**Characterising the functional kinetics of $OX_1$ antagonists using a novel fluorometric imaging plate reader (FLIPR) assay**

S. Groom[1,2], R. Mould[1], K. Bennett[1], M. Barnes[1], A. Brown[1]. [1]Heptares Therapeutics, Welwyn Garden City, UNITED KINGDOM, [2]Department of Pharmacy and Pharmacology, University of Bath, Bath, UNITED KINGDOM.

***Introduction:*** Mounting evidence has shown that a compound's kinetic properties can influence its duration of clinical action[1]. However, despite the potential benefits of tailoring the kinetic properties of preclinical compounds, optimisation of such parameters has been hindered by the limitations of traditional assay techniques. The aim of this study was to evaluate the kinetic properties of unlabelled orexin-1 receptor ($OX_1$) antagonists using a functional assay.

***Methods:*** The ability of $OX_1$ antagonists (80μM - 2.5nM) to inhibit the responses to an $EC_{80}$ of orexin-A ligand was tested in CHO cells stably expressing $OX_1$. Antagonist activity was assessed after a 5 minute antagonist preincubation period (37°C) and in coaddition with the orexin-A challenge. Antagonist potency was calculated using a modified Cheng-Prussoff equation and the difference in antagonist potency between the two assays ($\alpha fpK_B$) was used as an estimate of antagonist association rate ($k_{on}$). In order to characterise antagonist dissociation rate ($k_{off}$), Schild analysis was conducted by performing concentration-response curves of orexin-A (1μM - 31.6ρM) in the presence of the $OX_1$ antagonists ACT-462206 and suvorexant (800nM - 800ρM, 5 minute preincubation) (37°C). Kinetic profiling of unlabelled antagonists was generated in SF21 cell membranes expressing $OX_1$ using Motulsky-Mahan radioligand binding methods[1], using [$^3$H]-SB-674072.

***Results:*** Although for a number of antagonists, including ACT-462206 and almorexant, $\alpha fpK_B$ was in line with $k_{on}$, there was no coherent link observed between the two parameters across a wider range of antagonists. ACT-462206 displayed fully surmountable antagonism, whereas suvorexant caused concentration-dependent depression of the maximal orexin-A responses. The dissociation kinetics of suvorexant were much slower than those of ACT-462206, with $k_{off}$ values of 0.0068±0.0003min$^{-1}$ and 0.64±0.12min$^{-1}$ (mean±SEM, $n$=3) respectively.

***Conclusions:*** While studies into association rates yielded unclear results, pseudo-insurmountability in Schild experiments appeared to correlate with $k_{off}$, in line with previous studies[2]. This demonstrates that dissociation kinetics can be qualitatively assessed using a widely available assay technology, providing a functional platform to optimise for desirable compound kinetics early in the drug discovery process.

***References:***

(1)

- Dowling MR and Charlton SJ (2006). *Br J Pharmacol* **148**:927-937.

(2)

- Mould R *et al.* (2014). *Br J Pharmacol* **171**:351-363.

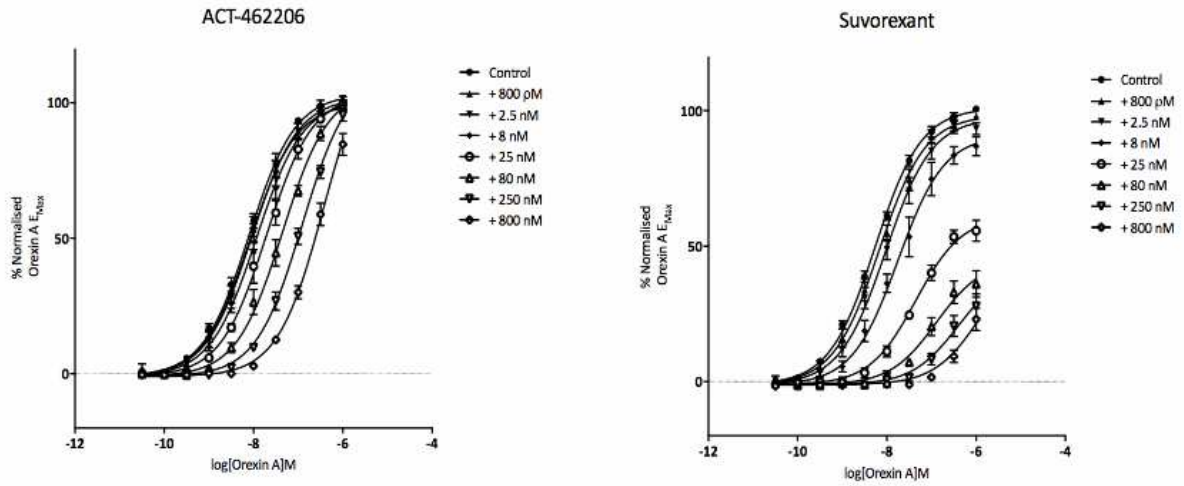


**Figure 1.** The effect of increasing concentrations of ACT-462206 and suvorexant on orexin-A concentration-response curves in CHO-$OX_1$ cells. Data are representative of mean±SEM, *n*=3.

| Compound | $OX_1$ Preincubation fp$K_B$ | $OX_1$ Coaddition fp$K_B$ | $\Delta$fp$K_B$ | $k_{on}$ ($\mu M^{-1}.min^{-1}$) |
|---|---|---|---|---|
| Almorexant | 7.37±0.15 | <4.10 | >3.27 | 0.27±0.02 |
| ACT-462206 | 7.69±0.12 | 7.20±0.15 | 0.49 | 19.45±2.41 |

**Table 1.** Antagonist activity in preincubation and coaddition FLIPR assays. Data are representative of mean±SEM, *n*=3.