), 1693 (C=O), 1651 (C=C), 1619, 1589, 1561, 1454, 1293 and 1243; δ_H (200 MHz; $CDCl_3$) 1.07 (3H, t, *J* 7.1, NCH_2CH_3), 1.49 (1H, dddd, $J_{7'B,8'A}$ 2.1, $J_{7'B,8'B}$ 6.4, $J_{7'B,6'}$ 6.4, J_{gem} 13.2, 7′B-H), 1.85–1.97 (1H, m, 8′A-H), 1.99–2.11 (2H, m, 4′B-H and 8′B-H), 2.23–2.38 (3H, m, 2′B-H and NCH_2CH_3), 2.65–2.67 (1H, m, 5′-H), 2.73–2.92 (1H, m, 7′A-H), 3.08 (1H, d, J_{gem} 10.3, 2′A-H), 3.31 (1H, d, J_{gem} 10.9, 4′A-H), 3.38 (3H, s, OCH_3), 3.42–3.47 (1H, m, 6′-H), 4.24 (2H, br s, OCH_2), 4.68 (1H, br s, 10′A-H), 4.87 (1H, br s, 10′B-H), 5.84 (2H, br, NH_2), 6.62–6.69 (2H, m, 3-H and 5-H), 7.25 (1H, td, *J* 7.8, 1.6, 4-H) and 7.86 (1H, dd, *J* 1.6, 8.2, 6-H); δ_C (50 MHz; $CDCl_3$) 12.3 (CH_3, NCH_2CH_3), 27.5 (CH_2, C-7′), 33.3 (CH_2, C-8′), 40.5 (quat., C-1′), 44.3 (CH, C-5′), 51.7 (CH_2, NCH_2CH_3), 53.4 (CH_2, C-4′), 55.7 (CH_3, OCH_3), 62.2 (CH_2, C-2′), 69.0 (CH_2, OCH_2), 82.5 (CH, C-6′), 104.0 (CH_2, C-10′), 110.3 (quat., C-1), 115.9 (CH, C-3), 116.4 (CH, C-5), 130.8 (CH, C-6), 133.9 (CH, C-4), 150.5 (quat., C-2), 151.2 (quat., C-9′) and 167.7 (quat., OC=O); *m/z* (EI) 344 (M^+, 45%), 329 (M−CH_3, 34), 313 (M−OCH_3, 13), 208 (M−$NH_2C_6H_4CO_2$, 100) and 120 ($NH_2C_6H_4CO$, 65); found: 344.2105. $C_{20}H_{28}N_2O_3$ (M^+) requires 344.2100.

5.1.8. (1″*S,5″*S**,6″*S**,3′ *R**)- and (1″*S**,5″*S**,6″*S**,3′*S**)-(3″-Ethyl-6″-methoxy-9″-methylidene-3″-azabicyclo[3.3.1]-non-1″-yl)methyl 2-(3′-methyl-2′,5′-dioxopyrrolidin-1′-yl)-benzoate 12.** The reaction was carried out according to the standard procedure[7] using (1′*S**,5′*S**,6′*S**)-(3′-ethyl-6′-methoxy-9′-methylidene-3′-azabicyclo[3.3.1]non-1′-yl)methyl 2-aminobenzoate. (200 mg, 0.580 mmol) and methylsuccinic anhydride (267 mg, 2.34 mmol), using 100% ethyl acetate as solvent for flash chromatography to afford **12** (216 mg, 84%) as a reddish oil. ν_{max} (NaCl)/cm^{-1} 2932 (C–H), 1782 (N–C=O), 1713 (C=O), 1603, 1493, 1454, 1262 and 1185; δ_H (200 MHz; $CDCl_3$) 1.04 (3H, t, *J* 7.0, NCH_2CH_3), 1.31–1.44 (4H, m, 3′-CH_3 and 7″B-H), 1.84–1.96 (2H, m, 4″B-H and 8″A-H), 2.01–2.08 (2H, m, 8″B-H and 2″B-H) 2.28 (2H, q, *J* 7.0, NCH_2CH_3), 2.49–2.55 (1H, m, 3′-H), 2.63–2.64 (1H, br s, 5″-H), 2.70–2.85 (1H, m, 7″A-H), 2.99–3.09 (3H, br m, 2″A-H and 4′-CH_2), 3.26 (1H, d, J_{gem} 11.0, 4″A-H), 3.34 (3H, s, OCH_3), 3.35–3.37 (1H, m, 6″-H), 4.17 (2H, br s, OCH_2), 4.61 (1H, br s, 10″A-H), 4.83 (1H, br s, 10″B-H), 7.24 (1H, d, *J* 7.7, 3-H) 7.50 (1H, t, *J* 7.6, 5-H), 7.64 (1H, t, *J* 7.6, 4-H) and 8.07 (1H, d, *J* 7.6, 6-H); δ_C (50 MHz; $CDCl_3$) 12.1 (CH_3, NCH_2CH_3), 16.1 (CH_3, 3′-CH_3), 27.3 (CH_2, C-7″), 33.2 (CH_2, C-8″), 35.0 (CH, C-3′), 36.7 (CH_2, C-4′), 40.4 (quat., C-1″), 44.2 (CH, C-5″), 51.7 (CH_2, NCH_2CH_3), 53.2 (CH_2, C-4″), 55.7 (CH_3, OCH_3), 61.8 (CH_2, C-2″), 69.9 (CH_2, OCH_2), 82.3 (CH, C-6″), 104.2 (CH_2, C-10″), 126.9 (quat., C-1), 129.1 (CH, C-5), 129.6 (CH, C-3), 131.1 (CH, C-6), 132.7 (quat., C-2), 133.2 (CH, C-4), 150.7 (quat., C-9″), 163.9 (quat., OC=O), 175.7 (quat., C-5′) and 179.6 (quat., C-2′); *m/z* (EI) 440 (M^+, 34%), 425 (M−CH_3, 52), 409 (M−OCH_3, 23), 224

