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Novel 1-(1-benzyl-1*H*-indol-3-yl)-*N*,*N*,*N*-trimethylmethanaminium iodides are competitive antagonists for the human α4β2 and α7 nicotinic acetylcholine receptors†

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This work presents the synthesis and the pharmacological characterization of a series of novel 1-(1-benzyl-1*H*-indol-3-yl)-*N*,*N*,*N*-trimethylmethanaminium iodide derivatives at the human (h) α7 and α4β2 nicotinic acetylcholine receptors (nAChRs). The inhibitory activity of the compounds was determined by Ca^{2+} influx assays on cells expressing either the hα7 or hα4β2 nAChR subtype. To determine whether the observed inhibitory activity is mediated by a competitive or non-competitive mechanism, additional radioligand binding assays were performed using [^{3}H]methyllycaconitine, [^{3}H]cytisine, and [^{3}H]imipramine. The results established that the compounds inhibit the nAChRs by a competitive mechanism and that the potencies are higher for the hα7 nAChR compared to that for the hα4β2 nAChR. Substitutions with oxygenated functional groups on the benzene ring increase the receptor selectivity. In particular, the hydroxyl derivatives **4b** and **4c** present the highest selectivity for the hα7 nAChR subtype. Molecular docking results indicate that the hydroxyl group forms a hydrogen bond with the carbonyl group at α7-Gln116, but not at β2-Phe115, supporting the observed receptor selectivity at the molecular level.

Introduction

Nicotinic acetylcholine receptors (nAChRs), members of the cys-loop ligand-gated ion channel superfamily, are formed by five subunits surrounding a central pore permeable to Na^+, K^+, and Ca^{2+} ions.[1] Depending on whether nAChRs are formed by only one or more than one subunit type, they can be divided into homomeric (*e.g.*, α7) and heteromeric (*e.g.*, α4β2) nAChRs. α7 and α4β2 nAChRs are the most abundant receptor subtypes in the brain. They are involved in important physiological functions[2] and in many relevant diseases, including neuropsychiatric, neurodegenerative, and pain-related diseases.[3] In the last decade, several laboratories have undertaken the synthesis of selective agonists for these two nAChR subtypes, but unfortunately, only a few are in clinical trials.[4,5] More recently, the interest in nAChR antagonists has increased because of their possible therapeutic uses.[6–8] In particular, α4β2 nAChR antagonists could be beneficial for the treatment of nicotine addiction,[7] whereas α7 nAChR antagonists could be beneficial for the treatment of cancer tumors[6] and organophosphorus nerve agent intoxication.[8]

Our laboratory has recently found that the indole ring might be a useful scaffold for the development of novel potent and selective nicotinic antagonists.[9] The most potent derivatives are those with the indole core attached to a short aminoalkyl substituent and bearing a medium-length substituent on its nitrogen (Fig. 1). Moreover, it has been shown that the

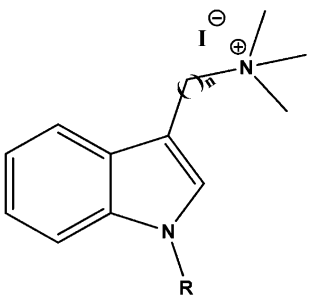

Fig. 1 Plausible pharmacophore for nAChR antagonists.[9] R = alkyl group.

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trimethylammonium group increases ligand affinity to nAChRs.[9–14] In this paper, we describe the synthesis of a series of novel 1-(1-benzyl-1*H*-indol-3-yl)-*N*,*N*,*N*-trimethylmethanaminium iodide derivatives by using a one-pot microwave heating procedure. Subsequently, the pharmacological activity of these compounds at human (h) α7 and α4β2 nAChRs was determined by Ca^{2+} influx assays. In addition, radioligand competition binding experiments were performed to determine the inhibitory mechanism of these compounds. Specifically, [^{3}H]methyllycaconitine ([^{3}H]MLA) and [^{3}H]cytisine were used as selective ligands for the α7 and α4β2 nAChR agonist sites, respectively, and [^{3}H]imipramine was used to test the interaction of these compounds with the nAChR ion channel.

