Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 551–559

BIOORGANIC & MEDICINAL CHEMISTRY

The Structural Basis for Kainoid Selectivity at AMPA Receptors Revealed by Low-Mode Docking Calculations

Luis M. Carcache, Jonierr Rodriguez and Kathleen S. Rein*

Department of Chemistry, Florida International University, 11200 SW 8th St., Miami, FL 33199, USA

Received 15 March 2002; accepted 26 August 2002

Abstract—The kainoids are a class of excitatory and excitotoxic pyrrolidine dicarboxylates that act at ionotropic glutamate receptors. The kainoids bind kainate receptors with high affinity and, while binding affinity is lower at AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors, they are active in functional assays at this receptor subtype as well. However, kainoids are only partial agonists at AMPA receptors. Currents evoked by kainoids have been described as either slowly desensitizing, partially desensitizing, or non-desensitizing. Recently acquired X-ray crystal structures of the ligand binding domain of the iGluR2, AMPA sensitive receptor suggest that differences in ligand–receptor interactions may influence functional properties of an agonist. In an effort to identify important ligand–receptor interactions of various kainoids, we have conducted a series of low-mode docking searches of AMPA agonists in the iGluR2 binding domain. Kainic acid exhibited alternate low-lying geometries, with loss of hydrogen bonds to domain 2, which may represent a dissociation route not available to other kainoids. The most potent of the kainoids are capable of forming hydrogen bonding interactions that span the two domains of the receptor. In particular, a hydrogen bond between the domoic acid C6′ carboxylic acid and Ser652 may prevent a peptide bond rotation that is associated with the desensitized state of the receptor.

Introduction

The kainoids are a class of pyrrolidine dicarboxylates that vary in the nature and configuration of the substituent at position 4 of the pyrrolidine ring and exhibit both excitatory and excitotoxic activity (Fig. 1). Kainic acid, a naturally occurring toxin isolated from the seaweed *Diginea simplex*,[1] is the parent of the class. Perhaps the most notorious of the kainoids is domoic acid, a marine environmental toxicant produced by the diatom, *Nitzschia pungens*. Domoic acid is responsible for a syndrome known as Amnesic Shellfish Poisoning[2] which is characterized by severe dementia and memory impairment.[3] Other naturally occurring kainoids include several congeners of domoic acid[4,5] as well as the poison mushroom constituents acromelic acids A–E.[6–8]

The excitotoxicity of the kainoids results from their ability to function as glutamate receptor agonists.[6,9] Glutamate is the principle neurotransmitter of the mammalian central nervous system and, as such, several subtypes of glutamate receptor are present in the CNS. The two major subclasses include the metabotropic and ionotropic groups. Glutamate gated ion channels are known to play critical roles in normal processes (learning and memory) as well as neurodegenerative processes (i.e., Alzheimer's and Parkinson's). The ionotropic glutamate receptors are further sub-divided according to their most potent agonist. They include the NMDA (*N*-methyl-D-aspartic acid) receptors, the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors and the kainate receptors. Taken together, the AMPA and kainate receptors are often referred to as the 'non-NMDA' receptors. To date, six NMDA receptor subunits (NR1, NR2A–2D and NR3A), four AMPA receptor subunits (iGluR1–4) and five kainate receptor subunits (KA1–2, iGluR5–7) have been cloned from rat, and characterized with respect to primary structure.[10–12]

While most kainoids are selective for the kainate receptor, many also have lower affinities for, yet are able to activate, AMPA receptors as well. Peak currents evoked by kainate at AMPA receptors are smaller than those evoked by AMPA and do not desensitize completely.[13]

*Corresponding author. Tel.: +1-305-348-6682; fax: +1-305-348-3772; e-mail: reink@fiu.edu

0968-0896/03/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0968-0896(02)00448-0

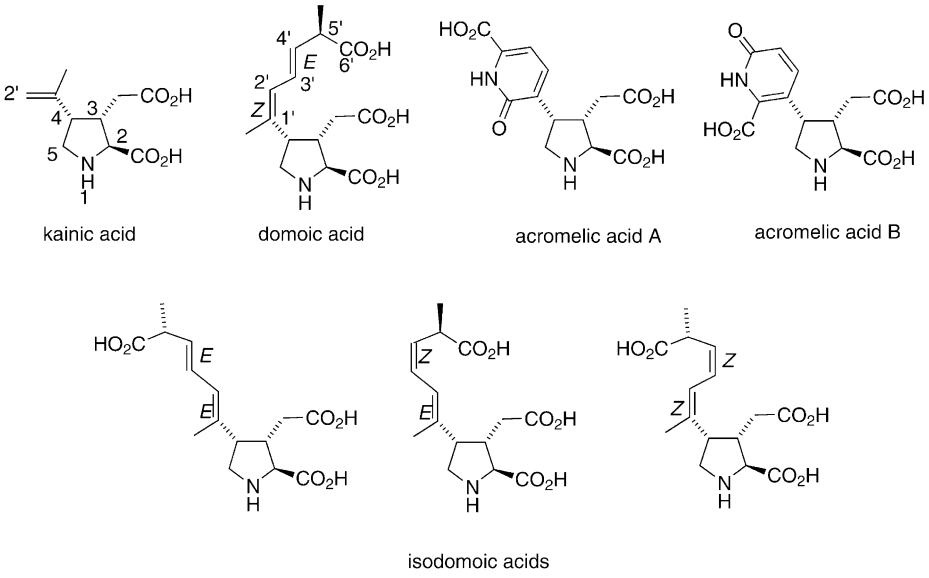

Figure 1. Structures of some naturally occurring kainoids.

