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Molecular dissection of tropisetron, an α7 nicotinic acetylcholine receptor-selective partial agonist

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Abstract

The α7 nicotinic acetylcholine receptor (nAChR)-selective partial agonist tropisetron is a conjugate of an indole and a tropane group. We tested compounds structurally related to either the indole or tropane domains of tropisetron on oocytes expressing human α7. α4β2, or α3β4 nAChR or rat $5HT_{3A}$ receptors. The simple compounds tropane and tropinone had α7-selective agonist activity comparable to that of tropisetron. Tropinone was more efficacious than tropisetron but 100-fold less potent. Some tropane compounds had antagonist activity on α3β4 nAChR but no effect on α4β2 nAChR. Some tropanes also affected the responses of 5HT3 receptors to serotonin. Tropisetron was more potent at inhibiting α3β4 receptors ($IC_{50} = 1.8 \pm 0.6$) than was tropane or tropinone, suggesting that the presence of the indole group has a large impact on the potency of tropisetron, both as an α7 agonist and as an α3β4 antagonist. The further reduced structures of dimethyl piperidinium and 1-methylpyrrolidine also had agonist activity on α7 receptors, suggesting that the minimal activating pharmacophore of these compounds, as with tetramethylammonium, may simply be the charged nitrogen, while additional structure elements impact subtype selectivity, potency, and efficacy. It has previously been reported that 5-hydroxyindole (5HI) can potentiate α7 receptor responses to acetylcholine (ACh). However, the site where 5HI binds to the receptor is not known. We tested the hypothesis that the tropisetron binding site might overlap the 5HI site and thereby produce a block of 5HI potentiation. Our results indicate that the indole portion of tropisetron is not likely to be binding to the same site where 5HI binds to potentiate α7 receptor responses since 5HI can greatly potentiate responses of tropisetron, tropinone, and other partial agonists such as 4OH-GTS-21.

Keywords: Alzheimer's disease; Drug development; *Xenopus* oocytes; Voltage-clamp

The α7-type nicotinic receptors of the brain have been suggested to be potential therapeutic targets for indications ranging from schizophrenia to Alzheimer's disease. This has led to the identification and development of several α7-selective agonists. One such selective agonist for α7 nicotinic acetylcholine receptor (nAChR) is tropisetron, which was originally identified as a 5HT3 receptor antagonist.

For some α7-selective agonists, a "core agonist" can be identified that will activate multiple nAChR subtypes. A modification of that nonselective agonist which makes it α7-selective may then represent a structural selectivity motif. For example, the addition of a benzylidene group to the non-selective agonist anabaseine creates selectivity for α7. For the endogenous α7-selective agonist choline, the apparent selectivity motif is the exposed hydroxyl, since both smaller and larger structurally related compounds (i.e. ethyltrimethyl ammonium and ACh, respectively [7]) are non-selective agonists. Consideration of the growing number of structurally unrelated molecules that have been identified as α7-selective agonists [1] suggests that there may be several structural motifs which can lead to α7-selectivity. In this work we investigate the basis for the α7-selectivity of the partial agonist tropisetron. Our initial hypothesis was that the basic selectivity motif for this large molecule (Fig. 1A) would be the conjugation of the large hydrophobic indole group to a small core agonist represented by the tropane portion of tropisetron. This motif would be analogous to the benzylidene conjuga-

