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Photoswitchable allosteric modulators for metabotropic glutamate receptors

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Abstract

Metabotropic glutamate receptors (mGlu) are a family of class C G protein-coupled receptors (GPCRs) with important biological functions and widespread expression. The mechanisms of mGlu activation and the development of allosteric modulators for these dimeric proteins have attracted singular attention including the use of light regulated ligands. Photopharmacology involves the integration of a photoactive moiety into the ligand structure that following specific illumination undergoes a structural rearrangement and changes its biological activity. The use of light-regulated allosteric ligands offers the opportunity to manipulate mGlu signalling with spatiotemporal precision, unattainable with classical pharmacological approaches. In this review, we will discuss some of the innovations that have been made in the allosteric photopharmacology of mGlu receptors to date. We discuss the prospects of these molecular tools in the control of mGluRs and the new perspectives in understanding mGlu mechanisms, pharmacology and (patho)physiology that can ultimately result in innovative drug discovery concepts.

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Abbreviations

mGlu, Metabotropic Glutamate; CNS, Centra Nervous System; PNS, Peripheral Nervous System; AM, Allosteric Modulator; PAM, Positive Allosteric Modulator; NAM, Negative Allosteric Modulator; mGluR, Metabotropic Glutamate Receptor; VFT, Vensu Fly Trap; TMD, Transmembrane Domain.

Introduction

Metabotropic glutamate receptors (mGlu) are mainly expressed in the central nervous systems (CNS), where they exert a modulatory role on neuronal excitability and synaptic plasticity. mGlu receptors can also be found in the peripheral nervous system (PNS) in tissues that receive glutamatergic innervation and also in other tissues and organs such as immune cells, liver or kidneys, among others [1]. Due to their physiological relevance in the CNS, mGlu receptors are considered potential drug targets for a variety of psychiatric and neurodegenerative disorders [1,2]. However, so far all drug candidates targeting mGlu receptors have failed in preclinical or clinical phases, either due to adverse effects or lack of efficacy [1,3–6]. One possible reason for these failures may be related to the broad expression of mGlu receptors in different regions of the nervous system, involving the regulation of multiple biological functions where they sometimes exert opposite roles [1]. Indeed, studies based on experiments in animal models have proposed that the modulation of some mGlu subtypes by conventional allosteric drugs may be used as treatments for several diseases related to specific locations of the nervous system [2]. However, the activity of these molecules in other areas of the body, where this specific mGlu subtype is also expressed and physiologically relevant, may counteract the beneficial effect or even lead to adverse effects. To bypass the aforementioned issues related to lack of local and temporal selectivity, novel approaches based on the use of light are currently under development [7]. One of these strategies consists in the use of ligands that include a photoresponsive molecular switch (also called photoswitch). In these molecules, derived from existing drugs, the pharmacological properties can be altered upon irradiation with light of different wavelengths (i.e. photoswitching) [8,9]. Azobenzene is the most widely used photoswitch for biological applications due to its relatively small size, photoisomerisation wavelength versatility, high rate of isomerisation and a large change in geometry between configurations. All these properties make azobenzene a good photochromic candidate to replace several moieties with two aromatic rings found in bioactive molecules, such as biarylamides, aryl phtalimides, biaryl

acetylenes or biaryl ethers. This strategy is known as 'azologisation' [10] and the resulting analogues can be defined as 'azologues'. In the end, this strategy allows the control of the drug in a specific location and a real-time modulation of its effects [8,11,12]. Currently, the use of light-regulated ligands targeting mGlu has demonstrated an accurate restriction of the effect to specific organs, tissues or even subcellular locations with strictly defined time applications [13–17]. However, it is worth noting that the final success of future photopharmacological approaches as therapeutic strategies is necessarily linked to the development of biocompatible illumination devices allowing the light to be delivered in the targeted region minimising the invasiveness of the procedure (see Table 1).

