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Cyclic non-opioid dynorphin A analogues for the bradykinin receptors

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ABSTRACT

Nerve injury and inflammation cause up-regulation of an endogenous opioid ligand, dynorphin A (Dyn A), in the spinal cord resulting in hyperalgesia via the interaction with bradykinin receptors (BRs). This is a non-opioid neuroexcitatory effect that cannot be blocked by opioid antagonists. Our systematic structure–activity relationships study on Dyn A identified lead ligands **1** and **4**, along with the key structural feature (i.e. amphipathicity) for the BRs. However, the ligands showed very low metabolic stability in plasma ($t_{1/2}$ <1 h) and therefore, in order to improve their metabolic stabilities with retained biological activities, various modifications were performed. Cyclization of ligand **4** afforded a cyclic Dyn A analogue **5** that retained the same range of binding affinity as the linear ligand with improved metabolic stability ($t_{1/2}$ >5 h) and therefore possesses the potential as a pharmacophoric scaffold to be utilized for drug development.

Chronic neuropathic pain is difficult to treat by current methods using opioids due to serious side effects such as tolerance and addiction which are indispensable for treatment.[1,2] Long term administration of opioids can even develop different types or more serious pain with prolonged use.[3] This is more likely caused by gene expression that is related to treatment attempts: nervous system adaptation/change.[4] From this perspective, it seems important to develop drugs through novel approaches considering the possible changes in pain pathways for efficient treatment of chronic pain states. Dynorphin A (Dyn A, H—Tyr[1]-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln[17]—OH), which is an endogenous ligand for three subtypes (mu, delta, and kappa) of opioid receptors, with a slight preference for the kappa opioid receptor (KOR), has been a good target to investigate possible system changes owing to its very distinctive biological roles in the pain pathway: neuroinhibitory (opioid) effects and neuroexcitatory (non-opioid) effects.[5–7] While Dyn A's neuroinhibitory effects are well-known with the opioid receptors, its non-opioid neuroexcitatory effects are not yet established despite its frequent observation in animal models. It was shown that under nerve injury and inflammation, up-regulated Dyn A or its fragments, specially [des-Tyr[1]]-Dyn A analogues, interact with the bradykinin receptors (BRs) in the central nervous system (CNS) to cause hyperalgesia.[8–13]

Therefore, in our earlier studies, we pursued systematic structure–activity relationship (SAR) studies to gain key insights into the structure for the central BRs and to utilize them to understand the uncertain non-opioid effects via the BRs.[14–17] Based on the results, ligands **1** and **4** were identified as lead ligands with good affinity along with a key structural feature for the BRs: amphipathicity. In in vivo tests, ligand **4** blocked Dyn A(2–13)-induced hyperalgesia and motor impairments in naïve animals and showed anti-hyperalgesic effects in L_5/L_6 spinal nerve ligation (SNL) animals in a dose-dependent manner.[14] Ligand **4** also inhibited Dyn A(2–13)-induced wide dynamic range (WDR) neuronal response in naïve animals and modulated WDR neuronal response to innocuous and noxious mechanical stimuli in SNL animals.[18] All of these effects were considered to be localized in the CNS because

Abbreviations: BBB, blood brain barrier; BK, bradykinin; Boc, *t*-butyloxycarbonyl; BR, bradykinin receptor; CNS, central nervous system; DALKD, [des-Arg[10], Leu[9]]-kallidin; DIPEA, diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; Dyn A, dynorphin A; Fmoc, fluorenylmethyloxycarbonyl; HBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate; HOBt, *N*-hydroxybenzotriazole; i.pl., intraplantar; MS, mass spectroscopy; Pbf, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; RP-HPLC, reversed-phase high performance liquid chromatography; SNL, spinal nerve ligation; SPPS, solid phase peptide synthesis; Pip, 2-phenylisopropyl; TFA, trifluoroacetic acid; TIS, triisopropylsilane; WDR, wide dynamic range.

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no peripheral activity was shown in in vivo tests via intraplantar (i.pl.) administration.[14] These results demonstrated that Dyn A structure-based BR antagonists can be developed for a therapeutic purpose to treat abnormal pain which can be caused by up-regulation of Dyn A in the CNS in chronic neuropathic pain states.

Even with high potency and affinity, ligand **4** showed very low metabolic stability in plasma and was completely degraded within 4 h of incubation ($t_{1/2}$ = 0.7 h). In an effort to improve metabolic stability and blood brain barrier (BBB) permeability, various modifications were performed on our lead ligands **1** and **4**. Here we report SAR results of cyclic Dyn A analogues at the BRs (Table 1).

To take advantage of cyclic peptide ligands, analogues **2** and **3** were first designed based on the structure of ligand **1**, which showed good binding affinity (IC_{50} = 78 nM) at the BRs, and in these structures, two Nle residues of ligand **1** were replaced by two allyl glycine residues to form a 17-membered carba ring via ring metathesis (Fig. 1).[19] The cyclization consumed, and thus buried, two hydrophobic chains in the ring, while allocating three Lys residues to position their side amino groups to be exposed due to the important role of positive charges in the BRs recognition. In contrast, cyclic ligands **5**–**7** retained hydrophobic alkyl chains exposed after a ring formation, which fulfill amphipathicity that is critical for interaction with the BRs. Originally, these cyclic ligands were designed based on the structure of linear ligand **4**, and a turn making Pro residue was replaced with a Glu residue for ring formation. The N-terminal amino group, which was shown not to be critical, was consumed for ring formation with a Glu residue. A central idea in the design was to enhance the turn structure around the Pro residue and to properly expose both positive charges and hydrophobic groups to satisfy ligand amphipathicity.

