The Novel Anxiolytic Compound BNC210 Reduces Stress on the Elevated Plus-maze in Pre-stressed Rats and Exhibits an Antidepressant Effect in the Rat Forced Swim Test

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Introduction

Generalised Anxiety Disorder (GAD) is a chronic, recurrent disease with a low rate of spontaneous remission that is characterised by excessive worry and interferes with the daily routine of those who suffer from it. The major pharmacologic agents currently used to manage patients with anxiety disorders include benzodiazepines, SSRIs and SNRIs. However, use of these agents is limited by a significant number of side-effects. Benzodiazepines produce sedation, motor defects, impairment of memory, dependence and tolerance. SSRIs and SNRIs have slow onset of action and side effects such as early agitation, sexual dysfunction and discontinuation syndrome. There is a clear medical need for fast-acting anxiolytic agents that lack the significant side-effects seen with current treatments.

We have identified the novel compound BNC210 and previously shown that it displays acute activity in several rodent models of anxiety (light dark box; mouse marble burying test; the elevated plus maze and isolation induced vocalizations in guinea pig pups). Chronic administration of BNC210 does not result in development of tolerance to its anxiolytic effect. Most importantly, BNC210 does not cause sedation, even at very high dose levels, does not affect motor co-ordination and does not impair cognitive function. Furthermore, chronic administration of BNC210 does not cause any signs of abuse liability. BNC210 exhibits excellent oral bioavailability and plasma pharmacokinetics that are consistent with once a day dosing.

Materials & Methods

FORCED SWIM TEST. The forced swim test (FST) (Porsolt et al, 1978 *Eur. J. Pharmacol*. **47**, 379-391), is the most widely used paradigm for evaluation of the potential antidepressant effect of compounds. Antidepressants have the ability to increase escape motivated behaviour and to decrease immobility time. The test is performed for 5 minutes in a glass cylinder (35cm High; 24cm Diameter) containing water to a depth of 25 cm and maintained at a temperature of 25±1°C. Immobility time is measured.

Doses of BNC210 were administered to rats p.o., 60 min prior to the swim test. Imipramine was the comparator and was administered i.p., 30 minutes prior to the swim test.

FORCED SWIM STRESS. Experimental conditions employed in the forced swim test were used to generate a stress response in rats with the aim of evaluating the ability of BNC210 to moderate anxiety in pre-stressed animals. Rats were exposed to 90 seconds of swim stress 5 minutes prior to being evaluated in the elevated plus-maze test.

ELEVATED PLUS MAZE. The elevated plus maze (EPM) (Pellow et al, 1985 *J. Neurosc. Meth*. **14**:149-167) is based on the conflict between the innate tendencies of rodents to explore novel environments and to avoid open, brightly lit areas. It is used to evaluate the relative anxiety status of rats. The 'plus-shaped' apparatus consists of four equal exploratory arms (45 × 10 cm) which are interconnected by a small platform (10 × 10 cm). Two arms are well lit and open and the other two are enclosed with 30 cm high walls and dimly lit. The apparatus is placed 66 cm above the floor. In this task the rat is placed in the centre of the maze from where it can walk down any of four runways. Rats prefer the closed arms but will venture out into the open arms. The amount of time spent in the open arms and the number entries into the open arms are recorded. "Anxious" rats will spend little time, and make very few entries, into the open arms. The apparatus is cleaned between each animal with 70% alcohol. Rats were exposed to 90 seconds of swim stress 5 minutes prior to being placed on the elevated plus-maze.

NON-PRECIPITATED WITHDRAWAL TEST. The aim of this assessment is to evaluate whether sudden cessation of drug treatment is associated with the occurrence of identifiable physical signs of withdrawal such as changes in body weight, food intake and body temperature. This method (Goudie et al, 1993 *NeuroReport* **4**:295-299) can be used to evaluate a wide variety of substances including those for which specific antagonists are not available. Rats received daily doses of BNC210, p.o for 14 consecutive days. Over the next 4-5 days following drug withdrawal, rats were measured for significant changes in food intake, body weight and body temperature.

Results

BNC210 REDUCES ANXIETY IN PRE-STRESSED RATS We have previously shown that BNC210 (0.1, 1, 10 and 50 mg/kg) has significant effects on anxiety-related behavior under basal conditions (Figure 1A and 1B).

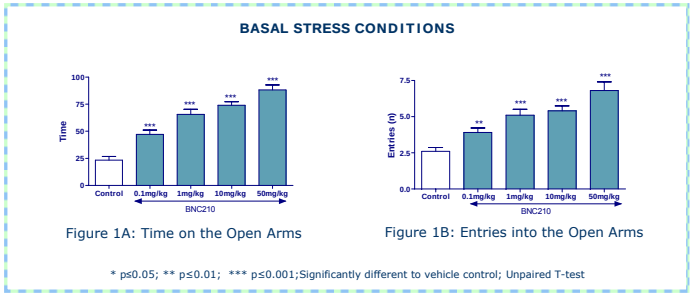


Figure 1A: Time on the Open Arms

Figure 1B: Entries into the Open Arms

* ps0.05; ** p≤0.01; *** p≤0.001;Significantly different to vehicle control; Unpaired T-test

Exposure to 90 seconds of swim stress significantly reduced the number of 'entries into' and 'time spent' on the open arms of the elevated plus-maze. This marked effect is indicative of increased levels of anxiety-related behaviour in the rats (Figure 2A and 2B)
Single doses of BNC210 at 1, 10 and 100 mg/kg reversed the swim stress-induced reduction in time and entries spent on the open arms. The BNC210 activity seen was dose dependent with the 100 mg/kg dose producing the largest benefit (Figure 2A and 2B).

