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# Indole- and indoline-based kainate analogues with antagonist activity at ionotropic glutamate receptors

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**Abstract**—A conformationally constrained, indole-based kainate analogue was designed based on Gouaux's X-ray structure of kainic acid bound to an iGluR2(S1S2) construct, a structural model for AMPA/kainate ionotropic glutamate receptors. In contrast to the parent kainic acid, a potent agonist, this compound, along with three structurally related analogues derived from synthetic intermediates, exhibited antagonist behavior towards KAR expressed in oocytes, a result that is rationalized by molecular modeling studies.


L-Glutamate represents the primary excitatory neurotransmitter in the mammalian central nervous system, playing an essential role in mediating basal excitatory synaptic transmission and many forms of synaptic plasticity, such as long-term potentiation and long-term depression, that are thought to underlie learning and memory.[1,2] Glutamate controls these functions by binding to ligand-gated ion channels (ionotropic receptors, iGluRs) and G-protein-coupled metabotropic receptors, initiating a conformational change in the cation-conducting pore and thus an influx of cations into the postsynaptic neuron.[3] The ionotropic glutamate receptors are subdivided into three categories, AMPARs, KARs, and NMDARs, based on the selective pharmacologic agonists α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA), kainic acid (kainate, KA), and *N*-methyl-D-aspartate (NMDA), that, respectively, activates each group with some specificity (Fig. 1). The study of the specific roles of the AMPARs and KARs has been hindered by the similarity between the two (40% sequence homology) and the presence of multiple subunits, iGluR1–4 for AMPARs and iGluR5–7, KA1–2 for KARs,[4] that comprise each group.

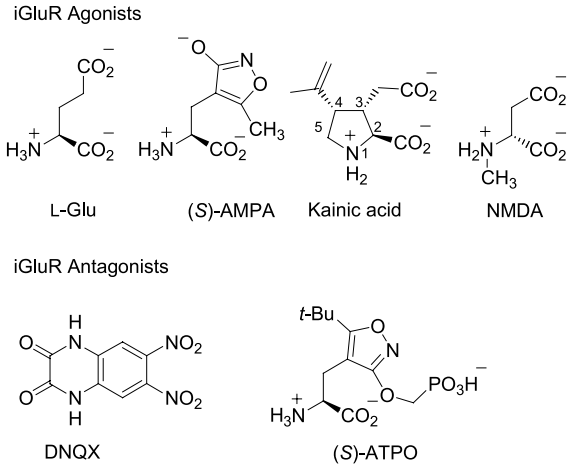


**Figure 1.** Examples of ionotropic glutamate receptor (iGluR) agonists and antagonist.

There have been many efforts to characterize the molecular interactions between the iGluRs and various agonists and antagonists with which they interact. A landmark in this area was the successful crystallization and structure determination by Gouaux et al.[5a] of the iGluR2 ligand-binding core (S1S2) complexed with kainic acid. This group has also reported X-ray structures of the iGluR2-S1S2 construct with several other agonists, including glutamate, AMPA, (*S*)-2-amino-3-[3-hydroxy-5-(2-methyl-2*H*-tetrazol-5-yl) isoxazol-4-yl]propionic acid (2-Me-Tet-AMPA), (*S*)-2-amino-3-(3-

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carboxy-5-methylisoxazol-4-yl)propionic acid (ACPA), (*S*)-2-amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl) propionic acid (ATPA), (*S*)-2-amino-3-(4-bromo-3-hydroxy-5-isoxazolyl)propionic acid (Br-HIBO), willardiines, as well as with the antagonists 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 2-amino-3-[5-*tert*-butyl-3-(phosphonomethoxy) 4-isoxazolyl]propionic acid (ATPO) (Fig. 1).[5] The crystal structures have provided insight into which iGluR2 residues are important for ligand affinity and specificity, and beyond that have afforded convincing evidence for the proposal that ligand-induced channel opening in the native receptor arises from closure of a 'hinge' between two domains (S1 and S2) of iGluR2.[5e] This hypothesis is based, in part, on a good correlation between agonist activity in native receptors with the degree of agonist-induced domain closure in the construct. A salient feature of this model is that full agonists induce full closure, partial agonists induce partial closure, and antagonists block closure—and hence channel opening—by interfering with crucial S1–S2 contacts that must form as the hinge closes.

