CHARACTERISATION OF PSNCBAM-1, A NOVEL ALLOSTERIC ANTAGONIST OF THE CANNABINOID TYPE 1 RECEPTOR WITH *IN VIVO* EFFICACY IN AN ACUTE RAT FEEDING MODEL

[1]Horswill, J.G., [1]Bali, U., [2]Shaaban, S., [1]Keily, J.F., [1]Babbs, A.J., [3]Cheetham, S., [1]Reynet, C., [1]Wong-Kai-In, P.[1]Prosidion Limited, Windrush Court, Watlington Road, Oxford, UK, OX4 6LT; [2]OSI Pharmaceuticals Inc., Bioscience Park Drive, Farmingdale, NY11735, USA; [3]Renasci Consultancy Limited, Pennyfoot Street, Nottingham NG1 1GF, UK. Prosidion Limited is a wholly owned subsidiary of OSI Pharmaceuticals, Inc.

Cannabinoid type 1 receptors (CB_1) are widely distributed in the brain and in peripheral tissues such as fat, liver, and intestine. There is considerable interest in the development of CB_1 antagonists for obesity treatment. Recently, the antagonist/inverse agonist SR141716A (rimonabant, Acomplia^{TM}) has been shown to significantly reduce body weight, waist circumference and triglyceride levels in obese patients (Pi-Sunyer *et al*., 2006). Modulation of the CB_1 receptor by compounds that bind to allosteric sites has recently been reported by Price *et al.* (2005). Here we describe the *in vitro* characterisation of a novel class of CB_1 allosteric antagonists, typified by PSNCBAM-1 (Figure 1) and also provide the first demonstration of the *in vivo* efficacy of a CB_1 allosteric antagonist in an acute rat feeding model.

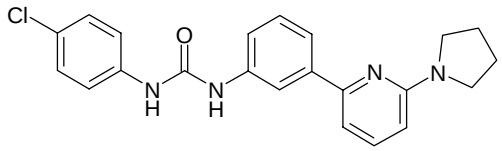

Figure 1. Structure of PSNCBAM-1

PSNCBAM-1 showed antagonism of CB_1 by inhibiting the agonist effect of 100 nM CP55940 in a human CB_1 (hCB_1) yeast reporter assay with an IC_{50} value of 45.2 ± 7.5 nM (n=3, mean ± s.e.m.). Interestingly, PSNCBAM-1 was inactive in yeast cells expressing constitutively active hCB_1, suggesting that the compound lacked inverse agonist properties. This was in contrast to SR141716A which reduced both CP55950 induced and constitutive CB_1 signalling with IC_{50} values of 22.5 ± 7.3 nM and 4.8 ± 0.4 nM respectively. In competition binding assays, PSNCBAM-1 paradoxically increased the binding of [^{3}H]CP55940 to HEK293-hCB_1 cell membranes by 58 ± 9 %, indicating positive modulation of agonist binding. Partial inhibition of [^{3}H]SR141716A binding was observed in similar experiments. Further characterisation in a mammalian functional assay confirmed the antagonist properties of the compound. PSNCBAM-1 inhibited CP55940 (50 nM) stimulated [^{35}S]GTPγS binding in HEK293-hCB_1 membranes with an IC_{50} value of 74.3 ± 12.7 nM, but not in HEK293-hCB_2 membranes. A Schild analysis using this assay revealed the functional antagonism of PSNCBAM-1 to be non-competitive. Taking these data together, we conclude that PSNCBAM-1 acts as an allosteric antagonist of CB_1.

Furthermore, in acute food intake studies in freely feeding male Sprague-Dawley rats (391 - 607 g), PSNCBAM-1 (30 mg/kg, i.p. using 5 % propyleneglycol / 5 % Tween 80 / 90% saline as vehicle) caused a significant 48 ± 7 % reduction in food intake over 24 hours, as compared to 48 ± 3 % reduction by 10 mg/kg, i.p. SR141716A (n=6, mean ± s.e.m., $P < 0.01$, ANOVA and Dunnett's test). Both PSNCBAM-1 and SR141716A were also found to decrease body weight significantly over 24 hours.

In conclusion, these results provide evidence that novel specific allosteric antagonists of CB_1, typified by PSNCBAM-1, may have the potential for use as anti-obesity agents.

Pi-Sunyer FX *et al.* (2006). *JAMA.* **295**: 761-775.
Price MR *et al.* (2005). *Mol Pharmacol* **68**: 1484-1495.