Cyclic opioid peptides

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Abstract: For decades the opioid receptors have been an attractive therapeutic target for the treatment of pain. Since the first discovery of enkephalin, approximately a dozen endogenous opioid peptides have been known to produce opioid activity and analgesia, but their therapeutics have been limited mainly due to low blood brain barrier penetration and poor resistance to proteolytic degradation. One versatile approach to overcome these drawbacks is the cyclization of linear peptides to cyclic peptides with constrained topographical structure. Compared to their linear parents, cyclic analogues exhibit better metabolic stability, lower off-target toxicity, and improved bioavailability. Extensive structure-activity relationship studies have uncovered promising compounds for the treatment of pain as well as further elucidate structural elements required for selective opioid receptor activity. The benefits that come with employing cyclization can be further enhanced through the generation of polycyclic derivatives. Opioid ligands generally have a short peptide chain and thus the realm of polycyclic peptides has yet to be explored. In this review, a brief history of designing ligands for the opioid receptors, including classic linear and cyclic ligands, is discussed along with recent approaches and successes of cyclic peptide ligands for the receptors. Various scaffolds and approaches to improve bioavailability are elaborated on and concluded with a discourse towards polycyclic peptides.

Keywords: cyclic peptides, polycyclic, opioid receptors, analgesia, central nervous system, blood brain barrier penetration, bioavailability.

1. INTRODUCTION

Opioids, which have been used clinically for over 5,000 years, deliver pharmacological effects, mostly analgesic, through three subtype opioid receptors: mu opioid receptor (MOR), delta opioid receptor (DOR), and kappa opioid receptor (KOR). The potential for optimizing opioid analgesics has launched decades of research and brought about novel compounds and a more in-depth understanding of opioid therapeutics. Discussed herein is a brief overview of a historical outlook on opioid receptors and their endogenous ligands and recent developments regarding cyclic opioid peptides.

In the mid-1970s and early 1980s the endogenous peptide ligands for the opioid receptors, enkephalin (ENK), endorphin (END), and dynorphin (DYN), were discovered [1-3]. Three precursor proteins, all of which are rendered in the nucleus and relocated to the terminals of nerve cells, are hydrolyzed by proteases to give these endogenous peptides [4]: 1) Proenkephalin yields four copies of Met-ENK and one copy of Leu-ENK, Met-ENK-Arg⁶-Phe⁷, and Met-ENK-Arg⁶-Gly⁷-Leu⁸ [5]; 2) Prodynorphin upon cleavage gives three Leu-ENK peptides, DYN A and B, and α - and β -neoendorphin (NEO) [6]; and 3) Proopiomelanocortin upon degradation produces β -END [7]. Deltorphin (DLT) and dermorphin (DER) were next to be isolated and were found to be DOR- and MOR-selective opioid peptides, respectively, isolated from the skin of frogs belonging to the genus *Phyllomedusa* and contain the D-form of the respective amino acid at the 2-position [8]. In the late 1990s, endomorphin (EM)-1 and -2 were discovered and found to exhibit both very high affinity and selectivity for the MOR [9].

1.1. Opioid receptors and ligands

Table 1: Endogenous opioid peptides and their selectivity for the opioid receptors

Ligand	Structure	Selectivity
ENKs	YGGFL	DOR > MOR
	YGGFM	DOR > MOR
	YGGFMRF	MOR > DOR > KOR
	YGGFMRGL	MOR > DOR > KOR
β -END	YGGFMTSEKSQTPLVTLF KNAIIKNAYKKGE	MOR > DOR
DYN A	YGGFLRRIRPKLWDNQ	KOR > MOR > DOR
DYN B	YGGFLRRQFKVVT	KOR > MOR > DOR
α -NEO	YGGFLRKYPK	KOR
β -NEO	YGGFLRKYP	KOR
EM-1	YPWF-NH ₂	MOR
EM-2	YPFF-NH ₂	MOR
DERs	YaFGYPS-NH ₂	MOR
	YaFGYPK	MOR
	YaFWYPN	MOR
DREK	YmFHLMD-NH ₂	DOR
DLT-1	YaFDVVG-NH ₂	DOR

DLT-2	YaFDVVG-NH ₂	DOR
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The MOR has been primarily targeted to bring about analgesia, but agonists have been known to also cause sedation, reduced blood pressure, decreased respiration, and constipation [10]. It has since been uncovered that chronic MOR activation can lead to serious side-effects such as tolerance and addiction [11, 12]. In rat models, the greatest density of MOR distribution can be found in the caudate putamen, nucleus accumbens, neocortex, globus pallidus, thalamus, and in the substantia gelatinosa [13]. The endogenous opioid ligands for the MOR are β -END, EM-1 and -2, the DERs, and the ENKs (Table 1) [14].

