Targeted In Vitro Pharmacology for GIPR/GLP-1R/GCGR in Weight Loss and Type 2 Diabetes Therapy

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Abstract

In recent decades, drug development has increasingly focused on obesity and type 2 diabetes, driven by the global rise in body weight and associated metabolic disorders. These conditions have become high-priority targets, leading to intense competition within the pharmaceutical industry. Current weight-loss drugs developed by major pharmaceutical companies primarily target the gut-brain axis and utilize incretin-based therapies, such as GIP, GLP-1, and glucagon receptor agonists—either as single agents or in combination. GIP/GLP-1-based therapies promote weight loss and type 2 diabetes by enhancing insulin secretion, delaying gastric emptying, and suppressing appetite. This approach reflects a growing scientific understanding of obesity and metabolic regulation. It also highlights advancements in drug discovery technologies, which now allow for the rapid screening of large compound libraries and efficient validation of their biological activity [1][2].

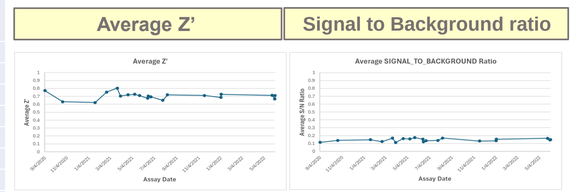
To meet the growing demands of the market, WuXi AppTec has recently upgraded our 370,000-compound small molecule diversity library, emphasizing drug-likeness, structural novelty, and rare chemotypes. In addition, we have developed a series of focused libraries, such as a 10,000-compound GPCR-targeted library which is designed to achieve higher success rates with fewer compounds screened. In response to the increasing research interest in weight loss and type 2 diabetes, WuXi AppTec has generated in-house cell lines expressing key biological targets including GIPR, GLP-1R, GLP-2R, and GCGR across multiple specie as human, mouse, rat, and monkey. These cell lines have been used to support structure–activity relationship (SAR) studies and high-throughput screening (HTS) using cAMP HTRF or radioligand binding assays in 96-, 384-, and 1536-well plate formats. Additionally, we have established GSIS (Glucose stimulated insulin secretion) assay in INS-1 832/3 cells and cAMP HTRF and GSIS assays in EndoC BH5 human pancreatic beta cells offering more physiological relevance insights than those derived from other immortalized cell lines.

In House GIP/GLP-1, GLP-2/Glucagon Receptors Engineered Cell Lines and SAR Screening

List of cell lines

Target	Receptor Species	Host Cell Line	Assay Format
GCGR	hGCGR	HEK293	Binding，cAMP，beta arrestin
	mGCGR	HEK293	Binding，cAMP
	rGCGR	HEK293	Binding，cAMP
	cynoGCGR	HEK293	Binding，cAMP
	rabbitGCGR	HEK293	cAMP
	dogGCGR	HEK293	cAMP
GIPR	hGIPR	CHO	Binding，cAMP，beta arrestin
	hGIPR	HEK293	Binding，cAMP
	mGIPR	HEK293	Binding，cAMP
	rGIPR	HEK293	Binding，cAMP
	cynoGIPR	HEK293	Binding，cAMP
	rabbitGIPR	HEK293	cAMP
	dogGIPR	HEK293	cAMP
GLP-1R	hGLP1R	HEK293	Binding，cAMP，beta arrestin, internalization
	mGLP1R	HEK293	Binding，cAMP
	rGLP1R	HEK293	Binding，cAMP
	cynGLP1R	HEK293	Binding，cAMP
	rabbitGLP1R	HEK293	cAMP
	dogGLP1R	HEK293	cAMP
GLP-2R	hGLP2R	CHO	cAMP

