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Design, synthesis and binding affinity of acetylcholine carbamoyl analogues

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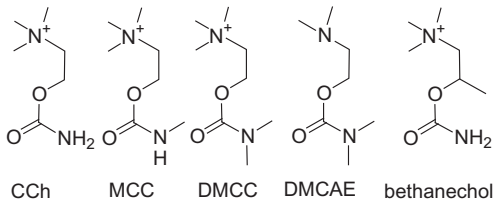
ABSTRACT

A series of acetylcholine carbamoyl analogues, cyclised at the carbamate moiety or at the cationic head or at both, were tested for binding affinity at muscarinic and neuronal nicotinic receptors (nAChRs). While no muscarinic affinity was found, submicromolar K_i values, similar to that of carbachol, were measured at $\alpha_4\beta_2$ nAChRs for the enantiomers of 5-dimethylaminomethyl- and 5-trimethylammoniomethyl-2-oxazolidinone, **2** and **2a**, and for (*S*)-*N*-methylprolinol carbamate (*S*)-**3**. Methylation of oxazolidinone nitrogen of **2** and **2a** and of *N*-methylprolinol nitrogen of (*S*)-**3** and, even more, hybridization of cyclic carbamate substructure (oxazolidinone) with cyclic cationic head (*N*-methylpyrrolidine) markedly lower the nicotinic affinity. Docking results were consistent with SAR analysis highlighting the interaction capabilities of (*R*)-**2a** and (*S*)-**3** and the negative effect of intracyclic nitrogen methylation and of double cyclisation.

The physiological effects of the neurotransmitter acetylcholine (ACh) are mediated by muscarinic ACh receptors (mAChRs) and nicotinic ACh receptors (nAChRs). Agonists at neuronal α4β2 and α7 nAChRs, in particular at the α4β2 subtype, are intensively studied nowadays because of their potential application in the therapy of a number of nervous-system disorders, such as Alzheimer's disease, schizophrenia, depression, attention deficit hyperactivity disorder, pain and tobacco addiction[1–5] In the treatment of this latter, varenicline, a partial α4β2 agonist, is widely used as an aid to smoking cessation[4] while the other prospective uses are currently investigated by developing new more potent and selective α4β2 ligands[6,7].

Much attention has been devoted to the replacement of ACh ester moiety by more stable carbamate group in order to differentiate the muscarinic/nicotinic agonist profile of the endogenous neurotransmitter and, furthermore, to gain α4β2 selectivity over α7 omomeric nAChR and other α and β subunits containing neuronal nAChRs [8–11].

Carbamoylcholine (or carbachol, CCh), which activates both muscarinic and neuronal nicotinic receptors, becomes a selective nicotinic ligand when methylated at the carbamic nitrogen (*N*-methylcarbamoylcholine, MCC) and the selectivity towards nicotinic over muscarinic receptors is further enhanced by double N-methylation (*N,N*-dimethylcarbamoylcholine, DMCC) (Table 1)[9]. The tertiary amine corresponding to DMCC, namely *N,N*-dimethylcarbamoyl-*N,N*-dimethylaminoethanol (DMCAE), loses the nicotinic selectivity displaying modest affinities at both nicotinic and muscarinic receptors (Table 1)[9] On the other hand, β-methylation of the choline portion converts CCh into a muscarinic agent[12] bethanechol, which is devoid of nicotinic activity.



The switch in nicotinic/muscarinic affinity and selectivity resulting from such simple modifications of ACh carbamoyl analogues has been explored through a number of analogues conformationally restricted at one of the two groups, carbamate or ammonium/amine[8,9,11] This notwithstanding, the cyclic carbamates **1**, **1a** and **2a**, formally obtained by mere cyclization of the carbamic moiety of DMCAE, DMCC, and MCC or bethanechol, respectively, as well as the quaternised carbamoyl prolinol **3a**,

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Table 1
Binding affinities of CCh, MCC, DMCAE, DMCC and bethanechol at native nAChRs (α4β2 and α7) and native mAChRs (M1/M5)

	α4β2-nAChR K_i μM	α7-nAChR K_i(μM)	M1/M5-mAChR K_i (μM)
CCh	0.750[a]	66[b]	0.005[a]
MCC	0.023[a]	44[a]	0.150[a]
DMCC	0.020[a]	N.D.	1.2[a]
DMCAE	5.7[a]	N.D.	18[a]
Bethanechol	N.D.	N.D.	0.097[c]

N.D. Not determined.
[a] See Ref. 9.
[b] See Ref. 13.
[c] See Ref. 12.

cyclic analogue of CCh, have never been synthesized or characterized for their nicotinic and muscarinic affinity (Fig. 1).

