Rational Design of a Novel AMPA Receptor Modulator Through a Hybridization Approach

Nicola Caldwell,[‖,‡] Jonathan E. Harms,[†,‡] Kathryn M. Partin[†] and Craig Jamieson*,[‖]

‖ Department of Pure and Applied Chemistry, University of Strathclyde, 295 Cathedral Street, Glasgow, G1 1XL, UK.

†Department of Biomedical Sciences, Colorado State University, Fort Collins, CO 80523-1617.

ABSTRACT: The α-amino-3-hydroxy-5-methyl-4-isoxazolepropanoic acid (AMPA) receptors are a family of glutamate ion channels of considerable interest in excitatory neurotransmission and associated disease processes. Here, we demonstrate how exploitation of the available X-ray crystal structure of the receptor ligand binding domain enabled the development of a new class of AMPA receptor positive allosteric modulators (**7**) through hybridization of known ligands (**5** and **6**), leading to a novel chemotype with promising pharmacological properties.

The α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors are a class of ligand gated ion channels that are ubiquitously expressed in the central nervous system (CNS). This sub-family of glutamate receptors is considered to be responsible for the vast majority of fast excitatory amino acid neurotransmission in the CNS.[1] A postulated function of AMPA receptors is in facilitating the process of synaptic plasticity and long-term potentiation (LTP), a use dependent potentiation of synaptic efficacy often considered to be responsible for encoding both learning and memory. Various classes of AMPA receptor modulators have demonstrated their potential in enhancing LTP; and accordingly are being considered as potential new treatments for a raft of diseases in the neurosciences area, including Alzheimer's Disease, schizophrenia, Parkinson's disease and Attention Deficit Hyperactivity Disorder.[2,3]

Four distinct genes (GluA1 to 4) encode subunits of the AMPA receptor, each consisting of four discernable domains: an *N*-terminal region, an extracellular glutamate binding site (ligand binding domain, LBD), a transmembrane region and a *C*-terminal domain.[4] Considerable advances have been made in solving the three-dimensional structure of the AMPA receptor. X-ray structures have been reported of protein complexes representing the LBD[5] and more latterly the full-length receptor.[6]

Based on the strong association with neurological disorders, significant effort has been invested in the identification of AMPA receptor positive modulators as cognition enhancers.[7] Pre-eminent among this class of small molecules are benzamide derivatives such as aniracetam (**1**),[8] the progenitor compound in this series; CX516 (**2**)[9] and CX614 (**3**),[10] Chart 1.
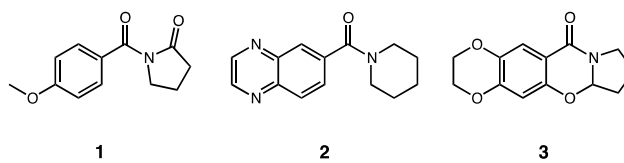

Chart 1. Benzamide derived AMPA receptor modulators

Although compounds such as **2** display promising activity in behavioral paradigms related to cognition,[11] relatively high doses (35 mg/kg) are required, attributable in part to the low *in vitro* potency of the compounds (e.g. EC_{50} = 150 μM in native tissue preparations for **2**).[9] In general, the low potency associated with this compound class can be rationalized through consideration of the available X-ray crystal structure of compound **3**[12] (Figure 1).
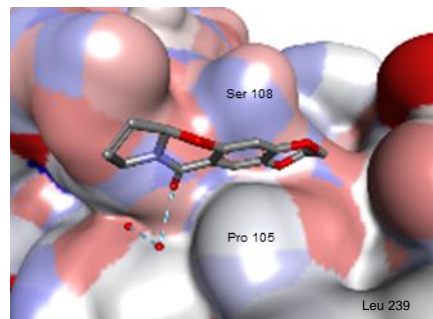

Figure 1. X-ray crystal structure of **3** in the Ligand Binding Domain of the GluA2 receptor with key interactions shown.

