**GPCR Photopharmacology**

Maria Ricart-Ortega1,2, Joan Font2 and Amadeu Llebaria1\*

*1 MCS, Laboratory of Medicinal Chemistry, Institute for Advanced Chemistry of Catalonia (IQAC-CSIC), Barcelona, Spain*

*2 IGF, CNRS, INSERM, University de Montpellier, F-34094 Montpellier, France*

[*maria.ricart@iqac.csic.es*](mailto:maria.ricart@iqac.csic.es)*,* *joan.font-ingles@igf.cnrs.f,* [*amadeu.llebaria@iqac.csic.es*](mailto:amadeu.llebaria@iqac.csic.es)

**Abstract**

New technologies for spatial and temporal remote control of G protein-coupled receptors (GPCRs) are necessary to unravel the complexity of GPCR signaling in cells, tissues and living organisms. An effective approach, recently developed, consists on the design of light-operated ligands whereby light-dependent GPCR activity regulation can be achieved. In this context, the use of light provides an advantage as it combines safety, easy delivery, high resolution and it does not interfere with most cellular processes. In this review we summarize the most relevant successful achievements in GPCR photopharmacology. These recent findings constitute a significant advance in research studies on the molecular dynamics of receptor activation and their physiological roles in vivo. Moreover, these molecules hold potential toward clinical uses as light-operated drugs, which can overcome some of the problems of conventional pharmacology.

**Keywords**

G protein-coupled receptors, photopharmacology, caged compounds, photochromic ligands, photoswitchable tethered ligands, photoswitchable orthogonal remotely tethered ligands

1. **Introduction**

Biological function takes place in an stringent regulated manner on a cell and organism level. From a molecular perspective, biology involves an accurate concatenation of chemical events in a defined temporal sequence and with strict spatial confinement. Understanding and manipulating this dynamic and precise machinery is an enormous scientific challenge. To do this, new technologies are necessary to operate at the intersection of chemistry, biology, physics and engineering to finely study the biological systems in a real-time mode. These tools must be able to connect an input signal controllable by the researcher to induce a specific biological response to be measured. Achieving molecular tools for the external conditional control of biomolecular processes is crucial for studying the molecular mechanisms and deciphering the dynamic complexity of biological activity in living systems. It is becoming evident that the molecular tools must operate at the same level of spatiotemporal resolution that has the endogenous system to be fully effective.[1](#_ENREF_1) Accordingly, the use of light provides an ideal external control element to induce a conditional stimulation allowing the activation and deactivation of drugs, molecular probes and endogenous biomolecules in biological environments. Light combines safety, easy delivery and high spatial and temporal resolution not interfering to most cellular processes, enabling significant advances in research studies and holding potential toward clinical uses in light-operated drugs, which can overcome some of the problems of conventional pharmacology.[2](#_ENREF_2), [3](#_ENREF_3)

G protein-coupled receptors (GPCRs) are the largest, most important and best-validated class of pharmaceutical target proteins. GPCRs drugs, which target this protein superfamily, account for the majority of best-selling medicines on the market and nearly half of all prescription pharmaceuticals. Thus, constitute the core of modern medicine. However, fundamental scientific questions on GPCR are not understood, including the mechanism of activation and receptor dynamics at the homo and heteromerisation and membrane and intracellular signaling. Moreover, important therapeutic challenges remain unsolved, including: the control of the drug action site, the time course of drug effect, and the fine-tuning of effects on target tissue. An appealing possibility to address these issues is using molecular probes and drugs with light-dependent properties (i.e. affinity and/or efficacy) to externally direct native protein activity. Administration of a light-regulated molecule in combination with illumination that is patterned in space and time would provide a novel degree of control and regulation of receptor action, precisely identifying connexions between specific receptor activity and biological, physiological or behavioral responses. The use of light technologies in neuronal GPCR research are of current interest and have been recently reviewed.[4](#_ENREF_4)

Three related scientific domains entangle light-technologies to control biological function: Optogenetics, Optogenetics pharmacology (also known as tethered photopharmacology) and Photopharmacology. Optogenetics is a technique that allows the control of a particular subset of neurons by light thanks to the exogenous expression of genetically encoded photoreceptors that can trigger electrical cell signals upon illumination. The optical control of biological signaling achieved with this technique has been a major area of scientific activity during the last decade and has revolutionised experimental neurobiology and is causing a great impact on physiology.[5](#_ENREF_5), [6](#_ENREF_6) Pure optogenetic approaches do not use chemical operations and were originally introduced as a tool to control the excitability of neurons. During the last few years, this has been expanded to cover other cellular functions, such as motility, proliferation and gene expression. Therefore, optogenetics is based on the genetic expression of light-sensitive proteins and the binding of the photoisomerisable molecule all-*trans* retinal, which is available in vertebrate organisms.[7](#_ENREF_7) The enormous impact, technical advantages and versatility demonstrated by optogenetics in animal research have raised expectations of direct transfer to therapeutic uses in humans.

Photopharmacology (also called optopharmacology) consists in the development of light-regulated small molecules acting as receptor agonists, antagonists or modulators.[8-11](#_ENREF_8) It offers a powerful complement and alternative to optogenetics[12](#_ENREF_12" \o "Kramer, 2013 #92) having the advantage of being operative on endogenous receptors without genetic manipulation and has been successfully used in cells and in living organisms.[13](#_ENREF_13) In addition, the third approach known as tethered photopharmacology that combines genetics and covalent light operated ligands has been developed for specific studies.[14](#_ENREF_14)

Photopharmacology operates over defined protein receptors and permits a real-time link between the activity of a specifically located receptor and a defined biological or physiological response in cells or living organisms. Unlike optogenetics, photopharmacology involves small molecules that can be validated and approved using standard drug development procedures. Moreover, this approach constitutes a single component that can be applied directly to wild type organisms, including humans.

Therefore, the optical control of proteins has opened up many research possibilities that can be applied to therapeutic uses. These new avenues are most likely to be met by photopharmacology, considering its competitive advantages compared to optogenetics and conventional pharmacology. Hence, in this review we will summarize the current status of irreversible and reversible light operated ligands for the control of GPCR receptor activity.

1. **Irreversible strategies**

The photopharmacology assisted by photoisomerisable compounds constitutes a suitable technique to achieve spatiotemporal control of the biological activity in an irreversible manner. The cage strategy was developed at the end of the 70’s involving the caged-ligands. These molecules contain a photoreactive moiety covalently attached to an active compound in a way that renders the ligand inert to its target receptor.

**Table 1.** Caged ligands for GPCRs.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Compound number** | **GPCR class** | **GPCR target** | **Compound name** | **Compound structure** | **Compound activity** |
| **1** | A | α1 | Caged-phenylephrine |  | Agonist |
| **2** | A | Adrenoceptor | Caged-epinephrine |  | Agonist |
| **3** | A | β1-2 | Caged-isoproterenol |  | Agonist |
| **4** | A | µ | Caged-(Leu)5-enkephalin |  | Agonist |
| **5** | A | µ | Caged-Dynorphin A |  | Agonist |
| **6** | A | µ | Caged-Naloxone |  | Antagonist |
| **7** | A | A2A | MRS7145 |  | Antagonist |

**Table 1.** Caged ligands for GPCRs (continued).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Compound number** | **GPCR class** | **GPCR target** | **Compound name** | **Compound structure** | **Compound activity** |
| **8** | C | mGlu | Caged-Glutamate |  | Agonist |
| **9** | C | mGlu | Caged-GABA |  | Agonist |
| **10** | C | mGlu5 | JF-NP-026 |  | NAM |

These ligands release the biologically active ligand irreversibly under suitable light exposure, allowing its fast and controlled localised delivery. Therefore, caged compounds are based on molecules whose biological activity is controlled by light through a photolytic conversion from an inactive to active state (*Figure 1*). A widely used approach on the design of successful caged compounds consists on the covalent attachment of a chemical substructure to a point of the active ligand which is relevant for its receptor recognition and biological activity. Therefore, the photochemical cleavage of the covalently attached substructure releases the active species.



**Figure 1.** An schematic representation of the uncaging strategy. The caged compound is inactive in dark condition due to the incorporation of a photolabile group which blocks the interactions between the molecule and the protein. Light illumination at suitable wavelength releases irreversibly the active specie and by-products coming from the photolabile group.