Table 1

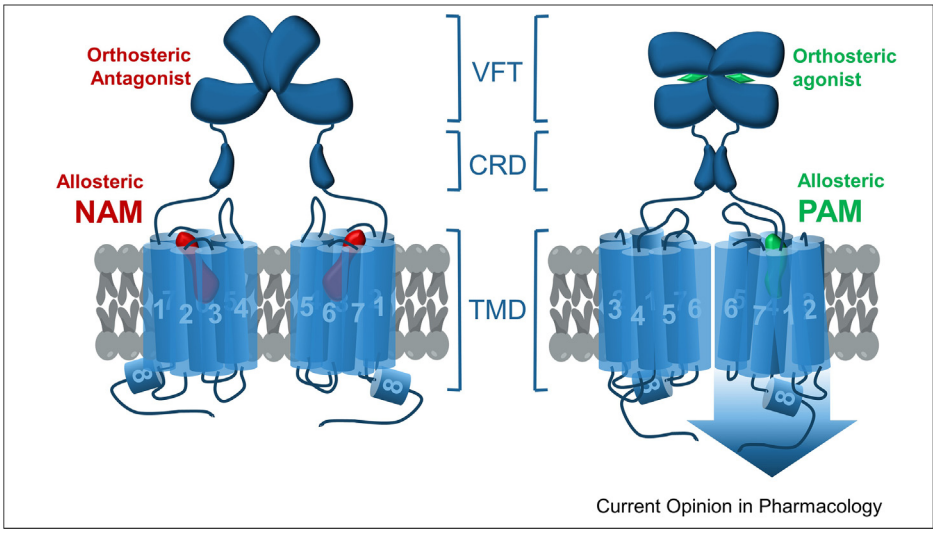
Summary of the current photoswitchable allosteric modulators for metabotropic glutamate receptors.

Group	Subtype	Photoswichable AM	Activity type
Group I	$mGlu_1$	Alloswitch family	NAM
	$mGlu_5$	Azo-Bispyridines	NAM
Group II	$mGlu_2$	aBINA	PAM
	$mGlu_3$		
Group III	$mGlu_4$	Optogluram	NAM
		OptogluNAM4.1	PAM
	$mGlu_6$	Optogluram	PAM
	$mGlu_7$	OptogluNAM4.1	NAM
	$mGlu_8$		

Metabotropic glutamate receptors belong to the class C of the G protein-coupled receptors (GPCRs) superfamily and are activated by glutamate, which is the main excitatory neurotransmitter in the vertebrate nervous system. mGlu family is divided in eight different subtypes, classified in three different groups depending on their function, synaptic location and sequence identity. Group I, containing subtypes 1 and 5 ($mGlu_{1,5}$), are mainly postsynaptic and bind to $G_{\alpha q}$ subunit. On the other hand group II, containing subtypes 2 and 3 ($mGlu_{2,3}$), and group III, containing subtypes 4, 7 and 8 ($mGlu_{4,7,8}$), bind to $G_{\alpha i/o}$ subunits and are mainly presynaptic, though Group II mGlu receptors can be expressed post-synaptically and in glia [1,2]. There is an additional mGlu subtype ($mGlu_6$), also belonging to group III, which represents an exception since it is mostly expressed post-synaptically in ON bipolar cell dendrites in retina.

mGlu receptors are constitutive dimers, linked by a disulphide bond, and activated by glutamate binding between two lobes forming a large extracellular domain called Venus flytrap (VFT). Agonist binding induces the approximation of these lobes and a closure of the cleft containing the binding site in this domain. This movement is followed by the rearrangement of the protomers, a relative rotation between the seven transmembrane domains (TMD) and a change in the conformation of one TMD that finally becomes activated (Figure 1) [18,19]. Orthosteric antagonists bind in the glutamate binding site blocking the VFT closure and preventing the receptor activation by agonists [20]. The receptor activity can also be potentiated or inhibited by allosteric modulators (AM), which generally bind to a lipophilic

Figure 1



Structural and dynamic features of mGlu receptors. mGlu receptors are expressed as strict dimers. Orthosteric agonists bind in the extracellular domain (VFT) stabilising its closed conformation and activating the receptor, whereas antagonists bind and block the VFT in the open conformation. In contrast, allosteric modulators bind in the transmembrane domain (TMD) and can be negative (NAM), if they stabilise the inactive conformation, or positive (PAM), thus stabilising the active conformation of one TMD.

allosteric pocket located at the TMD and stabilise its active (positive AM, PAM) or its inactive conformation (Negative AM, NAM). Moreover, these AM may possess intrinsic activity as allosteric agonists or inverse agonists. Allosteric modulators acting on mGlu receptors have generally been considered better candidates for drug development since they can cross the blood-brain barrier (BBB) more efficiently in comparison to the highly polar orthosteric ligands. Furthermore, they present a better selectivity profile due to lower evolutionary pressure over the allosteric binding pockets compared to the highly conserved orthosteric sites [21,22]. Still, finding selective AM between members of group II and III has demonstrated to be highly challenging, whereas some successful candidates has been discovered as orthosteric ligands [23].

