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# The 5-$HT_3$ Antagonist Tropisetron (ICS 205-930) is a Potent and Selective $\alpha$7 Nicotinic Receptor Partial Agonist

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This paper is dedicated to the memory of the outstanding chemist Robert A. Mack.

**Abstract**—The 5-$HT_3$ receptor antagonist tropisetron (ICS 205-930) was found to be a potent and selective partial agonist at $\alpha$7 nicotinic receptors. Two other 5-$HT_3$ receptor antagonists, ondansetron and LY-278,584, were found to lack high affinity at the $\alpha$7 nicotinic receptor. Quinuclidine analogues (**1** and **2**) of tropisetron were also found to be potent and selective partial agonists at $\alpha$7 nicotinic receptors. 

Both the $\alpha$7 nicotinic receptor ($\alpha$7 nAChR) and the 5-$HT_3$ receptor (5-$HT_3$R) have been implicated to play important roles in cognition and schizophrenia.[1,2] Whereas the $\alpha$7 nAChR receptor is a member of the ligand-gated ion channel family of nicotinic receptors,[3] the ligand-gated ion channel 5-$HT_3$R is unique within the serotonin family of receptors, since the other members of the 5-HT family are all serpentine G-protein coupled receptors.[4] While there are a number of structurally diverse 5-$HT_3$ ligands,[5] there exists a dearth of potent and/or selective ligands for the $\alpha$7 nAChR. Because of the evolutionary familial link between these two cationic channels,[2] it seemed reasonable that some 5-$HT_3$ receptor ligands might have affinity and functional activity at $\alpha$7 nAChRs, and vice versa.

This assumption led to a recent paper from our group that described the functional activity of weak $\alpha$7 nicotinic agonists at the 5-$HT_3$R.[6] In the current report, we describe the opposite scenario. Namely, we describe the activity of the potent 5-$HT_3$ antagonist tropisetron (Fig. 1), as a potent and selective $\alpha$7 nicotinic partial agonist. However, its nicotinic activity is not general for the class of 5-$HT_3$R antagonists, since two other high affinity 5-$HT_3$R antagonists, ondansetron and LY-278,584, had no $\alpha$7 nAChR activity.

We examined three 5-$HT_3$R antagonists as possible $\alpha$7 nAChR ligands: tropisetron, LY-278,584, and ondansetron. Tropisetron (ICS 205-930) and LY-278,584 were chosen because of structural similarity to a series of aryl quinuclidinyl carbamates, which we found to be potent $\alpha$7 nAChR partial agonists.[7] Ondansetron was tested since it is a prototypical representative of this class of drugs. As shown in Table 1,[8] tropisetron is a high affinity ligand for both th $\alpha$7 nAChR and 5$HT_3$R. In contrast, tropisetron has very low affinity for the other nicotinic subtypes tested ($\alpha$4$\beta$2, $\alpha$1$\beta$1$\gamma\delta$, and $\alpha$3, see Table 1). Neither ondansetron nor LY-278,584 demonstrated appreciable affinity for the $\alpha$7 nAChR. These results show that within the class of 5-$HT_3$ receptor antagonists, potent affinity for the $\alpha$7 nAChR can be found, but is not universal. Examination of the functional effects of tropisetron at the $\alpha$7 nAChR was performed using *Xenopus* oocytes.[9] Compared to acetylcholine and nicotine, tropisetron was a potent, but partial agonist at the $\alpha$7 nAChR (Table 2). Since the *N*-methyl quaternized analogue of tropisetron shares the quaternized amine functionality found in acetylcholine, it was expected to be a more efficacious $\alpha$7 nAChR agonist. However, while this compound was potent as an $\alpha$7 nAChR ligand, it was found to be less efficacious than tropisetron.

After finding the potent and selective $\alpha$7 nAChR activity of tropisetron, we wanted to examine the effect of different bicyclic amines on the nicotinic SAR. Specifically, since our interest in aryl quinuclidinyl carbamates

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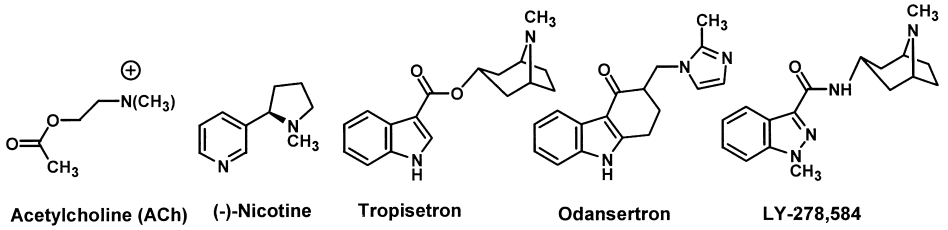