($M-C_{12}H_{10}O_3N$, 35), 216 ($C_{12}H_{10}O_3N$, 46) and 208 ($M-C_{12}H_{10}O_4N$, 100); found: 440.2316. $C_{25}H_{32}N_2O_5$ (M^+) requires 440.2311.

5.1.9. Ethyl (1*S,5*S**,6*R**)-3-ethyl-6-methoxy-9-methylidene-3-azabicyclo[3.3.1]nonane-1-carboxylate 13.** The reaction was carried out as described for **4**, with *n*-BuLi (0.90 ml, 2.2 mmol, 2.45 M solution in hexane), methyltriphenylphosphonium bromide (981 mg, 2.75 mmol) and **8** (294 mg, 1.09 mmol), using 9:1 hexane–ethyl acetate for flash chromatography to give **13** (194 mg, 67%) as a pale yellow oil. ν_{max} (NaCl)/cm^{-1} 2975 (CH), 1727 (ester, OC=O), 1651 (C=C), 1453 and 1253; δ_H (200 MHz; $CDCl_3$) 0.96 (3H, t, *J* 7.1, NCH_2CH_3), 1.20 (3H, t, *J* 7.0, OCH_2CH_3), 1.74–1.89 (1H, m, 7B-H), 2.00 (1H, ddd, $J_{8A,7B}$ 2.0, $J_{8A,7A}$ 6.4, J_{gem} 12.7, 8A-H), 2.09–2.34 (4H, m, 4B-H, 8B-H and NCH_2CH_3), 2.47 (1H, d, J_{gem} 10.6, 2B-H), 2.55–2.61 (1H, m, 5-H), 2.61–2.79 (1H, m, 7A-H), 2.95 (1H, d, J_{gem} 10.7, 2A-H), 3.16 (1H, d, J_{gem} 10.6, 4A-H), 3.26 (3H, s, OCH_3), 3.29–3.40 (1H, m, 6-H), 4.10 (2H, q, *J* 7.0, OCH_2CH_3), 4.49 (1H, br s, 10A-H) and 4.74 (1H, br s, 10B-H); δ_C (50 MHz; $CDCl_3$) 12.2 (CH_3, NCH_2CH_3), 14.0 (CH_3, OCH_2CH_3), 27.6 (CH_2, C-7), 32.9 (CH_2, C-8), 43.7 (CH, C-5), 49.9 (quat., C-1), 51.6 (CH_2, NCH_2CH_3), 53.3 (CH_2, C-4), 55.7 (CH_3, OCH_3), 60.3 (CH_2, OCH_2CH_3), 61.3 (CH_2, C-2), 82.0 (CH, C-6) 105.7 (CH_2, C-10), 149.1 (quat., C-9) and 173.8 (quat., OC=O); *m/z* (EI) 267 (M^+, 67%), 252 ($M-CH_3$, 90), 238 ($M-CH_2CH_3$, 49), 236 ($M-OCH_3$, 72), 222 ($M-OCH_2CH_3$, 21) and 58 (100); found: 267.1827. $C_{15}H_{25}NO_3$ (M^+) requires 267.1834.

