Identification of GLP1R agonists using a novel high throughput screening assay

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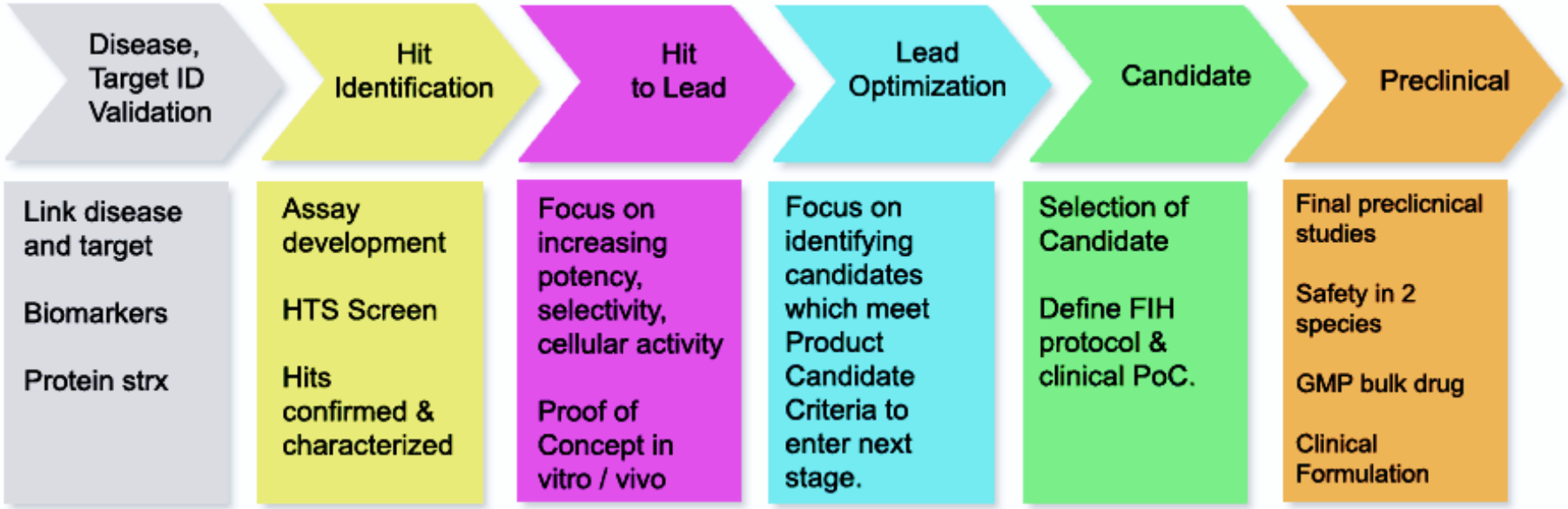
College of Pharmacy, Yonsei University

Contents

- High-throughput screening (HTS)
- HTS assays for identification of GLP1R agonists



http://www.acco.org/targeted-therapeutics/

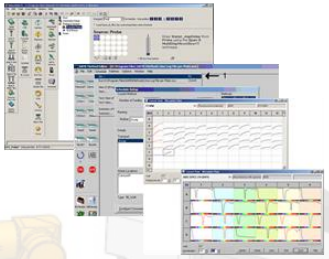
High-throughput screening (HTS)

A method for scientific experimentation especially used in drug discovery and relevant to the fields of biology and chemistry.

Robotics

Liquid handling devices

Sensitive detectors



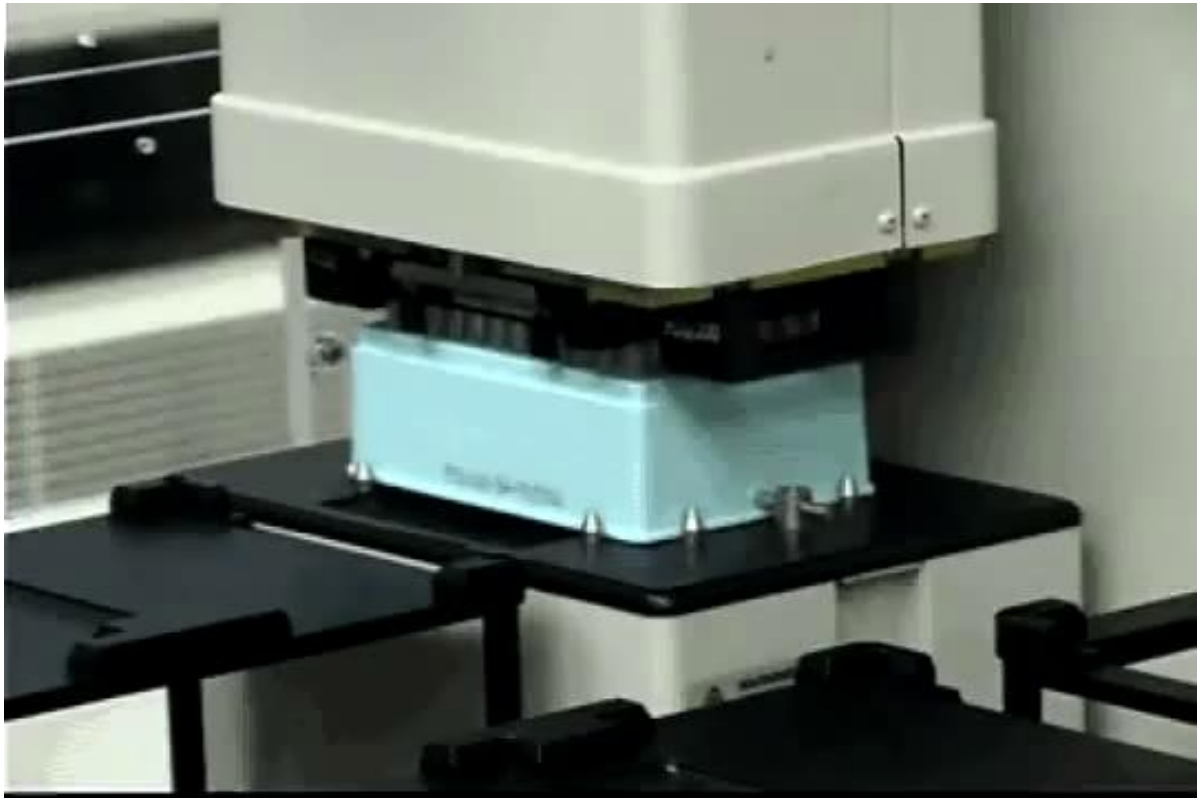
Data processing & control software

allows a researcher to quickly conduct millions of chemical, genetic or pharmacological tests.

identify target modulators using small molecule, gene, RNAi or CRISPR/Cas9 library

High-throughput screening (HTS)

✓ **Typically, HTS assays are performed in "automation-friendly" microplates with a 96, 384 or 1536 well format.**



High-throughput screening (HTS)

▣ Microplate reader

detects Fluorescence, Absorbance, Luminescence and Fluorescence Resonance Energy Transfer (FRET)

▣ High-content screening (HCS)

high-content analysis

Epi-fluorescence microscopy
Confocal microscopy

- **Ion channels / Receptors** (Fluorescence sensor)
- **Transcription** (Promoter assay)
- **Enzymes** (ELISA assay, Plate-based assay)
- **Cell growth** (MTT/XTT, Live/Dead cell staining)
- **Cell morphology** (High content confocal Imaging)
- **Protein-protein interactions** (FRET, Fluorescence polarization, Tripartite split-GFP complementation assay)

Workflow of HTS in Drug Discovery

► Target selection

Target identification & validation

- unmet medical need & a specific indication?
- intended patient population & market?
- relevant cellular or molecular targets?
- mode of action?
- risk & side effects?
- available relevant literature?
- competitive advantage?
- patents?
- appropriate assays - established or to be developed?

Workflow of HTS in Drug Discovery

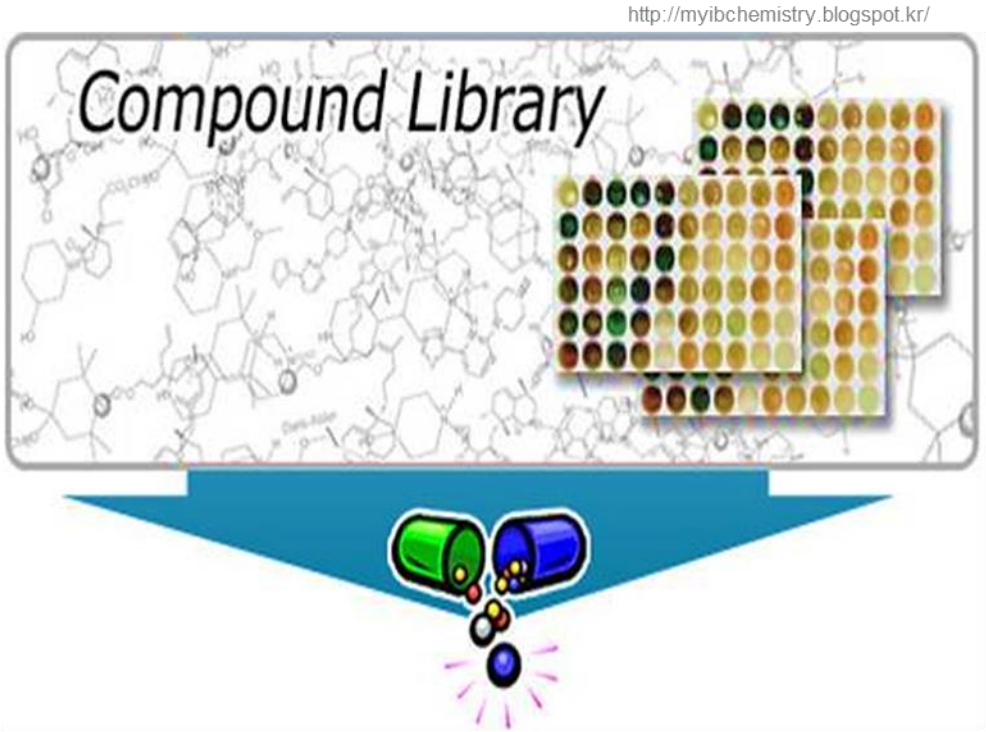
- **Target selection**
- **Assay development**

$$\text{Z-factor} = 1 - \frac{3(\sigma_p + \sigma_n)}{|\mu_p - \mu_n|}$$

mean (μ), standard deviation (σ), positive (p) and negative (n) controls

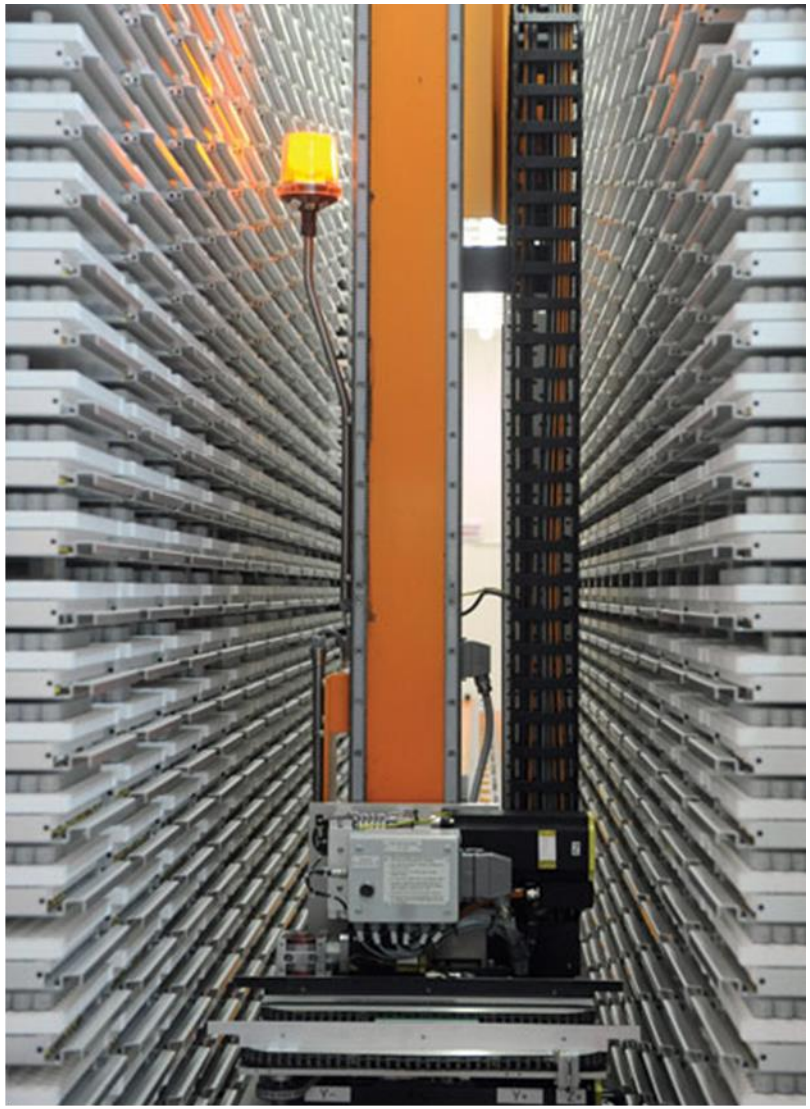
- **Primary screen with chemical libraries**

Chemical libraries



Part of the compound library at the Sanofi-aventis laboratory in Toulouse, France. More than 1 million compounds, stored in trays of vials, are kept here.

Nature 470, 42–43 (2011)



Chemical libraries

한국화합물은행 Korea Chemical Bank

ChemDiv The chemistry of cures℠

About Us

ChemDiv's Screening Libraries List

Discover Our Unique Collections

Autophagy library – 17,815 compounds

Epigenetics library – 30,431compounds

Library of modulators of Protein-protein interactions (PPI) – 110,055 compounds

Targeted Diversity library – 45,429 compounds

Ion Channels target platform library (2013 Y edition) – 16,952 compounds

Allosteric Kinases inhibitors library – 23,9

MDM2 targeted library – 22319 compour

Bcl2 PPI inhibitors library – 11,446 compo

Cancer Stem Cells targeted library – 20,2

CNS library – 33,173 compounds

C-Met library – 17,130 compounds

Updated Fragments library – 14,294 com

HDAC library – 20,914 compounds

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Chemical Compound Libraries

Aurora's chemical library is a collection of stocked chemicals used in high
as in chemical research.

Aurora offers about 1
predictability in silico
assessment and increa

Our targeted libraries
paradigm in molecular

- Our hit rate for pre
real bioassays.
- Features of our foc
  - High Affinity
  - Compliance wi
  - High Selectivit
  - Low Toxicity
  - Patentability
- Our database conta

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CHEM BRIDGE™

The G
Screen

ABOUT US | SCREENING LIBRARIES | BU

Screening Libraries

Key Facts
Diversity Libraries
Targeted & Focused Libraries
Fragment Library

ASINEX
inspired by Nature

Navigation Menu

- Building Blocks for PPI
- Fragments for PPI
- PPI Library
- Building Blocks for Macrocycles
- Macrocyclic Library
- Gram-Negative Antibacterial
- Lipid GPCRs
- Non-nitrogenous for CNS and PNS
- SAM/SAH
- Ion Channels
- α-helix mimetics
- Phenotypic Screening Set

Home > Libraries

Libraries

ASINEX's libraries consist of more than 600,000 compounds, encompas

ASINEX has synthesized more than 2,000 novel lead-like scaffolds over
Moreover, we experimentally test all our new compounds for solubility in

All ASINEX compounds are stored neat as a dry stock but can be re-forr

All ASINEX compounds have a minimum purity of 90%.

All ASINEX compounds have been characterized by either NMR and/or
compounds.