The first potent and selective peptidic MOR agonist to be rendered from ENK was Tyr-DAla-Gly-MePhe-Met(O)-ol (FK33-824) [15] and later the peptides Tyr-DAla-Gly-MePhe-Gly-ol (DAMGO) and the cyclic compound Tyr-c^{2,4}[DCys-Phe-DPen]-NH₂ (JOM-5) were developed and showed improved MOR selectivity and affinity (Figure 1) [16,17]. The DERs are potent and selective MOR agonists and have been derivatized over the years to yield other highly selective agonists such as Tyr-DArg-Phe- β Ala-OH (TAPA) [18] and Tyr-DArg-Phe-Lys-NH₂ (DALDA) [19]. MOR selective antagonists were also achieved by synthesizing somatostatin-like octapeptides [20]. The cyclic opioids DPhe-c^{2,7}[Cys-Tyr-DTrp-Arg-Thr-Pen]-Thr-NH₂ (CTAP) and DPhe-c^{2,7}[Cys-Tyr-DTrp-Orn-Thr-Pen]-Thr-NH₂ (CTOP) are commonly used selective MOR antagonists and have high affinity.

The DOR is a promising therapeutic target that may bring about analgesia with decreased respiratory depression, tolerance, and gastrointestinal effects [21]. Physiological responses incorporating the DOR include gastrointestinal transit, respiration, and a role in producing seizures [22,23]. Using autoradiographical techniques, it was shown that the greatest areas of opioid receptor densities within the central nervous system (CNS) reside in the olfactory tubercle, nucleus accumbens, and the caudate putamen [13]. Both Leu- and Met-ENK exhibit slight selectivity for the DOR over the MOR and DLTs are highly selective and potent at the DOR [8]. Classic DOR-selective agonists include DADLE [24] and DSLET [25] and the cyclic peptides DPDPE [26], DPLPE, and Tyr-c^{2,4}[DCys-Phe-DPen]-OH (JOM-13) [27]. DOR selective antagonists include H-Tyr-Tic-Phe-Phe-OH (TIPP) [28] and DALCE [29].

Figure 1. Selective cyclic opioid peptides

KOR ligands have potential beneficial properties including anti-inflammatory action, analgesia without encountering the serious side effects that are often associated with MOR agonists, and a therapeutic target for the treatment of cocaine addiction and HIV-1 encephalopathy [30,31]. However, KOR agonists are typically less effective in promoting analgesia in comparison

to MOR agonists and also have been shown to bring about dysphoria [32-34]. KOR agonists also cause the contraction of pupils, depress the flexor reflex, and bring about sedation [10]. Proposed endogenous ligands for the KOR are DYN and both α - and β -NEO. With the use of autoradiographical methodologies, the highest density of KOR distribution in rat brain is in the endopiriform nucleus and the medial preoptic area [35]. Both DYN A and DYN B and its truncation variants, with the exception of DYN-(1-6), express slight selectivity for KOR over MOR and DOR [14,36]. Derivatives of DYN A, including [DPro¹⁰]-DYN A-(1-11) [37], [NMeTyr¹, NMeArg⁷, DLeu⁸]-DYN A-(1-8)-ethylamide (E-2078) [38], and a number of analogues further modified from the Dyn A structure exude both potent and selective KOR activities. Numerous peptide antagonists have emerged at the KOR including the Mdp derivative of DYN A-(4-11)-NH₂ (Dynantin) and linear and cyclic variants of DYN A-(1-11)-NH₂ such as [AcPhe¹,Phe²,Phe³,Arg⁴,DAla⁸]-Dyn A-(1-11)-NH₂ (Arodyn) and c^{5,8}[BzlTyr¹,DAsp⁵,Dap⁸]-Dyn A-(1-11)-NH₂ (Zyklophin), respectively [39-41].

1.2. Peptide modifications

Peptides as therapeutics have potential advantages such as high specificity and selectivity for its target, straightforward synthesis for various modifications, and good biocompatibility. There are also disadvantages due to the potential of low metabolic stability, low BBB penetration, and high conformational flexibility, which can be handled through diverse approaches: modification of peptide backbone, incorporation of D-amino acids, cyclization of linear peptides, and introduction of prodrugs. Various approaches have been employed to limit the dynamic nature of peptides, and thus, deduce proper backbone and/or side-chain orientation for optimal receptor affinity and functional activity. Increasing the rigidity of a side chain at the β -position can result in a more constrained structure. Biological examples include Val, Ile, and Thr. Many unnatural amino acid derivatives such as β -methyl-2',6'-dimethyl tyrosine (Tmt), which has a restricted rotation around the C ^{β} -C ^{γ} bond, have been developed [42]. Modifications at the α -position of amino acids have also been discovered in nature such as Aib which is commonly used to elicit turn-structures. N-methylations also influence the conformational freedom of both the backbone and side-chain of an adjacent residue [43]. The use of N-methylation has been shown to increase membrane permeability by decreasing the number of intermolecular and intramolecular hydrogen bonds and increasing the peptide's lipophilicity.

