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Cyclopropyl-containing positive allosteric modulators of metabotropic glutamate receptor subtype 5

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ABSTRACT

Positive allosteric modulators (PAMs) binding to the transmembrane (TM) domain of metabotropic glutamate receptor 5 (mGluR5) are promising therapeutic agents for psychiatric disorders and traumatic brain injury (TBI). Novel PAMs based on a *trans*-2-phenylcyclopropane amide scaffold have been designed and synthesized. Facilitating ligand design and allowing estimation of binding affinities to the mGluR5 TM domain was the novel computational strategy, site identification by ligand competitive saturation (SILCS). The potential protective activity of the new compounds was evaluated using nitric oxide (NO) production in BV2 microglial cell cultures treated with lipopolysaccharide (LPS), and the toxicity of the new compounds tested using a cell viability assay. One of the new compounds, **3a**, indicated promising activity with potency of 30 μM, which is 4.5-fold more potent than its lead compound 3,3′-difluorobenzaldazine (DFB), and showed no detectable toxicity with concentrations as high as 1000 μM. Thus this compound represents a new lead for possible development as treatment for TBI and related neurodegenerative disorders.

Traumatic brain injury (TBI) is a highly prevalent neurodegenerative disorder with no proven neuroprotective therapies.[1] TBI induces chronic neuroinflammation associated with microglial activation,[2–4] which contributes to delayed neuronal cell death and functional disabilities.[5–10] Recent experimental evidence has shown that such secondary injury in the central nervous system (CNS) may last for months to even years, associated with progressive neurodegeneration.[11–13] Metabotropic glutamate receptor 5 (mGluR5) is commonly found in neurons and astrocytes, and is highly expressed in microglial cells.[3] Recent work has shown that activation of mGluR5 can effectively inhibit microglial activation as late as 1 month after experimental trauma.[10] Activation of mGluR5 can also block the neurotoxicity of activated microglia in vitro and in vivo.[10,14,15] Therefore mGluR5 has emerged as a promising neuroprotective drug target for TBI.

The structure of mGluR5 includes an N-terminal ligand-binding domain (LBD) and a seven-helical transmembrane (TM) domain. Although numerous orthosteric mGluR5 agonists are known,[16–19] none have been used in the clinic largely due to the challenge in identifying selective[20] and CNS permeable agonists of the receptor. Recent advances in the development of positive allosteric modulators (PAMs), by targeting the seven-helical trans-membrane (TM) of mGluR5, have provided new opportunities for discovery of therapeutic agents for TBI. Because the TM domains mGluRs are less conserved and the ligands to the hydrophobic TM domain do not require the charged amino acid character as for mGluR5 agonists, PAMs have greater potential to achieve specificity for mGluR5 and have a higher potential for CNS penetration compared to LBD binders. Numerous mGluR5 PAMs have been reported.[12,13,21–26] Of these mGluR5 PAM VU0360172 has shown promising in vivo efficacy for TBI,[15] and in vivo efficacy in rodent models for anxiety and psychosis.[27]

In previous studies we found that mGluR5 PAM 3,3′-difluorobenzaldazine (DFB, Fig. 1) showed potential protective activity (IC_{50} = for 136 μM NO production).[28] However, DFB, along with other tested PAMs, have limitations such as modest efficacy, significant cellular toxicity, and poor aqueous solubility. In addition, the azo group of DFB is light sensitive. Here we describe the design, synthesis and evaluation of mGluR5 PAMs (**1**–**3**) based on a *trans*-2-phenylcyclopropyl amide scaffold. The chemical structures of compounds **1**–**3** were chosen to mimic the planar (1*E*,2*E*)-1,2-dibenzylidenehydrazine core of DFB while maintaining favorable interactions with the receptor based on computer-aided drug design (CADD, see below). We hypothesize that improved PAMs

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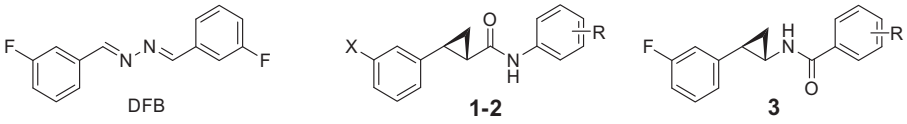

Figure 1. Chemical structures of DFB and compounds **1**–**3**. Only one of the two enantiomers of the racemic mixture is shown for compounds **1**–**3**.

can be achieved by replacing the azo linker of DFB with a photo stable *trans*-cyclopropyl amide group that is commonly used by natural and synthetic drugs (Fig. 1). The *trans*-cyclopropyl moiety is selected to break the planar configuration of the compounds. The efforts also included testing the effects of the orientation of the amide linker for the neuroprotective potency of new compounds.

