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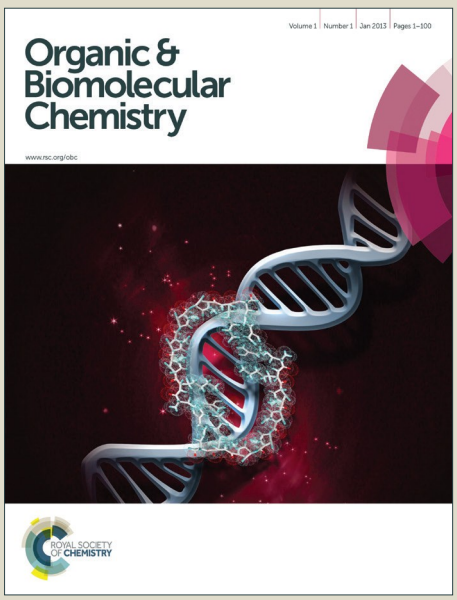

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COMMUNICATION

(7*S*)-Kaitocephalin as a potent NMDA receptor selective ligand

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Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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Structure-activity relationship (SAR) study of kaitocephalin known to be a potent naturally occuring NMDA receptor ligand was performed. This study led us to the discovery of (7*S*)-kaitocephalin as a highly selective NMDA receptor ligand. It displayed 22-fold higher degree of selectivity for NMDA receptor over kaitocephalin, though the binding affinity of (7*S*)-KCP [K_i = 29 nM] was 3.7-fold less potent than that of KCP [K_i = 7.8 nM].

Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels and mediate excitatory neurotransmission in the mammalian central nervous system. iGluRs are subdivided into three major subtypes: kainic acid (KA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and *N*-methyl-D-aspartate (NMDA) receptors according to their different affinities for external agonists; KA, AMPA, and NMDA (Fig. 1).[1] To elucidate the biological functions of iGluRs, many iGluR selective agonists and antagonists have been synthesized and successfully provided in the research areas of neuroscience and medicinal chemistry.

Among iGluR subtypes, NMDA receptors (NMDARs) are known to play an integral role in the regulation of signal transduction in the central nervous system of the brain such as memory and learning.[1,2] It has been indicated that neurodegenerative diseases such as Alzheimer's disease and Huntington's disease are implicated with NMDAR dysfunction.[2] It is speculated that, under these medical conditions, synaptic glutamate levels are raised and the excess amount of glutamate causes the prolonged NMDAR activation and consequential loss and damages of synapses and neurons by the excitotoxicity. Along this line, protection of the neurons from the glutamate overstimulation using NMDA antagonists has become a promising strategy for drug discovery of these neurodegenerative diseases.[2] One of the successful examples is memantine which is currently used to treat patients with Alzheimer's disease.

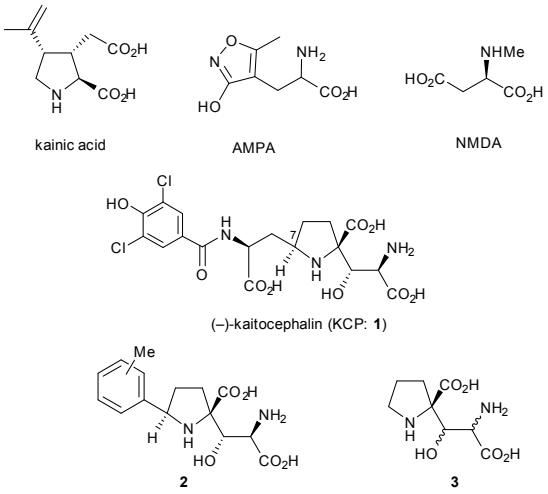

Fig. 1 Structures of iGluR ligands, kaitocephalin, and its analogues.

