Investigation of Hits against the GLP-1 Receptor Identified by Affinity Selection Mass Spectrometry: Hit Expansion, Structure-Activity Relationships, and Selectivity Profiling

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Abstract

A prior high-throughput affinity selection mass spectrometry (ASMS) screen of 50,000 compounds against the GLP-1 receptor expressed in copolymer-based nanodiscs using the NativeMP™ platform by Cube Biotech identified 34 confirmed hit compounds. Here we report the hit expansion and characterization of three distinct chemical series emerging from that screen. Structurally similar analogs were acquired from commercial sources. Three chemical scaffolds were characterized: a pyrazolinone/indazolone lactam series (20 analogs), an aminopyrimidine series (16 analogs), and a benzimidazole-amide series (11 analogs). Key structure-activity findings included: a 5–100× potency advantage for direct C4-aryl over aryl-ketone substituents in the lactam series; a 26-fold regiochemistry penalty for meta- vs. para-substitution on the pendant phenyl; dominant halogenation effects in the aminopyrimidine series (Br ≈ CF_3 > Cl >> H); and a 3× activity gain from a constrained piperidine linker in the benzimidazole-amide series. Selective GLP1R binders were subsequently profiled against two related off-targets GIPR and GCGR all in nanodisc format prepared by Cube Biotech. GCGR was the dominant cross-reactivity challenge (39% of compounds preferred GCGR). Halogenated compounds showed 3× better selectivity on average, and competition assays confirmed orthosteric binding within each series. This work illustrates the utility of nanodisc-based ASMS for rapid, label-free hit expansion and selectivity differentiation of membrane protein binders.

Background & Objectives

A previously described and presented ASMS screen of the GLP-1 Receptor identified 34 confirmed hits from a 50K compound diversity library. This study builds on that work by:

- Hit expansion of select chemotypes via chemical clustering (Butina, Tanimoto ≥ 0.20)
- SAR analysis using ASMS peak area as a proxy for binding affinity
- Selectivity profiling vs GIPR, GCGR and blank nanodisc

GLP1R: Type 2 Diabetes and Obesity Target

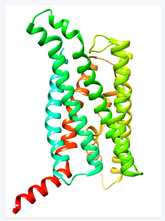
Target for Type 2 Diabetes and Obesity Treatment
GLP1R drugs manage blood sugar levels and promote weight loss

Enhanced Metabolic Health
GLP1R drugs can improve metabolic parameters beyond glucose and weight management, potentially benefiting patients with metabolic syndrome and associated conditions.

Drug Discovery at Momentum Bio and Cube Biotech
Discovery and development of compounds targeting GLP1R, from hit to lead

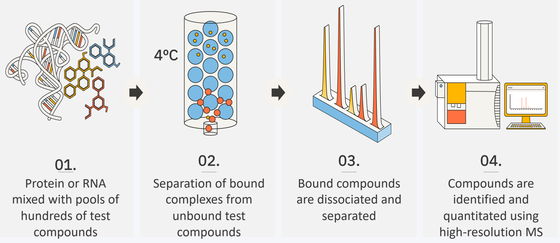
Cardiovascular Protection
Some GLP1R therapies reduce the risk of heart attack and stroke, which is valuable for patients with cardiovascular disease

Oral Availability
Small molecules can be taken orally, eliminating the need for injections and making treatment for diseases like type 2 diabetes and obesity more convenient.

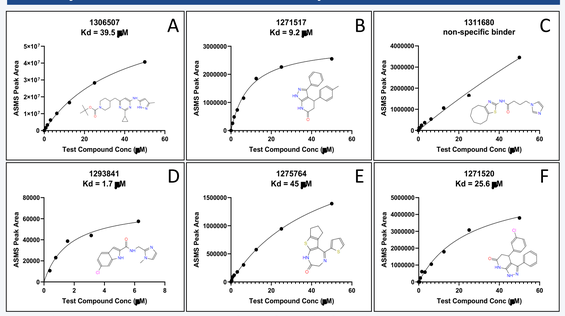


A relaxed-state 3D conformation of the GLP1 receptor (GLP1R) shown in cartoon representation (PDB: 8JIS; 2.46Å)

ALIS-ASMS Workflow



Affinity Determination — Dose-Response Curves

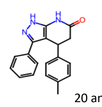
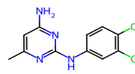
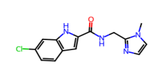


Example concentration response curves for confirmed hit from the ASMS HTS campaign comprising 50,000 diversity compounds from Momentum PS7 chemical library

