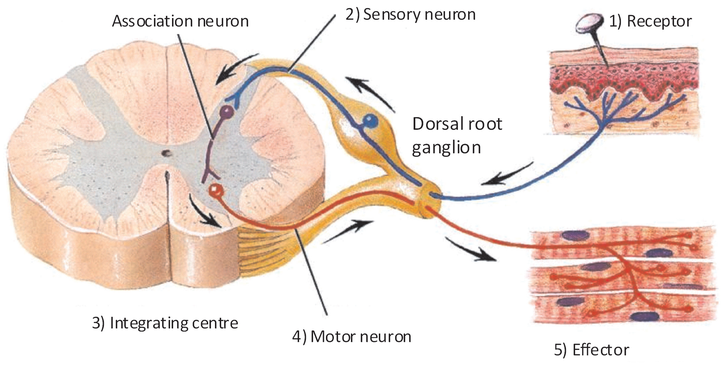
Identification and characterisation of novel positive allosteric modulators of the Galanin 2 Receptor

[1]Hayley Jones, [1]Paul Wright, [1]Jamie Cartland, [1]Catherine Kettleborough, [2] David Wynick and [1]Jeff Jerman.
[1]MRC Technology, Centre for Therapeutics Discovery, 1-3 Burtonhole Lane, Mill Hill, London, NW7 1AD
[2] University of Bristol, Medical Sciences Building, University Walk, Bristol, BS8 1TD

Neuropathic Pain and Galanin

- Chronic nerve damage or injury induces alterations in the primary sensory neurons in the dorsal root ganglion (DRG) and their central connections.
- This leads to spontaneous pain, which can result in the development of neuropathic pain (NP).
- The galanin system, particularly GalR2, is implicated in the regulation of pain response (nociception);
  - A dramatic 120-fold up-regulation in the levels of galanin is seen in the DRG after nerve injury
  - Demonstrated use of GalR2 specific peptide agonists in animal models
  - Use of transgenic mice with altered expression of the peptide or its receptors.
- Potentiation of galanin-induced peripheral GalR2 activity should ultimately lead to a marked reduction in nociceptive responses and offer novel therapeutics for NP.



Nociceptive Pathways

Positive Allosteric Modulation

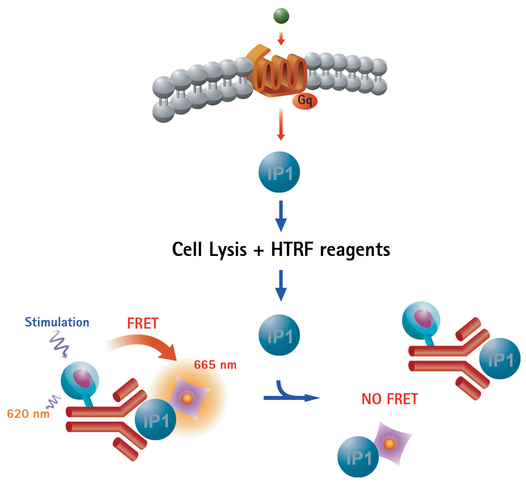
- In contrast to 'direct' orthosteric agonist activation, positive allosteric modulators of GalR2 could afford additional therapeutic advantage.
- These include;
  - Improved receptor-subtype selectivity
  - Retention of physiologically-controlled spatial and temporal resolution
  - A self-limiting saturability of effect
  - Exploitation of untapped chemical space.
- The use of functional assays has enabled simultaneous detection of both orthosteric and allosteric modulators but is associated with additional complexity in screening and follow-up.
- Novel and selective Postive Allosteric Modulators may provide novel therapies and IP for a range of unmet clinical needs.