Kaitocephalin (KCP) (**1**) was isolated from *Eupenicillium shearii* PF1191 by a screening program for glutamate receptor antagonists (Fig. 1).[3] KCP (**1**) is a structurally unique class of amino acid natural products and found to protect neuronal cell death of the cultured neurons from KA excitotoxicity.[3] In 2010, Miledi *et al.* examined preference for receptor subtypes by electrophysiological studies using the recombinant NMDARs, AMPA receptors (AMPARs), and KA receptors (KARs) expressed in *Xenopus laevis* oocytes. These studies revealed that KCP selectively antagonized the NMDARs (IC_{50} = 75 ± 9 nM), and weakly antagonized the AMPARs (242 ± 37 nM) and KARs (~100 μM).[4]

Due to the novel structural feature and potent NMDAR selective antagonist activity of KCP, KCP has received significant attention to be a new lead molecule for developing

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Electronic Supplementary Information (ESI) available: [Experimental details and spectra (1H, 13C NMR) for all products]. See DOI: 10.1039/x0xx00000x

potent neuroprotective agents. Since the discovery of KCP by Shin-ya and Seto *et al*.,[3] nine total synthesis of KCP have been achieved by seven research groups.[5–13] Structure-activity relationship (SAR) studies have been performed by two groups. Chamberlin *et al*. designed analogues **2** possessing the partial structural motif of KCP based on the putative ligand-KAR modelling study**.** These analogues were found to reduce the membrane current induced by 100 μM of KA at a concentration of 100 μM.[14] Kitahara *et al*. synthesized analogues **3** in which the substituent group at C7 of KCP was removed. The structurally simple analogues **3** resulted in decreasing neuroprotective effects against the KA-induced neurotoxicity than that of KCP (Fig. 1).[15]

We have established a facile synthetic route to supply a hundred milligrams of KCP.[9] Recently, the synthetic KCP was successfully provided to solve the three dimensional structure of the recombinant AMPAR (GluA2) ligand binding domain (LBD) bound to KCP (Fig. 2, complex **A**).[16] This analysis provides the key interaction of KCP with the AMPAR LBD. The glutamate-like right half moiety of KCP fits to the same binding site as that of the native neurotransmitter, glutamate. The carboxylic acid moiety at C9 in the left side chain could interact with the backbone amides of Y450 and G451. Based on the structural similarity of GluA2 to those of NMDARs (GluN1 and GluN2A), modelling studies of KCP with GluN1 and GluN2A were examined. The KCP-GluN2A LBD docking model is depicted in Fig 2 (model **B**). This model suggests that KCP could docks to the cleft of GluN2A LBD in a similar manner to that of complex **A**. Additional interactions between the N-benzoyl moiety with E517 and R692 are recognized in this docking model. We hypothesized that these interactions could account for the selective binding of KCP to NMDARs. In this paper, we would like to report SAR studies of KCP using five newly designed analogues **4**–**8** to elucidate the effect at C7 for the iGluR subtype selectivity (Fig. 3). Analogue **4** is the C7-epimer of KCP in which the position of the substituent at C7 of KCP is compelled in a different angle. To inspect the role of the carboxylate and the *N*-benzoylamide moieties at C9, respectively, analogues **5** and **6** were designed and synthesized. Analogues **7** and **8** correspond to the structurally simple analogues in which the substituent at C7 is removed. These new analogues were stereoselectively prepared and subjected to the iGluR binding assay using native iGluRs prepared from rat brain synaptic membrane.

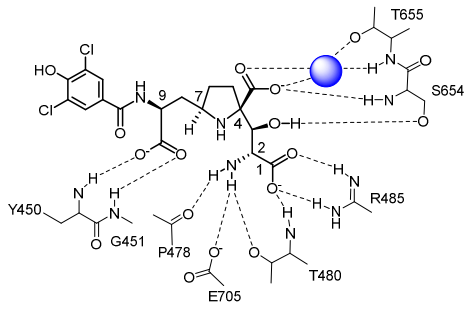


Fig. 2 Structures of KCP-iGluR ligand binding domain (LBD) complexes. Complex **A**: Crystal structure of KCP-AMPA receptor (GluA2) LBD [PDB code 4GXS], Model **B**: Docking model of KCP-NMDA (GluN2A) LBD [PDB code 2A5T]. Sphere: H_2O.