The binding site is formed through association of two distinct lobes (S1 and S2) held together by a peptide to give a C_2-symmetrical construct used for X-ray crystallography.[5] Examination of the biostructural data indicates that **3** makes no discernable interactions with the recep-

tor itself, and instead forms a single hydrogen bond with a network of water molecules. This limited interaction goes some way towards accounting for the modest potency (37 μM) associated with the compound and is believed to be conserved in the series. Based on all of the above, we reasoned that using compound **3** as a starting point, it would be possible to evolve a new lead series by designing more optimal interactions with the receptor through hybridization with an unrelated class of modulator.

We recently reported a class of thiophene-amide derived compounds (**4** and **5**),[13,14] which more fully occupy the receptor binding site, establishing additional interactions with the allosteric domain (Figure 2). The X-ray crystal structure of these compounds shows hydrophobic interactions of the pyrazole and benzothiophene rings with Pro and Leu residues, respectively. In addition, a hydrogen bonding interaction can be observed between the primary amide and a proximal Ser residue. This results in this class of compound having significantly improved potency with EC_{50} values in the low micromolar range (e.g., EC_{50} for **5** = 0.40 μM), as well as profound effects on desensitization and deactivation (*vide infra*). In a related study,[15] we have also demonstrated how the pyrazole motif could be used in a hybridization approach, combining features of unrelated classes of AMPA receptor modulators to furnish a new class of ligand.
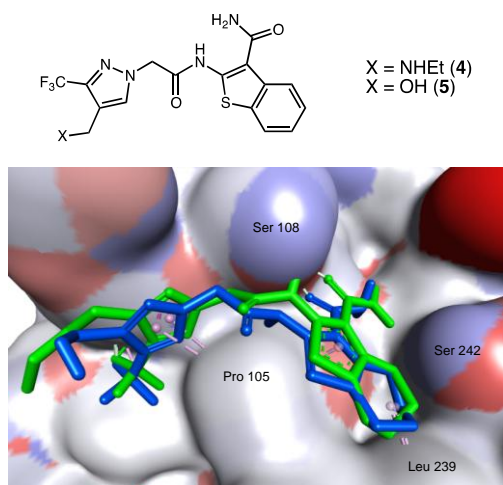

Figure 2. X-ray structure of compounds **4** (green) and **5** (blue) in the GluA2 LBD. Both compounds span the entire allosteric site, making interactions with distal hydrophobic pockets.

In this publication, we illustrate how this approach can be extended to generate a novel series of AMPA receptor modulators though combination of the benzamide and thiophene amide derived systems. From consideration of the binding modes of both the benzamide series and the more recent thiophene-derived systems, we concluded that hybridizing elements of both series could lead to a novel chemotype with enhanced potency compared to the progenitor benzamide series. The basic design hypothesis is outlined in Chart 2.

The pyrazole fragment from amide **5** was chosen for inclusion in the new template based on its highly con-

served binding mode as determined through previous biostructural work,[13,15] thus providing confidence of how it is likely to interact with the receptor as part of a hybridized series.

From consideration of the benzamide portion, we elected to base our hybrid compound on the tricyclic benzamide-derived scaffold represented by **6** (Chart 2). This progenitor compound which is formally related to both **2** and **3** has previously been reported as a modulator of the AMPA receptor,[15] and was selected as the basis of this approach as it offered greater synthetic tractability.
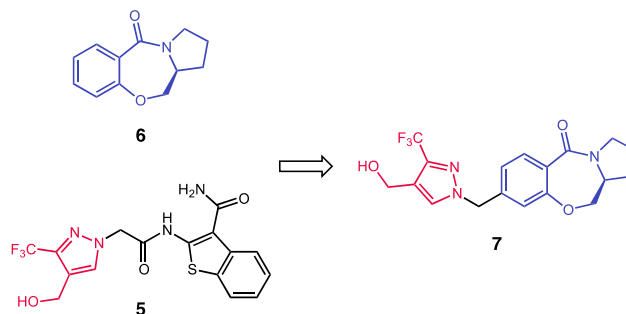

Chart 2. Hybridization strategy adopted combining known classes of modulators, 5 and 6.