Currents evoked by domoic acid have been described as non-desensitizing.[14] Currents induced by acromelic acid A have been described as more 'AMPA-like' than those induced by kainate.[15]

Dissociation constants for the majority of kainoids at the AMPA receptor are typically in the low micromolar range (depending on the specific receptor). However, binding affinities do not correlate well with functional EC_{50} values.[14,16] The affinity of domoic acid for AMPA receptors is roughly equivalent to, or only slightly better than, kainic acid (Table 1). However, activity studies indicate that domoic acid is a much more potent agonist at both AMPA and kainate receptors.[16] Unlike kainic acid, domoic acid is thought to induce neurotoxicity through interaction with *both* the kainate and AMPA receptors.[17] Acromelic acid A is the only naturally occurring kainoid with a dissociation constant in the low nanomolar range (Table 1) and it is the most potent of the kainoids at AMPA receptors. Additionally, acromelic acid B is less potent than acromelic acid A at AMPA receptors.[18]

The development of a model for kainoid selectivity at non-NMDA receptors has been hampered by the lack of a three-dimensional model of iGluRs. Computer models based on homologies between iGluRs and bacterial periplasmic amino acid binding proteins predicted the structure of the ligand binding core with remarkable accuracy.[19–21] More recently, crystal structures of the extracellular ligand binding core of iGluR2 in the apo form and co-crystallized with kainic acid, AMPA and glutamate have become available.[22,23]

Table 1. Dissociation constants for kainoids at AMPA receptors

Compd	K_d (nM)
Kainic acid	2800,[a] 1237[b]
Acromelic acid A	16,[c] 39[b]
Domoic acid C5′ *R*	2500,[a] 306,[b] 499[c]
Isodomoic acid C5′ *S*	5700[a]
Isodomoic acid (*EZ*)	12,000[a]
Isodomoic acid (*ZZ*)	17,000[a]
Isodomoic acid (*EE*)	32,000[a]

[a]Hampson, 1992.
[b]Kwak et al. *Neurosci. Lett.* **1992**, *139*, 114
[c]Smith et al. *Br. J. Pharmacol.* **1992**, *105*, 83.

The iGluR binding domains are composed of extracellular N-terminal segment S1 and extracellular loop S2 separated by two transmembrane domains. The expression of a construct linking S1 and S2 (from iGluR2) with concomitant deletion of the membrane spanning domains provided a soluble protein with binding affinities similar to the native receptor that formed ordered crystals when bound to kainic acid.[23] The crystal structures revealed that the binding region is composed of two domains. Domain 1 formed from S1 and the 33 C-terminal amino acids of S2. Domain 2 is composed of the N-terminal 134 amino acids of S2. Glutamate receptor agonists bridge these two domains promoting domain closure. It was postulated that the isopropylidene group of kainic acid, which extends into the solvent, acts as a wedge between domains, preventing further domain closure. Crystallization of the binding core in the apo form and with AMPA and glutamate supported this hypothesis since domain separation was found to increase in the order AMPA $\approx$ glutamate $<$ kainate $<$ apo.

The resolution of a three-dimensional model for the ligand binding core of iGluR2 co-crystallized with several ligands and in various stages of closure not only provided a tremendous advance in our understanding of the mechanism of ligand binding and gating of ionotropic glutamate receptors, but will no doubt prove to be a tremendous advantage in the structure-based design of ligands of pharmacological interest in the study of many neurological disorders. It also presents a unique opportunity to identify the molecular basis of *selectivity* of the kainoids for their non-NMDA receptor subtypes or for the extreme variability in potency and desensitization rates of different agonists.

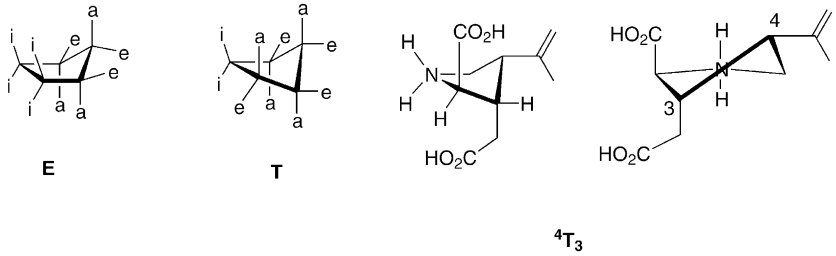

Figure 2. Envelope (E) and half-chair (T) conformations of cyclohexane. Two views of the $^4T_3$ conformations of kainic acid (right). The superscript and subscript refer to the out of plane atoms. The superscript refers to the atom on the same face of the pyrrolidine ring as the C2 carboxylic acid.

The application of the low-mode docking search to the docking of ligand–receptor pairs has recently been described.[24,25] This approach addresses the complex problem of docking a flexible ligand into a flexible receptor. The low-mode conformational search (LMCS) method consists of a Monte Carlo (MC) step in which the atoms in the freely flexible parts of the structure are moved along a trajectory that is consistent with the low frequency normal modes of vibration for that part of the structure. In low-mode *docking* searches, ligands are additionally subject to explicit translations and rotations. Thus both conformational *and* orientational space is sampled. Each Monte Carlo step is followed by energy minimization (EM) of the geometrically perturbed structure. We report here the results of the low-mode docking search of kainic acid, acromelic acids A and B, domoic acid, and several isomers of domoic acid in the ligand binding core of iGluR2, when co-crystallized with kainic acid (PDB Id: 1GR2). These computations reveal the basis for the high affinity of acromelic acid A when compared to other kainoids and were able to predict the relative selectivities of domoic acid isomers which exhibit a very narrow range of binding affinities. Furthermore, these calculations suggest a molecular basis for the non-desensitizing nature of currents evoked by domoic acid.

Results

Five-membered rings may occupy one of two conformations possessing symmetry (Fig. 2), the envelope (E) and the twist (T). Each atom may assume any position in the various conformations. Thus ten envelopes and ten twists, along with intermediate geometries along the pseudorotational path, are available to kainoids. The conformation of kainic acid within the ligand binding core of iGluR2 corresponds to $^4T_3$.[26] This structure served as the starting geometry for low-mode docking searches of kainic acid and other kainoids in iGluR2.

Ten structures within a 5 kcal/mol energetic window (Table 2) were identified for kainic acid. These ten structures represented four unique ring conformations: $^4T_3$ (four side-chain rotomers), $^3T_4$ (four side-chain rotomers), E_5 (which is closely related to $^4T_3$) and ^1E each represented only once. The RMS for superposition of the five ring atoms on the crystal structure, as well as the comparison of the side chain orientation, reveal that structure 2 corresponds most closely to that of kainic acid bound to iGluR2.