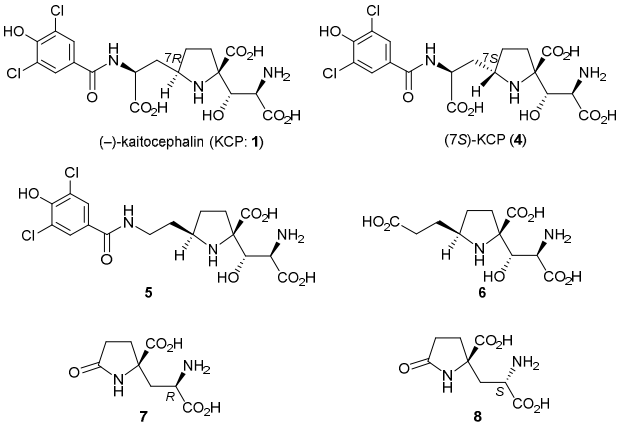
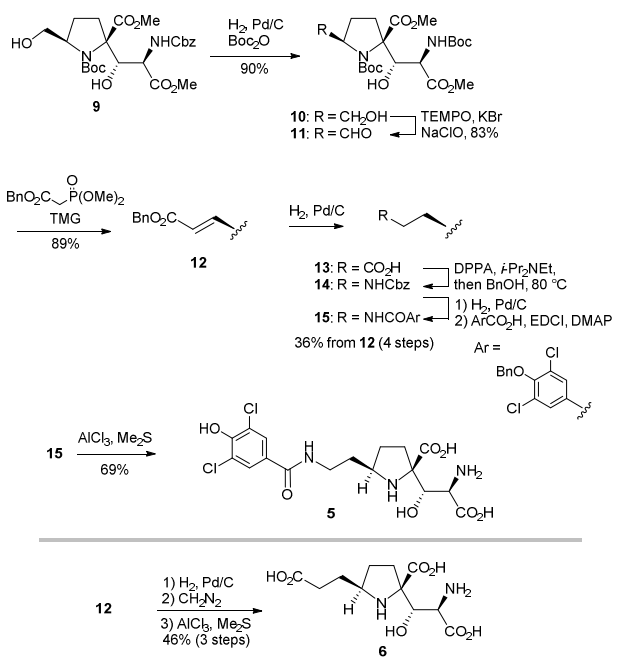


Fig. 3 Structures of KCP (**1**) and its analogues **4**–**8**.

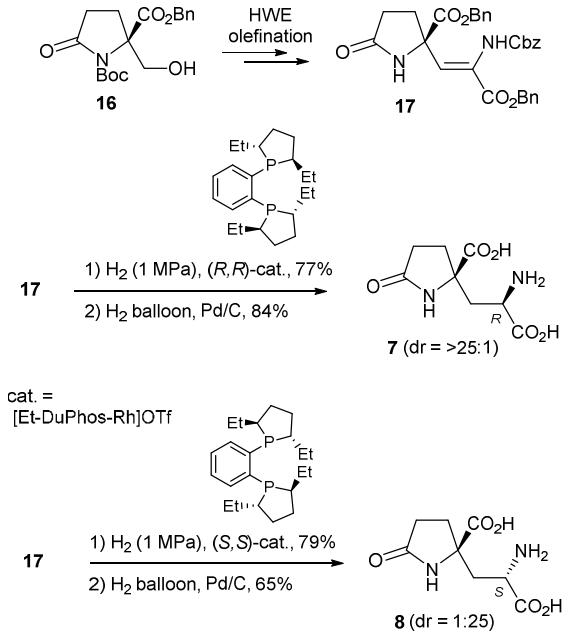
(7*S*)-KCP (**4**) was prepared according to our previous method.[7] Analogues **5** and **6** were synthesized starting from **9** (Scheme 1).[9] Carbamate **9** was transformed to *E*-α,β-unsaturated ester **12** via **10** in good yield over three steps. The hydrogenation reaction of **12** using H_2/Pd-C resulted in the removal of the benzyl group and the saturation of the double bond to give carboxylic acid **13**. Treatment of **13** with DPPA developed by Shioiri *et al.*[17] gave the corresponding isocyanate which was successively trapped with benzyl alcohol to provide benzyl carbamate **14**. After the removal of the Cbz group of **14,** the resulting amine was condensed with 3,5-dichloro-4-benzyloxybenzoic acid to give amide **15** (36% yield from **12** in 4 steps). Amide **15** was converted to analogue **5** in 69% yield by the global deprotection of Boc, benzyl, and ester methyl groups using $AlCl_3$ and Me_2S.[7] Analogue **6** was prepared from **12** in 3 steps via hydrogenation and deprotection reactions (Scheme 1).

