Functional high throughput screening for identification of human OX_2 antagonists

Vanessa Burlon, Geoffroy Ronvaux, Sébastien Blanc, Nadège Pugnet, Delphine Colette, Sophie Brogniet & Jerome Bernard

Contact: jbernard@euroscreen.com, Tel: +32 71 348 508, www.euroscreen-fast.com - **Euroscreen FAST, Adrienne Bolland 47, 6041 Gosselies, Belgium**

Euroscreen FAST, a Business Unit of Euroscreen SA, rue Adrienne Bolland 47, 6041 Gosselies, Belgium

EUROSCREEN fast
FUNCTIONAL ASSAYS & SCREENING TECHNOLOGIES

Introduction

Orexins, which were initially identified as endogenous peptide ligands for two orphan G-protein coupled receptors, have been shown plays key role in the control of eating, sleeping and rewarding (reviewed in Civelli, 1998). Recently, two orexin receptor antagonists achieved clinical proof-of-concept for the treatment of insomnia (Roecker & Coleman, 2008). With these exciting findings, the search for Orexin (or hypocretin) receptor antagonists for the treatment of sleep and neurological disorders has recently increased in intensity in the pharmaceutical industry. Here we present the assay development and validation for high throughput functional screening of Orexin 2 (OX_2) receptor, using Aequorin assay (further information and methods available upon request).

Results

Aequorin assay was selected as primary screening assay for these Gq coupled receptors. Moreover such functional assay allowed to screen simultaneously for agonists and antagonists. Even if some slow acting agonists could be missed in such flash kinetics assays, the screening conditions for antagonists were well validated with reference compounds (Fig. 1, Hirose et al., 2003). Different timing were evaluated and screening were performed either with 15 or 30 min incubation. A miniscreen was performed on two independent days with the same set of 5,000 compounds (Fig. 2) and confirmed that the test was very robust and suitable for high throughput screening. The screening of Euroscreen library was performed with a throughput of 15,000 compounds/day and gave a Z' of 0,77 in agonist mode (Fig. 3) and 0.69 in antagonist mode (Fig. 4) with a very good stability for reference compounds (Fig. 6). All the positive were directly confirmed and tested in parallel for counterscreening with endogenous $P2Y_2$ (Fig. 5) and for specificity with OX_1 (Fig. 5). Such confirmation step allowed to discard non specific compounds. Additional assays were developed for characterization of positives and hit to lead programs, including radioligand binding assays (Fig. 7), IPOne HTRF functional assay (Fig. 8) and rat OX_1 and OX_2 aequorin assays (Fig. 9). This screening allowed to identify several chemotypes with some highly potent compounds (Fig. 10).

Figure 1: OX_1 (top) & OX_2 (bottom) aequorin assays

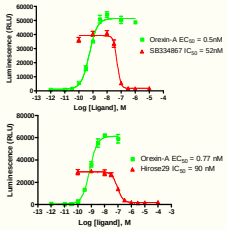
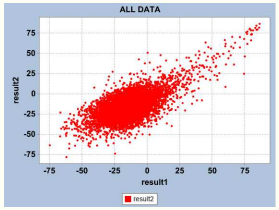


Figure 2: Validation of OX_2 HTS with a miniscreen



Fig; 3: Aequorin OX_2 agonist screening

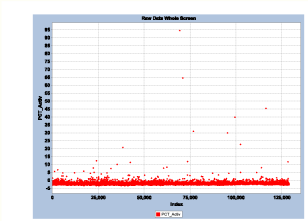


Fig. 4: Aequorin OX_2 antagonist screening

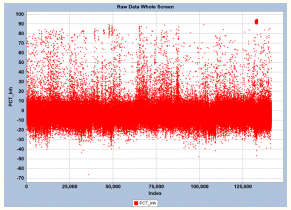


Fig. 5: Aequorin counterscreening (top) & specificity screening (bottom)

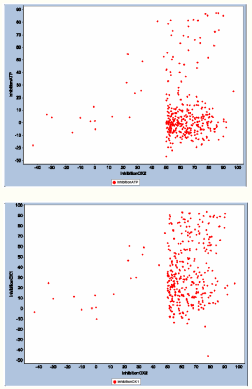


Fig. 6: Reproducibility of OX_2 assay

	Average (nM)	StDev	n
Orexin-A	0,48	0,335	21
Orexin-B	0,40	0,281	20
Hirose 29	295	135	21

The compound labeled 29 in the publication of Hirose et al. was synthesized by Euroscreen's chemists and used as a reference antagonist (Bioorg Med Chem Lett. 2003, 13:4497-9)
Hirose 29 gave an IC50 < 100 nM when tested at 0.3 nM OXA

Fig. 7: OX_2 radioligand binding assay

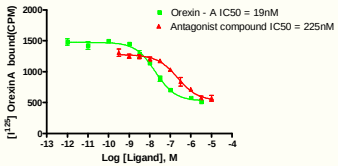


Fig. 8: OX_2 IPOne™ assay

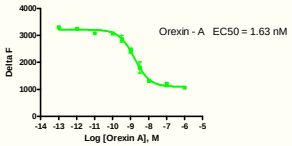


Fig. 9: Rat OX_1 & OX_2 functional assays

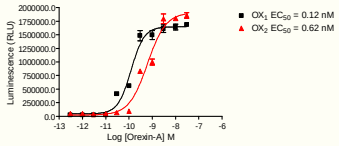
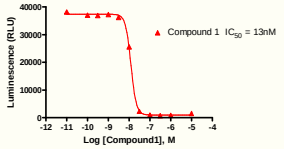


Fig. 10: Example of OX_2 Hit



Conclusions

OX_1 and OX_2 high throughput functional assays were successfully developed and used for screening of Euroscreen & customers libraries. Here we present the data obtained with Euroscreen library and the use of various assays for further characterization of hits that are now in lead optimization

References
Civelli (1998) FEBS Lett. 430:55-8
Hirose et al. (2003) Bioorg Med Chem Lett. 13:4497-9
Roecker & Coleman (2008) Curr Top Med Chem. 8:977-87

On Target