Docking of the proposed hybrid compound **7** using the GOLD algorithm[17] into the GluA2 LBD gave a favorable pose (Figure 3), with similar hydrophobic interactions with the allosteric domain being observed as for progenitor compounds, **4** and **5**. An additional hydrophobic interaction was observed with the trifluoromethyl group, providing confidence that synthesis of related analogues would furnish new templates capable of interacting with the receptor.
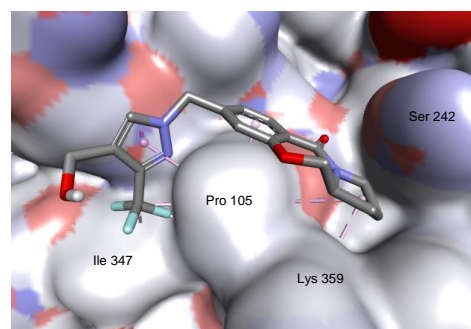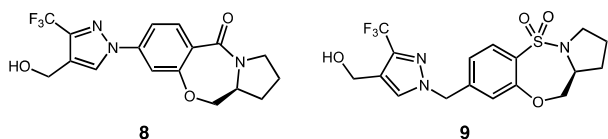

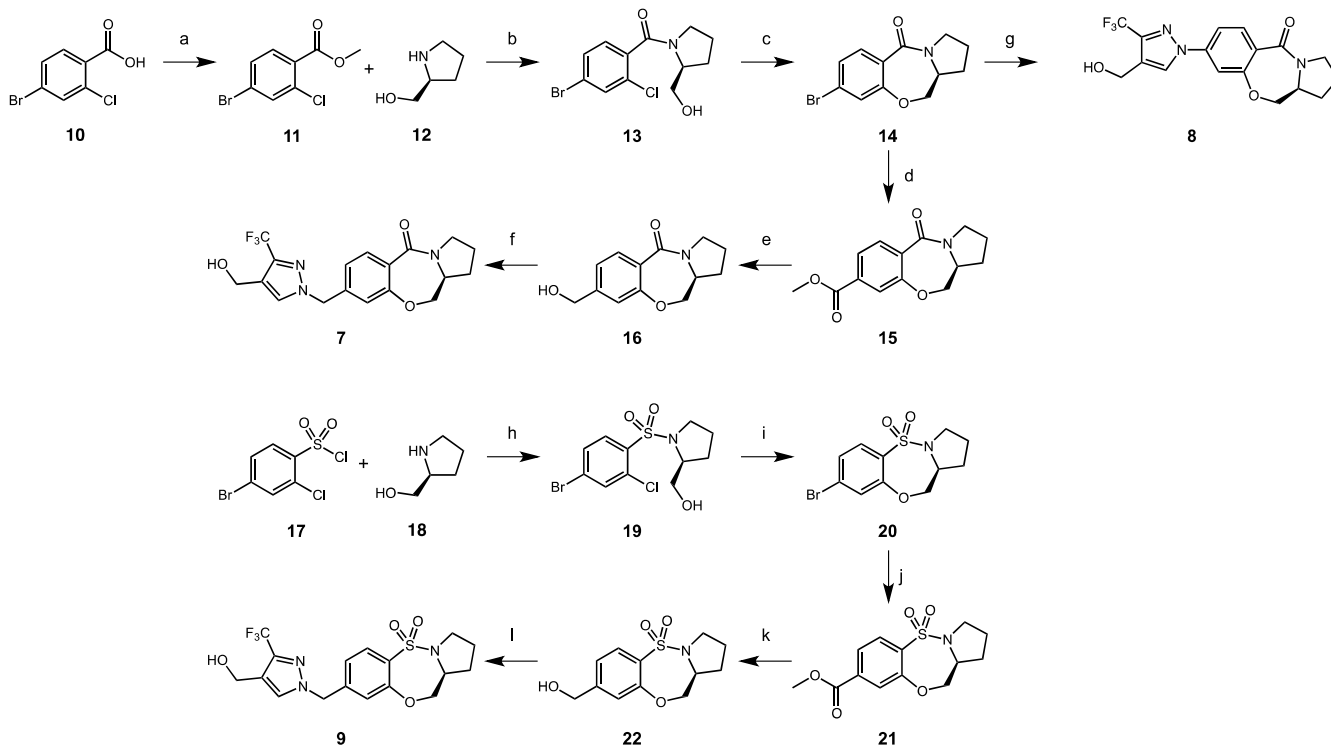**Figure 3.** Docked pose of proposed benzamide hybrid compound **7** in the GluA2 LBD.