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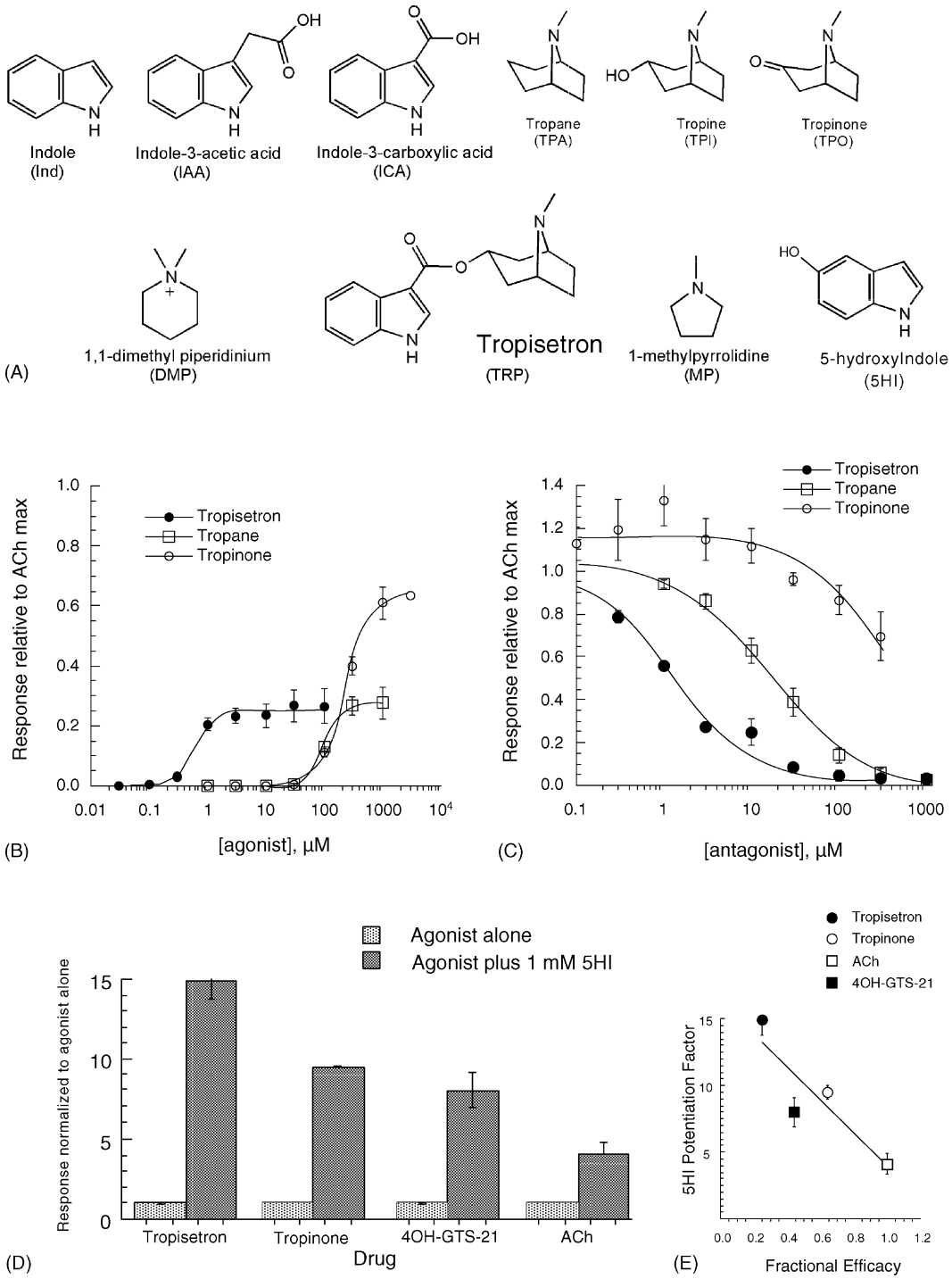

Fig. 1. (A) Structure of tropisetron and its subdomain analogs are shown along with the abbreviations used in the text and Table 1. (B) Concentration–response curves for tropisetron, tropane, and tropinone on human α7 receptor expressed in oocytes. Data are calculated as net charge and normalized to ACh maximum responses. Each point represents the average ± S.E.M. of at least four oocytes. The tropisetron data was taken from Papke et al. (2003). (C) The inhibition of ACh-evoked responses with the co-application of tropisetron, tropane, or tropinone at increasing concentrations. Data were calculated as the ratio of the peak response to the co-application of ACh and antagonist to the peak response of ACh alone in the same cell. Each point represents the average ± S.E.M. of at least four oocytes. The tropisetron data was taken from Papke et al. (2003). (D) 5-Hydroxy-indole (5HI) potentiation of α7 responses to full and partial agonists. Control responses to 1 μM tropisetron, 3 μM 4OH-GTS-21, 30 μM ACh, or 100 μM tropinone were obtained in the absence of 5HI. The running buffer was then switched to one containing 1 mM 5HI and the drugs were re-applied with 5HI. The net charge of the responses obtained in the presence of 5HI are plotted relative to the net charge responses obtained in the absence of 5HI. Each point represents the average ± S.E.M. of at least four oocytes. (E) The graph illustrates the inverse relationship between agonist efficacy and the magnitude of 5HI potentiation (taken from panel D). The efficacy of ACh was defined as 1, the efficacies of tropisetron and tropinone are the I_{max} values from Table 2 and the efficacy of 4OH-GTS-21 is as reported in Papke and Papke [9].

