Identification of a novel quinoxaline-based small molecule dual glucagon-like peptide-1 receptor and glucagon receptor allosteric modulator

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Introduction Glucagon-like peptide-1 receptor (GLP-1R) and glucagon receptor (GCGR) are attractive treatment targets for Type 2 diabetes mellitus (T2DM)[(1)]. These two Class B G protein-coupled receptors can be activated by various endogenous ligands, such as glucagon, glucagon-like peptide-1 (GLP-1) and oxyntomodulin (OXM, a C-terminal extended form of glucagon); all of which possess insulinotropic actions[(2)]. Current GLP-1R-based treatments involve peptide drugs which are natural or modified version of GLP-1 and these are clinically efficacious[(3)]. However, these only come as injectables, as such suffer from poor patient compliance and higher production cost. Hence, oral GLP-1R treatment therapies have been prompted, leading to the search of small molecule GLP-1R agonists or positive allosteric modulators (PAMs).

Method BETP[(4)] and Compound 2[(5)], which are GLP-1R agonist-positive allosteric modulators, were used as chemical queries against 3 purchasable chemical libraries. A novel quinoxaline-based compound was identified and characterised as a GLP-1R PAM in cAMP bioassays. 9 analogues based on 80% structural similarity of this lead chemical scaffold were further explored. Allosteric activities on GLP-1R and GCGR endogenous ligands were characterised using homogenous TR-FRET LANCE® cAMP detection kit (PerkinElmer®) in Chinese Hamster Ovary-K1 (CHO) cells stably expressing GLP-1R (CHO-GLP-1R), CHO cells stably expressing GCGR (CHO-GCGR) and rat insulinoma (INS-1) gastric inhibitory polypeptide receptor (GIPR) knock-out cells.

Results Only analogue 249 with a modification on the quinoxaline-ring scaffold, enhanced OXM-mediated cAMP accumulation in GLP-1R in CHO-GLP-1R cells (pEC_{50} values increased from 8.10 ± 0.07 to 8.71 ± 0.09, $p<0.01$, $n\geq 3$, Fig. 1) and INS-1 GIPR knock-out cells (pEC_{50} values increased from 7.62 ± 0.08 to 8.11 ± 0.13, $p<0.001$, $n\geq3$, Fig. 2). It also potentiated OXM-mediated cAMP accumulation in GCGR in CHO-GCGR cells (pEC_{50} values increased from 9.19 ± 0.11 to 10.24 ± 0.09, $p<0.0001$, $n\geq3$, Fig. 3).

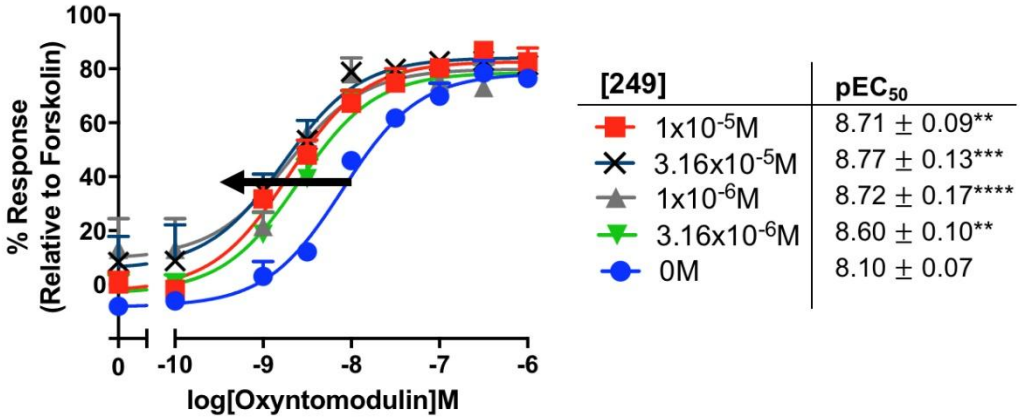

Fig. 1: Analogue 249 shows a concentration-dependent positive allosteric modulation on OXM-mediated cAMP accumulation in CHO-GLP-1R cells. Statistical significance compared with OXM (**, $p<0.01$; ***, $p<0.001$, ****, $p<0.0001$) was determined by one-way ANOVA with Dunnett's test, $n\geq3$.