Results and discussion

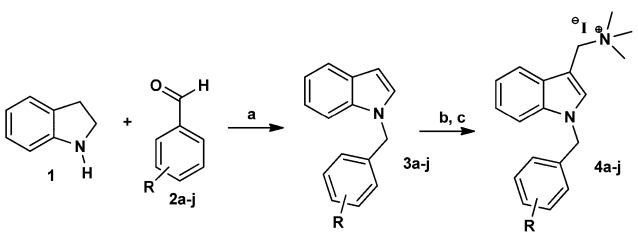
Chemical synthesis

The classical synthesis of N^1-benzylindole derivatives involves the deprotonation of the N–H group present in the indole ring and the subsequent coupling with a benzyl halide.[9] This strategy is efficient when it is not necessary to protect the functional groups. However, when acidic functional groups are present (*e.g.*, phenol group), the synthesis needs two additional steps: protection and deprotection of the functional group. To avoid these additional steps, we used a one-step microwave heating strategy that permits the coupling of indoline with different benzaldehydes producing N^1-benzylindole derivatives without the need for protecting protocols.[15,16]

Scheme 1 illustrates the synthesis of compounds **4a–j** in three steps. First, treatment of indoline (**1**) with substituted benzaldehydes (**2a–j**) in toluene under microwave heating conditions[15,16] gave N^1-benzylindoles (**3a–j**). The subsequent Mannich reaction with dimethylamine and formaldehyde followed by quaternization using CH_3I produced the desired compounds **4a–j** (see Fig. 2 for their structures).

Ca^{2+} influx assays

The functional and binding properties of compounds **4a–j** were subsequently determined on the hα7 and hα4β2 nAChRs using procedures published elsewhere (see ESI†).[17–20] The inhibitory potencies (IC_{50}) of our compounds were determined in GH3-hα7 (Fig. 3A) and HEK293-hα4β2 (Fig. 3B) cells by Ca^{2+} influx assays. The results indicate that the compounds inhibit the hα7 nAChR with potencies 2–15 times higher than that for the hα4β2 nAChR (Table 1). Interestingly, the compound **4j** showed no activity on the hα4β2 subtype at concentrations as high as 100 μM (Fig. 3B), suggesting that this compound as well as compounds **4b** and **4c** are more selective antagonists for the hα7 nAChR.



Scheme 1 *Reagents and conditions*: (a) toluene, microwave heating, 30 min; (b) aqueous $(CH_3)_2NH$, aqueous HCHO, acetic acid, rt, 16 h; (c) CH_3I, acetone, 16 h.

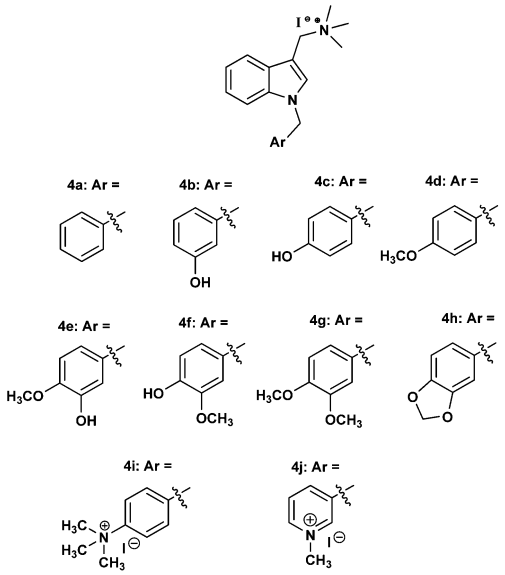


Fig. 2 Chemical structures of the novel 1-(1-benzyl-1*H*-indol-3-yl)-*N*,*N*,*N*-trimethylmethanaminium iodide compounds, **4a–j**.