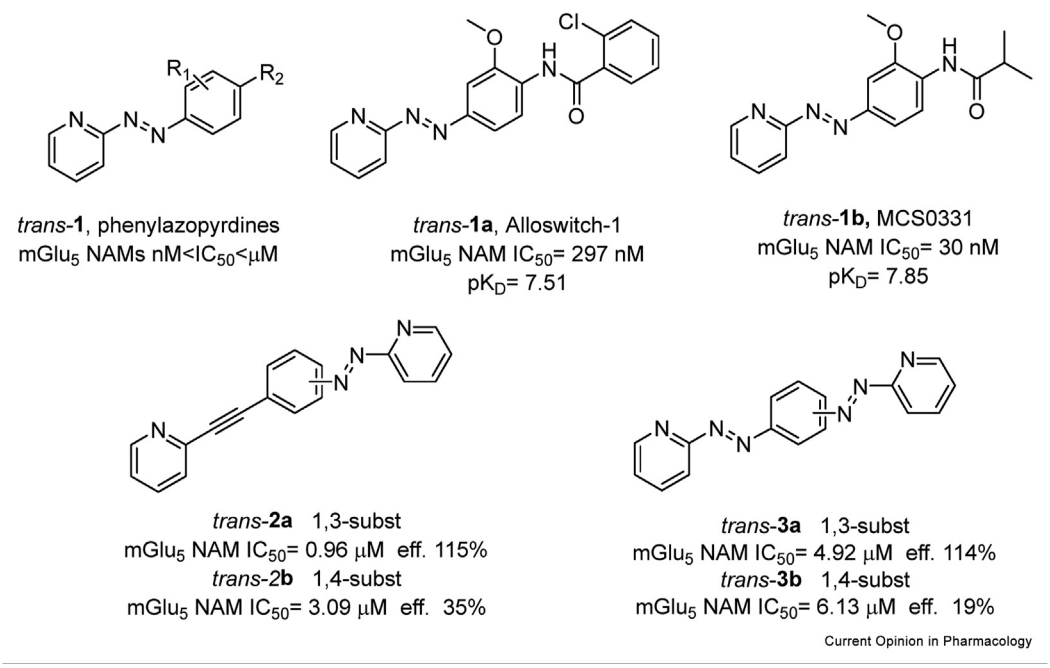
Metabotropic glutamate receptors have been a hot topic in the development of photopharmacological approaches. Indeed, these receptors were the first GPCR family to be targeted by photoswitchable ligands in 2013 using photochromic tethered glutamate derivatives, which bind to a mutated version of the VFT of $mGlu_2$ receptor [24]. One year later, the first allosteric modulator with photoswitchable properties was reported [25] and several others has been developed to date [26,27]. In this review, we will present current advances on the development of photoswitchable allosteric modulators for mGlu receptors. Moreover, we will comment on future directions and perspectives for phototherapeutic approaches to improve the selectivity profile that may inspire innovative drugs targeting metabotropic glutamate receptors.

Photoswitchable AM for group I mGlu receptors

Currently, the group of photoswitchable ligands targeting group I mGlu mainly comprises alloswitch-1 (**1a**) and its analogues (**1**), which are based on the phenylazopyridine scaffold (Figure 2). These molecules are potent selective NAMs of $mGlu_5$ receptor and possess allosteric inverse agonist activity. They were the first allosteric modulators to be used in GPCR photopharmacology and their biological properties have been deeply characterised in vitro and in vivo [14,25]. All these drugs, which behave as *trans* active compounds, exhibit differential pharmacological properties before and after violet light illumination.