Within a 20 kJ/mol energetic window, the pyrrolidine ring of acromelic acids A and B adopts only the $^4T_3$ conformation when docked in the S1S2 receptor (Tables 3 and 4). Six geometries are available to the acromelic acid A/receptor pair, while only three are available to the acromelic acid B/receptor pair. The various structures differ in kainoid side-chain orientation and in the orientation of the ligand relative to the receptor. As one might have anticipated, additional interactions, between the side chain of the ligand and the receptor, were identified for both acromelic acids A and B, although the interactions are not identical for these two ligands (Tables 3 and 4). The two lowest energy structures for acromelic acid A differ in energy by only 0.22 kcal/mol and share some common features. Structure 1 forms hydrogen bonds between the piperidone ring NH and Glu402 (δ-carboxylate) on domain 1 and between the piperidone ring carboxylate and Thr686 (backbone NH) on domain 2. Structures 2 and 3 are distinguished from 1 by a 180° C4 side-chain rotation, resulting in the loss of the hydrogen bond to Glu402, but the hydrogen

Table 2. Conformations of kainic acid in the iGluR2 receptor

Conf.	RMS[a] (Å)	Relative energy (kcal/mol)	C3–4–1′–2′ dihedral angle (crystal = 120°)
$^3T_4$	0.337	0	90°
$^4T_3$	**0.048**	**0.28**	**118°**
$^3T_4$	0.351	0.38	30°
$^4T_3$	0.055	2.52	−85
$^4T_3$	0.053	2.62	−45
^1E	0.241	2.99	100
$^3T_4$	0.337	3.47	−129
$^3T_4$	0.349	3.73	32
E_5	0.164	3.77	114
$^4T_3$	0.047	4.84	118

[a]For superposition of the five ring atoms on the crystal structure conformation. The ligand bound conformation of kainic acid is shown in bold.

Table 3. Conformations of acromelic acid A in the iGluR2 receptor

Entry	Conf.	Relative energy (kcal/mol)	Side-chain interactions
1	$^4T_3$	0	NH to Glu402, CO_2^- to **Thr686**
2	$^4T_3$	0.22	CO_2^- to **Thr686**
3	$^4T_3$	2.04	CO_2^- to **Thr686**
4	$^4T_3$	3.70	
5	$^4T_3$	3.78	
6	$^4T_3$	4.03	Lys449

Domain 2 residues are shown in bold.

bond to Thr686 remains. No hydrogen bonds are formed between the receptor and the piperidone ring in the two lowest energy structures of acromelic acid B. Structure 3 forms hydrogen bonds from Gly451 and Tyr450 to the ring carboxylate, but only at the expense of critical hydrogen bonds to NH of the kainate ring (Pro478 and Glu705).

The low-mode docking search of domoic acid in iGluR2 revealed that only the $^4T_3$ and the closely related E_3 conformations were represented within a 25 kJ/mol window, with the $^4T_3$ significantly lower in energy (Table 5). Likewise, less variability was observed in the C3–C4–C1′–C2′ dihedral angle, which ranged only from −76° to −92° in domoic acid compared to −129° to 118° in the kainic acid structures. Notable are numerous electrostatic interactions between the domoic acid side chain and the amino acid residues in all of the structures identified. In the two lowest energy conformations, the C6′ carboxylate interacts with several residues. Domain spanning hydrogen bonds to Gly451, Tyr450 (backbone NH) and Ser652 (side chain OH) were observed in the two low energy structures. Higher energy conformations exhibited interactions with Arg485 and Tyr450. Isomers of domoic acid adopt nearly identical conformations both in the ring and the side chain. However, far fewer geometries are observed for the C5′ diastereomer of domoic acid (Table 6). Electrostatic interactions between the C6′ carboxylate and Lys449, Tyr450 and Ser652 were identified in the two low energy structures of the C5′ diasteroemer. Some re-orientation within the binding pocket to accommodate less than optimal stereoisomers was observed. With the exception of the *EE* isomer (the poorest ligand) the side chain of all domoic acid isomers interacted through hydrogen bonds with the receptor.

Table 4. Conformations of acromelic acid B in the iGluR2 receptor

Entry	Conf.	Relative energy (kcal/mol)	Side-chain interactions
1	$^4T_3$	0	None
2	$^4T_3$	0.42	None
3[a]	$^4T_3$	0.74	CO_2^- Gly451 and Tyr450
4	$^4T_3$	5.64	Outside of binding site

[a]Hydrogen bonds to kainate NH and Pro478 and Glu705 are lost.

Table 5. Conformations of domoic acid in the iGluR2 receptor

Entry	Relative energy (kcal/mol)	Conf.	Side-chain interactions C5′ carboxylate
1	0	$^4T_3$	Gly451, Tyr450, **Ser652**
2	0.24	$^4T_3$	Gly451, Tyr450, **Ser652**
3	2.09	E_3	Gly451
4	2.44	E_3	Gly451
5	2.96	E_3	Gly451, Arg485
6	3.00	$^4T_3$	Gly451, Tyr450
7	3.32	$^4T_3$	Gly451, Tyr450
8	3.66	$^4T_3$	Gly451, Tyr450
9	3.98	$^4T_3$	Tyr450, Arg485
10[a]	4.49	$^4T_3$	Gly451, Tyr450, **Ser652**
11[b]	4.69	E_3	Gly451, Tyr450, Ser652
12	4.71	$^4T_3$	Tyr450
13	4.93	E_3	Tyr450

[a]Hydrogen bond to Pro478 is lost.
[b]Hydrogen bond to **Glu705** is lost. Domain 2 residues are shown in bold.

