RATIONAL DESIGN OF SUBTYPE-SELECTIVE ORTHOSTERIC AGONISTS FOR GROUP III METABOTROPIC GLUTAMATE RECEPTORS

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A COLLABORATIVE APPROACH WITH THE UNIVERSITÉ PARIS DESCARTES

Group III metabotropic glutamate receptors (mGlu) have been shown to be potential targets for numerous central nervous system (CNS) diseases. Selectively modulating their activity offers strong therapeutic potential for Parkinson's disease, pain, epilepsy, schizophrenia and anxiety. However, previous attempts at subtype-selective orthosteric modulators have met limited success due to either a lack of specificity or bioavailability. To address this, the group of Dr. Francine Acher at the Université Paris Descartes explored the possibility of developing new subtype-selective agonists for group III mGlu. Docking studies of various candidate molecules carried out in BIOVIA Discovery Studio were used to corroborate experimental results and elucidate their underlying mechanisms of action. The docking studies revealed three potential binding sites: the primary glutamate binding site and two chloride-binding sites. It was further revealed that variations in the side chains bridging these separate binding sites between different subtypes could sterically modulate this binding. Subsequent *in vivo* studies confirmed the therapeutic potential of subtype-selective ligands generated from the *in silico* findings. As a result, the deeper understanding of the differences in the binding mode between different mGlu subtypes offers a compelling basis for the design and optimization of future drug candidates.

INTRODUCTION

G protein-coupled receptors (GPCRs), a broad family of integral membrane proteins which translate extracellular stimuli into intracellular signals, constitute one of the largest protein families, accounting for approximately 4% of the protein-coding genome[1]. Their diversity of function and widespread expression in human tissue makes them a prime candidate for drug targets: as of 2017 20-30% of FDA-approved treatments target GPCRs[1]. Metabotropic glutamate (mGlu) receptors, which belong to the Class C GPCR subfamily, modulate a wide range of synaptic activities in the central nervous system (CNS)[2]. These receptors in turn possess eight subtypes (mGlu1-8) which have been classified by their sequence similarity, transduction mechanism and pharmacological profile into three groups. Due to their tendency to presynaptically inhibit neuronal activity, receptors from groups II and III exhibit strong potential as therapeutic targets for pathologies where an excess of neurotransmitter release has been implicated, including Parkinson's disease, anxiety, epilepsy, addiction, and pain[3].

While subtype-selective positive (PAMs) and negative allosteric modulators have been previously identified via high-throughput screening[4], developing subtype-selective orthosteric ligands for mGlu receptors has proven difficult due to the high degree of conservation in the endogenous ligand binding site. Class C GPCRs are noted for their structure: a large bilobate extracellular domain which acts as the glutamate binding site, known as the "Venus Flytrap" domain (VFD); a core domain which consists of seven transmembrane helices (7TM), the hallmark of all GPCRs; and a cysteine-rich domain which connects the two. Class C receptors also function as obligatory dimers (Figure 1). Previous attempts to create PAMs for mGlu receptors by targeting the 7TM domain have received mixed success: while animal models utilizing mGlu PAMs have demonstrated anti-Parkinsonian activity[5], their hydrophobicity could limit their metabolic stability. Orthosteric agonists offer nontrivial therapeutic potential as evidenced in clinical trials and can cross the blood-brain barrier[6]; their oral bioavailability can also be improved with synthesis via prodrugs[7].

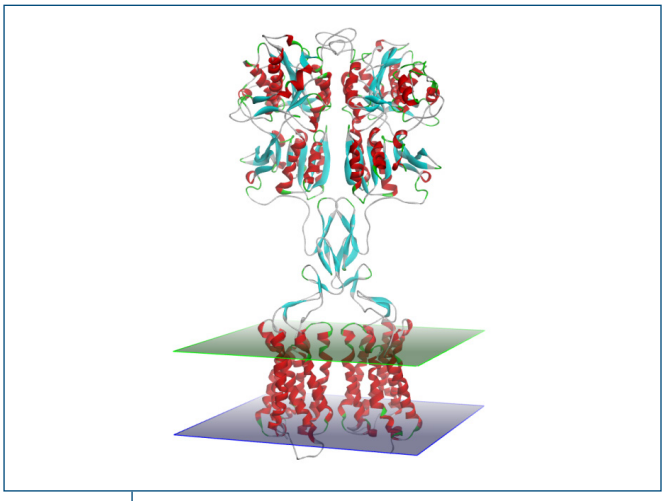

Figure 1: A Class C GPCR dimer in the active "closed" conformation.