Compound Clustering Summary

	N	Hit	Scaffold	Area Range
A	20	1271517	Pyrazolinone/Indazolone Lactam	0.4K–103K
B	16	1038635	Aminopyrimidine (chloro-aryl)	10K–2.1M
C	11	1293841	Benzimidazole-amide	0.4K–104K

Three HTS Hits Selected as Starting Points for Hit Expansion

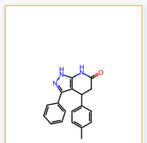
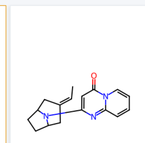
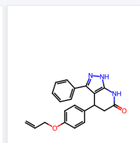
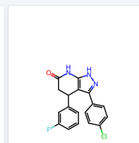
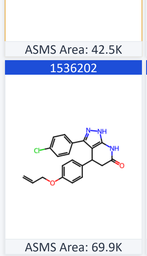
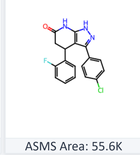
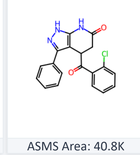
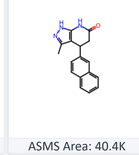
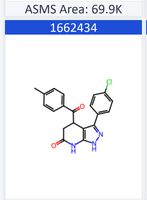
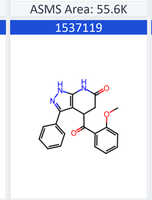
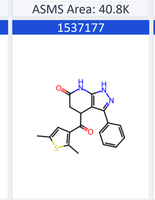
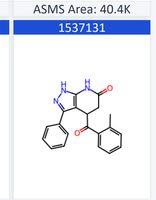
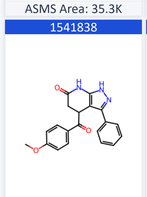
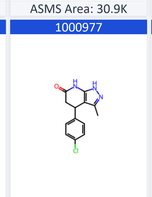
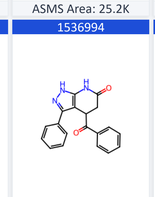
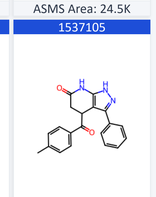
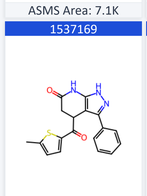
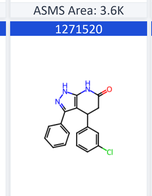
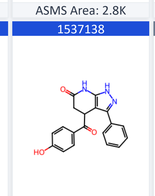
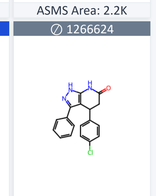
★ 1271517	★ 1038635	★ 1293841
		
20 analogs	16 analogs	11 analogs
A — Pyrazolinone series	B — Aminopyrimidine series	C — Benzimidazole series
Pyrazolinone/Indazolone lactam	Aminopyrimidine (chloro-aryl)	Benzimidazole-amide
Area: 42.5K | Score: 98.4	Area: 190.5K | Score: 99.3	Area: 33.8K | Score: 98.7

Cluster A — Pyrazolinone / Indazolone Lactam Series (20 Cpds)

Core Scaffold — Cluster A

Pyrazolinone lactam fused indazole/pyrazole. Key positions:
C4: Aryl substituent (direct vs ketone linker defines activity)
C5: Pendant aryl ring (para-position critical)
N1: Indazole N-H (essential)
C6: Halogen sub-pocket (additive ~2×)

● Key substituent ● Favourable ● Detrimental ● Core scaffold

★ 1271517	1151192	1266629	1273094
			
ASMS Area: 42.5K	ASMS Area: 103.1K	ASMS Area: 79.4K	ASMS Area: 78.7K
1536202	1273111	1662436	1110901
			
ASMS Area: 69.9K	ASMS Area: 55.6K	ASMS Area: 40.8K	ASMS Area: 40.4K
1662434	1537119	1537177	1537131
			
ASMS Area: 35.3K	ASMS Area: 30.9K	ASMS Area: 25.2K	ASMS Area: 24.5K
1541838	1000977	1536994	1537105
			
ASMS Area: 7.1K	ASMS Area: 3.6K	ASMS Area: 2.8K	ASMS Area: 2.2K
1537169	1271520	1537138	∅ 1266624
			
ASMS Area: 1.9K	ASMS Area: 1.6K	ASMS Area: 415	*Non-binder*

Clusters B & C — Hit Expansion Overview

Cluster B — Aminopyrimidine Series (16 compounds, incl. 1 non-binder)