Specifically, the IC_{50} of alloswitch-1 (**1a**) shifts from 297 nM in dark conditions to 1.5 μM under violet light and it is highly selective with regard to other mGlu subtypes, other GPCRs and several channels and transporters [14,25]. Similarly, alloswitch-1 analogues within the phenylazopyridines family (**1**) display an IC_{50} ranging from nanomolar to micromolar for $mGlu_5$ receptors in dark conditions. Interestingly, several alloswitch-1 analogues can be employed to fine-tune the activity of $mGlu_5$ receptor using light of different wavelengths [14]. Additionally, some phenylazopyridines of this series

Figure 2

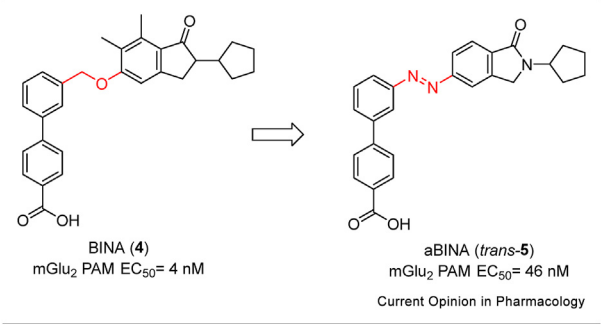


Structures of reported photoswitchable allosteric ligands for $mGlu_5$ receptor in *trans* configurations with the corresponding potencies and affinities (IC_{50} and pK_D).

evoked an over-activation or an increased signalling of $mGlu_5$ receptors upon ligand inactivation. This effect was also observed in behavioural experiments with zebrafish larvae and tadpoles, where animal locomotion was monitored and photocontrolled by the concomitant effect of light and photoswitchable active molecules. Moreover, alloswitch-1 (**1a**) and also other phenylazopyridines (**1**) photoisomerised using 2-photon excitation allowing to perform $mGlu_5$ photopharmacological experiments in precise compartments of cultured cells and brain slices [28]. Additionally, these molecules exhibited light-dependent reversible analgesic effects in rodents, which could be regulated by violet illumination in the periphery or the CNS [14].

The observed photo-induced loss of NAM activity of the phenylazopyridines (**1**) was assumed to be associated to a reduced ligand binding of the *cis* isomer to $mGlu_5$. However, a remaining question to be answered was whether photoisomerisation could occur not only when the ligand is in solution but also when bound to the receptor. Computational docking and molecular dynamics simulations led to propose that the *cis* isomer of alloswitch-1 (**1a**) would have a different binding mode, reducing its binding affinity and stability when compared with the *trans* isomer. These results suggest that the ligand photoisomerisation could be possible while bound at the allosteric pocket of the $mGlu_5$ receptor [29]. In a recent report, the mechanism of action of alloswitch-1 and a related phenylazopyridine (**1a**, MCS0331 **1b**, Figure 2) were extensively investigated by functional assays, Mass Spectrometry (MS) and radioligand binding assays [16]. Supporting the hypothesis from computational studies [29], the authors observed that the dissociation rate constants of these ligands under violet light illumination were significantly faster than that measured in the dark for the *trans* isomers, suggesting that photoisomerisation takes place also inside the ligand binding pocket to give a *cis* isomer that dissociates faster. Therefore, *cis* isomer ligands are capable of temporarily bind to the receptor, albeit with reduced affinity and shorter residence times [16].

Figure 3



The azologisation of BINA afforded the first PAM for $mGlu_2$ receptors. The active *trans* configuration is depicted with the corresponding potency (EC_{50}).

Another series of four NAMs of $mGlu_5$ receptor based on the phenylazopyridine scaffold was also reported. These were extended molecules comprising an ethynylpyridine and a phenylazopyridine (**2a-b**, Figure 2) or phenylbisazopyridines (**3a-b**, Figure 2) [30]. The observed $mGlu_5$ NAM efficacy depended on the substitution pattern of the central ring supported by computational modelisation. For these compounds, the authors also observed that NAM potencies and efficacies were reduced upon photoisomerisation. However, the low photoinduced shift on these pharmacological properties make them weak candidates for further photopharmacological characterisation.

Photoswicthable AM for group II mGlu receptors

Very recently, aBINA (**5**) has been reported as a photoswitchable PAM for $mGlu_2$ (group II) with allosteric agonist activity (Figure 3) [31]. An azologisation strategy based on the $mGlu_2$ PAM BINA (**4**, Figure 3) was used. The activity of aBINA (**5**) on $mGlu_2$ was characterised using electrophysiology methods with cells expressing GIRK channels. *Trans*-aBINA (**5**) was found to be a potent PAM with an EC50 of 46 nM that was reduced upon photoisomerisation with 340 and 380 nm light, whereas the activity of the *trans* isomer was recovered with wavelengths ranging from 400 to 600 nm light. Additionally, aBINA (**5**) showed selectivity versus $mGlu_{1/3/4/7}$ and allowed the control with light of the spontaneous firing of culture primary cortical neurons [31].