5.1.10. (1*S,5*S**,6*R**)-(3-Ethyl-6-methoxy-9-methylidene-3-azabicyclo[3.3.1]non-1-yl)methanol 14.** The reaction was carried out as described for **9**, with **13** (215 mg, 0.80 mmol) and lithium aluminium hydride (61 mg, 1.61 mmol), using 1:1 hexane–ethyl acetate for flash chromatography to give **14** (171 mg, 94%) as a clear oil. ν_{max} (NaCl)/cm^{-1} 3422 (OH), 2922 (CH), 1651 (C=C), 1352 and 1240; δ_H (200 MHz; $CDCl_3$) 0.99 (3H, t, *J* 7.1, NCH_2CH_3), 1.56–1.73 (2H, m, 7B-H and 8A-H), 1.94 (1H, d, J_{gem} 11.3 4B-H) 2.13 (1H, dd, $J_{8B,7B}$ 3.6, J_{gem} 10.8, 8B-H), 2.25 (2H, q, *J* 7.1, NCH_2CH_3), 2.46–2.49 (2H, m, 2B-H and 5-H), 2.60–2.70 (2H, m, 7A-H and OH), 2.89 (1H, d, J_{gem} 11.3, 4A-H), 2.93 (1H, d, J_{gem} 9.9, 2A-H), 3.24 (3H, s, OCH_3), 3.43 (1H, d, *J* 2.7, 6-H), 3.50 (2H, br s, OCH_2), 4.54 (1H, br s, 10A-H) and 4.72 (1H, br s, 10B-H); δ_C (50 MHz; $CDCl_3$) 12.3 (CH_3, NCH_2CH_3), 26.1 (CH_2, C-7), 31.1 (CH_2, C-8), 41.7 (quat., C-1), 45.7 (CH, C-5), 51.8 (CH_2, NCH_2CH_3), 55.8 (CH_3, OCH_3), 57.7 (CH_2, C-4), 62.5 (CH_2, C-2), 68.4 (CH_2, CH_2OH), 82.8 (CH, C-6), 104.1 (CH_2, C-10) and 150.4 (quat., C-9); *m/z* (EI) 225 (M^+, 40%), 210 ($M-CH_3$, 62), 208 ($M-OH$, 41), 194 ($M-CH_2OH$, 35) and 58 (100); found: 225.1734. $C_{13}H_{23}NO_2$ (M^+) requires 225.1729.