Discussion

Kainic acid

As a test of the computational method, an LMCS/MOLS search of kainic acid in the ligand binding core of S1S2 was performed. The receptor was represented as a fixed shell within which various kainic acid orientations and conformations were sampled. Although numerous starting geometries were used and explicit translations and rotations were performed, kainic acid remained essentially in the starting position maintaining *most* of the critical interactions, with only minor rotations observed in the higher energy conformations. Structure 2, at only 0.28 kcal/mol above the global minimum, closely resembles the conformation identified in the crystal structure. The identification of the crystallographically determined geometry among the low lying structures is generally considered as an indicator of the success of the docking calculation.[24] The dihedral angle between the substituents at the C2 and C3 positions of the pyrrolidine ring in conformation $^4T_3$ is 152° and the isopropylidene group is roughly coplanar to the pyrrolidine ring. This arrangement allows the *anti-periplanar* carboxylate groups to act as a tether between the two domains via electrostatic interactions. Important hydrogen bonding interactions identified in the crystal structure are also maintained in structure 2. These interactions include, on domain 1, Arg485 (guanidinium NH to the α-carboxylate of kainate), Thr480 (NH to the α-carboxylate of kainate), Pro478 (carbonyl oxygen to kainate NH) and on domain 2, Glu705 (γ-carboxylate to kainate NH), Ser654 (NH to the α-carboxylate of kainate) and Thr655 (NH and OH to the γ-carboxylate of kainate). The arrangement of the isopropylidene groups allows the ligand to slip into the narrow cleft between domains (Fig. 3). By contrast, the C2 and C3 substituents are *synclinal* (dihedral angle of 85°) to one another in the $^3T_4$ conformation and the isopropylidene group is roughly perpendicular to the plane of the pyrrolidine ring. In this arrangement the γ-carboxylate no longer interacts with Thr655. The

Table 6. Conformations of domoic acid C5′ diastereomer in the iGluR2 receptor

Entry	Conf.	Relative energy (kcal/mol)	Side-chain interactions C5′ carboxylate
1[a]	$^4T_3$	0	Lys449, Tyr450, **Ser652**
2	$^4T_3$	0.36	Lys449, Tyr450, **Ser652**
3[b]	$^4T_3$	4.30	Arg485
4	$^4T_3$	4.36	Arg485
5	$^4T_3$	4.55	Arg485, Gly451

[a]Hydrogen bond between kainate NH and **Glu705** is lost.
[b]Hydrogen bonds between kainate NH and Pro478 and **Glu705** are lost. Domain 2 residues are shown in bold.

hydrogen bond formed between kainate and Thr655 is critical to kainate binding as demonstrated by mutational studies on related kainate binding proteins. Mutation of the corresponding Thr247 of a goldfish kainate binding protein[27] and the corresponding Ser267 of a chick kainate binding protein[28] completely abolishes affinity for kainic acid. Thus when kainic acid is bound in the $^3T_4$ conformation, the receptor may more readily revert to the apo form with subsequent dissociation of the kainic acid. The availability of this low-lying geometry, with concomitant loss of a critical hydrogen bond to domain 2, may provide a route to domain opening and subsequent agonist release that is not available to other kainoids. The $^3T_4$ conformation is accessible only to kainic acid because the side chain is small. For the acromelic and domoic acids, this conformation would extend the larger side chain into the 'floor' of the receptor (domain 2). Thus the pyrrolidine ring of these larger kainoids is locked into the optimal binding geometry in the bound state, by virtue of the larger C4 side chain. None of the other kainoids examined in this study enjoy as much conformational freedom within the S1S2 ligand binding core as kainic acid. This observation may be significant since a recent LMCS/MOLS study of cytochrome P450 inhibitors correlated decreased conformational mobility with increased activity.[29]

Acromelic acids A and B

Acromelic acid A is the only naturally occurring kainoid that exhibits low nanomolar binding to the AMPA receptor (Table 1). A particularly noteworthy outcome of the LMCS/MOLS search is the identification of the hydrogen bonds between Glu402 (domain 1), Thr686 (domain 2) and the piperidone ring (Fig. 4a). A hydrogen bond between Glu402 (δ-carboxylate) and Thr686 (side-chain hydroxyl) identified in the crystal structure remains intact when acromelic acid A is bound to the receptor. Thus the additional domain spanning interactions of acromelic acid A serve to enhance already existing interdomain contacts. A 180° C4 side-chain rotation (structures 2 and 3) eliminates the hydrogen bond between NH and Glu402 but the hydrogen bond to Thr686 remains. Parenthetically, molecular dynamics simulations of S1S2 opening in the presence of kainate[30] suggest that interactions between kainate and domain 1 remain intact during the entire process. Interactions with domain 2 are the first to be disrupted, suggesting that these interactions are weaker. Thus, any additional interactions with domain 2 (i.e., Thr686) may be most critical in enhancing activity of the kainoids. While further domain closure, which is observed with glutamate and AMPA, may be precluded for steric reasons, these domain spanning interactions, present in the three lowest energy structures, must enhance binding by preventing domain separation and reversion to the apo form.

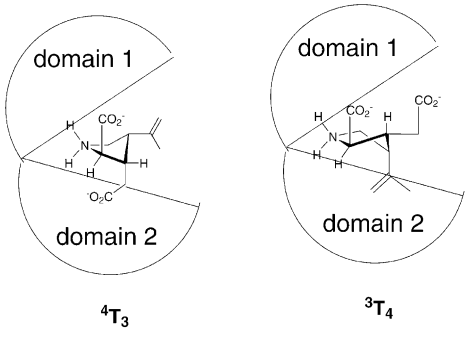

Figure 3. Model for two conformations ($^4T_3$ and $^3T_4$) of kainic acid in the S1S2 receptor.