Analogues **7** and **8** were prepared starting from the known alcohol **16**[18] via α,β-dehydroamino acid ester **17** (Scheme 2). The key diastereoselective hydrogenation of **17** was achieved under the reagent controlled conditions using chiral DuPhos-Rh catalysts[19] in a highly selective manner. According to the empirical role of the asymmetric hydrogenation using chiral



Scheme 1 Synthesis of analogues **5** and **6**.

Rh catalysts,[20] the newly created stereogenic centers of **7** and **8** were putatively assigned as *R* and *S*, respectively. Removal of the protecting groups with the H_2/Pd-C gave (*R,R*)-**7** and (*R,S*)-**8**, respectively.



Scheme 2 Synthesis of **7** and **8** from **16**.

Binding affinities of KCP (**1**) and its analogues **4**–**8** for iGluRs were evaluated using native iGluRs prepared from rat brain synaptic membrane (Table 1 and Fig. 4).[21,22] Radio-labelled ligands: [^{3}H]KA for KARs, [^{3}H]AMPA for AMPARs, and [^{3}H]CGP 39653 for NMDARs were employed in this assay. The binding affinity of KCP and its analogues **4**–**8** using native iGluRs was tested for the first time in this study. KCP (**1**) showed potent binding affinity for NMDARs at K_i = 7.8 nM.

Table 1 K_i values of **1** and **4**–**8** for NMDA, AMPA, and KA receptors using ^{3}H-labelled ligands[a] and rat brain synaptic membrane.

	K_i value[b] (nM)			AMPA K_i/NMDA K_i
Compound	NMDA	AMPA	KA	ratio
KCP (**1**)	7.8 ± 0.8	590 ± 54	14000 ± 3300	76
(7*S*)-KCP (**4**)	29 ± 4	49000 ± 14000	>10^5	1700
5	510 ± 50	3000 ± 1200	>10^5	5.9
6	1800 ± 210	1600 ± 600	55000 ± 19000	0.89
7	5900 ± 1300	56000 ± 18000	26000 ± 7200	9.5
8	9100 ± 1400	38000 ± 9800	7500 ± 1100	4.2

[a] ^{3}H-labelled ligands: [^{3}H]CGP 39653 for NMDARs, [^{3}H]AMPA for AMPARs, and [^{3}H]KA for KARs. [b] K_i values are indicated as the mean ±SE for three determinations.