Figure 2. Cyclizations through the formation of covalent bonds

The cyclization of linear peptides enhances proteolytic stability and bioavailability by limiting the peptide's dynamic nature, reducing its hydrogen bonding capability, increasing lipophilicity, and lowering its hydrodynamic radius [44,45]. Therefore, the cyclic peptides become able to adopt better defined conformations compared to their linear variants which typically occur in a conformational equilibrium, and thus, pay lower entropy when binding to their respective receptors. Limiting the number of conformations of a peptide can enhance the ability to orient the backbone and/or side-chain(s) properly for target receptors.

Constraints of cyclic peptides can be attained through the formation of covalent bonds such as lactones, lactams, lanthionine, disulfides, thioethers, etc. that span from the N-terminus to C-terminus, N-/C-terminus to side-chain, or side-chain to side-chain, the latter being the most common (Figure 2). For the synthesis of cyclic peptides, orthogonal protection strategies are necessary to accomplish regioselective cyclization in both solid and solution phase synthesis.

1.3. Cyclic peptides

1.3.1. MOR:

The literature has supported the notion that ligands for the opioid receptors need a positively charged nitrogen atom that interacts with the receptor forming a salt bridge. However, it has been demonstrated that cyclic analogues of β -casomorphin, (CHO-Dmt- $c^{2,5}$ [DOrn-2-Nal-DPro-Gly] and Dhp- $c^{2,5}$ [DOrn-2-Nal-DPro-Gly]), lack the protonated nitrogen atom, and yet exhibit opioid activities as DOR ($K_e = 16$ and 120 nM, respectively, in the MVD) and MOR ($K_e = 220$ and 240 nM, respectively, in the GPI) antagonists, but did not interact with the KOR [46]. The Dhp-derivatives were the first neutral compounds not to possess the positively charged nitrogen atom for the opioid receptors. Cyclic EM-1 analogues with a glycine residue as a linker, $c^{N,C}$ [L/DTyr-L/DPro-L/DTrp-L/DPhe-Gly-], were also devoid of the positive charge due to the N-terminal amino group being incorporated in the cyclization [47]. These compounds displayed a decreased affinity for the MOR in respect to EM-1 and only the compound $c^{N,C}$ [Tyr-DPro-DTrp-Phe-Gly] showed high MOR affinity ($K_i = 34$ nM) as an agonist.

Cyclic EM-2 analogues were synthesized to investigate the effect of ring size on MOR affinity and selectivity [48]. Tyr- $c^{2,5}$ [DLys-Phe-Phe-Asp]-NH₂, a 17-membered ring, showed high MOR affinity in the picomolar range, but reduced selectivity dramatically (4-fold) by enhancing KOR affinity. The incorporation of DOrn² decreased the ring size to 16, maintaining high MOR affinity with a little increased selectivity (24-fold). The introduction of D-2,4-diaminobutyric acid (Dab) at position 2 reduced the ring size to 15 and dramatically decreased MOR affinity. The incorporation of DDap², affording a 14-membered ring, resulted in a highly selective MOR ligand with high affinity in the picomolar range. Another 14-membered cyclic analogue, Tyr- $c^{2,4}$ [DLys-Phe-Asp]-NH₂, gave a similar biological profile to the DDap²-analogue, suggesting the optimal ring size for MOR affinity and selectivity to be 14. The cyclic EM-2 analogue Tyr/Dmt- $c^{2,5}$ [DLys-Xxx-Yyy-Asp]-NH₂ was also modified at position 3 or 4 using Phe, Phe(4-F), Phe(2,4-difluoro), or Phe(4-CF₃) [49]. All compounds exhibited picomolar affinities at the MOR with the exception of the Phe(4-CF₃)³ containing analogues which only interact with the KOR. An analogue with Nal³ was also prepared to improve bioactivity but showed slightly lower antinociceptive activity after peripheral and also central administration than the parent cyclic peptide [50]. Analogues that had Dmt instead of Tyr did not greatly improve affinities for the opioid receptors unlike parent linear analogues. Cyclic EM-2 analogues based on the scaffold Tyr- $c^{2,5}$ [DLys-Xxx-Tyr-Gly] were modified at position 3 and the analog containing a Phe³ exhibited high binding affinity and 3-fold more potent agonist activity than morphine [51]. NMR study suggested that a trans conformation at D-Lys²-Xxx³ is crucial for high affinity, selectivity, and functional activity at the MOR. In a stability test, EM-2 was degraded within 90 min whereas the cyclic EM-2 analogues remained unchanged (less than 7% degradation). The most potent analogues contained the Phe(4-F) or Phe(2,4-difluoro) moiety and showed full MOR agonist with slight KOR agonist activity and a strong antinociceptive effect by intracerebroventricular or intraperitoneal injection, indicating high potential of BBB penetration. To further constrain the analogues, DLys was substituted with cis- or trans-4-aminocyclohexyl-D-alanine (D-ACAla) to use as a bridge moiety [52]. This series of analogues gave decent affinities for the MOR and interestingly those with the trans-D-ACAla showed high affinity for the DOR unlike the cis-isomer and other analogues. EM-2 analogues containing cAmp instead of Pro² were also

