Dapiglutide is a dual agonist on human GLP-1- and GLP-2-receptors with a biased and prolonged signaling profile at the GLP-1R

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Introduction & Background

- Dapiglutide, a potential first-in-class therapy targeting obesity and low-grade inflammation, is designed for activating both GLP-1 and GLP-2 receptors (GLP-1R and GLP-2R).
- In a MAD trial[1], once-weekly s.c. injection of dapiglutide up to 6 mg for 4 weeks was well-tolerated in healthy participants and showed dose-dependent body weight loss up to a mean 4.3%[1], and dose-dependent reductions of plasma glucose and insulin (data not shown)[1].
- At the cellular level, GLP-1R activation by native GLP-1 induces formation of its major second messenger cAMP and recruitment of β-arrestin.
- Recent studies hypothesized that biased agonists displaying lack of β-arrestin recruitment at the GLP-1R are beneficial in controlling blood glucose and body weight loss in DIO mice[2-4].

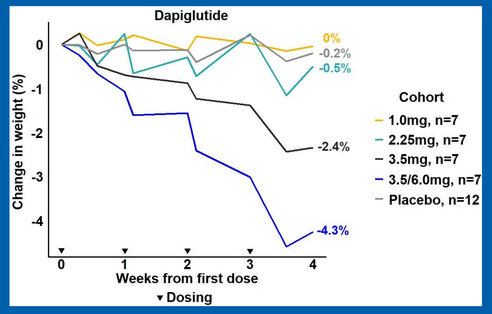


Fig a: Dose dependent body weight loss by dapiglutide

References:

1. Olsen MB, Hovelmann U, Griffin J, Knudsen KM, Eriksson PO, Agersnap MA. Dapiglutide, a once-weekly GLP-1R/GLP-2R dual agonist, was safe and well tolerated and showed dose-dependent body weight loss over four weeks in healthy subjects. *Diabetes* 2022;71(Supplement_1):335-OR.
2. Hinds C E et al. Abolishing β-arrestin recruitment is necessary for the full metabolic benefits of G protein-biased glucagon-like peptide-1 receptor agonists. Diabetes Obes Metab. 26:65 (2024).
3. Jones B et al. Targeting GLP-1 receptor trafficking to improve agonist efficacy. Nat Commun. 2018;9(1):1602.
4. Lucey M et al. Disconnect between signaling potency and in vivo efficacy of pharmacokinetically optimized biased glucagon-like peptide-1 receptor agonists. Mol Metab. 2020;37:100991.

Study Objectives

- To determine the binding affinity of dapiglutide at recombinantly expressed GLP-1- and GLP-2 receptors.
- To investigate the in vitro profile of dapiglutide, emphasizing on GLP-1R signaling bias and the cellular signaling consequences.

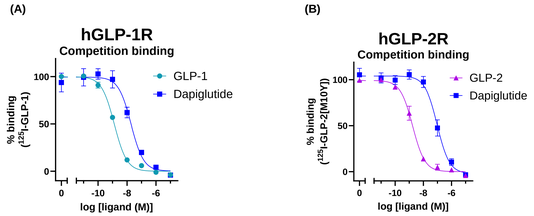
Methods

- Receptor binding assays using 125-iodinated radioligands were applied to estimate binding affinities of dapiglutide for human GLP-1R and GLP-2R recombinantly expressed in COS-7cells.
- The human GLP-1R in vitro signaling profile was investigated in a kinetic cAMP formation assay and for recruitment of β-arrestin in HEK293 cells.
- Receptor internalization was measured following agonist stimulation of a YFP-tagged human GLP-1R expressed in HEK293 cells

Results

- Initial in vitro characterization indicated that dapiglutide is more potent for binding to human GLP-1R over GLP-2R, with estimated binding affinities of 38 nM and 102 nM, respectively (see Fig 1).
- At the human GLP-1R, dapiglutide showed signaling bias, displaying full agonist activity in cAMP formation, whilst having significantly blunted response to β-arrestin recruitment, resulting in less agonist-receptor internalization compared to GLP-1 (see Fig 2).
- Further in vitro characterization showed that dapiglutide-induced cAMP formation kinetics persisted for up to 12 hours unlike native GLP-1, potentially reflecting signaling bias and lack of receptor desensitization (see Fig 3).

