In this study, we described the properties of a thiazolylidene analog, A-716096, to elucidate biochemical neurotransmitter release, electrophysiological activity and behavioral effects *in vivo*. A-716096 was found to potentiate ACh-evoked α7 nAChR currents in oocytes expressing rat or human α7 nAChRs, but did not potentiate other nAChR subtypes (hα4β2 and hα3β4) in FLIPR-based Ca^{2+} influx assays. A-716096 enhanced agonist-evoked phospho-ERK in PC12 cells as well as α7 nAChR-evoked [^{3}H] NE release in SH-SY5Y cells. Like other α7 PAMs (NS1738, TQS and PNU-120596), A-716096 did not displace the binding of either [^{3}H] MLA or [^{3}H] A-585539 to rat cortical membranes. Co- or pre-application of A-716096 amplified choline-evoked α7-like current responses in hippocampal CA1 slices and enhanced synaptic inhibitory activity in dentate gyrus in electrophysiological studies. Consistent with *in vitro* data, *in vivo* administration of A-716096 in mice increased dose-dependently phosphorylation of the downstream signaling protein, CREB. *In vivo* evoked-potential EEG recordings revealed that A-716096 improved sensory gating in DBA2 mice, a strain that exhibits natural deficits on this pre-attention measure. Taken together, these results demonstrate that A-716096 is a valuable tool compound with which to further study mechanism of action and other physiological effects of PAMs including modulation of synaptic effects and signaling mechanisms critical for learning and memory.

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2.12

2,2-Dimethylcyclopropyl-benzamides: Novel positive allosteric modulators of α7 nAChRs

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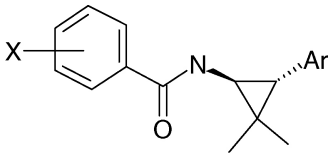
Activation of α_7 nicotinic acetylcholine receptor (nAChR-α_7) has been shown by pre-clinical and clinical evidence to improve cognitive function and a number of partial or full agonists are in advanced clinical trials, among them GTS-21, EVP-6124, AZD-0328 and R34787/MEM3454. Positive allosteric modulators of this ion channel offer the option of enhancing the endogenous signal of acetylcholine while avoiding the rapid desensitization and long-lasting deactivation caused by agonists. A prototypic agent in this class is PNU-120596. The identification and early optimization of a novel series of 2,2-dimethylcyclopropyl-benzamides as positive modulators of the nAChR-α_7 channel is described.



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2.13

In vitro and *in vivo* characterization of PheTQS, a novel α7 nAChR positive allosteric modulator

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PheTQS ((3aR, 4S, 9bS)-4-(4-methylphenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide, WO2004098600) facilitated nicotine (10 μM)-evoked responses in GH4C1 cells stably expressing human α7 nAChRs with a $pEC_{50} = 7.4 \pm 0.2$ and exhibited >30-fold selectivity vs. the 5-HT_3 receptor and α4β2 and α1, α3-containing nAChRs. PheTQS also facilitated ACh (300 μM)-evoked currents in rat cultured hippocampal neurons with a $pEC_{50} = 7.0 \pm 0.2$ to a maximum of 6191±955% of control response charge, but did not activate the receptor in the absence of ACh at up to 3 μM. Bath application of PheTQS (1 μM) mediated an MLA-sensitive potentiation of currents evoked by local ejection of ACh (1 mM) onto CA1 stratum radiatum interneurons in rat hippocampal slices. PheTQS exhibited good brain exposure following oral administration (Brain C_{max} = 1540 ng/g, 10 mg/kg p.o.) but had no affect on activity measures up to 32 mg/kg in the rat. PheTQS (30 mg/kg, p.o.) elicited a significant increase in extracellular levels of dopamine in the prefrontal cortex and significantly increased c-Fos immunoreactivity in the central nucleus of the amygdala and the shell of the nucleus accumbens. PheTQS (10 mg/kg, i.v.) enhanced auditory gating in anaesthetised DBA2 mice and this effect was abolished by prior administration of α-bungarotoxin (1.25 nmol, i.c.v.). PheTQS (10 and 30 mg/kg, p.o.) attenuated deficits in pre-pulse inhibition in isolation-reared rats and significantly improved performance in a rat novel object recognition task. Thus, PheTQS is a selective α7 nAChR positive allosteric modulator which exhibits efficacy in rodent sensory gating and cognition models suggesting potential therapeutic utility in psychiatric disorders.

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2.14

Dual allosteric modulators of neuronal nicotinic-acetylcholine and GABAA receptors

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We have designed a molecule that incorporates selective negative allosteric modulation of $GABA_A$ α5 receptors and positive modulation of α7 neuronal nicotinic receptors (nAChRs). This molecule termed 2-228 represents the first known compound with putative cognition enhancing properties derived from simultaneous modulation of both $GABA_A$ and nAChRs. The research goal is to develop a positive allosteric modulator of α7 nAChRs that

is also a negative allosteric modulator of $GABA_A$ α5 receptors with more drug-like attributes than 2-228. The proposed molecule will selectively activate α7 nAChRs over other neuronal nAChRs (e.g. α4β2, α3β4). In addition, the proposed molecule selectively inhibits activity mediated by $GABA_A$ α5 receptors relative to other $GABA_A$ subunit containing receptors (e.g. α1, α2, α3). 522-054 is an analog of 2-228 that has a similar dual allosteric profile as 2-228 but with improved absorption. 522-054 is active in the radial arm maze (RAM) and 5-choice serial reaction (5-CSR) model at doses that suggests synergy between α7 nAChRs and $GABA_A$ α5 for cognition may be possible to design into one molecule.