tion of anabaseine, which is the basis for the α7-selectivity of 4OH-GTS-21 and related benzylidene anabaseines [8]. In order to test this hypothesis we tested a selection of indoles, tropanes and two cyclic amines (Fig. 1A) on human α7 receptors and two other model neuronal AChRs expressed in *Xenopus* oocytes, the α3β4 subunit combination, which is often used to model ganglionic nAChR, and the α4β2 subunit combination, representative of the high affinity nicotine receptor of the brain [12].

The source of the 4OH-GTS-21 was Taiho Pharmaceuticals (Tokyo, Japan), and tropisetron was supplied by Memory Pharmaceuticals. All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Mature (>9 cm) female *Xenopus laevis* African frogs (Nasco, Ft. Atkinson, WI) were used as a source of oocytes. Prior to surgery, the toads were anesthetized by placing the animal in a 1.5 g/l solution of MS222 (3-aminobenzoic acid ethyl ester) for 30 min. Oocytes were removed from an incision made in the abdomen.

In order to remove the follicular cell layer, harvested oocytes were treated with 1.25 mg/ml collagenase from Worthington Biochemical Corporation (Freehold, NJ) for 2 h at room temperature in calcium-free Barth's solution (88 mM NaCl, 1 mM KCl, 2.38 mM $NaHCO_3$, 0.82 mM MgSO4, 15 mM HEPES (pH 7.6), 0.1 mg/ml gentamicin sulfate). Subsequently, stage 5 oocytes were isolated and injected with 50 nl (5–20 ng) each of the appropriate subunit cRNAs. Recordings were made 5–15 days after injection.

Experiments were conducted using OpusXpress 6000A (Axon Instruments/Molecular Devices, Union City, CA, USA). OpusXpress is an integrated system that provides automated impalement and voltage-clamp of up to eight oocytes in parallel. Both the voltage and current electrodes were filled with 3 M KCl. Cells were voltage-clamped at a holding potential of −60 mV. Data were collected at 50 Hz and filtered at 20 Hz for α7 responses and 5 Hz for other subtypes. Drug applications alternated between ACh controls and experimental agonists. Applications were 12 s in duration followed by 181-s washout periods.

Responses of α7 receptors were calculated as net charge [9]. Each oocyte received two initial control applications of 300 μM ACh, then an experimental drug application, and then a follow-up control application of 300 μM ACh, a concentration which is sufficient to evoke a maximal net charge responses [9]. The control ACh concentrations for α3β4 and α4β2 receptors were 100 μM and 10 μM, respectively. In other experiments (not shown) these concentrations were determined to be the EC_{15} and EC_{22}, respectively. The control concentration of the $5HT_{3A}$ receptors was 3 μM, which was the EC_{18}. Responses to experimental drug applications were calculated relative to the preceding ACh (or 5HT) control responses in order to normalize the data and compensating for the varying levels of channel expression among the oocytes. Means and standard errors were calculated from the normalized responses of at least four oocytes for each experimental concentration. For concentration–response relations, data derived from net charge analyses were plotted using Kaleidagraph 3.0.2 (Abelbeck Software; Reading, PA), and curves were generated from the Hill equation,

$$\text{Response} = \frac{I_{\max}[\text{agonist}]^n}{[\text{agonist}]^n + (\text{EC}_{50})^n}$$

where $I_{\max}$ denotes the maximal response for a particular agonist/subunit combination, and n represents the Hill coefficient. $I_{\max}$, n, and the EC_{50} were all unconstrained for the fitting procedures. For IC_{50} calculations the Hill slope was constrained to equal −1.

