Characterization of novel type I positive allosteric modulators of the α7 nicotinic acetylcholine receptor.

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INTRODUCTION

Positive allosteric modulation of the α7 nicotinic acetylcholine receptor (α7 nAChR) offers a promising therapeutic strategy for cognitive enhancement in disorders including Alzheimer's disease and schizophrenia. Compared with agonists, α7 nAChR positive allosteric modulators (PAMs) amplify transmission without affecting intrinsic signalling patterns or desensitizing the receptor, potentially providing a broader therapeutic range. In literature, α7 nAChR PAMs have been categorized as either type I or type II depending on their effect on the rate of desensitization relative to acetylcholine (ACh). Type I PAMs are typified by an increase in the amplitude of the acetylcholine response, with minimal effect on desensitization kinetics. We sought to identify a series of novel type I α7 nAChR PAMs using a screening paradigm of electrophysiology and an animal model of cognition. Herein, we describe three novel type I α7 nAChR PAMs produced in a medicinal chemistry campaign with the objective to generate potent and orally efficacious compounds.

MATERIALS AND METHODS

***In vitro* assessment** α7 PAM activity was tested using stable cell lines expressing human or rat α7nAChR/GH4C1 by Ca^{2+} flux and electrophysiology. Potentiation of an EC_{20} response to nicotine or ACh, by 3 μM of each PAM was measured by fluorescence detection and Patchliner® (Nanion Technologies).[1] Dose responses were obtained with manual patch-clamp recordings using a fast-application system (Dynaflow®; Cellectricon, Sweden). Allosteric or agonist activity of BL010343 on related receptors was evaluated in a membrane potential fluorescence assay at 3, 10, 30, 50 μM (α1 nAChR in human TE671 cells, α3 nAChR in human SH-SY5Y cells , α4β2 in HEK cells. Agonists for PAM activity were epibatidine on α1 and α3 and nicotine for α4β2.

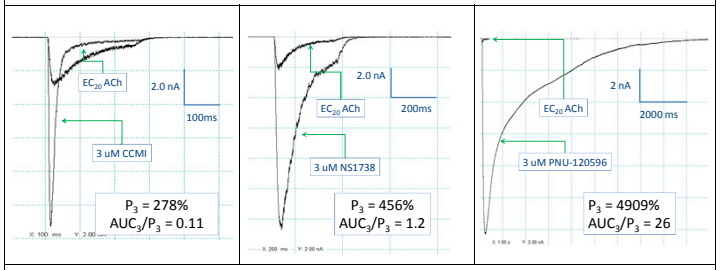
***In vivo* characterization** The compounds were evaluated in the T-maze Continuous Alternation Task (T-CAT),[2] which tested the ability to reverse memory and cognitive deficits induced by the muscarinic AChR antagonist, scopolamine (1 mg/kg, i.p.) administered 20 minutes prior to test. Data presented as percent of spontaneous alternation over 14 free-choice trials within 10 minutes; n=10-20 mice. Compounds were administered 60 minutes prior to test. PAMs tested were BNC1942 and BNC1977 (3 and 30 mg/kg i.p.) BL010343 (3, 10 and 30 mg/kg p.o.) Donepezil (0.3 mg/kg, i.p.) reversal of scopolamine–induced deficit was used to benchmark BL010343. Statistical analyses were performed using the student's t-test. P values indicate significant differences to scopolamine treatment: ^p≤0.05, ^^p≤0.01, ^^^p ≤ 0.0001. P values representing significant difference to vehicle treatment: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.0001.

RESULTS

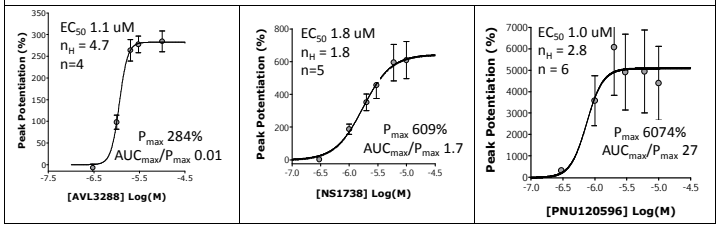
Evaluation of reference compounds – quantitation of effect on desensitization

AVL-3288 "Type I"	NS1738 "Type I"	PNU-120596 "Type II"

A Overlay of compound PAM at effect (3 uM) relative to ACh (EC_{20})