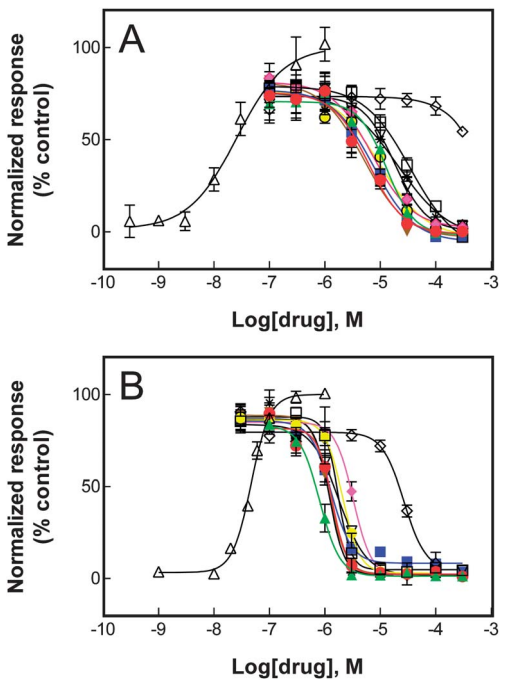


Fig. 3 Inhibitory effect of compounds shown in Table 1 on (±)-epibatidine-induced Ca^{2+} influx in HEK293-hα4β2 (A) and GH3-hα7 (B) cells. The EC_{50} values for (±)-epibatidine (*A*) are (A) 30 ± 5 nM (n = 21) and (B) 52 ± 4 nM (n = 17), respectively. The symbols are: **4a** (●), **4b** (▲), **4c** (□), **4d** (■), **4e** (♦), **4f** (●), **4g** (✻), **4h** (▼), **4i** (∇), and **4j** (◊). The plots are representative of three determinations, where the error bars correspond to the standard deviation (SD). The calculated IC_{50} and n_H values are summarized in Table 1.

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Table 1 Inhibitory potencies for the studied compounds on the hα4β2 and hα7 nAChRs determined by Ca^{2+} influx experiments[a]

Compound	hα4β2		hα7		hα4β2/hα7 ratio
	IC_{50} (μM)	n_H	IC_{50} (μM)	n_H	
4a	4.2 ± 1.5	1.02 ± 0.28	2.3 ± 0.9	2.15 ± 0.20	1.9
4b	14.7 ± 1.2	1.32 ± 0.35	1.0 ± 0.2	1.81 ± 0.02	14.7
4c	24.9 ± 8.0	1.05 ± 0.01	2.2 ± 0.4	2.24 ± 0.39	11.3
4d	4.4 ± 2.5	1.02 ± 0.13	2.0 ± 0.6	2.25 ± 0.40	2.2
4e	9.5 ± 4.3	0.94 ± 0.05	3.4 ± 0.2	2.24 ± 0.37	2.8
4f	9.1 ± 5.2	0.93 ± 0.25	2.2 ± 0.1	2.46 ± 0.29	4.1
4g	14.8 ± 5.0	1.13 ± 0.31	2.2 ± 0.8	2.03 ± 0.22	6.7
4h	3.9 ± 1.0	1.23 ± 0.07	1.3 ± 0.2	2.00 ± 0.10	3.0
4i	20.0 ± 0.4	1.45 ± 0.22	2.7 ± 0.8	2.03 ± 0.23	7.4
4j	>100	—	41.3 ± 15.5	2.16 ± 0.01	—

[a] The IC_{50} and n_H values were obtained from Fig. 3A and B, respectively.

Radioligand binding assays

Our radioligand binding results clearly indicate that the compounds are competitive antagonists for the hα7 (Fig. 4A) and hα4β2 (Fig. 4B) nAChR subtypes. More specifically, compounds **4b** and **4c** inhibit [^{3}H]MLA and [^{3}H]cytisine binding to hα7 and hα4β2 nAChR agonist sites, respectively, in the 0.6–2.9 μM concentration range, whereas compound **4b** inhibits [^{3}H]imipramine binding to AChR ion channels (Fig. 4C) only at concentrations higher than 100 μM (Table 2).