Aside from providing a paradigm for agonist and antagonist activities, this structural information serves as the basis for the design of new ligands that might interact with iGluRs with greater selectivity. The native receptors consist of multiple subtypes, as discussed above, that share a high level of sequence homology that results in considerable overlap of ligand binding specificity;[6,7] for example, kainic acid binds not only to receptors comprised of the KAR subtypes but also to the AMPA family that includes iGluR1–4. Employing a combination of structure-based ligand design, homology modeling, and library synthesis, we have embarked on a project to identify new subtype-selective iGluR agonists and antagonists, and in this communication we report initial results on a newly identified class of antagonists based on the structure of the classic agonist kainic acid.

## 1. Design and synthesis

The activity of kainic acid analogues toward the KARs depends heavily on the nature of the C-4 substituent. For example, if the isopropenyl appendage in kainate is hydrogenated, the resultant analogue dihydrokainate (DHK) loses activity at the AMPARs and KARs, and becomes a moderately active inhibitor at the sodium-dependent transporter; conversely, the absence of a C-4 appendage results in an analogue that is active as an NMDA receptor agonist.[8] This sensitivity toward C-4 substituent changes, together with the Gouaux model of the iGluR2–kainate complex, provides a basis for designing new kainate analogues that might pharmacologically differentiate among the iGluR subtypes. In this communication, we describe our preliminary studies on a series of kainate-like analogues that are constrained to resemble bound conformation (Fig. 2), with an isopropenyl methyl group surrogate **R** that eventually can be structurally varied (R = H in these initial studies). Many kainic acid analogues have been reported previously, including those modified at C3–C5,[9] and others with various linkers as conformational constraints.[10] In addition, indole 2-carboxylates, including **2**, have been reported to bind to the glycine site on the NMDA receptors.[11]

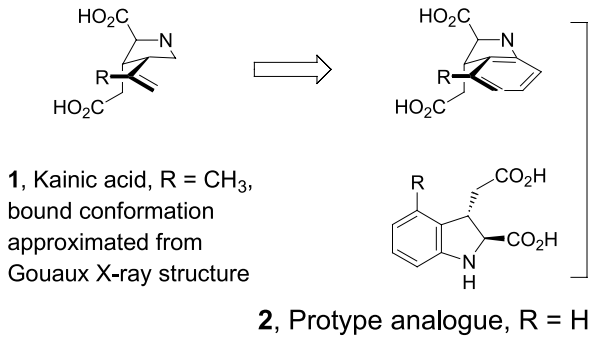


**Figure 2.** Proposed conformationally constrained kainate analogue **2**.

The parent structure **2** was conceived by inspection of a Gouaux model (Fig. 3A), noting in particular the disposition of the five-membered ring substituents in the bound kainic acid, which appeared to be reinforced by allylic strain between the isopropenyl group and the C-3 carboxymethyl side chain. Mindful of the fact that reduced basicity resulting from an *N*-aryl substitution on the α-amino group in the analogue could thwart the plan on account of the potential reduction in receptor affinity of an unprotonated amino group, there appeared to be ample space for a phenyl ring that could replace an isopropenyl group and at the same time bias the pyrrolidine ring toward any desired conformation (although the pyrrolidine envelope in the analogue would clearly be less pronounced than that shown; see Fig. 3B for energy-minimized structures). In fact, filling the 'empty space' in the vicinity of E402 and P478 with the aryl ring appeared to provide additional favorable van der Waals interactions in our preliminary modeling studies.[12]

The synthesis of dihydroindoline **2** commenced with the formation of the 2,3-disubstituted indole **3** (Fig. 4), which had previously been converted into similar dihydroindoline derivatives.[13] Condensation of phenylhydrazine hydrochloride with ketoglutaric acid provided the corresponding phenyl hydrazone, which underwent Fischer cyclization in the presence of polyphosphoric acid to generate indole **3**.[14] Attempts at hydrogenating indole **3** were unsuccessful, but the corresponding Boc-protected indole was smoothly reduced to give the racemic *cis*-dihydroindoline **4**; it has been proposed that electron-withdrawing *N*-substituents facilitate hydrogenations in such systems by reducing the electron density of the indole ring.[14] Epimerization of the *cis*-indoline with KO(*t*-Bu) afforded a mixture of *trans*- and *cis*-dihydroindolines (8:1 ratio) in a total yield of 75%. The *trans*-indoline **5** was separated and then converted into the designed ligand **2** by deprotection and saponification. Meanwhile, synthetic intermediates **3** and **4** were deprotected and saponified to generate additional analogues, indole **6** and the *cis*-dihydroindoline **7**, respectively. A fourth structurally related analogue, **8**, was also readily available from an abortive earlier route to **2**; Friedel–Crafts reaction of the indole-2-carboxylate,