explored [53]. This Pro derivative contains a γ -amino-n-butyric acid moiety. The amino group at the γ position of the Pro ring affords a new side chain to tail cyclization without much variance from EM-2. The compound Tyr-c^{2,4}[cAmp-Phe-Phe] elicited 69-fold lower affinity ($K_i = 660$ nM) than EM-2 at the MOR and had no affinity for the KOR and DOR. No agonist activities were shown in the GPI and MVD assays. Stereoisomers were also explored by looking at DPhe³ and both DPhe³ and DPhe⁴ analogues [54]. These compounds gave moderate binding to the MOR as the DPhe³ variant was 20-fold more selective for the MOR over the DOR, but had abysmal to scarce affinities at the DOR and KOR.

Morphiceptin (Tyr-Pro-Phe-Pro-NH₂), a selective MOR ligand, is structurally similar to EM-2. Constrained analogues of EM-2 and morphiceptin were synthesized via cyclization of an amide bond rendered from side-chain amino and carboxy groups at positions 2 and 5 [55]. Tyr-c^{2,5}[Asp-Phe-Phe-Lys]-NH₂ and Tyr-c^{2,5}[Asp-Phe-DPro-Lys]-NH₂ exhibited a greater analgesic effect compared to that of EM-2 in the *in vivo* test after intracerebroventricular administration. The analgesic effects of these compounds were of longer duration than that of EM-2, but did not show significant affinities at opioid receptors. The two cyclic analogues also had no impact on locomotor activity in mice. These results may indicate that these compounds are prodrugs and become pharmacologically active post enzymatic or non-enzymatic reactions.

The cyclic tetrapeptide Tyr-c^{2,4}[DLys-Phe-Ala] has picomolar potency for MOR and DOR as was illustrated in the MVD and GPI assays, respectively [56]. Substitution at position 4 using a number of branched amino acids afforded compounds with high potencies ($IC_{50} = 0.11 - 6.3$ nM), but substitution with an Asp residue reduced biological activity significantly ($IC_{50} = 79$ and 74 nM in the GPI and MVD, respectively) [57]. The loss of MOR activity is considered to be caused by the negative charge on the Asp residue, which is incompatible with the topography of the receptor, while the DOR is known to accommodate the negative charge in DLT-2. It also has been known that DOR pharmacophoric structures are stabilized via hydrophobic interactions from side chains, and thus it is suggested that the Asp residue, having perturbed the hydrophobic face, distorts its optimal conformation for DOR recognition [26]. In Tyr-c^{2,4}[DDab-Phe-Ala] and Tyr-c^{2,4}[DDab-Phe ψ [CH₂NH]Ala], Dab² and pseudo peptide bond modifications lost activities at MOR (31-200 fold) and DOR (74-470 fold) in respect to Tyr-c[DLys-Phe-Ala].

Side-chain to side-chain cyclic DER/HLT analogues using the scaffold c^{2,4}(-CH₂COCH₂-)(DXxx², Yyy⁴)DER(1-4)-NH₂ where Xxx is Lys or Orn and Yyy is Lys, Orn, Dab, or Dap were synthesized via formation of an ureido group [58]. Compounds with ring sizes of 14-17 exhibited high dual agonist potency (ex. Orn² and Dab⁴: $IC_{50} = 1.6$ and 1.3 nM in the GPI and MVD assay, respectively) for MOR and DOR, but had no explicit selectivity. NMR studies showed that conformational freedoms of the side chains in Tyr¹ and Phe³ residues along with the ring structures in 14-18 membered rings exist in solution. Therefore, the biologically active conformation may be brought about in the process of peptide-receptor interplay.

Cyclic ENK analogues with 3-(2,6-dimethyl-4-carbamoylphenyl)propanoic acid (Dcp) or 3-(2,6-dimethyl-4-hydroxyphenyl)propionic acid (Dhp), which do not possess an α -amino group necessary for opioid agonist activity, in place of Tyr¹, were synthesized [59]. The analogue, Dcp-c^{2,5}[DCys-Gly-Phe(4-NO₂)-DCys]-NH₂, displayed highly selective MOR affinity (MOR/DOR/KOR = 1/9/350) in the nanomolar range with potent MOR antagonist activity ($K_e = 3.2$ nM, MOR/DOR/KOR = 1/19/45). Interestingly, the Dcp substitution for Dhp increased the MOR selectivity without changing the affinity. β -methylation of Dhp was also investigated to adjudicate its effect on opioid activity by using (3*S*)- or (3*R*)-Mdp [60]. The (3*S*)-Mdp isomer displayed higher antagonist potencies ($K_e=1.4, 55, \text{ and } 5.8$ nM for MOR, DOR, and KOR, respectively) in functional assays and higher binding affinities for the three receptors relative to the Dhp-analogue. Conversely, the (3*R*)-Mdp