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2.15

Effects of 4R,6R-cembratriene diol on human α7 nicotinic acetylcholine receptor

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Neuronal nicotinic acetylcholine receptors (nAChR) have been targeted for developing drug treatments in a wide variety of illnesses and conditions that affect humans. The α7 nAChR was reported to have a role in the Alzheimer's disease, Parkinson's disease, schizophrenia, Tourette's syndrome, and anxiety disorders. The 4R, 6R-cembratriene diol (4R) is a cyclic diterpenoid that displays neuroprotective properties by a mechanism involving the α7 nAChR. The present work was undertaken to study the effects of 4R on human α7 nAChR expressed in SHSY5Y cells (obtained from Novartis Pharma AG). Using α[^{125}I]bungarotoxin binding assay, we determined that the level of expression was 1.5 pmoles receptor per mg protein. Whole-cell patch-clamp recordings indicated that nicotinic agonist (300 μM ACh, 80 μM nicotine, 1.6 mM choline and 1 μM epibatidine) evoked inward currents with amplitudes of 50-1500pA. Currents induced by 300 μM ACh and 1 μM epibatidine were totally inhibited by 10 nM methyllycaconitine (MLA), a selective α7 antagonist. 4R displayed a complex pattern of effects on the current evoked by 300 μM ACh. 4R inhibited the response to ACh at low concentration (30 nM 4R; 50% inhibition) and at high concentration (30 μM 4R; 95% inhibition), but not intermediate concentrations (1-10 μM 4R, no inhibition). 10 nM MLA totally inhibited the current remaining in the presence of 30 nM 4R, but only partially the current observed in the presence of 10 μM 4R. These results are consistent with the interpretation that 4R acts both as an inhibitor and as a positive modulator of human α7 nAChR.

Conflict of interest: Vesna Eterovic and Richard Hann have patents related to the use of Cembranoids; SHSY5Y cells expressing alpha7 nAChRs were obtained from Novartis Pharma AG.

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2.16

Characterization of type I and type II positive allosteric modulators of α7 nicotinic acetylcholine receptors

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Positive allosteric modulators (PAMs) of nicotinic acetylcholine receptors (nAChRs) have attracted considerable interest. They are useful experimental tools to study the pharmacological and biophysical properties of nAChRs. In addition, it has been suggested that they may have potential therapeutic use in the treatment of cognitive deficits associated with disorders such as schizophrenia and Alzheimer's disease. An extensive series of compounds have been identified that act as selective positive allosteric modulators of α7 nAChRs. All potentiate peak agonist-evoked responses but differences have been reported in their effect on receptor desensitization. These compounds have been designated as being either "type I" or "type II" potentiators, on the basis of their differing effects on receptor desensitization [1,2]. Type I compounds (e.g. LY-2087101 and NS-1738) have little or no effect on the rate of desensitization, whereas type II compounds such as PNU-120596 dramatically reduce rates of receptor desensitization. We have previously obtained evidence to indicate that positive allosteric modulators of α7 nAChRs may bind to an intrasubunit transmembrane site [3]. We have now extended those studies with the aim of determining whether all α7-selective positive allosteric modulators share a common binding site. In part, these studies have been prompted by recent evidence suggesting that potentiation by some allosteric modulators (e.g. NS-1738) may be influenced by additional nAChR domains [4] (findings that have been reproduced in our own lab). In order to examine this question, we have performed studies with a series of chimeric and mutated nAChRs expressed in *Xenopus* oocytes. A variety of experimental approaches have been used, including those designed to investigate competitive ligand binding, as well as studies examining receptor modification by cysteine-reactive reagents. Data obtained from these studies supports the hypothesis that both type I modulators (such as LY-2087101 and NS-1738) and type II modulators (such as PNU-120596) interact competitively on α7 nAChRs at a common allosteric site.

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Section 3. Nicotine addiction and smoking

3.1

Chronic nicotine exposure differentially alters gene expression in VTA from adolescent and adult rats

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Smokers who begin in adolescence are at a higher risk of developing dependence than those who begin as adults. We have previously demonstrated that adolescent male Sprague Dawley rats have a distinct pattern of expression of the three major neuronal nicotinic acetylcholine receptors (nAChR) subtypes and differential response to chronic nicotine treatment in multiple brain regions compared to their adult counterparts [1]. A different pattern of CNS nicotinic receptor expression may play a role in the initiation of smoking among adolescents. Furthermore, the distinct pattern of responses of nAChR subtypes to nicotine during adolescence may contribute to the higher daily consumption and decreased probability of cessation observed in smokers who initiate tobacco use during adolescence. We used a similar chronic nicotine exposure model to examine the effects of chronic nicotine treatment on whole genome expression in the ventral tegmental area (VTA). Adolescent and