A convergent interest in these compounds arose from our studies on novel nicotinoids, in which the *N*-methyl-2-pyrrolidinyl residue is directly linked to hydrogen bond acceptor and π-electron-rich (HBA-π) groups alternative to nicotine 3-pyridyl[14–17] Among these, somewhat unexpectedly, very modest nicotinic affinities are displayed at α4β2 and α7 neuronal nAChRs by all the stereoisomers of 5-(*N*-methyl-2-pyrrolidinyl)-2-oxazolidinone **4** (Fig. 1), though consisting of a typical HBA-π group for nicotinoids, such as oxazolidinone[15,16] Compound **4**, unlike compounds **1**–**3**, is conformationally restricted at both the groups, the carbamate and the tertiary amine being both cyclic. Therefore, **1**–**3** represent also the attempt to restore the interaction capability of the two substructures by alternately making one of them linear and thus the whole molecule less rigid. Furthermore, we considered the pyrrolidinyl oxazolidinone **5**, which is a positional isomer of **4**, resulting from shifting 2-pyrrolidinyl from C(5) to C(4) of oxazolidinone, that is from exchanging O and NH positions in this heterocycle (Fig. 1).

The title compounds were prepared in unichiral form. In particular, we studied both the enantiomers of the oxazolidinone derivatives **1**, **1a**, **2** and **2a**, while limiting our investigation to the stereoisomers having *S* configuration at the pyrrolidine in the case of compounds **3**, **3a** and **5**. As shown in Scheme 1 (path A), (*S*)-**1** was prepared from (*R*)-**2** by methylation of oxazolidinone nitrogen through reaction with sodium hydride and iodomethane in DMF[18] Successive treatment with iodomethane in DCM transformed the tertiary amine (*S*)-**1** into the quaternary ammonium (*R*)-**1a**[19] By

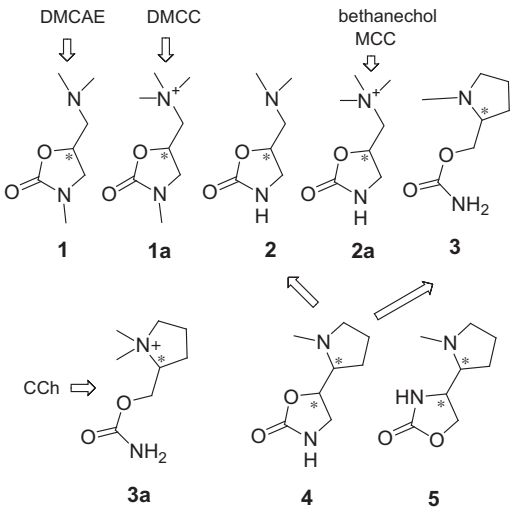
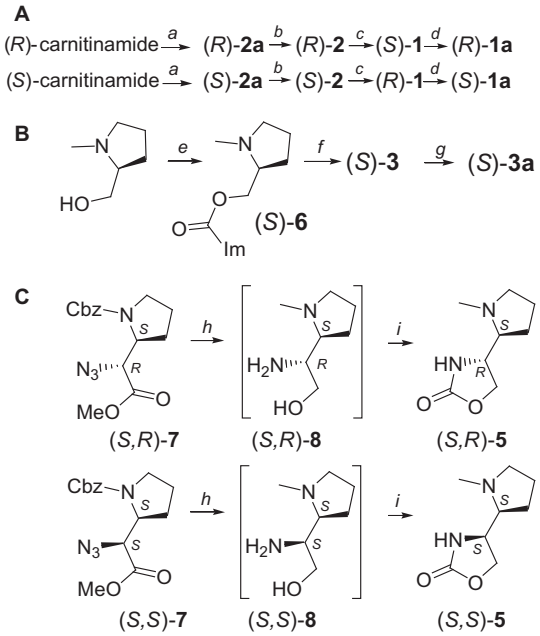


Figure 1. Chemical structures of the designed ACh carbamoyl analogues.