QC of hGLP-1R cAMP HTRF SAR screening

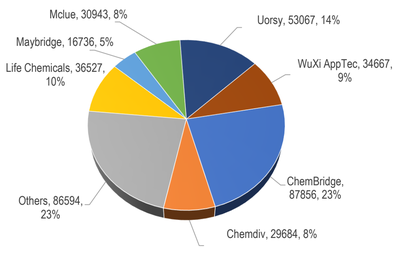


List of in-house cell lines and example QC of SAR screening with hGLP-1R cell. WuXi AppTec has established GIPR/GLP-1R/GLP-2R/GCGR engineered cell lines in various species. Assays have been developed in 384-, 1536-well plate format for cAMP HTRF assay and 96-, 384-well format for radioligand binding assay. Right panel showed representative QC data of hGLP-1R cAMP HTRF SAR screening. Z' was between 0.6-0.8 and signal to background ratio was sable over the runs. GLP-1R reference compound showed consistent potency and efficacy throughout the course, which was indicated by the value of moving MSR (2.1) and MSD (11.6).

WuXi AppTec Diversity and Focused Libraries Offer Flexible Choice for Hit-Finding

Small Molecule Diversity Library (370K)*New*

- **Curated selection** of diverse drug-like molecules from commercial sources and WuXi AppTec's in-house collection:
  - ✓**Prioritization** of drug-likeness, novelty, rare chemotypes
  - ✓PAINS **100%** excluded
  - ✓**>85%** structures meet strict filters, including REOS and Kazius filters
- **High diversity** with an average Tanimoto similarity of **0.1249**:
  - ✓Containing **200K** unique Bemis-Murcko scaffolds, with an average of 1-2 compounds per scaffold
  - ✓Including **167K** reduced graph clusters



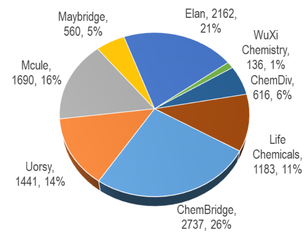
- **95% RO5** compliance
- **86%** of compounds fall within the ideal **250 – 450** molecular weight range for hit optimization
- **31.4% (130K)** of compounds feature high **Fsp3** ≥0.4
- Flexible choice of library size for screen, as 30k, 90k subsets

Focused Library List

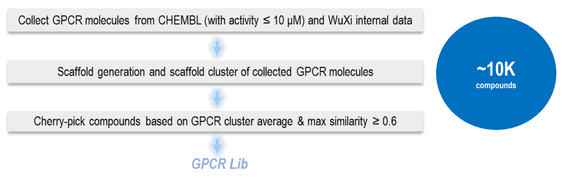
GPCR Library Design

Filter by

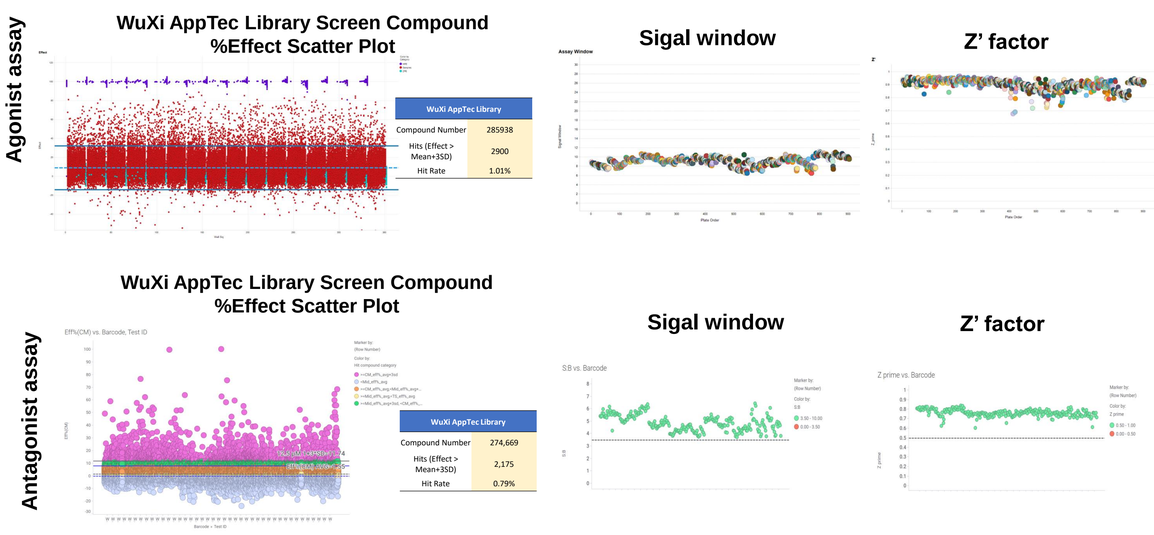
- Pan Assay Interference Compounds (PAINS)
- WuXi AppTec structural filters, based on MedChem experience