Dual HTS for Galanin 2 Receptor Agonists and PAMs

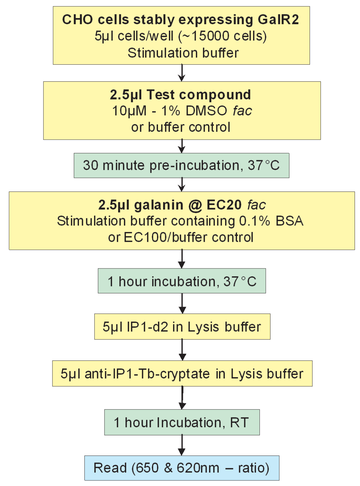
- We have undertaken a high-throughput screen of GalR2 to identify novel Neuropathic Pain therapeutics.
- A robust HTRF® functional IP1 assay (Cisbio) was configured using CHO cells stably expressing GalR2 (GE Healthcare).
- Pre-incubation of cells with a sub-maximal concentration of the galanin agonist sensitised the HTS to the simultaneous detection of both agonists and PAMS.
- ~85K compounds of the MRCT collection were screened at a final assay concentration of 10µM and assay performance criteria were met or exceeded.

HTRF® IP-One Assay

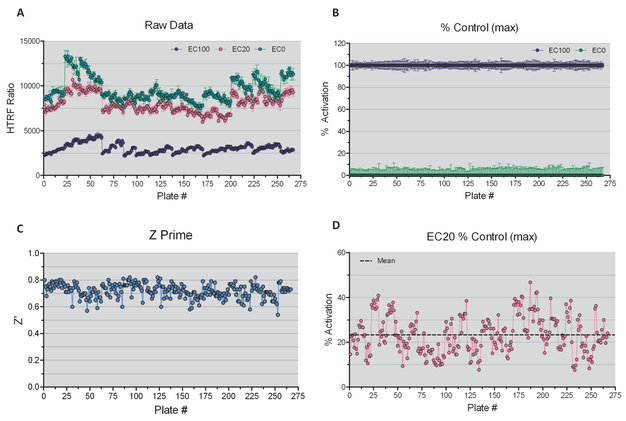
The assay is based on a competitive immunoassay principle whereby free IP1 competes against IP1-d2 (HTRF® acceptor) for binding to anti-IP1 Cryptate conjugate (HTRF® donor). The signal is inversely proportional to IP1 levels in the cell with maximum FRET obtained in the absence of IP1.



HTS Assay Protocol

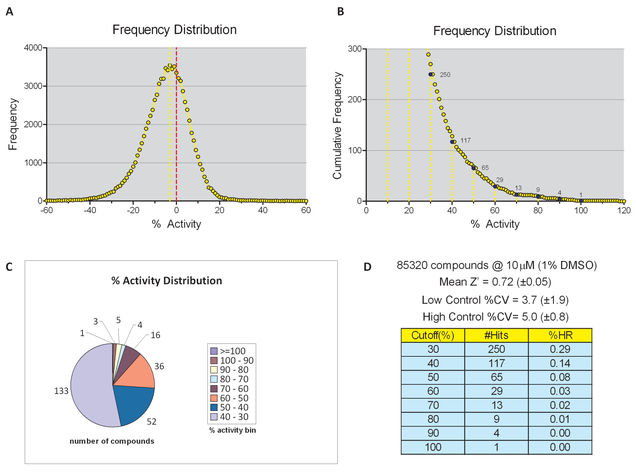


HTS Assay Performance



- Signal magnitude was largely consistent across test occasion and for the entire duration of the screen (A).
  - Data were normalised to high (EC100) and low (EC20) controls (B).
  - Z prime values consistently exceeded plate pass/fail acceptance criteria (Z'≥0.5) (C).
- Throughout the HTS the response to Galanin (nominally EC20) was very consistent despite the complexity of handling this sticky peptide, with an across screen average of 23.3% activity.

HTS Assay Statistics

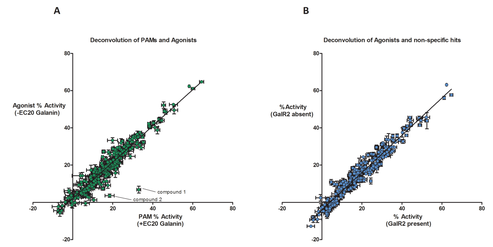


Cutoff(%)	#Hits	%HR
30	250	0.29
40	117	0.14
50	65	0.08
60	29	0.03
70	13	0.02
80	9	0.01
90	4	0.00
100	1	0.00

- In keeping with good assay performance and low control well CVs, the activity of test compounds followed a normal distribution and was sharply centred around 0%. A very slight negative skew likely reflected the presence of inhibitors in the compound collection (A).
- Selected activity cut-offs provided a range of hit rates, albeit on the low side (B&C).
- Overall assay performance statistics were acceptable (D).