This project, a joint collaboration between BIOVIA and the Université Paris Descartes, seeks to identify subtype-selective orthosteric ligands for group III mGlu receptors. This collaboration has resulted in the disclosure of LSP4-2022, a novel orthosteric ligand specific to mGlu4[8] via interaction with two potential sites modulated by chloride ions ***in vivo***[3,9]. Taken together, these results provide a compelling basis for the design of future subtype-selective ligands to tackle various CNS diseases and a method for identifying subtype-selective ligands for other receptors.

METHODOLOGY

Homology Modeling

Homology models of the VFD of mGlu4 and mGlu8 were generated with the MODELER component within BIOVIA Discovery Studio to assess the protein-ligand interactions between the various subclasses of mGlu receptors. Human, mouse and rat mGlu sequences were sourced from the UniProt database[10] and aligned with BIOVIA Discovery Studio[3]. X-ray structures of mGlu2[11] and mGlu5 bound to glutamate were used to further refine the glutamate binding pocket for mGlu4 and mGlu8, respectively[3].

Docking Studies of LSP4-2022 and Other Ligands

All calculations were performed in BIOVIA Discovery Studio. Models were further used for docking studies, both rigid (CDOCKER) and flexible (GOLD). In these simulations, residues from chloride binding sites 1 and 2 were selected for their probable role in creating the binding specificity for mGlu4: Arg60, Lys74, Thr108, Ser110, Ser157, Ser160, Arg258, Asn286, Glu287, and Ser313[3]. Molecular dynamics simulations were calculated within Discovery Studio utilizing NAMD and CHARMM. Once the trajectory for each interaction was equilibrated, individual snapshots were assessed using Discovery Studio and submitted to energy minimization[3,8].

RESULTS

Target Analysis

Previous work[12] had identified (R)-PCEP (3-amino-3-carboxypropyl-2'-carboxyethylphosphinic acid), a new agonist of mGlu4 as determined via virtual screening. A subsequent study measuring the EC50 of (R)-PCEP and its derivatives against recombinant mGlu4 determined that the (S)-enantiomer was more potent than (R)-PCEP[12] (Figure 2). Homology modeling and docking showed that the distal carboxylate group could interact with a new site, which naturally binds a chloride ion (Figure 3, site 1).

Further work[9] has also shown that there is a second chloride site in the cleft of the VFD near the glutamate orthosteric site in mGlu4 and other mGluRs besides mGlu2, bridging the two lobes in the closed active state (Figure 3). While site 1 is conserved between mGlu receptors and is found within a pocket only in lobe 1, site 2 binds to both. This allows residues on separate lobes, Ser160 and Asn286, to form H-bonds, potentially stabilizing the binding of the active "closed" state for the VFD[3]. This suggests that functional groups which can mimic a chloride ion, such as the distal carboxylate in (S)-PCEP, can serve a similar role; molecular dynamics simulations revealed that site 2 binding can occur for some agonists[9].

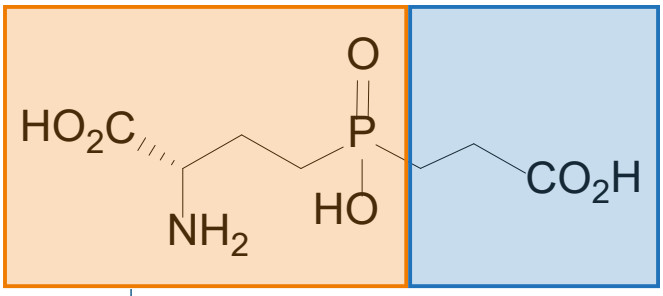

Figure 2: (S)-PCEP. This molecule shows a glutamate like part (orange). The distal group (blue) was modified to design and synthesize the optimized derivatives depicted in this study.

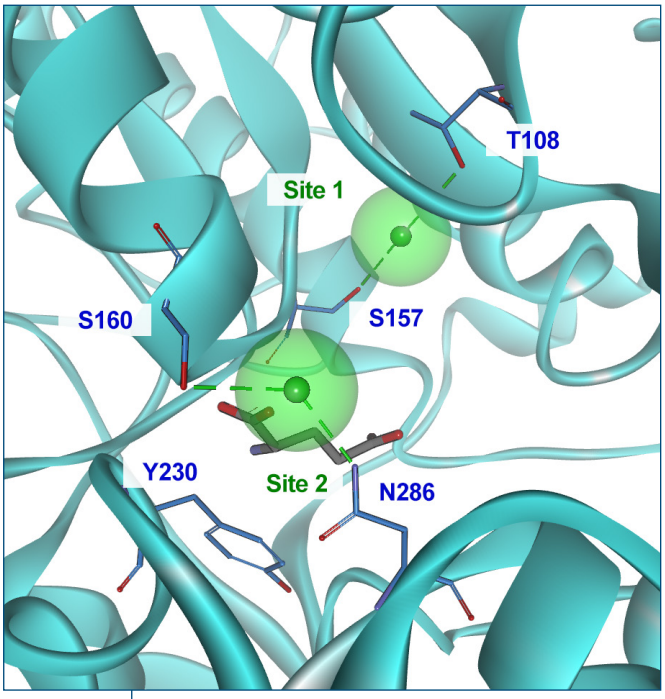

Figure 3: Homology model of the mGlu4 VFD binding glutamate (gray) with the two chloride ions in sites 1 and 2 (green).