Design principle



High-Throughput Cell-Based cAMP HTRF Assay of GIPR Agonist and Antagonist Screening



High-throughput screening of agonists or antagonists of hGIPR. High-throughput cell-based assays was performed to screen 270k WuXi AppTec compounds for agonists of GIPR in 384-well plate format or antagonists in 1536-well plate format. The data confirm the robustness and sensitivity of this platform for profiling functional GIPR agonists or antagonists in a cellular context.

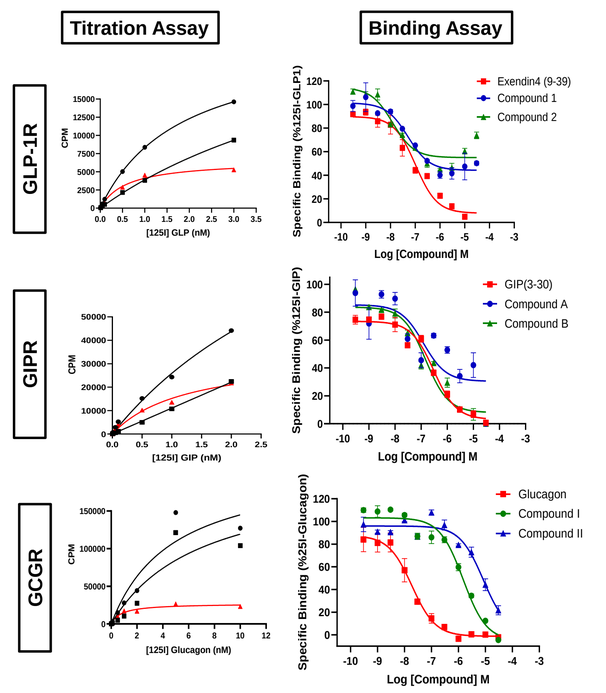
Radioligand Binding assay : Specificity of Binding of hGIPR/GLP-1R/GCGR Cell Membrane Preparation

Assay development protocol
Membrane titration
Bead titration (if SPA requested)
Assay buffer optimization
Manual vs. automation optimization
Radioligand titration
Reference dose curve
Production screening (dual replicate dose curves)

Reagents
Membranes: purchased or in house generated
Radioligand:
Commercially available vs. Custom
Commercial
-[125I] and [35S] radioligand time strict
-[3H] purchased anytime
Custom
4-6 weeks for manufacturing
SPA vs. Filtration method
Disposable plates (1-3 weeks)

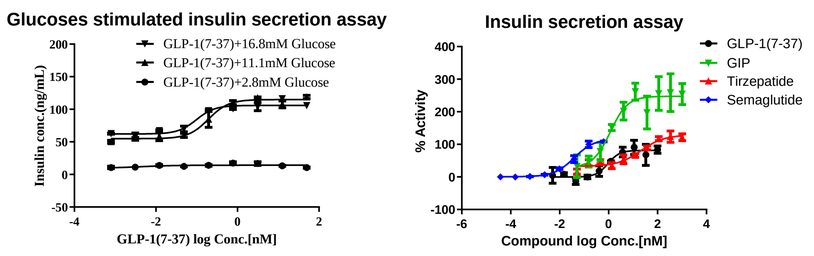
Assay format
96 well plate (SPA and filtration):
Most commonly requested
384 well plate (SPA):
Seldom requested by clients