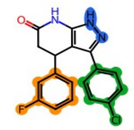
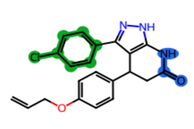
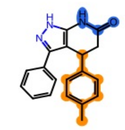
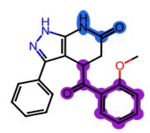
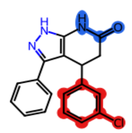
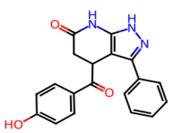
16 analogs screened around original hit 1038635. Halogenation is the dominant SAR driver — para-Br and CF_3 deliver 10–50× improvement. Best compound: 1104065 (diphenylamino, 2.1M area, best in class). Regiochemistry critical: ortho-OMe/para-Cl (514K) vs swapped isomer (42K) = 12× penalty.

Cluster C — Benzimidazole-Amide Series (11 compounds, incl. 2 non-binders)

11 analogs screened around original hit 1293841. Best compound: 1272018 (piperidine-constrained linker, 104K, 3× gain over hit). Benzimidazole core irreplaceable (pyrrole swap = 80× loss). EWG at C5/C6 required; OMe → 5× loss. 2 non-binders confirm scaffold essentials.

Structure–Activity Relationships: Cluster A (Pyrazolinone/Indazolone Lactam)

Two Distinct Sub-Series Within Cluster A
Sub-series 1: Direct aryl at C4 — top binders (3.6K–103K). **Sub-series 2:** Aryl ketone at C3 — weaker (0.4–31K). Direct-aryl is 5–100× more potent.

★ Top binder	Top binder	★ Original Hit
		
1273094	1536202	1271517
Area: 78.7K | Score: 99.6	Area: 69.9K | Score: 97.6	Area: 42.5K | Score: 98.4
Ketone sub-series	**Meta-Cl (weak)**	**Weakest binder**
		
1537119	1271520	1537138
Area: 30.9K | Score: 98.4	Area: 1.6K | Score: 72.0	Area: 415 | Score: 10.3

C4 direct aryl essential — ketone penalised 5–100×
Direct C–C bond to pendant aryl at C4 is 5–100× more potent than a ketone linker. Within the ketone series, ortho-substitution rescues activity: ortho-OMe (31K) vs unsubstituted (2.8K) via preferred rotamer.

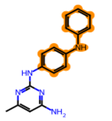
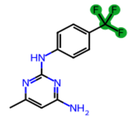
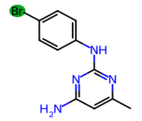
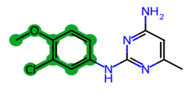
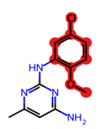
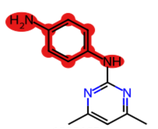
Para-substitution on pendant phenyl strongly preferred
Para-OAllyl (79K) and para-Cl are optimal. Meta-Cl (1271520) drops 42K→91.6K — a 26× loss from a single regiochemical change. Pocket is stringently defined.

Para-Cl on indazole ring is consistently additive (~2×)
Adding para-Cl to the indazole provides ~2× gain regardless of pendant aryl substitution. Likely a discrete halogen-bond or lipophilic contact in a sub-pocket.

Ortho-substitution rescues ketone sub-series
Ortho-OMe ketone (1537119, 31K) is 11× better than unsubstituted phenyl ketone (1536994, 2.8K). Ortho group enforces a dihedral mimicking the direct-aryl geometry.

High TPSA penalised — hydrophobic binding pocket
Top binders: avg TPSA 57 Å² vs 76 Å² bottom quartile. H-bond donors also lower (1.5 vs 2.3). Consistent with a hydrophobic, solvent-excluded binding site.

Structure–Activity Relationships: Cluster B (Aminopyrimidine Series)

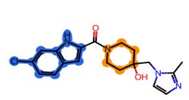
Best in class	CF_3 (top 3)	Para-Br
		
1104065	1006677	1006653
Area: 2.11M | 99.8	Area: 691.9K | 98.6	Area: 537.0K | 99.5
Correct regio.	**Swapped regio. ⚠**	**No halogen**
		
1024731	1006673	1235165
Area: 514.8K | 99.2	Area: 42.0K | 96.7	Area: 32.6K | 98.6

Halogenation is the dominant driver
Br = CF_3 > Cl >> H. 10–50× gains over unhalogenated. Best compound 1104065 (diphenylamino, 2.1M) is the highest area in the entire dataset.