Given the high level of structural information available at the outset of our synthesis campaign, we envisaged a focused chemistry effort, targeting specific compounds in order to ascertain the feasibility of our hybridization approach. Accordingly, in addition to compound **7** we selected the analogs shown in Chart 3 for preparation.

Chart 3. Additional hybrid compounds targeted for synthesis.

Based on the modeling data shown in Figure 3, a methylene linker unit was considered to be essential in order to achieve optimal interaction with the allosteric site.

Scheme 1.[a] Preparation of Target Compounds

[a]Reagents and conditions: (a) (1) AcCl, MeOH, 0 °C (2) 100 °C, 87% (b) 10 mol% BEMP, MeCN, 25 °C, 92%; (c) NaH, DMF, 120 °C, 70%; (d) *trans*-bis(acetato)bis[*o*-(di-*o*-tolylphosphino)benzyl]dipalladium(II), [HP(^{t}Bu)$_3$]BF$_4$, DBU, Mo(CO)$_6$, MeCN/MeOH, 120 °C (μW), 62%; (e) LiAlH$_4$, THF, 25 °C, 54%; (f) (1) SOCl$_2$, CH$_2$Cl$_2$, 25 °C (2) (3-(trifluoromethyl)-1*H*-pyrazol-4-yl)methanol, K$_2$CO$_3$, DMF, 60 °C, 26% (over 2 steps); (g) (1) 3-(trifluoromethyl)-1*H*-pyrazole-4-carbaldehyde, CuI, *trans*-1,2-diaminocyclohexane, K$_2$CO$_3$, 1,4-dioxane, 180 °C (μW) (2) NaBH$_4$, MeOH, 25 °C, 11% (over 2 steps); (h) Et$_3$N, CH$_2$Cl$_2$, 25 °C, 98%; (i) as (c), 72%; (j) *trans*-bis(acetato)bis[*o*-(di-*o*-tolylphosphino)benzyl]dipalladium(II), [HP(^{t}Bu)$_3$]BF$_4$, DBU, Mo(CO)$_6$, MeCN/MeOH, 110 °C, 87%; (k) as (e), 70%; (l) as (f), 22% (over 2 steps)

The site contains a 'saddle' region in the center of the C$_2$-symmetrical domain, therefore a flexible linker was included in the design of compound **7**. The corresponding fused analogue **8** was targeted as a control, with this more rigid structure not anticipated to be accommodated in the allosteric site. The sulfonamide hybrid **9** was selected in order to probe spatial requirements in the region of the hydrophobic pocket. Additionally, previous work on benzamide related derivatives had indicated that sulfonamide derivatives could be tolerated in this region of the molecule,[18] as well as other sulfonamide derived compounds which target hydrogen bond interactions with Pro105.[19,20]

Target compounds **7 – 9** were prepared as shown in Scheme 1. Base-mediated amidation of the benzoate ester **11**[21] followed by S$_N$Ar cyclization furnished tricyclic intermediate **14**. This could be converted to target compound **8** in two steps (copper-mediated arylation and

subsequent reduction). Alternatively, **14** could be subjected to a carbonylation procedure with Herrmann's catalyst,[22] followed by a sequence of reduction, cholorination and alkylation to furnish the requisite pyrazole derivative **7**. The corresponding sulfonamide system **9** was accessed in an analogous fashion, starting from the aryl sulfonyl chloride building block **18**.

Each of the analogs were then examined in an electrophysiological assay in order to assess their efficacy as positive allosteric modulators of the AMPA receptor. In this study, two separate splice variants of the GluA2 receptor, termed flip and flop were employed. These variations can lead to differences in the kinetic and pharmacological properties of the channel and particularly manifest in the allosteric site where an N754S mutation occurs.[7] Based on this, we sought to explore the pharmacology of our nascent compounds against both splice variants.

