Glucagon-like peptide-1 receptor (GLP-1R) is a G-protein coupled receptor that is present in insulin-secreting beta cells, and GLP-1(7-37) amide and (7-37) with 5-terminal truncation. Their active forms of GLP-1 are deactivated by the further fragmentation with peptidases. The defining action is augmentation of glucose-induced insulin secretion following Its primary screen of natural products was then performed under high throughput screening conditions, followed by the creation of a homogenous cell-based binding assay using Tag-lite technology. The assay was automated using a non-contact liquid dispenser commonly used in high-throughput settings. Detection of the two fluorescent emissions was accomplished simultaneously using the matched PMT of an HTS multi-mode microplate reader. Optimization experiments were performed to validate the procedure to use for automated assay processing. A small primary screen of natural products was then performed under high throughput screening conditions, followed by the creation of dose-response curves using known GLP-1R ligands.

Figure 1 – H9C2 cells were transiently transfected with a pZAP-GFP-GLP-1 plasmid (P3APCGLP-1) for 24 hrs prior to being labeled with SnAP-LumIF (SNAP-FLC). Then, labeled cells were frozen. A 1% DMSO/1X concentration was then created in one instrument. The instrument was used to dispense red labeled agonist and the red emitting labeled ligand. Competition between non-labeled compounds and the Exendin-4 (9-36)amide is present in insulin-secreting beta cells. Intact GLP-1(1-37) is produced by post-translational processing of proglucagon precursor and converted into active forms of GLP-1 (7-37) amide and (7-37) with 5-terminal truncation. Their active forms of GLP-1 are deactivated by the further fragmentation with peptidases.

Materials

BioTek Instrumentation

Figure 2 – GLP-1 receptor cells are dispensed into 384-well plates. Compounds to be tested and a fluorescent derivative of Exendin-4, Exendin-4 red-LOX233, are then added sequentially to the plate. Upon binding of the fluorescent ligand to the GLP-1 receptor, a time resolved fluorescence resonance energy transfer (TR-FRET) occurs between the lumifluor donor and the DCP2 and the red emitting labeled ligand. Competition between non-labeled compounds and the Exendin-4 red-LOX233 leads to a decrease in TR-FRET signal.

BioTek Instrumentation

Figure 3 – Precision® Microplate Dispensing System. The Precision combines an 8-channel pipetting head and an 8-channel bulk reagent dispenser in one instrument. The instrument was used to dilute the Natural Product Library and transfer the final 4X concentrations to the V-SM-well assay plates.

BioTek Instrumentation

Figure 4 – Matrix® Microplate Dispenser. The dispenser offers fast, accurate non-contact plate dispensing rapidness through two peristaltic and two syringe pumps, with volume ranges from 1-3000 µL. The peristaltic pump on the instrument was used to dispense cryopreserved cells, and detection solution to the V-SM-well assay plates.

Materials

Glucagon GLP-1 Receptor Ligand Binding Assay, Tag-lite GLP-1 transformed, Glucagon GLP-1 (3-37)amide (Catalog No. 1117301P), Tag-lite buffer (Tb) (Catalog No. LAMB36), and GLP-1 receptor red agonist were provided by Cistibo US, Inc.

Components

The Screen-Well® Natural Product Library, v. 7.2 (Catalog No. BML001; www.Screen-Well.com). The Screen-Well® Natural Product Library is a high-throughput screening platform that allows for automated assay processing. A small primary screen of natural products was then performed under high throughput screening conditions, followed by the creation of dose-response curves using known GLP-1R ligands.

Table 1 – IC50 values for positive inhibitor compounds. IC50 values from dose response curves generated for the three compounds demonstrating positive inhibition of red agonist receptor binding agree with values or information previously published.

Conclusions

1. The Tag-lite Glucagon GLP-1 Receptor Ligand Binding Assay provides an easy-to-use, cell-based format for detecting ligands of the GLP-1 receptor.

2. The Glucagon-GLP-1 Receptor Ligand Binding Assay provides a small primary screen of natural products was then performed under high throughput screening conditions, followed by the creation of dose-response curves using known GLP-1R ligands.

3. The combination of assay, automation, and rapid, high-quality detection creates a robust process for high-throughput screening of potential GLP-1 receptor modulators.