isomer gave much lower activities at the all receptors. Positions 2 and/or 5 of the analogue Tyr-c^{2,5}[DCys-Gly-Phe(4-NO₂)-DCys]-NH₂ were substituted with Hmc to evaluate both steric and hydrophilic effects on opioid receptor affinity and selectivity and all analogues were found to be weak agonists at MOR and DOR with very low affinities in the micromolar range relative to the parent peptide. These results indicate that increased bulkiness on the Cys residue is not tolerated for receptor interaction and overall had little effect on MOR/DOR selectivity.

Dicarba analogues were scrutinized using MOR selective cyclic DER, Tyr-c^{2,4}[DCys-Phe-Cys]-NH₂ [61]. Both cis and trans olefin containing analogues slightly decreased potency in the GPI and retained potency in the MVD assays relative to their ethylene and disulfide variants, but overall only slight MOR selectivity was observed over the DOR.

1.3.2. DOR:

Side-chain to side-chain cyclization of ENK analogues, Tyr-DXxx-Gly-Phe-Yyy-NH₂ where Xxx is DLys or DOrn and Yyy is Lys, Orn, Dab, or Dap, afforded 17-20 membered rings through a urea moiety [62]. The 18-membered ring analogue cyclized between DLys² and Dap⁵ had high increases in MOR and DOR agonist activities (IC₅₀ = 0.21 and 0.65 nM in the GPI and MVD assay, respectively) compared to Leu-ENK. All analogues displayed significant agonist activities in the functional assays, but did not exhibit substantial selectivity. Ring size influenced the activities of the analogues as an 18-membered ring was observed to be the most active in the assays [63]. N-terminal amidated ENK analogues cyclized through a substituted guanidine or thiourea bridge at positions 2 and 5, providing a 15-membered ring, and positions 2 and 6, giving a 22-membered ring, were synthesized [64, 65]. All analogues were MOR agonists with slight selectivity and the 15-membered ring analogues showed a significant variation of their activity in relation to the bridge substitution. The thiourea bridge in the small ring size series resulted in the very potent MOR/DOR agonist (IC₅₀ = 1.8 and 2.4 nM in the GPI and MVD assays). Analogues incorporating the bulky Bcp residue in place of Tyr¹ of the non-selective cyclic ENK analogue Tyr-c^{2,5}[DCys-Gly-Phe(4-NO₂)-DCys]-NH₂, MOR selective DALDA, Tyr-DArg-Phe-Lys-NH₂, and KOR selective analogue, Dyn A-(1-11)-NH₂ were synthesized [66]. These analogues retained high MOR affinity, but showed very different receptor selectivity compared to parent analogues. The Bcp analogue of the cyclic ENK turned out to be a very potent MOR agonist (IC₅₀ = 0.23 nM in the GPI) with picomolar affinity and high selectivity.

Dicarba analogues of cyclic ENKs Tyr-c^{2,5}[DCys-Gly-Phe-D/LCys]-NH₂ using allylglycine at positions 2 and 5, olefinic variants, and the parent Cys derivatives were studied [67]. The L-isomer at position 5 showed well-rounded MOR/DOR agonist activity (IC₅₀ = 1.0 and 3.2 nM) and attenuated KOR affinity with high stability. The D-analogues slightly decreased MOR/DOR agonist activity in respect to the cyclic DCys²/DCys⁵ parent (6-9 fold). ENK dicarba analogues containing the free acid to help improve selectivity for the DOR over the MOR were adjudicated [68]. Olefin containing analogues showed a more potent biological profile than their reduced variants owing to their increase in ring rigidity while the cis and trans isomers showed little variation from one another. The olefin analogues were found to be strong agonists at both MOR and DOR in functional assays including the [³⁵S]GTPγS assay (EC₅₀ = 2.4-2.7 nM at MOR and 0.83-0.88 nM at DOR. High affinity and selectivity for the DOR was accomplished by using a lanthionine bridge in the scaffold of Tyr-c^{2,5}[DAla/Val-Gly-Phe-D/LAla]-OH [69]. Incorporation of DVal² resulted in increase of DOR selectivity over MOR (170-680 fold). The analogue cyclized between DVal² and L/DAla⁵ produced subnanomolar analgesic potencies (ED₅₀ = 0.12, 0.26 nM) in the *in vivo* assay, which may be due to the potent DOR activity and improved stability. All lanthionine-bridged compounds had significantly lesser antinociceptive ED₅₀ values compared to DPDPE, but had increased potency relative to morphine following spinal delivery.