We investigated the functionality of tropane and indole components of tropisetron by testing the reduced structures shown in Fig. 1A. We hypothesized that agonist functionality would be restricted to just one of these domains and that the other domain would not be necessary for agonist activity but might perhaps provide the basis for the α7-selectivity of the conjugated indole-tropone. The probe compounds, related structurally to either the indole or tropane domains of tropisetron, were tested for basic functionality on oocytes expressing human α7, α4β2, or α3β4 nAChR, or rat 5HT3 receptors. We tested for agonist activity by applying the drugs alone at relatively high concentrations (100 μM and 1 mM) and compared the data to the responses to either ACh or 5HT at saturating concentrations. To evaluate potential antagonist activity, the probe compounds were co-applied at 100 μM and 1 mM with the control agonist (ACh or 5HT) and those responses were compared to the responses obtained when the control agonist was applied alone. The results of these experiments are summarized in Table 1. The tropane compounds which showed activity at high concentrations were also investigated with full CRC studies (Fig. 1B and C).

Interestingly, we found that the simple compounds tropane and tropinone had α7-selective agonist activity comparable to that of tropisetron. Tropinone was even more efficacious than tropisetron, although both tropinone and tropane were

Table 1
Qualitative screening of tropisetron and sub-structural analogs (see Fig. 1A for drug abbreviations)

Receptor	IND	IAA	ICA	TRP	TPA	TPI	TPO	DMP	MP
α7	N	N	N	30% PA	25% PA	N	64% PA	>60% PA[a]	>10% PA[a]
α3β4	N	N	N	An	An	An	An	An	An
α4β2	N	N	N	An	An	An	N	N	>10% PA[a]
5HT3A	>20% PA[a]	N	>20% PA[a]	An	N	An	N	An	An

PA: partial agonist (percent values refer to %ACh or $5HT_{max}$); An: antagonist; N: neither agonist nor antagonist.
[a] Percent maximum ACh (or 5HT) response obtained when the experimental drug was applied at 1 mM.

Table 2
Values from the curve fits in Fig. 1

	Activation of α7			Inhibition of α3β4 (IC_{50}, μM)
	EC_{50} (μM)	*n*	I_{max}	
Tropisetron	0.6 ± 0.1	2.7 ± 0.5	0.25 ± 0.01	1.8 ± 0.6
Tropane	103 ± 3	3.2 ± 0.3	0.28 ± 0.01	18 ± 0.9
Tropinone	230 ± 4	1.9 ± 0.1	0.64 ± 0.01	350 ± 96

approximately 100-fold less potent than tropisetron (Fig. 1B, Table 2). The simple tropane compounds had antagonist activity on α3β4 nAChR but relatively little effect on α4β2 or 5HT3 receptors. Notably, tropisetron was more potent at inhibiting α3β4 receptors than were tropinone and tropane (Fig. 1C and Table 2), suggesting that the presence of the indole group has a large impact on the potency of tropisetron, both as an α7 agonist and as an α3β4 antagonist.

The simpler structures of dimethyl piperidinium iodide (DMP) and 1-methylpyrrolidine (MP) also had agonist activity, suggesting that the minimal activating pharmacophore of these compounds, as with TMA may simply be the charged nitrogen with at least three attached carbons, while the additional structure impacts subtype selectivity, potency, and efficacy. Interestingly, amongst the nAChR subtypes tested, DMP but not MP was α7-selective. A number of additional small amines were tested. The compounds, 1-methylpiperidine, 1-(2-hydroxyethyl) pyrrolidine, 1-(2-hydroxyethyl) piperidine, and 1-methyl-3-piperidinol had no detectable activity on the nAChR tested (data not shown). Trimethylammonium had agonist activity at 1 mM for α4β2 and α7 receptors, but not α3β4 receptors. However, the efficacy of trimethylammonium was only about a third of that of tetramethylammonium on α4β2 and α7 receptors, indicating the crucial importance of the quaternary nitrogen for the activity of these reduced structures. The preference for quaternization may reflect a combination of providing a fixed positive charge with additional hydrophobic interactions between alkyl groups and the binding site, since trialkylamines which are not quaternary are fully protonated under the experimental conditions employed.

