Identification and characterisation of novel positive allosteric modulators of the Galanin 2 Receptor

Hayley Jones, Jeff Jerman, MRC Technology, London
David Wynick, University of Bristol, Bristol, UK
Catherine Wark, Carl Peters, Ph.D., BMG LABTECH, Cary, NC

Application Note 236, Rev. 06/2013

- An HTRF IP1-based functional HTS has been used to screen the MRCT 100K compound collection
- The detection for this screening is simplified using the HTRF optic module for the PHERAstar
- Strong statistical results such as Z' and % CV indicate this approach is useful for HTS

Introduction

Chronic nerve damage or injury induces alterations in the primary sensory neurons in the dorsal root ganglion (DRG) and their central connections (Figure 1).

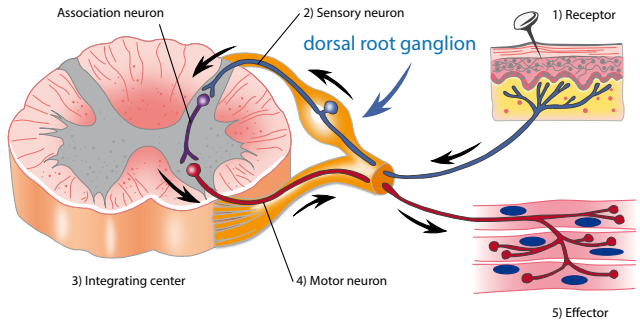

Fig. 1: *Nociceptive Pathways*

The physiological effects of galanin are mediated by activation of galanin receptors (GalR1, GalR2 and GalR3) which inhibit adenylyl cyclase. GalR2 additionally mediates intracellular calcium mobilisation. The galanin system, particularly GalR2, is implicated in the regulation of nociception, in part based on a dramatic 120-fold up-regulation in the levels of galanin seen in the DRG after nerve injury. Potentiation of galanin-induced peripheral GalR2 activity should ultimately lead to a marked reduction in nociceptive responses and offer novel therapeutics for neuropathic pain.

In contrast to 'direct' orthosteric agonist activation, positive allosteric modulators (PAMs) of GalR2 could afford additional therapeutic advantage. These include; improved receptor-subtype selectivity, retention of physiologically-controlled spatial and temporal resolution, a self-limiting saturability of effect and exploitation of untapped chemical space. Confirmed and selective PAMs may provide novel therapies and IP for a range of unmet clinical needs.

We have undertaken a high-throughput screen of GalR2 to identify novel Neuropathic Pain therapeutics. This screen employed the MRCT 100K compound collection, a selection of drug-like molecules from commercial libraries which includes over 10,000 compounds which target protein-protein interaction.

Assay Principle

A robust HTRF® functional IP1 assay (Cisbio) was used for screening. The assay is based on a competitive immunoassay principle whereby free IP1 competes against IP1-d2 (HTRF® acceptor) for binding to anti-IP1 Cryptate conjugate (HTRF® donor). The signal is inversely proportional to IP1 levels in the cell with maximum FRET obtained in the absence of IP1 (Figure 2).

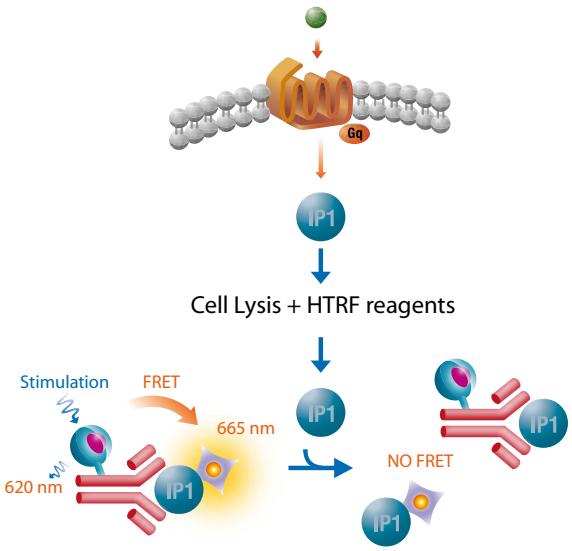

Fig. 2: *Cisbo IP-One HTRF assay*

Materials and Methods

- ☐ CHO cells stably expressing GalR2 (GE Healthcare)
- ☐ IP-One HTRF® assay kit (Cisbio)
- ☐ White 384-well small volume plates (Greiner)
- ☐ PHERAstar microplate reader with HTRF optic module [620/665] (BMG LABTECH)

The assay was configured using CHO cells stably expressing GalR2. Pre-incubation of cells with a sub-maximal concentration of the galanin agonist sensitised the HTS to the simultaneous detection of both agonists and PAMs. The MRCT 100K compound collection was screened at a final assay concentration of 10 µM.

