Allosteric optical control of a class B G protein-coupled receptor

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Abstract: *Allosteric regulation promises to open up new therapeutic avenues by increasing drug specificity at G protein-coupled receptors (GPCRs). However, drug discovery efforts are at present hampered by the inability to precisely control the allosteric site. Here, we describe the design, synthesis and testing of* ***PhotoETP****, a light-activated positive allosteric modulator of the glucagon-like peptide-1 receptor (GLP-1R), a class B GPCR involved in the maintenance of glucose homeostasis in humans.* ***PhotoETP*** *potentiates Ca^{2+}, cAMP and insulin responses to glucagon-like peptide-1 and its metabolites following illumination of cells with blue light. Thus,* ***PhotoETP*** *provides a blueprint for the production of small molecule class B GPCR allosteric photoswitches, and may represent a useful tool for understanding positive cooperativity at the GLP-1R.*

The incretin hormone glucagon-like peptide 1 (GLP-1) is released from enteroendocrine L-cells in the intestine,[1] from where it binds cognate receptors to promote pancreatic beta cell survival, insulin release and weight loss.[2] For these reasons, incretin-mimetics based on GLP-1 have become widely-prescribed drugs for the restoration of normal glucose levels during type 2 diabetes (T2D),[3] a socioeconomically costly syndrome affecting almost 400 million individuals worldwide.[4]

The glucagon-like peptide 1 receptor (GLP-1R) is a class B GPCR primarily coupled to adenylate cyclase activity and 3'-5'-cyclic adenosine monophosphate (cAMP) accumulation,[5] as well as intracellular Ca^{2+} fluxes.[6] Recently, an allosteric site has been described for this receptor, allowing the fine modulation of orthosteric ligand binding.[7] Thus, the ligand-dependent allosteric activator 4-(3-(benzyloxy)phenyl)-2-(ethylsulfinyl)-6-(trifluoromethyl)pyrimidine (BETP) potentiates Ca^{2+} mobilization in response to GLP-1(7-36)NH_2,[7-8] the active amidated form of GLP-1. By contrast, BETP amplifies cAMP generation in response to GLP-1(9-36)NH_2,[7-8] a metabolite and weak partial GLP-1R agonist. Such interactions are therapeutically-desirable, since drugs that target the GLP-1R allosteric site may improve receptor specificity, thereby reducing side effects.[7-9] However, their investigation is at present hindered by a lack of specific research tools for the fine control of allosterism and receptor movement. Photopharmacology is well-suited to this task, since it relies on the properties of light to precisely deliver drug activity in space and time.[10]

Herein, we describe the development and testing of **PhotoETP**, a light-activated positive allosteric modulator that allows optical control of GLP-1R signaling and insulin secretion using blue light (Figure 1A).