The caging technique uses light illumination to generate active compounds controlled in time, location and amplitude. Thereby, abrupt or localised changes in the concentration of active species can be induced with controlled wavelengths. Avoiding at the same time all undesired effects caused by the active ligand in other localisations. However, there is a major limitation on this approach considering that this photorelease does not enable a reversible control over the drug’s activity. Even though, photolysis of caged compounds is one of the most accessible and robust techniques to examine the fast kinetics or spatial heterogeneity of biochemical responses in such systems.[15](#_ENREF_15" \o "Adams, 1993 #73)

In order to be useful in biological experiments, the cage moiety must satisfy (at least partially) several criteria: It should render inert biomolecules to the biological system used, release the active molecule in high yield at sufficient speed by photolysis at light wavelengths that are non-hazardous to the biological process and any photoproduct other than the desired compound should not interact or interfere with this biological system.[16](#_ENREF_16" \o "Falvey, 2005 #78)

The first example of caged compounds application in biological systems, was reported by Hoffman and collaborators describing rapid releasing of ATP from a protected analogue (*Figure 2A*).[17](#_ENREF_17" \o "Kaplan, 1978 #76) These cutting-edge experiments constituted the first evidence of caged compounds activated with light in living cells.[18](#_ENREF_18" \o "Ellis-Davies, 2007 #66) Another clear example was developed to test in *Limulus* ventral photoreceptors for uncoupling phototransduction blocking initial cascade reactions such as rhodopsin excitation or G protein activation.[19](#_ENREF_19" \o "Faddis, 1992 #117)

Since then, a variety of caged compounds have been improved and synthesised, even macromolecules such as peptides and proteins (*Figure 2B*). G-actin was the first protein to be covalently caged by modifying an specific amino acid residue. Posteriorly, the same approach has been used to cage many other proteins.[20-22](#_ENREF_20" \o "Marriott, 1994 #75)

Remarkably, this methodology not only intended to cage organic compounds, but also inorganic cations such as calcium (*Figure 2C*). Photolabile derivatives of high-affinity calcium chelators have been developed, decreasing the affinity for calcium upon illumination, thus uncaging some of the bound calcium.[23](#_ENREF_23" \o "Ellis-Davies, 2003 #68)

Moreover, the caging technique has also been applied to the development of different photoactivatable neurotransmitters acting in different classes of GPCRs such as class C including glutamate (*Figure 2D*) and GABA or class A in adrenergic receptors.[24](#_ENREF_24" \o "Muralidharan, 1995 #51)



**Figure 2.** Structures and photochemistry of different caged compounds. (**A**) Photolysis of caged-ATP with nitrobenzylphotolabile group. (**B**) Photochemical cleavage of a peptide/protein containing 2-nitroglycine. (**C**) NP-EGTA cage Ca2+ by high affinity binding, photolysis breaks calcium coordination sphere in two, yielding low-affinity fragments that release the bound calcium. (**D**) Photolysis of neurotransmitter glutamate using indolinephotolabile groups.

Thereby, there are several caged ligands targeting **class A GPCRs**. Muralidharan and Nerbonne[24](#_ENREF_24" \o "Muralidharan, 1995 #51), synthesised caged derivatives of **adrenergic receptor** **agonists** such as **phenylephrine** (Table 1, compound 1), **epinephrine** (Table 1, compound 2) or **isoproterenol** (Table 1, compound 3)(*Figure 3*). The study reveals different kinetics of photolysis attaching the photolabile group at two different parts of the agonist’s scaffold. Moreover, they attempt to define the structural-activity relationship concerning the residual activity from the inactive caged compounds.

Further studies using caged compounds were applied on **µ-opioid receptors**. Sabatini and Banghart used two different strategies to overcome the lack of suitable reagents for spatiotemporal delivery of neuropeptide opioids. In the first approach, they developed photoactivable analogues of two opioid peptides: **(Leu5)-enkephain** (Table 1, compound 4) and the 8 amino acid form of **Dynorphin A** (Table 1, compound 5). These peptides are functionally inactive prior to photolysis, upon UV-light a photorelease of (Leu5)-enkephalin is triggered and this peptide was shown to activate µ-opioid receptor.[25](#_ENREF_25" \o "Banghart, 2012 #104) In a more recent follow-up paper, the same authors described novel caging approaches for (Leu5)-enkephalin (Table 1, compound 4). They showed different caging sites in the peptide scaffold that reduce the residual activity prior to photolysis. Thus, they reached a more effective photocontrol of the GPCR activity.[26](#_ENREF_26" \o "Banghart, 2018 #105)

One year after, the spatiotemporal dynamics of opioid signalling in the brain still remain poorly defined. Therefore, they generate a competitive antagonist caged derivative from Naloxone to reveal deactivation kinetics of opioid signalling. Photolysis of **caged-Naloxone** (Table 1, compound 6)in slices of rat locus coeruleus promoted a rapid inhibition of ionic currents evoked by multiple agonists of µ-opioid receptor. On this study, different agonists were tested, concluding that some MOR agonist yielded deactivation rates that are limited by G-protein signalling, whereas others appeared limited by agonist dissociation. Therefore, the choice of agonist determines which features of receptor signalling is unmasked by caged-Naloxone photolysis.[27](#_ENREF_27" \o "Banghart, 2013 #77)



|  |  |  |
| --- | --- | --- |
| **Compound** | **R1** | **R2** |
| **3a**  **3b**  **3c**  **3d** | OMe  OMe  OCH2(CH3)2  H | H  OMe  H  CF3 |

**Figure 3.** (**A**) Schematic uncaging of two different caged-phenylephrine at 355 nm. Muralidharan and Nerbonne demonstrated the rapid photolysis for caged-phenylephrine directly attached to the amine group (**1**) in comparison with the phenol one (**2**). (**B**) Caged-phenylephrine **1** was already tested resulting in the loss of agonist activity. Therefore, the same caging strategy was improved with isoproterenol (**3**). Unfortunately, this compound showed residual β-adrenergic receptor activity. To determine whether structural variations in the photolabile group could remove the residual β-agonist activity of **3**, substituents onto the 2-nitrobenzyl group had been introduced, generating caged compounds from **3a** to **3d**. Moreover, a different approach was tried attaching the photolabile group to one of the phenolic OH group (**4**). Despite the synthetic efforts, the physiological studies revealed that all of these compounds still retained undesired β-receptor activity.

Recently, Ciruela and collaborators showed a local targeting of **adenosine A2A receptor** to treat movement disorders.[28](#_ENREF_28) In this study, was developed a new light-sensitive caged adenosine antagonist **MRS7145** (Table 1, compound 7) by blocking with a photoactive coumarin the 5-amino group of the selective antagonist SCH442416.[29](#_ENREF_29) Photoactivation at 405 nm of MRS7145 induced the local release of the active antagonist, resulting in A2AR ligand binding. MRS7145 was able to preclude A2AR-mediated cAMP accumulation in a light and dose-dependent manner. They also assessed the efficacy of this compound released by light in animal behaviour experiments. Light was delivered using a dual fiber-optic cannula, observing an increase of spontaneous locomotor activity after administration of SCH442416. Whereas MRS7145 alters significantly spontaneous locomotion only upon illumination at 405 nm. In addition, two animal models that mimicked two Parkinson disease symptoms, namely rigidity and tremor, were tested. Similarly to previous results, SCH442416 showed a light-independent anticataleptic and antitremulous activity. In contrast, MRS7145 was only able to reverse catalepsy and tremulous activity upon illumination at 405 nm.

**Class C GPCRs**, or glutamate family of GPCRs, were also targeted by caged compounds. Therefore, photoactivation of **caged glutamate** (Table 1, compound 8) which is a widely used caged neurotransmitter in physiology. Many caged glutamate derivatives have been published using different caging strategies.[18](#_ENREF_18" \o "Ellis-Davies, 2007 #66) It was greatly improved in spatial and temporal resolution of synaptic connectivity mapping in neuronal networks and had been used also to study the function of mGluRs.[30](#_ENREF_30" \o "Callaway, 1993 #62)

The use of caged-glutamate opened new possibilities to adapt this approach with other neurotransmitters such as aspartate, glycine and GABA.[31](#_ENREF_31" \o "Wilcox, 1990 #60), [32](#_ENREF_32" \o "Wieboldt, 1994 #74) Yuste and co-workers described a selective photorelease of γ-aminobutyric acid (GABA) using a novel **caged-GABA** (Table 1, compound 9). It uses ruthenium inorganic complexes as the photolabile group which could be excited at visible ranges. Therefore, it provides better penetration, less photo-toxicity and faster photolytic kinetics compared to those attached to UV-light sensitive moieties.[33](#_ENREF_33" \o "Rial Verde, 2008 #64) Unfortunately, the use of these caged-glutamate or caged-GABA is somehow limited due to the lack of subtype selectivity. This led to the development of caging selective compounds.

Recently, a novel caged compound selective for **mGlu5** **receptor** was described by Llebaria and Ciruela groups. They synthesised the photoactive compound **JF-NP-026** (Table 1, compound 10) based on the mGlu5 negative allosteric modulator (NAM) Raseglurant. Thereby, they obtained the caged compound through the chemical binding of the NAM to a DEACM coumarin photoactive moiety. Consequently, they verified the photochemical cleavage upon violet illumination controlling the mGlu5 receptor activity both in cultured cells and in primary neurons. Additionally, the *in vivo* analgesic effect of JF-NP-026 was established in neuropathic and acute inflammatory pain murine models. Therefore, JF-NP-026 was unable to promote antinociception without illumination whereas peripheral illumination at hind paw or brain illumination at thalamus induced analgesia. Remarkably, illumination at other locations resulted ineffective on analgesia indicating the specific location of mGlu5 receptors in pain transmission.[34](#_ENREF_34" \o "Keywood, 2009 #65), [35](#_ENREF_35" \o "Font, 2017 #67)

1. **Reversible strategies**

Controlling the activity of biomolecules in a reversible manner using light is an enthralling challenge that enables protein regulation with high spatial and temporal resolution. This strategy results in compounds with localised therapeutic effects and precise dosing patterns minimizing potential side effects. Thereby, a variety of synthetic photoswitches have been designed to allow reversible modifications to their structure upon light illumination (*Figure 4*). Photoswitches can be classified in two distinct groups, depending on their isomerisation mechanism. Those that, upon light illumination, are interconverted between *cis* and *trans* isomers (azobenzenes, stilbenes and hemithioindigos) and those that are photoconverted between open and closed forms (spiropyrans, diarylethenes and fulgides). However, photocontrolled biomolecules have to accomplish specific requirements in order to be considered pharmacologically promising. Both chemical structures have to be stable and non-toxic before and after illumination on aqueous solution. Moreover, the wavelength of isomerisation needs to be compatible with biological systems. Finally, the trigger event must affect the pharmacological ligand activity on the target receptor.[36](#_ENREF_36" \o "Beharry, 2011 #12)