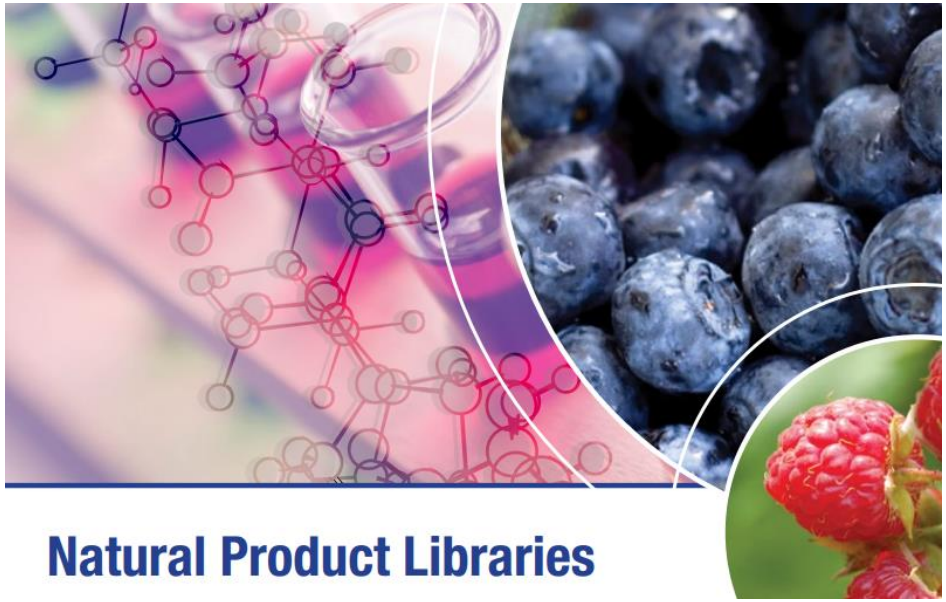
High probability of refills is secured for the most recent high-value librarie

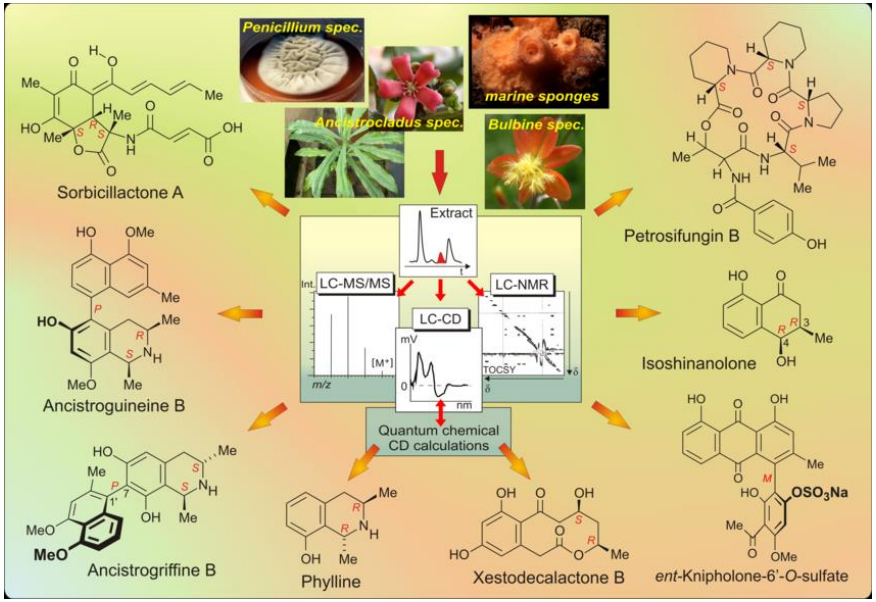
ASINEX is willing to support your follow-up chemistry programs by offeri
availability to ensure the possibility of the resupply of any particular comp

Building Blocks for protein-protein interactions

Protein-protein interactions (PPIs) have great potential as therapeutic tar
screening such targets is presumably linked to the lack of small molecule
the PPI interface. Compounds generally do not have the geometry and s
To help address these issues, ASINEX has developed a range of Buildin
structures with multiple substituents, for targeting PPIs ... read more

Chemical libraries

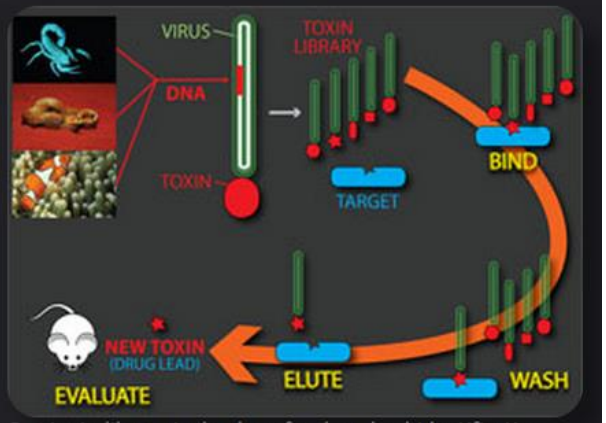




http://www-organik.chemie.uni-wuerzburg.de/

Screen Millions of Toxins

At the University of Chicago, Zoltan Takacs co-invented the Designer Toxins technology to create toxin libraries. Each library contains thousands to millions of native and engineered animal toxins using computational animal venom genomics and molecular methods. The libraries are screened on a target — a molecule that decides the fate of a disease. Those toxins that are the most selective for the target are isolated, pharmacologically verified, and become drug templates. It's like trying out a million keys at once and picking the one that opens a lock for which no one had a key before. Control over the lock, means having control over the disease.



Our toxin library technology for drug-lead identification.

Chemical libraries

LOPAC®1280 – The Library of Pharmacologically Active Compounds

The power and pe… …ounds (LOPAC1280) is assured. This b… …pacts impacts most signaling pathway…

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Product Categories

Screening Libraries › Kinase Inhibitor Library

Kinase Inhibitor Library

A unique collection of 355 kinase inhibitors for high throughput…

Catalog No. L1200 ★★★★★ Reviews (5)

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Pre-dissolved in DMSO	
100uL/well(10mM solution)	USD 5800
250uL/well(10mM solution)	USD 8900

Selleck USA Tel: +1-832-582-8158 sales@selle…

Workflow of HTS in Drug Discovery

- Target selection
- Assay development

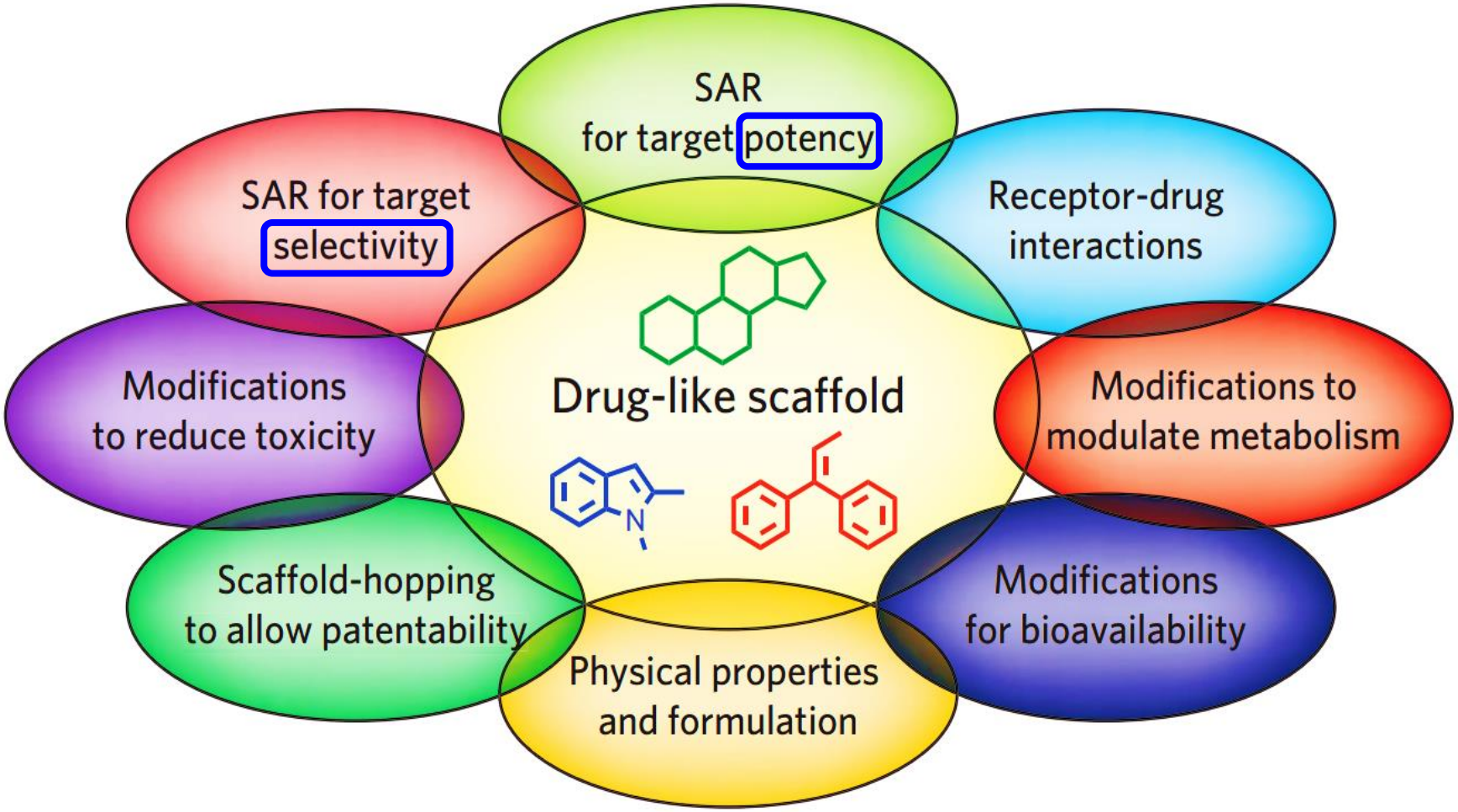
$$\text{Z-factor} = 1 - \frac{3(\sigma_p + \sigma_n)}{|\mu_p - \mu_n|}$$

mean (μ), standard deviation (σ), positive (p) and negative (n) controls

- Primary screen with chemical libraries
- Secondary screen with primary hits using more sensitive assay
- Characterization of hit compounds
- Structure Activity Relationships (SAR)

Workflow of HTS in Drug Discovery

► Structure Activity Relationships (SAR)



Nature Chemical Biology **6**, 476–479

Workflow of HTS in Drug Discovery

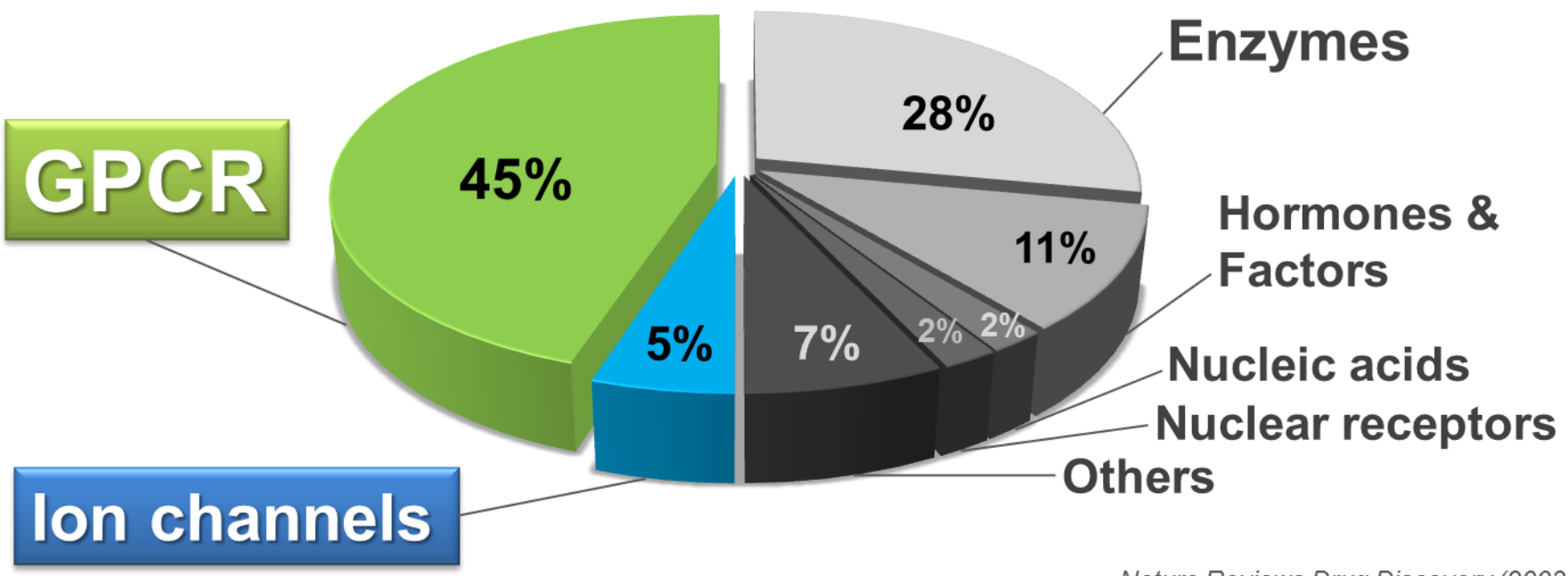
- Target selection
- Assay development

$$\text{Z-factor} = 1 - \frac{3(\sigma_p + \sigma_n)}{|\mu_p - \mu_n|}$$

mean (μ), standard deviation (σ), positive (p) and negative (n) controls

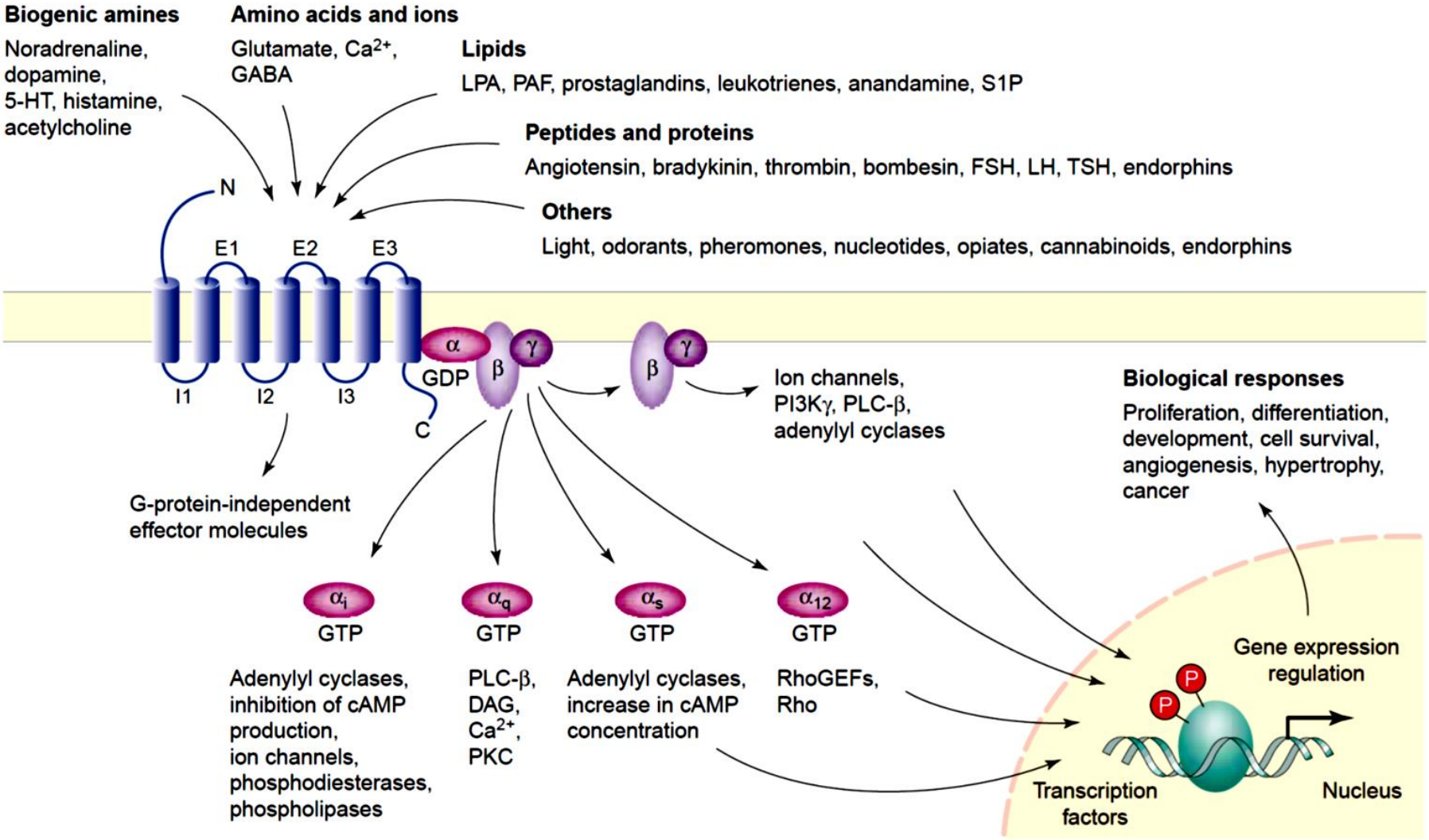
- Primary screen with chemical libraries
- Secondary screen with primary hits using more sensitive assay
- Characterization of hit compounds
- Structure Activity Relationships (SAR)
- Determination of drug potency and selectivity
- Apply to cell or animal model

Therapeutic target classes



Nature Reviews Drug Discovery (2003)

GPCR (G-protein coupled receptor)



TRENDS in Pharmacological Sciences

GPCR (G-protein coupled receptor)

A

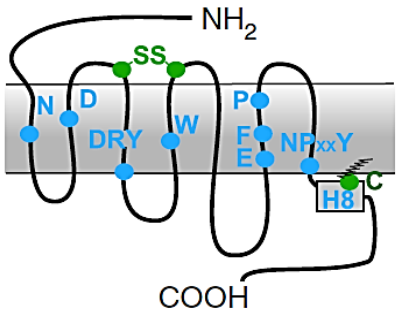
Class A Rhodopsin-like

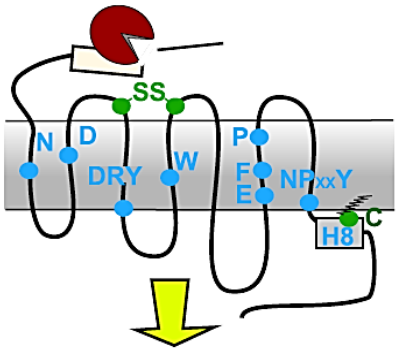
Amine
Rhodopsins
Olfactory
Prostanoid
Nucleotide-like
Cannabinoid
Peptide
Hormone protein
GnRH