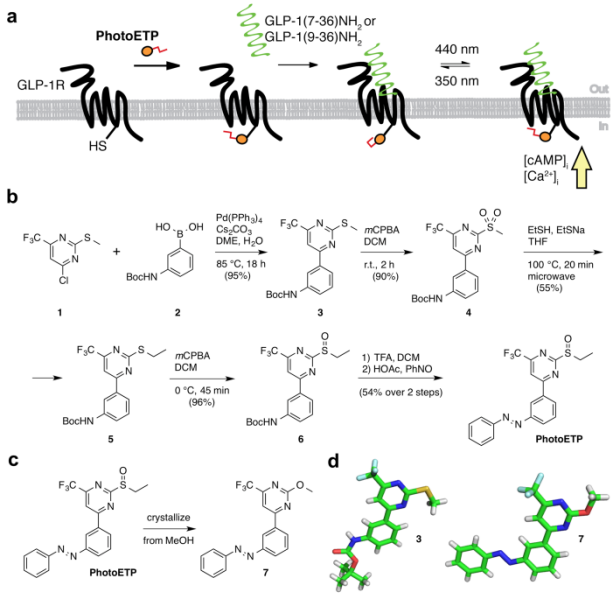


Figure 1. Logic and synthesis of **PhotoETP**. a) An azobenzene is installed on the positive allosteric modulator, BETP, to produce **PhotoETP**. This allows Ca^{2+} and cAMP responses to GLP-1 and its inactive metabolites to be reversibly potentiated following illumination with UV or blue light. b) Six-step synthetic pathway for production of **PhotoETP**. c) Crystallization of **PhotoETP** to its methoxy counterpart (**7**) using MeOH. d) Crystal structures for **PhotoETP** congener **7** (CDCC: 1420305) and its precursor bisaryl thioether **3** (CDCC: 1420306).

We set out to confer photoswitching on the GLP-1R allosteric site by subjecting BETP to our azologization strategy[11] (Figure 1B) (Figure S1). By coupling commercially available chloropyrimidine **1** and boronic acid **2** under Suzuki-Miyaura conditions, bisaryl thioether **3** was obtained in 95%. After oxidizing the sulfur atom with *m*CPBA to its sulfone counterpart **4** in 90%, it was exchanged in an aromatic substitution with β-mercaptoethanol to give ethyl thioether **5** in 55%. Subsequent oxidation with one equivalent of *m*CPBA gave access to sulfoxide **6** (96%), which was deprotected with TFA before undergoing Mills reaction with nitrosobenzene to produce

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PhotoETP in 54% over two steps. Attempts to crystallize **PhotoETP** from MeOH yielded compound **7**, providing further evidence for the electrophilicity of **PhotoETP** and its congeners (Figure 1C). Crystals suitable for x-ray crystallography were obtained for both **3** and **7** (Figure 1D).

The UV/Vis spectrum of **PhotoETP** under illumination with λ = 440 nm (*trans*, blue) and λ = 330 nm (*cis*, gray) demonstrated the presence of wavelength-dependent switching (Figure 2A), as expected for a *meta*-azobenzene system (Figure 2B). Photoswitching could be repeated over several cycles with reasonably fast kinetics (τ_{cis} = 204.2 ± 7.3 sec; τ_{trans} = 54.5 ± 2.9 sec) and without obvious fatigue (Figure 2C), and the *cis*- and *trans*-isomers were separated by LC-MS analysis (Figure 2D). Together, these features of **PhotoETP** provided a basis for the allosteric photocontrol of GLP-1R activity.

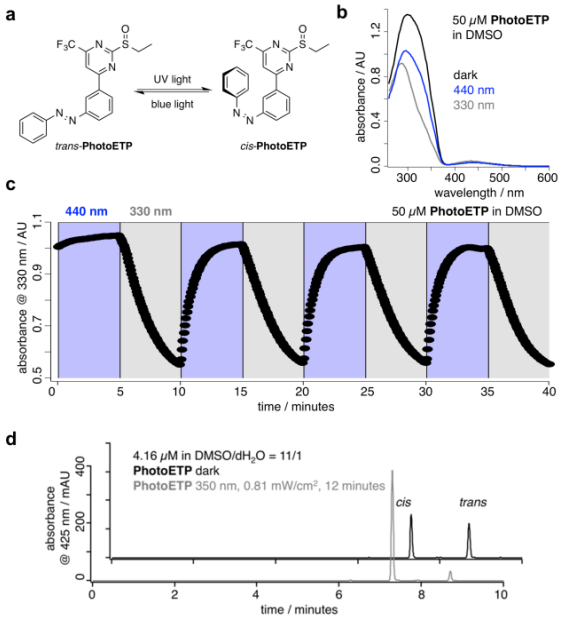


Figure 2. Characterization of **PhotoETP**. a) Isomerization of **PhotoETP** between its *trans*- and *cis*- states using blue light or UV, respectively. b) UV-Vis spectra of **PhotoETP** in DMSO following illumination with λ = 440 nm (blue), λ = 330 nm (grey) or under dark conditions (black). c) Robust photoswitching between *cis*- and *trans*-**PhotoETP** induced with λ = 440 nm and λ = 330 nm, respectively. d) LC-MS trace of **PhotoETP** in the dark (black) and after UV light (λ = 350 nm) exposure (gray).