Agonist Structure

The identification of two potential binding sites – the glutamate orthosteric site and the chloride binding sites – provided a key consideration for designing subtype-selective ligands. The enantiomer of the initial virtual high throughput screening (vHTS) hit, (S) PCEP, interacts with chloride site 1; however, it did not show any subtype selectivity. This is likely due to a lack of interaction with the variable side chains surrounding the chloride binding sites as suggested by subsequent docking and mutagenesis experiments[13]. As a result, dualsteric ligands (i.e. ligands which can bind at both sites) could provide a basis for subtype selectivity[3].

Further chemical optimization via docking studies of (S)-PCEP led to the development of aromatic derivatives such as LSP1-2111 (Figure 4, top). Indeed, this compound proved to be mGlu4 subtype preferential. Further improvements provided LSP4-2022 (Figure 4, bottom), that is subtype 4 selective[8].

The structures of LSP4-2022 and LSP1-2111 possess multiple advantages for further optimization. Their aromatic ring offers multiple substitution sites to improve the performance of the ligand. Additionally, the distal carboxylate of LSP4-2022 is flexible enough to reach and fully interact with both chloride binding sites (Figure 5). This is supported by its strong potency to activate mGlu4: whereas mGlu4 activation by glutamate is normally highly sensitive to chloride concentration, LSP4-2022 has been shown to activate mGlu4 even at low chloride concentration[9].

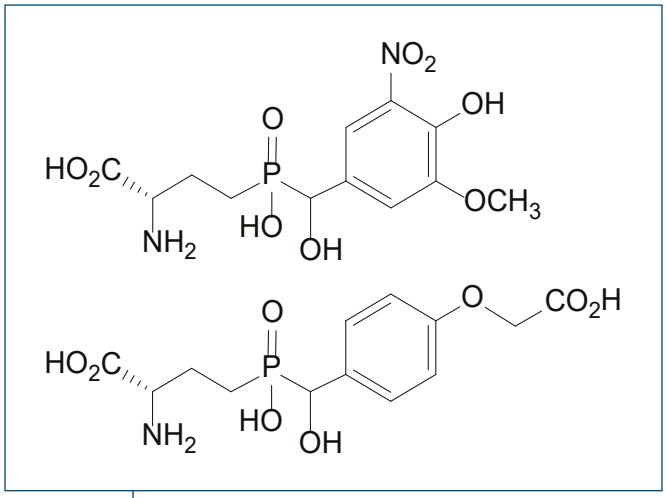

Figure 4: LSP1-2111 (top) and LSP4-2022 (bottom).

Characterization of the Agonist Binding Mode in mGlu4 vs. mGlu8

Assessments of the interactions of LSP4-2022 and LSP1-2111 on mGlu4 and mGlu8 have demonstrated selective agonist activity, with an EC50 ratio for mGlu4/mGlu8 of over 250 and 30, respectively. While their glutamate binding sites are 91% conserved, Ser157 and Gly158 in mGlu4 are changed to Ala154 and Ala155 in mGlu8. Docking studies of homology models of mGlu4 and mGlu8 revealed that the amino acid and phosphinic acid portions of LSP1-2111 behave similarly between them; however, the methyl side group of Ala155 in mGlu8 creates hydrophobic π-interactions with the phenyl group of LSP1-2111, preventing its nitro group from creating stabilizing H-bonds with chloride site 2. This could be further supported by H-bond interactions between the nitrophenolate group and Ser107 in mGlu8, which stabilizes the docking of the nitro group outside of site 2. As a result, these interactions could explain the experimentally observed specificity for LSP4-2022 and LSP1-2111[3].