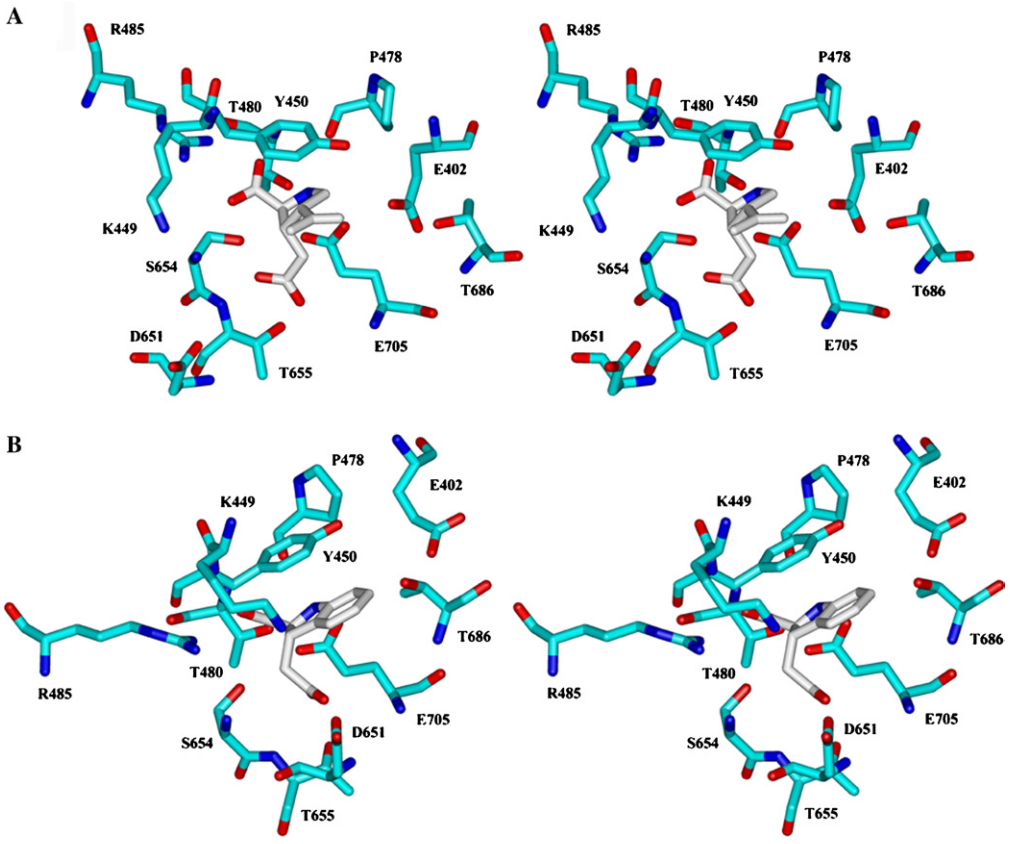


**Figure 3.** (A) Stereoview of kainic acid in the binding pocket of iGluR2S1S2 construct, from a Gouaux X-ray structure.[5] (B) Stereoview of indoline analogue **2** docked and minimized in the binding pocket of iGluR2S1S2 construct.

