Three novel Type II positive allosteric modulators of the α7 nicotinic acetylcholine receptor demonstrate safe, effective cognitive enhancement in mice and rats.

O'Connor S.[1], Andriambeloson E.[2], Coles C.[1], Huyard B.[2], Grishin A. A.[1], Kolesik P.[1], Kolev Y.[1], Huyard B.[2], Wagner S.[2] and Harvey A.[1]

1. Bionomics Limited, 31 Dalgleish Street, Thebarton SA 5031, Australia
2. Neurofit SAS, Parc d'Innovation, Boulevard Sebastien Brant, Illkirch, 67400, France

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INTRODUCTION

Positive allosteric modulation (PAM) of the α7 nicotinic acetylcholine receptor (nAChR) represents a promising therapeutic target for cognitive impairment in Alzheimer's disease and schizophrenia. Compared with agonists, α7 nAChR PAMs amplify transmission without affecting intrinsic signalling patterns or desensitising the receptor. We describe three novel Type II α7 PAMs produced in a focussed medicinal chemistry campaign with the objective of making potent and orally efficacious compounds.

METHODS

***IN VITRO* ASSESSMENT** of compounds was performed in a Ca^{2+} flux assay which measured potentiation of an EC_{20} nicotine response (Dunlop 2007); and by electrophysiology, where potentiation of an EC_{20} ACh response by 3uM of each PAM was measured using a Patchliner® (Nanion). More detailed characterisation of each compound was performed with conventional patch-clamp recordings using a fast-application system (Dynaflow®, Cellectricon, Sweden). All experiments were performed in stable cell lines expressing human or rat α7nAChR/GH4C1.

***IN VIVO* CHARACTERISATION** was performed using the mouse T-maze Continuous Alternation Task (T-CAT) (Spowart-Manning & van der Staay, 2004) and the rat Novel Object Recognition (NOR) (Ennaceur & Delacour, 1988). Both models explored the ability of the compounds to reverse a memory deficit induced by scopolamine. Each compound was compared to vehicle and scopolamine treated animals for their reversal of the scopolamine–induced memory deficit.

T-MAZE: Scopolamine (1 mg/kg) and Donepezil (0.3 mg/kg) were administered to mice i.p., 20' prior to testing. BNC1881 and AVL-3288 (1 mg/kg) were administered i.p.; BNC2591 and BL010362 were administered orally, 1 hour prior to testing. Percent of spontaneous alternation was measured over 14 free-choice trials or after 10 min had elapsed; n=10-20 mice.

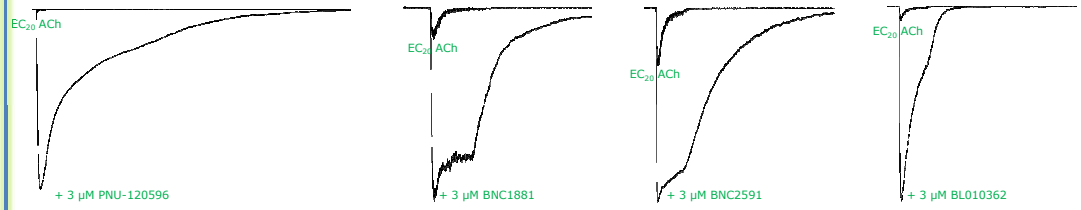
NOR: Scopolamine (1 mg/kg and Donepezil (0.3 mg/kg) were administered to rats i.p., 20' prior to testing. BNC1881 and CCMI (1 mg/kg) were administered i.p.; BNC2591 and BL010362 were administered orally, 1 hour prior to testing. For each animal, the time taken to explore familiar object A (tA) and novel object B (tB) was recorded during a 10 minute period and the recognition index (RI) determined using the formula RI = tB/(tA + tB) x 100; n=12-22 rats

STATISTICAL ANALYSES: were performed using the student's t-test. P values indicating significant difference to scopolamine treatment: ^p≤0.05, ^^ p ≤ 0.01, ^^^ p ≤ 0.0001.

P values representing significant difference to vehicle treatment only: * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.0001

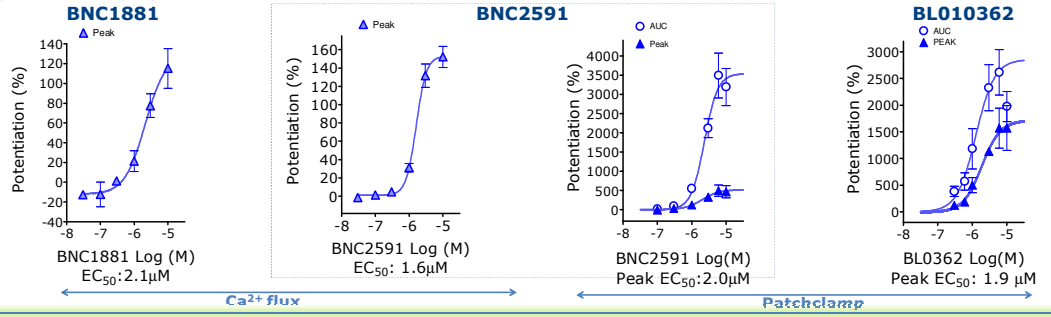
RESULTS

1. The novel compounds are Type II PAMs and have been compared with a Type II example from the literature, PNU-120596 (Hurst, 2007). Patch-clamp recordings show that 3μM of each compound potentiates the EC_{20} ACh peak current and area under the curve, and delays receptor desensitisation.