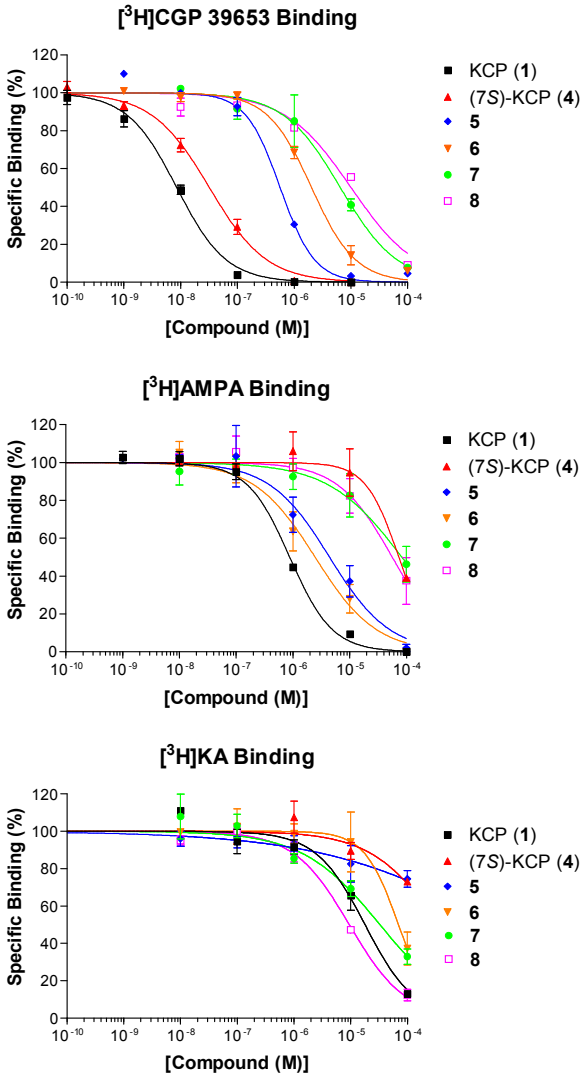


Fig.4 Displacement of the specific ^{3}H-labelled ligand binding (NMDA, AMPA, and KA receptors) to rat synaptic membrane by increasing concentrations of **1** and **4**–**8**. Each point is the mean of triplicate determinations.

On the other hand, those for AMPARs (590 nM) and KARs (14000 nM) were significantly lowered. The characteristic iGluR selectivity [NMDARs > AMPARs > KARs] observed in our study shows good agreement with the results reported by Miledi *et al*. It is noteworthy that the value at 7.8 nM is superior to those of other known NMDA antagonists.[1a]

In our surprise, (7*S*)-KCP (**4**) revealed potent binding affinity for NMDARs at K_i = 29 nM. It was only 3.7-fold less potent than that of KCP. In addition, the degree of selectivity for NMDARs of (7*S*)-KCP [AMPA K_i/NMDA K_i ratio: 1700] was found to be 22-fold greater than that of KCP [AMPA K_i/NMDA K_i ratio: 76].

Furukawa *et al.* reported the structural insights into competitive antagonism in NMDA receptors based on the X-ray structure analysis of the GluN1/GluN2A NMDA receptor-glycine/NMDA antagonists (AP-5 and PPDA) complexes (PDB code 4NF5 and 4NF6, Fig. 5).[23] These analyses revealed that binding of antagonists (PPDA and AP-5) to GluN2A results in opening of the bilobed structure of the GluN2A LBD compared to the glutamate bound form. Our structure analysis of KCP-iGluR complexes indicates that the large aryl group could place around the top of the binding cleft opposite to the deeper glutamate binding site. It is assumed that NMDARs could accommodate the substituent at C7 of (7*S*)-KCP in the opened cleft.

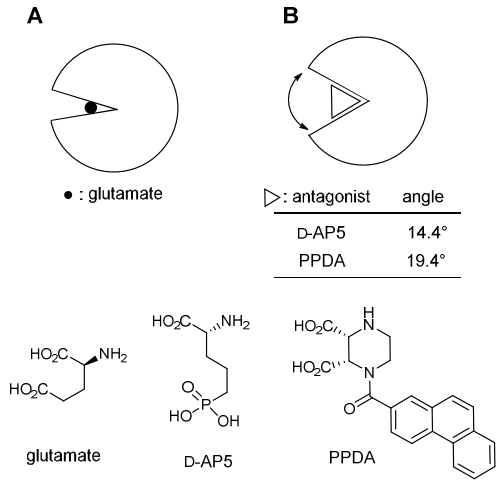


Fig. 5 Domain opening structures of Ligand-bound LBDs reported by Furukawa *et al*.[23] **A**: Glutamate (agonist)-GluN2A LBD. **B**: Antagonists (AP-5 & PPDA)-GluN2A LBD [PDB code 4NF5 and 4NF6].

In Figure 6, the expanded view of the binding site for KCP-GluA2 LBD and a tentative (7*S*)-KCP-GluA2 complex model are depicted. The side chain at C9 of (7*S*)-KCP (**4**) could turn to the backbone of Y450 and G451 if the glutamate-like motif of the right side chain of **4** could bind to the glutamate binding site (Fig. 6, **B**). It is assumed that the resulting steric repulsion and/or loss of interaction between the carboxylic acid at C9 and the backbone militate against the binding of **4** to AMPARs to lower the binding affinity. Further modelling and SAR studies are currently under investigation.