It has previously been reported that 5HI can potentiate α7 receptor responses to ACh [2,13]. However, the site where 5HI binds to the receptor is not known. Since the indole-containing agonist tropisetron is 100-fold more potent as an agonist than the simple tropane compounds, it seems likely that there may be a specific site where the indole subdomain of tropisetron binds. This site would necessarily be close to the agonist binding site where binding of the tropane nitrogen produces channel activation. Is the tropisetron-indole site also the 5HI binding site? If so, then channel activation by tropisetron might produce a block of 5HI potentiation. We tested this hypothesis by measuring the ability of 5HI to potentiate activation of human α7 receptors by ACh, 4OH-GTS-21, tropisetron, and tropinone. We first applied 30 μM ACh, 3 μM 4OH-GTS-21, 1 μM tropisetron, or 100 μM tropinone (roughly the EC_{50} concentrations for each of these drugs) in the absence of 5HI and then re-applied each of the agonists in the presence of 1 mM 5HI. As shown in Fig. 1D, responses to each of these drugs were significantly increased in the presence of 5HI. These results indicate that the indole portion of tropisetron is not likely to be binding to the same site where 5HI binds to potentiate α7 receptor responses, since 5HI can greatly potentiate tropisetron responses. It is interesting to note that responses to the partial agonists were potentiated more than the response to the reference "full" agonist ACh. In fact, to a large degree, the weaker the partial agonist, the greater the potentiation (see Fig. 1E).

Tropisetron was initially developed as an antagonist of $5HT_3$ receptors [11], which are members of the same superfamily of ligand-gated ion channels as α7 nAChR. More recently, tropisetron was identified as a selective agonist of α7 nAChR [4], and we have shown that it is also an antagonist of non-α7 nAChR [10]. This activity profile is similar to that of benzylidene anabaseine α7-selective partial agonists such as 4OH-GTS-21 [3,10]. In the case of the benzylidene anabaseines, the anabaseine portion of the molecule is a "core agonist", sufficient to activate α7 nAChR as well as other nAChR subtypes and the addition of the benzylidene group is responsible for loss of agonist activity at subtypes other than α7 [8]. Our initial hypothesis was that the selectivity of tropisetron for activating α7 receptors would have a similar structural basis, that is that tropanes might have non-selective agonist activity and that conjugation of the indole group would preclude activation of subtypes other than α7. However, our data indicate that simple tropanes are themselves α7-selective agonists, so the basis for tropisetron α7 selectivity must lie within the tropane structure and not in the indole conjugation. A bridged ring structure is common to tropanes and other α7-selective agonists such as AR-R17779 [6] and TC-1698 [5], suggesting that the bridged ring may be a structural motif which can generate α7-selectivity. Our data suggest that if a "core agonist" can in fact be identified in the tropisetron structure, it may be as rudimentary as a simple charged nitrogen with a suitable surrounding framework of alkyl groups.

Although simple tropanes and tropisetron do not activate non-α7 nAChR, they apparently bind to non-α7 AChR, since they can function as competitive antagonists. It is interesting that the potency of tropane compounds, both as α7 agonists and antagonists of other nAChR, is enhanced by indole conjugation. Our data show that the effect of indole conjugation on tropisetron potency is not due to an effect of the indole group at the site where 5HI can bind to potentiate α7 responses. Our data also suggest that as a potentiator, 5HI can obviate some factors limiting the efficacy of partial agonists since the potentiated responses to partial agonists are equivalent to the potentiated responses of full agonists.

Studies of α7 nAChR pharmacology have identified several classes of structurally diverse selective agonists. Variously large or small structural modifications have been identified which can convert a relatively nonselective ("core") agonist into one which selectively activates α7 receptors. Such

modifications to the agonist may be associated with "selectivity motifs" that are effective in the α7 binding site but exclude either binding and/or activation of other nAChR subtypes. The structural elements of the α7 binding site which match these motifs may be considered "selectivity filters". The structural diversity of α7-selective agonists suggests that there are multiple selectivity motifs and, correspondingly, multiple selectivity filters. However, there is more to a good therapeutic candidate than just subtype selectivity; issues of potency, efficacy, and blood–brain permeation are also important. We show that even though the indole portion of tropisetron is not important for α7 selectivity, it does greatly impact the potency of the drug. Likewise in the family of benzylidene anabaseine agonists, specific side groups on the benzylidene moiety account for a large range of differences in potency and efficacy [8]. Through modeling the α7 agonist binding site it should be possible to advance future development of nAChR-based therapeutics by matching the selectivity motifs of selective agonists to the structural features of the receptor.

