BNC210 Is A Novel, Fast Acting Compound With Potent Anxiolytic Activity And No Side Effects

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Introduction

Clinically significant anxiety disorders remain one of the most widespread health problems in the world. Generalised Anxiety Disorder (GAD) is a chronic, recurrent disease characterised by excessive worry which interferes with the daily lives of those who suffer from it. The major pharmacologic agents currently used to manage patients with GAD include Benzodiazepines, SSRIs and SNRIs. The therapeutic benefits offered by these agents are counterbalanced by significant side-effects.

There is a clear medical need for fast-acting anxiolytic agents that :

lack the side-effects seen with current treatments and offer the same or greater therapeutic benefits

We have undertaken a targeted medicinal chemistry approach, directed towards improving the anxiolytic profile of select quinolones known to exhibit central nervous system (CNS) effects, to discover novel agents that will satisfy this medical need. This effort has led in the identification of BNC210, a compound with a unique profile *in vivo* which suggests a novel mode of action.

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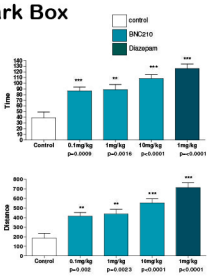
BNC210 has an Anxiolytic Effect in the Mouse Light Dark Box

BNC210 has a significant effect on the performance of mice in this model as demonstrated here with doses of 0.1 - 10mg/kg (PO). We have previously shown that the minimum effective dose is 0.01mg/kg (PO) for the Time and Distance parameters. At 10mg/kg, the magnitude of the anxiolytic response with BNC210 is not significantly different from diazepam. Data represents mean ± SEM. n=10 mice. p values calculated using Fisher's Protected Least Significant Difference test



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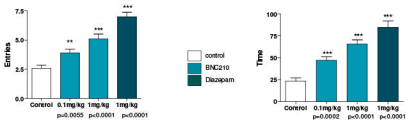
BNC210 Displays Anxiolytic Activity in the Rat Elevated Plus Maze



BNC210 was examined in the Rat Elevated Plus Maze at doses of 0.1 and 1mg/kg; PO. The number of entries into and time spent on the open arms of the maze was significantly increased by both doses. The minimum effective dose in this model was 0.1mg/ kg. Data represents mean ± SEM. n=10 rats, p values calculated using Fisher's Protected Least Significant Difference test.

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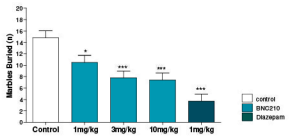
Anxious Marble Burying Behaviour in Mice is Reduced by BNC210



Mice have an innate tendency to bury objects lying on the surface of the cage. The Marble Burying model of anxiety has been validated with several anxiolytic compounds including Diazepam. BNC210 significantly reduced marble burying behaviour by up to 51% at a dose of 10mg/kg and a clear dose response was seen for the dose range used in the experiment. Data represent mean ± SEM. n=10 mice, *p=<0.05, **p<0.001 (Fishers Protected Least Significant Difference test).

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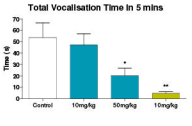
BNC210 Reduces the Duration of Separation Induced Vocalizations in a Guinea Pig Model of Anxiety



BNC210 significantly reduced total vocalization time by guinea pig pups at 50mg/kg (IP). The anxiolytic profile of BNC210 in this model differentiates it from other anxiolytic compounds and supports the concept that it has a novel mechanism of action. Data represents mean ±SEM. n=10 guinea pig pups, *p=<0.05, **p<0.01, Fishers Protected Least Significant Difference test.

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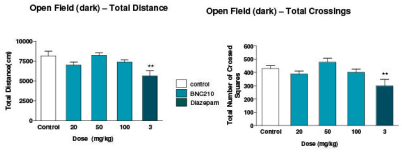
BNC210 is Not Sedating in the Mouse Open Field (Dark)



BNC210 does not effect spontaneous locomotor activity in the Open Field (dark) at doses of 20, 50 or 100mg/kg (PO). In contrast, the sedative side effect of diazepam is clearly observed at a dose of 3mg/kg (PO). Data represent mean ± SEM. n=10 mice, **p=<0.01 (Fishers Protected Least Significant Difference test).

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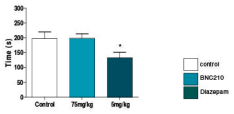
BNC210 Does Not Affect Motor Coordination in Mice at High Doses



There is no difference in performance on the Rotarod between vehicle treated mice or BNC210 treated mice (75mg/kg(PO). In contrast, 5mg/kg (PO) of diazepam significantly reduces the ability of mice to stay on the rotating rod. Data represents mean ± SEM. n=10 mice, *p=<0.05 (Fishers Protected Least Significant Difference test).

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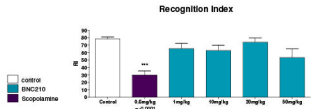
BNC210 has been Evaluated in the Rat Object Recognition Test for Memory Impairment



BNC210 was evaluated in the rat Object Recognition test at doses ranging from 1 to 50mg/kg; PO. No significant effect on short term memory was seen as indicated by the Recognition Index (RI) ((Time B/(Time A+B))*100. We have previously shown that in the rat elevated plus maze BNC210 had an anxiolytic effect at 0.1mg/kg which represents a therapeutic window of >500x. Scopolamine demonstrated strong memory impairment at 0.6mg/kg. Data represents mean ± SEM. n=10 rats, p values calculated using Fishers Protected Least Significant Difference test.