We next examined whether **PhotoETP** was able to yield optical control over GLP-1(9-36)NH_2-induced cAMP generation. Similarly to BETP, *trans*-**PhotoETP** potentiated cAMP rises in response to GLP-1(9-36)NH_2 (EC_{50} (*trans*-**PhotoETP**) = 163.3 nM) (EC_{50} (BETP) = 99.5 nM). Importantly, this activity could be switched off using UV illumination to induce *cis*-isomer formation (Figure 3A) (unable to calculate EC_{50}). The extent of photoswitching is similar to that recently reported for an allosteric modulator of the metabotropic glutamate receptor mGluR5, a class C GPCR.[12] Effects of **PhotoETP** on cell viability were determined in islets using necrosis and apoptosis assays in the dark. At the concentration used throughout the present study, **PhotoETP** did not induce significant necrosis (Figure 3B) or apoptosis (Figure 3C), as measured using propidium iodide incorporation and terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL), respectively. By contrast, treatment with staurosporine or thapsigargin positive controls resulted in large increases in necrotic and apoptotic indices (Figure 3B,C). Furthermore, levels of cleaved caspase-3, an enzyme involved in the proteolytic cleavage of critical intracellular effectors including poly (ADP-ribose) polymerase, were unaffected during incubation with **PhotoETP** (Figure S2).

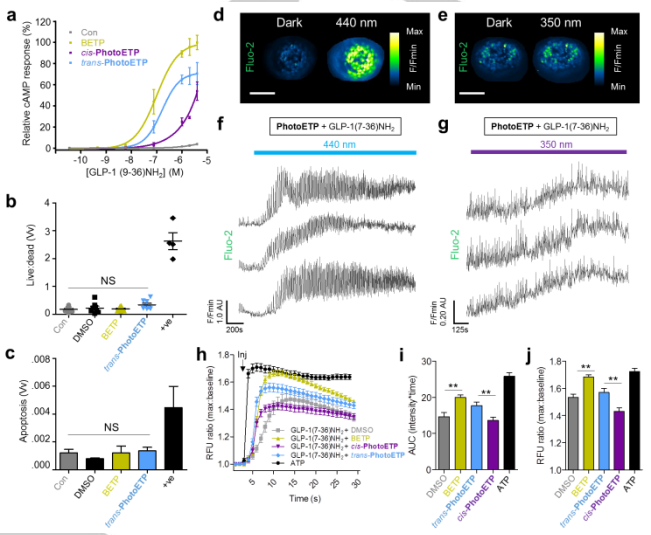


Figure 3. Optical control of cAMP and Ca^{2+} signals. a) Illumination with λ = 440 nm, but not λ = 350 nm (UV), optically modulates GLP-1(9-36)NH_2-induced cAMP generation in CHO-GLP-1R cells (*n* = 4 repeats). b) Incubation of MIN6 beta cells with **PhotoETP** for 3 hr does not alter cell viability (*n* = 8 repeats) (+ve = positive control; staurosporine or thapsigargin). c) As for b) but apoptosis (*n* = 3 repeats). d-g) Illumination with λ = 440 nm, but not λ = 350 nm, modulates GLP-1(7-36)NH_2-induced Ca^{2+} rises in intact **PhotoETP**-treated islets (*n* = 9 recordings from 3 animals). h-j) As for a-d, but in MIN6 beta cells (*n* = 8 repeats) (RFU = relative fluorescence units; AUC = area under the curve). In all cases, GLP-1(7-36)NH_2 was co-applied at 10 nM in the presence of 8-11 (islets) or 17 (MIN6) mM D-glucose. **PhotoETP** and BETP were applied at 50 μM. NS, non-significant or **P<0.01 versus *cis*-**PhotoETP** or DMSO; one-way ANOVA. Values represent mean ± s.e.m. Scale bar, 75 μm.

