Development of subtype-selective photoswitchable positive allosteric modulators for mGlu receptors

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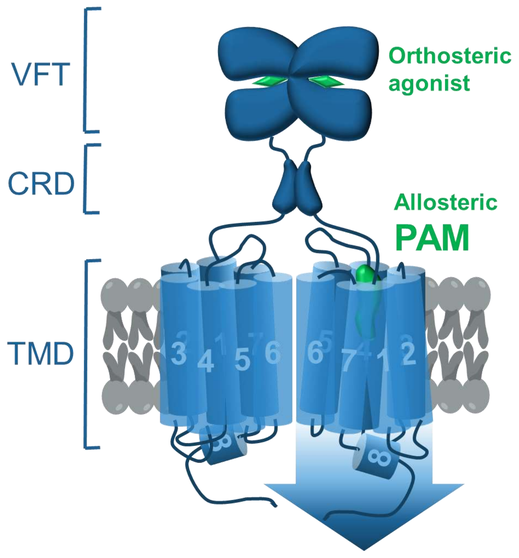
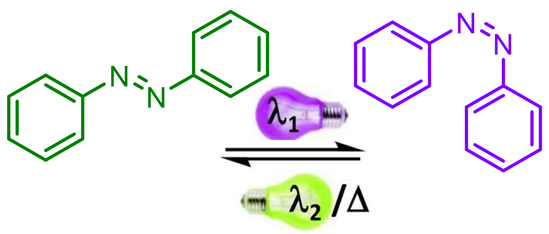
1. mGlu receptors



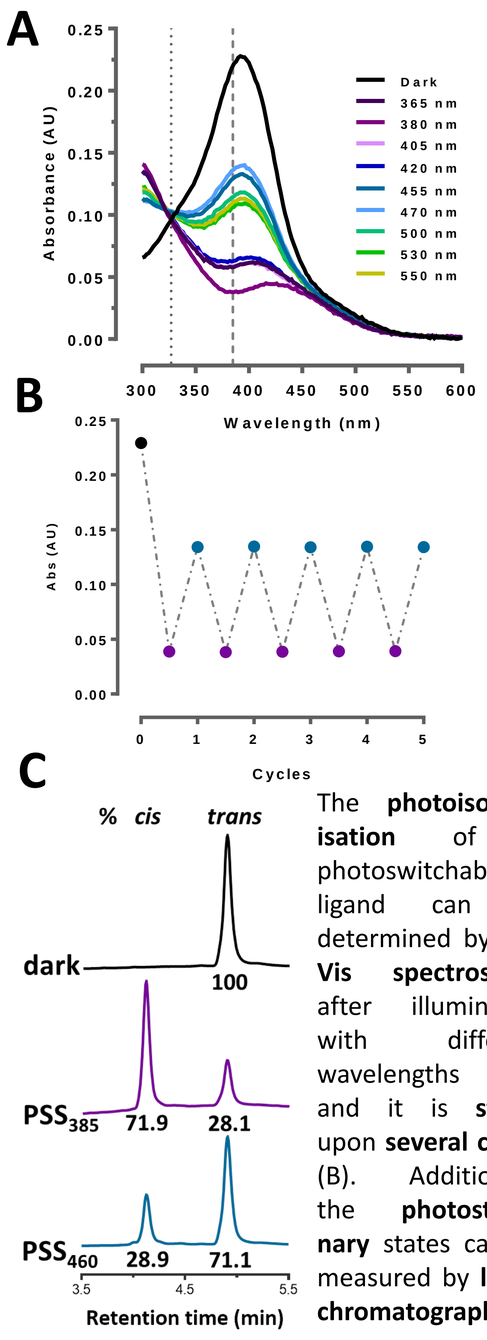
Metabotropic glutamate receptors (mGlu) belong to the class C of **GPCRS)** that form constitutively dimers. Glutamate, bind in the extracellular part. However, **allosteric ligands** can bind the **transmembrane domain** (TMD).[1]