To facilitate ligand design we undertook CADD analysis of the PAM binding region of the mGluR5 TM domain. CADD analysis involved the site identification by ligand competitive saturation (SILCS) approach[29] on a homology model of the TM domain of mGluR5 derived from the mGluR1 crystal structure (PDB: 4OR2).[30] SILCS calculations and ligand modeling used the CHARMM36 and CGenFF force field along with the programs MODELLER, CHARMM, and GROMACS. SILCS is a method that maps the functional group affinity patterns of a protein. The method accounts for both protein flexibility and desolvation contributions by running molecular dynamics (MD) of the protein in an aqueous solution of the small solute molecules representative of different chemical functional groups.[31] To sample the partially occluded ligand binding pocket of the mGluR5, we applied an extension of the SILCS method that involves an iterative Grand Canonical Monte Carlo/MD (GCMC/MD) methodology.[32,33] From the simulations, discretized probability distributions of the fragment atoms that are normalized by their bulk values are obtained and then converted to free energies based on a Boltzmann distribution, yielding Grid Free Energy (GFE) FragMaps. The maps thus represent the 3D free energy distribution of functional group binding at the ligand binding pocket and may be used both qualitatively and quantitatively to direct ligand design. In the current work, eight representative solutes with different chemical functionalities: benzene, propane, acetaldehyde, methanol, formamide, imidazole, acetate and methylammonium were chosen to probe the ligand binding pocket of mGluR5. Benzene and propane serve as probes for nonpolar functionalities. Methanol, formamide, imidazole and acetaldehyde are neutral molecules that participate in hydrogen bonding. The positively charged methylammonium and negatively charged acetate molecules serve as probes for charged donor and acceptors, respectively. The voxel occupancies of the eleven atom types were merged in the following manner to create five generic FragMap types: (1) generic nonpolar, APOLAR (benzene and propane carbons); (2) generic neutral hydrogen bond donor, HBDON (methanol, formamide and imidazole polar hydrogens); (3) generic neutral hydrogen bond acceptor, HBACC (methanol, formamide, and acetaldehyde oxygen and imidazole unprotonated nitrogen) (4) positive donor, POS (methylammonium polar hydrogens); and (5) negative acceptor, NEG (acetate oxygens). The FragMaps used in the present work were those prepared for our previous study, which includes details of the computational methods.[32,34]

Favorable FragMap affinities were found near residues R647, L743, T780 and W784, previously identified through mutational studies to be important for ligand binding and activity.[35] Presented in Figure 2A is DFB docked into the PAM binding site using Autodock-Vina[36] directed by the SILCS FragMaps. The phenyl moiety overlaps with the APOLAR FragMaps in the proximity of L743, W784 and V805 (marked A2 in Fig. 2B) indicating this region of the model to be important for binding.

This information motivated the design of the novel scaffolds (compounds **1**–**3**) based on a *trans*-2-phenylcyclopropyl amide scaffold. Docking of **1** into the SILCS FragMaps was then performed with the resulting orientation shown in Figure 2B. In addition to the overlap of the phenyl ring with the APOLAR FragMaps is the overlap of the cyclopropyl moiety and of the amide carbonyl oxygen with a HBACC FragMap, interactions that may improve binding. A collection of 15 derivatives of **1**, **2** and **3** were then designed and synthesized based on the overlap with FragMaps in the region of the hydrophobic cavity and the donor and acceptor FragMaps in the proximity of T780, S804 and S808 (Table 1). Quantitative predictions of the binding of DFB, compound **1**, and its derivatives in the pocket were then performed using Ligand Grid Free Energy (LGFE) scoring.[32] LGFE is based on the overlap of atoms in the ligand functional moieties with their respective GFE FragMap types and was calculated as a Boltzmann averages over 25 runs with 200,000 steps of MC sampling of each of the ligands in the field of FragMaps. Individual MC sampling was performed for all the possible enantiomers of the *ortho*- and *meta*-substituted compounds. Presented in Table 1 are the resulting LGFE scores. Notably, all the designed compounds were predicted to have improved affinity over DFB, indicating that the design strategy would yield improved analogs. The following section describes the synthesis of all the compounds in Table 1 and subsequent biological evaluation.

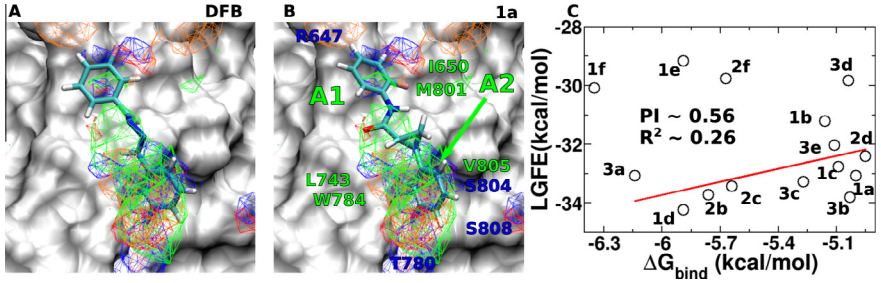

Figure 2. FragMaps overlaid on the PAM binding site of mGluR5 with ligands (A) DFB, (B) Compound **1a**. Receptor atoms occluding the view of the binding pocket were removed to facilitate visualization. The color for nonpolar (APOLAR), neutral donor (HBDON), neutral acceptor (HBACC), negative acceptor (NEG) and positive donor (POS) FragMaps are green, blue, red, orange and cyan, respectively. APOLAR, HBACC and HBDON FragMaps are set to a cutoff of −0.5 kcal/mol, while NEG and POS are set to −1.2 kcal/mol. Distinct FragMap affinities that overlap with the functional groups of the ligands are indicated by arrows colored the same as the FragMaps. (C) Satisfactory correlation was observed between the LGFE and the ΔG_{bind} when ligands **1e**, **1f** and **2f** were not considered in the R^2 and PI calculations.