A series of *N*-ureidoethylamides designed from DLT(1-4) were synthesized and an analogue Tyr-c^{2,4}[DLys-Phe-Dab]-CH₂CH₂NHCONH₂ had a stronger antinociceptive response than that of morphine and was resistant to enzymatic degradation [70]. *N*-guanidinylated cyclic DLT(1-4) analogues were assessed to determine the guanidinylation's effect on MOR/DOR affinities and biological availability. Both series of compounds exhibited balanced nanomolar affinities for the MOR and DOR and yielded high enzymatic stabilities. Hybrid ENK/DLT analogues cyclized via a urea bridge with DLys/DOrn at position 2 and Lys, Orn, Dab, or Dap at position 5, giving 17- to 20-membered rings, showed high potencies in the nanomolar range in the GPI and MVD assays [71]. Increased selectivity was observed for the DOR due to a loss of affinity for the MOR.

As an efficient tool, cyclization has been applied to all kinds of peptides with diverse biological profiles. Besides the cyclic opioid ligands, cyclic multifunctional ligands in which an opioid pharmacophore is combined with a non-opioid pharmacophore were synthesized to enhance biological activity and stability. The linear compound Tyr-DAla-Gly-Phe-Met-Pro-Leu-Trp-NH-[3',5'-(CF₃)₂Bzl] (TY027), a MOR/DOR agonist/NK-1 antagonist, was cyclized through a disulfide bond at positions 2 and 5 using a DCys² and a DCys⁵ or L/DCys⁷ [72]. Positions 2 and 5 were selected for cyclization on the basis of the SAR results showing the important roles of Tyr¹, Gly³, Phe⁴, Pro⁶, and Trp⁸ at the opioid and NK-1 receptors. β -turn structures were observed at DAla²-Met⁵ and from the Pro⁶- to C-terminal benzyl group. Cyclization at positions 2 and 5 was considered to stabilize this structural element, whereas cyclization at positions 2 and 7 could potentially ablate the β -turn involving the C-terminus. An analogue cyclized at positions 2 and 7 improved stability dramatically. A cyclic analogue Tyr-c^{2,7}[DCys-Gly-Phe-Nle-Pro-DCys]-Trp-NH-[3',5'-(CF₃)₂Bzl] was a selective DOR agonist with potent NK1 antagonist activity. Another cyclic analogue of a multifunctional ligand is an opioid agonist/CCK antagonist [73]. Among the cyclic analogues, Tyr-c^{2,5}[DCys-Gly-Trp-Cys]-Asp-Phe-NH₂ showed the highest potency with very good selectivity for the DOR. NMR studies displayed similar structural features to both opioid and CCK receptor ligands at the N-terminal side. However, disulfide cyclization was considered to cause poor interaction with CCK receptors due to a tight, unfavorable constraint at the C-terminal side, and thus, a cyclic lactam may be a better option for CCK receptor based on the ring size and flexibility.

1.3.3. KOR:

Analogues of Tyr-c^{2,4}(SCH₂CH₂S)[DCys-Phe-DPen]-NH₂ (JOM-6) and JOM-13 that are selective for the MOR and DOR, respectively, were synthesized to uncover the steric, lipophilic, and electronic effects of the amino acid at the 3rd position on KOR activity [74]. Furthermore, substitution of DPen⁴ with the more flexible DCys was also explored. Cyclization was achieved through the formation of disulfides or ethylene dithioethers at positions 2 and 4. Replacing Phe³ with aliphatic side-chain containing amino acids did not result in KOR recognition, but those substituted with Cha did exhibit low nanomolar affinity for both MOR and DOR. The effect of having basic residues at this position afforded compounds that had severely reduced binding affinities at the opioid receptors. DPen⁴ replacement with DCys⁴ while retaining Phe³ was also explored to deduce whether the geminal methyls of DPen may interfere with KOR interactions. Cyclization achieved by an ethylene dithioether bridge gave little KOR binding improvement relative to JOM-6, but when the cyclization incorporated a disulfide, KOR binding increased by approximately 70-fold relative to JOM-5 (Tyr-c^{2,4}[DCys-Phe-DPen]-NH₂) and presented nanomolar affinity to all opioid receptors. Phe³ substitutions with other aromatic amino acids were performed to investigate lipophilicity, size, electronic properties, and spatial orientation effects. The majority of dithioether bridged compounds reduced affinities at the KOR relative to the disulfide analogue. DPhe³, bulky derivatives, and substituents that could participate in hydrogen bonding, yielded little to no noticeable binding to the KOR. These results suggest that aromaticity at the 3rd position is critical for KOR binding along with the MOR and DOR, disulfide-bridged compounds are preferred by the KOR and DOR whereas

the ethylene dithioether-bridged compounds prefer the MOR, and an amide at the C-terminus is selective for MOR and KOR as opposed to a carboxylate which favors DOR. Also, substitution of DPen⁴ with DCys⁴ greatly enhances KOR affinity and maintains high MOR and DOR affinity.