2. Azobenzene



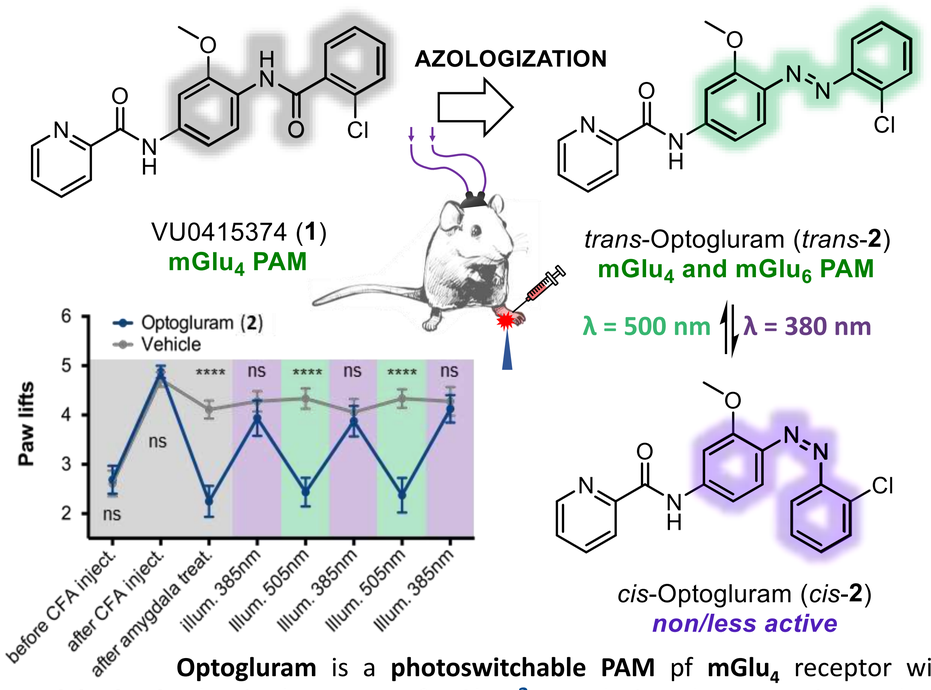
Azobenzene is a photochromic molecule that can **isomerise** upon illumination with different wavelengths, changing geometry and polarity. If azobenzene is inserted in the structure of a **GPCR ligand**, we obtain a **photoswitchable ligand,** which has different activities upon illumination wavelength.[2]

3. Photochemistry

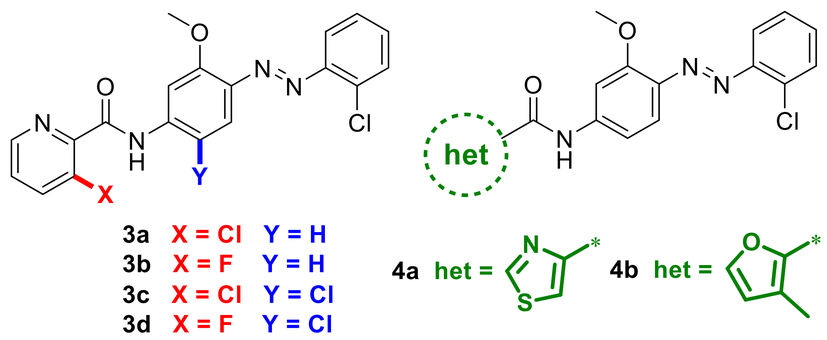


The **photoisomerisation** of a photoswitchable ligand can be determined by **UV-Vis spectroscopy** after illuminating with different wavelengths (A) and it is **stable** upon **several cycles** (B). Additionally the **photostationary** states can be measured by **liquid chromatography**