Thyrotropin-releasing hormone and Secretagogue
Melantonin
Viral
Sphingolipid and LPA (EDG)
Leukotriene B4 receptor
Hydroxycarboxylic acid receptor
Ecdysis triggering hormone receptor
Class A Orphan/other

Protease-activated receptors

PAR1	Thrombin
PAR2	Mmp1
PAR3	APC
PAR4	plasmin
	cathepsin G
	trypsin



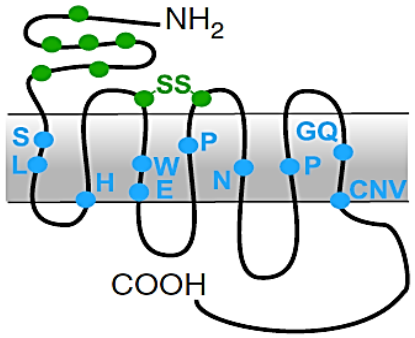


SIGNALING

Class B Secretin-like

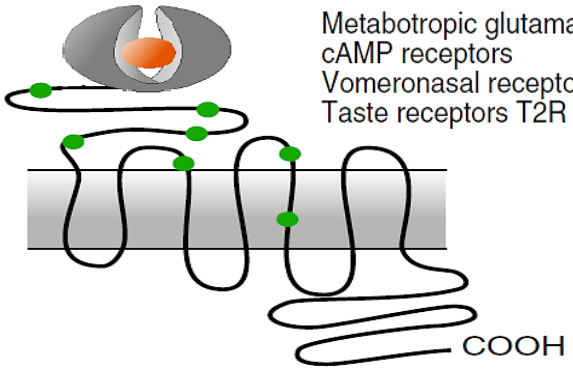
Parathyroid hormone receptor
Glucagon receptor
Calcitonin receptor
Growth hormone releasing hormone receptor
Secretin receptor
GPR56
GPR116
Lathrophilin receptor

● **GLP1R**



Class C-F

Metabotropic glutamate/pheromone
cAMP receptors
Vomeronasal receptors
Taste receptors T2R

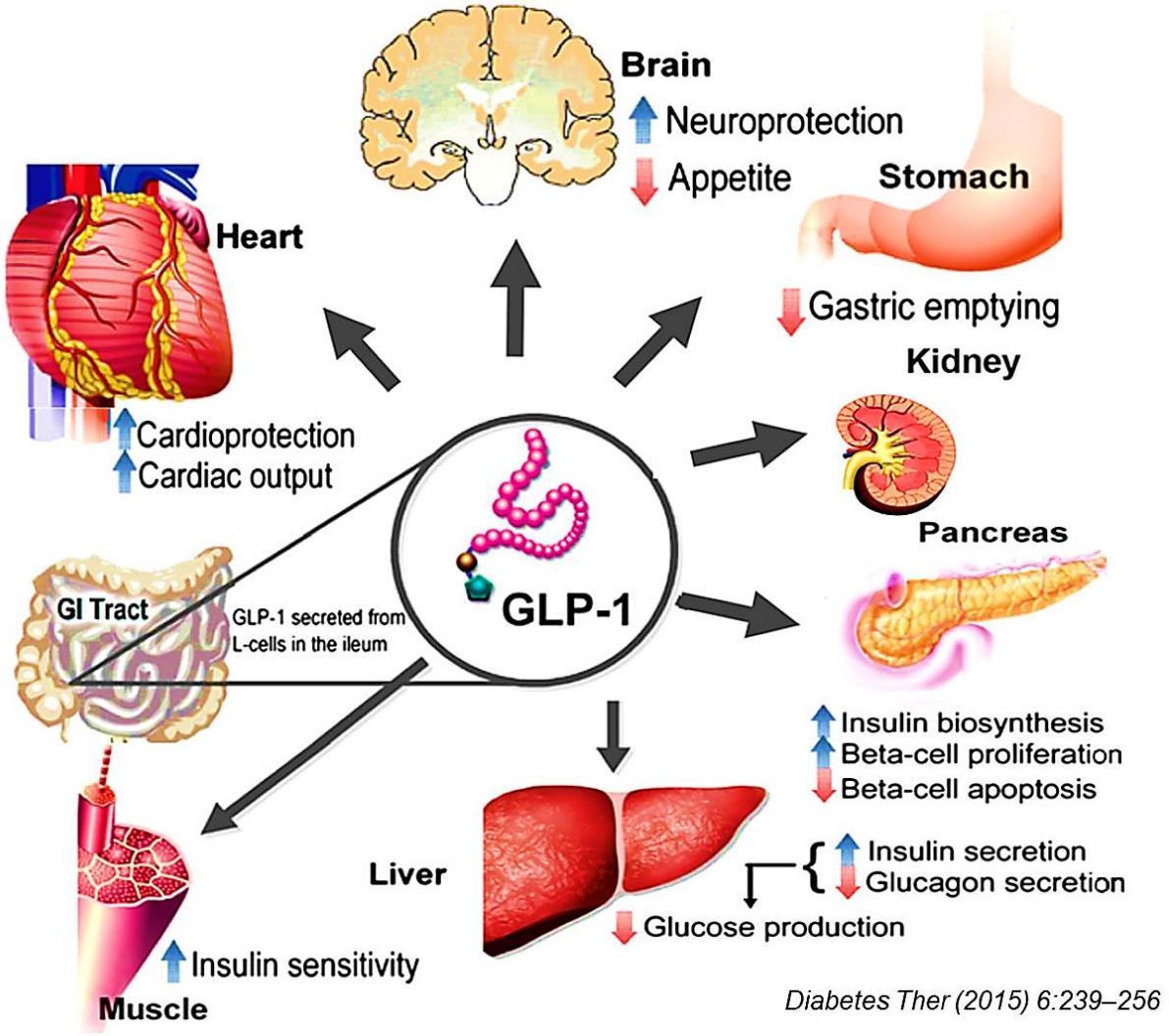


- The gene family of **Rhodopsin-like GPCRs**, constituting for **26.8% of all FDA-approved drugs**.
- 367 GPCRs with endogenous ligands - 87 human GPCRs that are drugged already → **280 GPCRs?**

JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 287, NO. 16, pp. 12787–12796, April 13, 2012

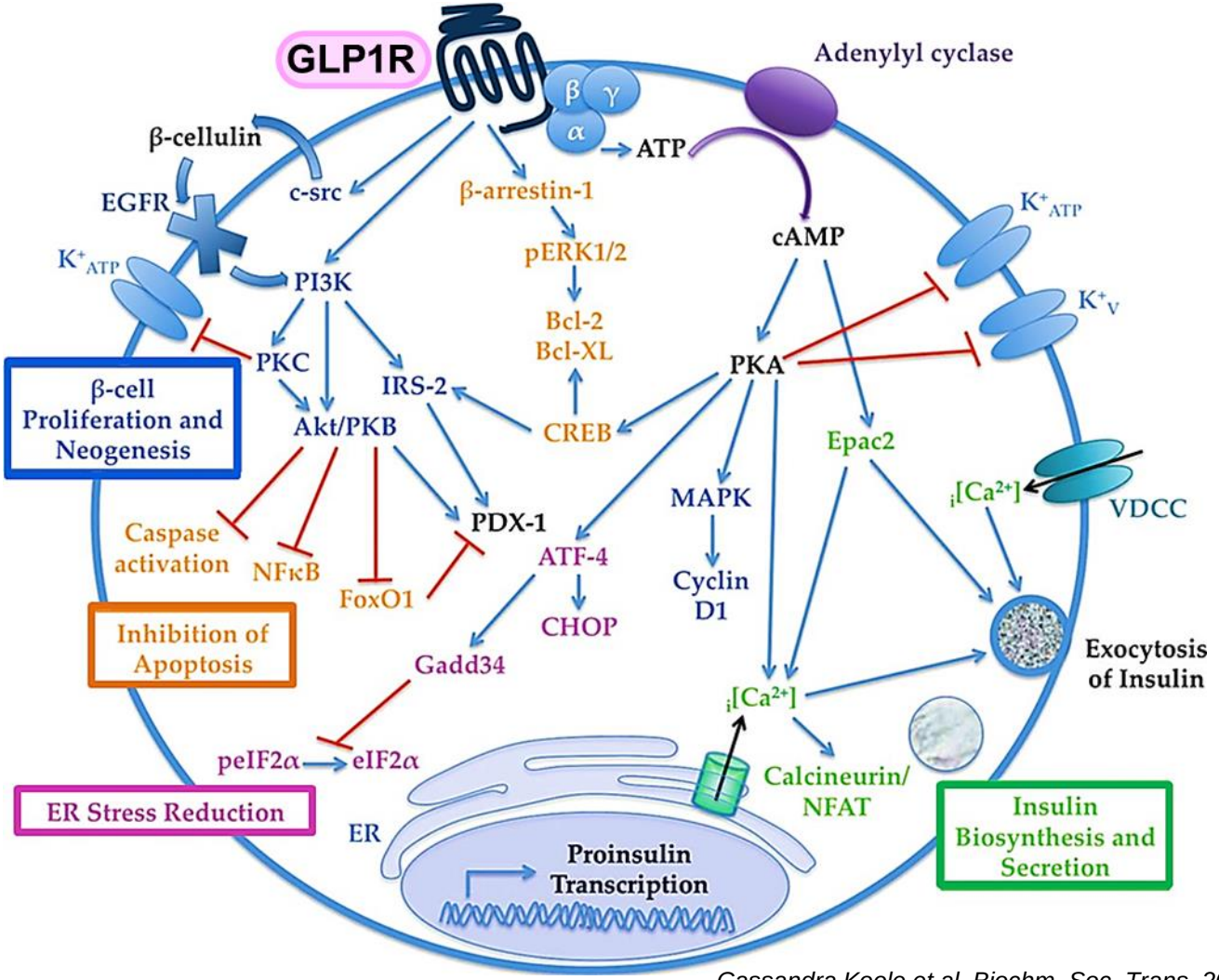
HTS assays for identification of GLP1R agonists

Glucagon-Like Peptide 1 Receptor (GLP-1R, GLP1R)



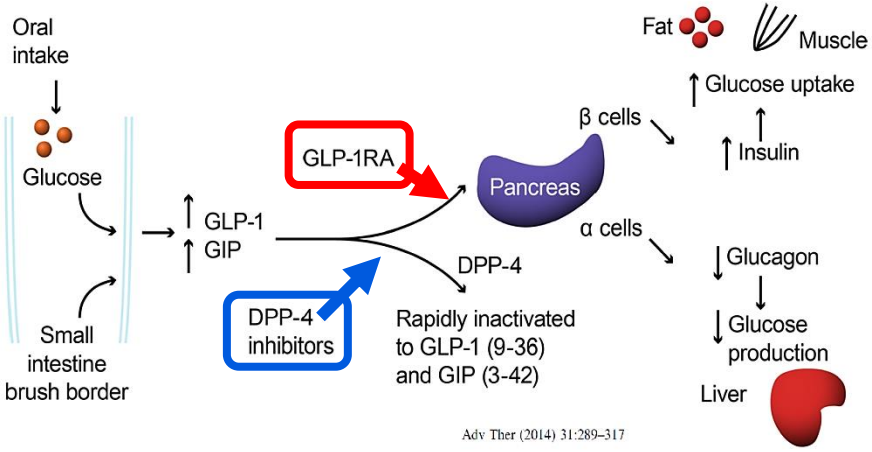
Diabetes Ther (2015) 6:239–256

Effects of GLP1R activation on pancreatic β-cells



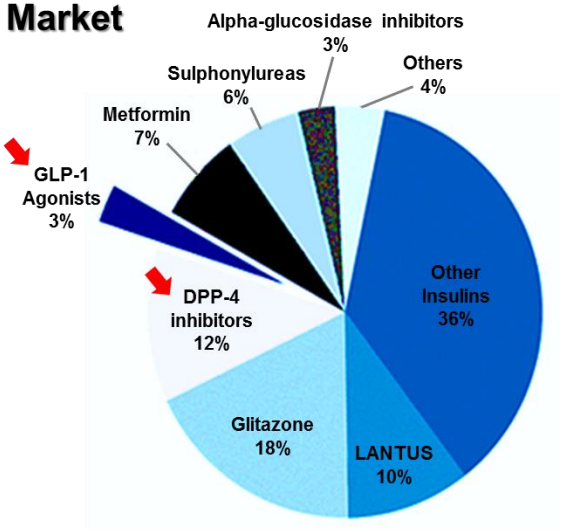
Cassandra Koole et al. Biochm. Soc. Trans. 2013;41:172-179

GLP-1 *vs* Dipeptidyl peptidase 4 (DPP-4)

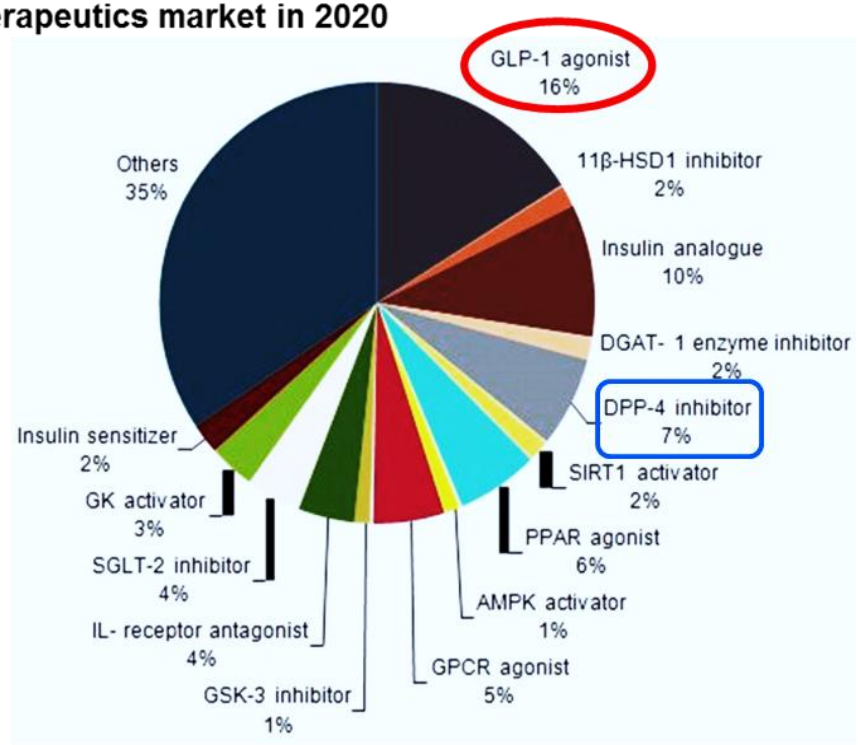


Worldwide Diabetes Market

- **GLP-1s represent significant growth opportunity within worldwide diabetes market**
- **VICTOZA (daily GLP-1) U.S. sales of $263 million in first year of launch; worldwide sales > $1 billion expected in CY 2011**



The expected global type 2 diabetes therapeutics market in 2020



Source: Type 2 Diabetes - Global Drug Forecasts and Treatment Analysis to 2020 (ASDReports, 2012)

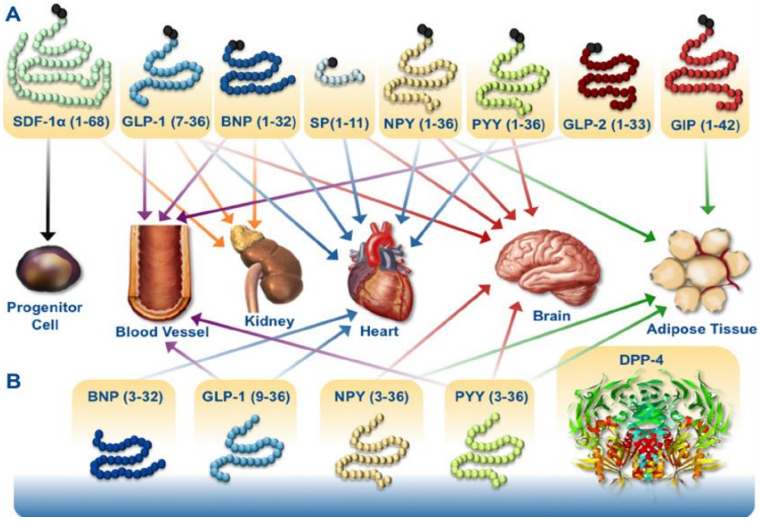
Comparing antihyperglycemic agents

	Relative A1C lowering	Change in body weight	Overall risk of hypoglycemia	Cost
DPP-4 inhibitor	↓↓	Neutral to ↓	Rare	$$$
GLP-1 receptor agonists	↓↓ to ↓↓↓	↓↓	Rare	$$$$
Insulin	↓↓↓	↑↑	Yes	$-$$$$
Meglitinides	↓↓	↑	Yes	$$
Sulfonylureas	↓↓	↑	Yes	$

