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