Hit Confirmation

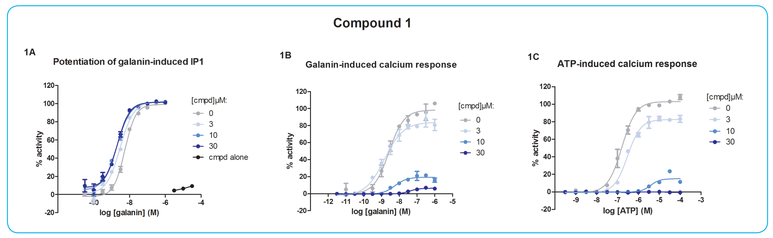
- Using a hit cut-off at 30% activity, above EC20, 250 compounds were selected for confirmation studies.
- Hits were re-screened at a single concentration (10µM) to confirm activity in the presence and absence of EC20 of Galanin (A).
- Compounds were also tested in the absence of receptor (B).

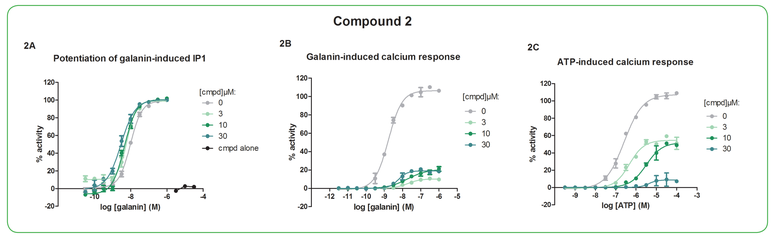


- Analysis shows a lack of discrete populations of putative agonists and PAMs.
- Compounds active only in the presence of native agonist were classified to be PAMS, of which there are two.

Hit Deconvolution

- The putative PAM-like hits (compounds 1 and 2) were further validated to confirm discrete mechanism of action, utilizing full concentration-response curve and leftward-shift assays, to determine efficacy and potency.
- These were tested for potentiation of IP1 signal and in calcium mobilization assays (Molecular Devices, Calcium 5 dye. Protocol as described previously; moleculardevices.com).





- Moderate 0.5 log unit leftward-shifts of the galanin concentration response curves were observed for compounds 1 and 2 at 10µM (1A and 2A).
- However, calcium assay data for both compounds were not consistent with this when induced with galanin (1B and 2B)
- Similarly, ATP-mediated signals were inhibited by both compounds (1C and 2C) and to a similar degree to that of galanin.
- Conflicting data may reflect technical difference in the assays e.g. IP1 accumulation over 60mins vs transient calcium mobilization over approximately 3mins.
- Alternatively, apparent reduction of signal max in the calcium assays may reflect different sensitivities to the compounds themselves e.g. toxicity and/or mechanism of potentiation.
- Testing of further structural analogues of compounds 1 and 2 (data not shown) did not significantly improve the calcium assay profile.
- The exact reason(s) for these discrepancies remain unknown.

Summary and Outlook

- An HTRF IP1-based functional HTS has been used to screen ~85K compounds of the MRCT collection.
- The screen hit rate was relatively low (0.3% at 30% activity cutoff), although reconfirmation rates in PAM mode were high (78.4%).
- The vast majority of putative PAMS were also active in both agonist mode and in WT cells; suggesting only two specific PAMs were identified.
- Unfortunately, despite further validation and small scale medicinal chemistry efforts, the PAM mechanism of action could not be confirmed.
- These data highlight the importance of timely and informative hit validation studies in multiple modalities for GPCR and PAM targets.
- Further screening against a distinct 80K compound library is ongoing.
- It is hoped despite the paucity of chemical starting points and the apparent lack of small molecule modulators of GalR2, PAMs will provide novel therapeutics for the treatment of Neuropathic Pain.

info@tech.mrc.ac.uk mrctechnology.org