Photoswitchable AM for group III mGlu receptors

Regarding group III mGlu receptors, photoswitchable azobenzene PAMs and NAMs have been reported to date. As many group III allosteric modulators binding to the mGlu transmembrane domains, these molecules showed cross-activity between the different mGlu subtypes. This is a consequence of a high degree of structural and sequence homology of their allosteric binding pockets and reflects the difficulty of obtaining subtype-selective modulators [32]. Optogluram (**7**) is a potent PAM of both $mGlu_4$ and $mGlu_6$ and has some PAM activity on $mGlu_8$. It was originally designed using the azologisation strategy based on VU0415374 (**6**, Figure 4) and resulted in the first photoswitchable PAM for mGlu receptors with an EC_{50} in the nanomolar range, whereas upon violet light illumination the potency was significantly reduced. This compound also showed efficacy without addition of an orthosteric agonist in a similar mode than VU0415374 (**6**), even if this was not described in the original report. This could be compatible with an allosteric agonist activity, but a positive modulation of ambient glutamate cannot be discarded.

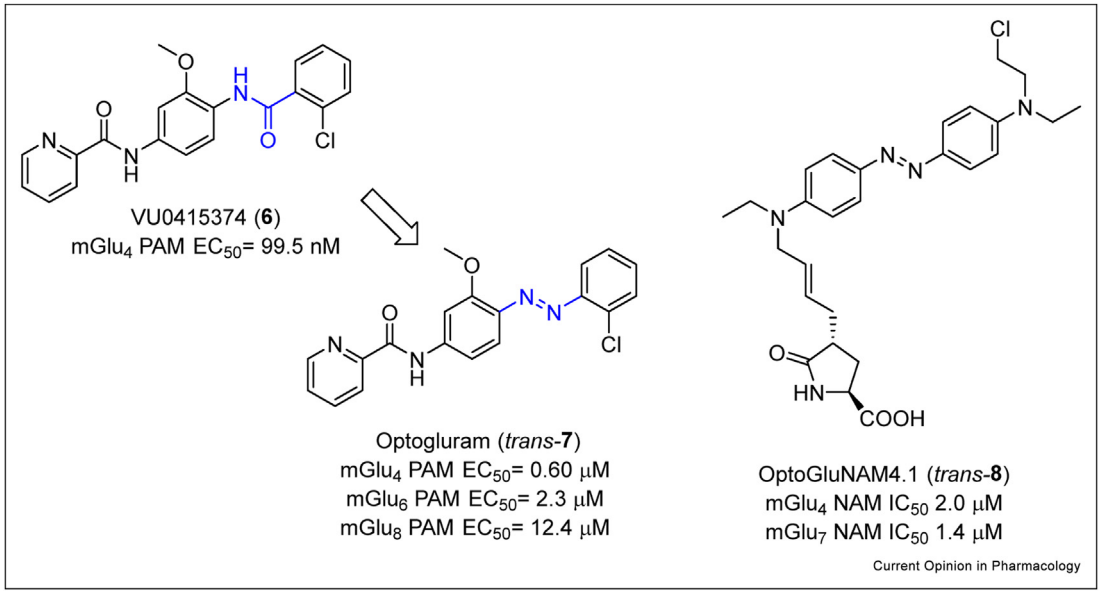
Very interestingly, Optogluram (**7**) was studied in vivo in a murine model of persistent inflammatory pain allowing for the optical '*on/off*' control of pain sensation. Importantly, this photopharmacological approach allowed to uncover the specific role of $mGlu_4$ receptors localised in the amygdala in this pathological situation. Indeed, optogluram (**7**) produced acute and reversible analgesic peripheral responses, as well as anxiolytic and antidepressive effects in mice with persistent inflammatory pain in the dark, whereas those effects where significantly reduced in few minutes upon illumination of the amygdala with light at 380 nm. Strikingly, the analgesic effects of the molecule could be activated again with a 500 nm light, an effect that could be reproduced for several '*on/off*' cycles in the same animal [15].