4. mGlu$_4$: Optogluram as the first photo-PAM

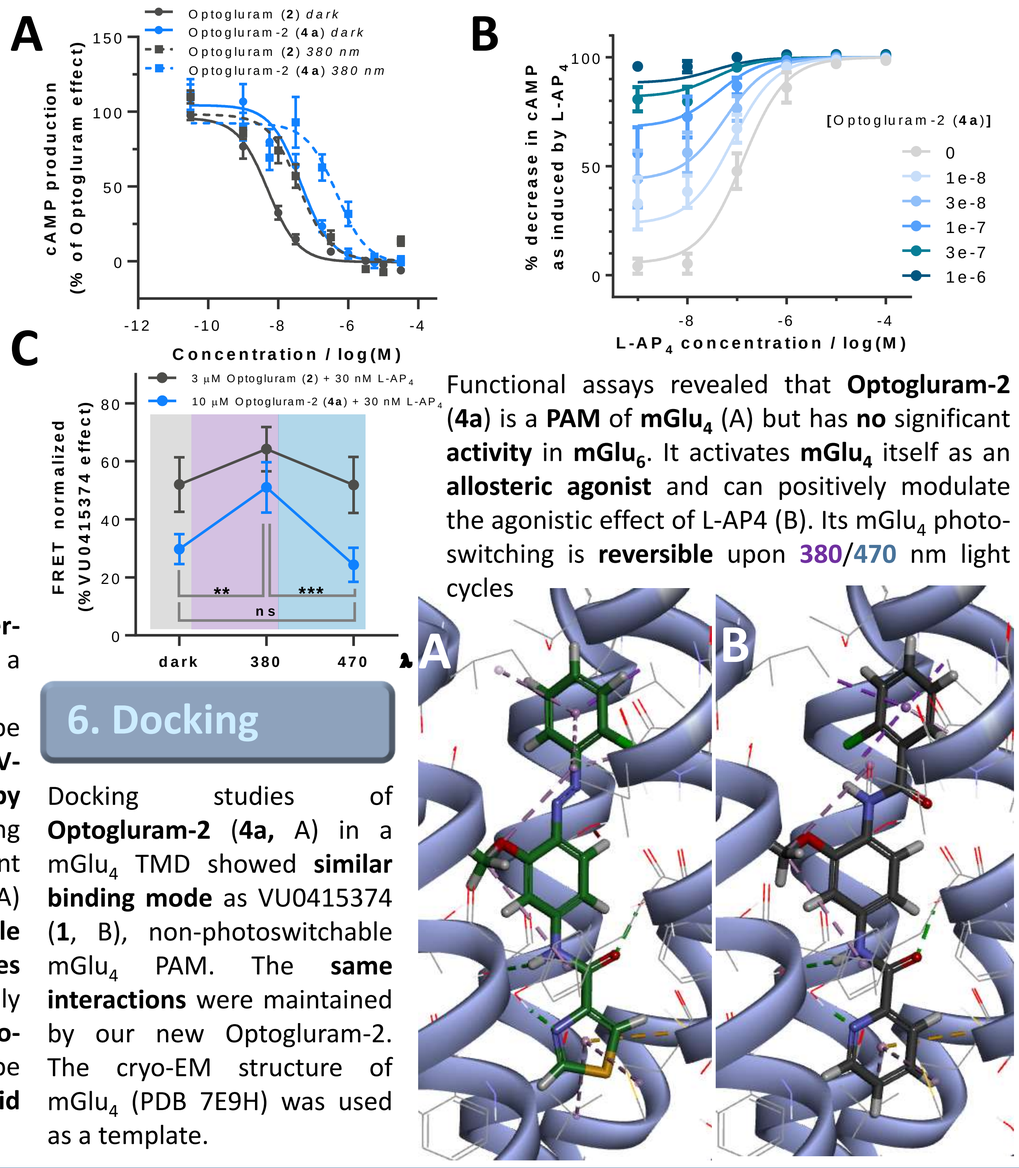


Optogluram is a **photoswitchable PAM** pf **mGlu$_4$** receptor with **activity in vivo** but is also a **PAM of mGlu$_{6/8}$**.[3] We designed a new series to obtain a photoswitchable PAM of mGlu$_4$ with **selectivity** over the **mGlu receptors**.



5. mGlu$_{4/6}$ pharmacological assays

	rat mGlu$_4$ cAMP pEC$_{50}$ ± SEM		rat mGlu$_4$ IP pEC$_{50}$ ± SEM		human mGlu$_4$ IP pEC$_{50}$ ± SEM		human mGlu$_6$ IP pEC$_{50}$ ± SEM		human mGlu$_8$ IP pEC$_{50}$ ± SEM	
Cpd.	*trans*	PSS$_{380}$	*trans*	PSS$_{380}$	*trans*	PSS$_{380}$	*trans*	PSS$_{380}$	*trans*	PSS$_{380}$
2	**8.4± 0.1**	**7.4± 0.2**	**6.4± 0.2**	**5.9± 0.2**	**6.0± 0.1**	**5.5± 0.2**	**5.3± 0.0**	**4.7± 0.0**	**5.3± 0.1**	**4.5± 0.2**
3a	6.6± 0.1	5.5± 0.2	5.1± 0.1	<4.5	5.2± 0.1	<4.5	<4.5	<4.5	4.5± 0.3	<4.5
3b	7.6± 0.2	7.0± 0.3	5.9± 0.2	5.4± 0.2	5.7± 0.1	4.9± 0.3	4.7± 0.2	<4.5	4.7± 0.1	<4.5
3c	n.t.	n.t.	n.t.	n.t.	<4.5	<4.5	<4.5	<4.5	n.t.	n.t.
3d	n.t.	n.t.	n.t.	n.t.	<4.5	<4.5	<4.5	<4.5	n.t.	n.t.
4a	**7.7± 0.2**	**6.4± 0.2**	**6.0± 0.1**	**5.4± 0.0**	**5.7± 0.1**	**5.2± 0.1**	**<4.5**	**<4.5**	**<4.5**	**<4.5**
4b	7.1± 0.4	5.9± 0.1	5.3± 0.2	5.0± 0.2	5.3± 0.1	<4.5	<4.5	<4.5	4.7± 0.2	<4.5

