Development of a fluorescent ligand competition binding assay for the gastrin releasing peptide BB2 receptor

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\textbf{Introduction.} Gastrin releasing peptide (GRP) and its G protein coupled receptor BB2 are implicated in pain perception, gastrointestinal regulation, chemoattraction, and tumour proliferation\textsuperscript{(1,2)}. Establishing structure activity relationships for BB2 ligands has previously involved radiolabelled agonist binding studies. We report development of a novel BB2 fluorescent ligand (SM1292; BODPIY630/650-[DPhe\textsuperscript{6}, DAla\textsuperscript{11}]Bombesin\textsuperscript{6-13}-NH\textsubscript{2}), based on the amide of BIM26226\textsuperscript{(3)}, for use in a whole cell BB2 competition binding assay for BB2 based on high content imaging.

\textbf{Methods.} HEK293 cells stably expressed SNAP-tagged human BB2 receptor (HEK BB2); fluo4 calcium mobilisation and fluorescent ligand assays were as described\textsuperscript{(4, 5)}. Binding assays used cells on 96 well imaging plates pre-labelled with 0.1\textmu M SNAP-surface AF488 (30min, 37\textdegree C, DMEM). Cells were incubated with SM1292 (0.1-1000nM) plus or minus competing peptides (10\textsuperscript{-11}-10\textsuperscript{-6}M) in HEPES buffered saline solution / 0.1% BSA / H33342 (2\mu g ml\textsuperscript{-1}). After 30min at 37\textdegree C, H33342 nuclear, SNAP-labelled BB2 and BODIPY630/650 ligand images were acquired using an MDC IX Ultra plate-reader; binding was quantified by granularity analysis\textsuperscript{(4)}. Calcium concentration response relationships, saturation binding analysis, and inhibitor competition curves were fitted to individual duplicate or triplicate experiments using GraphPad Prism v7. pEC\textsubscript{50}/pK\textsubscript{D}/pK\textsubscript{i} values (Cheng-Prusoff corrected from IC\textsubscript{50}) are mean±s.e.m. from 3 - 4 experiments.

\textbf{Results.} In calcium mobilization assays, the unlabelled ligand (DPhe\textsuperscript{6}, DAla\textsuperscript{11}]Bombesin\textsuperscript{6-13}-NH\textsubscript{2}) was a competitive antagonist of GRP signaling (control pEC\textsubscript{50} 9.45±0.18), with a pK\textsubscript{D} of 7.2 estimated by Schild analysis. Confocal imaging revealed that SM1292 binding was predominantly distributed to the plasma membrane of HEK BB2 cells and colocalised with SNAP-labelled BB2; this binding was inhibited by the presence of 1\mu M GRP. SM1292 saturation analysis in the absence or presence of 1\mu M GRP, estimated its pK\textsubscript{D} as 7.32±0.18. Competition binding using 10nM SM1292 generated pKi estimates for GRP (8.35±0.22) and neuromedin B (6.74±0.41).

\textbf{Conclusions.} Characterization of SM1292 demonstrates that fluorophore modification of DPhe\textsuperscript{6}, DAla\textsuperscript{11}]Bombesin\textsuperscript{6-13}-NH\textsubscript{2} can be achieved whilst retaining nanomolar affinity for the BB2 receptor, and the SM1292 suitability for binding experiments was confirmed by obtaining the affinities for competing ligands expected for BB2\textsuperscript{(2)}. Supported by a Brazilian CAPES fellowship (#041/2014, LFM), and a Wellcome Trust Vacationship Scholarship (DN).