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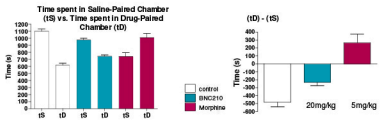
BNC210 is Not Addictive in Mice in the Conditioned Place Preference Test



At a dose of 20mg/kg (PO), which is 200x the minimum therapeutic dose, BNC210 does not increase the time mice spend in the drug-paired compartment of the Conditioned Place Preference apparatus when allowed free exploration. In contrast, mice treated with 5mg/kg of morphine develop a clear preference for the drug-paired compartment. Data represent mean ± SEM. n=10 mice.

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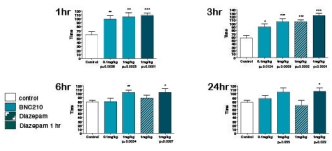
Duration of the Anxiolytic Effect of BNC210 in the Mouse Light Dark Box After Administration of an Acute Dose



BNC210 and diazepam were assessed at 1, 3, 6 and 24 hours in the light dark box. At the 6 hour time point, mice treated with1mg/kg (PO) of BNC210 still spent significantly more time in the lit area whereas the diazepam treated mice (1mg/kg; PO) were similar to control animals. Data represents mean ± SEM. n=10 mice, p values calculated using Fisher's Protected Least Significant Difference test.

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Physicochemical and Pharmacokinetic Properties

BNC210 has physicochemical properties that are consistent with being a "good drug"

- **MW: = 418**
- **PSA: 75 Å²**
- **FRB: 4**
- **logD pH7.4 = 2.71**
- **Number of steps in synthesis: 5**
- **Number of Chiral Centres: 0**

BNC210 is orally bio-available and is suitable for 1x daily dosing (rat pharmacokinetics)

- **T1/2 = 6.2 h**
- **Tmax = 60 min**
- **%F = 69%**
- **Plasma Protein Binding: 77% Bound**
- **Microsome Metabolism**
- **Low to Intermediate metabolism rates**
- **No significant species difference in metabolism**
- **No species differences in metabolites (rat, dog, human)**

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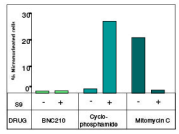
Safety Studies

Assessment of interaction with hERG

A hERG binding assay was done in HEK-293 cells with the reference compound astemizole. 10µM of BNC210 gave no inhibition of astemizole binding.

Evaluation of genotoxicity in the micronucleus assay

BNC210 does not cause genotoxicity in the micronucleus assay at concentrations up to 500µm



CYP Inhibition

CYP Isoform	BNC210 IC50 (µM)
CYP1A2	>30
CYP2C9	>30
CYP2C19	>30
CYP2D6	>30
CYP3A4	>30

IC_{50} values for BNC210 activity against major drug-metabolising CYP enzymes in human liver microsomes (substrate specific approach)

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BNC210 is Very Safe

- BNC210 has been evaluated in Acute and 7 Day Toxicology studies in rats and dogs
- BNC210 is well tolerated at all dose levels.
- No mortality, morbidity or other clinical signs of toxicity have been observed
- No abnormalities found on necropsy

Acute Rat

	Doses:
3Males, 3Females /dose	100
GLP with14 day Recovery	300
Weight and Food Consumption	1000
Necropsy	2000

Acute Dog

	Doses:
1Male, 1Female	50
Weight and Food Cons.	150
TK and Clinical Pathology	450

7-Day Rat

5Males, 5Females /Dose	Doses:
Weight and Food Consumption	100
TK(9M,9F/dose)	300
Clinical Pathology	600
Necropsy	

7-Day Dog

	Doses:
2Males, 2Females /dose	25
Weight and Food Consumption	100
TK and Clinical Pathology	400
Necropsy	

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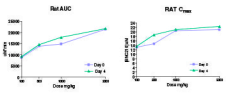
Pharmacokinetics of BNC210 in Rat and Dog Studies

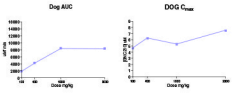
RAT Study Parameters

5 day repeat dosing at 100, 600,1000, 2000mg/kg 9 Females/dose; 3 Day Recovery; Weight; Food Consumption; Toxico kinetics; Clinical Pathology; Necropsy



DOG Study Parameters

Single dosing at 100, 400,1000, 2000 mg/kg; 1Male and 1 Female/dose; Weight; Food Consumption; Toxico kinetics; Clinical Pathology;



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BNC210 Summary

- **Potent anxiolytic activity in multiple models and species with efficacy as low as 0.01mg/kg**
- **MTD is >1000mg/kg**
- **Not sedating**
- **No effect on motor coordination**
- **No memory impairment**
- **No abuse liability**
- **No effect on CYP450 enzymes or hERG**
- **Good physicochemical properties & PK**
- **Good oral bioavailability and potential for 1x daily oral dosing**
- **Novel proprietary compound**

No potential conflicts of interest