Another scaffold, Tyr-c^{2,5}[DCys-Phe-Phe-X]-NH₂, was employed in an attempt to improve KOR affinity, where X = D- or L-Cys/Pen bridged by S(CH₂)_nS (n = 0, 1, 2) [75]. KOR affinities were improved in cyclic tetrapeptides compared to tripeptides along with a C-terminal Cys substitution, although stereochemistry of this residue and the type of cyclization have little impact on the KOR. Even though these ligands exhibit high binding affinities for the KOR, they are not selective. It is significant to note that compounds Tyr-c^{2,5}[DCys-Phe-Phe-Cys]-NH₂, Tyr-c^{2,5}[DCys-Phe-Phe-DCys]-NH₂, and Tyr-c^{2,5}[DCys-Phe-Phe-DCys]-NH₂, bridged by -SCH₂S-, -SS-, and -SCH₂S-, respectively, have low picomolar affinities for MOR. Dithioether bridged analogues of Tyr-c^{2,5}[DCys-Phe-Phe-Cys]-NH₂, which possesses high affinity for the MOR and DOR, partial agonism for DOR and full agonism for MOR and KOR, were synthesized to increase selectivity, efficacy, and affinity at the MOR [76]. Analogues having 1-Nal and 2-Nal at positions 3 and 4 showed MOR agonist activity with significantly decreased DOR efficacy (E_{max} < 25%) in the [³⁵S]GTPγS assay. The compound Tyr-c^{2,5}(-SCH₂S-)[DCys-Phe-2-Nal-Cys]-NH₂ had nanomolar affinity at the three receptors and was an agonist for MOR and a partial agonist or antagonist for the DOR, contingent upon the type of assay employed. It was also shown to be a full agonist for the KOR.

Analogues of Dyn A (1-11)-NH₂ were cyclized at various positions. Side-chain to side-chain cyclization of c^{2,5}[DAsp²,Dap⁵]Dyn A (1-11)-NH₂ and substitutions at position 3 were evaluated [77]. Modifications at position 3 displayed high affinities for the KOR and MOR, and high KOR selectivity, but were not tolerated for the DOR. The DALa³-substituted analogue compared to Ala³ and the parent peptide, gave higher KOR affinity and selectivity (KOR/MOR/DOR = 1/18/660). The DALa³ analogue also was a potent full KOR agonist (EC₅₀ = 0.54 nM) identified by the adenylyl cyclase assay. Cyclic analogues in most cases yielded higher affinities for MOR relative to their linear parents. L/DTrp³ and Pro³ substitution in the cyclic variant decreased potency by up to 390-fold arbitrated by the adenylyl cyclase assay. In order to maintain basicity at the N-terminus and constrain Tyr¹ in DYN A, an acetyl linker between the N-terminus and the side-chain of Lys was implemented in Dyn A (1-11)-NH₂ [78]. Considering that positions 3 and 5 of Dyn A have been shown to not be critical for opioid activity, cyclizations spanned from the head to the 3rd or 5th position which result in 15- and 21-membered ring, respectively. Stereochemical effects at these positions were also explored by using L/DLys. The conformational constraint and the N-terminal α-acetamide group caused prominent loss of affinity for the opioid receptors.

Analogues of Dyn A (1-11)-NH₂ were cyclized at positions 2 and 5 or 5 and 8 through an allyl glycine [79]. The positioning and stereochemistry of the cyclization impacted both KOR affinity and selectivity. C^{2,5}(-CH=CH-) [DALa²,Ala⁵]Dyn A (1-11)-NH₂ in the cis and trans configuration maintained their affinities and selectivity for the KOR, MOR, and DOR in respect to the parent peptide. C^{5,8}(-CH=CH-)[Ala⁵,Ala⁸]Dyn A (1-11)-NH₂ cis and trans analogues yielded low nanomolar affinities to the KOR and MOR and showed slight selectivity for KOR. Although C^{2,5}(-CH=CH-)[Ala²,Ala⁵]Dyn A (1-11)-NH₂ exhibited attenuated affinity for the opioid receptors compared to Dyn A (1-11)-NH₂, they were more selective for the KOR than the parent peptide.