Azobenzenes constitute a widely used light-sensitive moiety in the generation of photo-controllable biomolecules as they satisfy most of the criteria mentioned previously. An azobenzene contains a diazene (HN=NH) derivative where both hydrogens are replaced by phenyl groups. Under dark conditions, the photoisomerisable group exists in most of the cases in a thermally stable *trans* configuration, which under illumination at the appropriate wavelength reversibly isomerises to the *cis* state. Reversible conversion to the thermodynamic isomer can take place either by thermal relaxation with variable kinetics or by illumination with light in a very fast process. Several studies indicate *trans-*azobenzenes take up an extended planar conformation. On the contrary, *cis*-azobenzenes adopt a bent disposition with the two phenyl rings twisted ~ 55° out of the plane from the azo group. As a consequence, the *cis* isomer is shorter than the *trans* one by ~ 3.5 Å.[36-38](#_ENREF_36)

The absorption spectra of an unsubstituted *trans*-azobenzene presents two main bands, a strong π-π\* near 320-350 nm and a very weak n-π\* transition between 400-450 nm. On the other hand, the unsubstituted *cis*-isomer shows an increase on the n-π\* band and shorter wavelength bands at 280 nm and 250 nm. Therefore, the absorption spectra of the two isomers present noticeable differences but at the same time overlap to a certain degree. Substituents on the phenyl rings also enable clear modulation of the absorption spectra of the isomers. Consequently, the best isomeric ratio will be reached as less superposition exist between the *trans* and *cis* absorption spectra. Nevertheless the percentages are approximately 80% *cis* or 100% *trans*, under white light or dark condition, respectively. Additionally, thermal relaxation produces 100% of thermodynamic isomer.[36](#_ENREF_36), [37](#_ENREF_37), [39](#_ENREF_39)



**Figure 4.** Synthetic photoswitches: (**A**) azobenzenes, (**B**) stilbenes, (**C**) hemithioindigos, (**D**) spiropyrans, (**E**) diarylethenes and (**F**) fulgides. The *trans* isomer or open form is drawn on the left and the *cis* isomer or closed form on the right.

Thermal isomerisation rates depend on the chemical and electronic structure of the azobenzene and its environment. Therefore, these properties have an important effect on the half-life of the *cis* isomer, which can range from nanoseconds to several days, weeks or months. Moreover, the substituents on the phenyl rings also have an effect on the isomerisation rates. Thus, for a specific azobenzene under defined illumination conditions (intensity, wavelength) and environment (solvent, concentration, pH, presence of interacting molecules, etc), the so-called photostationary state can be reached. Azobenzenes in this state are found in a kinetic equilibrium resulting from equal rates of interconversion between *cis* and *trans* isomers, where isomer ratio remains constant in time. Selecting suitable wavelengths, a high ratio of each isomer in the photostationary state can be achieved.[40-43](#_ENREF_40" \o "Weston, 2014 #26)

The design of photoregulated ligands for proteins necessarily involves an affinity structural element that is recognised selectively by the target protein. A photoactive group, usually an azobenzene, is also needed that can be reversibly photoisomerised from *trans* to *cis* configurations by using suitable wavelengths. In this way, a photoregulation of the protein activity can be achieved by switching the azobond (N=N) configuration with light illumination. When the resulting isomers bind to the protein with different affinities or result in different efficacies, it is possible to regulate the protein biological activity with light. These ligands can be administered to cells, tissues or organisms inducing a real-time control of the protein activity with spatiotemporal precision.



**Figure 5.** Common strategies on photopharmacology (**A** and **B**) and optogenetic pharmacology (**C** and **D**). (**A**) photochromic ligands (PCLs), (**B**) photoswitchable affinity labels (PALs), (**C**) photoswitchable tethered ligands (PTLs) and (**D**) photoswitchable orthogonal remotely tethered ligands (PORTLs).

To sum up, this photoinduced isomerisation leads to a remarkable change in the azobenzene’s physical, chemical and pharmacological properties that can be translated to a precise spatiotemporal control of biological activity. These photoswitchable molecules can be classified depending on their interaction with the target protein which can be endogenous or genetically engineered. Diffusible ligands are known as photochromic ligands (PCLs) and target wild type proteins (*Figure 5A*). On the other hand, ligands tethered covalently to the target are called tethered ligands (TLs) and they can bind to either natural (*Figure 5B*) or modified proteins (*Figures 5C-D*). Tethered ligands, in turn, can be divided on: photoswitchable tethered ligands (PTLs), bioorthogonal ligands tethering (BOLTs), photoswitchable orthogonal remotely tethered ligands (PORTLs), unfused photoswitchable orthogonal remotely tethered ligands (uPORTLs), photoswitchable affinity labels (PALs) and antibody-photoswitch conjugates (APCs).[13](#_ENREF_13" \o "Hüll, 2018 #4), [14](#_ENREF_14" \o "Leippe, 2017 #91), [44](#_ENREF_44" \o "Goudet, 2018 #15) However for GPCRs only PCLs, PTLs and PORTLs have been developed.

Therefore, we classify the reversible strategies on two main groups: photochromic ligands (PCLs) and tethered ligands (TLs).

* 1. **Photochromic ligands (PCLs)**

Photochromic ligands are freely diffusible ligands linked to a light-sensitive moiety, commonly azobenzenes as we have previously described. Therefore, illumination with a suitable wavelength reversibly change the structure and physicochemical properties of the molecule. This reversible changes can lead to significant differences on the pharmacological properties of the two distinct states. Thereby, the ligand structural modification induced by light can have a noticeable effect on the affinity and potency or efficacy of the molecule for its native target.[44](#_ENREF_44" \o "Goudet, 2018 #15)

Up to date, the development of photopharmacology (also called optopharmacology) has substantially evolved and it has a high potential to decipher fundamental biology aspects. In the last few years, photoswitchable molecules have been developed based in peptides, lipids, carbohydrates, as well as drugs and small molecule ligands to control cell activity of enzymes transporters and pumps, ion channels, nucleic acids and cytoskeleton machinery.[36-38](#_ENREF_36" \o "Beharry, 2011 #12) We refer the reader to other reviews on photochromic ligands for an overview in different types of light-regulated molecules. Herein, we will review the most relevant PCLs designed to photocontrol the superfamily of cell surface receptors named G-Protein Coupled Receptors (GPCRs).

There are several diffusible photoswitchable ligands targeting **class A GPCRs**. The **muscarinic acetylcholine** (mACh or M) **receptors** were the first GPCRs from the rhodopsin family that were successfully controlled by light. At the beginning of the 80’s, a pioneering work of Erlanger and collaborators employed the photoisomerisable compound 3,3’-*bis*-[α-(trimethylammonium)methyl] azobenzene (**Bis-Q**; Table 2, compound 11) to study the response to muscarinic agents in frog myocardium.[45](#_ENREF_45) Previously, Bis-Q had been tested in nicotinic acetylcholine receptors acting as agonist in the *trans* configuration.[46](#_ENREF_46), [47](#_ENREF_47) Thereby, using radioligand binding assays they found that Bis-Q inhibits the binding of the antagonist [3H]N-methylscopolamine ([3H]NMS) to muscarinic receptors on frog ventricle homogenates. The calculated dissociation constant (Kd) for *cis*-Bis-Q was 15-20 μM, whereas the *trans* isomer showed a Kd value threefold higher. The close fit of the competition binding curves to simple mass-action isotherms suggested that *cis* and *trans* molecules interact competitively with [3H]NMS. Moreover, electrophysiological studies confirmed that *trans*-Bis-Q inhibited the carbachol-induced currents better than the *cis* isomer. Therefore, in this study they demonstrated that Bis-Q isomers are bound to muscarinic receptors and that its action is well described as competitive antagonism. They observed small changes in the pharmacological properties of both isomers and that was attributed to the obtention of isomeric mixtures upon light illumination rather than toward the pure *cis* or *trans* isomers.[45](#_ENREF_45" \o "Nargeot, 1982 #23)

Recently Decker, Hoffmann, Holzgrabe and colleagues took a step forward, synthetizing, characterizing and testing *in vitro* a photoswitchable dual steric ligand targeting the type 1 muscarinic acetylcholine receptor. They designed the bitopic ligand termed **BQCAAI** (Table 2, compound 12) by connecting the agonist iperoxo to the positive allosteric modulator BQCA through an azobenzene linker. The most relevant feature of this ligand is the opposite pharmacological behaviour observed for the two isomers. *Cis*-BQCAAI acts as an antagonist under 366 nm while *trans*-BQCAAI is an agonist under dark condition or 455 nm.[48](#_ENREF_48" \o "Agnetta, 2017 #13)