Regiochemistry: 12× penalty for a simple swap
Ortho-OMe/para-Cl (1024731, 514K) vs para-OMe/ortho-Cl (1006673, 42K). Same atoms, different positions — binding pocket is spatially stringent.

Bicyclic tail sub-series: distinct chemotype
1095779, 1147707, 1080458 lack the aniline-pyrimidine pharmacophore yet bind well — may access the same site via an alternative mode.

Structure–Activity Relationships: Cluster C (Benzimidazole-Amide Series)

Best in class	★ Original Hit	F analog
		
1272018	1293841	1335417
103.8K | 99.8	33.8K | 98.7	31.7K | 98.3
OMe (weak) ⚠	**Pyrrole (dead)**	**Lead — constrained linker**
1272495	1253450	1272018b
7.1K | 75.5	432 | 14.3	103.8K | 99.8

Constrained linker delivers 3× gain
1272018 (103K) exceeds hit 1293841 (34K) 3×. Piperidine ring locks N-methyl imidazole in optimal geometry — highest-priority lead

EWG at C5/C6 required; EDG kills activity
Cl and F are equipotent at C5. OMe at C6 (1272495, 7K) is 5× weaker — electron donation reduces N-mediated binding contacts.

Benzimidazole core is irreplaceable
Pyrrole swap (1253450) = 432 area — ~80× collapse. Fused bicyclic aromatic system essential for π-stacking or H-bond acceptor contacts

GLP1R Selectivity Screening: GLP1R, GIPR, GCGR

GLP1R, GIPR, and GCGR are three closely related Class B1 GPCRs sharing a common architecture: a large N-terminal extracellular domain (ECD) for peptide hormone recognition, a seven-transmembrane (7-TM) helical bundle, and an intracellular tail mediating Gs protein coupling. The receptors share substantial transmembrane sequence identity: approximately 44% between GLP1R and GIPR, and ~48% between GLP1R and GCGR reflecting their common evolutionary origin. The high structural similarity of their transmembrane binding pockets, especially between GLP1R and GCGR is the primary reason selective small molecule engagement of any one receptor over the others remains a central challenge in drug discovery for this target family.

Target Receptor Structures — 7-TM Topology (GLP1R, GIPR, GCGR)

45 compounds screened in parallel as singletons against GLP1R, GIPR, GCGR and blank nanodisc.
Selectivity = $\log_2$(GLP1R/off-target). Values >0 = GLP1R preferred.
22/44 compounds GLP1R-selective. GCGR is the dominant challenge (39% of compounds prefer GCGR).

Top GLP1R-Selective Compounds

1311531 composite: 12.30	1299341 composite: 5.72	1271520 composite: 3.23	1660920 composite: 3.19
vs GIPR 0.8 | vs GCGR 18.1 | vs Blank 18.1	vs GIPR 0.5 | vs GCGR -2.3 | vs Blank 18.9	vs GIPR 0.6 | vs GCGR 2.2 | vs Blank 6.9	vs GIPR 1.0 | vs GCGR 2.8 | vs Blank 5.8
LogP 4.28 TPSA 46.1 MW 321.4 fCSP3 0.44 Halo 1	LogP 3.45 TPSA 65 MW 389.9 fCSP3 0.35 Halo 1	LogP 4.2 TPSA 57.8 MW 323.8 fCSP3 0.11 Halo 1	LogP 3.86 TPSA 57.8 MW 303.4 fCSP3 0.16 Halo 0
GLP1R 275K GIPR 163K GCGR 0 Blank 0	GLP1R 499K GIPR 354K GCGR 2.38M Blank 0	GLP1R 578K GIPR 385K GCGR 125K Blank 5K	GLP1R 499K GIPR 256K GCGR 73K Blank 9K
1293685 composite: 3.08	**1273451 composite: 3.00**	**1663759 composite: 2.86**	**1275764 composite: 2.86**
vs GIPR 0.5 | vs GCGR 3.0 | vs Blank 5.7	vs GIPR 1.1 | vs GCGR 6.7 | vs Blank 1.2	vs GIPR 0.5 | vs GCGR 1.5 | vs Blank 6.6	vs GIPR 0.5 | vs GCGR 0.9 | vs Blank 7.2
LogP 3.9 TPSA 47.6 MW 369.4 fCSP3 0.32 Halo 1	LogP 3.13 TPSA 49.8 MW 288.4 fCSP3 0.47 Halo 0	LogP 5.09 TPSA 66.3 MW 433.5 fCSP3 0.35 Halo 1	LogP 3.09 TPSA 41.5 MW 288.4 fCSP3 0.29 Halo 0
GLP1R 268K GIPR 197K GCGR 33K Blank 5K	GLP1R 241K GIPR 110K GCGR 2K Blank 106K	GLP1R 2.19M GIPR 1.54M GCGR 794K Blank 22K	GLP1R 274K GIPR 192K GCGR 146K Blank 2K

