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Structure–activity relationship study at C9 position of kaitocephalin

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ABSTRACT

Kaitocephalin (KCP) isolated from *Eupenicillium shearii* PF1191 is an unusual amino acid natural product in which serine, proline, and alanine moieties are liked with carbon–carbon bonds. KCP exhibits potent and selective binding affinity for one of the ionotropic glutamate receptor subtypes, NMDA receptors (K_i = 7.8 nM). In this study, new structure–activity relationship studies at C9 of KCP were implemented. Eleven new KCP analogs with different substituents at C9 were prepared and employed for binding affinity tests using native ionotropic glutamate receptors. Replacement of the 3,5-dichloro-4-hydroxybenzoyl group of KCP with a 3-phenylpropionyl group resulted in significant loss of binding affinity for NMDARs (K_i = 1300 nM), indicating an indispensable role of the aromatic ring of KCP in the potent and selective binding to NMDARs. Other analogs showed potent binding affinity in a range of 11–270 nM. These findings would directly link to develop useful chemical tools toward imaging and labeling of NMDARs.

Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels which mediate excitatory neurotransmission in the mammalian central nervous system.[1] Extensive efforts have been devoted to elucidate the function of iGluRs because of their crucial role in higher order brain functions such as memory and learning. iGluRs are grouped into three major subtypes: kainate receptors (KARs), *N*-methyl-D-aspartate receptors (NMDARs), and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) by their selective binding abilities of kainic acid (KA: **3**), NMDA (**4**), and AMPA (**5**) for each iGluR subtype.[2] These subtype selective ligands as well as naturally occurring iGluR ligands such as domoic acid (**6**), quisqualate (**7**), dysiherbaine (**8**), and NPTX-594 (**9**) have been widely employed as a useful tool for neurobiological studies to investigate functions of each iGluR subtype by selective activation and inactivation of iGluRs.[3] In addition, these ligands have successfully served for X-ray structure analysis to understand the gating mechanism how these ligands open or close iGluRs in the molecular structure level (Fig. 1).[4]

Kaitocephalin (**1**: KCP) was isolated form *Eupenicillium shearii* PF1191 by a screening program of naturally occurring neuroprotecting agents which suppressed KA-induced excitotoxicity.[5] It was speculated that KCP would antagonize iGluRs to display the remarkable neuroprotecting effect. Miledi et al. investigated pharmacological details of KCP and proved that KCP was found to be potent antagonists of NMDARs (IC_{50} = 75 ± 9 nM) and AMPARs (IC_{50} = 242 ± 37 nM) by electrophysiological studies.[6] We have recently evaluated subtype selectivity and binding affinity of KCP for each iGluR subtype using native iGluRs prepared from rat synaptic membrane.[7] Our study showed that KCP was found to be a potent and selective ligand for native NMDARs (K_i = 7.8 nM) over AMPARs (K_i = 590 nM) and KARs (K_i = 14,000 nM).

Recently, we examined the structure analysis of **1** bound to the ligand binding domain (LBD) of iGluR subtype, GluA2 by X-ray analysis (Fig. 2A)[8] and computational docking studies of **1** with a NMDAR subtype, GluN2A. These results provided several interesting conformational and binding features of the KCP-iGluR complexes: (i) the ligand–protein complex forms an open-bilobed structure in which the structural feature is found in other ligand–protein complexes of iGluR antagonists bound to iGluRs, (ii) the glutamate like moiety (C1–C4 in KCP) is buried in the glutamate binding site, (iii) the benzoyl alanine side chain at C7 lies in the cavity of the opened bilobe far from the glutamate binding site, (iv) interactions between the side chain at C7 and the amino acid residues G486, H485, R692, and E517 of GluN2A are predicted by the docking model of KCP bound to GluN2A (Fig. 2B).

Further structure–activity relationship (SAR) studies using KCP analogs **10**–**12** were performed to screen the key pharmacophore

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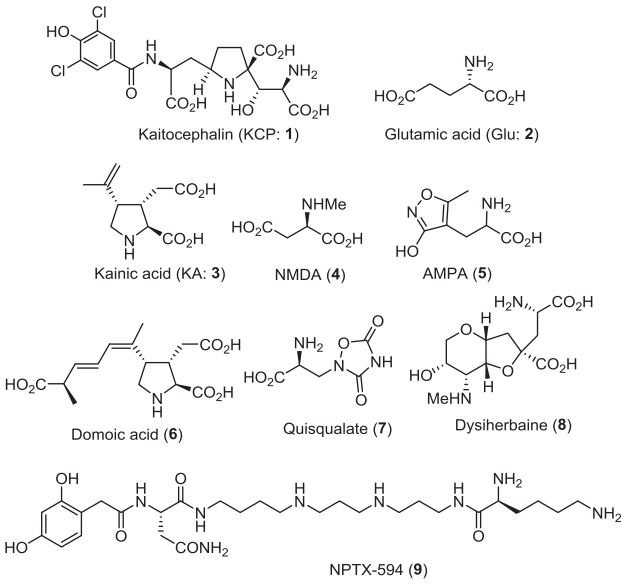


Figure 1. Structures of ionotropic glutamate receptor ligands: kaitocephalin (**1**), glutamate (**2**), kainic acid (**3**), NMDA (**4**), AMPA (**5**) and other naturally occurring ligands (**6**)–(**9**).

at C7 (Fig. 3).[7] Analogs **10**–**12** revealed much lower affinity for NMDARs than KCP (**1**), indicating that powerful contribution of the aromatic ring and the carboxylic acid at C9 for the potent binding to NMDARs. These experimental results are consisted with the assumption obtained by docking model study in which these functional groups would cooperatively interact with NMDARs as shown in Figure 2B.