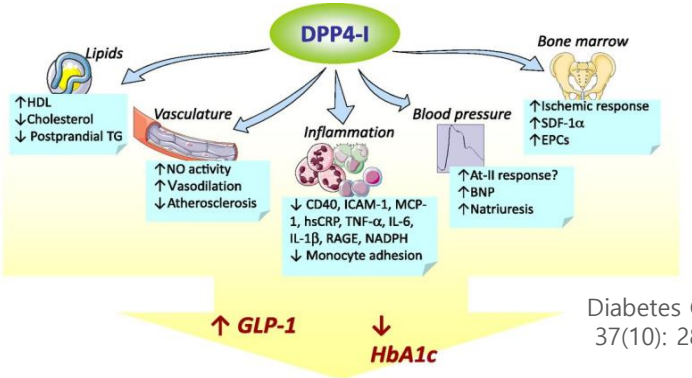
CDA 2013 Clinical Practice Guidelines

DPP-4 substrates regulate many biological function

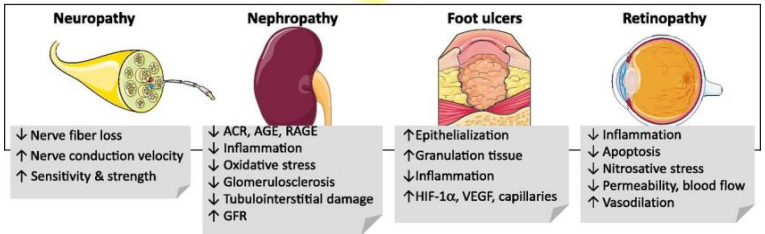
	Substrates	Biological effect
Hormones	GLP-1	Inactivation
	GLP-2	Inactivation
	GIP	Inactivation
	Glucagon	Inactivation
	GHRH	Inactivation
	PACAP	Inactivation
	Petide YY	Change in receptor preference
Vasoactive peptides	Bradykinin	Change in receptor preference
	VIP	Inactivation
	BNP	Change in receptor preference or Inactivation
Neuropetides	NPY	Change in receptor preference
	β-casomorphins	Inactivation
	Endomorphins	Change in receptor preference
	Substance P	Inactivation
Chemokines	CCL3 (MIP-1α)	Enhanced activity
	CCL4 (MIP-1β)	Change in receptor preference
	CCL5 (RANTES)	Change in receptor preference
	CCL11 (Eotaxin)	Inactivation
	CCL22 (MDC)	Change in receptor preference
	CXCL6 (GCP-2)	No changes
	CXCL9 (MIG)	Inactivation
	CXCL10 (IP-10)	Inactivation, CXCR3 antagonist
	CXCL11 (I-TAC)	Inactivation, CXCR3 antagonist
	CXCL12 (SDF-1α)	Inactivation, CXCR4 antagonist



http://care.diabetesjournals.org



Diabetes Care 2014; 37(10): 2884-2894.



The GLP1R agonists currently available & in development

Drug	Brand name	Dosing frequency	US FDA approval status	EMA approval status	Phase III clinical trial program
Exenatide	Byetta®	Twice daily	Approved 28 April 2005	Approved 20 November 2006	AMIGO
Liraglutide	Victoza®	Daily	Approved 25 January 2010	Approved 30 June 2009	LEAD
Exenatide	Bydureon®	Weekly	Approved 26 January 2012	Approved 17 June 2011	DURATION
Lixisenatide	Lyxumia® (Europe)	Daily	Submitted Withdrawn 12 September 2013	Approved 1 February 2013	GetGoal
Albiglutide	Tanzeum® (US) Eperzam® (Europe)	Weekly	Approved 15 April 2014	Approved 23 January 2014	HARMONY
Dulaglutide		Weekly	Submitted	Submitted	AWARD

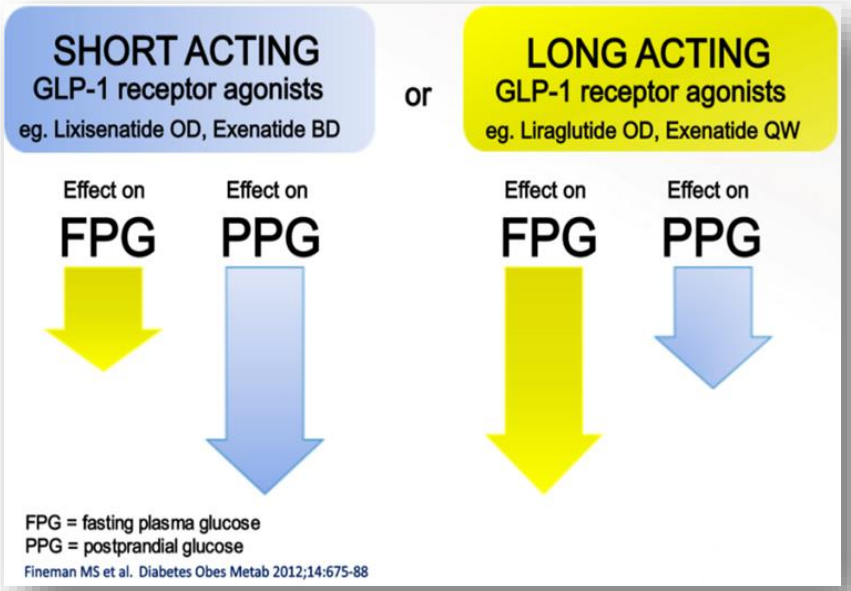
Abbreviations: US FDA, United States Food and Drug Administration; EMA, European Medicines Agency.

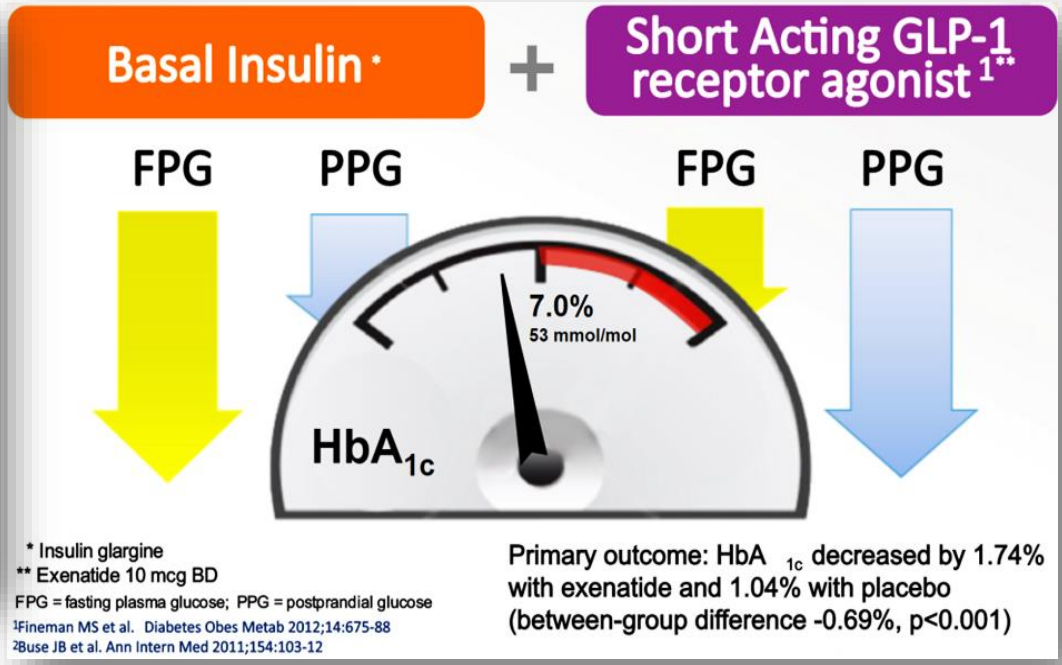
Ther Adv Endocrinol Metab. 2015 Feb; 6(1): 19–28.

Exenatide LAR Weekly Kit (Bydureon)

- 4 parts (Single dose tray)
  - Needle
  - Vial Connector
  - Syringe (Diluent)
  - Vial (Powder)
- Complex preparation
- Dose can be given in the thigh, abdomen, or back of the upper arms
- Dose must be given immediately
- Push down on plunger until it stops

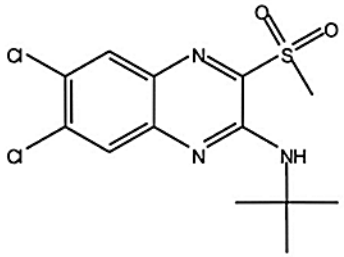
Effects of SA & LA GLP-1 on regulation of plasma glucose levels



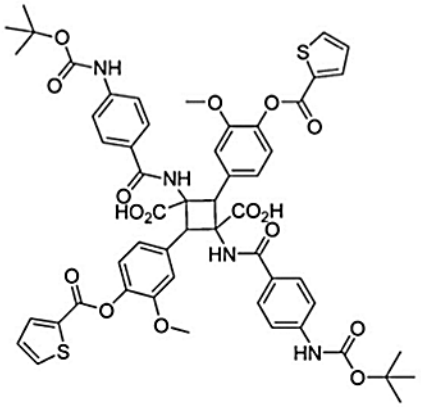


Small-molecule agonists of GLP1R

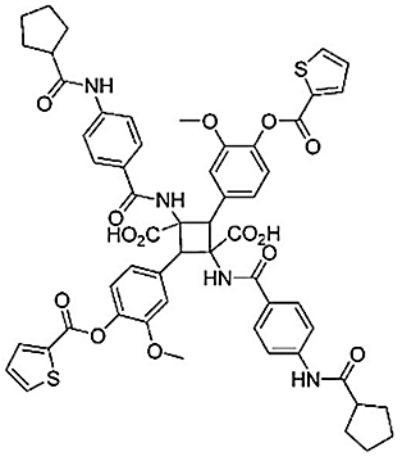
Compound 2



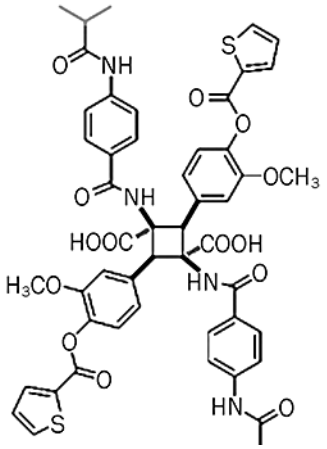
Boc5



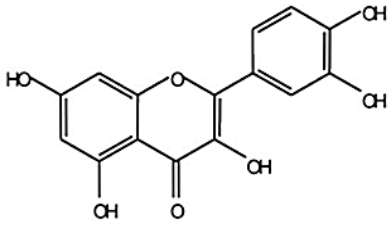
S4P



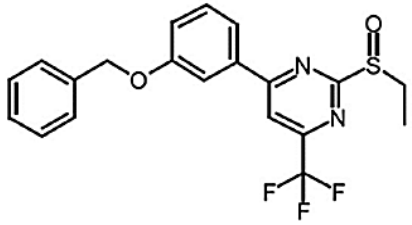
WB4-24



Quercetin

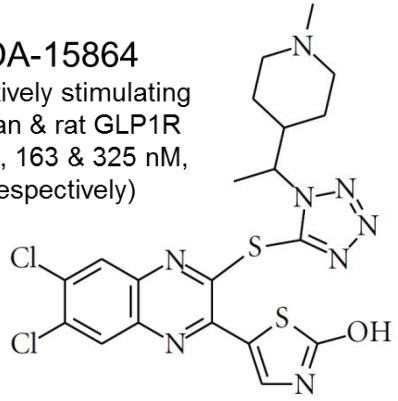


Compound B/BETP



DA-15864

selectively stimulating human & rat GLP1R (EC_{50}, 163 & 325 nM, respectively)

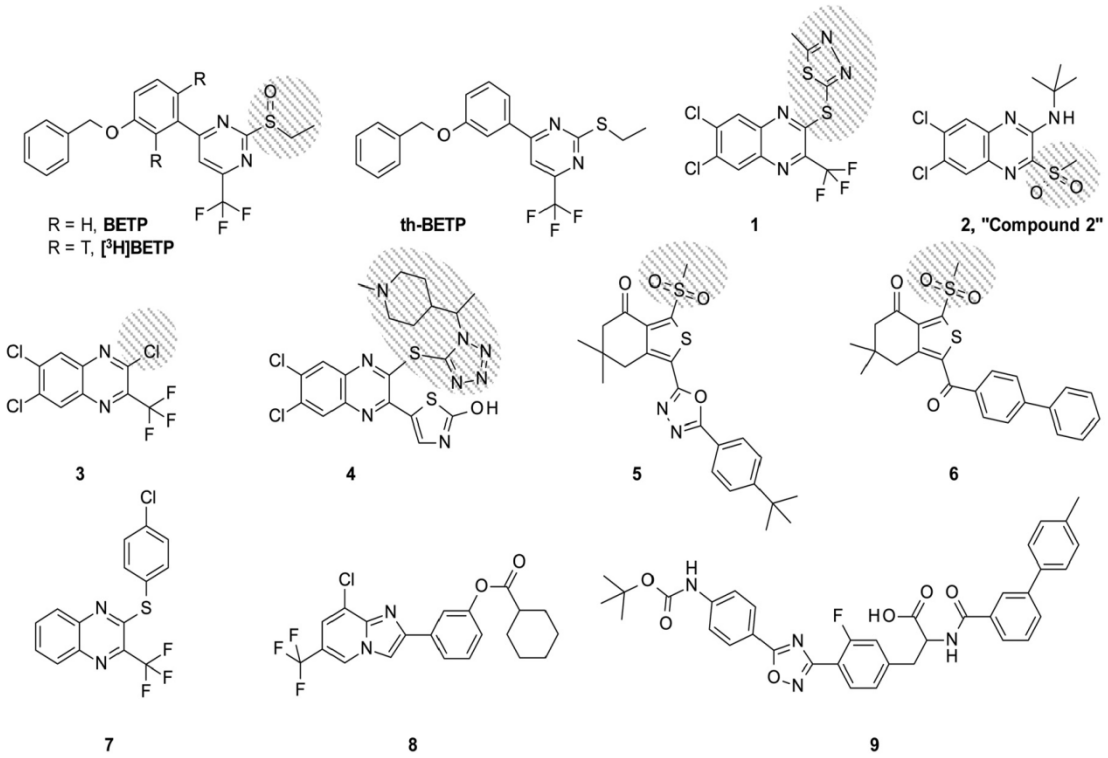


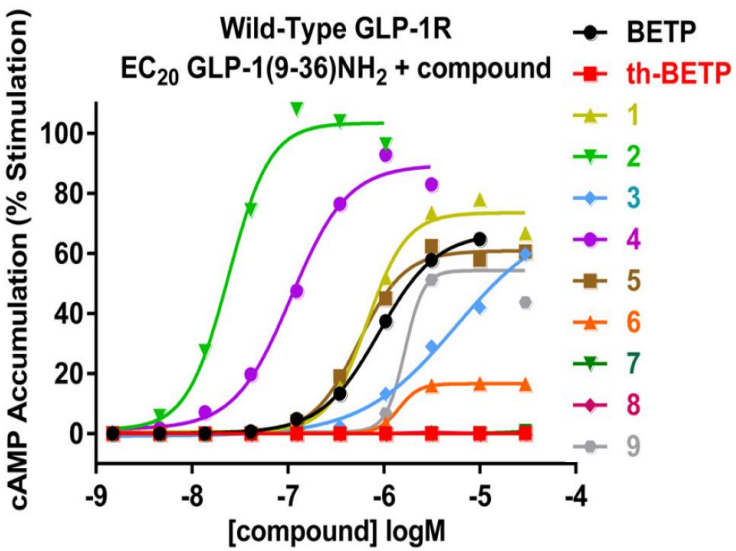
Exp Diabetes Res. 2012;2012:709893. / Acta Pharmacol Sin. 2012 Feb;33(2):148-54. / Cassandra Koole et al. Biochm. Soc. Trans. 2013;41:172-179 / WO2011/122815

Small-molecule agonists of GLP1R

Positive Allosteric Modulation of the GLP1R by Diverse Electrophiles

J Biol Chem. 2016; 291(20):10700-15.