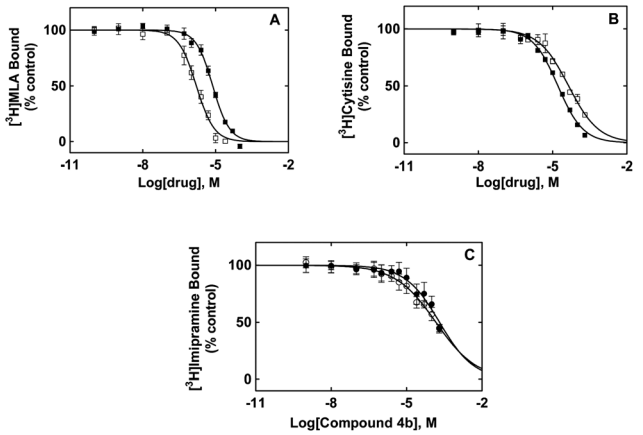

Fig. 4 Ligand interaction with different binding sites at the hα7 and hα4β2 nAChRs. Inhibition of (A) [^{3}H]MLA binding to hα7 nAChR agonist sites and (B) [^{3}H]cytisine binding to hα4β2 nAChR agonist sites mediated by compounds **4b** (□) and **4c** (■), respectively. (C) Inhibition of [^{3}H]imipramine binding to hα7 (○) and hα4β2 (●) nAChR ion channels mediated by compound **4b**.

The Hill coefficient (n_H) values for the hα4β2 nAChR are close to unity, suggesting that the ligand-induced [^{3}H]cytisine binding inhibition is mediated by a non-cooperative mechanism (Table 2). In addition, the n_H values for the hα7 nAChR are closer to two, suggesting that the ligand-induced [^{3}H]MLA binding inhibition is mediated by a cooperative mechanism and thus that the compounds bind to more than one [^{3}H]MLA binding site. This result is in agreement with the fact that the hα7 nAChR has five putative binding sites and it needs three of them to be occupied for full activation.[21] In contrast, the n_H value for the binding of compound **4b** to the [^{3}H]imipramine site at the hα7 nAChR is close to 0.5, suggesting a negative cooperative mechanism, and thus, supporting an allosteric mode of inhibition.[22] These results support the idea that these compounds inhibit hα7 and hα4β2 nAChRs by a competitive mechanism and rule out the possibility of a noncompetitive mechanism. Our results indicate that compounds with a trimethylammonium group attached to the indole ring by a methylenic linker (see Fig. 1) behave as nAChR competitive antagonists, which is in agreement with trends reported in the literature for other quaternary ammonium compounds.[9–14]

The presence of a variety of functional groups on the benzene ring was evaluated to establish both the optimal functional group and its position on this aromatic ring (Table 1). The initial results indicate that **4a**, a compound without substitution on the benzene ring, has inhibitory potency in the micromolar range but with only negligible hα7/hα4β2 selectivity. Subsequent result analyses of compounds with substitutions on the benzene ring indicated that they are more selective. For example, compounds **4b** and **4c**, containing only one hydroxyl group on the benzene ring, are the most selective antagonists for the hα7 subtype. Interestingly, the methylation of the hydroxy group present in **4b** to furnish **4d** maintains similar potency but decreases the observed receptor selectivity.

Additional substitutions (*e.g.*, hydroxy, methoxy, or methylendioxy) on the benzene ring were next examined. The results indicate that the antagonist activity of these compounds (*i.e.*, **4e**, **4f**, **4g**, and **4h**) at hα4β2 nAChRs improved, diminishing consequently the hα7 nAChR selectivity. Finally, we centered our attention to dicationic compounds because it has been

Table 2 Ligand binding affinities for the agonist and ion channel binding sites from hα7 and hα4β2 nAChRs[a]

Radioligand	nAChR sites	Ligand	hα7		hα4β2	
			K_i (μM)	n_H	K_i (μM)	n_H
[^{3}H]MLA	Agonist	**4b**	0.6 ± 0.1	1.18 ± 0.11	—	—
		4c	2.9 ± 0.2	1.26 ± 0.10	—	—
[^{3}H]Cytisine	Agonist	**4b**	—	—	1.6 ± 0.2	0.72 ± 0.06
		4c	—	—	0.6 ± 0.1	0.86 ± 0.04
[^{3}H]Imipramine	Ion channel	**4b**	145 ± 36	0.54 ± 0.08	112 ± 22	0.75 ± 0.12

[a] The K_i and n_H values were obtained from Fig. 4A–C, respectively.