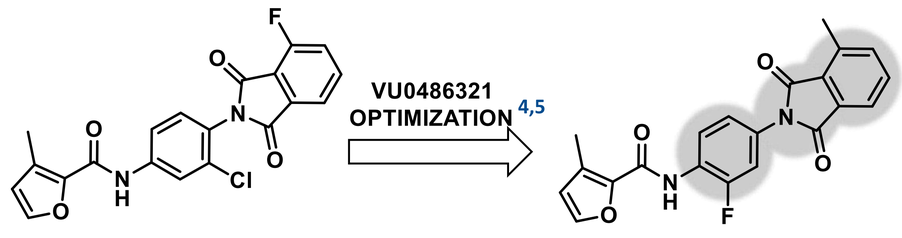


Functional assays revealed that **Optogluram-2** (**4a**) is a **PAM** of **mGlu$_4$** (A) but has **no** significant **activity** in **mGlu$_6$**. It activates **mGlu$_4$** itself as an **allosteric agonist** and can positively modulate the agonistic effect of L-AP4 (B). Its mGlu$_4$ photoswitching is **reversible** upon **380**/**470** nm light cycles

6. Docking

Docking studies of **Optogluram-2** (**4a,** A) in a mGlu$_4$ TMD showed **similar binding mode** as VU0415374 (**1**, B), non-photoswitchable mGlu$_4$ PAM. The **same interactions** were maintained by our new Optogluram-2. The cryo-EM structure of mGlu$_4$ (PDB 7E9H) was used as a template.

