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A Combined Bioinformatics and Chemoinformatics Approach for Developing Asymmetric Bivalent AMPA Receptor Positive Allosteric Modulators as Neuroprotective Agents

Haijun Chen^{a,†}, Cheng Z. Wang^{a,†}, Chunyong Ding^a, Christopher Wild^a, Bryan Copits^b, Geoffrey T. Swanson^b, Kenneth M. Johnson^a, and Jia Zhou^a

Jia Zhou: jizhou@utmb.edu

^aChemical Biology Program, Department of Pharmacology and Toxicology, Center for Addiction Research, University of Texas Medical Branch, Galveston 77555, TX (USA)

^bDepartment of Molecular Pharmacology and Biological Chemistry, Northwestern University Feinberg School of Medicine, Chicago 60611, IL (USA)

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As the cost of research and development in pharmaceutical drugs rises significantly, it is imperative that clinical candidates should be designed with an improved probability of success.^[1] Bridging the gap between chemical and biological space would make the quantum leap in the efficiency of drug discovery, and effective utilization of bioinformatics and chemoinformatics is becoming recognized as a valuable component of drug design.^[2] The number of crystal structures of proteins or protein-ligand complexes has been growing at an approximately exponential rate.^[3] Analysis and comparison of the tertiary structures of a protein complexed with different small ligands can be utilized to guide the rational drug design with the aim of exploring further chemical space to identify novel fragments of ligands.^[4] These new fragments or scaffolds may provide a range of more efficient starting points that can be further developed. Generally, a sizable compound library can be generated from such fragments by optimization of their physicochemical properties including absorption, dissolution, metabolic stability, plasma protein binding, distribution, elimination, toxicological profiles, cost of synthesis, and other pharmaceutical properties.^[5] The compounds identified to comply with Lipinski's drug-like "rule of five"^[6] and Hann and Oprea's lead-like criteria^[7] are then selected for further molecular docking. The promising compounds can be extracted using suitable algorithms that may efficiently and accurately predict their conformations.^[8] In this communication, we applied a combined bioinformatics and chemoinformatics approach to the cost-effective design and identification of novel asymmetric bivalent AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor positive allosteric modulators with remarkable potency *in vitro* and efficacy *in vivo* for preventing neuroapoptosis.

Correspondence to: Jia Zhou, jizhou@utmb.edu.

[†]These authors contribute equally to this work.

Supporting information for this article is available on the WWW under <http://www.chemmedchem.org> or from the author.

Despite two decades of research on AMPA receptors and significant advances in characterization of this major subtype of ionotropic glutamate receptors (iGluRs) in the central nervous system (CNS),^[9] a rationally designed drug acting as AMPA receptor positive allosteric modulator has yet to reach the market.^[10] Several significant challenges, including the limited chemical diversity of currently available ligands,^[11] lack of efficient evaluation methods,^[10] and potential toxicity profiles of some potentiators at high doses^[12] have prevented progression of any candidates into large scale Phase III patient trials. However, many diverse ligands are known to bind to allosteric sites,^[13] and a substantial number of crystal structures with different ligands of the GluA2 isoform of the AMPA receptors^[14] are available from the Protein Data Bank (PDB), a tremendous resource that provides a variety of tools for accessing data in the PDB archive to examine, manipulate, and compare three-dimensional molecular structures.^[15] By bridging available chemical and biological data with a particular emphasis on drug design, we were inspired to explore more chemical diversity space utilizing a combined approach and our new functional assays of neuroprotection with thinking outside of the box.