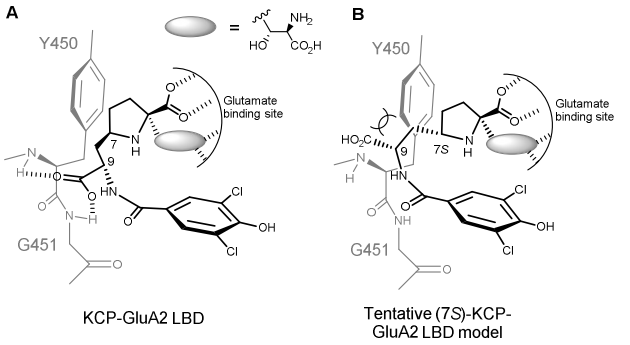


Fig. 6 A: Expanded view of the binding site for KCP-GluA2 LBD (PDB code 4GXS). **B**: Tentative (7*S*)-KCP-GluA2 LBD binding model.

Analogues **5**–**8** revealed poor binding affinity to NMDARS (65~1167-fold less potent than that of KCP). These results prove the indispensable role of the left side chain moiety at C7 for the specific binding to NMDARs and experimentally support our hypothesis on the presence of favourable interactions predicted by the docking model **B** (Fig. 2). The synergistic effects of the glutamate like moiety and the left side chain moiety is crucial for the specific binding affinity.

Although analogues **4**–**8** displayed lower binding affinity for AMPARs, analogues **5** and **6** possessing either the carboxylic acid moiety or benzoyl moiety at C9 revealed almost the same potency (**5**: 3000 nM, **6**: 1600 nM) as that of KCP (**1**: 590 nM). On the other hand, the binding affinity of **4**, **7**, and **8** for AMPARs was significantly decreased. These results indicated a favourable interaction between the benzoyl group at C9 and AMPARs might be involved.

Conclusion

An important role of the side chain moiety at C7 of KCP for the specific binding to NMDARs has been proved by the present SAR study using five new KCP analogues. We discovered (7*S*)-KCP (**4**) as a highly NMDAR selective ligand with potent binding affinity at 29 nM. It displayed 22-fold higher degree of selectivity for NMDA receptor over kaitocephalin. (7*S*)-KCP would be an advanced lead for the design of NMDA selective ligand and drug discovery of neuroprotecting agents. The present SAR studies provide useful information about the role of the hydrophobic aryl group and its feasibility as a tuneable site for further derivatization. Pharmacological characterization of KCP and its analogues, and studies in view of chemical biology of natural products[24] are currently underway.

Acknowledgement

TS gratefully acknowledges financial supports from Scientific Research of Innovative Areas, Chemical Biology of Natural Products, the Ministry of Education, Culture, Sports, Science and Technology (No. 23102009) and the Japan Society for the Promotion of Science (KAKENHI Nos. 23228001, 25282233). YY gratefully acknowledges financial supports by the Sasagawa Scientific Research Grant from The Japan Science Society.

Notes and references

‡The preparation of rat synaptic membrane was carried out in accordance with the National Institute of Advanced Industrial Science and Technology (AIST) guidelines for life science experiments.

1 (a) S. F. Traynelis, L. P. Wollmuth, C. J. McBain, F. S. Manniti, K. M. Vance, K. K. Ogden, K. B. Hansen, H. Yuan, S. J. Myers and R. Dingledine, *Pharmacol. Rev*., 2010, **62**, 405; (b) J. N. C. Kew and J. A. Kemp, *Psychopharmacology,* 2005, **179**, 4; (c) R. Dingledine, K. Borges, D. Bowie and S. F. Traynelis, *Pharmacol. Rev*., 1999, **51**, 7.

2 (a) S. Li, A. H. C. Wong and F. Liu, *Front. Cell. Neurosci*., 2014, **8**, 125; (b) T. W. Lai, S. Zhang and Y. T. Wang, *Prog. Neurobiol*., 2014, **115**, 157; (c) P. Paoletti, C. Bellone and Q. Zhou, *Nat. Rev. Neurosci.,*2013, **14**, 383; (d) S. E. Lakhan, M. Caro and N. Hadzimichalis, *Front. Psychiatry*, 2013, **4**, 52; (e) D. T. Monaghan and D. E. Jane, "Pharmacology of NMDA Receptors" in *Biology of the NMDA Receptor*, ed. A. M. Van Dongen, CRC Press, 2009, Chapter 12.