For the synthesis of the cyclic analogues, chain elongations were performed by standard solid phase peptide synthesis (SPPS) using fluorenylmethyloxycarbonyl (Fmoc) chemistry with 2-phenylisopropyl (Pip), 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf), and *t*-butyloxycarbonyl (Boc) group as a side protecting group for Glu, Arg, and Lys, respectively, on Fmoc-Lys(Boc)-attached Wang resin in high yields (overall yields >40%). Each coupling reaction used 3 equiv 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HBTU)/3 equiv *N*-hydroxybenzotriazole (HOBt)/6 equiv diisopropylethylamine (DIPEA) for 50 min at rt and each Fmoc-deprotection used 20% piperidine/*N*,*N*-dimethylformamide (DMF) for 20 min at rt. After chain elongation, ring closing metathesis was performed under microwave (100 °C) using second generation Grubbs catalyst in dichloromethane (DCM) containing 10% of 0.4 M LiCl in DMF solution for 1 h (Scheme 1).[20] Crude dicarba cyclic peptide–resin, of which the half portion was cleaved by a 90% trifluoroacetic acid (TFA) cocktail solution containing 5% thioanisole, 3% ethanedithiol, and 2% anisole and purified by preparative reversed-phase high performance liquid chromatography (RP-HPLC, 10–50% of acetonitrile in 20 min) to afford pure dicarba cyclic analogue **2**. The other half was reduced by Wilkinson's hydrogenation method using cat. $Rh(PPh_3)_3Cl$ in 90% DCM and 10% MeOH at rt for 1 day, cleaved by the TFA cocktail, and finally purified by RP-HPLC to afford more than 95% pure saturated dicarba cyclic analogue **3**.[20] For the synthesis of cyclic analogues **5**–**7**, a Pip group on a Glu residue was

Table 1
Binding affinities of cyclic Dyn A analogues at BRs in rat brain

No	Structure	Ring size	BR [^{3}H]BK[a]	
			Log[IC_{50}][b]	IC_{50} (nM)
1[c]	H—Nle-Lys-Pro-Lys-Nle-Lys—OH	—	−7.11 +0.14	78
2	H—c^{1,5}(—*cis*CH=CH—)[Ala-Lys-Pro-Lys-Ala]-Lys—OH	17	—	n.c.
3	H—c^{1,5}(—CH_2CH_2—)[Ala-Lys-Pro-Lys-Ala]-Lys—OH	17	—	n.c.
4[c]	H—Phe-Leu-Arg-Ile-Arg-Pro-Lys—OH	—	−7.16 +0.09	69
5	c^{N,6}[Phe-Leu-Arg-Ile-Arg-Glu]-Lys—OH	20	−6.72 +0.13	191
6	c^{N,6}[Lys-Leu-Arg-Ile-Arg-Glu]-Lys—OH	20	−6.52 +0.15	302
7	c^{N,5}[Leu-Arg-Ile-Arg-Glu]-Lys—OH	17	−5.82 +0.09	1510

n.c.: no competition.
[a] Radioligand competition assays were carried out using [^{3}H]BK in rat brain membranes at pH 6.8.
[b] Logarithmic values determined from the nonlinear regression analysis of data collected from at least two independent experiments in duplicate using GraphPad Prism 6.
[c] Reference #14, [^{3}H]DALKD.

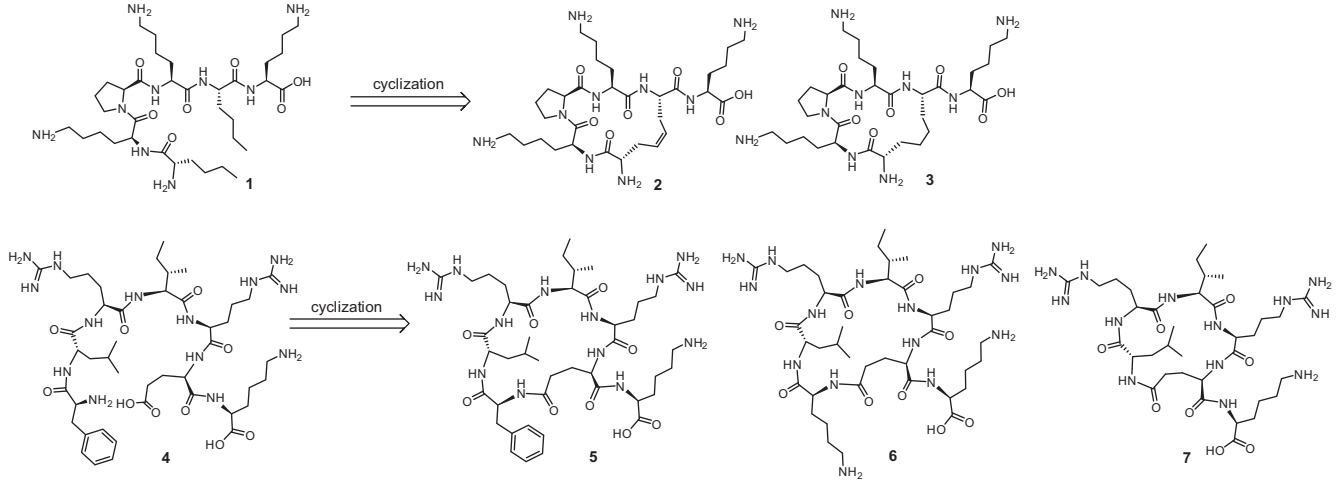

Figure 1. Cyclizations of amphipathic Dyn A analogues.