The number of geometries accessible to acromelic acid B becomes limited by virtue of moving the ring carboxylate to the 2-position, closer to the narrow 'hinge' region of the receptor. Unlike acromelic acid A, the two lowest energy structures do not hydrogen bond to receptor through the piperidone ring (Fig. 4b). Furthermore, inspection of Figure 4b reveals that a critical hydrogen bond from the α-carboxylate of acromelic acid B to Ser654 is lost. The ring carboxylate is hydrogen bonded to Gly451 and Tyr450 (both belonging to domain 1) in structure 3, but only at the expense of critical hydrogen bonds to NH of the kainate ring (Pro478 and Glu705). Since these (latter) interactions are common to all ligands (kainate, AMPA and glutamate), we believe they must be critical to domain closure and their loss leads to domain separation. The LMCS/MOLS method does not currently allow backbone movement of the protein. Thus the consequence of the loss of the critical hydrogen bond to Glu705 on the degree of domain closure cannot be predicted with this technique. However, recent molecular dynamics simulations of the conformational changes that take place during S1S2 domain separation indicate that the early stages of domain opening are correlated with loss of the hydrogen bond between the ligand (glutamate or kainate) NH and Glu705,[30] supporting the hypothesis that this interaction is critical to maintaining domain closure. Also noteworthy is the fact that the additional interactions of acromelic acid B are with domain 1, and may not help to promote domain closure. Structure 4 of the acromelic acid B/receptor pair lies outside of the 5 kcal/mol energetic window that we consider for discussion purposes, but is included here for completeness. In this structure, acromelic acid B no longer resides in the binding site, but is rather bound to the outer surface of the receptor. No such observation was made for acromelic acid A within the 6 kcal/mol window that was sampled (or for any of the other kainoids studied), suggesting that the acromelic acid B/receptor pair lies in a shallow potential energy well when compared to other kainoids. Thus LMCS/MOLS correctly predicts that acromelic acid B is a poorer ligand relative to acromelic acid A.

Domoic acid in iGluR2

The binding affinities for kainic acid and domoic acid at AMPA receptors are similar. However, binding affinities do not correlate well with functional EC_{50} values, as AMPA receptors respond to these two agonists with markedly distinct kinetics.[14,16] These differences must be attributable to different intermolecular interactions which can be identified by performing a conformational/orientational analysis of domoic acids in iGluR2.

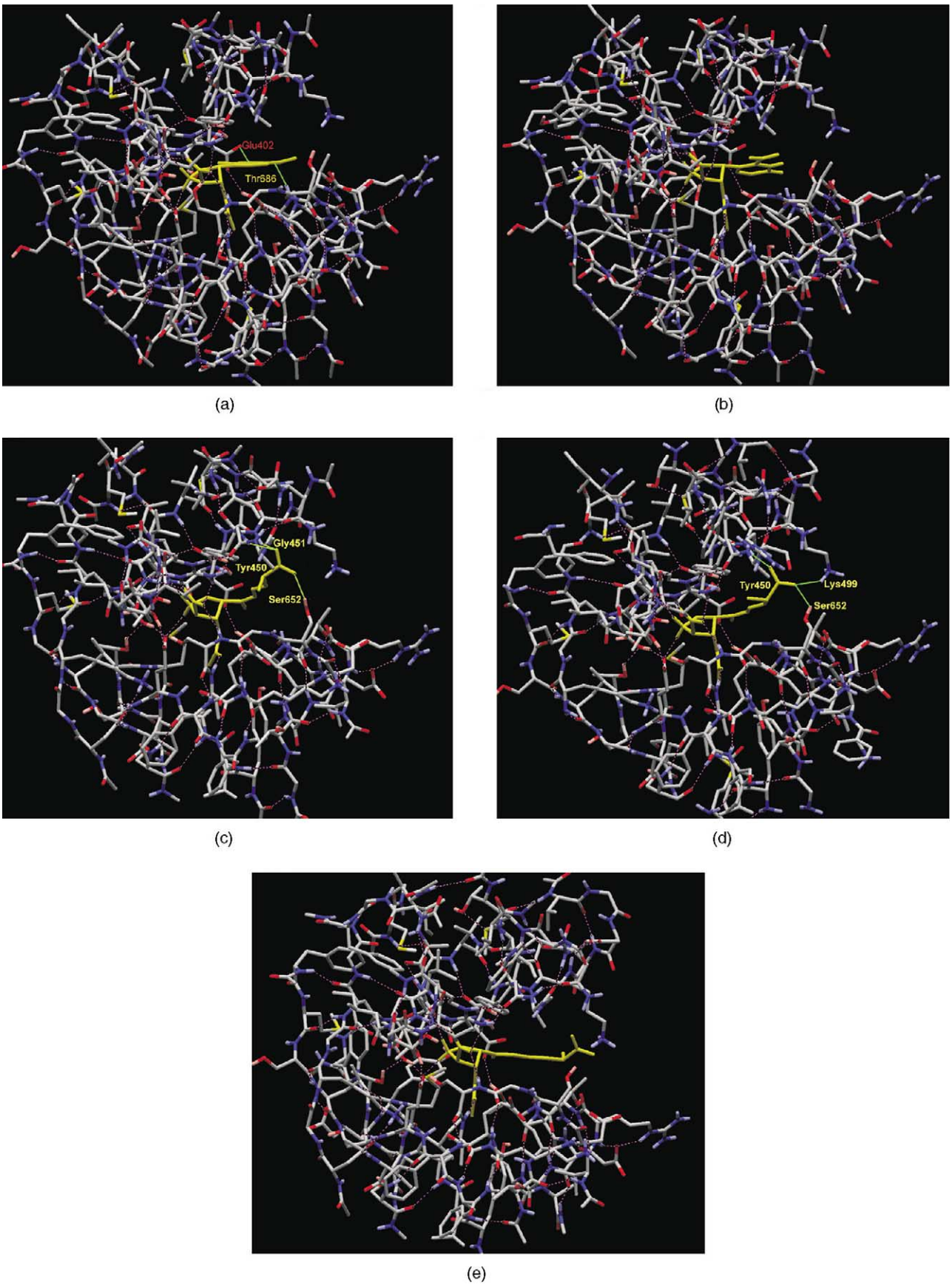

Figure 4. Global minimum conformations of kainoids in the iGluR2 S1S2 receptor. Dashed purple lines represent hydrogen bonds within the receptor. Green lines represent hydrogen bonds between the ligand and receptor. (a) Acromelic acid A. Note hydrogen bonding interactions between the acromelic acid side chain and Glu402 and Thr686. (b) Acromelic acid B. Note loss of hydrogen bond to Ser654. (c) Domoic acid. (d) C5′ diastereomer of domoic acid. (e) Domoic acid *EE* isomer.