On the other hand, OptogluNAM4.1 (**8**, Figure 4) was the first reported NAM of $mGlu_4$. Moreover, this molecule contains a blue light-activated, fast-relaxing azobenzene group that provides light-control with only one wavelength with low phototoxicity [13]. The *trans* isomer of OptogluNAM4.1 blocked the $mGlu_4$ activation in the dark and application of 430–470 nm light induced the photoisomerisation to the less active *cis* isomer, thus allowing the activation of $mGlu_4$ receptors by orthosteric agonists. In addition to its effects on $mGlu_4$, OptoGluNAM4.1 (**8**) showed also a light–dependent partial NAM effect on $mGlu_7$. This molecule covalently binds the receptor through a nitrogen mustard (2-chloroethyl aniline), which was hypothesised to react with nucleophilic residues of the $mGlu_4$ receptors (*e.g.* lysines and cysteines) [33]. This covalent binding was suggested by the observation that the antagonistic effect persisted after thorough washing steps in cell cultures. This work was pioneering the study of photoswitchable compounds using methods based on the locomotion monitorisation of zebrafish larvae under different illumination conditions. Interestingly, $mGlu_4$ NAM OptoGluNAM4.1 (**8**) and $mGlu_5$ NAM alloswitch-1 (**1a**) induced opposite effects in zebrafish larvae behaviour, which is consistent with the opposite roles of these receptors in the synapse [2,15]. Later, OptoGluNAM4.1 (**8**) was applied in rodent cerebellar slices at the parallel fibre-Purkinje cell synapse to study the role of $mGlu_4$ receptors in excitotoxic conditions simulating the early phases of cerebellar ischemia [17]. All these results, reveal OptoGluNAM4.1 (**8**) as a singular tool compound useful to study native $mGlu_4$ receptor activity under different physiopathological conditions both in brain tissues or in vivo.

Future directions: establishing a complete photoswitchable toolbox and quest of light-on compounds

Despite the efforts in medicinal chemistry and pharmacology to obtain photoswitchable AM for mGlu receptors, there is still considerable work to complete the photopharmacological toolbox. The AM currently reported cover all the mGlu subtypes, except $mGlu_1$ and $mGlu_3$, with either PAMs or NAMs. However, it would be desirable to provide both photoswitchable PAMs and NAMs for each mGlu subtypes. Obtaining mGlu allosteric modulators selective among the other subtypes might be a burden, specially between the group III subtypes, but future research directions may cover this

Figure 4



Structures of the current reported photoswitchable allosteric ligands for Group III mGlu receptors in their *trans* configurations with the corresponding potencies (EC_{50}). Optogluram resulted from the application of an azologisation approach to a known $mGlu_4$ PAM (VU0415374).

issue to provide better drugs to the scientific community. Additionally, pharmacological characterisation of these AMs in physiologically relevant heterodimers may also provide valuable information since it is known that mGlu ligands can have different pharmacological profiles between homo and heterodimers [34,35].

On the other hand, all the mGlu azobenzene-based allosteric modulators described up to date are more active in their *trans* configuration. From the therapeutic point of view and as tool compounds for research, active *cis* isomers would be more convenient, since these compounds would remain inactive until the isomerisation to the *cis* isomer is triggered by light in a specific location and duration. However, no mGlu *cis* active azobenzene has been described to date. Recent advances in GPCR structural biology have allowed the elucidation of the transmembrane domains of several mGlu subtypes. The planar and long azobenzene moiety can accommodate very well in the lypophyic allosteric pockets found in these receptors, as recently shown in the structure of alloswitch-1 in complex with the $mGlu_5$ TMD [36]. This allosteric binding pocket is found in a similar position as the ortosteric binding site in class A GPCRs but the mGlu allosteric binding sites are reported to be deeper and narrower [37,38]. Indeed, most allosteric ligands for mGlu receptors, with few exceptions, are typically small and compact [32] compared to some class A, which can accommodate ligands with large molecular weights or even large peptides and proteins. Therefore, it is difficult to determine if a bulkier and bent *cis* azobenzene would be able to bind in such allosteric pocket. In fact, several azobenzene compounds binding in *cis* configurations have been reported for class A GPCRs [39–43], in contrast to the absence of light-on ligands for class C GPCRs.