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References

[1] W.H. Bunnelle, M.J. Dart, M.R. Schrimpf, Design of ligands for the nicotinic acetylcholine receptors: the quest for selectivity, Curr. Top. Med. Chem. 4 (2004) 299–334.

[2] D. Gurley, E.W. Harris, C. Li, E.C. Johnson, T. Lanthorn, 5-Hydroxyindole potentiates the nicotinic acetylcholine receptor alpha7 subtype, Soc. Neurosci. Abstr. 716.15 (2000).

[3] D.A. Gurley, T.H. Lanthorn, Nicotinic agonists competitively antagonize serotonin at mouse 5-HT3 receptors expressed in *Xenopus* oocytes, Neurosci. Lett. 247 (1998) 107–110.

[4] J.E. Macor, D. Gurley, T. Lanthorn, J. Loch, R.A. Mack, G. Mullen, O. Tran, N. Wright, J.C. Gordon, The 5-HT3 antagonist tropisetron (ICS 205-930) is a potent and selective alpha7 nicotinic receptor partial agonist, Bioorg. Med. Chem. Lett. 11 (2001) 319–321.

[5] M.B. Marrero, R.L. Papke, B.S. Bhatti, S. Shaw, M. Bencherif, The neuroprotective effect of TC-1698, a novel alpha7 selective ligand, is prevented through angiotensin II activation of a tyrosine phosphatase, J.P.E.T. 309 (2003) 16–27.

[6] G. Mullen, J. Napier, M. Balestra, T. DeCory, G. Hale, J. Macor, R. Mack, J. Loch III, E. Wu, A. Kover, P. Verhoest, A. Sampognaro, E. Phillips, Y. Zhu, R. Murray, R. Griffith, J. Blosser, D. Gurley, A. Machulskis, J. Zongrone, A. Rosen, J. Gordon, (−)-Spiro[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin-2′-one], a conformationally restricted analogue of acetylcholine, is a highly selective full agonist at the alpha 7 nicotinic acetylcholine receptor, J. Med. Chem. 43 (2000) 4045–4050.

[7] R.L. Papke, M. Bencherif, P. Lippiello, An evaluation of neuronal nicotinic acetylcholine receptor activation by quaternary nitrogen compounds indicates that choline is selective for the a7 subtype, Neurosci. Lett. 213 (1996) 201–204.

[8] R.L. Papke, E.M. Meyer, S. Lavieri, S. Bollampally, T. Papke, B. Horenstein, J.K.P. Papke, Effects at a distance in alpha7 nAChR selective agonists: benzylidene substitutions regulate potency and efficacy, J. Neuropharm. 46 (2004) 1023–1038.

[9] R.L. Papke, J.K.P. Papke, Comparative pharmacology of rat and human alpha7 nAChR conducted with net charge analysis, Br. J. Pharmacol. 137 (2002) 49–61.

[10] R.L. Papke, G. Rose, J.K.P. Papke, Activity of a7-selective agonists at nicotinic and serotonin 5HT3 receptors expressed in *Xenopus* oocytes, Bioorg. Med. Chem. Lett. 14 (2004) 1849–1853.

[11] B.P. Richardson, G. Engel, P. Donatsch, P.A. Stadler, Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs, Nature 316 (1985) 126–131.

[12] P. Whiting, J. Lindstrom, Purification and characterization of a nicotinic acetylcholine receptor from rat brain, Proc. Natl. Acad. Sci. U.S.A. 84 (1987) 595–599.

[13] R. Zwart, G. De Filippi, L.M. Broad, G.I. McPhie, K.H. Pearson, T. Baldwinson, E. Sher, 5-Hydroxyindole potentiates human alpha 7 nicotinic receptor-mediated responses and enhances acetylcholine-induced glutamate release in cerebellar slices, Neuropharmacology 43 (2002) 374–384.