When compared to kainic acid, domoic acid enjoys significantly less conformational mobility in both the side chain and the pyrrolidine ring when docked in the S1S2 receptor. For domoic acid, only the $^4T_3$ and the closely related E_3 conformations were observed (Table 5). Furthermore, LMCS/MOLS indicates that the isopropylidene group of kainic acid enjoys a significant degree of conformational mobility. For domoic acid, the C3–C4–C1′–C2′ torsion angle occupied a narrow range of −76° to −92° in all conformations. The torsion angle between C3 of the pyrrolidine ring and the side-chain methyl is 60° (*synclinal*) in the kainic acid crystal structure, whereas in domoic acid it is *anticlinal*. While this may seem an apparent contradiction, it is consistent in that the larger of the two groups is *synclinal* in both instances. We interpret these observations as a natural consequence of the greater steric demand of the larger side chain.

The *synclinal* torsion angles extend the side chain of domoic acid into the cleft between the two domains of the receptor, rather than into the solvent. This arrangement allows the domoic acid side chain to interact with residues on the S1S2 receptor. The most notable features of domoic acid binding are the additional, domain spanning, electrostatic interactions between the C6′ carboxylate and Gly451, Tyr450 (backbone NH) and Ser652 (side-chain OH) (Fig. 4c). The additional interaction with Ser652 on domain 2 may be the most critical in terms of enhancing the activity of domoic acid over kainic acid at AMPA receptors. In Armstrong's analysis of the S1S2 crystal structures, a roughly 180° *trans* peptide bond flip between Asp651 and Ser652 between the kainate bound form and the AMPA bound form was noted. This rotation was also observed in molecular dynamics simulations of backbone motions of the receptor.[31] It was suggested that this peptide bond reorientation modulates receptor deactivation and agonist release. It is further believed that the geometry of the AMPA bound state corresponds to the desensitized state. We suggest that the hydrogen bond between the domoic acid C6′ carboxylate and Ser652 inhibits desensitization by preventing this peptide bond rotation. Stabilization of the conducting state contributes to the enhanced toxicity of domoic acid over other glutamate receptor agonists. It is interesting to note that the corresponding residue in kainate receptors (iGluR5–7) is aspartic acid, which would not be expected to hydrogen bond to the domoic acid side chain. It is not likely that full domain closure of iGluR2 is possible when bound to any kainoid. However, domoic acid is able to act as a bridge, replacing at least some of the domain spanning, electrostatic interactions which are present in the fully closed form. When the S1S2 receptor is in its fully closed form, Gly451 (NH) is hydrogen bonded to Ser652 (C=O).[22] Thus two features of domoic acid binding, the inaccessibility of the $^3T_4$ conformation and the additional hydrogen bonding interactions with the C4 side chain, stabilize the domoic acid–iGluR2 pair in the conducting state compared to the kainate–iGluR2 pair.

Table 7. Estimated relative docking energies for domoic acid isomers

Agonist	Relative docking energy (kcal/mol)	Relative docking energy[a] (kcal/mol)
Domoic acid	0	0
C5′ diastereomer	0.78	0.81
Isodomoic acid *EZ*	−5.11	−2.41
Isodomoic acid *ZZ*	−2.52	−4.51
Isodomoic acid *EE*	−11.05	−8.93

[a]Explicit rotations of Ser652 and Lys449 side chains included in the docking search.

Isodomoic acids in iGluR2

We next turned our attention to several isomers of domoic acid for which binding studies at AMPA receptors have been reported. These isomers adopt conformations that are identical to domoic acid in the bound state. Only the $^4T_3$ and related E_3 ring conformations were observed and the side-chain methyl and C3 are *anticlinal* in all instances. However, there is a great deal of conformational mobility about the C4′–C5′ bond and this is where most differences were found. We first examined the C5′ diastereomer, which is predicted to be only a slightly poorer ligand for AMPA receptors than domoic acid. In the global minimum structure of domoic acid, the C5′ methyl extends into the solvent. Inversion of the C5′ stereocenter directs the C5′ methyl toward domain 1 (specifically Arg485) (Fig. 4d). Because of this, we had anticipated that inversion of the C5′ stereocenter would preclude C6′ interactions with the receptor. However, the lowest energy structure of this diastereomer is nearly identical to that found for domoic acid, including similar (but not identical) side-chain hydrogen bonding interactions (Table 6). Due to a slight rotation (clockwise as viewed in Fig. 4d) of the C5′ diastereomer in the receptor, to accommodate the inversion at C5′, the hydrogen bond to Gly451 is lost. However, it is compensated for by a hydrogen bond to Lys449. In contrast, the energy minimum structure for isodomoic acid *EE* (the poorest of the domoic acid ligands) forms no hydrogen bonds from the domoic acid side chain to the receptor.

Docking energies are compared for the global minima of domoic acid, the C5′ diastereomer, and double bond isomers in Table 7. Inspection of Table 7 suggests that LMCS/MOLS can predict trends in binding affinities when differences are large. However, LMCS/MOLS has predicted inverse relative affinities for domoic acid and its C5′ diastereomer as well as for the *ZZ* and *EZ* isomers. Thus we were prompted to refine our docking search and include rotations of side chains of selected residues of the receptor. The two residues (Lys449 and Ser652) were chosen because they extend into the binding cleft and were seen in the previous search to interact with the side chains of domoic acid and its isomers via hydrogen bonds. We felt that variations in receptor side-chain conformations had the potential to significantly influence the outcome of the conformational searches for these isomers. Inspection of the results detailed in Table 7 reveals that this is indeed the case. The global minimum conformation of each isomer is significantly lower (6–11 kcal/mol) when side chain

movement is permitted. With the exception of the C5′ diastereomers (which are quite close in terms of binding affinity) LMCS/MOLS now predicts the correct relative affinities for *all other isomers* (including the *ZZ* and *EZ* isomers), both in terms of total energy relative to domoic acid and in terms of estimated docking energy.