Receptor binding profile of dapiglutide



	hGLP-1R K_i (nM)	hGLP-2R K_i (nM)
Dapiglutide	38.1	102
GLP-1	2.78	-
GLP-2	-	2.84

Figure 1: Binding and estimated binding affinities of dapiglutide for (A) human GLP-1R and (B) human GLP-2R. Dapiglutide displaces radioactively labeled ^{125}I-GLP-1 and ^{125}I-GLP2(M10Y) from cells recombinantly expressing human GLP-1 and GLP-2Rs, respectively, as observed for the endogenous agonists GLP-1 and GLP-2. Datapoints represent mean ± SEM from 6 independent experiments. K_i values were calculated using the Cheng-Prussov equation.

Dapiglutide shows blunted β-arrestin-2 recruitment and internalization at GLP-1R

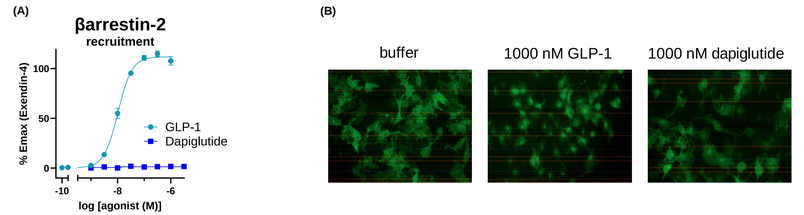


Figure 2: Dapiglutide does not recruit β-arrestin and induce less GLP-1R internalization in comparison to GLP-1. (A) Whereas GLP-1 potently recruits βArrestin-2 to the GLP-1R, the effect of dapiglutide is completely blunted. (B) Likewise, dapiglutide less efficiently internalizes GLP-1R tagged with a c-terminal YFP fluorescent protein at saturating 1000 nM concentrations compared to GLP-1, which shows clear formation of intracellular punctuae. Data in (A) represent mean ± SEM from 7 (GLP-1) or 3 (dapiglutide) independent experiments. Pictures in (B) are representative of 4 independent experiments.

Dapiglutide induces a prolonged cAMP response over time

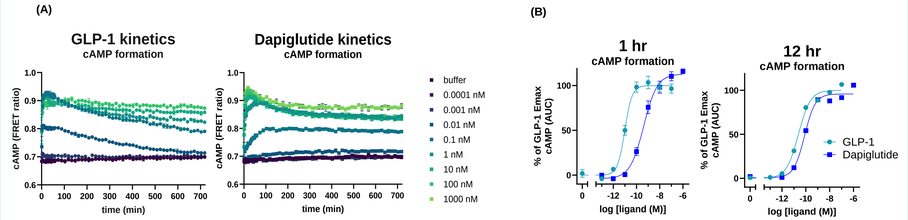


Figure 3: Dapiglutide shows a prolonged cAMP response over time to increase its potency towards that of GLP-1. (A) Monitoring intracellular cAMP levels over 12 hours show persistent increases upon dapiglutide stimulation whereas GLP-1 induced cAMP levels gradually decrease. (B) Consequently, the potency of dapiglutide increases relative to GLP-1 when comparing total cAMP formed at 1- and 12-hours following stimulation. Data in (A) are representative kinetic curves performed in triplicate of which AUCs are calculated as mean ± SEM from 3-6 independent experiments and plotted as a function of the agonist concentration.

Conclusions

- Dapiglutide is more potent for binding to the human GLP-1R compared to the human GLP-2R.
- Dapiglutide does not recruit β-arrestin to the GLP-1R and shows a prolonged cAMP response over time.
- The unique biased signaling profile at GLP-1R combined with additional GLP-2R activity may translate into an efficacious weight management therapy in individuals with obesity.