AMPA receptor potentiation by an exogenous ligand is a product of its impact on two processes: deactivation and desensitization. Deactivation involves the channel being at rest following dissociation of the endogenous ligand (glutamate), whereas desensitization involves the closure of the channel with glutamate still bound. For the AMPA receptors, these processes occur on a very rapid timescale (1-2 milliseconds and 10 milliseconds, respectively) and are pivotal in shaping the duration and amplitude of response to glutamate at a synapse. Inhibition of either or both of these responses will lead to potentiation of the receptor. Accordingly, we sought to determine the effects of our novel compounds against both desensitization and deactivation. Compounds were tested at a single concentration of 30 μM, with the highly potent analog **5** (which was previously shown to be effective against both processes and tested here at a concentration of 100 μM[14]) used for comparison. Earlier efforts from our laboratories[23] have shown how comparing electrophysiological profiles within a series can be used to determine relative efficacy and affinity of individual exemplars using a single test concentration.

GluA2 flip or flop receptor cDNA was transiently-transfected and expressed in HEK 293 cells. Outside-out membrane patches containing homomeric GluA2 were pulled and positioned within the control stream of a two-barrel flowpipe. This stream was then rapidly switched to the adjacent compound-containing stream for 1 millisecond or 500 milliseconds to record receptor deactivation or desensitization, respectively.

As demonstrated previously,[14] compound **5** was effective at modulating deactivation of both flip ($p<0.05$) and flop ($p<0.05$) GluA2 homomers (Figure 4$_A$), one of a limited number of compounds which demonstrates a modulatory effect on flip deactivation. Similarly, though not affecting desensitization kinetics (Figure 4$_B$, flip: $p=0.82$, flop: $p=0.85$**),** compound **5** increased the steady-state current observed with receptor desensitization on both isoforms (Figure 4$_C$, flip, flop: $p<0.001$). Based on our recent analysis of decay current produced by the onset of densistization,[23] behavior of this type is indicative of a compound with low affinity and low efficacy.

By comparison, compound **7** was significantly more effective than **5** at modulating deactivation of flop isoforms ($p<0.001$), even at the lower concentrations tested, but no longer showed an effect on flip GluA2 ($p=0.64$, **Fig 4$_A$**). This flop isoform specific effect of **7** was also observed for desensitization kinetics, displaying a slowed onset of desensitization for flop receptors ($p<0.001$) not observed on flip isoforms ($p=0.77$) or with compound **5** (**Fig 4$_B$**). Despite this isoform specificity relating to onset of desensitization, compound **7** showed an enhanced steady-state current on both flip ($p<0.001$) and flop ($p<0.001$) receptors (**Fig 4$_C$**). Our previous study[23] suggests that slowing of desensitization together with robust block of steady-state current is indicative of a compound with high efficacy and high affinity. In this regard, compound **7** represents a significant advance on compound **5** (*vide supra*).

These apparently detached patterns of receptor kinetics were further observed with the hybridized compound **9**. This compound showed no observable effect on deactivation kinetics (flip: $p=0.64$, flop: $p=0.46$, **Fig 4$_A$**) or steady-state current (flip: $p=0.24$, flop: $p=0.25$ **Fig 4$_C$**), but demonstrated a most pronounced effect on desensitization kinetics specific to the flop isoform ($p<0.001$, **Fig 4$_B$**), with a slight effect on flip receptors ($p=0.05$). Finally, there was no observed modulation by compound **8** on either isoform for any tested measure (deactivation: flip: $p=0.12$, flop: $p=0.96$; desensitization: flip: $p=0.54$, flop: $p=0.87$; SS/Peak: flip: $p=0.20$, flop: $p=0.55$).

