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Insights into the interaction of negative allosteric modulators with the metabotropic glutamate receptor 5: Discovery and computational modeling of a new series of ligands with nanomolar affinity

Andrew Anighoro [a,†], Davide Graziani [b,†], Ilaria Bettinelli [b], Antonio Cilia [b], Carlo De Toma [b], Matteo Longhi [b], Fabio Mangiarotti [b], Sergio Menegon [b], Lorenza Pirona [b], Elena Poggesi [b], Carlo Riva [b], Giulio Rastelli [a,*]

[a] Life Sciences Department, University of Modena and Reggio Emilia, Via Campi 103, 41125 Modena, Italy
[b] Drug Discovery Department, Recordati S.p.A. Via Civitali 1, 20148 Milan, Italy

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ABSTRACT

Metabotropic glutamate receptor 5 ($mGlu_5$) is a biological target implicated in major neurological and psychiatric disorders. In the present study, we have investigated structural determinants of the interaction of negative allosteric modulators (NAMs) with the seven-transmembrane (7TM) domain of $mGlu_5$. A homology model of the 7TM receptor domain built on the crystal structure of the $mGlu_1$ template was obtained, and the binding modes of known NAMs, namely MPEP and fenobam, were investigated by docking and molecular dynamics simulations. The results were validated by comparison with mutagenesis data available in the literature for these two ligands, and subsequently corroborated by the recently described $mGlu_5$ crystal structure. Moreover, a new series of NAMs was synthesized and tested, providing compounds with nanomolar affinity. Several structural modifications were sequentially introduced with the aim of identifying structural features important for receptor binding. The synthesized NAMs were docked in the validated homology model and binding modes were used to interpret and discuss structure–activity relationships within this new series of compounds. Finally, the models of the interaction of NAMs with $mGlu_5$ were extended to include important non-aryl alkyne $mGlu_5$ NAMs taken from the literature. Overall, the results provide useful insights into the molecular interaction of negative allosteric modulators with $mGlu_5$ and may facilitate the design of new modulators for this class of receptors.

1. Introduction

Glutamate, the major excitatory neurotransmitter in the central nervous system, acts on two distinct classes of receptors: ionotropic glutamate receptors to elicit fast excitatory responses, and metabotropic glutamate receptors (mGlu) to modulate synaptic transmission.[1] Ionotropic glutamate receptors have been classified into NMDA, AMPA and kainic acid receptor subtypes, according to the ability of ligands to bind to the different receptor sites and to produce distinct physiological effects.[2] Since the mid-1980s, evidences of the existence of a large family of metabotropic glutamate receptors coupled to effector systems through GTP-binding proteins have been reported. The mGlus belong to class C G-protein coupled receptors (GPCRs), and are characterized by an unusually large extracellular amino-terminal domain (ATD) (~500–600 amino acids) with no sequence homology to other families of GPCRs. Although the sequence homology among class C members is low (about 20% amino acid identity), these receptors are structurally related. O'Hara et al. observed that the ATD of $mGlu_1$ shows some degree of similarity with a family of bacterial periplasmic amino acid-binding proteins,[3] in particular the leucine-, isoleucine- and valine binding protein (LIVBP).[4] Based on the crystal

Abbreviations: DMAC, *N*,*N*-dimethylacetamide; DMF, *N*,*N*-dimethylformamide; LiHMDS, lithium bis(trimethylsilyl)amide; $mGlu_5$, metabotropic glutamate receptor subtype 5; NAM, negative allosteric modulator; GPCR, G-protein coupled receptor; TFA, trifluoroacetic acid; TLC, thin layer chromatography; MD, molecular dynamics; rt, room temperature.

* Corresponding author. Tel.: +39 059 2058564; fax: +39 059 2055131.
E-mail address: giulio.rastelli@unimore.it (G. Rastelli).
† These authors contributed equally.

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structure of LIVBP, they proposed a bilobal structure for the agonist-binding pocket of mGlu receptors in which the glutamate is bound in a 'venus flytrap' mechanism. To date, eight metabotropic glutamate receptor subtypes have been cloned and classified into three groups on the basis of sequence similarity, agonist and antagonist binding profiles, and preferred coupling to signal transduction pathways. Group I receptors ($mGlu_1$ and $mGlu_5$) preferentially couple via Gq/11 proteins to stimulate phospholipase C activity but are differentially localized and mediate distinct physiological functions.[5–8] Activation of these receptors elicits highly distinct Ca^{2+} responses at cellular level. $mGlu_1$ primarily elicits a peak-plateau type Ca^{2+} response, whereas $mGlu_5$ leads to oscillatory changes in intracellular Ca^{2+} concentration in both recombinant and native (e.g., astrocyte) cell environment.[9–13] Group II receptors ($mGlu_2$ and $mGlu_3$) are negatively coupled to cAMP production and are not stimulated by L-(±)-2-amino-4-phosphono butyric acid (L-AP4), and finally group III receptors ($mGlu_4$, $mGlu_6$, $mGlu_7$, and $mGlu_8$) are negatively coupled to cAMP production but are activated by L-AP4.[1,14]

Because of their critical role as modulators of synaptic transmission, ion channel activity, and synaptic plasticity,[15,16] mGlu receptors are implicated in major neurological disorders such as Alzheimer's and Parkinson's disease as well as depression, schizophrenia, anxiety, and pain.[17,18] The ongoing interest in $mGlu_5$ as a drug target is borne out by the large number of companies focused on the discovery of new ligands and new chemotypes acting as negative allosteric modulators of this receptor. Clinical studies that received major consideration include drugs for the treatment of the L-DOPA induced dyskinesia in Parkinson's disease (PD-LID), anxiety, depressive disorders, and the treatment of Fragile X syndrome (FXS). $mGlu_5$ antagonists involved in phase II clinical trial studies include AFQ056/mavoglurant (Novartis) for PD-LID and FXS, ADX48621/Dipraglurant (Addex Therapeutics) for PD-LID, and RG7090/Basimglurant (Roche) for FXS and major depressive disorders. Very recently, Novartis and Roche announced the suspension of the development of mavoglurant and basimglurant for Fragile X syndrome. The other trials are still on-going, but so far none of the $mGlu_5$ candidates have progressed to phase III. In conclusion, despite the extensive efforts, the identification of potent, selective and clinically efficacious candidates does still represent a highly desirable goal.