Anti-Parkinsonian Activity of LSP1-2111 and LSP4-2022

In addition to the *in silico* studies of the characteristics of LSP1-2111 and LSP4-2022, *in vivo* studies of the potential of its anti-Parkinsonian activity were assessed via its ability to inhibit induced Parkinsonism in rats[8,14]. The first example[14] explored the ability of LSP1-2111 to impact denervation of dopamine

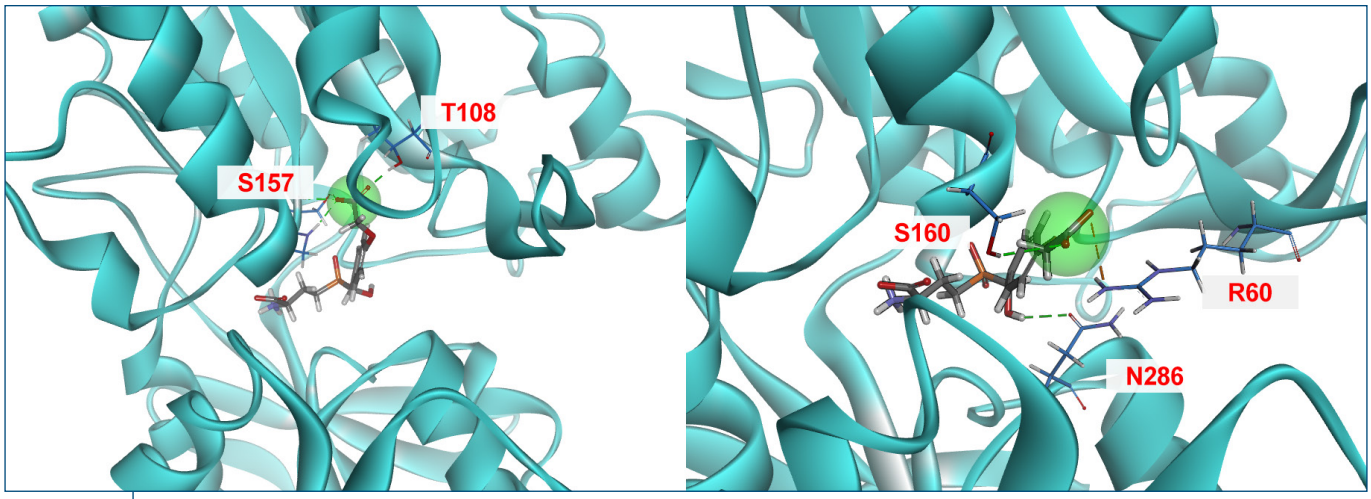

Figure 5: Docking poses for LSP4-2022 at the two chloride sites: site 1 (left) and site 2 (right). Key interaction residues in both sites are specified in red.

receptors by 6-hydroxydopamine (6-OHDA) in rats. Akinesia was measured via the rat's reaction time: subjects were to release a lever within 600 ms of a cue light flashing. Prior to testing, rats were trained on this task for three months. Following the training, subjects were assessed for a week, given a bilateral injection of 6-OHDA in the dorsal striatum of their brains, and given 7 days to recover before postoperative testing. Intrapalladial injections of varying concentrations of LSP1-2111 were given to the subjects and underwent the test, with mean reaction times being recorded for each dose. In each case, LSP1-2111 both lowered the mean reaction time and the number of "delayed" responses compared to postoperative and zero dose results, even returning their performance to preoperative levels.

In a second test, haloperidol was used to induced catalepsy in rats[8,14]. 60 minutes after an intraperitoneal injection of haloperidol, the animals received either an intracerebroventricular or systemic injection of LSP1-2111 or LSP4-2022. The animals were then administered a horizontal bar test: each animal was timed how long it took to step down from a horizontal bar at 30 minute intervals. In both cases, the mean "step down time" was significantly reduced at t=30, with a generally sustained inhibition of cataleptic activity through t=90 for LSP4-2022[8] and t=120 for LSP1-2111[14]. These tests demonstrate the capability of LSP1-2111 and LSP4-2022 to not only inhibit Parkinsonian behavior but also highlights their ability to cross the blood-brain barrier[8,14].

DISCUSSION AND CONCLUSION

Molecular dynamics simulations helped to explain experimentally observed results by elucidating stable conformations of various ligands docked with mGlu receptors. This confirmed the role of the distal carboxylate in LSP4-2022 and the nitro group of LSP1-2111 as mimics of chloride ions, which supported the lower sensibility of mGlu4 to variations in chloride concentration. The ability for these ligands to interact with the second chloride binding site and stabilize the active "closed" conformation of mGlu4 demonstrated their potency as orthosteric ligands, while the inhibition of this binding due to Ala155 and Ser107 in mGlu8 corroborated experimentally observed subtype specificity. Further *in vivo* work extended these results to show the anti-Parkinsonian behavior of these ligands. Together, these findings support the conclusion that LSP4 2022 and derivatives are powerful subtype-selective orthosteric ligands for mGlu4[3]. Furthermore, this study provides a compelling example for the rational design of subtype-selective orthosteric ligands from vHTS hit to lead compound via a joint approach of modeling, chemical synthesis and bio-assays.

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