One possible way to circumvent this problem is the use of caged ligands. A recent example is JF-NP-26, the caged version of the $mGlu_5$ NAM raseglurant, which is an inactive compound that includes a coumarin group undergoing photolysis upon violet illumination. This powerful approach allowed the photorelease of a potent NAM (i.e. raseglurant) with high precision for the local control of the receptor in animal models of pain [44]. Another effective approach, may be the use of cyclic azobenzene photoswitches, also known as benzodiazocines, which have a thermodynamically stable *cis* isomer. Their photoisomerisation properties are opposite to the regular azobenzene. That is, UV light (385 nm) is used to switch from the thermodynamically stable *cis* isomer to the metastable *trans* form, and the back isomerisation to the *cis* configuration can be achieved with green light (525 nm) [45,46]. This approach allows to obtain photoswitchable ligands inactive in the dark, able to be specifically activated in the selected location and time. Despite being a photopharmacological approach with potential to be used in AM for mGlu receptors, no examples are reported in the literature.

Challenges for in vivo application

The major challenge in photopharmacology is the translation of the photochemical and photophysical properties of light-regulated ligands to physiological effects in complex biological systems. Certainly, a critical aspect to consider for in vivo experiments is the illumination device selected for the application. This selection will mainly depend on the targeted tissue and the organism. For instance, external illumination can be easily applied to relatively transparent small animal models, such as tadpoles or zebrafish larvae. On the other hand, the illumination systems for photopharmacological procedures in bigger organisms such as rodents or humans may be more complex depending on the target tissue. Organs, such as the skin, the auditory system or the eye may be suitable for external illumination. However, other internal organs, such as the brain, usually require implantable systems involving surgery.

Since most of mGlu receptors are expressed in the CNS, some of the cited studies in this review have pioneered this translation to in vivo models. Indeed, alloswitch-1 was the first azo-compound in which the photoswitchable activity was tested in tadpoles using external illumination [25]. Both, OptogluNAM4.1 and alloswitch-1 were used to develop one of the first medium-throughput in vivo screening of light-regulated ligands by monitoring the motility of zebrafish larvae upon different illumination conditions [13,14]. Additionally, AM for mGlu receptors also pioneered the application of photopharmacological tools in mammals using optical fibres implanted in the brain of mice. This equipment was originally developed for optogenetic stimulation. For example, allowsitch-1, optogluram and JP-NP-26 were able to modulate pain-related behaviours in mice depending on the illumination conditions, establishing new paradigms for the control of analgesia and innovative tools for the study of nociceptive pathways [15,44].

Since photopharmacology is based on the synchronised use of photoresponsive molecules and light, its application in more complex organisms requires the development of suitable devices to specifically illuminate a target organ or tissue [47]. Fortunately, as a consequence of the advent of optical imaging, microscopy and optogenetics, the development of bioelectronic devices and wireless illumination systems for optical regulation and light delivery is in constant progress and can be adapted and used in photopharmacology [48,49]. In addition, the experience gained with other biological and medical applications of light, such as photodynamic therapy, can serve as a guide for photopharmacology translation to patients. In any case, the progress of in vivo and clinical photopharmacology will always

depend on the development of novel and efficient illumination devices that might be employed in the near future, as nowadays deep brain stimulation or even the commonly used cardiac pacemakers are being clinically employed for localised electrical stimulation.

Conclusions

During the last decade, photopharmacology has been consolidated in the academic research as a new discipline between chemistry, biology, physics and engineering. Several proteins have been targeted with photocontrolled ligands, such as ion channels, enzymes and GPCRs. Specifically, photopharmacology of metabotropic glutamate receptors has been prominent in the field with several novel photoswitchable allosteric modulators covering a great part of the mGlu family. Some allosteric modulators have been validated in vitro and in native tissues, but also in several animal models, such as tadpoles, zebrafish larvae and rodents. Strikingly, in some cases this approach has helped to decipher complex biological mechanisms, such as the role of some receptors in pain transmission. However, all the reported azobenzene-based allosteric modulators are active in *trans* configuration, which is the thermodynamically stable isomer. Thus, these AM are active in the dark and can be inactivated with light. With no doubt, the opposite approach is conceptually and therapeutically more interesting since it makes use of an inactive drug that will be activated in a specific location of the organism with light. This would allow a higher control of the drug therapeutic activity. Therefore, future research lines in development of light-sensitive AM will need to consider this question. Moreover, the development of novel photoswitchable allosteric modulators to cover positive and negative modulation of the complete set of the mGlu subtypes is an interesting scientific challenge, for which the final aim would be obtaining a full photopharmacological toolbox to control the activity of mGlu receptors with high precision using light technologies.

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Conflict of interest statement

None declared.

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