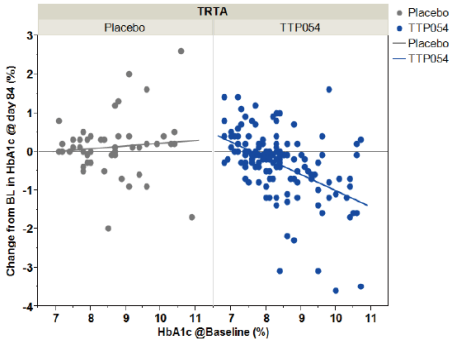


Small-molecule agonists of GLP1R

vTv's GLP-1R Agonists Program Genesis

- Coordinated utilization of data from cell EC50 assays, liver microsome stability, and pharmacokinetics led to potent lead series which resulted in 2 clinical candidates

In a 3 month phase 2 study, TTP054 showed significant reduction in A1 c



TTP-595 (TTProbe™)

EC_{50} 45 µM

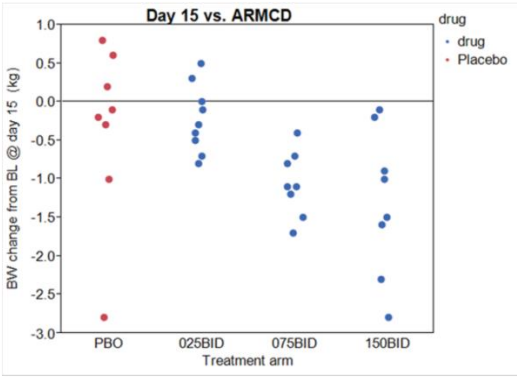
First Generation Series

Second Generation Series

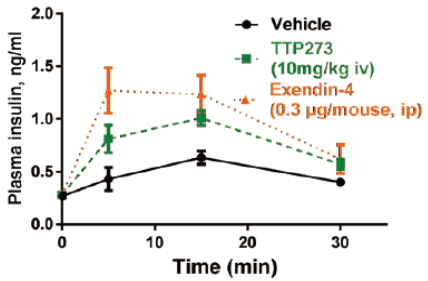
TTP054
EC_{50} 40 nM
(Clinical POC)

TTP273
EC_{50} 5 nM
(Clinical POM)
Phase 2 ongoing

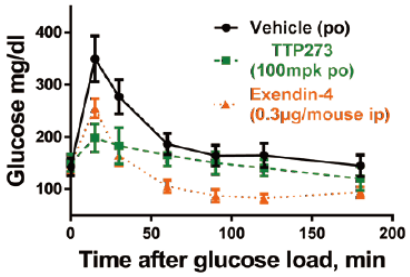
TTP273-102 Phase 1b T2D on Metformin 2w



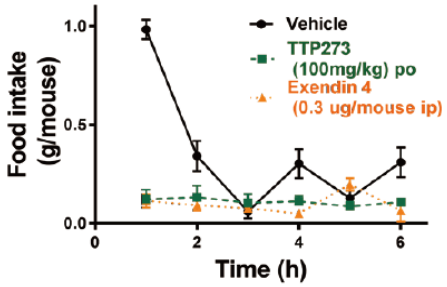
Enhances insulin secretion



Decreases glucose



Decreases food intake



(Rodent: EC_{50}34nM; 34% activation)

GLP1R Structure & GLP1 Binding Site

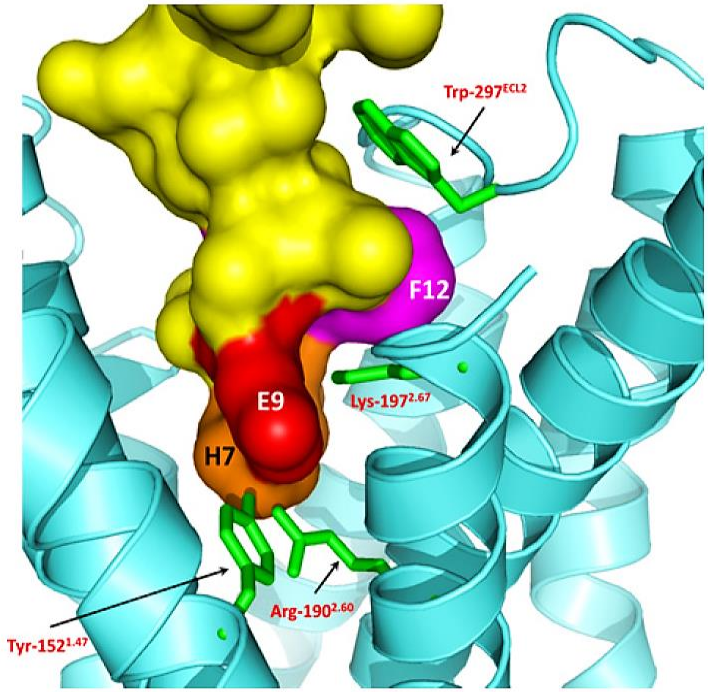
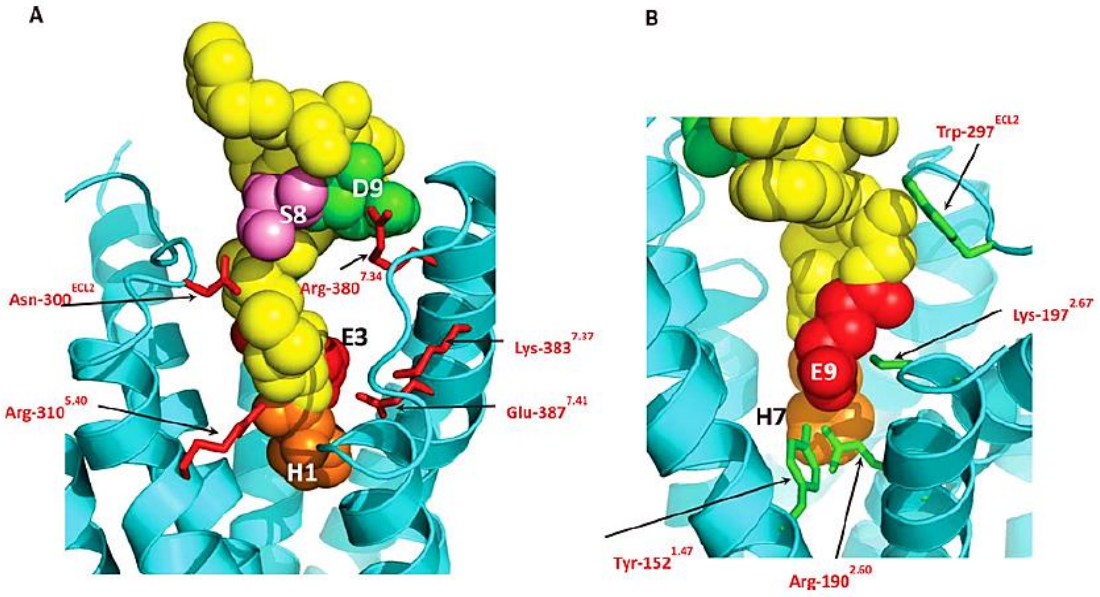


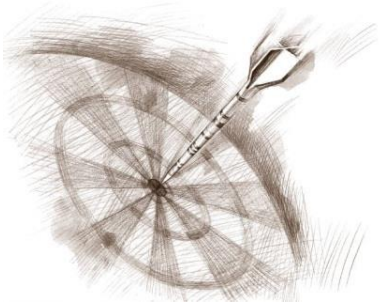
Figure 5 A side view of the GLP-1-docked GLP-1R model from between TM1 and TM2
GLP-1R is shown in cartoon form in cyan, with the side chains of four residues highlighted by mutagenesis in the literature (Table S1) shown as green sticks. The ligand is shown with its surface in yellow, with three residues highlighted by colour and single residue codes.



Views of the GLP-1R model docked with "model 11" from pdb code 2N0I
(**A**) View of the GLP-1R model from between TM5 and TM6 for comparison with Figure 4. (**B**) View of the GLP-1R model from between TM1 and TM2 for comparison with Figure 5. The ligand is the cyclic constrained synthetic 11-residue analogue of GLP-1 based on model 11 of pdb code 2N0I, and is shown as space-fill in yellow, but with four conserved residues highlighted by colour and single residue codes.

Second extracellular loop of human glucagon-like peptide-1 receptor (GLP-1R) has a critical role in GLP-1 peptide binding and receptor activation. J. Biol. Chem. 2012, 287;3642–3658

Biosci Rep. 2016 Feb; 36(1): e00285.

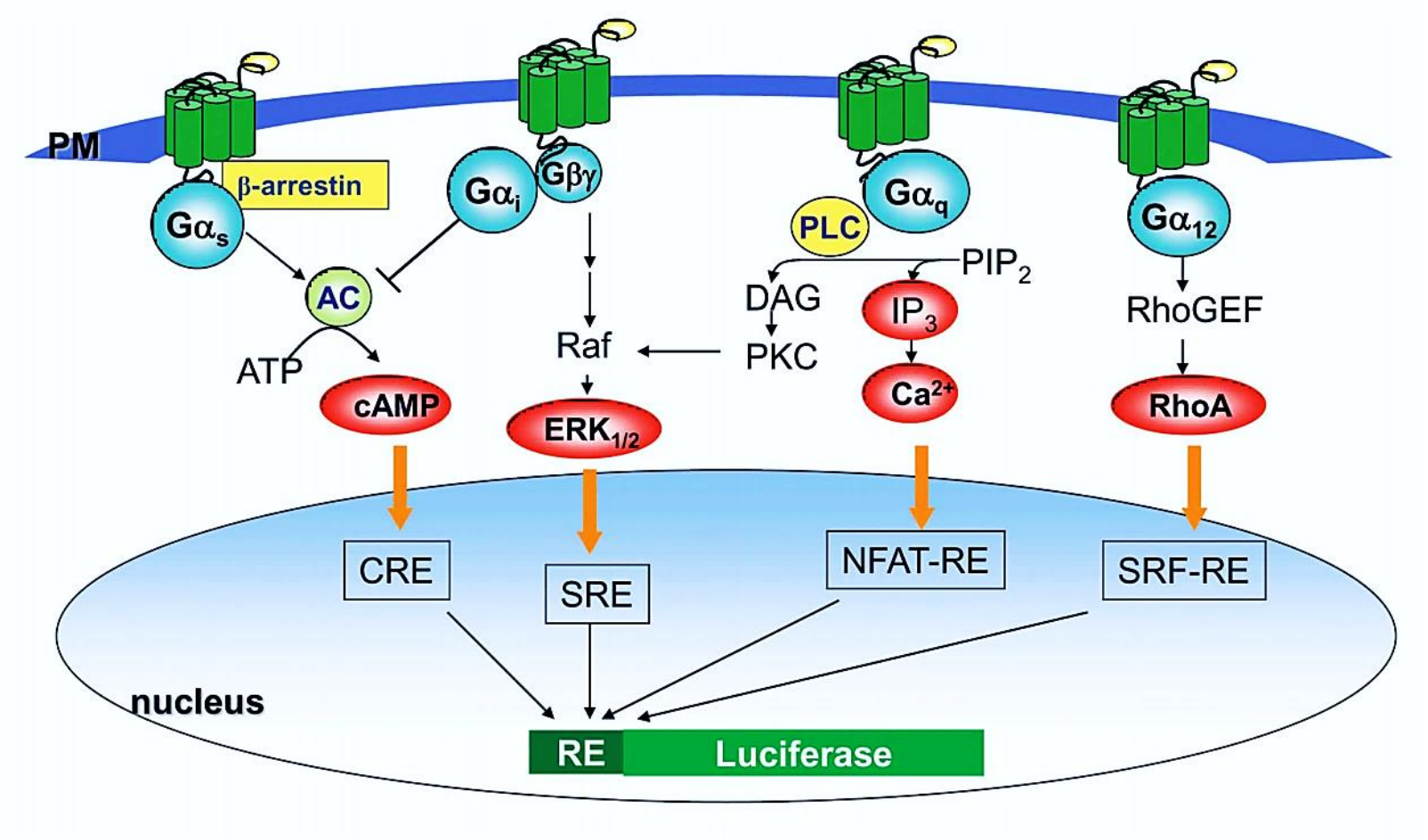


Aims

Identification of potent & specific small-molecule agonists of GLP1R for treatment of type 2 diabetes

How to identify novel GLP1R agonists?

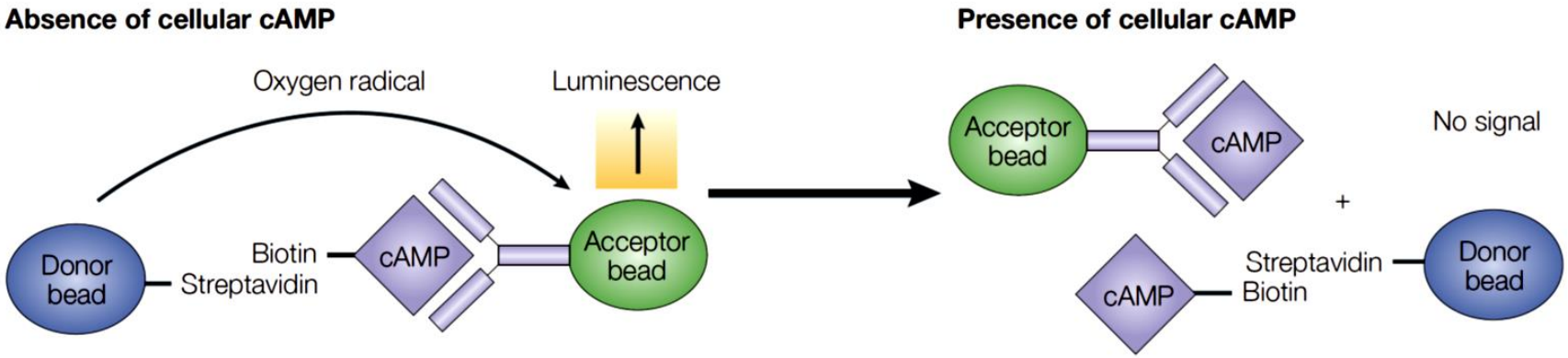
HTS assays for identification of GPCR modulators



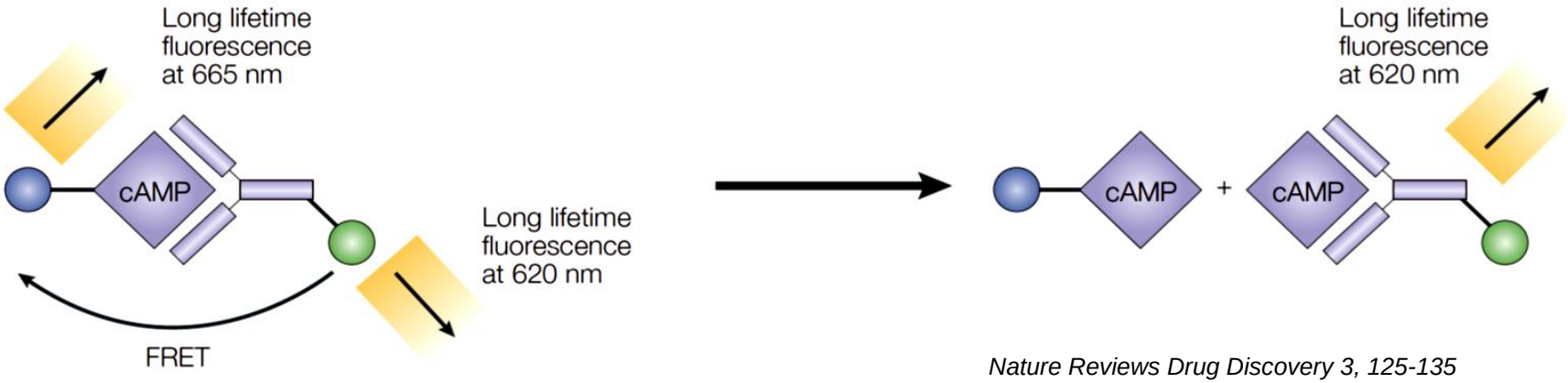
Current Chemical Genomics, 2010, 4, 84-91

HTS assays for identification of GPCR modulators

- **The amplified luminescence assay (AlphaScreen)**



- **Time-resolved fluorescence resonance energy transfer (TR-FRET)**

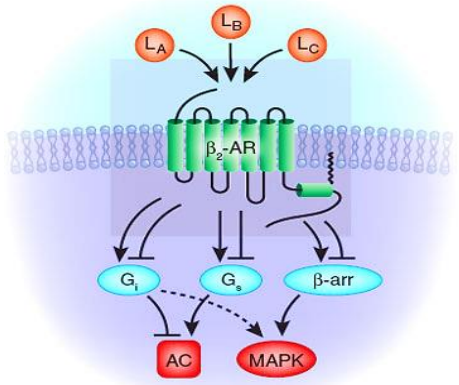


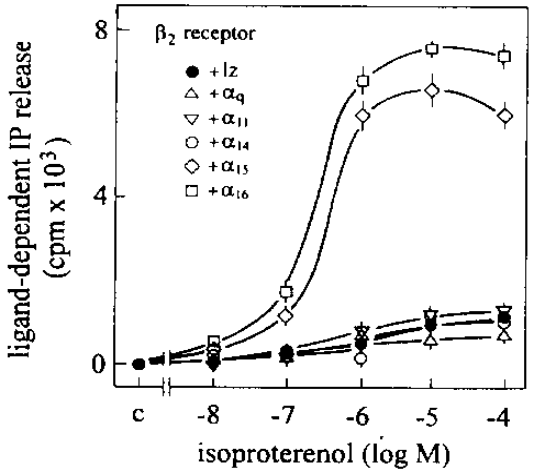
Nature Reviews Drug Discovery 3, 125-135

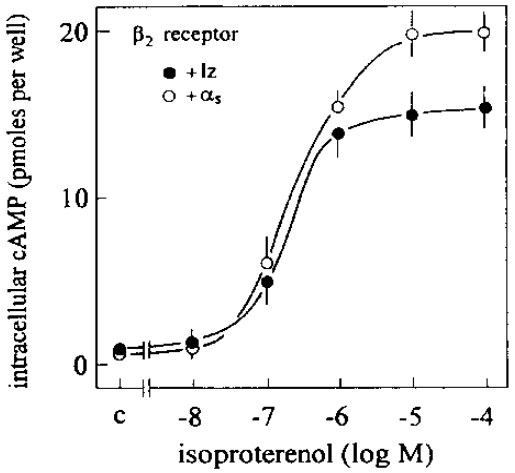
HTS assays for identification of GPCR modulators

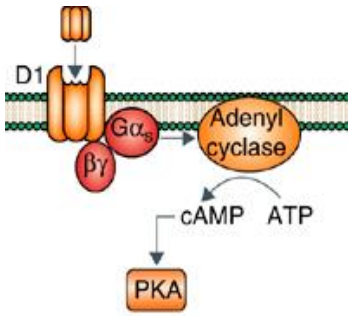
G alpha 15 and G alpha 16 couple a wide variety of receptors to phospholipase C

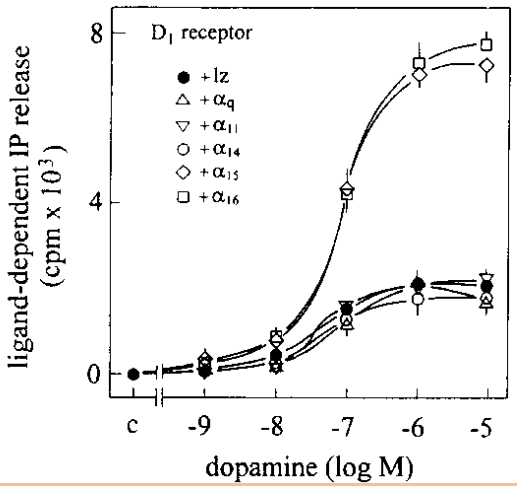
J Biol Chem. 1995 ;270(25):15175-80.