Conclusion

A recent analysis of the kinetics of conformational motions associated with binding of ligands to the S1S2 binding domain suggests that a two-step process is in effect.[32] First is a rapid association (locking) whose kinetics are influenced by domain 1 mutations. This is followed by a slower conformational change (locking) whose kinetics are influenced by domain 2 mutations. These observations are supported by molecular dynamics simulations of S1S2 domain *opening* which also suggest a two-step process in which interactions with domain 2 are the first to be disrupted.[30] The most obvious and striking difference between kainic acid and other kainoids revealed by this study is the greater conformational freedom available to kainic acid when bound to the S1S2 receptor when compared to the other kainoids. In particular, the identification of the $^3T_4$ conformation for kainic acid in four out of ten of the low-lying geometries (with loss of a critical hydrogen bond to Thr655) suggests a low-energy pathway for ligand release and domain separation that would not be available to acromelic acids or domoic acids. Since ligand binding is coupled to channel gating, these differences in binding modes alone could dramatically alter the kinetics of channel gating for these agonists. Furthermore, kainoids that are most potent at AMPA receptors are capable of forming interdomain interactions that kainic acid is not capable of. We suggest that interactions with domain 2 may be most significant. It is generally believed that the conducting state of ionotropic glutamate receptors correlates to the partially closed receptor, while the fully closed form correlates to the desensitized state.[22,33] It has been suggested that binding assays measure binding to the fully closed state which explains the lack of correlation between binding and activity. Currents evoked by kainoids at AMPA receptors are partially or non-desensitizing. When bound to agonists like acromelic acid A and domoic acid, the receptor cannot fully close (desensitize). We suggest that kainoids that have significantly higher potencies at AMPA receptors possess–additional ligand–receptor interactions that stabilize the *conducting* state. For acromelic acid A, domain spanning hydrogen bonds from the side chain to Glu402 and Thr686 were identified. The domoic acid side chain bridged domains via interactions with Gly451, Tyr450 and Ser652. The hydrogen bond to Ser652 may be critical in preventing a peptide bond flip that is associated with full domain closure and desensitization.

In conclusion, we have completed a low-mode conformational analysis of a number of kainoids in the crystal structure of the binding site of the iGluR2 receptor. These calculations were performed with two goals in mind. First, to test the validity of the computational method by analyzing a ligand–receptor pair for which a crystal structure is available and by comparing a number of isomers whose relative affinities are known. Second, to identify the factors that contribute to the vastly different responses of AMPA receptors to kainoids and to the selectivity of the iGluR2 receptor for various kainoids. Ideally, all calculations should be performed in the presence of explicit water molecules. Unfortunately, this increases the computational cost dramatically. However, it is important to keep in mind that we are *not* performing protein modeling. For the majority of our calculations, the protein is a fixed shell within which the conformational and orientational space of the *ligand* is sampled. In a few instances we allow limited protein side-chain motion. But in no instance have we permitted protein backbone movement. Further, the fixed protein shell is derived from the crystal structure when bound to kainic acid. It is certainly not unreasonable to assume that the three-dimensional structure of this *same receptor*, when bound to other kainoids, will be very similar to this, if not identical. There are, however, two water molecules that bridge kainic acid and the receptor and may indeed influence our calculations. These two water molecules serve to help anchor the γ-carboxylate group via bridging hydrogen bonds to NH of Glu705 and the carbonyl oxygen of Ser652. Other hydrogen bonds to the γ-carboxylate, present in the crystal structure (NH groups of Ser654 and Thr655), were identified in our calculations. In spite of the omission of these two water molecules, the crystal structure conformation of kainic acid was returned among the low energy conformations. However, their absence probably contributes to variability in the orientation of the γ-carboxylate and to the identification of the $^3T_4$ conformation as the global minimum. Indeed, we speculate that if these two water molecules are included, our calculations would return the $^4T_3$ conformation as the global minimum. Nevertheless, the other kainoids, with larger side chains, are unable to adopt the $^3T_4$ conformation for steric reasons. Thus the outcome in the presence of these water molecules, and the conclusions drawn from this work, would very likely not be altered. The ability of LMCS/MOLS to identify the crystal structure among the low lying geometries *and* to reliably predict the relative affinities of a series of isomers that are quite similar in their binding affinities (K_d's ranging from 2.5 to 32 μM) lends confidence to the results obtained with this method.

Experimental

The crystal structure (Protein Data Bank Id: 1GR2) was downloaded as a PDB file from the Protein Data Bank. Residue numbers referred to in this work correspond to those of iGluR2 (SwissProt accession number: P19491). A fixed shell of residues within 10 Å of the ligand was used as the receptor. To adapt the crystal structure to the AMBER* force field explicit hydrogen atoms were added, then deleted. All crystallographically determined water molecules were removed to simplify the calculations. In preparation for docking searches, ligands were

placed in the binding site in various conformations and orientations. Amino acid side chains and ligands were subjected to energy minimizations using the AMBER* united atom force field with the programs MacroModel (v. 6.5) and Batchmin (v. 6.5)[34] running on a Silicon Graphics O_2 workstation. No solvation was employed; however, a distance dependent dielectric constant of $4r$ was used.

Calculations for low-mode docking searches were performed as described above. In addition ligands were subjected to explicit translation/rotations, using the Batchmin MOLS command, randomly varied from 0.1–0.5 Å and 0–90° respectively, to ensure efficient orientational sampling of all allowed binding modes (LMCS/MOLS). In some instances, movement of a limited number of amino acid side chains was permitted (with the explicit Batchmin TORS commands) but backbone movements were not allowed. A global search approach was undertaken in which the starting geometry for each Monte Carlo/Energy Minimization (MC/EM) cycle is taken randomly from the pool of structures found thus far, and the one selected is the one that has been used the least number of times previously as a starting structure. A minimum of one thousand, but not more than five thousand, MC/EM cycles were performed for each ligand–receptor pair. Gradient convergence to 0.05 kJ/Ang-mol RMS and an energy window for saved structures of 25 kJ/mol was employed. However, for discussion purposes only those conformations within a 5 kcal/mol (20.92 kJ/mol) window are considered. Each of the low-lying structures was identified a minimum of five times but typically more than 10 times. Docking energies were estimated as the energy difference between the receptor–ligand complex when the ligand is bound to the receptor in its global minimum energy conformation and orientation, and the sum of the energies calculated for the separated ligand (in its bound global minimum energy conformation) and the receptor.