3 K. Shin-ya, J.-S. Kim, K. Furihata, Y. Hayakawa and H. Seto, *Tetrahedron Lett.,* 1997, **38**, 7079.

4 A. Limon, J. M. Reyes-Ruiz, R. G. Vaswani, A. R. Chamberlin and R. Miledi, *ACS Chem. Neurosci*., 2010, **1**, 175.

5 D. Ma and J. Yang, *J. Am. Chem. Soc*., 2001, **123**, 9706. The synthesis of (2*S*)-isomer of **1** was reported.

6 H. Watanabe, M. Okue, H. Kobayashi and T. Kitahara, *Tetrahedron Lett*., 2002, **43**, 861.

7 M. Kawasaki, T. Shinada, M. Hamada and Y. Ohfune, *Org. Lett*., 2005, **7**, 4165.

8 R. G. Vaswani and A. R. Chamberlin, *J. Org. Chem*., 2008, **73**, 1661.

9 M. Hamada, T. Shinada and Y. Ohfune, *Org. Lett*., 2009, **11**, 4664.

10 S. Yu, S. Zhu, X. Pan, J. Yang and D. Ma, *Tetrahedron*, 2011, **67**, 1673.

11 K. Takahashi, D. Yamaguchi, J. Ishihara and S. Hatakeyama, *Org. Lett*., 2012, **14**, 1644.

12 W. Lee, J.-H. Youn and S. H. Kang, *Chem. Commun*., 2013, **49**, 5231.

13 P. Garner, L. Weerasinghe, I. V. Houten and J. Hu, *Chem. Commun*., 2014, **50**, 4908.

14 R. G. Vaswani, A. Limon, J. M. Reyers-Ruiz, R. Miledi and A. R. Chamberlin, *Bioorg. Med. Chem. Lett*., 2009, **19**, 132.

15 M. Okue, H. Kobayashi, H. Watanabe and T. Kitaraha, *The 43rd Symposium on the Chemistry of Natural Products symposium papers*, 2001, p133.

16 A. H. Ahmed, M. Hamada, T. Shinada, Y. Ohfune, L. Weerasinghe, P. P. Garner and R. E. Oswald, *J. Biol. Chem*., 2012, **287**, 41007.

17 K. Ninomiya, T. Shioiri and S. Yamada, *Tetrahedron*, 1974, **30**, 2151.

18 T. Shinada, H. Yoshida and Y. Ohfune, *Synthesis*, 2009, 3751.

19 (a) M. J. Burk, *Acc. Chem. Res*., 2000, **33**, 363; (b) M. J. Burk, J. E. Feaster, W. A. Nugent and R. L. Harlow, *J. Am. Chem. Soc*., 1993, **115**, 10125.

20 K. V. L. Crépy and T. Imamoto, *Adv. Synth. Catal*., 2003, **345**, 79

21 (a) R. Sakai, G. T. Swanson, K. Shimamoto, T. Green, A. Contractor, A. Ghetti, Y. Tamura-Horikawa, C. Oikawa and H. Kamiya, *J. Pharmacol. Exp. Ther*., 2001, **296**, 650; (b) K. Shimamoto, B. Lebrun, Y. Yasuda-Kamatani, M. Sakaitani, Y. Shigeri, N. Yumoto and T. Nakajima, *Mol. Pharmacol*., 1998, **53**, 195; (c) D. E. Murphy, E. W. Snowhill and M. Williams, *Neurochem. Res*., 1987, **12**, 775.

22 The experimental procedure of receptor binding assay and calculation of K_i values are described in details on supporting information.

23 A. Jespersen, N. Tajima, G. Fernandez-Cuervo, E. C. Garnier-Amblard, H. Furukawa *Neuron,* 2014, **81**, 366.

24 (a) M. Schenone, V. Dančík, B. K. Wagner and P. A. Clemons, *Nat. Chem. Biol*., 2014, **9**, 232. (b) S. Rizzo and H. Waldmann, *Chem. Rev*., 2014, **114**, 4621. (c) M. Ueda, *Chem. Lett.,* 2012, **41**, 658. (d) K. C. Nicolaou, C. R. H. Hale, C. Nilewski and H. A. Loannidou, *Chem. Soc. Rev.*, 2012, **41**, 5185.