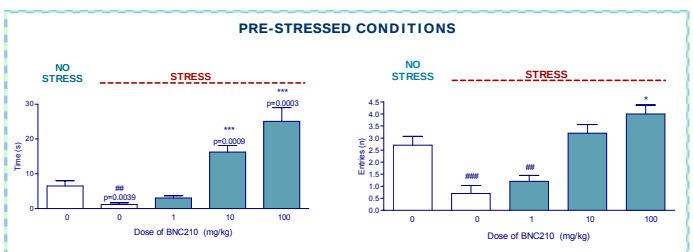


Figure 2A: Time on the Open Arms

Figure 2B: Entries into the Open Arms

* ps0.05; ** p≤0.01; *** p≤0.001;Significantly different to vehicle control; Unpaired T-test

BNC210 EXHIBITS ANTIDEPRESSANT ACTIVITY IN THE RAT FORCED SWIM TEST The potential activity of BNC210 as an antidepressant was evaluated in the forced swim test. BNC210 was administered to rats at 10, 20, 30 and 100 mg/kg 1 hour prior to exposure to the forced swim test. Significantly reduced immobility time compared to the vehicle treated rats was observed for the 100mg/kg dose (Figure 3A). The lower doses of BNC210 were not active. Chronic administration of BNC210 for 14 days at 10, 30 and 100 mg/kg/day resulted in a half log increase in potency with the dose of 30 mg/kg also causing a significant reduction in immobility time (Figure 3B). BNC210 has antidepressant effects in the acute rat FST which are augmented following chronic administration for 14 days. In accord with clinically active anti-depressants, doses of BNC210 reduce the total time of relative immobility in this test and sensitization develops to the effects of repeat administration.

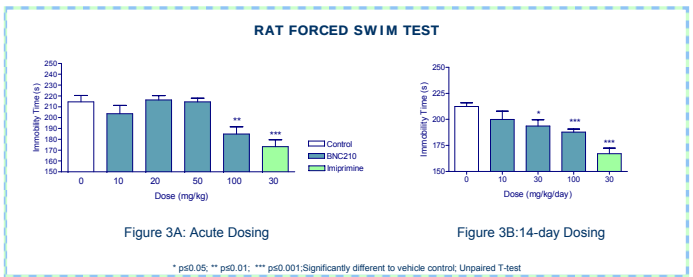
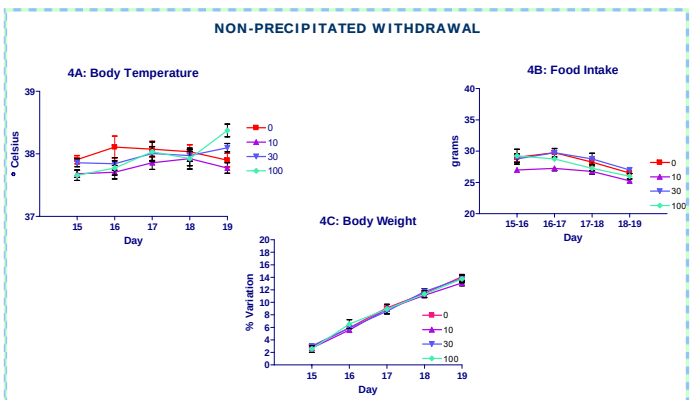


Figure 3A: Acute Dosing

Figure 3B:14-day Dosing

* ps0.05; ** ps0.01; *** ps0.001;Significantly different to vehicle control; Unpaired T-test

BNC210 DOES NOT PRODUCE SIGNS OF WITHDRAWAL FOLLOWING A 14-DAY DOSING PERIOD Rats treated chronically with opioids, benzodiazepines or SSRIs display adverse physical effects after non precipitated withdrawal of the drugs. We assessed the potential consequences of abrupt cessation of dosing with BNC210 following 14 days of treatment at O, 10, 30 and 100 mg/kg/day. Withdrawal of BNC210 treatment did not produce changes in rat body temperature, weight gain or food consumption (Figure 4A, 4B and 4C) for the duration of the post-treatment period (5 days) indicating that repeated dosing with BNC210 does not cause the development of physical dependence to the drug and is consistent with its suitability for chronic use.



Discussion & Conclusions

►In addition to its effects on innate anxiety levels, BNC210 is effective in reducing stress-induced anxiety.
► BNC210 exhibits antidepressant activity in the rat Forced Swim Test. The activity displayed by BNC210 was similar in magnitude to Imipramine. BNC210 antidepressant activity increased considerably following repeat administration for 14 days.
►Withdrawal of BNC210 treatment following 14-day repeat dosing does not result in physical adverse events usually seen following withdrawal of opioids, benzodiazepines or SSRIs.
►The activity profile seen with BNC210 in these preclinical tests expands its therapeutic potential to treatment of generalized anxiety disorder with co morbid depression.
►The safety and pharmacokinetics of BNC210 in healthy volunteers is currently being evaluated in a Phase I clinical trial.