Next, photoswitching of intracellular Ca^{2+} dynamics was assessed using **PhotoETP** directly in beta cells residing within intact islets of Langerhans. Whereas *cis*-**PhotoETP** (λ = 350 nm) was largely without effect, the *trans*-isomer (λ = 440 nm) potentiated GLP-1(7-36)NH_2-induced Ca^{2+} rises (Figure 3D-G), as for cAMP. The latter could be abolished using either low glucose (Figure S3A,B) or the specific GLP-1R antagonist, exendin 9-39 (Figure S3C,D). In all cases, results in islets were replicated using MIN6 beta cells subjected to high-throughput Ca^{2+} screens (Figure 3H-J). Using batch-incubated islets, *trans*-**PhotoETP** potently amplified GLP-1(9-36)NH_2-induced insulin secretion (Figure 4A), whereas *cis*-**PhotoETP** was less effective as an allosteric modulator (Figure 4A). By contrast, BETP, *cis*-**PhotoETP** and *trans*-**PhotoETP** all augmented insulin secretion in response to GLP-1(7-36)NH_2 by almost 2-fold (Figure 4B), with no evidence of photocontrol.

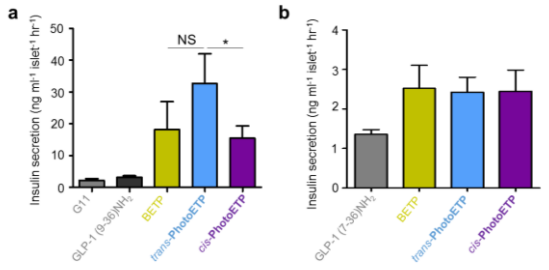


Figure 4. Optical control of insulin secretion. a) *trans*-**PhotoETP** is more effective than *cis*-**PhotoETP** at potentiating GLP-1(9-36)NH_2-induced insulin secretion. b) *trans*-**PhotoETP** and *cis*-**PhotoETP** similarly potentiate GLP-1(7-36)NH_2-induced insulin secretion. In all cases, GLP-1(7-36)NH_2 was co-applied at 10 nM in the presence of 11 mM D-glucose. **PhotoETP** and BETP were applied at 50 µM. NS, non-significant or *P<0.05 versus *cis*-**PhotoETP** or BETP; one-way ANOVA. Values represent mean ± s.e.m.

PhotoETP was almost 20% *cis*-enriched under benchtop conditions (^{1}H-NMR; see SI), whereas the UV-Vis spectra revealed a more pronounced π-π* band in the dark (see Figure 2B). This finding can further be explained by consulting a model of the glucagon receptor mutant F345C bound to BETP, where a twisted conformation of the benzylether is found, being the lowest energy state.[7] Such a conformation may also be adopted by *cis*-**PhotoETP** due to its higher affinity for covalent binding. However, in contrast to BETP, which can reorganize its molecular shape in response to orthosteric ligand binding, **PhotoETP** would remain trapped in its *cis*-state until illumination to induce *trans*-isomerization. Although the exact isomer ratio at the receptor is difficult to determine empirically, such properties may nonetheless afford fine control over photoswitching, with dark, 440 nm and 350 nm leading to graded Ca^{2+} responses (Figure S4). Further studies using rigid *E*- and *Z*-stilbene bioisosteres of **PhotoETP** will be required to better delineate the mechanisms involved.