The electrophysiological data presented above indicates that for meaningful activity at the GluA2 receptor an appropriate spacer must be incorporated into the hybridized compounds (c.f. compounds **7** and **8**). This observation is consistent with our earlier design hypothesis informed through consideration of the X-ray structure of the allosteric site. The new hybrid compound **7** is able to robustly block the process of desensitization against both flip and flop receptor isoforms, showing encouraging levels of efficacy as an AMPA receptor modulator and compares favorably with progenitor compounds such as **2**, **3** and **5**.
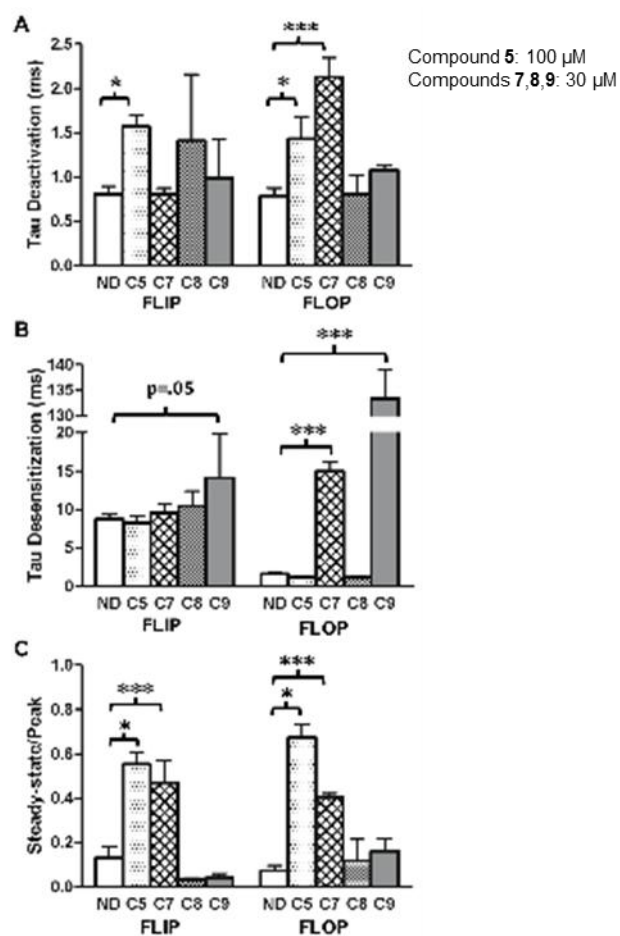

Figure 4. Electrophysiology data for tested compounds in both flip and flop receptors showing: **A** deactivation; **B** desensitization; and **C** steady-state/peak ratios.

The subtype preference observed with compound **7** for slowing of on-set of desensitization and deactivation is not readily explained through use of the available biostructural data and attempts to generate biostructural data have so far not proven to be fruitful. It can be remarked, however, that this subtype preference has been observed for progenitor compounds (e.g. **3**), and so is likely to be a function of the benzamide chemotype.[10]

Lastly, the sulfonamide analogue **9**, despite slowing on-set of desensitization does not exhibit any measurable degree of efficacy as a desensitization blocker as evidenced by its negligible effect on the steady-state to peak ratio. This indicates a strong preference for the benzamide moiety as a preferred chemotype.

In summary, hybridization of two distinct lead series with contrasting levels of affinity and efficacy has furnished a new chemotype with a high amplitude of modulation of the AMPA receptor. This new chemotype represented by compound **7** compares very favourably with highly potent modulators (e.g. **5**) identified previously in terms of tonic potency, affinity and efficacy. The use of biostructural information has greatly enabled the design process and led to the expedient generation of new tools with which to further interrogate the biology of the AMPA receptor.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures (synthesis and pharmacology) and spectral data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

* (C.J.) Tel: +44-141-548-4830. Email craig.jamieson@strath.ac.uk

Notes

The authors declare no competing financial interests.

Author Contributions

‡These authors contributed equally. All authors have given approval to the final version of the manuscript.

ABBREVIATIONS

AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CNS, Central Nervous System; GluA, ionotropic Glutamate Receptor; LBD, Ligand Binding Domain; LTP, Long Term Potentiation.

REFERENCES

(1) Kew, J. N. C. and Kemp, J. A. Ionotropic and metabotropic glutamate receptor structure and pharmacology. *Psychopharmacology* **2005,** *179*, 4-29.