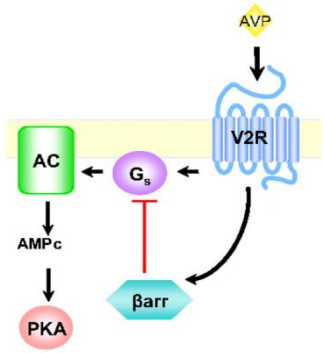


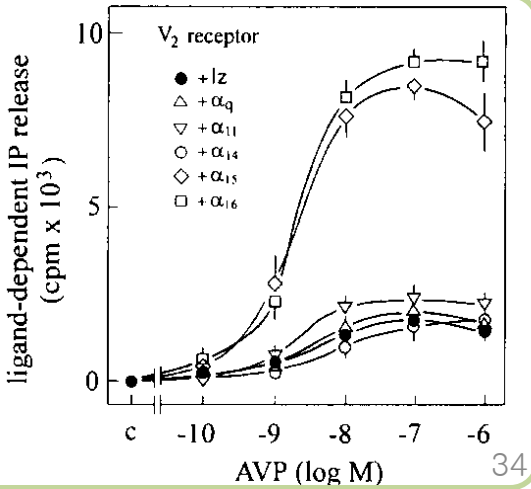




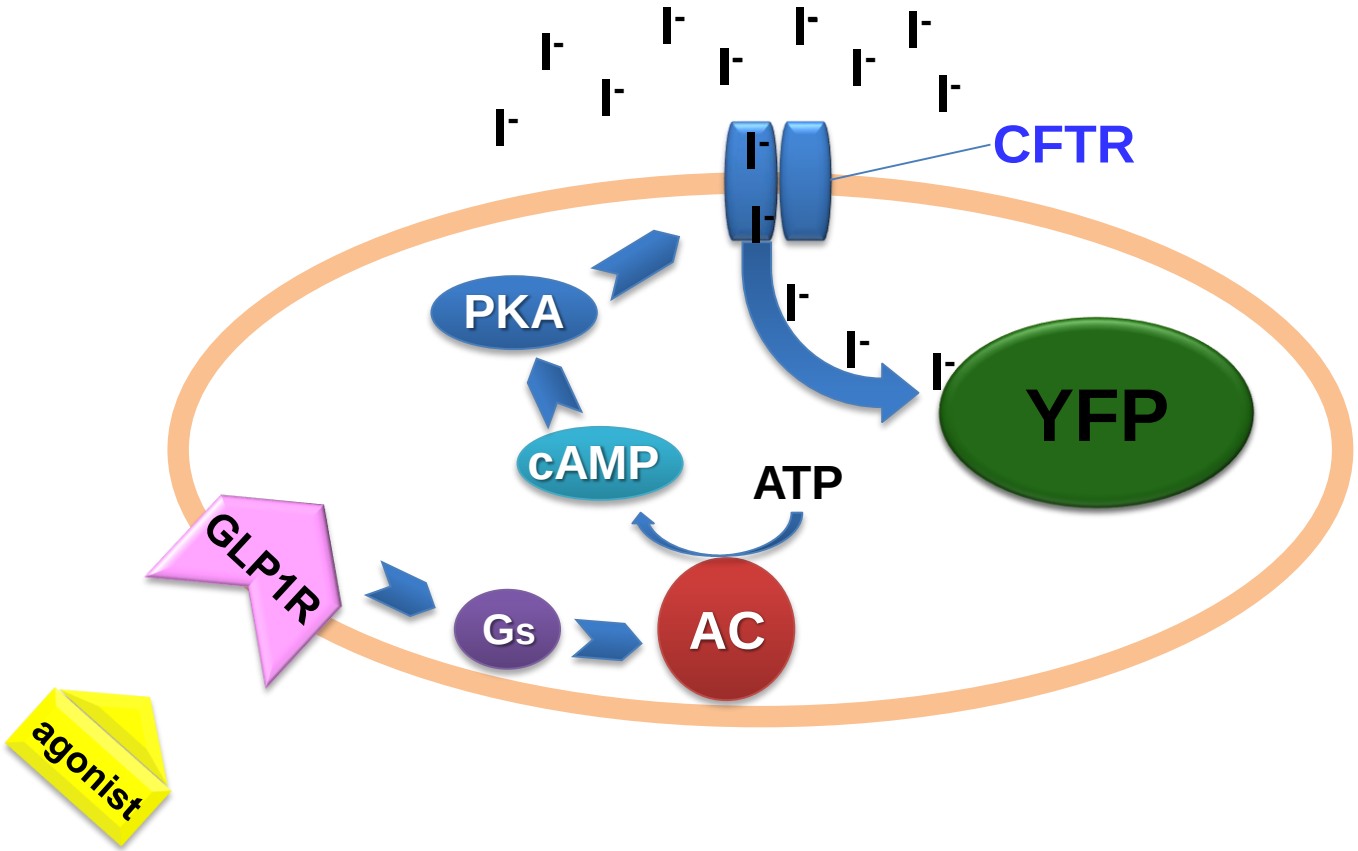








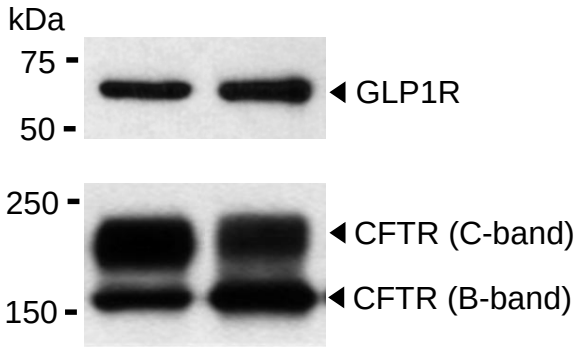
Establishment of a cell-based HTS assays for identification of GLP1R agonist



Establishment of a cell-based HTS assays for identification of GLP1R agonist

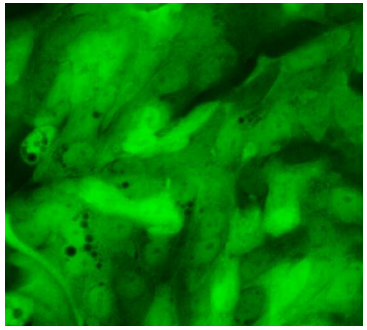
A

Calu-3-GLP1R-CFTR-YFP cell

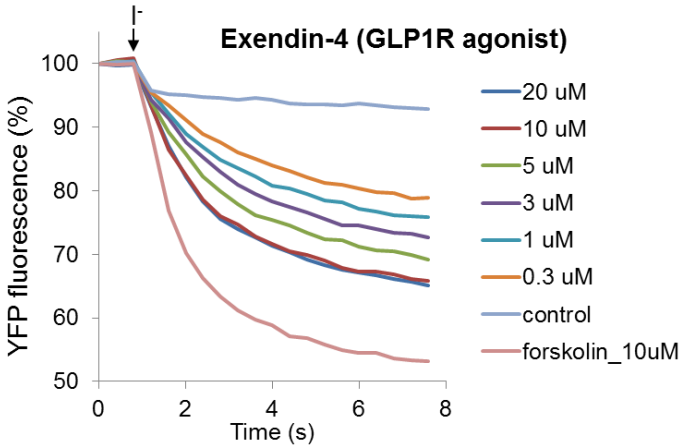


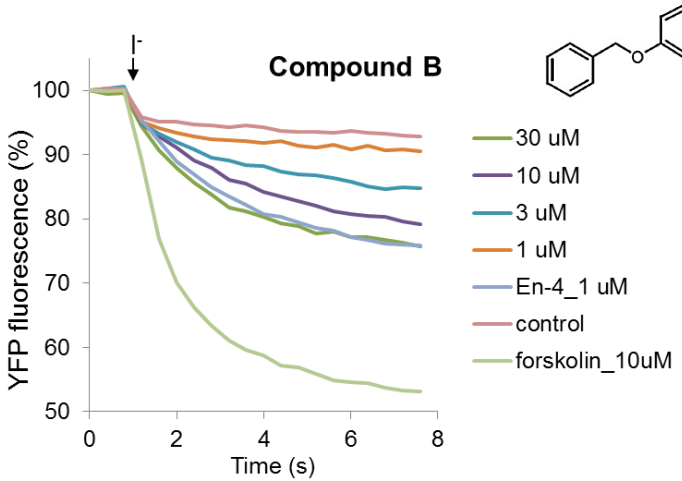
B

Calu-3-GLP1R-CFTR-YFP cell



C





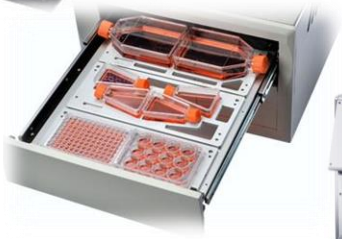
In House HTS Facilities

Cell culture room

Fluorescence Microplate reader

Chemical library ~110,000 compounds

Robotic liquid handler



IncuCyte™ ZOOM

Microplate dispenser

FACS

Q-TOF, NMR, LC/MS, HPLC

Microplate sealer

Confocal microscope

HTS Assay Procedure

seed the cells in **96 well microplate** and incubate the cells for 48h

GLP1R, CFTR & YFP expressing cells

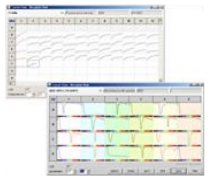
add **chemical compounds** in each well and then incubate the cells for 10 min at 37°C

Automated liquid handler

Run a screening assay

Microplate reader

evaluate effect of each compound on GLP1R activity using microplate reader and data processing software



identify novel "Hit" compounds

Strategy for the identification of specific GLP1R agonists

Primary screening with ~60,000 compounds

26 hits

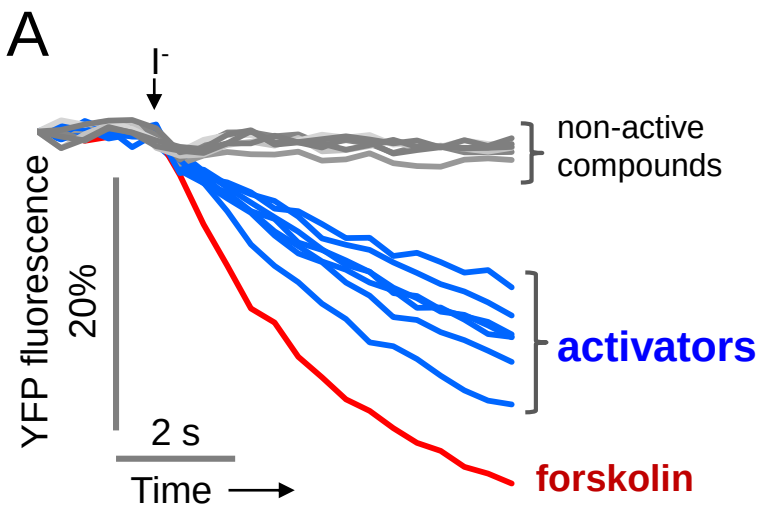
Second screening / CFTR assay

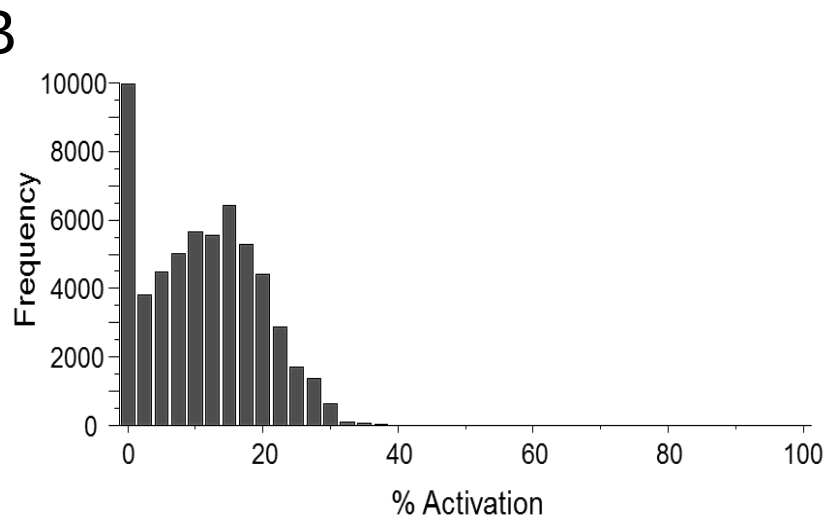
9 hits

Dose response analysis of 9 hits

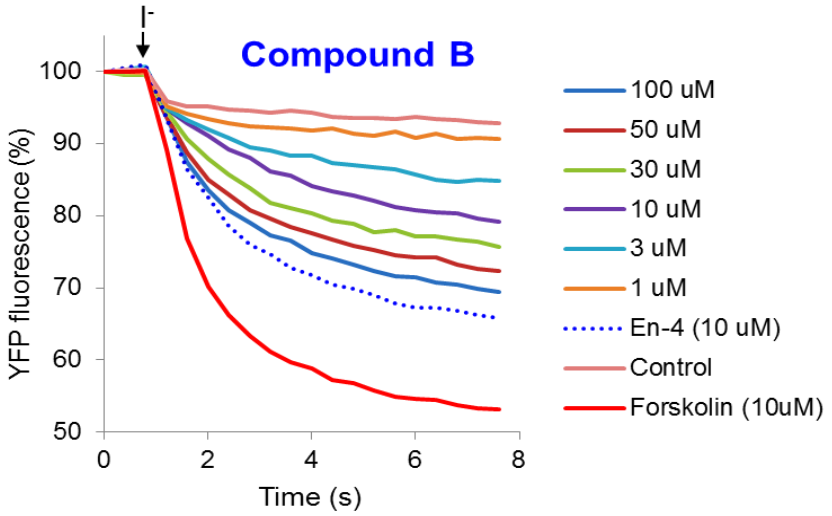
3 novel potent & selective agonists of GLP1R

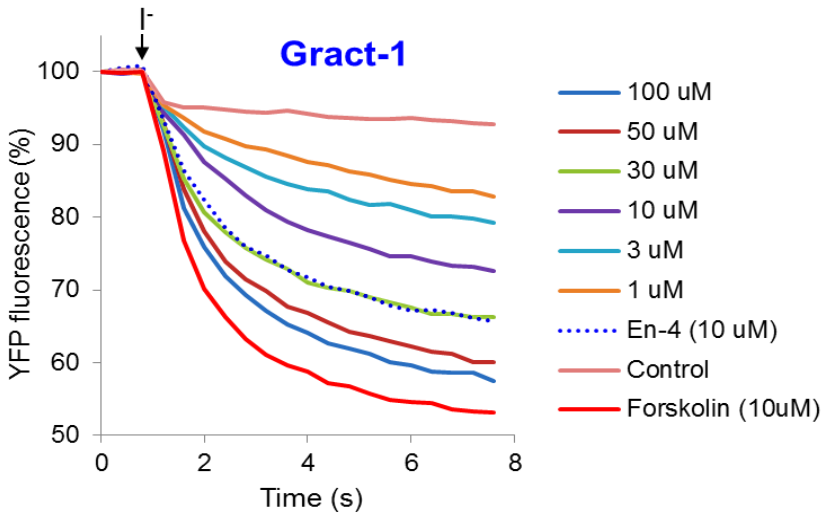
Representative traces of YFP fluorescence & HTS summary