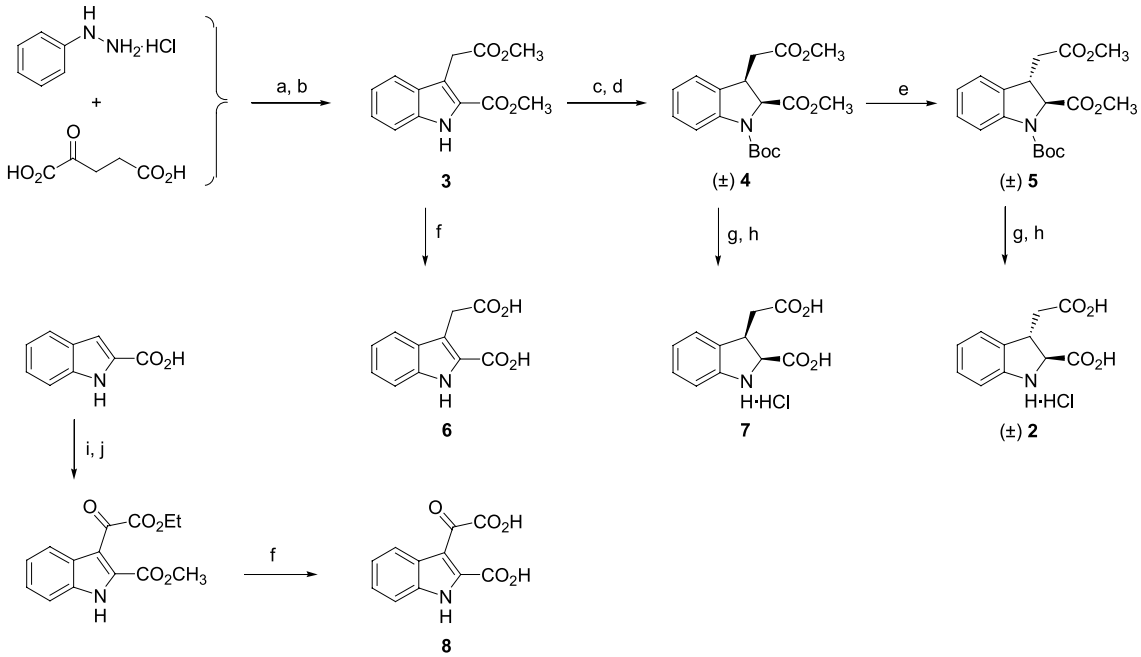


**Figure 4.** Synthesis of indoline/indole ligands **2**, **6**, **7**, and **8**. Reagents and conditions: (a) $H_2O$, rt; (b) HCl, PPA, MeOH (66%, two steps); (c) $Boc_2O$, DMAP, $Et_3N$ (77%); (d) Pd/C, $H_2$, AcOH (85%); (e) KO(*t*-Bu), followed by 1*N* AcOH (66%); (f) LiOH, THF/$H_2O$, followed by 1*N* AcOH (90% for **6**, 80% for **8**); (g) TFA; (h) LiOH, THF/$H_2O$, followed by 1*N* HCl (40% for **2**, 50% for **7**, two steps); (i) MeOH, AcCl; (j) $AlCl_3$, ethyl malonyl chloride (64%, two steps).

prepared from the commercially available indole-2-carboxylic acid, followed by saponification provided the indole analogue **8**.[13] All four analogues—the target *trans*-dihydroindoline **2** and the structurally related compounds **6**, **7**, and **8**[15]—were prepared in ample quantities for bioassays.

## 2. Biological assays

The activities of compound **2** and the three structurally related analogues **6**–**8** were screened in oocytes injected with native RNA from rat cerebral cortex.[16] Potential agonist activity was assayed based on the membrane current generated at concentrations of 100 μM for each analogue against varying concentrations (10, 30, and 100 μM) of kainic acid as control (Fig. 5A). The same compounds were screened for antagonist activity by measurements of 50 μM kainate-induced membrane currents inhibited by the presence of each analogue (100 μM) (Fig. 5B). For comparison, the very potent benchmark antagonist DNQX was also assayed in this system. Experiments were highly reproducible in six oocyte preparations, with a standard error of only a few percent, as indicated by the error bars in Figure 5B.

In the agonist activity assay, there was no current observed for any of the analogues at concentrations up to 100 μM, while the control agonist kainic acid induced currents at concentrations as low as 10 μM. In contrast,