5 µl of cells/well (15,000 cells) were dispensed and 2.5 µl compound or buffer control was added to 384 well low volume white plates. Following a 30 min incubation at 37°C, 2.5 µl of galanin was added at a maximal concentration of 1 µM (EC100) or an above-minimal concentration of 3.16 nM (EC20). Test samples received buffer containing 0.1% BSA. Plates were incubated for 1 hr at 37°C. Subsequently, 5 µl of each HTRF reagent were added to each well and a 1 hr incubation at room temperature performed. Plates were then read using the PHERAstar microplate reader.

PHERAstar instrument settings

Readings were performed using the HTRF optic module that excites at 337 nm and detects emission at 665/620 nm simultaneously. Using a focal height of 12.3 mm, integration was performed for 400 micro seconds (integration time) starting at 50 micro seconds (integration start) using 400 flashes per well. A ratio multiplier of 10,000 was employed.

Data calculation

Data normalization was performed by calculating the ratio of the raw data obtained at 620 and 665 nm:

Ratio = (665/620)*10000

This ratio was expressed relative to EC100 and EC20 such that EC20 = 0% and EC100 = 100% (Figure 3). Hits were selected using a 30% response cut off or 30% above EC20.

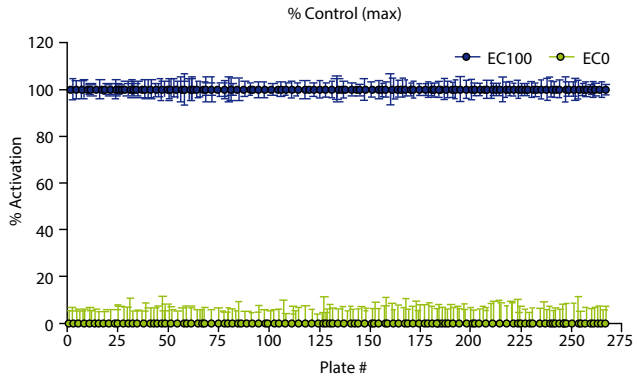

Fig. 3: *Data were normalised to high (EC100) and low (EC20) controls*

Results and Discussion

The HTS assay performed well as indicated by the robust and consistent Z' data (Figure 4). For 85320 compounds screened, a mean Z' of 0.72 (± 0.05) was obtained.

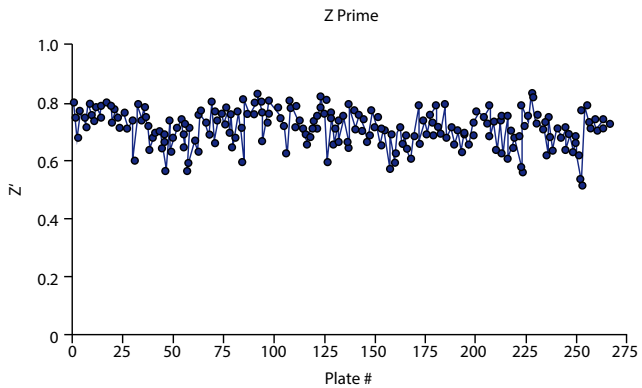

Fig. 4: *Z prime values consistently exceeded plate pass/fail acceptance criteria (Z' › 0.5)*

Furthermore, the %CV for the control wells is low: Low Control %CV = 3.7 (± 1.9); High Control %CV = 5.0 (±0.8).

Table 1 shows the number of hits depending on cutoff. 250 compounds are selected for confirmation studies.

Table 1: *Number of hits attained during screening.*

Cutoff (%)	#Hits
30	250
40	117
50	65
60	29
70	13
80	9
90	4
100	1

Conclusion

The screen gave a fairly low hit rate (0.3% at 30% activity), with the majority confirmed in PAM mode (data not shown). However, most compounds were also active agonists (data not shown). Further studies are underway against an additional 80 K library. The same approach with the PHERAstar (Fig. 5) can be used as the results shown here validate this application.

Fig. 5: *BMG LABTECH's multidetection microplate reader PHERAstar*

Germany:	**BMG LABTECH GmbH**	**Tel: +49 781 96968-0**
Australia:	BMG LABTECH Pty. Ltd.	Tel: +61 3 59734744
France:	BMG LABTECH SARL	Tel: +33 1 48 86 20 20
Japan:	BMG LABTECH JAPAN Ltd.	Tel: +81 48 647 7217
UK:	BMG LABTECH Ltd.	Tel: +44 1296 336650
USA:	BMG LABTECH Inc.	Tel: +1 877 264 5227
Internet:	**www.bmglabtech.com**	**applications@bmglabtech.com**