Previously, Ciruela, Gorostiza and Jacobson designed a efficacy photoswitch adenosine-based compound, a visible light-switchable ligand. They concluded that both **MRS5543** (Table 2, compound 13) isomers showed agonist activity against subtype 3 adenosine receptor. Whereas, the *cis*-MRS5543 acted as an antagonist and the *trans* functioned as a partial agonist on HEK293T cells expressing the **adenosine A2A receptor**. These results were further confirmed by molecular modelling studies.They described a slightly different interaction of both isomers of MRS5543 with A3R and A2R which can be related to structural differences in the EL3 region of these receptors.[49](#_ENREF_49" \o "Bahamonde, 2014 #36)

A different member of class A GPCRs, the **μ-opioid receptors** (MOR), have also been controlled by light through a photoswitchable agonist. A fentanyl derivative called Photofentanyl-2 (**PF2**; Table 2, compound 14) was synthesised, which showed to be thermally stable in aqueous solution under both dark and light conditions. The μ-opioid receptors were activated in presence of *trans*-PF2 which predominates in the dark or under illumination with 420-480 nm light. In contrast, MOR activation is abrogated by switching back 360 nm light given that the *trans*-PF2 isomer is interconverted to the less-active *cis*-PF2 isomer.[50](#_ENREF_50" \o "Schönberger, 2014 #6)

Focusing on the **dopamine receptors** (DRs), Isacoff and Trauner designed the first *cis-*active compounds using an azobenzene derivative of the well-known DR ligand PPHT. In a cAMP accumulation assay, the *cis*-AP was 1.5- and 3-fold more potent than its *trans* isomer at D1R and D2R, respectively. Similarly, in a D2R-mediated arrestin recruitment assay, *cis*-AP was 4-fold more potent than *trans*-AP exposed to 460 nm light or dark condition. Moreover, they evaluated **AP** (Table 2, compound 15) binding to D2 receptor in an HTRF binding assay finding that *cis*-AP displayed 3-fold higher affinity than *trans* isomer. Thus, the *cis*-AP is an agonist which shows higher affinity towards the dopamine receptors than the *trans*-AP.[51](#_ENREF_51" \o "Donthamsetti, 2017 #95)

On the other hand, König and collaborators succeeded in synthesizing dithienylethenes and fulgide based photochromic ligands targeting the dopamine receptors.

**Table 2.** Photochromic ligands for GPCRs.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Compound number** | **GPCR class** | **GPCR target** | **Compound name** | **Compound structure** | **Active isomer** | **Compound activity** |
| **11** | A | M1-5 | BisQ |  | *trans* | Antagonist |
| **12** | A | M1 | BQCAAI |  | *trans*  *cis* | Agonist  Antagonist |
| **13** | A | A2A | MRS5543 |  | *trans*  *cis* | Partial agonist  Antagonist |
| **14** | A | µ | PF2 |  | *trans* | Agonist |
| **15** | A | D1-2 | AP |  | *cis* | Agonist |
| **16** | A | D2 | Compound 29 |  | open | Agonist |
| **17** | A | D2 | Compound 52 |  | closed | Agonist |
| **18** | A | FFA1 | FAAzo-10 |  | *trans* | Agonist |

**Table 2.** Photochromic ligands for GPCRs (continued).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Compound number** | **GPCR class** | **GPCR target** | **Compound name** | **Compound structure** | **Active isomer** | **Compound activity** |
| **19** | A | CB1 | Azo-THC-3 |  | *cis* | Partial  agonist |
| **20** | A | CB1 | Azo-THC-4 |  | *trans* | Partial  agonist |
| **21** | A | H3 | VUF14738 |  | *cis* | Agonist |
| **22** | A | H3 | VUF14862 |  | *trans* | Antagonist |
| **23** | A | CXCR3 | VUF16216 |  | *trans*  *cis* | Antagonist  Agonist |
| **24** | B | GLP-1R | LirAzo |  | *trans*  *cis* | Agonist  Agonist |
| **25** | B | GLP-1R | PhotoETP |  | *trans* | PAM |
| **26** | C | mGlu5 | Alloswitch-1 |  | *trans* | NAM |

**Table 2.** Photochromic ligands for GPCRs (continued).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Compound number** | **GPCR class** | **GPCR target** | **Compound name** | **Compound structure** | **Active isomer** | **Compound activity** |
| **27** | C | mGlu4 | OptoGluNAM4.1 |  | *trans* | NAM |
| **28** | C | mGlu4 | OptoGluram |  | *trans* | PAM |

They evaluated the pharmacological activity of the cyclopentene-DTE **29** (Table 2, compound 16) and the fulgimide-based pair **52** (Table 2, compound 17) through inositol phosphate (IP) accumulation assays and β-arrestin recruitment assays in transiently co-transfected HEK293T cells. At nanomolar range, the 29-open was more potent than 29-closed, isomerising under 312 nm and 410 nm respectively. Contrarily, the fulgimide-based pair52exhibited inverse activation properties. The closed state under visible light presented 4-fold higher activity.[52](#_ENREF_52" \o "Lachmann, 2017 #29)

Additionally, modulation of the **free fatty acid receptor 1** (FFA1), also known as GPR40, has been achieved through photoswitchable fatty acids. Trauner, Schultz and Hodson research groups designed **FAAzo-10** (Table 2, compound 18), a light-sensitive derivative of Gw-9508. Differently to previous studies, *trans-*FAAzo-10 acts as a potent agonist against GPR40 expressed in HeLa cells under blue light or in the dark. After 375 nm illumination, *cis* isomer concentration increases leading to a decrease in [Ca2+]i.[53](#_ENREF_53" \o "Frank, 2017 #38)

Regarding the **cannabinoid receptor 1** (CB1), Frank, Carreira, Trauner and co-workers designed four photoswitchable Δ9-tetrahydrocannabinol derivatives (*azo*-THCs) based on the synthesis of 3-Br-THC compound. *Azo*-THC-1 and *azo*-THC-2 were inactive while *azo*-THC-3 and *azo*-THC-4 emerged as photoswitchable partial agonist for CB1. They demonstrated by whole-cell electrophysiology and FRET-based cAMP assay that *cis*-**azo-THC-3** (Table 2, compound 19) and *trans*-**azo-THC-4** (Table 2, compound 20) presented higher agonist efficacy than its *trans* or *cis* isomer, respectively. In addition, docking of the *azo*-THC compounds corroborated that the binding of *cis*-azo-THC-3 and *trans*-azo-THC-4 to the CB1 target presented highly favourable scores.[54](#_ENREF_54" \o "Westphal, 2017 #8)

Recently, Leurs and Wijtmans have established a structure-activity relationship (SAR) synthesizing 16 photoswitchable antagonists targeting the **histamine 3 receptor** (H3R). At first, the compounds were photochemically characterised. Thereafter, they performed radiolabeling competition binding experiments acquiring a series of moderate to highly potent H3R ligands. Considering these results, the *meta*-substituted **VUF14738** (Table 2, compound 21) and the *para-*substituted **VUF14862** (Table 2, compound 22) were selected as key compounds. *In vitro* and *in vivo* studies showed significant differences on the pharmacological properties of VUF14738 before and after violet light illumination, with the compound being more active on the *cis* state. On the contrary, VUF14862 acted as *trans*-active antagonist. The opposite pharmacological effects of the two compounds were further explained through molecular modelling studies.[55](#_ENREF_55" \o "Hauwert, 2018 #39)

Finally, a recent publication related to class A GPCRs reports the optical control of **chemokine receptor CXCR3** by small photoswitchable molecules. A series of azobenzene-containing ligands was synthetised building on the scaffold of the biaryl CXCR3 ligands. The *ortho*-Br atom of the azobenzene moiety was maintained while Y were tailored (*Figure 6*). Radiolabeling binding assays were used to confirm that both isomers bound to CXCR3. *Trans* analogues demonstrated similar submicromolar affinity values, whereas, the *cis* analogues showed affinity values increasing with augmented size and electron-donating properties of substituent Y. Subsequently, they corroborated that the *trans* compounds behaved as antagonists or very weak partial agonists through [35S]GTPγS functional assay. On the other hand, upon 360 nm illumination, *cis* analogues acted as agonists with variable efficacies (α). Electrophysiology experiments verified that **VUF16216** (Table 2, compound 23) gives a real-time efficacy trigger from antagonism to agonism

(*Figure 6,* ***2e***).[56](#_ENREF_56)



**Figure 6.** Structure of CXCR3 efficacy photowitchable ligands. The efficacy (α) of the ligands **2a-e** depends on the nature of the Y substituents. Adapted from Gómez-Santacana et al.[56](#_ENREF_56)

**Class B GPCRs**, or secretin family GPCRs, are activated by peptide ligands such as hormones, neuropeptides and peptide autocrine factors.[57](#_ENREF_57) Of the existing fifteen receptors, only the **glucagon-like peptide-1 receptor** (GLP-1R) has been optically controlled. Incretin mimetics have emerged as suitable drugs for the management of type 2 diabetes (T2D), even though this therapeutic approach causes considerably side effects. For this reason, Trauner, Hodson and Hoffmann-Röder applied photopharmacology concepts to chemically modify the stabilised GLP-1 analogue liraglutide. Thereby, an azobenzene comprising the amino acid AMPP was incorporated to liraglutide yielding **LirAzo** (Table 2, compound 24), a photoswitchable agonist. As previous computational studies predicted, the binding mode of the two distinct isomers to GLP-1R was fairly similar which could potentially lead to an inefficient molecular trigger.[58](#_ENREF_58), [59](#_ENREF_59) However, the *trans*-LirAzo was able to induce higher increase on intracellular calcium concentration compared to the *cis* isomer. On the other hand, upon 350 nm light illumination, the *cis*-LirAzo favoured cAMP generation better than the *trans* ligand. Interestingly, both secondary messengers converge to common downstream signalling which amplifies insulin secretion. Nevertheless, the *cis*-LirAzo was more effective on the secretion of insulin stimulated by glucose than the *trans*-LirAzo. As a conclusion to their work, they suggested that cAMP is the major driving force of incretin-potentiated insulin secretion.[60](#_ENREF_60" \o "Broichhagen, 2015 #47)