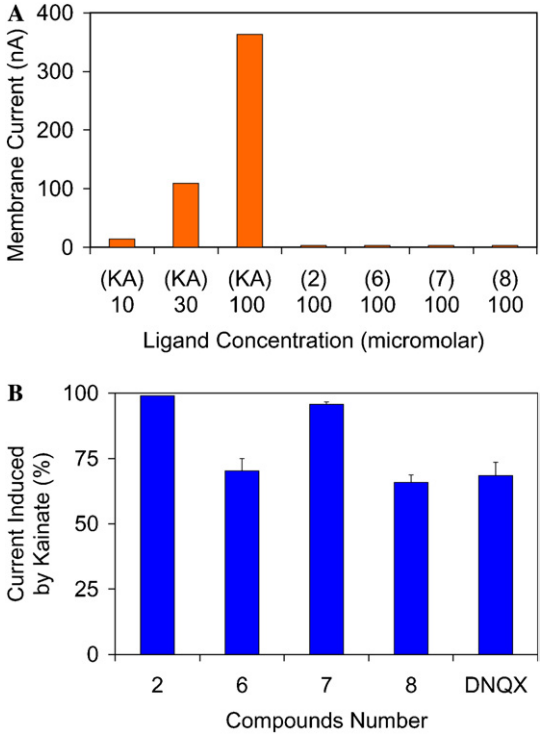


**Figure 5.** Assays of indoline/indole ligands **2**, **6**, **7**, and **8** in oocytes injected with native RNA from rat cerebral cortex. (A) Agonist activity assay: membrane current generated from the analogues compared to kainic acid control. (B) Antagonist activity assay: percentage of current induced by kainic acid (50 μM) in the presence of the analogues (100 μM); no analogue = 100%; DNQX was tested at 0.1 μM.

the antagonist assay showed that ligands **6** and **8** at 100 μM inhibited approximately one-third of the current induced by 50 μM kainic acid. While ligands **2** and **7** show little or no activity at these 100 μM concentrations, blocking only 2% and 5% of the current, respectively, they do block currents (7% and 12%, respectively) at lower concentrations (30 μM) of kainic acid (data not shown). For comparison, the very potent antagonist DNQX blocked 30% of the current at a concentration of 0.1 μM.

## 3. Discussion

The results of the biological assays of compounds **2**, **6**, **7**, and **8** clearly show that they are KAR antagonists rather than agonists, despite the fact that **2** was designed around the structure of the agonist kainic acid, and that **2** has previously been reported to be inactive in KAR-binding assays.[11b] The antagonist activity of these analogues prompted us to dock them in the binding site of the iGluR2S1S2 construct, but beginning with the structure of the complex of the known antagonist DNQX,[8] rather than with the kainic acid complex (the L-isomers of **2** and **7** were modeled, by analogy to natural kainate). Docked and minimized as before,[11] the analogues adopt orientations similar to DNQX in the active site (Fig. 6; for clarity, only analogue **6** is shown) in which the respective phenyl groups push residues E402, P478, T686, and E705 farther apart compared with agonist complex iGluR2S1S2–kainic acid. As mentioned above, Gouaux has postulated that domain closure is crucial for activation of the receptor, but the binding of the 7-nitro group of DNQX to T686 blocks the interaction of T686 with E402, which is a critical contact in this process. Similarly, the phenyl group of each analogue blocks this contact and perhaps also stabilizes the open state of the clamshell-like S1S2 core, both of which could contribute to the observed antagonist activity.

Interestingly, all four analogues show antagonist behavior at similar potencies, although indole **6** and **8** are somewhat more potent than either the designed indoline analogue **2** or its *cis*-isomer **7**. In addition, the results suggest that binding affinities for **6** and **8** are only marginally lower than that of kainic acid itself, but approximately 1000-fold less than that of the very potent, nonselective antagonist DNQX (the potencies could be higher for enantiomerically pure analogues). In retrospect, the antagonist behavior of these compounds is perhaps not surprising, given the fact that these bicyclic aromatic compounds are somewhat reminiscent of the DNQX structure and appear to assume very similar orientations in the binding site to that of DNQX. Specifically, a comparison of the probable binding interactions for each ligand suggests that **6** and DNQX generally form better contacts than does the L-indoline **2**. The α-carboxyl group of all ligands forms hydrogen bonds with the guanidinium group of R485 and the main chain NH of T480. Also, the α-amino group of the ligands can interact with the carbonyl group of P478, the hydroxy group of T480, and the side-chain carboxylate of E705. Some of these important interactions appear to be absent in