**Figure 1.**

**Table 1.** Binding affinities[a,b]

| Compound | α7 nAChR $K_i$ (nM) | 5-$HT_3$R $K_i$ (nM) | α4β2 nAChR $K_i$ (nM) | α3 (PC12) nAChR $K_i$ (nM) | α1β1γδ nAChR $K_i$ (nM) |
|---|---|---|---|---|---|
| (−)-Nicotine | 480 ± 30 (6) | 71,000 ± 25,000 (3) | 2.3 ± 0.2 (6) | 220 ± 20 (4) | 5500 ± 440 (13) |
| Tropisetron | 6.9 ± 2.4 (3) | 5.3 ± 3.0 (3) | 55,000 ± 28,000 (3) | 16,000 ± 2400 (3) | 27,000 (2) |
| Quaternized tropisetron | 6.4 ± 1.2 (3) | 7.2 ± 1.7 (3) | 7800 ± 1000 (3) | 2300 ± 520 (3) | 3000 (1) |
| LY-278,584 | >10,000 (3) | 24 ± 11 (3) | 38,000 (2) | 67,000 (1) | Not done |
| Ondansetron | >3000 (3) | 12 ± 6 (3) | 46,000 (2) | Not done | Not done |
| **1** | 2.3 ± 0.6 (3) | 1.1 ± 0.2 (3) | 2600 (2) | Not done | Not done |
| **2** | 8.8 ± 1.7 (3) | 10 ± 4 (3) | 14,000 (2) | Not done | Not done |

[a]Number of determinations in parentheses.
[b]SEM for $n \geq 3$.

led us to test tropisetron at α7 nAChR, we examined the replacement of the azabicyclo[3.2.1]heptane amine in tropisetron with the azabicyclo[2.2.2]hexane amine, quinuclidine. The syntheses of these analogues are shown in Scheme 1. Activation of indole-3-carboxylic acid with carbonyldiimidazole, followed by treatment of the imidazolyl amide with 3-quinuclidinol using DMAP as a catalyst afforded the quinuclidin-3-yl indole-3-carboxylate (**1**).

Using methodology we developed for the regioselective acylation of the indole nitrogen,[10] we also prepared the quinuclidin-3-yl indole-1-carboxylate (**2**). This preparation also involved the use of carbonyldiimidazole, but it was used directly on indole itself in the presence of a catalytic amount of DMAP to form the imidazol-1-yl indol-1-yl urea, which when reacted with quinuclidin-3-ol afforded **2**.

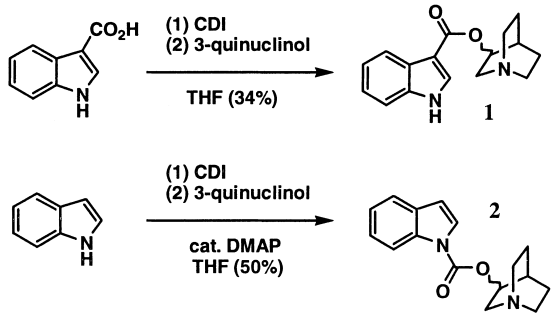
Table 1 summarizes the in vitro pharmacology of these analogues of tropisetron. Clearly quinuclidine substitutes well for the azabicyclo[3.2.1]heptane found in tropisetron. Also, the position of attachment of the carboxylate function (i.e., C3 for **1** and tropisetron, N1 for **2**) did not seem to have any effect on the affinity of these analogues for the α7 nAChR. In addition, there was little (if any) stereogenic differentiation between the enantiomers of **1** and **2** although in both cases the (*S*)-enantiomer seemed to be slightly more potent than the (*R*)-enantiomer (data not presented). The only difference seen in this series of tropisetron analogues was that indole-3-carboxylate (**1**) was a slightly more efficacious agonist than the indole-1-carboxylate (**2**, Table 2). Whereas one could describe both tropisetron and **2** as weak partial agonists, **1** appeared to be a true partial agonist for the α7 nAChR. We are presently using these compounds in in vivo models to see if this difference can be measured and observed. In summary, quinuclidine of either stereogenicity is minimally an equivalent replacement for the azabicyclo[3.2.1]heptane found in tropisetron. However, the exact regiomeric analogue of tropisetron (i.e., attachment of the carboxylate at C3 of the indole) using quinuclidine afforded an α7 nAChR agonist (**1**) with greater intrinsic agonist activity than tropisetron. This compound represents an important pharmacological tool for the study of α7 nAChRs.