(2) Marenco, S. and Weinberger, D. R. Therapeutic potential of positive AMPA receptor modulators in the treatment of neuropsychiatric disorders. *CNS Drugs* **2006,** *20*, 173-185.

(3) Zarate, J. and Manji, H. K. The Role of AMPA receptor modulation in the treatment of neuropsychiatric diseases. *Exp. Neurology* **2008,** *211*, 7-10.

(4) Mayer, M. L. and Armstrong, N. Structure and Function of Glutamate Receptor Ion Channels. *Ann. Rev Physiol* **2004,** *66*, 161-181.

(5) Armstrong, N.; Sun,Y.; Chen, G. Q.; Gouaux, E. Structure of a glutamate-receptor ligand-binding core in complex with kainate. *Nature* **1998,** *395*, 913-917.

(6) Sobolevsky, A. I.; Rosconi, M. P.; Gouaux, E. X-ray structure, symmetry and mechanism of an AMPA-subtype glutamate receptor. *Nature* **2009,** *462*, 745-756.

(7) Grove, S. J. A.; Jamieson, C.; Maclean, J. K. F.; Rankovic, Z. Positive Allosteric Modulators of the AMPA receptor. *J. Med. Chem.* **2010,** *52*, 7044-7053.

(8) Ito, I.; Tanabe, S.; Kohda, A.; Sugiyama, H. Allosteric potentiation of quisqualate receptors by a nootropic drug aniracetam. *J. Physiol.* **1990,** *424*, 533-544.

(9) Arai, A. C.; Xia, Y. F.; Rogers, G.; Lynch, G.; Kessler, M. Benzamide-type AMPA receptor modulators from two subfamilies with distinct modes of action. *J. Pharm. Exp. Ther.* **2002,** *303,* 1075-1085.

(10) Arai, A. C.; Kessler, M.; Rogers, G.; Lynch, G. Effects of the potent ampakine CX614 on hippocampal and recombinant AMPA receptors. *Mol. Pharmacol.* **2000,** *58*, 802-813.

(11) Hampson, R. E.; Rogers, G.; Lynch, G.; Deadwyler, S. A. Facilitative effects of the ampakine CX516 on short-term memory in rats. *J. Neurosci.* **1998,** *18*, 2748-2763.

(12) Jin, R.; Clark, S.; Weeks, A. M.; Dudman, J. T.; Gouaux, E.; Partin, K. M. Mechanism of positive allosteric modulators action on AMPA receptors. *J. Neurosci.* **2005,** *25*, 9027-9036.

(13) Jamieson, C.; Campbell, R. A.; Martin, F.; Moir, E. M.; Morrow J. A.; Pantling, J.; Rankovic, Z.; Smith, L.; Cumming, I. A., Gillen, K. J.; Gillespie, J.; Kazemier, B.; Kiczun, M.; Lamont, Y.; Lyons, A. J.; Maclean, J. K. F. A novel series of positive modulators of the AMPA receptor: structure-based lead optimization. *Bioorg. Med. Chem. Lett.* **2010,** *20*, 6072-6075.

(14) Harms, J. E.; Benveniste, M.; Maclean, J. K. F.; Partin, K. M.; Jamieson, C. Functional analysis of a novel positive allosteric modulator of AMPA receptors derived from a structure-based drug design strategy. *Neuropharmacology* **2013,** *64*, 45-52.

(15) Jamieson, C.; Maclean, J. K. F.; Brown, C. I.; Campbell, R. A.; Gillen, K. J.; Gillespie, J.; Kazemier, B.; Kiczun, M.; Lamont, Y.; Lyons, A. J.; Moir, E. M.; Morrow, J. A.; Pantling, J.; Rankovic, Z.; Smith, L. Structure based evolution of a novel series of positive modulators of the AMPA receptor. *Bioorg. Med. Chem. Lett.* **2011,** *21*, 805-811.