Scheme 1. Reagents, conditions: and yields. Path A: synthesis of oxazolidinone derivatives **1** and **1a**; (a) see Ref. 22; (b) see Ref. 22; (c) NaH, DMF, −15 °C, 10 min and CH_3I (1 equiv), 10 °C, 30 min, 71% [(*S*)-**1**] and 75% [(*R*)-**1**]; (d) CH_3I, DCM, room temperature, 1 h, quantitative yield. Path B: synthesis of pyrrolidine derivatives (*S*)-**3** and (*S*)-**3a**; (e) carbonyldiimidazole (1 equiv), anhydrous CH_3CN, room temperature, 1 h; (f) NH_3 conc., CH_3CN, room temperature, 1 h, 79% (overall yield *N*-methylprolinol → (*S*)-**3**); (g) CH_3I, DCM, room temperature, 1 h, quantitative yield. Path C: synthesis of pyrrolidinyl oxazolidinones (*S,R*)-**5** and (*S,S*)-**5**; (h) $LiAlH_4$, THF, −10 °C and room temperature, 30 min; (i) carbonyldiimidazole, THF, room temperature, 3 h, 47% (overall yield methyl 1-Cbz-2-pyrrolidinyl-α-azidoacetate → (*S,R*)-5) and 51% (overall yield methyl 1-Cbz-2-pyrrolidinyl-α-azidoacetate → (*S,S*)-5).

the same sequence, we obtained the *R* enantiomer of **1** and the *S* enatiomer of **1a** from (*S*)-**2** (Scheme 1, path A)[20,21].

As recently reported[22] (*R*)-**2** and (*S*)-**2** were prepared by N-demethylation of the respective quaternary ammoniums (*R*)-**2a** and (*S*)-**2a**, synthesized from (*R*)-carnitinamide chloride[23,24] and (*S*)-carnitinamide chloride[23,24] respectively, or by alkylating dimethylamine with the tosyl ester of (*R*)- and (*S*)-5-hydroxymethyl-2-oxazolidinone[25,26] respectively.

(*S*)-**3** and (*S*)-**3a** were synthesized from (*S*)-*N*-methyl-prolinol, which was converted into imidazolyl carbamate (*S*)-**6** by treatment with carbonyldiimidazole[27] Successive reaction with NH_3 gave *N*-methyl-L-prolinol carbamate (*S*)-**3** with loss of imidazole[28] and quaternization of (*S*)-**3** with iodomethane afforded (*S*)-**3a** (Scheme 1, path B).[29]

The two pyrrolidinyl oxazolidinones (*S,R*)-**5** and (*S,S*)-**5**, epimers at the oxazolidinone stereogenic carbon, were synthesized from the two diastereoisomers of methyl 1-Cbz-2-pyrrolidinyl-α-azidoacetate with *S* configuration at the pyrrolidine stereocenter, (*S,R*)-**7** and (*S,S*)-**7**[30] by lithium aluminium hydride reduction of the ester, azido and Cbz groups to alcohol, amine and methyl, respectively, and conversion of the two crude β-aminoalcohols (*S,R*)-**8** and (*S,S*)-**8**[31] to cyclic carbamates by treatment with carbonyldiimidazole (Scheme 1, path C)[32,33]

We evaluated the affinity towards the α4β2 and the α7 subtypes of nAchRs present in rat cortex (α4β2) or hippocampus (α7) membranes by binding studies using, as ligands, [^{3}H]-epibatidine and [^{125}I]-αBungarotoxin respectively[34] [^{3}H]NMS equilibrium binding parameters were measured in membrane homogenate derived from CHO cells stably transfected with the human muscarinic receptors (hM_1–hM_5)[35] The K_i values are listed in Table 2.