Recently, the crystal structure of the transmembrane domain of $mGlu_1$ in complex with the negative allosteric modulator 4-Fluoro-*N*-[4-[6-(isopropylamino)pyrimidin-4-yl]-1,3-thiazol-2-yl]-*N*-methylbenzamide (FITM)[19] has been reported. The structure, which was the first of a class C GPCR, provided important insights into the architecture of the seven-transmembrane (7TM) domain of $mGlu_1$ and revealed the location of the allosteric modulator binding site, providing a key framework for understanding molecular recognition and enabling the structure-based design of new modulators for this class of receptors.

Here we report a homology model of the 7TM domain of $mGlu_5$ based on the crystal structure of the high sequence identity (77%) $mGlu_1$ template. Moreover, we describe the complexes of $mGlu_5$ with MPEP and fenobam (Scheme 1), two established negative allosteric modulators (NAMs), predicted by means of docking and molecular dynamics simulations. The structural models were validated using mutagenesis data available in the literature, and subsequently corroborated by the recently described crystal structure of $mGlu_5$ in complex with mavoglurant.[20] Finally, the validated models were used to predict the binding mode of a newly synthesized series of NAMs based on arylpropiolic acid piperazine amides and 4-(3-arylprop-2-ynylidene)piperidine scaffolds[21] (Scheme 1) and to discuss structure–activity relationships within this class. The structures of these compounds were elaborated starting from the piperazine propionamide scaffold (compound **5** in Scheme 1) reported by Euro-Celtique[22] (now Purdue Pharma) by replacing the amide group with a double bond, followed by terminal aryl optimization. To this aim, a subset of NAMs synthesized during our medicinal chemistry efforts[21] was carefully selected by including molecules with key structural variations and different activities. In particular, key compounds were selected in consideration of their suitability to investigate and probe the effects of the substitution pattern, conformational requirements, and other features of the $mGlu_5$ allosteric pocket relevant for receptor binding. The compounds were docked into the receptor structure and the resulting binding modes and structure–activity relationships were used to further elaborate and probe the model of the interaction of NAMs with the allosteric pocket. The reduction of the acetoamido group of compound **5** (rat K_i 0.6 nM, human K_i 0.5 nM) provided compound **12** with significantly reduced binding affinity in both rat and human species (rat K_i 117.4 nM, human K_i 60.7 nM). Reestablishing planarity with the introduction of a double bond, such as in compound **13** (rat K_i 0.6 nM, human K_i 0.4 nM), restored high affinity for $mGlu_5$ and excluded the possibility that a hydrogen bond acceptor mapped on the carbonyl was strictly necessary for binding. After terminal aryl optimization potent (nanomolar) and selective NAMs of $mGlu_5$ were obtained. Finally, the model of the interaction of NAMs with $mGlu_5$ was further extended by docking clinically-important non-aryl alkyne $mGlu_5$ NAMs taken from the literature.

2. Results and discussion

2.1. Putative MPEP and fenobam binding modes in the $mGlu_5$ homology model

A refined homology model of the 7TM domain of $mGlu_5$ was built on the basis of the recently reported $mGlu_1$ crystal structure template[19] (see experimental methods for details). The two receptors share high sequence identity (77%). Then, docking of MPEP and fenobam in the homology model was performed, and the predicted binding poses were compared with the mutagenesis data available for these two ligands. Mutagenesis studies suggest that fenobam, *N*-(3-chlorophenyl)-*N*′-(4,5-dihydro-1-methyl-4-oxo-1*H*-imidazole-2-yl)urea (compound **3** in Scheme 1) and MPEP, 2-methyl-6-(phenylethynyl)pyridine, (compound **1**) share the same or at least a partially overlapping binding site.[23–27] The Y659V, W785A, F788A, and A810V mutations abolished MPEP and fenobam binding. In addition, P655S, S658C, and T781A mutations severely impaired fenobam binding but left MPEP binding almost unaffected. Remarkably, in the generated model the residues mentioned above clearly define the walls of a pocket having a size and shape able to accommodate the two NAMs. Interestingly, superposition of the $mGlu_1$ crystal structure with the $mGlu_5$ homology model shows that the area encompassed by these key residues overlaps only partially with the binding pocket of FITM, i.e. the NAM co-crystallized with $mGlu_1$ (Fig. 1A). For instance, the fluorophenyl ring of FITM, which points to the bottom of the pocket toward the intracellular side, is found in proximity of W785, F788 and T781 but is ~8 Å distant from A810 and S658, and the substituted pyrimidine ring, which extends toward the opposite extracellular side, is >5 Å distant from other residues identified as important by mutagenesis of $mGlu_5$. Compared to $mGlu_1$, $mGlu_5$ exhibits an additional sub-pocket recently proposed to become accessible in $mGlu_5$ because of the serine to proline 655 replacement.[19,20,28] Interestingly, we observed that A810, one of the residues lining this intracellular sub-pocket, is a valine in $mGlu_1$. As a consequence of the bulkier side chain, the pocket is