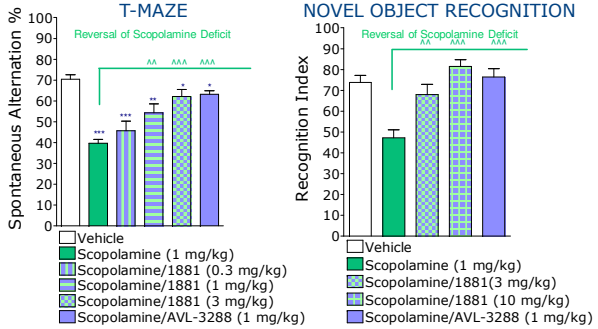


Compound	PNU-120596	BNC1881	BNC2591	BL010362
Potentiation of EC_{20} ACh BY 3μM	4908.5 ± 1771.7%	421 ± 86%	360 ± 54%	1130 ± 68%
RATIO AUC:PEAK	26.42	5.56	5.64	2.06

2. EC50 values are in the 1-2 μM range. Full dose responses were obtained for BNC1881 and BNC2591 in a Ca^{2+} flux assay and for BNC2591 and BL010362 using patch-clamp recordings with Dynaflow®. Good correlation was seen between the values obtained from each method (BNC2591:1.6 μM (Ca^{2+}) and 2.0 μM (Patch-clamp). Interestingly, improvement in the percentage of potentiation (efficacy) across the compounds was not paralleled by potency as seen by the similar EC_{50} values for BNC2591 vs. BL010362 (2.0 vs. 1.9 μM).



3. BNC1881 (i.p.) reverses cognitive impairment and fully restores memory in mice and rats.



BNC1881	
MED in T-maze	1 mg/kg; i.p.
Efficacy in T-Maze (% Vehicle Control)	88% (3 mg/kg)
MED in NOR	3 mg/kg; i.p.
Efficacy in NOR (% Vehicle Control)	110% (10 mg/kg)

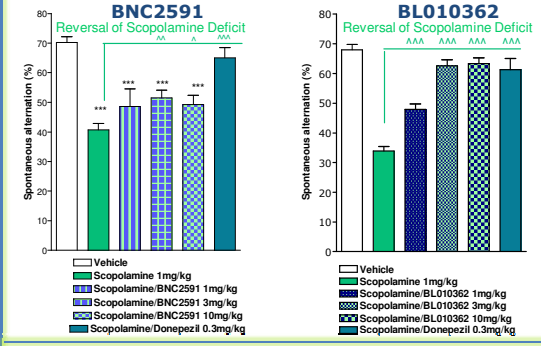
(AVL-3288, Type 1 PAM (Ng et al., 2007)

BNC2591 and BL010362 are orally active compounds with improved *in vivo* efficacy

4. BNC2591 is orally active with potency and efficacy similar to BNC1881. Pro-cognitive activity was demonstrated in the T-maze where BNC2591 significantly restored spontaneous alternation in scopolamine treated mice at 3 and 10 mg/kg, p.o.

5. BL010362 is orally active with superior efficacy to BNC1881, BNC2591 and Type II PAMS described in the literature. BL010362 demonstrates:

- Significant reversal of scopolamine deficit with MED≤1 mg/kg; p.o. (p≤ 0.0001).
- Full restoration of memory with ≥3mg/kg; po.
- Doses ≥3mg/kg; p.o. are as effective as Donepezil
- No 'bell-shaped' dose response seen – an effect associated with α7 agonists



CONCLUSIONS

- We have developed and characterised a series of Type II PAMS and demonstrate their progression to orally active compounds with increased potentiation of the peak ACh response and improved efficacy *in vivo*
- Pro-cognitive activity has been demonstrated in mouse and rat models
- BL010362 performs better than Donepezil
- Compounds do not exhibit the 'bell-shaped' dose response
- A broad range of Type II channel kinetics has been shown, from PNU-120596-like effects to those more typical of compounds at the Type I end of the PAM spectrum
- All compounds have micromolar EC_{50} values but vary in their ability to potentiate a 3μM ACh response from 360% to ~1100%