The data presented here outline a straightforward synthetic strategy for the production of a blue light-activated positive allosteric modulator, which enables photocontrol of GLP-1R activity via a feedforward loop encompassing the orthosteric site (Figure 1A). Although similar 'alloswitches' have been described for ionotropic and metabotropic mGluRs,[12-13] this is the first demonstration of their use in a therapeutically-relevant class B GPCR. Using a combination of Ca^{2+}, cAMP and insulin assays in CHO-GLP-1R and MIN6 cells, as well as islets of Langerhans, we are able to show that **PhotoETP** allows photoswitching of responses to GLP-1(7-36)NH_2 and its less active breakdown product, GLP-1(9-36)NH_2, with similar potency to native BETP. Notably, **PhotoETP** displays unusual behavior in cells, where it possesses an enriched *cis*-content when interacting with its target in the dark. Indeed, the more active *trans*-isomer has to be photochemically induced by irradiation with blue light. Due to these properties, **PhotoETP**, together with the recently described signal-biased GLP-1R photoswitch **LirAzo**,[14] may allow the precise dissection of allosteric-orthosteric cooperativity, molecular movement and binding.

Both BETP and **PhotoETP** were more effective at potentiating GLP-1(9-36)NH_2- compared to GLP-1(7-36)NH_2-induced insulin secretion. This suggests that cAMP rather than Ca^{2+} is the primary driver of the 'incretin effect', and is consistent with previous results obtained using **LirAzo**.[14] Intriguingly, optical control of insulin release could only be observed in GLP-1(9-36)NH_2 and **PhotoETP**-treated islets, where blue light provoked a two-fold higher response than UV illumination. Although the exact reasons for this remain unknown, it may reflect the inability to detect relatively small isomer-induced differences in intracellular Ca^{2+} versus cAMP concentration at the level of secretion in islets.

BETP was susceptible to UV-A- but not white light-induced reactions, thus acting as a poor control for photoswitching purposes (see SI for a description of the results) (Figure S5-S8). In contrast, **PhotoETP** was remarkably robust. This protective effect stems from the azobenzene unit, which preferentially harvests UV-A photons with its π-π*-band to undergo isomerization. In other words, by installing an azobenzene moiety onto BETP, side reactions can be quenched and the resulting molecule stabilized. Nevertheless, the UV-A-induced rearrangement of BETP to its sulfenic ester counterpart *via* a Meisenheimer complex, and the accompanying transformation, is in itself an interesting finding (Fig. S5,S6). Although related rearrangements of sulfoxides have been reported,[15] sulfenic esters have not been isolated as products due to the low (UV-C) wavelengths used in these experiments. Such rearrangements are relevant for drug activity, as best exemplified by acid-activation of the irreversible proton pump inhibitor omeprazole (Prilosec).[16]

In summary, we showcase **PhotoETP**, a light-activated modulator for the allosteric optical control of GLP-1R function, and highlight the requirement to run parallel control experiments with benchmark drugs in photopharmacology. **PhotoETP**, or optimized derivatives thereof, may be useful in drug discovery programs aimed at unraveling the complexity of allosterism and class B GPCR signaling.

Experimental Section

Experimental details including synthesis, spectroscopic and spectrometric characterization, and biology can be found in the Supporting Information.

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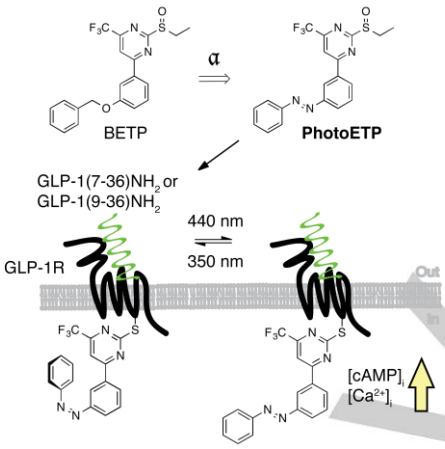
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Entry for the Table of Contents (Please choose one layout)

Layout 1:

COMMUNICATION

Blues and twos Using an ‘azologued’ version of BETP, the GLP-1 receptor allosteric site can be turned into a synthetic photoswitch, allowing levels of cAMP, Ca^{2+} and insulin to be optically controlled using blue light.



Johannes Broichhagen, Natalie R. Johnston, Helena Meyer-Berg, Yorrick von Ohlen, Ben J. Jones, Stephen R. Bloom, Guy A. Rutter, Dirk Trauner, David J. Hodson**

Page No. – Page No.

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