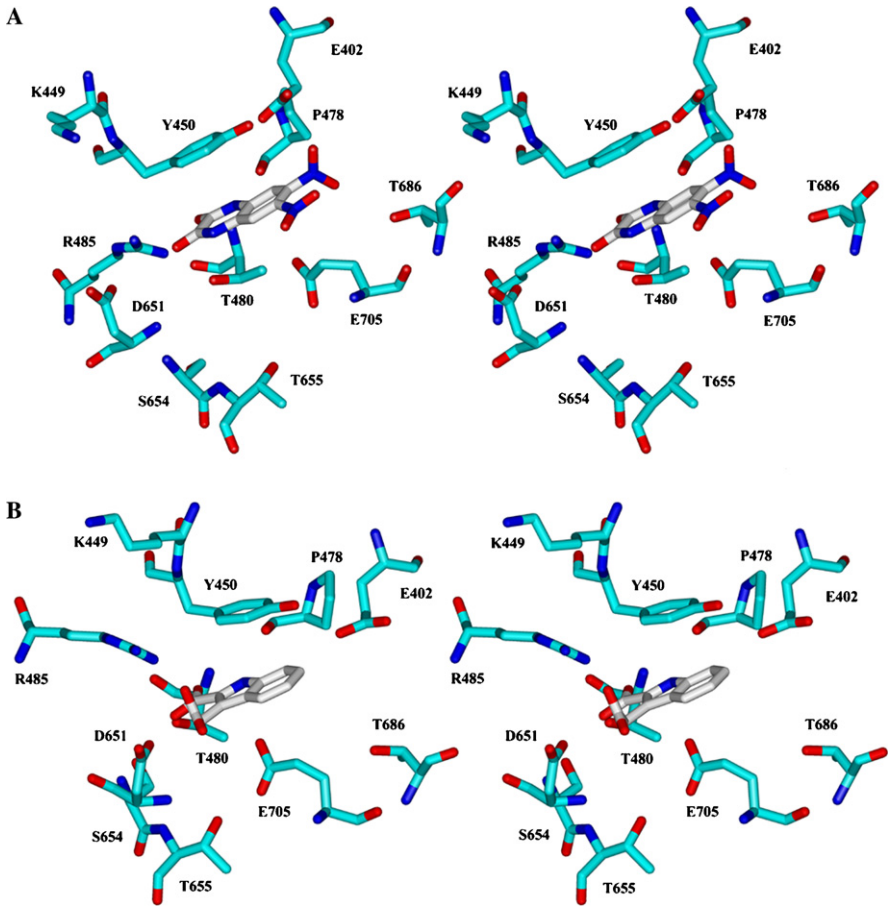


**Figure 6.** (A) Stereoview of antagonist DNQX in the binding pocket of iGluR2S1S2 construct X-ray structure. (B) Stereoview of indole analogue **6** docked and minimized in the binding pocket of an iGluR2S1S2 construct.

indoline **2** complex, as residues E705, R485, T480, and P478 are several Angstroms farther from the ligand in the minimized structures of these complexes. Likewise, in the *cis*-indoline **7** complex, the essential residues are even farther removed. On the other hand, most of these interactions are maintained in the indole complexes **6** and **7** (not shown), as they are in the DNQX complex. It also appears that a stronger π–π interaction between **6** and the aryl ring of Y450 may contribute significantly to ligand binding, as compared to the indoline **2** complex, in which Y450 is pushed farther away by its ligand α-carboxylate (not shown). While any computational study is inherently speculative, we believe that these models provide a reasonable working hypothesis for the observed antagonist activity of this family of analogues, and further studies are underway based on this model.

## 4. Conclusion

A conformationally constrained kainate analogue was designed based on Gouaux's X-ray structure of an iGluR2S1S2 construct complexed with kainic acid. It was synthesized in racemic form and, along with three other structurally related analogues, was screened for activity in oocyte assays. Although the analogues were originally designed as kainate-like agonists, their indole-based structures, instead, resulted in antagonist activity. A refined computational model suggests a mode of binding that is reminiscent of that found among the non-selective quinoxalinedione antagonists, such as DNQX. The change to antagonist behavior (compared to kainate) likely results, as proposed by Gouaux for DNQX, from additional interactions of the indole aryl ring that block the interdomain closure necessary for channel opening. This indole structure, aside from being an interesting antagonist in its own right, also serves as a core scaffold for library synthesis of potential iGluR receptor subtype antagonists, guided by our homology models of iGluR1, 3–7 complexes (unpublished), and the recently reported crystal structures of iGluR5 and iGluR6 ligand binding cores.[17]

## Acknowledgments

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