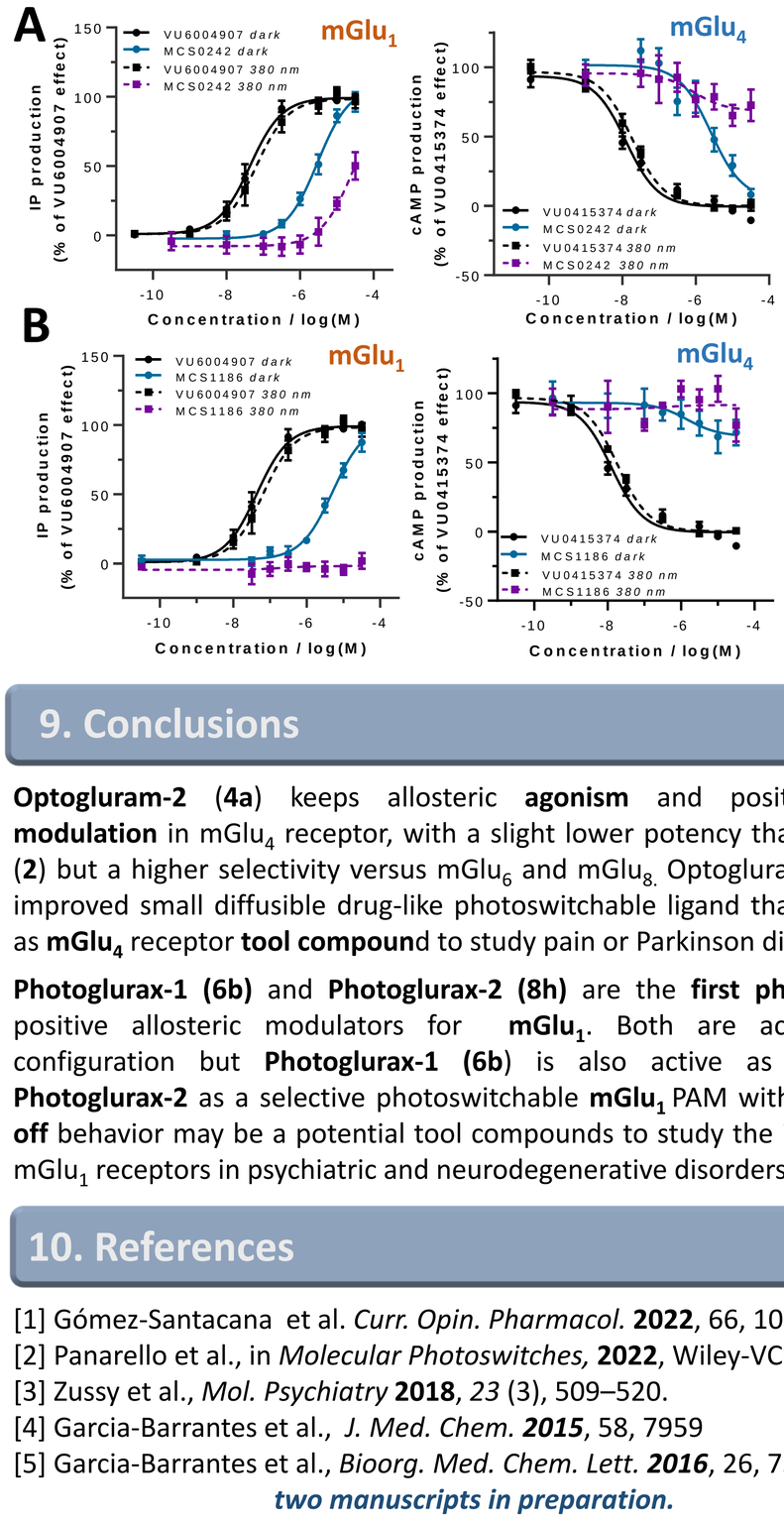
7. mGlu$_1$: development of photo-PAMs



Two series of azo-analogs of VU6004907 were synthesised to have photoswitchable **mGlu$_1$ PAMs** in trans configuration with **no mGlu$_4$ activity**

8. mGlu$_{1/4}$ pharmacological assays

	mGlu$_1$ IP pEC$_{50}$ ± SEM		mGlu$_4$ cAMP pEC$_{50}$ ± SEM			mGlu$_1$ IP pEC$_{50}$ ± SEM		mGlu$_4$ cAMP pEC$_{50}$ ± SEM	
Cpd.	trans	PSS$_{380}$	trans	PSS$_{380}$	Cpd.	trans	PSS$_{380}$	trans	PSS$_{380}$
5a	<4.5	<4.5	<4.5	<4.5	**8c**	5.3 ± 0.1	<4.5	6.0 ± 0.3	<4.5
5b	<4.5	<4.5	<4.5	<4.5	**8d**	5.3 ± 0.1	<4.5	6.0 ± 0.2	<4.5
6a	4.9 ± 0.2	4.6 ± 0.2	5.5 ± 0.2	<4.5	**8e**	<4.5	<4.5	-	-
6b	**5.6 ± 0.1**	**4.6 ± 0.1**	**5.8 ± 0.1**	**<4.5**	**8f**	<4.5	<4.5	-	-
7	4.5 ± 0.3	<4.5	5.4 ± 0.1	<4.5	**8g**	<4.5	<4.5	-	-
4b	<4.5	<4.5	7.1 ± 0.4	5.9 ± 0.1	**8h**	**5.3 ± 0.1**	**<4.5**	**<4.5**	**<4.5**
8a	5.0 ± 0.0	<4.5	5.8 ± 0.2	<4.5	**8i**	<4.5	<4.5	-	-
8b	4.9 ± 0.2	<4.5	5.9 ± 0.2	<4.5					



Functional assays revealed two hits: **Photoglurax-1** (**6b**) is a **PAM** of both **mGlu$_1$** and **mGlu$_4$** in the dark and loses activity upon 380-nm activity (A) and **Photoglurax-2** (**8h**) which is only active as **PAM** of **mGlu$_1$** in the dark (B).

9. Conclusions

Optogluram-2 (**4a**) keeps allosteric **agonism** and positive allosteric **modulation** in mGlu$_4$ receptor, with a slight lower potency than Optogluram (**2**) but a higher selectivity versus mGlu$_6$ and mGlu$_8$. Optogluram-2 (**4a**) is an improved small diffusible drug-like photoswitchable ligand that can be used as **mGlu$_4$** receptor **tool compoun**d to study pain or Parkinson disease in vivo.

Photoglurax-1 (6b) and **Photoglurax-2 (8h)** are the **first photoswitchable** positive allosteric modulators for **mGlu$_1$**. Both are active in *trans* configuration but **Photoglurax-1 (6b**) is also active as **mGlu$_4$ PAM**. **Photoglurax-2** as a selective photoswitchable **mGlu$_1$** PAM with excellent **on-off** behavior may be a potential tool compounds to study the implications of mGlu$_1$ receptors in psychiatric and neurodegenerative disorders.

10. References

[1] Gómez-Santacana et al. *Curr. Opin. Pharmacol.* **2022**, 66, 102266
[2] Panarello et al., in *Molecular Photoswitches,* **2022**, Wiley-VCH, Ch37
[3] Zussy et al., *Mol. Psychiatry* **2018**, *23* (3), 509–520.
[4] Garcia-Barrantes et al., *J. Med. Chem.* ***2015***, 58, 7959
[5] Garcia-Barrantes et al., *Bioorg. Med. Chem. Lett.* ***2016***, 26, 751.

two manuscripts in preparation.

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