Acknowledgements

This work was supported by NIH NIEHS ES07938, ES05705. LMC is grateful to NIH for a minority supplement. The authors wishes to thank Dr. Wayne Guida for many helpful discussions.

References and Notes

1. Ueno, Y. N. H.; Ueyanagi, J.; Morimoto, H.; Nakamori, R.; Matsuoka, T. *J. Pharm. Soc. Japan* **1955**, *75*, 807.
2. Quilliam, M. A.; Wright, J. L. *Anal. Chem.* **1989**, *61*, 1053 A.
3. Clayton, E. C.; Peng, Y. G.; Means, L. W.; Ramsdell, J. S. *Toxicon* **1999**, *37*, 1025.
4. Wright, J. L.; Bird, C. J.; de Freitas, A. S.; Hampson, D.; McDonald, J.; Quilliam, M. A. *Can. Dis. Wkly. Rep.* **1990**, *16* (Suppl. 1E), 21.
5. Zaman, L.; Arakawa, O.; Shimosu, A.; Onoue, Y.; Nishio, S.; Shida, Y.; Noguchi, T. *Toxicon* **1997**, *35*, 205.
6. Shinozaki, H.; Ishida, M.; Okamoto, T. *Brain Res.* **1986**, *399*, 395.
7. Fushiya, S. S. S.; Kera, Y.; Nozoe, S. *Heterocycles* **1989**, *34*, 1277.
8. Kono, K. S. H.; Matsumoto, T. *Tetrahedron Lett.* **1983**, *24*, 939.
9. Krogsgaard-Larsen, P.; Hansen, J. J. *Toxicol. Lett.* **1992**, *64–65* (Spec No), 409.
10. Sommer, B.; Seeburg, P. H. *Trends Pharmacol. Sci.* **1992**, *13*, 291.
11. Werner, P.; Voigt, M.; Keinanen, K.; Wisden, W.; Seeburg, P. H. *Nature* **1991**, *351*, 742.
12. Ozawa, S.; Kamiya, H.; Tsuzuki, K. *Prog. Neurobiol.* **1998**, *54*, 581.
13. Stern-Bach, Y.; Russo, S.; Neuman, M.; Rosenmund, C. *Neuron* **1998**, *21*, 907.
14. Hampson, D. R.; Manalo, J. L. *Nat. Toxins* **1998**, *6*, 153.
15. Watkins, J. C.; Krogsgaard-Larsen, P.; Honore, T. *Trends Pharmacol. Sci.* **1990**, *11*, 25.
16. Hollmann, M.; O'Shea-Greenfield, A.; Rogers, S. W.; Heinemann, S. *Nature* **1989**, *342*, 643.
17. Larm, J. A.; Beart, P. M.; Cheung, N. S. *Neurochem. Int.* **1997**, *31*, 677.
18. Ishida, M.; Shinozaki, H. *Br. J. Pharmacol.* **1991**, *104*, 873.
19. O'Hara, P. J.; Sheppard, P. O.; Thogersen, H.; Venezia, D.; Haldeman, B. A.; McGrane, V.; Houamed, K. M.; Thomsen, C.; Gilbert, T. L.; Mulvihill, E. R. *Neuron* **1993**, *11*, 41.
20. Stern-Bach, Y.; Bettler, B.; Hartley, M.; Sheppard, P. O.; O'Hara, P. J.; Heinemann, S. F. *Neuron* **1994**, *13*, 1345.
21. Lampinen, M.; Pentikainen, O.; Johnson, M. S.; Keinanen, K. *EMBO. J.* **1998**, *17*, 4704.
22. Armstrong, N.; Gouaux, E. *Neuron* **2000**, *28*, 165.
23. Armstrong, N.; Sun, Y.; Chen, G. Q.; Gouaux, E. *Nature* **1998**, *395*, 913.
24. Kolossvary, I.; Guida, W. C. *J. Comput. Chem.* **1999**, *20*, 1671.
25. Kolossvary, I. G.; Wayne, C. *J. Am. Chem. Soc.* **1996**, *118*, 5011.
26. The superscript and subscript refer to the out of plane atom(s). A superscripted atom is out of plane oriented toward the same face of the ring as the C2 carboxylate, whereas a subscripted atom is oriented toward the opposite face. Thus the $^4T_3$ conformation is closely related to, and intermediate between, the ^4E and E_3 conformations.
27. Wo, Z. G.; Chohan, K. K.; Chen, H.; Sutcliffe, M. J.; Oswald, R. E. *J. Biol. Chem.* **1999**, *274*, 37210.
28. Paas, Y.; Eisenstein, M.; Medevielle, F.; Teichberg, V. I.; Devillers-Thiery, A. *Neuron* **1996**, *17*, 979.
29. Keseru, G. M.; Kolossvary, I. *J. Am. Chem. Soc.* **2001**, *123*, 12708.
30. Mendieta, J.; Ramirez, G.; Gago, F. *Proteins* **2001**, *44*, 460.
31. Arinaminpathy, Y.; Sansom, M. S.; Biggin, P. C. *Biophys. J.* **2002**, *82*, 676.
32. Abele, R.; Keinanen, K.; Madden, D. R. *J. Biol. Chem.* **2000**, *275*, 21355.
33. Varney, M. A.; Rao, S. P.; Jachec, C.; Deal, C.; Hess, S. D.; Daggett, L. P.; Lin, F.; Johnson, E. C.; Velicelebi, G. *J. Pharmacol. Exp. Ther.* **1998**, *285*, 358.
34. Chang, G. G.; Wayne, C.; Still, W.; Clark *J. Am. Chem. Soc.* **1989**, *111*, 4379.