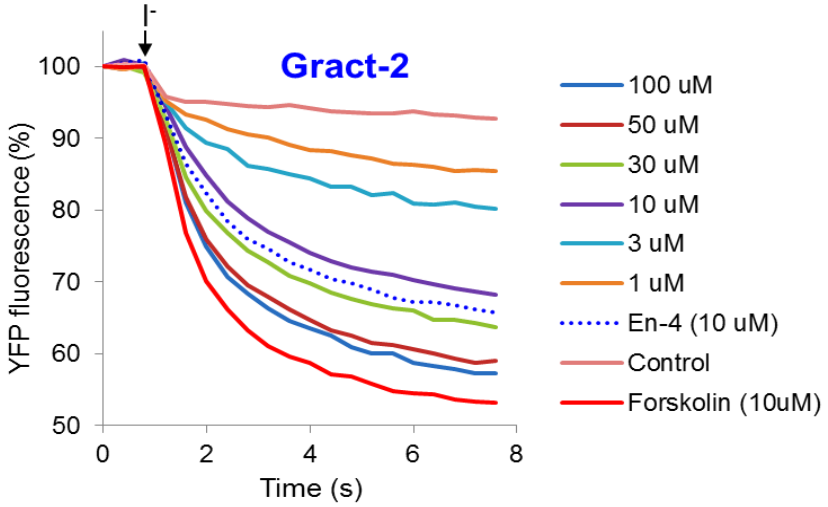


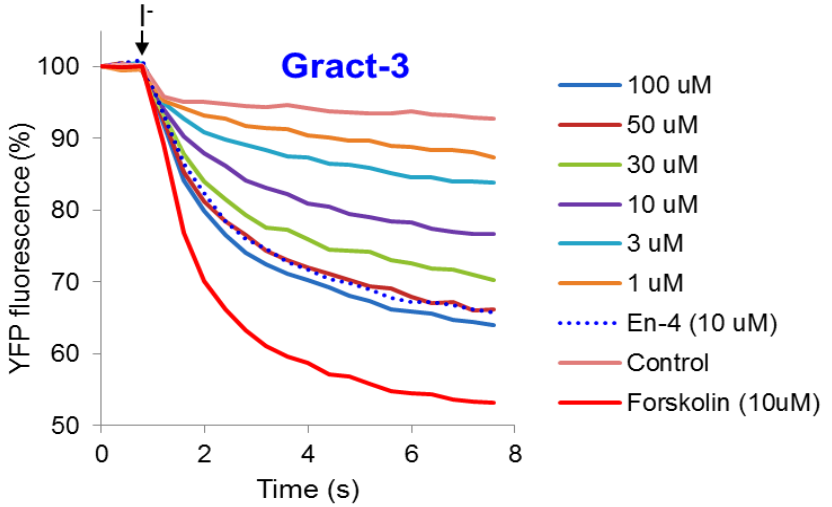


Effect of hit compounds on GLP1R-mediated activation of CFTR in the Calu-3 cells

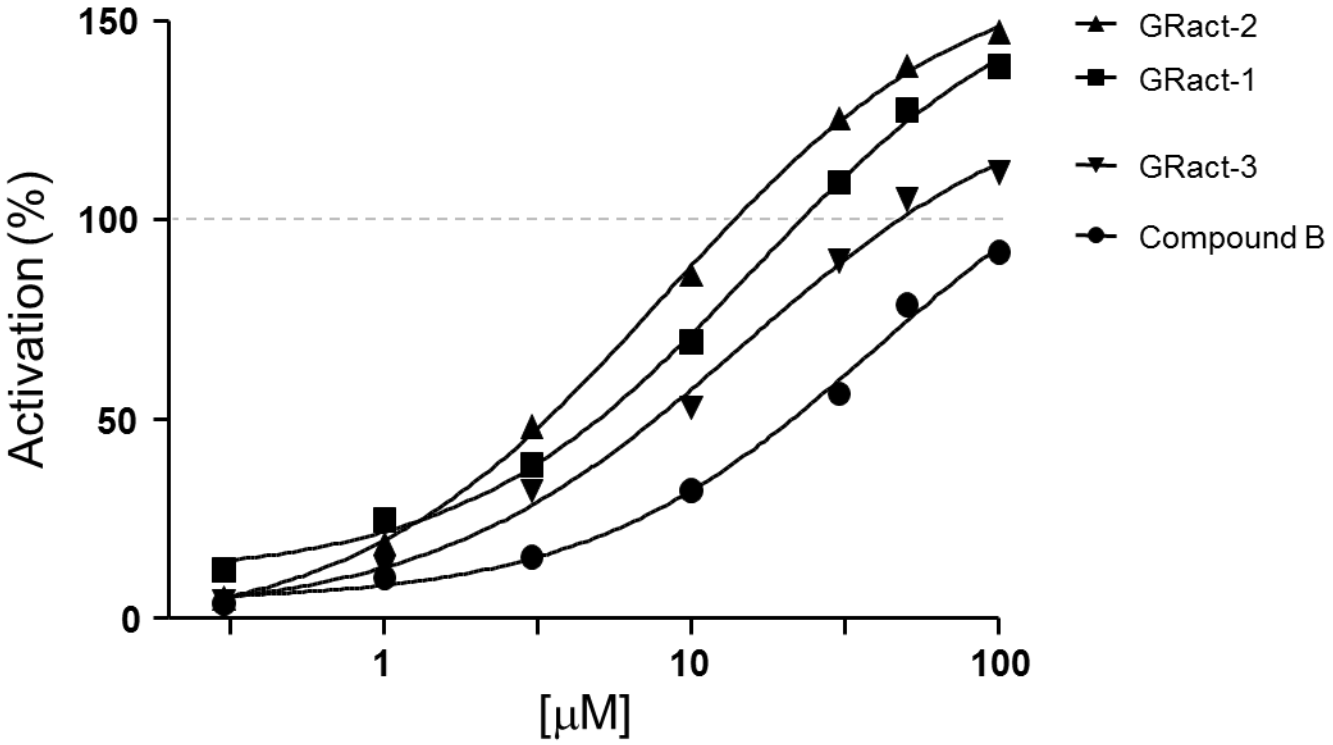




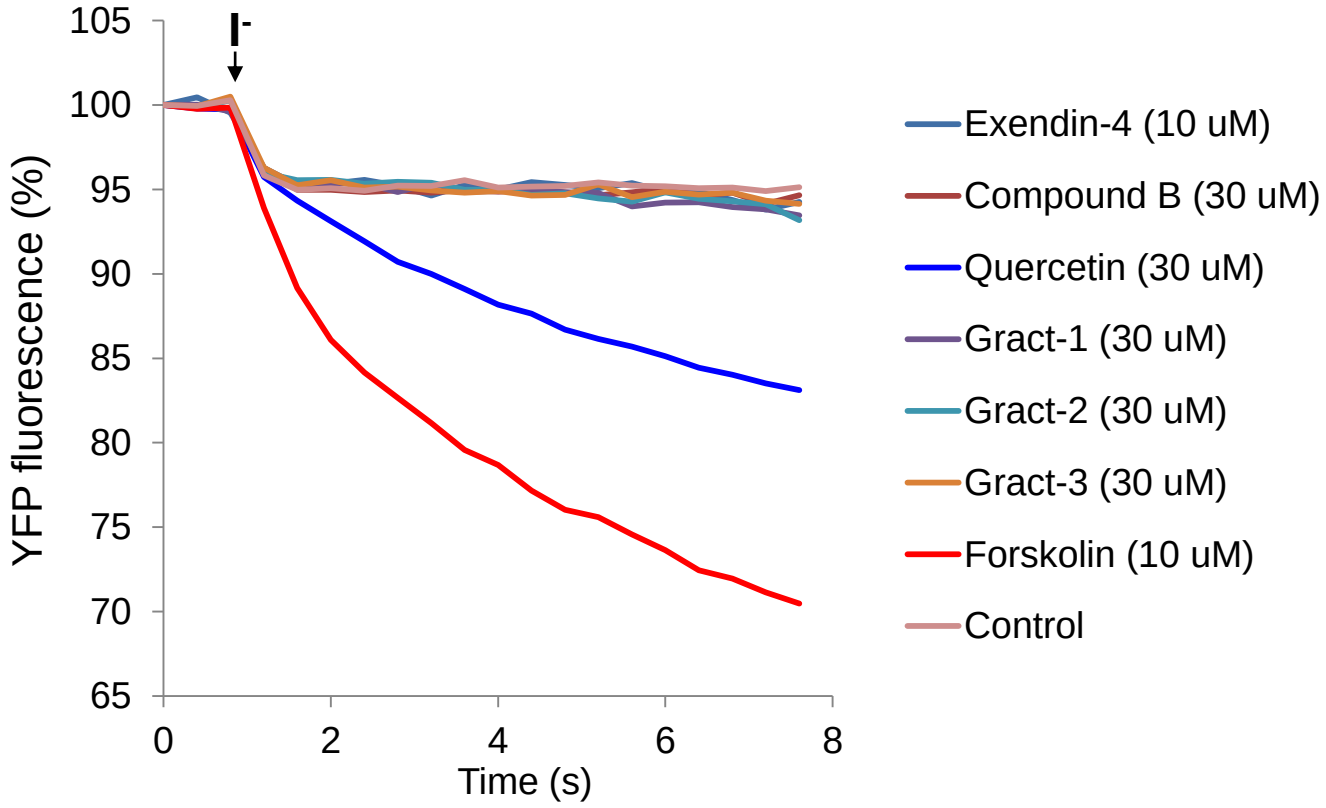




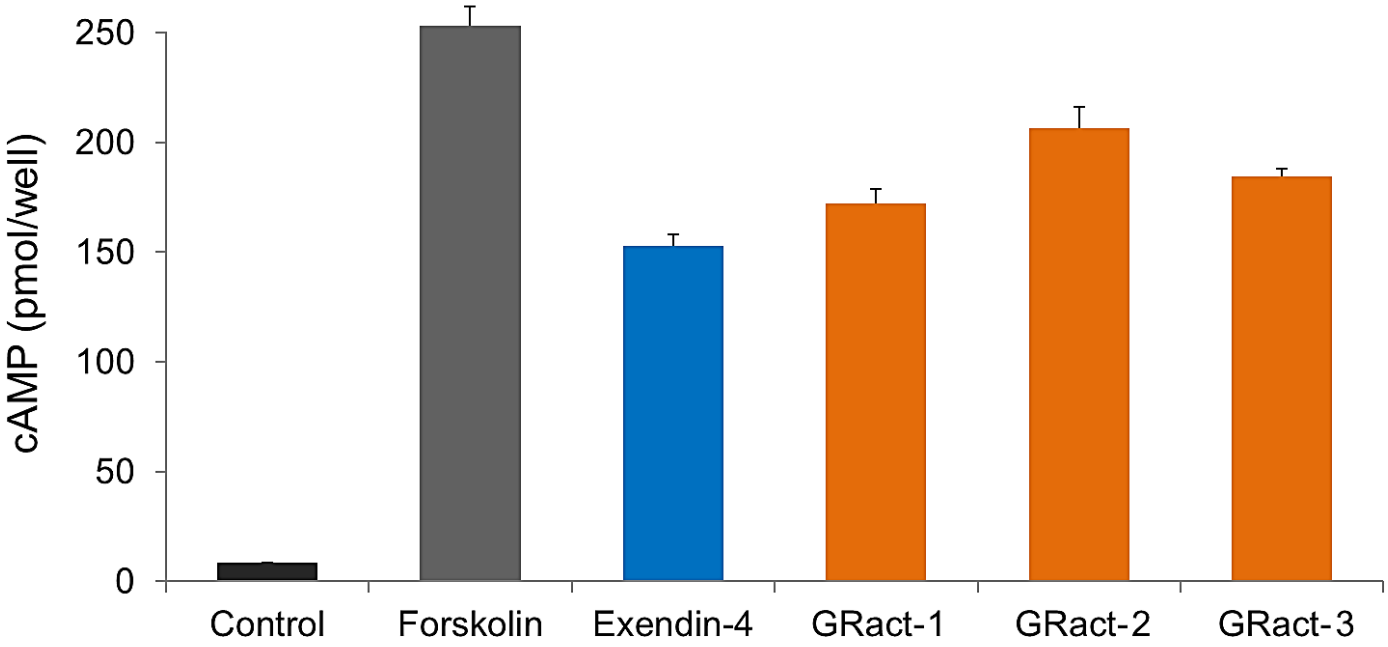
Effect of hit compounds on GLP1R-mediated activation of CFTR in the Calu-3 cells



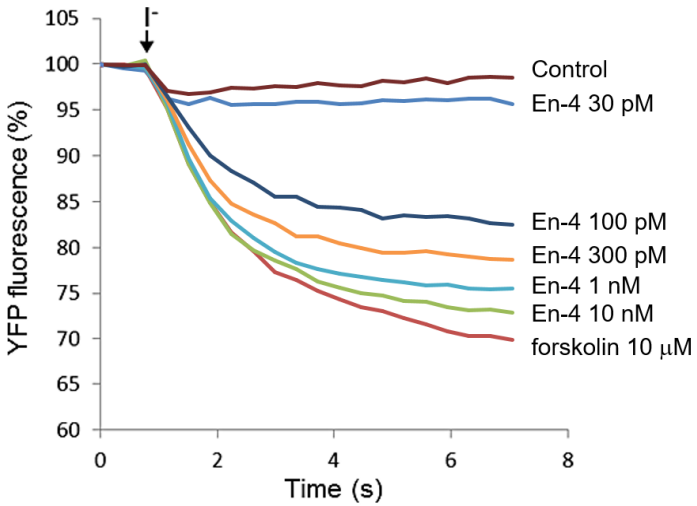
Effect of GLP1R agonists on CFTR activity in CHO-CFTR cells not expressing GLP1R

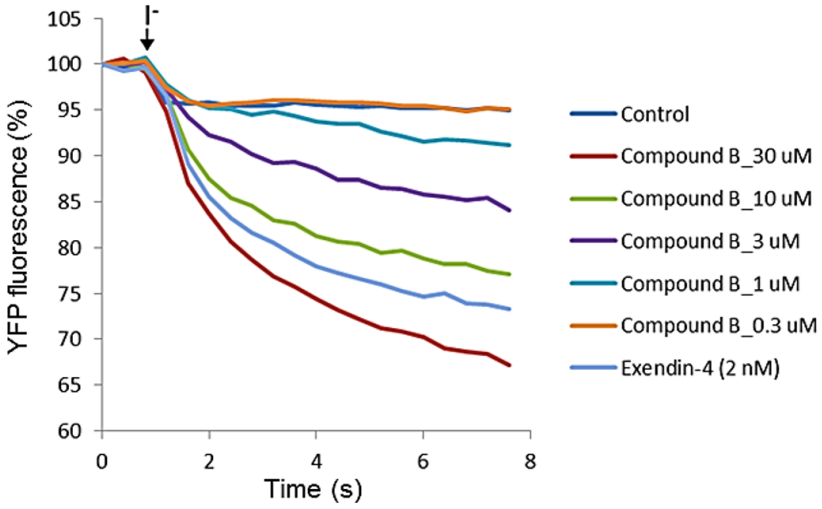


Effect of Exendin-4 & GRacts on intracellular cAMP levels in GLP1R expressing Calu-3 cells



Effect of GLP1R agonists on GLP1R-mediated CFTR activation in CHO-CFTR-GLP1R cells