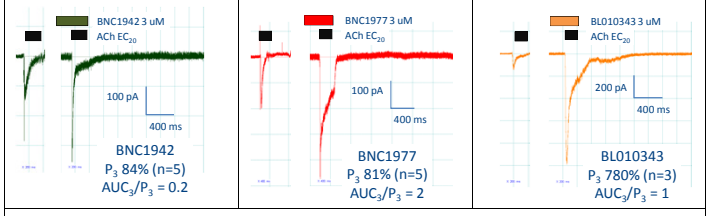
B Concentration-response curves of compounds in presence of ACh (EC_{20})



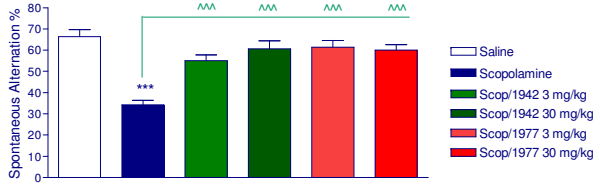
Three reference compounds were evaluated using our electrophysiology platform to generate a uniform dataset to compare with our compounds. Further, we wanted to establish a simple metric for the definition of novel α7 nAChR PAMs as either type I or II in a screening paradigm. **A** AVL-3288 and NS1738 (type I PAMs) and PNU-120596 (type II)[3,4] were tested at 3 μM. The AUC alone was not sufficient to define the effect on desensitization, since the peak potentiation (P_3) varied almost 20-fold. We used the ratio of normalized AUC_3/P_3 as a simple means to categorize PAMs as type I (AUC_3/P_3<2). **B** On testing the concentration–response, the reference compounds gave similar potencies (1.0-1.8 μM) and a range of potentiation (284%-6074%). The ratio of AUC/P at maximal effect was comparable to the 3 μM ratio.

Screening of novel compounds

C Electrophysiology screen (Patchliner® 3 μM compound at ACh EC_{20})



D In vivo screen (T-CAT, compound dosed IP at 3 and 30 mg/kg)

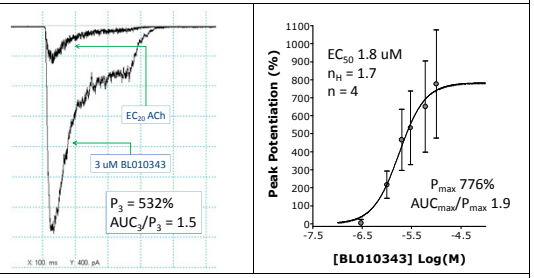


In our drug discovery program, we aimed to establish the correlation between *in vivo* effect for type I PAMs. Accordingly, we set early evaluation points as the effect on α7 nAChR using an electropysiology screen at 3 μM and reversal of cognitive deficit in the T-CAT model. **C,D** Two early examples of a chemical series of type I PAMs, BNC1942 and BNC1977, showed that 81-84% potentiation translated into efficacy in the T-CAT model. Advancement of the chemical series to improve the ADME profile, yielded BL10343, a strongly potentiating type I PAM, which was selected for in-depth profiling.

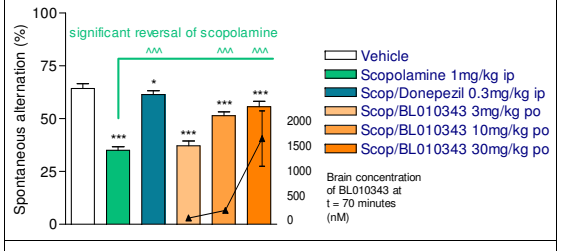
RESULTS

Characterization of BL010343 – a potent, selective orally efficacious type I α7 nAChR PAM

Kinetics and concentration-response curve



Oral efficacy in T-CAT with high brain exposure



No agonist or allosteric effect on related receptors

Receptor	Agonism at 3,10, 30 or 50 uM	PAM effect at 3, 10, 30 or 50 uM
α1 nAChR	X	X
α3 nAChR	X	X
α4β2 nAChR	X	X

CONCLUSION

- The ratio of AUC/peak potentiation was established as a metric to categorize α7 nAChR PAMs as type I with single concentration screening conditions.
- Combined *in vitro* and *in vivo* screening revealed a new series of type I PAMs.
- From this approach, we identifed BL010343, a potent and selective type I PAM with oral efficacy in the T-CAT model.

REFERENCES

1. Dunlop et al; (2007) Biochemical Pharmacology; 74: 1172-1189.
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3. Ng et al; (2008) Proc Nat Acad Sci; 104(19): 8059-8064.
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