Recently, an allosteric pocket has been discovered for the glucagon-like peptide-1 receptor. Allosteric modulators offer a vast number of potential advantages for GPCR drug development, and therefore they are employed to modulate the action of native agonists. **PhotoETP** (Table 2, compound 25) has been described as the first blue-light-activated positive allosteric modulator (PAM) for GLP-1R. This compound acts as a trans-active compound (λ=440 nm), which has been confirmed through a combination of Ca2+ and cAMP cell-based assays.[61](#_ENREF_61" \o "Broichhagen, 2016 #88) Moreover, they hypothesised that PhotoETP is possibly a covalent ligand. This idea arises as a consequence to the fact that this ligand is based in BETP structure, a non-photoswitchable PAM which covalently modifies the cysteines 347 and 438 in GLP-1R.[62](#_ENREF_62" \o "Nolte, 2014 #89)

**Class C GPCRs**, or glutamate family GPCRs, comprise twenty-two receptors classified in four subgroups (GABA-B, TAS1/CASR, Glutamate and RAIG).[63](#_ENREF_63) A common characteristic of glutamate family GPCRs is the dimerisation of the receptors at the cell surface, either as homodimers or heterodimers.[64](#_ENREF_64) Class C GPCRs are complex machines which contain a transmembrane domain (TMD) with seven alpha helixes connected to a large extracellular venus fly trap domain (VFT). All the subgroups, except GABA-B receptors, present a cysteine-rich domain (CRD) between the TMD and the VFT. The orthosteric pocket is located in the VFT, whereas the allosteric binding site is situated in the TMD. [65](#_ENREF_65" \o "Goudet, 2009 #45), [66](#_ENREF_66" \o "Nicoletti, 2011 #46)

Herein, we focus on metabotropic glutamate receptors (mGluRs) considering that they were the first GPCRs to be optically controlled by allosteric photoswitchable ligands. The mGluRs can regulate several neuronal and glia functions. Thus, they have an essential role in the regulation of neuronal excitability and synaptic transmission.[66](#_ENREF_66), [67](#_ENREF_67) Thereby, achieving spatio-temporal control over mGluR activity is essential to better understand the function and dynamics of these GPCRs either in cells or in living organisms. There are eight different subtypes of mGluRs classified in three different groups on the basis of sequence similarities, agonist pharmacology and G-protein effector coupling. Group I subtypes (mGlu1 and mGlu5) are mainly postsynaptic and bind to Gq subunit. Whereas, group II (mGlu2 and mGlu3) and group III (mGlu4, mGlu6, mGlu7 and mGlu8) are mainly presynaptic and bind to Gi/o subunit.[68](#_ENREF_68" \o "Flor, 2012 #82), [69](#_ENREF_69" \o "Lindsley, 2016 #83)

Llebaria, Gorostiza and collaborators designed **Alloswitch-1** (Table 2, compound 26), the first potent and selective photoswitchable NAM of the **mGlu5 receptor**. This azobenzene ligand presents photoswitchable properties, while maintaining its drug-like characteristics. Illumination by green light yields the *trans* isomer while illumination with violet light leads to the *cis* isomer of the ligand. Functional assays of IP accumulation showed significant differences on the pharmacological properties of the compound before and after violet light illumination. They noticed that the compound was more active in the *trans* state. Moreover, they corroborated the mentioned results through real-time calcium imaging experiments with rat cortical astrocytes expressing endogenously mGlu5 receptor. Remarkably, *in vivo* studies using tadpoles and zebrafish illustrated important light regulated effects on the locomotion of the animals. In the absence of illumination, *trans*-Alloswitch-1 was found to exert an inhibitory action on the zebrafish motility compared to the untreated controls. Thereafter, they noticed through rodent *in vivo* pain assays that the local photoisomerisation of Alloswitch-1 from *trans* to *cis* configuration in the amygdala can control the analgesic effect of the compound in peripheral tissues.[70](#_ENREF_70), [71](#_ENREF_71)

It is currently unknown whether this photo-induced loss of NAM activity is attributed to a loss of ligand binding to mGlu5 or due to a more subtle effect such as a change in binding mode or a ligand-induced effect on receptor conformation. Computational docking and molecular dynamics simulations have shown that the photoswitch of Alloswitch-1 alters the ligand’s binding mode, reducing its binding affinity and stability. However, molecular simulations have also suggested that binding of the *cis* isomer is possible, as well as a theoretical “instantaneous” photoswitching of the ligand while it is bound at the allosteric pocket of the mGlu5 receptor.[72](#_ENREF_72" \o "Dalton, 2016 #48), [73](#_ENREF_73" \o "Gómez-Santacana, 2017 #49) In conclusion, further experimental evidence is required to support one of both hypotheses.

More recently, they established a structure-activity relationship (SAR) synthesizing a **family of NAMs** targeting the mGlu5 receptor. The phenylazopyridine derivatives showed a variety of photoisomerisation properties, depending on the nature of the *azo*-substituents. Thereby, almost all the compounds exhibited significant differences on their pharmacological properties before and after violet light illumination. *In vitro* and *in vivo* studies demonstrated that all the analogues acted as *trans* active compounds.[71](#_ENREF_71" \o "Gómez-Santacana, 2017 #37)

Llebaria, Goudet, Gorostiza and colleagues introduced a blue-light-controlled negative allosteric modulator of **mGlu4 receptor**, termed **OptoGluNAM4.1** (Table 2, compound 27). Single-cell calcium imaging experiments demonstrated that the *trans* isomer blocked the mGlu4 activation. On the contrary, the *cis-*OptoGluNAM4.1 restored the intracellular calcium responses under 430 nm light illumination. Thereafter, they noticed through mouse behavioural studies that the compound was able to block the analgesic effect induced by an orthosteric agonist. Additionally, *in vivo* studies using zebrafish also showed important light regulated effects on the locomotion of the animals. In the absence of illumination, *trans*-OptoGluNAM4.1 increased the free-swimming distance over untreated control animals, consistent with the effect of the *trans* isomer on mGlu4. The distance is reduced to a similar level to that of untreated controls upon illumination with blue light in accordance with the lack of effect of *cis*-OptoGluNAM4.1 on the receptor. Remarkably, they suggested that OptoGluNAM4.1 could be a covalent ligand considering that the ligand has a chemical reactivity group that can potentially bind covalently to the protein receptor.[74](#_ENREF_74" \o "Rovira, 2016 #85)

On the contrary, **Optogluram** (Table 2, compound 28) is the first photoswitchable positive allosteric modulator for the mGlu4 receptor reported to date. The photoisomerisation process was stable and reversible upon repetitive illumination cycles at violet (λ=380 nm) and green (λ=500 nm) light. Therefore, functional assays for IP accumulation illustrated an enhancement of mGlu4 activity by *trans*-Optogluram. While the *cis* isomer showed a lack of effect on mGlu4 potency. Additionally, *in vivo* studies using a murine model of persistent inflammatory pain showed that the optical “on/off” control of amygdala localised mGlu4 receptors produced acute and reversible analgesic peripheral responses, as well as anxiolytic and anti-depressive effects in mice.[75](#_ENREF_75" \o "Zussy, 2016 #87)

* 1. **Tethered ligands (TLs)**

Light-sensitive tethered ligands are constituted by photochromic compounds covalently attached to the target through a bioconjugation reaction. The target can be an endogenous protein or a genetically modified receptor. In this strategy, the activatable molecules cannot diffuse away, and this leads to a fast, reproducible and spatially acute protein response. Therefore, these light-regulated moieties can reversibly change their structure allowing the activation and deactivation of proteins within milliseconds. Notably, these bistable tethered molecules can be classified depending on the attachment site and the length of the tether moiety, as we have previously described (*see Figure 5*).[12](#_ENREF_12" \o "Kramer, 2013 #92), [14](#_ENREF_14" \o "Leippe, 2017 #91)

The first conceived tethered ligands were the **photoswitchable tethered ligands** (**PTLs**).[76](#_ENREF_76" \o "Lester, 1980 #94), [77](#_ENREF_77" \o "Chabala, 1986 #93) These small chemical compounds are maleimide-azobenzene derivatives that react with a single genetically modified natural aminoacid, commonly a cysteine residue. Thus, the mentioned mutation introduces a slight variation in the structure, trafficking and function of the native protein. In addition, the photoswitch moiety comprises the greatest part of the tether and is located closely to the binding site.[12](#_ENREF_12" \o "Kramer, 2013 #92), [14](#_ENREF_14" \o "Leippe, 2017 #91) The PTLs were first reported for voltage-gated and ligand-gated ion channels.[12](#_ENREF_12" \o "Kramer, 2013 #92) Subsequently, this approach has been applied to class A[51](#_ENREF_51" \o "Donthamsetti, 2017 #95) and C[78](#_ENREF_78" \o "Levitz, 2013 #96) GPCRs.