In this study, we performed an advance SAR study focusing on the benzoyl group at C9 of KCP (Fig. 4). New analogs **14a**–**17a** in which the hydroxyl and chlorides on the benzoyl group were removed or replaced with other functional groups were employed to assess effects of these functional groups on the potency and selectivity. The corresponding (9*R*)-analogs **13** and **14b**–**17b** were also employed to evaluate tolerance of the cavity of the binding site where the benzoyl group fits. 3-Phenylpropionamide analogs **18a** and **18b** were designed to test whether the distal aromatic ring could take part in the preferential ligand–protein interactions or not.

KCP analogs **14a** and **14b** were prepared in one step by a reductive dechlorination reaction of a mixture of KCP (**1**) and (9*R*)-KCP (**13**) under the hydrogenation reaction conditions in the presence of *i*-Pr_2Net (Scheme 1). The diastereomeric mixture was purified by a C_{18}-reversed phased HPLC to give (9*S*)-**14a** and (9*R*)-**14b**, respectively.

Analogs **17a** and **17b** were prepared in a similar manner to our previous synthetic method for the efficient total synthesis of KCP (**1**)[9] (Scheme 2). Commercially available 3-chloro-4-hydroxybenzoic

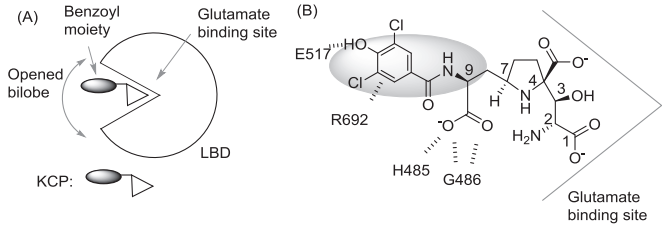


Figure 2. (A) KCP bound to GluA2 ligand binding domain (LBD), (B) Docking model of KCP bound to GluN2A.

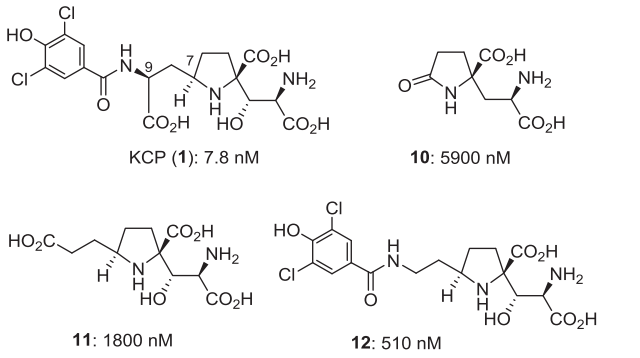


Figure 3. Binding affinity of KCP and its analogs for NMDARs prepared from rat synaptic membranes (K_i values). [^{3}H]CGP 39653 was used as a competitive ligand.

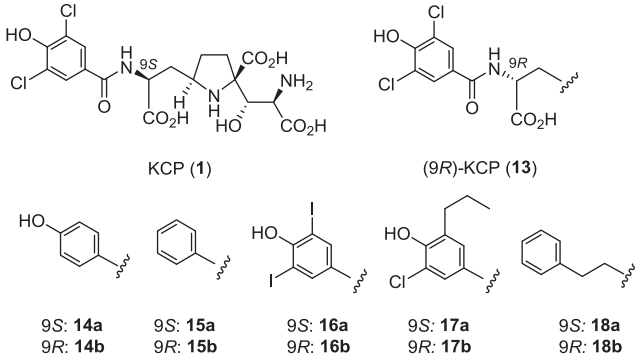
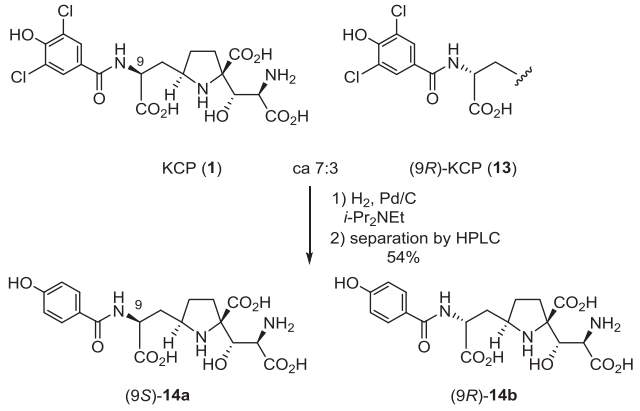


Figure 4. Structures of KCP analogs.



Scheme 1. Synthesis of **14ab** from **1** and its (9*R*)-isomer (**13**).

acid (**19**) was converted to allyl benzoate **20** in 82% yield via the Claisen rearrangement reaction. Compound **20** was subjected to benzyl etherification and hydrolysis reactions to provide the acid **21** which was condensed with ammonium salt **23** prepared from **22**[9] to give phosphonate **24** in 66% yield. Aldehyde **25**[9] was subjected to the olefination reaction with **24** in the presence of DBU/NaI to give *E*-**26** in a highly selective manner (*E*:*Z* = > 95:5). Hydrogenation of *E*-**26** in the presence of a Rh catalyst under H_2 allowed the selective reduction of the dehydroamino acid and allyl group to afford a 7:3 mixture of (9*S*)- and (9*R*)-**27** in 72% yield without the loss of the chloride atom. Global deprotection of **27** was achieved under the conditions using $AlCl_3$ and Me_2S. The resulting crude mixture of **17a** and **17b** was purified by an ion-exchange chromatography using Dowex 50W followed by a C_{18}-reversed phased HPLC to give (9*S*)-**17a** and (9*R*)-**17b**, respectively. The stereochemistry at C9 of **17a** and **17b** was