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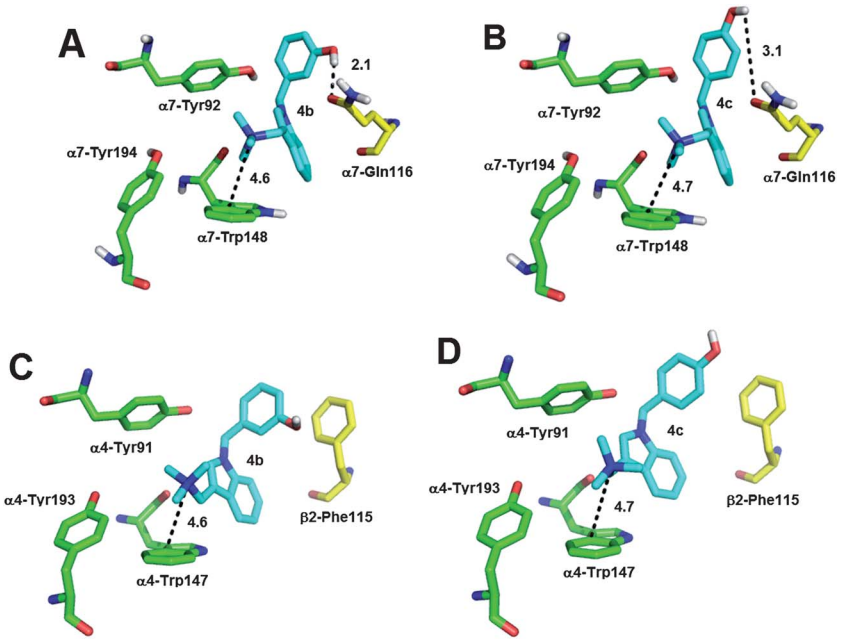

Fig. 5 Molecular interactions of compounds **4b** and **4c** with the (A and B) α7 and (C and D) α4β2 nAChR orthosteric sites. Residues from the principal and complementary components are colored green and yellow, respectively, whereas the ligands are colored cyan. Distances in dashed lines are in Angstrom (Å).

reported that these types of compounds are potent antagonists for different nAChR subtypes.[23,24] The results show that the *p*-trimethylammonium derivative (*i.e.*, **4i**) presents a 7-fold higher selectivity for the hα7 nAChR compared to the hα4β2 nAChR, whilst the *m*-methylpyridinium derivative (*i.e.*, **4j**) is inactive at the hα4β2 nAChR and shows low activity at the hα7 nAChR. Although the hα7 nAChR inhibition is low, the lack of activity at the hα4β2 nAChR opens the door for the development of potentially selective hα7 nAChR antagonists structurally related to this *m*-methylpyridinium derivative.

Molecular docking[25] of compounds **4b** and **4c** to the nAChR orthosteric sites explained the observed receptor selectivity (Fig. 5A–D). The results indicate that the trimethylammonium group forms cation–π interactions with the aromatic box at both nAChR subtypes. Nevertheless, the hydroxyl group (*meta* and *para* positions at the benzyl moiety of **4b** and **4c**, respectively) forms a hydrogen bond with the carbonyl group from the hα7-Gln116 side chain at the complementary component (Fig. 5A and B). Interestingly, this hydrogen bond is absent in the hα4β2 nAChR because this residue is replaced by β2-Phe115 which does not have the polar group to interact with the ligand hydroxyl moiety (Fig. 5C and D). Our results coincide with other publications indicating that hα7-Gln116 is a key residue to define the selectivity between α7 and α4β2 nAChR subtypes.[17,26,27]

Conclusions

Taken together, the results confirm the importance of the indole ring as a novel moiety for the development of competitive antagonists for hα7 and hα4β2 nAChR subtypes. More importantly, hydroxyl substitutions on the benzene ring from N^1-benzyl-3-trimethylmethanammonium iodides improve hα7 nAChR selectivity by forming a hydrogen bond with the polar group only present in hα7-Gln116. This increased selectivity could be exploited for the treatment of disorders where hα7 nAChRs are implicated such as cancer tumors[6] and organophosphorus nerve agent intoxication.[8]

Acknowledgements

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