HTS results in CHO-CFTR-GLP1R-YFP cells

Primary screening with 50,000 compounds

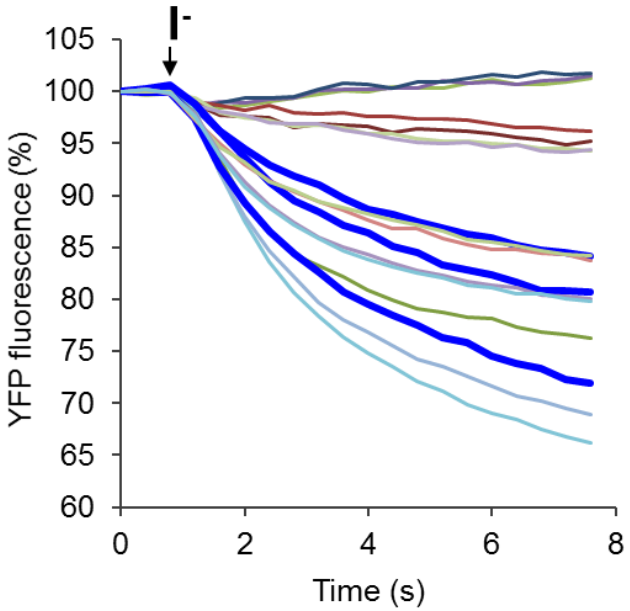
40 hits

Second screening / CFTR assay

3 hits

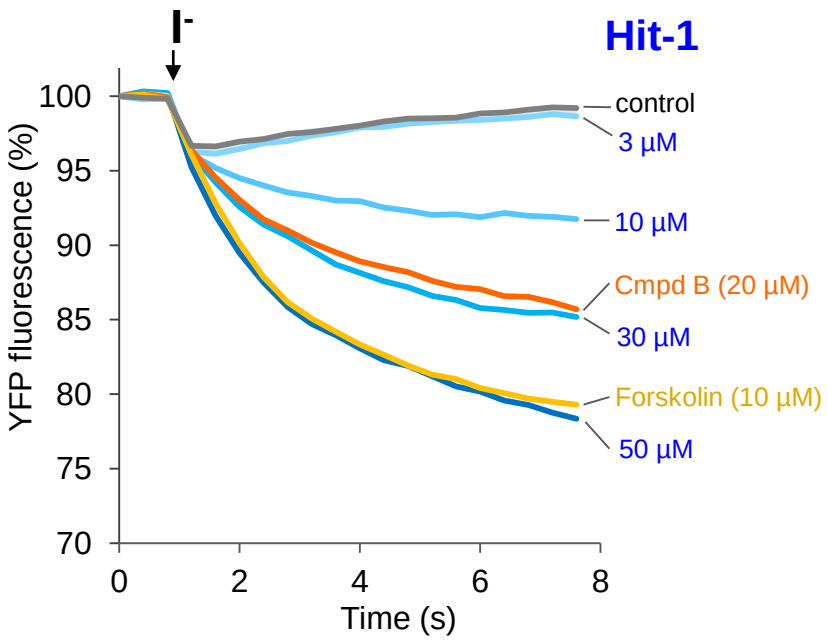
Dose response analysis

3 novel agonists (1 class) of GLP1R

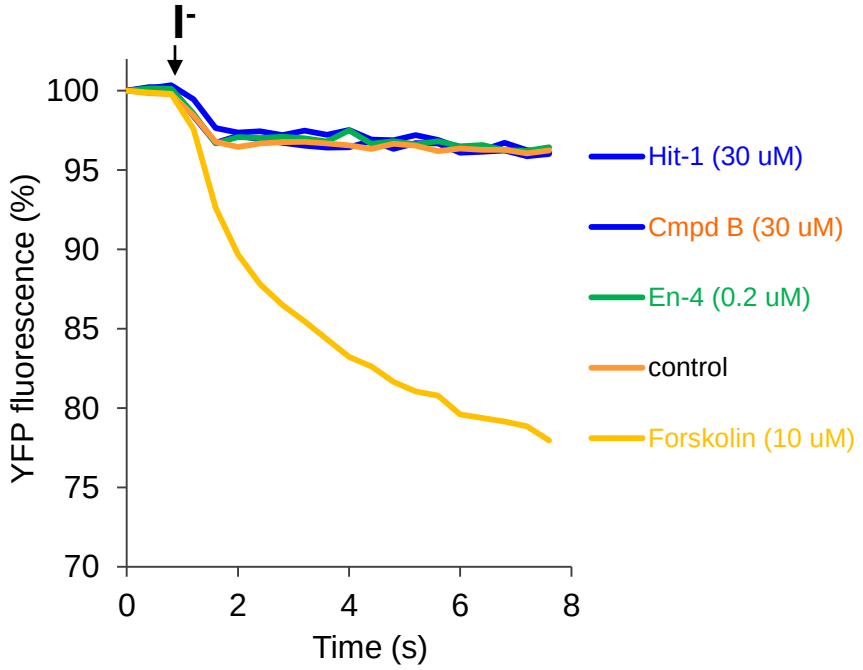


Effect of GLP1R agonists on GLP1R-mediated CFTR activation in CHO-CFTR-GLP1R cells

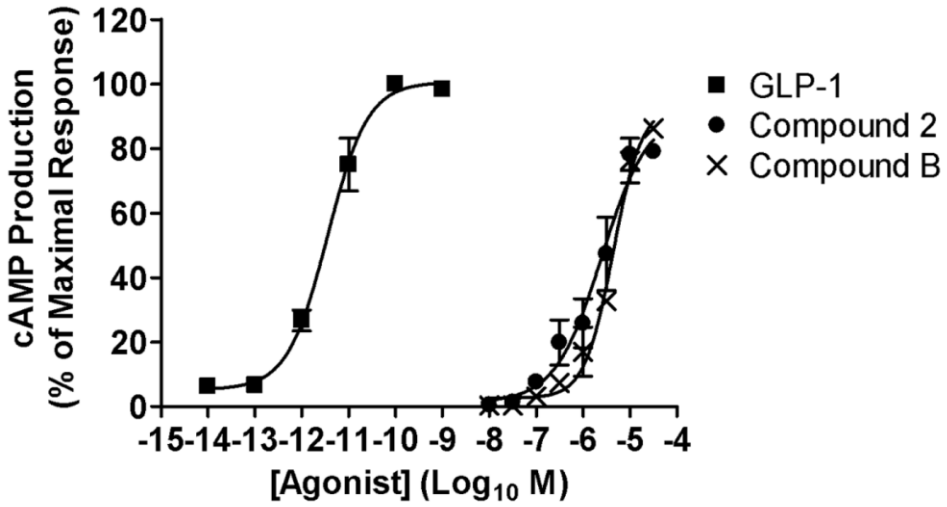
CHO_CFTR_GLP1R

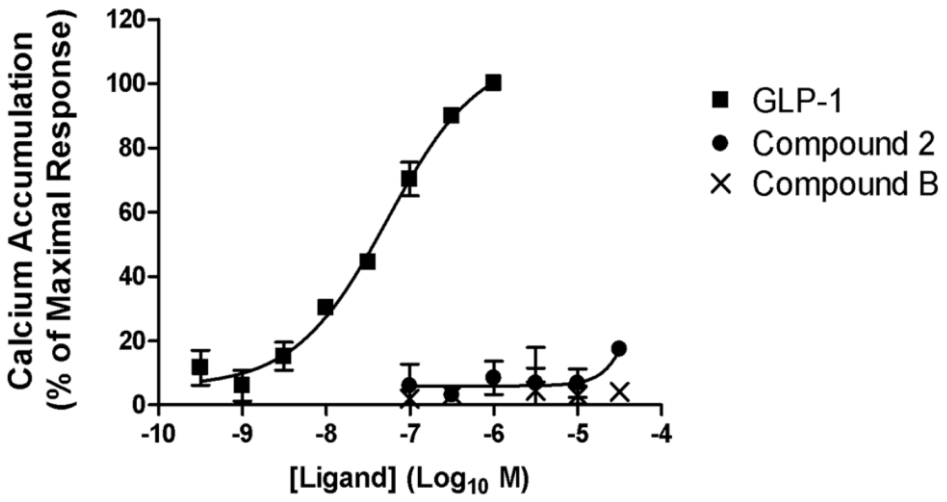


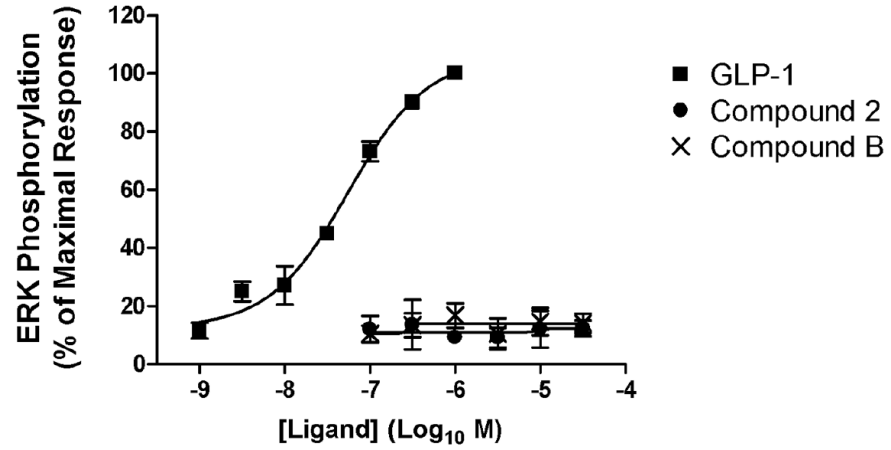
CHO_CFTR

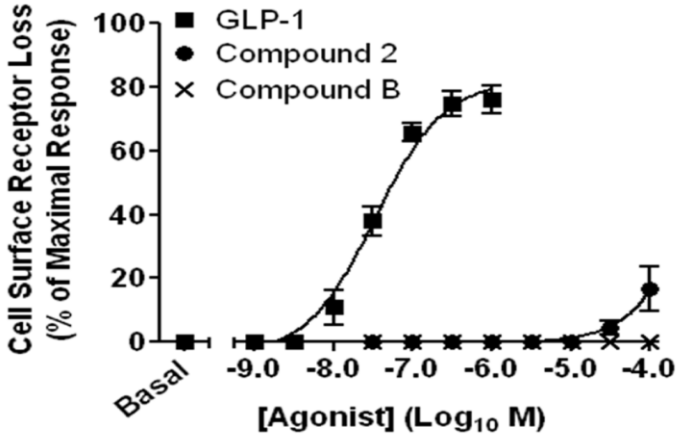


In human GLP1R (hGLP1R) expressing cells, **compounds 2 and B** induced cAMP production but caused **no intracellular Ca^{2+} accumulation**, **ERK phosphorylation** or **hGLP1R internalisation**. *PLoS One. 2016; 11(4): e0154229.*

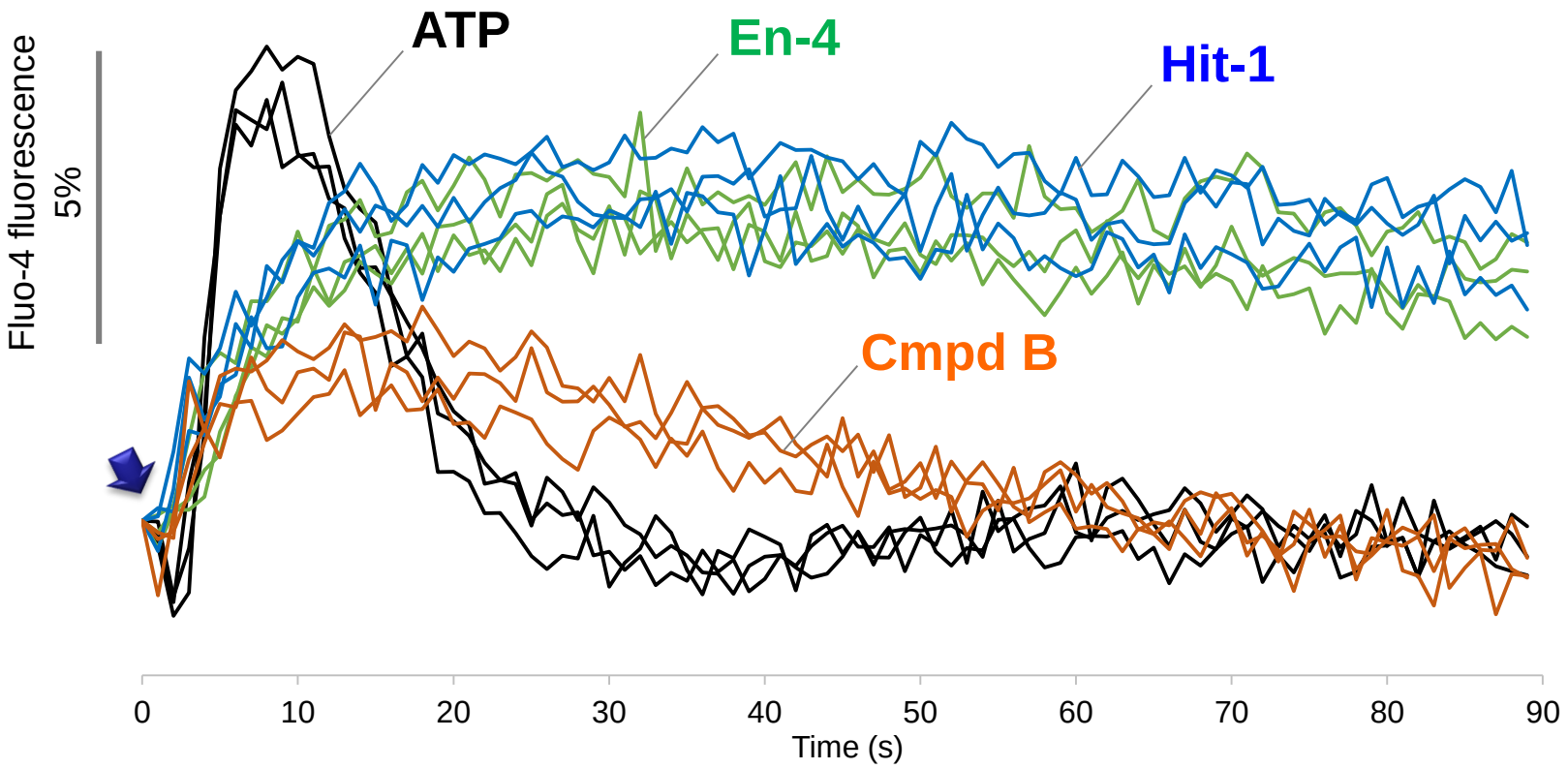




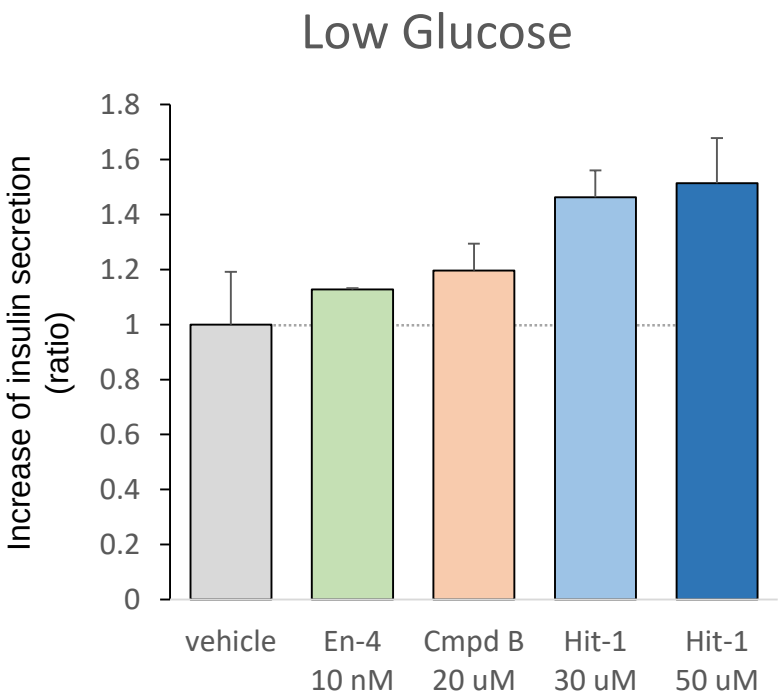


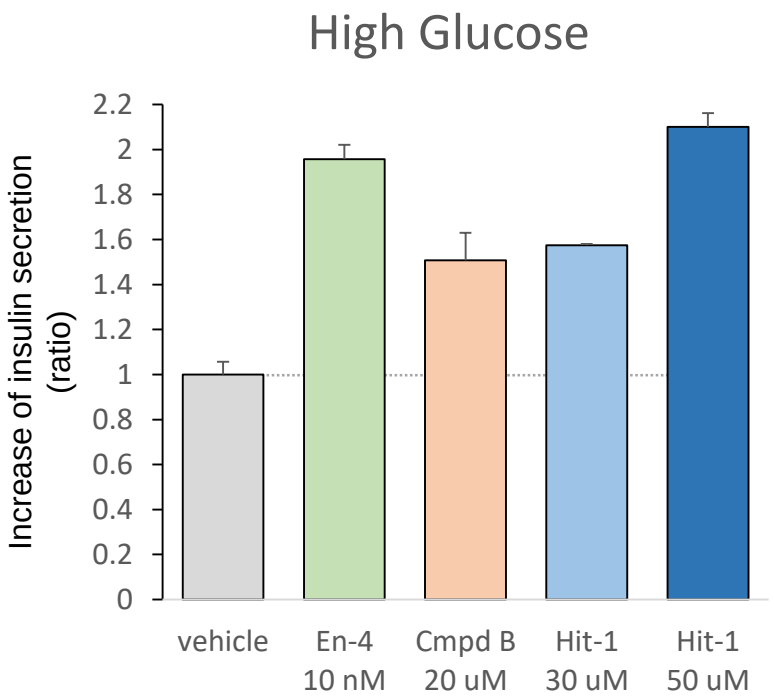


Effect of GLP1R agonist on $[Ca^{2+}]_i$ in CHO-CFTR-GLP1R cells



Effect of GLP1R agonist on insulin secretion in MIN6 cells





Summary

- High-throughput screening of ~110,000 compounds yielded **3 novel agonists (1 class) of GLP1R**.
- Hit-1 showed **GLP1R-mediated CFTR activation** in a dose-dependent manner in CHO-K1 cells expressing CFTR & GLP1R.
- Hit-1 **induced intracellular calcium increase** in CHO-K1 cells expressing CFTR & GLP1R.
- Hit-1 **increased insulin secretion** in MIN6 cells.
- Hit-1 may be **a good starting material for the development of small-molecule GLP1R agonist.**

Acknowledgment

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THANK YOU