5.1.11. (1′*S,5′*S**,6′*R**)-(3′-Ethyl-6′-methoxy-9′-methylidene-3′-azabicyclo[3.3.1]non-1′-yl)methyl 2-aminobenzoate.** The reaction was carried out according to the standard procedure[7] using **14** (276 mg, 1.22 mmol), *N*-(trifluoroacetyl)anthranilic acid (**11**) (864 mg, 3.71 mmol), 4(dimethylamino)pyridine (76 mg, 0.62 mmol), 1,3-dicyclohexylcarbodiimide (763 mg, 3.70 mmol) and sodium borohydride (94 mg, 2.47 mmol), using 3:2 hexane–ethyl acetate as solvent for flash chromatography to afford the title compound (320 mg, 76%) as a pale yellow oil. ν_{max} (NaCl)/cm^{-1} 3464 and 3370 (NH_2), 2918 (CH), 1689 (C=O), 1617, 1589, 1560, 1454, 1294 and 1243; δ_H (200 MHz; $CDCl_3$) 1.04 (3 H, t, *J* 7.1, NCH_2CH_3), 1.66–1.77 (1H, m, 7′B-H), 1.88 (1H, m, 8′A-H), 2.06 (1H, d, J_{gem} 9.6, 4′B-H), 2.17–2.34 (4H, m, 2′B-H, 8′B-H and NCH_2CH_3), 2.59 (1H, br s, 5′-H), 2.67–2.86 (1H, m, 7′A-H), 2.98 (1H, d, J_{gem} 9.6, 4′A-H), 3.06 (1H, d, J_{gem} 10.6, 2′A-H), 3.33 (3H, s, OCH_3), 3.52 (1H, m, 6′-H), 4.24 (2H, br s, OCH_2), 4.72 (1H, br s, 10′A-H), 4.86 (1H, br s, 10′B-H), 5.72 (2H, br, NH_2), 6.61–6.68 (2H, m, 3-H and 5-H), 7.26 (1H, td, *J* 7.2, 1.6, 4-H) and 7.84 (1H, dd, *J* 1.6, 8.6, 6-H); δ_C (50 MHz; $CDCl_3$) 12.4 (CH_3, NCH_2CH_3), 26.2 (CH_2, C-7′), 31.6 (CH_2, C-8′), 40.7 (quat., C-1′), 45.7 (CH, C-5′), 51.8 (CH_2, NCH_2CH_3), 55.9 (CH_3, OCH_3), 57.5 (CH_2, C-4′), 63.0 (CH_2, C-2′), 69.5 (CH_2, OCH_2), 82.7 (CH, C-6′), 104.8 (CH_2, C-10′), 110.8 (quat., C-1), 116.2 (CH, C-3), 116.6 (CH, C-5), 131.1 (CH, C-6), 134.0 (CH, C-4), 149.9 (quat., C-2), 150.5 (quat., C-9′) and 168.0 (quat., OC=O); *m/z* (EI) 344 (M^+, 50%), 329 ($M-CH_3$, 32), 313 ($M-OCH_3$, 12), 208 ($M-NH_2C_6H_4CO_2$, 100) and 120 ($NH_2C_6H_4CO$, 67); found: 344.2092. $C_{20}H_{28}N_2O_3$ (M^+) requires 344.2100.

5.1.12. (1″*S,5″*S**,6″*R**,3′*R**)- and (1″*S**,5″*S**,6″*R**,3′*S**)-(3″-Ethyl-6″-methoxy-9″-methylidene-3″-azabicyclo[3.3.1]-non-1″-yl)methyl 2-(3′-methyl-2′,5′-dioxopyrrolidin-1′-yl)-benzoate 15.** The reaction was carried out according to the standard procedure[7] using (1′*S**,5′*S**,6′*R**)-(3′-ethyl-6′-methoxy-9′-methyidene-3′-azabicyclo[3.3.1]non-1′-yl)methyl 2-aminobenzoate (40 mg, 0.116 mmol) and methylsuccinic anhydride (53 mg, 0.465 mmol) using 100% ethyl acetate as solvent for flash chromatography to afford **15** (41 mg, 80%) as an orange oil. ν_{max} (NaCl)/cm^{-1} 2932 (C–H), 1781 (N–C=O), 1715 (C=O), 1602, 1492, 1453, 1259 and 1187; δ_H (200 MHz; $CDCl_3$) 1.00 (3H, t, *J* 7.1, NCH_2CH_3), 1.24–1.45 (4H, m, 3′-CH_3 and 7″B-H), 1.60–1.72 (2H, m, 4″B-H and 8″A-H), 2.02–2.03 (1H, m, 8″B-H), 2.21–2.28 (3H, m, 2″B-H and NCH_2CH_3), 2.56–2.76 (3H, br m, 3′-H, 5″-H and 7″A-H), 3.02–3.17 (4H, br m, 2″A-H, 4″A-H and 4′-CH_2), 3.30 (3H, s, OCH_3), 3.51 (1H, br s, 6″-H), 4.19 (2H, s, OCH_2), 4.65 (1H, br s, 10″A-H), 4.84 (1H, br s, 10″B-H), 7.24 (1H, d, *J* 7.6, 3-H), 7.51 (1H, t, *J* 7.6, 5-H), 7.65 (1H, t, *J* 7.6, 4-H) and 8.08 (1H, d, *J* 7.6, 6-H); δ_C (50 MHz; $CDCl_3$) 12.3 (CH_3, NCH_2CH_3), 16.3 (CH_3, 3′-CH_3), 26.1 (CH_2, C-7″), 31.4 (CH_2, C-8″), 35.2 (CH_2, C-4′), 36.9 (CH, C-3′), 40.6 (quat., C-1″), 45.6 (CH, C-5″), 51.7 (CH_2, NCH_2CH_3), 55.9 (CH_3, OCH_3), 57.4 (CH_2, C-4″), 62.8 (CH_2, C-2″), 70.3 (CH_2, OCH_2), 82.6 (CH, C-6″), 104.8 (CH_2, C-10″), 127.2 (quat., C-1), 129.3 (CH, C-5), 129.8 (CH, C-3), 131.4 (CH, C-6), 132.8 (quat., C-2), 133.4 (CH, C-4), 149.3 (quat., C-9″), 164.2 (quat., OC=O), 175.7 (quat., C-5′) and 179.9 (quat., C-2′); *m/z* (EI) 440 (M^+, 22%), 425 ($M-CH_3$, 46), 409 ($M-OCH_3$, 17), 224 ($M-C_{12}H_{10}O_3N$, 18), 216