Several classes of AMPA receptor positive allosteric modulators that have been resolved in crystal structures with the GluA2 isoform of the AMPA receptors are shown in Figure 1, including pyrrolidinones (aniracetam and piracetam),^[16] benzothiadiazides (cyclothiazide, CTZ),^[17] benzamides (CX614),^[16a] sulfonamides (LY404187, **6** and **7**),^[18] indazole based derivatives (**8**),^[11] and phenyl-1,4-bis-carboxythiophene (CMPDB).^[19] Detailed overlay analysis (Figure 1a, methods described in the Supporting Information) of these complexed crystal structures (Figure 1b) reveals that some fragments from potent ligands or existing drug candidates have key interactions with three pockets of AMPA receptors (Figure 1c). For example, the piracetam scaffold (**2**) and chloro group of CTZ (**3**) bind to the pocket S1.^[16b] The bicyclo[2.2.1]hept-5-en-2-yl moiety of CTZ (**3**) and the ethyl group of CMPDB (**9**) interact with the hydrophilic pocket S2.^[19,20] Furthermore, it was also revealed from our analysis that the *N*-(2-phenylpropyl)methanesulfonamide moiety could effectively reach the deep cavity and accommodate the most space in the pocket S3 (Figure 1c). Therefore, based upon this favourable pharmacophore as one binding site around S3, we envisioned that it might be possible to generate a novel bivalent (containing two binding domains), or even multivalent (containing multiple binding domains) ligands with additional fragments around the other two pockets S1 and S2 thereby accessing more biological space. The proof of this principle was illustrated by the symmetric dimeric biarylpropylsulfonamide **6** and CMPDB **9**.^[18b,19] It is thought that allosteric modulators of AMPA receptors reduce channel desensitization and deactivation by opposing dissociation of ligand-binding domain dimer interfaces.^[21,22] Therefore, developing asymmetric bivalent ligands targeting two or multiple binding domains, especially those present in functionally relevant AMPA receptor heterodimers, represents a unique strategy in drug discovery and has great potential to obtain highly potent and specific AMPA receptor allosteric modulators. Based on these findings, we have designed a group of bivalent ligands by merging different scaffolds with privileged fragments, followed by structural tuning and optimization addressing binding within two or three pockets simultaneously (Figure 1c and Table S1 in the Supporting Information). After calculation of the physicochemical properties of members of this focused compound library, the extracted compounds were evaluated using the AutoDock Vina algorithm, which could predict the conformation more efficiently and accurately (This algorithm was found to have higher predictive capability than alternatives; see Figure S1 in the Supporting Information).^[23] The molecular docking results suggest that these newly designed compounds should have excellent binding affinity to AMPA receptors (see Table S1 in the Supporting Information). Two compounds, **HJC0122** and **HJC0124**, with the most desirable physicochemical properties and highest predicted binding affinities were synthesized (see Supporting Information for synthesis and characterization) and pharmacologically evaluated using a battery of assays to validate our new approach.

We first evaluated these two molecules using a functional assay that we have exploited based on the previous studies from several laboratories, including ours, which demonstrated that NMDA (*N*-methyl-D-aspartic acid) receptor antagonists induce apoptosis during critical developmental stages.^[24–29] Caspase-3 activation has been defined as a key player in PCP (phencyclidine)-induced developmental neuroapoptosis. NMDA receptor blockade-induced caspase-3 activation in postnatal rodent brain has been reported by many laboratories.^[26, 29–33] Our own studies have shown that acute PCP administration to postnatal day 7 (PND7) rats induces expression of cleaved caspase-3. Further investigation has demonstrated that AMPA reduced PCP-induced caspase-3 activity in the superficial cortical layers (2–4) of DIV10 (10 days *in vitro*) slices in a dose-dependent manner with a maximal effect at 10 μ M, which blocked about 75% of the caspase-3 activity induced by PCP (3 μ M) (see Figures S2 and S3 in the Supporting Information). The effect of this saturation dose was blocked by NBQX (10 μ M, a specific AMPA receptor antagonist).^[29] Using this functional assay, **HJC0122** and **HJC0124** (Figure 2a) significantly inhibited PCP-induced caspase-3 activities in organotypic slices of rat cortex with IC₅₀ values of 12.9 nM and 2.6 nM, respectively (Figure 2b). Using the same assay, both aniracetam and CTZ, two previously reported AMPA receptor positive allosteric modulators (structures shown in Figure 1b), modulate the effect of AMPA in organotypic slices (approximate IC₅₀ 1–3 μ M, see Figure S4 in the Supporting Information). It was noteworthy that LY451395^[34] with the same chemotype as **5** and **6** and advanced into phase II human clinical trials, showed an IC₅₀ value of 33.9 nM (Figure 2b). Our new ligands are at least 200-fold more potent than CTZ and aniracetam, and at least 3-fold potent than LY451395. Modeling studies also suggested that the pyrrolidine-1-carbonyl moiety of **HJC0122** could fit well into the hydrophilic pocket S1, and accommodate more space than previous ligands (Figures 1c and 2c).

