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 Ca²⁺ flux assay which measured potentiation of an EC₅₀ nicotine response (Donlop 2007) and by electrophysiology, where potentiation of an EC₅₀ ACh response by 3 μM 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 (Dynaflo®, Cellnetix, 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 Staa, 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.

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 3 μM of each compound potentiates the EC₅₀ ACh peak current and area under the curve, and delays receptor desensitisation.

2. EC50 values are in the 1-2 μM range. Full dose responses were obtained for BNC1881 and BNC2591 in a Ca²⁺ 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 (Ca²⁺) 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 EC₅₀ 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.

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 EC₅₀ values but vary in their ability to potentiate a 3 μM ACh response from 360% to ~1100%