*Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol16Issue1abst169P.pdf*

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

D. Nesheva[1], L. F. Medeiros[1], H. Verli[2], R. Roesler[3], S. N. Mistry[4], N. D. Holliday[1]. [1]School of Life Sciences, University of Nottingham, Nottingham, UNITED KINGDOM, [2]Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, BRAZIL, [3]Department of Pharmacology, Universidade Federal do Rio Grande do Sul, Porto Alegre, BRAZIL, [4]School of Pharmacy, University of Nottingham, Nottingham, UNITED KINGDOM.

**Introduction.** Gastrin releasing peptide (GRP) and its G protein coupled receptor BB2 are implicated in pain perception, gastrointestinal regulation, chemoattraction, and tumour proliferation (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$^{6}$, DAla$^{11}$]Bombesin$^{6\text{-}13}$-$NH_2$), based on the amide of BIM26226 (3), for use in a whole cell BB2 competition binding assay for BB2 based on high content imaging.

**Methods.** HEK293 cells stably expressed SNAP-tagged human BB2 receptor (HEK BB2); fluo4 calcium mobilisation and fluorescent ligand assays were as described (4, 5). Binding assays used cells on 96 well imaging plates pre-labelled with 0.1µM SNAP-surface AF488 (30min, 37℃, DMEM). Cells were incubated with SM1292 (0.1-1000nM) plus or minus competing peptides ($10^{-11}$-$10^{-6}$M) in HEPES buffered saline solution / 0.1% BSA / H33342 (2µg $ml^{-1}$). After 30min at 37℃, 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(4). Calcium concentration response relationships, saturation binding analysis, and inhibitor competition curves were fitted to individual duplicate or triplicate experiments using GraphPad Prism v7. $pEC_{50}$/$pK_D$/$pK_i$ values (Cheng-Prusoff corrected from $IC_{50}$ ) are mean±s.e.m. from 3 - 4 experiments.

**Results.**In calcium mobilization assays, the unlabelled ligand (DPhe$^{6}$, DAla$^{11}$]Bombesin$^{6\text{-}13}$-$NH_2$) was a competitive antagonist of GRP signaling (control $pEC_{50}$ 9.45±0.18), with a $pK_D$ 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 1µM GRP. SM1292 saturation analysis in the absence or presence of 1µM GRP, estimated its $pK_D$ 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).

**Conclusions.** Characterization of SM1292 demonstrates that fluorophore modification of DPhe$^{6}$, DAla$^{11}$]Bombesin$^{6\text{-}13}$-$NH_2$ 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 (2). *Supported by a Brazilian CAPES fellowship (#041/2014, LFM), and a Wellcome Trust Vacationship Scholarship (DN).*

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