Inspired by these findings, **HJC0122**, which has excellent water solubility (approximately 1.6 mg/mL for its HCl salt), was selected for further characterization in assays that included glutamate-induced calcium influx and electrophysiological recording of recombinant receptor currents. Intracellular Ca²⁺ transients measured in a FLIPR assay demonstrated that **HJC0122** enhanced glutamate (100 μ M)-evoked calcium influx in a concentration-dependent fashion (see Figure S5 in the Supporting Information) in DIV9 dissociated primary rat forebrain neurons with an EC₅₀ value of 6.5 nM (% E_{max} = 170 $\pm$ 22), which is significantly more potent than reference compounds (EC₅₀ = 93.3 nM, % E_{max} = 169 $\pm$ 17 for LY451395, EC₅₀ = 0.64 μ M for **6**,^[18b] and EC₅₀ = 0.15 μ M for LY404187^[34]). Glutamate-evoked currents also were recorded from recombinant GluA1_i/GluA2_i receptors and GluA4_i AMPA receptors expressed in HEK293-T/17 cell in whole-cell patch clamp recordings. We found that **HJC0122** slowly potentiated steady-state glutamate-evoked currents (see Figure S6 in the Supporting Information), while having no effect on GluK2 kainate receptor currents (a negative control). No effect on rapid entry into desensitization was observed for these AMPA receptors. This “resensitization” effect is qualitatively similar to that previously observed for the biarylpropylsulfonamide LY404187^[18a,35] and further confirmed that **HJC0122** acts as an AMPA receptor positive allosteric modulator.

We then investigated whether our selected compound **HJC0122** could reduce PCP-induced cleaved caspase-3 immunoreactive neurons in an *in vivo* model.^[29] The results demonstrated that **HJC0122** pretreatment *in vivo* (0.1–10 mg/kg, s.c.) significantly reduced PCP (10 mg/kg)-induced cleaved caspase-3 immunoreactive neurons in frontal cortex (M2/1) of PND7 rats in a dose-dependent manner (Figure 3). **HJC0122** at 10 mg/kg was found to prevent 85% of PCP (10mg/kg)-induced caspase-3 activation (Figure 3E). The good cLogP and TPSA values (Table S1 in the Supporting Information) as well as *in vivo* efficacy of **HJC0122** suggest that the molecules of this new chemotype are likely capable of penetrating the blood-brain barrier (BBB). Moreover, **HJC0122** did not show significant signs of toxicity even at the dose of 30 mg/kg (s.c.).

In summary, two asymmetric bivalent AMPA receptor positive allosteric modulators **HJC0122** and **HJC0124** have been identified with high potency and efficacy in preventing neuroapoptosis (both *in vitro* and *in vivo*) through a combined bioinformatics and chemoinformatics approach. Several key contributions have been made in this work. First, developing small molecules by potentiating AMPA receptors to prevent neuroapoptosis represents a unique strategy that has not heretofore been attempted. Second, we have applied a novel approach by bridging the available chemical and biological data in an effort to integrate them with a particular emphasis on drug design toward the identification of asymmetric bivalent or multivalent AMPA receptor ligands with enhanced potency and efficacy. Third, the newly identified small molecules demonstrated marked neuroprotective effects and may act as novel pharmacological probes and potential therapeutic modalities for glutamatergic hypofunction and its related neurological and psychiatric disorders, which include schizophrenia and Alzheimer's disease. Given the ever-increasing number of crystal structures in the public domain, a combined bioinformatics and chemoinformatics approach has the potential to provide valuable insights into the rational drug design.^[36] We believe that the described approach of using online databases as well as public algorithms would open up new avenues and expedite cost-effective small-molecule drug discovery in yielding molecules with enhanced potency, efficacy and drug-like properties.

Experimental Section

Full details of the protocols used to design, synthesize and evaluate the compounds described are given in the Supporting Information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

AMPA receptor positive allosteric modulators and overlay analysis. a) Overlay of the nine positive allosteric modulators with structures available from the PDB. b) Chemical structures of selected AMPA receptor positive allosteric modulators. c) General combined bioinformatics and chemoinformatics approach for drug design schemes.

Figure 2.

Newly designed asymmetric bivalent AMPA receptor positive allosteric modulators as neuroprotective agents. a) Chemical structures of **HJC0122** and **HJC0124**. b) **HJC0122** and **HJC0124** modulated the effect of AMPA (1 μ M) on PCP (3 μ M)-induced caspase-3 activity in a concentration-dependent fashion, and displayed more potent activity than LY451395 (in Phase II human clinical trials). c) Predicted binding mode of **HJC0122** (pink) to the GluA2 dimer interface.

Figure 3.

Cleaved caspase-3 immunoreactive neurons in layer 2 of M2/1 cortices of postnatal day 7 (PND7) rats 9 h after Saline (A) or PCP (10 mg/kg, s.c., B–E) without (A–B) or with (C–E) pretreatment (30 min prior to PCP) of **HJC0122** (C, 0.1 mg/kg; D, 1 mg/kg; E, 10 mg/kg, s.c.). Note that **HJC0122** reduced PCP (10 mg/kg)-induced cleaved caspase-3 immunoreactive neurons in a dose-dependent fashion.