Least GLP1R-Selective Compounds: High Off-Target Binding

1104701 composite: -3.05	1299453 composite: -1.57	1298002 composite: -1.22	1306217 composite: -1.22
vs GIPR -4.0 | vs GCGR -5.5 | vs Blank 0.3	vs GIPR 0.3 | vs GCGR -3.6 | vs Blank -1.5	vs GIPR -0.0 | vs GCGR -2.4 | vs Blank -1.2	vs GIPR -0.1 | vs GCGR -2.3 | vs Blank -1.4
LogP 4.04 TPSA 45.3 MW 326.4 Halo 0	LogP 3.89 TPSA 50.5 MW 391.5 Halo 0	LogP 4.11 TPSA 75.6 MW 373.5 Halo 0	LogP 4.86 TPSA 64.2 MW 442.5 Halo 1
GLP1R 2K GIPR 31K GCGR 86K Blank 2K	GLP1R 163K GIPR 131K GCGR 1.92M Blank 448K	GLP1R 39K GIPR 40K GCGR 209K Blank 89K	GLP1R 1.80M GIPR 1.86M GCGR 8.63M Blank 4.62M

1. Blank nanodisc is the most informative discriminator
25/44 compounds (57%) bind GLP1R at least 4× more than blank nanodisc. This is the cleanest selectivity signal. Compounds with low blank binding likely bind via a specific, defined mode rather than non-specific lipophilic partitioning into the nanodisc membrane. This axis shows essentially no correlation with any single descriptor, suggesting it is driven by specific binding contacts rather than bulk physicochemical properties.

2. GCGR is the dominant off-target challenge
17 of 44 compounds (39%) show higher GCGR signal than GLP1R ($\log_2 < 0$). GCGR (glucagon receptor) is the closest structural relative of GLP1R, making cross-reactivity unsurprising. LogP shows the strongest (modest) correlation vs GCGR (r=+0.17): higher LogP slightly favours GLP1R selectivity. GCGR-selective compounds have notably lower aromatic ring count (avg 2.1 vs 2.7), suggesting flat, aromatic scaffolds may be less discriminating between these two receptors.

3. Halogen presence is consistently associated with GLP1R selectivity
Halogenated compounds show mean composite selectivity of +1.93 vs +0.63 for non-halogenated (3× difference). The effect is most pronounced in the blank nanodisc axis (avg +5.1 vs +2.5 $\log_2$), suggesting halogens promote specific binding rather than simply increasing lipophilicity. This is consistent with halogen bonding or tight hydrophobic contacts at defined GLP1R binding pockets not present on GCGR/GIPR.

4. Low TPSA / low aromatic ring count favours selectivity
Compounds with TPSA < 50 Å² show mean composite selectivity of +1.81 vs +0.84 for higher TPSA compounds. The negative correlation of aromatic ring count (r = −0.19) is the strongest single descriptor effect: highly aromatic, flat compounds tend to bind non-selectively. This may reflect pan-assay interference or non-specific hydrophobic binding. Compounds with higher sp^3 character (fCSP3 ≥ 0.35) average +1.34 vs +0.89 selectivity — 3D character modestly benefits selectivity.

Conclusions

Small molecule engagement of the GLP-1 receptor using affinity selection mass spectrometry (ASMS) proved to be a productive starting point for drug discovery, with a 50,000 compound diversity screen identifying three structurally distinct hit series that could be expanded and characterized in depth. Hit expansion across 52 analogs established clear structure-activity relationships within 3 scaffolds. Selectivity profiling against the closely related GIPR and GCGR confirmed that GLP1R-selective binding is achievable (22 of 44 compounds showed net GLP1R preference) but that GCGR cross-reactivity, driven by ~48% transmembrane sequence identity, represents the primary differentiability challenge.
Taken together, this work demonstrates that nanodisc-based ASMS is a powerful and efficient platform for the iterative characterization of membrane protein binders, from primary hit identification through analog profiling and selectivity differentiation, without the need for labeled compounds, surface immobilization, or extensive assay development, and that it is directly applicable to other GPCRs and membrane-embedded targets where selectivity among structurally homologous family members is a key challenge.

COI Statement: The authors of this poster work at contract research organizations that offer protein production and mass spec based analytical services.