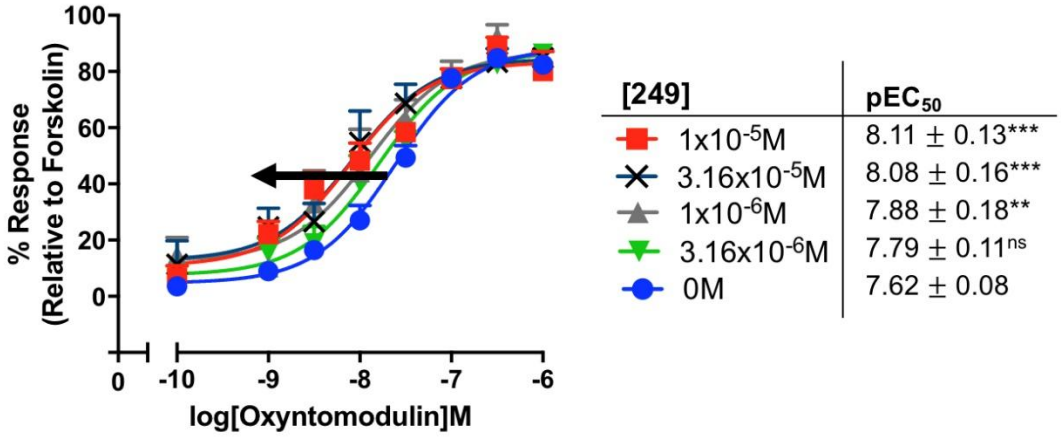

Fig. 2: Analogue 249 shows a concentration-dependent positive allosteric modulation on OXM-mediated cAMP accumulation in INS-1 GIPR knock-out cells. Statistical significance compared with OXM (**, p<0.01; ***, p<0.001; ns, non-statistically significant) was determined by one-way ANOVA with Dunnett's test, n≥3.

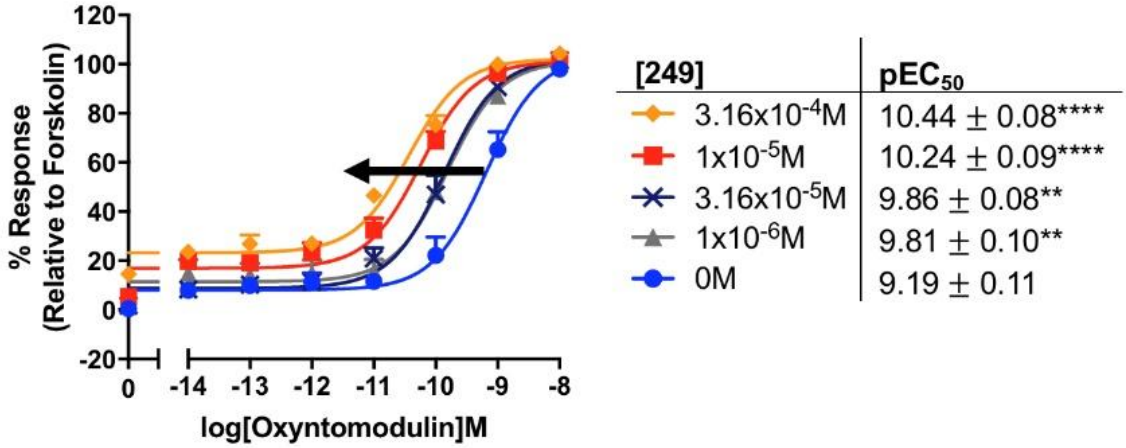

Fig. 3: Analogue 249 shows a concentration-dependent positive allosteric modulation on OXM-mediated cAMP accumulation in CHO-GCGR cells. Statistical significance compared with OXM (**, p<0.01; ****, p<0.0001) was determined by one-way ANOVA with Dunnett's test, n≥3.

Conclusions Here we show how a modification on the quinoxaline-ring scaffold affects allosteric modulation in GLP-1R and also in GCGR, an observation that is unseen in other quinoxaline-based GLP-1R PAMs. Hence, analogue 249 may serve as an experimental tool to examine allosteric modulation in these two receptors and a novel OXM-based T2DM treatment.

References

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