Focusing on **family A GPCRs**, Trauner, Isacoff and co-workers extended this strategy to dopamine **D1** and **D2 receptors**. They synthesised a PTL based on the synthetic DAR agonist PPHT, named **MAP** (Table 3, compound 29). After a screening of several mutants, they observed that *trans*-MAP acted as an inverse agonist when tethered to cysteines to either EL1 or EL2 of D2 receptor. On the contrary, *trans*-MAP was an inverse agonist or antagonist when attached to cysteines in EL1 or EL2 of D1 receptor, respectively.[51](#_ENREF_51" \o "Donthamsetti, 2017 #95)

However, these are not the first photoswitchable tethered ligands reported in the literature regarding GPCRs. The same research groups had previously designed PTLs targeting **mGlu2**, **mGlu3** and **mGlu6 receptors**. In vitro assays, illustrated that *cis*-D-MAG-1 (λ=380 nm) induced antagonism which was reversed by illumination at 500 nm. On the other hand, *cis*-D-MAG-0 acted as an agonist under 380 nm. Thereby, **LimGluR2-block** (Table 3, compound 30) was termed the **D-MAG-1** anchored to mutant S302C. Whereas, the L300C substitution in combination with **D-MAG-0** was called **LimGluR2** (Table 3, compound 30).

Remarkably, despite the limited homology between mGlu2 and mGlu3 or mGlu6 receptors, photo-control can be generalised within the mGluR family. Consequently, the *cis*-**D-MAG-0** attached at cysteine of mGluR3 that is homologous to mGlu2R’s L300 yielded **LimGluR3** (potent agonist; (Table 3, compound 31)). On the contrary, the *cis*-D-MAG-0 anchored at the homologous cysteine of mGlu6 gave **LimGluR6-block** (antagonist; (Table 3, compound 32)).[78](#_ENREF_78" \o "Levitz, 2013 #96)

Lately, Isacoff and colleagues achieved two-photon (2P) photoswitching of **mGlu3 receptor**. They designed a new mGluR-specific PTL named **D-MAG0460** (Table 3, compound 33). This photoswitchable tethered ligand was able to induce mGluR3-dependent cascade signalling by 2P photoactivation, increasing the spatial precision.[79](#_ENREF_79" \o "Carroll, 2015 #97)

**Table 3.** Photoswitchable tethered ligands for GPCRs.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Compound number** | **GPCR class** | **GPCR target** | **Compound name** | **Compound structure** | **Active isomer** | **Compound activity** |
| **29** | A | D1  D2 | MAP |  | *trans*  *trans* | Inverse agonist /  Antagonist  Inverse agonist |
| **30** | C | mGlu2 | D-MAG-0  (LimGluR2)  D-MAG-1  (LimGluR2-block) |  | *cis*  *cis* | Agonist  Antagonist |
| **31** | C | mGlu3 | D-MAG-0  (LimGluR3) |  | *cis* | Agonist |
| **32** | C | mGlu6 | D-MAG-0  (LimGluR6-block) |  | *cis* | Antagonist |
| **33** | C | mGlu3 | D-MAG-0460  (LimGluR3460) |  | *cis* | Agonist |

The second covalent strategy described on GPCRs was the **photoswitchable orthogonal remotely tethered ligand** (**PORTL**). Thereby, PORTLs are large bioconjugation motifs composed of a reactivity moiety connected to a light-sensitive ligand through a long, water-soluble and flexible linker (polyethylene glycol (PEG)). In addition, the reactivity scaffold is anchored far from the ligand binding pocket to a self-labeling tag (SNAP-tag[80](#_ENREF_80" \o "Keppler, 2002 #98), CLIP-tag[81](#_ENREF_81" \o "Gautier, 2008 #99) or HALO-tag[82](#_ENREF_82" \o "Los, 2008 #100)). Remarkably, these tags are chemically stable in aqueous solution and each reaction tag is bioorthogonal.[14](#_ENREF_14" \o "Leippe, 2017 #91)

Notably, this approach overcomes the restrictions presented by PTLs. Thus, there are many reactive cysteines on the cell surface, decreasing the selectivity of bioconjugation. Furthermore, maleimides are unstable in aqueous solution, limiting the technique to extracellular applications.

The **mGlu2 receptor** was the first GPCRs successfully controlled by a PORTL. Thereby, Trauner and collaborators synthesised two sets of benzylguanine-azoglutamates (BGAGs) through click cycloaddition reaction. After tested the ability of compounds to photoactivate SNAP-mGlu2 receptor, **BGAG0/4/8/12** (Table 4, compound 34) and **BGAG12(460)**(Table 4, compound 35) were selected. *In vitro* assays illustrated that the *cis-*BGAG0/4/8/12 activates the receptor activity under 380 nm light illumination. On the contrary, the receptor was inactivated in presence of *trans-*BGAG0/4/8/12 upon 500 nm light illumination. Additionally, the red-shifted version termed BGAG12(460), activated the protein after illumination with blue light (420-470 nm).

Furthermore, they demonstrated the ability to orthogonally optically control two different receptors (mGluR2 and GluK2) in the same cell by the SNAG-mGluR2 and the photoswitchable tethered maleimide-based LiGluR strategy.[83](#_ENREF_83" \o "Broichhagen, 2015 #101) Remarkably, the SNAG-mGluR2 system was used for the restoration of vision in blind mice.[84](#_ENREF_84" \o "Berry, 2017 #102)

More recently, the optical control of mGlu2 receptor through the two mentioned covalent tethering strategies have been reached. *In vitro* assays indicated that *cis-*D-MAG-0 and *cis-* BGAG12 photoactivated the mGlu2 receptor under 380 nm. Whereas mGluR2 deactivation was induced on the *trans* state of both isomers.

Additionally, they extended the PORTL toolbox to the mGlu3, mGlu6, mGlu7 and mGlu8 receptors. They founded that the **BGAG0** (Table 4, compound 36) targeting the **mGlu3 receptor** acted as *cis* active agonist (λ=380 nm). By contrast, the **BGAG12** (Table 4, compound 37) derivatives activated three of the four **group III mGluRs (mGlu6-8)** on *trans* state (λ=460 nm).

Moreover, two families of benzylcytosine-azoglutamates (BCAGs) were synthesised. After tested the ability of molecules to photoactivate CLIP-**mGlu2 receptor**, **BCAG12** (Table 4, compound 38) and **BCAG12(460)** (Table 4, compound 39) were selected. Both PORTLs acted as *cis* on compounds upon UV light illumination.

Finally, they demonstrated the dual optical control of group II and III mGluRs. Therefore, they sequentially photoactivated **CLIP**-**mGlu2** and **SNAP-mGlu7** receptors through the *cis-***CLIP460-mGluR2** and the *trans-***SNAG-mGluR7** systems under 590 nm or 500 nm light flash. The λ values used are target-dependent.[85](#_ENREF_85)

As we have mentioned before, there are covalent tethering strategies that target unmodified receptors. Although, these approaches have not yet applied to G-Protein Coupled Receptors. Remarkably, Llebaria and collaborators achieved a chemical strategy to covalently conjugate a photoswitch ligand to native kainate receptor channel GluK1.[86](#_ENREF_86" \o "Izquierdo-Serra, 2016 #116)

**Table 4.** Photoswitchable orthogonal remotely tethered ligands for GPCRs.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Compound number** | **GPCR class** | **GPCR target** | **Compound name** | **Compound structure** | **Active isomer** | **Compound activity** |
| **34** | C | mGlu2 | BGAG0/4/8/12  (SNAG-mGluR2) |  | *cis* | Agonist |
| **35** | C | mGlu2 | BGAG12(460)  (SNAG460-mGluR2) |  | *cis* | Agonist |

**Table 4.** Photoswitchable orthogonal remotely tethered ligands for GPCRs (continued).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Compound number** | **GPCR class** | **GPCR target** | **Compound name** | **Compound structure** | **Active isomer** | **Compound activity** |
| **36** | C | mGlu3 | BGAG0  (SNAG-mGluR3) |  | *cis* | Agonist |
| **37** | C | mGlu6-8 | BGAG12  (SNAG-mGluR6-8) |  | *trans* | Agonist |
| **38** | C | mGlu2 | BCAG12  (CLIP-mGluR2) |  | *cis* | Agonist |
| **39** | C | mGlu2 | BCAG12(460)  (CLIP460-mGluR2) |  | *cis* | Agonist |

1. **Conclusions and future perspectives**

As balanced to conventional pharmacology, the use of light operated protein ligands offers unique possibilities to regulate the activity of GPCRs in their native environments with high spatiotemporal control. In particular, optogenetic pharmacology and photopharmacology can greatly improve the current tools to study GPCR mechanism of action, as well as, their involvement in specific physiological and biological responses at different experimental levels including cells, tissues or living organisms. The approaches here included, show its potential to decipher molecular mechanism and kinetic aspects of GPCR receptor activation. Photopharmacology also hold great therapeutic potential of GPCRs allowing a precise modulation of receptors and their associated signaling in disease. In the near future, it is expected an increase of the photomolecular tools for many GPCRs to understand fundamental questions of these receptors at different biological levels, including signaling, subcellular localisation or anatomical distribution. Characterisation of molecular tools and their capacity to stimulate endogenous in vivo signaling to define GPCR roles is an exciting field almost unexplored. Developing advances in photonics, chemistry and biological technology will promote the creation of many light operated tools for specific optogenetics and photopharmacology approaches.