Instruments available
Pneumatic transfer pipettors
Integra platform
Multidrop
Brandel 96 well filtration system
Plate centrifuge
Scintillation counter

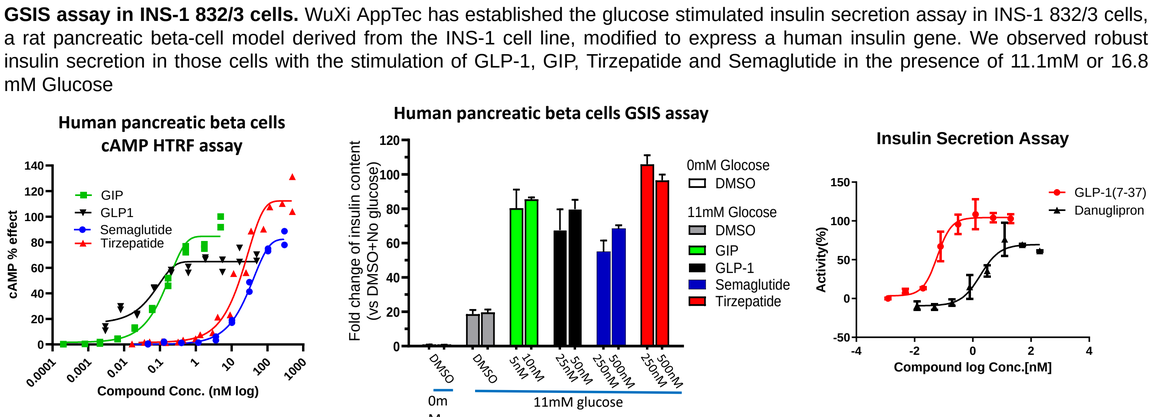


Workflow of radioligand binding assay and representative data
WuXi AppTec has established radioligand binding assay with cell membrane prep purchased or in house generated. [125I], [35S] and [3H] radioligand are available dependent on the plate format of 96- or 384-well plate. Right panel, representative data of in-house GIP/GLP1/Glucagon cell membrane preparation titration and binding assay. Each membranes showed specific binding activity with Exendin4 EC50=41.1nM, GIP (3-30) EC50=0.1nM, Des Glucagon EC50=6.12nM. Data represented Mean±SEM

GSIS assay and/or cAMP HTRF assay in INS-1 832/3 cells or Human Pancreatic Beta Cells



GSIS assay in INS-1 832/3 cells. WuXi AppTec has established the glucose stimulated insulin secretion assay in INS-1 832/3 cells, a rat pancreatic beta-cell model derived from the INS-1 cell line, modified to express a human insulin gene. We observed robust insulin secretion in those cells with the stimulation of GLP-1, GIP, Tirzepatide and Semaglutide in the presence of 11.1mM or 16.8 mM Glucose



cAMP HTRF assay and GSIS assay in EndoC BH5 human pancreatic beta cells. cAMP HTRF assay and GSIS assay have been successfully set up at Wuxi AppTec in EndoC BH5 cells, a ready-to-use, > 99% pure population of functional human pancreatic beta cells. We demonstrated dose dependent manner of stimulation of cAMP and Insulin release by GIPR or GLP-1R agonists in EndoC BH5 cells.

References

1. Timo D. Müller, Matthias Blüher, Matthias H. Tschöp and Richard D. DiMarchi Anti-obesity drug discovery: advances and challenges, Nature Revlew, vol 21, March 2022

2. Brian Finan and Jonathan D. Douros, GLP-1/GIP/glucagon receptor triagonism gets its try in humans, Cell Metabolism 34, January 4, 2022

Summary

In summary, our validated assay platforms consistently generate high-quality, reproducible data with robust QC standards. As a result, WuXi AppTec offers powerful capabilities for hit identification, hit confirmation, and hit-to-lead optimization, leveraging both our proprietary compound libraries and customized collections to meet the rapidly expanding needs of the weight loss and type 2 diabetes drug discovery market.

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