In conclusion, we have found that the 5-$HT_3$R antagonist tropisetron is a potent and selective partial agonist for the α7 nAChR. Replacement of the azabicyclo[3.2.1]heptane found in tropisetron with quinuclidine afforded a ligand (**1**), which was as potent and selective in binding and more efficacious as a partial agonist than tropisetron at α7 nAChRs. Given the paucity of selective ligands for the α7 nAChRs, these compounds should prove to be useful as tools to study that ligand-gated ion channel.

It should be noted that tropisetron was studied extensively in human clinical trials and is available in Europe for the treatment of emesis.[11] Its unremarkable safety profile[12] can now be interpreted as demonstrating the safety of *both* a 5-$HT_3$ receptor antagonist and an α7 nicotinic receptor partial agonist. Furthermore, the discovery that tropisetron is also a potent partial agonist for α7 nAChRs gives cause for the re-examination of both clinical and preclinical findings with this com-

**Table 2.** Functional activity

| Compound | α7 nAChR Efficacy (%) | α7 nAChR $EC_{50}$ (nM) |
|---|---|---|
| Acetylcholine | 100 | 230,000 ± 4000 |
| (−)-Nicotine | 53 ± 6 | 38,000 ± 5000 |
| Tropisetron | 36 ± 2 | 1300 ± 200 |
| Quaternized Tropisetron | 13 ± 1 | 280 ± 40 |
| **1** | 42 ± 1 | 580 ± 80 |
| **2** | 17 ± 7 | 4800 ± 3000 |




**Scheme 1.**

pound, particularly when tropisetron displayed pharmacological effects different from other 5-$HT_3$R antagonists. In one notable study, it was reported that tropisetron and ondansetron had qualitatively different results in learning and memory paradigms in rats.[13] Thus, the results reported in our paper give reason to re-examine such studies with tropisetron to ascertain whether the results were the effect of 5-$HT_3$ antagonism or α7 nicotinic partial agonism.

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8. (a) SEM for values determined ≥3 times. Binding assay conditions: α7nAChR: 5 nM [$^{125}$I]α-bungartoxin, rat hippocampal membranes, 0.1 mg/mL BSA, buffer (120 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 50 Tris, pH 7.4; mM), 2 h, 20 °C. (b) α4β2nAChR: 3 nM [$^3$H]nicotine, rat cortical membranes, buffer, 1 h, 4 °C. (c) α3nAChR: 0.1 nM [$^3$H]epibatidine, PC12 cell membranes, 1 h, 20 °C. (d) α1β1γδnAChR: 1 nM [$^{125}$I]α-bungartoxin, $BC_3H1$ cell membranes, buffer, 2 h, 37 °C. (e) $HT_3$R: 0.5 nM [$^3$H]zacopride, rat small bowel muscularis membranes, 150 mM NaCl, 50 mM Tris, pH 7.4, 1 h, 37 °C.
9. Potency and intrinisic activity values were determined by measuring current activation in *Xenopus* oocytes expressing rat α7 nAChRs (Fig. 2). Oocytes were injected with cRNA coding for nAChR α7. Oocytes were used 3–10 days post-injection. 100% intrinsic activity was defined for each egg by current elicited by 3 mM acetylcholine. Currents were measured from baseline to peak. Oocytes were washed for 5 min after each application of agonist to allow receptors to fully recover from desensitization. Data from individual experiments were fit by the logistic equation to estimate $EC_{50}$ and efficacy. Data in Table 2 are averages of three or more experiments ±SEM.

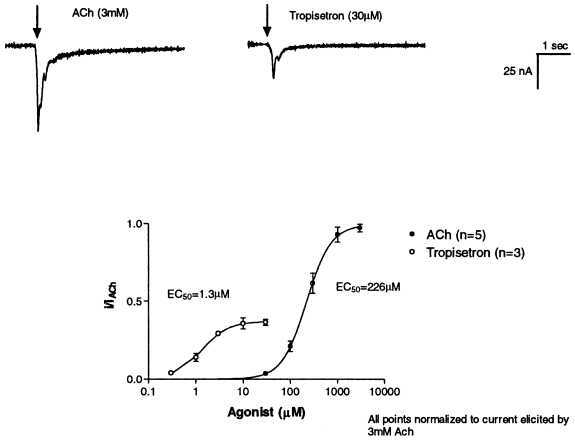


**Figure 2.** The effect of acetylcholine and tropisetron on nAChR α7. Top panel, representative traces of current elicited in oocytes expressing mouse nAChR α7 (see above). Traces shown are from the same oocyte; superfusion of acetylcholine and tropisetron begins at arrow (5 min between agonist applications). Bottom panel, concentration–response curve to acetylcholine and tropisetron. Data are fit by the logistic equation.

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