These molecular tools and associated technologies also have translational potential, providing transformational approaches for more precise drug therapeutics getting closer to the spatiotemporal regulation of neurotransmitters, hormones and other types of endogenous biomolecules targeting GPCRs.

**Acknowledgements**

We acknowledge financial support from the ERANET Neuron LIGHTPAIN and MAGNOLIA projects, the Spanish Ministry of Science, Innovation and Universities (CTQ2014-57020-R and CTQ2017-89222-R), cofinanced by the European Regional Development Fund (FEDER) and the Catalan Government (2017SGR01604).

**References**

1. Ross, B., Mehta, S. & Zhang, J. Molecular Tools for Acute Spatiotemporal Manipulation of Signal Transduction. *Current opinion in chemical biology* **34**, 135-142 (2016).

2. Kienzler, M.A. & Isacoff, E.Y. Precise modulation of neuronal activity with synthetic photoswitchable ligands. *Current opinion in neurobiology* **45**, 202-209 (2017).

3. Ankenbruck, N., Courtney, T., Naro, Y. & Deiters, A. Optochemical Control of Biological Processes in Cells and Animals. *Angewandte Chemie International Edition* **57**, 2768-2798 (2017).

4. Spangler, S.M. & Bruchas, M.R. Optogenetic approaches for dissecting neuromodulation and GPCR signaling in neural circuits. *Current Opinion in Pharmacology* **32**, 56-70 (2017).

5. Deisseroth, K. Optogenetics: 10 years of microbial opsins in neuroscience. *Nature neuroscience* **18**, 1213-1225 (2015).

6. Weitzman, M. & Hahn, K.M. Optogenetic approaches to cell migration and beyond. *Current opinion in cell biology* **0**, 112-120 (2014).

7. Deisseroth, K. Optogenetics. *Nature Methods* **8**, 26 (2010).

8. Lerch, M.M., Hansen, M.J., van Dam, G.M., Szymanski, W. & Feringa, B.L. Emerging Targets in Photopharmacology. *Angewandte Chemie International Edition* **55**, 10978-10999 (2016).

9. Broichhagen, J., Frank, J.A. & Trauner, D. A Roadmap to Success in Photopharmacology. *Accounts of Chemical Research* **48**, 1947-1960 (2015).

10. Velema, W.A., Szymanski, W. & Feringa, B.L. Photopharmacology: Beyond Proof of Principle. *Journal of the American Chemical Society* **136**, 2178-2191 (2014).

11. Gorostiza, P. & Isacoff, E.Y. Optical Switches for Remote and Noninvasive Control of Cell Signaling. *Science* **322**, 395 (2008).

12. Kramer, R.H., Mourot, A. & Adesnik, H. Optogenetic pharmacology for control of native neuronal signaling proteins. *Nature neuroscience* **16**, 816-823 (2013).

13. Hüll, K., Morstein, J. & Trauner, D. In Vivo Photopharmacology. *Chemical Reviews* (2018).

14. Leippe, P., Koehler Leman, J. & Trauner, D. Specificity and Speed: Tethered Photopharmacology. *Biochemistry* **56**, 5214-5220 (2017).

15. Adams, S.R. & Tsien, R.Y. Controlling Cell Chemistry with Caged Compounds. *Annual Review of Physiology* **55**, 755-784 (1993).

16. Falvey, D.E. Dynamic Studies in Biology:  Phototriggers, Photoswitches, and Caged Biomolecules Edited by Maurice Goeldner (Université L. Pasteur Strasbourg, France) and Richard Givens (University of Kansas, USA). Wiley-VCH GmbH & Co. KGaA:  Weinheim. 2005. xxviii + 558 pp. $259.00. ISBN 3-527-30783-4. *Journal of the American Chemical Society* **127**, 16747-16747 (2005).

17. Kaplan, J.H., Forbush, B. & Hoffman, J.F. Rapid photolytic release of adenosine 5'-triphosphate from a protected analog: utilization by the sodium:potassium pump of human red blood cell ghosts. *Biochemistry* **17**, 1929-1935 (1978).

18. Ellis-Davies, G.C.R. Caged compounds: photorelease technology for control of cellular chemistry and physiology. *Nature methods* **4**, 619-628 (2007).

19. Faddis, M.N. & Brown, J.E. Flash photolysis of caged compounds in Limulus ventral photoreceptors. *The Journal of General Physiology* **100**, 547 (1992).

20. Marriott, G. Caged Protein Conjugates and Light-Directed Generation of Protein Activity: Preparation, Photoactivation, and Spectroscopic Characterization of Caged G-Actin Conjugates. *Biochemistry* **33**, 9092-9097 (1994).

21. Chang, C.-y., Fernandez, T., Panchal, R. & Bayley, H. Caged Catalytic Subunit of cAMP-Dependent Protein Kinase. *Journal of the American Chemical Society* **120**, 7661-7662 (1998).

22. Curley, K. & Lawrence, D.S. Photoactivation of a Signal Transduction Pathway in Living Cells. *Journal of the American Chemical Society* **120**, 8573-8574 (1998).

23. Ellis-Davies, G.C.R. in Methods in Enzymology 226-238 (Academic Press, 2003).

24. Muralidharan, S. & Nerbonne, J.M. Photolabile “caged” adrenergic receptor agonists and related model compounds. *Journal of Photochemistry and Photobiology B: Biology* **27**, 123-137 (1995).

25. Banghart, M.R. & Sabatini, B.L. Photoactivatable neuropeptides for spatiotemporally precise delivery of opioids in neural tissue. *Neuron* **73**, 249-59 (2012).

26. Banghart, M.R., He, X.J. & Sabatini, B.L. A Caged Enkephalin Optimized for Simultaneously Probing Mu and Delta Opioid Receptors. *ACS Chemical Neuroscience* **9**, 684-690 (2018).

27. Banghart, M.R., Williams, J.T., Shah, R.C., Lavis, L.D. & Sabatini, B.L. Caged Naloxone Reveals Opioid Signaling Deactivation Kinetics. *Molecular Pharmacology* **84**, 687 (2013).

28. Taura, J. et al. Remote control of movement disorders using a photoactive adenosine A2A receptor antagonist. *Journal of Controlled Release* **283**, 135-142 (2018).

29. Todde, S. et al. Design, Radiosynthesis, and Biodistribution of a New Potent and Selective Ligand for in Vivo Imaging of the Adenosine A2A Receptor System Using Positron Emission Tomography. *Journal of Medicinal Chemistry* **43**, 4359-4362 (2000).

30. Callaway, E.M. & Katz, L.C. Photostimulation using caged glutamate reveals functional circuitry in living brain slices. *Proceedings of the National Academy of Sciences of the United States of America* **90**, 7661-7665 (1993).

31. Wilcox, M. et al. Synthesis of photolabile precursors of amino acid neurotransmitters. *The Journal of Organic Chemistry* **55**, 1585-1589 (1990).

32. Wieboldt, R., Ramesh, D., Carpenter, B.K. & Hess, G.P. Synthesis and Photochemistry of Photolabile Derivatives of .gamma.-Aminobutyric Acid for Chemical Kinetic Investigations of the .gamma.-Aminobutyric Acid Receptor in the Millisecond Time Region. *Biochemistry* **33**, 1526-1533 (1994).

33. Rial Verde, E.M., Zayat, L., Etchenique, R. & Yuste, R. Photorelease of GABA with Visible Light Using an Inorganic Caging Group. *Frontiers in Neural Circuits* **2**, 2 (2008).

34. Keywood, C., Wakefield, M. & Tack, J. A proof-of-concept study evaluating the effect of ADX10059, a metabotropic glutamate receptor-5 negative allosteric modulator, on acid exposure and symptoms in gastro-oesophageal reflux disease. *Gut* **58**, 1192-1199 (2009).

35. Font, J. et al. Optical control of pain in vivo with a photoactive mGlu(5) receptor negative allosteric modulator. *eLife* **6**, e23545 (2017).

36. Beharry, A.A. & Woolley, G.A. Azobenzene photoswitches for biomolecules. *Chemical Society Reviews* **40**, 4422-4437 (2011).

37. Merino, E. & Ribagorda, M. Control over molecular motion using the cis–trans photoisomerization of the azo group. *Beilstein Journal of Organic Chemistry* **8**, 1071-1090 (2012).

38. Szymański, W., Beierle, J.M., Kistemaker, H.A.V., Velema, W.A. & Feringa, B.L. Reversible Photocontrol of Biological Systems by the Incorporation of Molecular Photoswitches. *Chemical Reviews* **113**, 6114-6178 (2013).

39. Bandara, H.M.D. & Burdette, S.C. Photoisomerization in different classes of azobenzene. *Chemical Society Reviews* **41**, 1809-1825 (2012).

40. Weston, C.E., Richardson, R.D., Haycock, P.R., White, A.J.P. & Fuchter, M.J. Arylazopyrazoles: Azoheteroarene Photoswitches Offering Quantitative Isomerization and Long Thermal Half-Lives. *Journal of the American Chemical Society* **136**, 11878-11881 (2014).

41. Garcia-Amorós, J., Díaz-Lobo, M., Nonell, S. & Velasco, D. Fastest Thermal Isomerization of an Azobenzene for Nanosecond Photoswitching Applications under Physiological Conditions. *Angewandte Chemie International Edition* **51**, 12820-12823 (2012).