($C_{12}H_{10}O_3N$, 64) and 208 ($M-C_{12}H_{10}O_4N$, 100); found: 440.2318. $C_{25}H_{32}N_2O_5$ (M^+) requires 440.2311.

5.2. Pharmacology

5.2.1. Expression of nicotinic AChRs in *Xenopus* oocytes by cytoplasmic injection of cRNA. *Xenopus laevis* were anaesthetised with 0.17% ethyl 3-aminobenzoate and a lobe of an ovary was removed and rinsed with oocyte releasing buffer, OR2 (82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2{\cdot}6H_2O$, 5 mM HEPES, pH 7.5). It was then treated with Collagenase A (2 mg/ml or OR2, Bohringer Manheim) for 2 h. The released oocytes were rinsed in modified frog Ringer solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2{\cdot}6H_2O$, 1.8 mM $CaCl_2$, 5 mM HEPES, 2.5 mM pyruvate, 0.5 mM theophylline, 50 ng/ml gentamycin, pH 7.5). Stage V–VI oocytes were collected and stored in this buffer.

Rat α7, α4, α3, β4 and β2 pcDNA were provided by Associate Professor Jim Boutler (Neuropsychiatric Institute, University of California, Los Angeles, USA); Plasmid containing the α7 was linearised with restriction enzyme Sma1, similarly α3 and α4 were linearised with ECOR1; β2 and β4 were linearised with Hind3 and Xho-I, respectively. cRNA was synthesised using the 'mMessage mMachine' kit from Ambion (Austin, TX, USA). cRNA was mixed in a ratio of 1α3:1β4 and 2α4:β2) and injected into defolliculated oocytes at a concentration of 50 ng/50 nl. Oocytes were stored for 2–5 days at 18 °C.

5.2.2. Electrophysiology. Receptor activity was measured by two electrode voltage clamp recording using a Geneclamp 500 amplifier (Axon Instruments, Foster City, CA, USA), a MacLab 2e recorder (AD Instruments, Sydney, NSW, Australia) and Chart Version 4.0.1 program. Oocytes were voltage clamped at −60 mV and continuously superfused with frog Ringer solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2{\cdot}6H_2O$, 1.8 mM $CaCl_2$, 5 mM HEPES) supplemented with 1 μM atropine. For receptor activation measurements, the indicated concentrations of drug were added to the buffer solution. Test compounds were applied to oocytes at intervals of 15–20 min to minimise effects of desensitisation.

5.2.3. Data analysis. The amplitude of the current (I) recorded in response to each drug was normalised to the maximum amplitude (Im) of the current response to ACh (α7, 3 mM; α4β2, 30 μM; α3β4, 300 μM). Normalised concentration-response data were analysed using the GraphPad 'Prism' version 3.02, plotted on a semi-logarithmic scale and analysed using the Gaussian distribution equation. The equation used was

$$Y = (\text{Area}/[\text{SD}(2\text{pi})^{0.5}]\exp(-0.5(X-\text{mean})\text{SD})^2)$$

The EC_{50} value (the concentration resulting in half of the maximum normalised response) is determined by fitting data from the individual oocytes using sigmoidal fit (variable slope)—GraphPad 'Prism' (3.02) and expressed as mean ± SEM. IC_{50} (the inhibition dose that blocks half of the maximum normalised response) is determined in a similar way to the EC_{50} value. The equation used was

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom})/1 + 10^{(\log \text{EC}_{50} - X)\text{Hillslope}}$$

Where X is the logarithm of concentration; Y is the response; Y starts at the bottom and goes to top with a sigmoidal shape. This is identical to the 'four parameter logistic equation'.

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