(16) Bursi, R.; Erdemli, G.; Campbell, R.; Hutmacher, M. M.; Kerbusch, T.; Spanswick, D.; Jeggo, R.; Nations, K. R.; Dogterom, P.; Schipper, J.; Shahid, M. Maximum Tolerated Dose Evaluation of the AMPA Modulator Org 26576 in Healthy Volunteers and Depressed Patients. *Psychopharmacology* **2011,** *218*, 713-724.

(17) Jones, G.; Willett, P.; Glen, R.C.; Leach, A.R.; Taylor, R.; Development and validation of a genetic algorithm for flexible docking. *J. Mol. Biol.* **1997,** *267*, 727-748.

(18) Ward, S. E.; Harries, M.; Aldegheri, L.; Austin, N.E.; Ballantine, S.; Ballini, E.; Bradley, D. M.; Bax, B. D.; Clarke, B. P.; Harris, A. J.; Harrison, S. A.; Melarange, R. A.; Mookherjee, C.; Mosley, J.; Dal Negro, G.; Oliosi, B.; Smith, K. J.; Thewlis, K. M.; Woollard, P. M.; Yusaf, S. P. Integration of Lead Optimization with Crystallography for a Membrane-Bound Ion Channel Target: Discovery of a New Class of AMPA Receptor Positive Allosteric Modulators. *J. Med. Chem.* **2011,** *54*, 78-94.

(19) Ornstein, P. L.; Zimmerman, D. M.; Arnold, B. M.; Bleisch, T. J.; Cantrell, B.; Simon, R.; Zarrinmayeh, H.; Baker, R. S.;

Gates, M.; Tizzano, J. P.; Bleakman, D.; Mandelzys, A.; Jarvie, K. R.; Ho, K.; Deverill, M.; Kamboj, R. K. Biarylpropylsulfonamides as Novel, Potent Potentiators of 2-Amino-3- (5-methyl-3-hydroxyisoxazol-4-yl)- propanoic Acid (AMPA) Receptors. *J. Med. Chem.* **2000**, *43*, 4354-4358.

(20) Patel, N. C.; Schwarz, J.; Hou, X. J.; Hoover, D. J.; Xie, L.; Fliri, A. J.; Gallaschun, R. J.; Lazzaro, J. T.; Bryce, D. K.; Hoffman, W. E.; Hanks, A. N.; McGinnis, D.; Marr, E. S.; Gazzard, J. L.; Hajos, M.; Scialis, R. J.; Hurst, R. S.; Schaffer, C. L.; Pandit, J.; O'Donnell, C. J. Discovery and Characterization of a Novel Dihydroisoxazole Class of
α‑Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) Receptor Potentiators. *J. Med. Chem.* **2013**, *56*, 9180-9191.

(21) Caldwell, N.; Jamieson, C.; Simpson, I.; Tuttle, T., Organobase-catalyzed amidation of esters with amino alcohols, *Org. Lett.* **2013**, *15*, 2506-2509.

(22) Herrmann, W. A.; Brossmer, C.; Ofele, K.; Reisinger, C. P.; Priermeier, T.; Beller, M.; Fischer, H. Palladacycles as Structurally Defined Catalysts for the Heck Olefination of Chloro- and Bromoarenes. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1844-1848.

(23) Weeks, A. M.; Harms, J. E.; Partin, K. M.; Beneveniste, M. Functional insight into development of positive allosteric modulators of AMPA receptors. *Neuropharmacology* **2014**, *85*, 57-66.
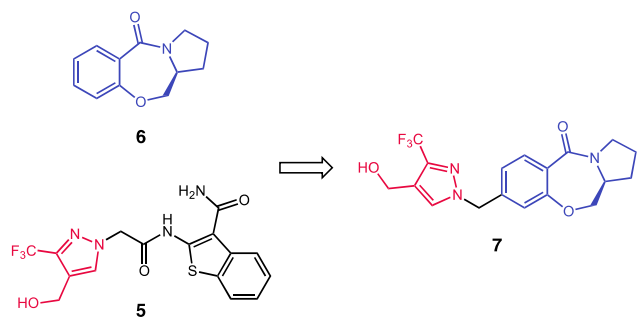
Table of Contents Graphic