42. Ciccone, S. & Halpern, J. CATALYSIS OF THE CIS-TRANS ISOMERIZATION OF AZOBENZENE BY ACIDS AND CUPRIC SALTS. *Canadian Journal of Chemistry* **37**, 1903-1910 (1959).

43. Bortolus, P. & Monti, S. Cis-trans photoisomerization of azobenzene. Solvent and triplet donors effects. *The Journal of Physical Chemistry* **83**, 648-652 (1979).

44. Goudet, C., Rovira, X. & Llebaria, A. Shedding light on metabotropic glutamate receptors using optogenetics and photopharmacology. *Current Opinion in Pharmacology* **38**, 8-15 (2018).

45. Nargeot, J. et al. A photoisomerizable muscarinic antagonist. Studies of binding and of conductance relaxations in frog heart. *The Journal of General Physiology* **79**, 657 (1982).

46. Bartels, E., Wassermann, N.H. & Erlanger, B.F. Photochromic Activators of the Acetylcholine Receptor. *Proceedings of the National Academy of Sciences of the United States of America* **68**, 1820-1823 (1971).

47. Chabala, L.D., Gurney, A.M. & Lester, H.A. Dose-response of acetylcholine receptor channels opened by a flash-activated agonist in voltage-clamped rat myoballs. *The Journal of Physiology* **371**, 407-433 (1986).

48. Agnetta, L. et al. A Photoswitchable Dualsteric Ligand Controlling Receptor Efficacy. *Angewandte Chemie International Edition* **56**, 7282-7287 (2017).

49. Bahamonde, M.I. et al. Photomodulation of G Protein-Coupled Adenosine Receptors by a Novel Light-Switchable Ligand. *Bioconjugate Chemistry* **25**, 1847-1854 (2014).

50. Schönberger, M. & Trauner, D. A Photochromic Agonist for μ-Opioid Receptors. *Angewandte Chemie International Edition* **53**, 3264-3267 (2014).

51. Donthamsetti, P.C. et al. Optical Control of Dopamine Receptors Using a Photoswitchable Tethered Inverse Agonist. *Journal of the American Chemical Society* **139**, 18522-18535 (2017).

52. Lachmann, D. et al. Photochromic Dopamine Receptor Ligands Based on Dithienylethenes and Fulgides. *Chemistry – A European Journal* **23**, 13423-13434 (2017).

53. Frank, J.A. et al. Optical control of GPR40 signalling in pancreatic β-cells †Electronic supplementary information (ESI) available. See DOI: 10.1039/c7sc01475a. *Chemical Science* **8**, 7604-7610 (2017).

54. Westphal, M.V. et al. Synthesis of Photoswitchable Δ9-Tetrahydrocannabinol Derivatives Enables Optical Control of Cannabinoid Receptor 1 Signaling. *Journal of the American Chemical Society* **139**, 18206-18212 (2017).

55. Hauwert, N.J. et al. Synthesis and Characterization of a Bidirectional Photoswitchable Antagonist Toolbox for Real-Time GPCR Photopharmacology. *Journal of the American Chemical Society* **140**, 4232-4243 (2018).

56. Gómez-Santacana, X. et al. Photoswitching the Efficacy of a Small-Molecule Ligand for a Peptidergic GPCR: from Antagonism to Agonism. *Angewandte Chemie International Edition* **57**, 11608-11612 (2018).

57. Hoare, S.R.J. Mechanisms of peptide and nonpeptide ligand binding to Class B G-protein-coupled receptors. *Drug Discovery Today* **10**, 417-427 (2005).

58. Underwood, C.R. et al. Crystal structure of glucagon-like peptide-1 in complex with the extracellular domain of the glucagon-like peptide-1 receptor. *J Biol Chem* **285**, 723-30 (2010).

59. Siu, F.Y. et al. Structure of the human glucagon class B G-protein-coupled receptor. *Nature* **499**, 444-9 (2013).

60. Broichhagen, J. et al. Optical Control of Insulin Secretion Using an Incretin Switch. *Angewandte Chemie (International Ed. in English)* **54**, 15565-15569 (2015).

61. Broichhagen, J. et al. Allosteric Optical Control of a Class B G‐Protein‐Coupled Receptor. *Angewandte Chemie (International Ed. in English)* **55**, 5865-5868 (2016).

62. Nolte, W.M. et al. A potentiator of orthosteric ligand activity at GLP-1R acts via covalent modification. *Nature Chemical Biology* **10**, 629 (2014).

63. Fredriksson, R., Lagerström, M.C., Lundin, L.-G. & Schiöth, H.B. The G-Protein-Coupled Receptors in the Human Genome Form Five Main Families. Phylogenetic Analysis, Paralogon Groups, and Fingerprints. *Molecular Pharmacology* **63**, 1256 (2003).

64. Kniazeff, J., Prézeau, L., Rondard, P., Pin, J.-P. & Goudet, C. Dimers and beyond: The functional puzzles of class C GPCRs. *Pharmacology & Therapeutics* **130**, 9-25 (2011).

65. Goudet, C. et al. Metabotropic receptors for glutamate and GABA in pain. *Brain Research Reviews* **60**, 43-56 (2009).

66. Nicoletti, F. et al. Metabotropic glutamate receptors: From the workbench to the bedside. *Neuropharmacology* **60**, 1017-1041 (2011).

67. Niswender, C.M. & Conn, P.J. Metabotropic Glutamate Receptors: Physiology, Pharmacology, and Disease. *Annual review of pharmacology and toxicology* **50**, 295-322 (2010).

68. Flor, P.J. & Acher, F.C. Orthosteric versus allosteric GPCR activation: The great challenge of group-III mGluRs. *Biochemical Pharmacology* **84**, 414-424 (2012).

69. Lindsley, C.W. et al. Practical Strategies and Concepts in GPCR Allosteric Modulator Discovery: Recent Advances with Metabotropic Glutamate Receptors. *Chemical reviews* **116**, 6707-6741 (2016).

70. Pittolo, S. et al. An allosteric modulator to control endogenous G protein-coupled receptors with light. *Nat Chem Biol* **10**, 813-5 (2014).

71. Gómez-Santacana, X. et al. Illuminating Phenylazopyridines To Photoswitch Metabotropic Glutamate Receptors: From the Flask to the Animals. *ACS Central Science* **3**, 81-91 (2017).

72. Dalton, J.A.R. et al. Shining Light on an mGlu5 Photoswitchable NAM: A Theoretical Perspective. *Current Neuropharmacology* **14**, 441-454 (2016).

73. Gómez-Santacana, X. et al. Positional isomers of bispyridine benzene derivatives induce efficacy changes on mGlu5 negative allosteric modulation. *European Journal of Medicinal Chemistry* **127**, 567-576 (2017).

74. Rovira, X. et al. OptoGluNAM4.1, a Photoswitchable Allosteric Antagonist for Real-Time Control of mGlu4 Receptor Activity. *Cell Chemical Biology* **23**, 929-934 (2016).

75. Zussy, C. et al. Dynamic modulation of inflammatory pain-related affective and sensory symptoms by optical control of amygdala metabotropic glutamate receptor 4. *Molecular Psychiatry* **23**, 509 (2016).

76. Lester, H.A., Krouse, M.E., Nass, M.M., Wassermann, N.H. & Erlanger, B.F. A covalently bound photoisomerizable agonist. Comparison with reversibly bound agonists at electrophorus electroplaques. *The Journal of General Physiology* **75**, 207-232 (1980).

77. Chabala, L.D. & Lester, H.A. Activation of acetylcholine receptor channels by covalently bound agonists in cultured rat myoballs. *The Journal of Physiology* **379**, 83-108 (1986).

78. Levitz, J. et al. Optical Control of Metabotropic Glutamate Receptors. *Nature neuroscience* **16**, 507-516 (2013).

79. Carroll, E.C. et al. Two-photon brightness of azobenzene photoswitches designed for glutamate receptor optogenetics. *Proceedings of the National Academy of Sciences of the United States of America* **112**, E776-E785 (2015).

80. Keppler, A. et al. A general method for the covalent labeling of fusion proteins with small molecules in vivo. *Nature Biotechnology* **21**, 86 (2002).

81. Gautier, A. et al. An Engineered Protein Tag for Multiprotein Labeling in Living Cells. *Chemistry & Biology* **15**, 128-136 (2008).

82. Los, G.V. et al. HaloTag: A Novel Protein Labeling Technology for Cell Imaging and Protein Analysis. *ACS Chemical Biology* **3**, 373-382 (2008).

83. Broichhagen, J. et al. Orthogonal Optical Control of a G Protein-Coupled Receptor with a SNAP-Tethered Photochromic Ligand. *ACS Central Science* **1**, 383-393 (2015).

84. Berry, M.H. et al. Restoration of patterned vision with an engineered photoactivatable G protein-coupled receptor. *Nature Communications* **8**, 1862 (2017).

85. Levitz, J. et al. Dual optical control and mechanistic insights into photoswitchable group II and III metabotropic glutamate receptors. *Proceedings of the National Academy of Sciences of the United States of America* **114**, E3546-E3554 (2017).

86. Izquierdo-Serra, M. et al. Optical control of endogenous receptors and cellular excitability using targeted covalent photoswitches. *Nature Communications* **7**, 12221 (2016).