To acquire KOR antagonist activity, Ac[Lys²,Trp³,Trp⁴,DALa⁸]-Dyn A (1-11)-NH₂ (venorphin) was investigated [80]. A cyclic analogue, c^{N,5}[Trp³,Trp⁴,Glu⁵]-Dyn A (1-11)-NH₂, was prepared by cyclizing the N-terminus to the side-chain of Glu⁵ and exhibited nanomolar affinity at the KOR with high selectivity over the MOR (12-fold). Antagonist activity was adjudicated

from an adenylyl cyclase assay as this was the first cyclic KOR antagonist that lacks the N-terminal amino group that still holds affinity for the opioid receptors. C-terminal modifications of Dyn A (1-11) were investigated to assess if efficacy would be impacted [41,81,82]. Zyklophin showed low nanomolar affinity and high selectivity for the KOR along with nanomolar antagonist potency *in vitro*. Zyklophin is a KOR selective antagonist *in vivo*, capable of crossing the BBB to antagonize KOR, and does not elicit any agonist activity, whether administered centrally or peripherally, as was shown in the warm-water tail-withdrawal assay. Antagonist activity lasts less than 12 hours after initial systemic administration. Zyklophin's ability to deter stress-induced restoration of cocaine-seeking behavior when administered systemically provides further evidence that it crosses the BBB.

The fungus *Ctenomyces serratus* ATCC15502 was identified to produce the KOR antagonist c[DPro-Phe-Trp-Phe] (CJ-15,208) with high affinity and selectivity [83]. Both L/DTrp isomers yielded the same nanomolar affinity to the KOR but showed different biological profiles on Ala scans [84]. The DTrp isomer did not possess KOR agonist activity but Ala substitution at any position increased antinociceptive activities in the tail-flick assays, which were diminished by nor-BNI, a KOR antagonist, suggesting that the activity is mediated through the KOR. However, the Ala substitution of LTrp isomer brought about antinociception through the MOR as was evidenced by attenuated effects when using MOR selective opioid receptor antagonists [85,86]. Binding affinities of selected cyclic opioid ligands at MOR, DOR, and KOR are shown in Table 2.

Table 2: Binding affinities of cyclic opioid ligands at MOR, DOR, and KOR.

Structure	K_i (nM)			Reference
	MOR	DOR	KOR	
c ^{N,C} [Tyr-DPro-DTrp-Phe-Gly]	34 ± 2 ^a	-	-	47
Tyr-c ^{2,5} [Asp-Phe-Phe-Lys]-NH ₂	289 ± 45 ^a	>1000 ^b	>1000 ^c	55
Tyr-c ^{2,5} [Asp-Phe-DPro-Lys]-NH ₂	514 ± 98 ^a	>1000 ^b	>1000 ^c	55
Tyr-c ^{2,5} [DLys-Phe-Phe-Asp]-NH ₂	0.56 ± 0.03 ^{a,d}	280 ± 2 ^{d,e}	2.4 ± 0.2 ^{d,f}	48
Tyr-c ^{2,5} [DCys-Phe-Phe-DCys]-NH ₂	0.05 ± 0.01 ^g	0.4 ± 0.09 ^g	1.6 ± 0.5 ^g	75
Tyr-c ^{2,5} [DCys-Phe-2-Nal-Cys]-NH ₂	1.2 ± 0.3 ^g	11 ± 6.4 ^g	5.9 ± 0.8 ^g	76
Tyr-c ^{2,5} [DLys-Phe-Tyr-Gly]	0.99 ± 0.14 ^a	39 ± 4 ^h	4450 ± 1245 ^c	51
Tyr-c ^{2,5} [cis-D-ACAla-Phe-Phe-Asp]-NH ₂ ⁱ	3.2 ± 0.0 ^{a,d}	>1000 ^{d,e}	-	52
Tyr-c ^{2,5} [trans-D-ACAla-Phe-Phe-Asp]-NH ₂	2.1 ± 0.0 ^{a,d}	3.9 ± 0.0 ^{a,d}	-	52
Tyr-c ^{2,5} (-SCH ₂ S-)[DCys-Phe-2Nal-Cys]-NH ₂ ⁱ	0.47 ± 0.20 ^g	0.48 ± 0.20 ^g	1.3 ± 0.4 ^g	76
Tyr-c ^{2,4} [DLys-Phe-Asp]-NH ₂	0.21 ± 0.02 ^{a,d}	461 ± 625 ^{d,e}	684 ± 59 ^{d,f}	48
Tyr-c ^{2,4} (-NHCONH-)[DLys-Phe-Dab]-CH ₂ CH ₂ NHCONH ₂ ⁱ	1.7 ± 0.0 ^a	9.1 ± 0.7 ^b	-	70
Tyr-c ^{2,4} [DCys-Phe-DCys]-NH ₂	1.3 ± 0.3 ^g	16 ± 4 ^g	39 ± 2 ^g	74
Tyr-c ^{2,4} (-SCH ₂ CH ₂ S-)[DCys-Phe-DPen]-NH ₂ ⁱ	0.17 ± 0.02 ^g	12.0 ± 1.40 ^g	2650 ± 401 ^g	74
CHO-Dmt-c ^{2,5} [-DOrn-2-Nal-DPro-Gly] ⁱ	218 ± 28 ^a	32.8 ± 1.6 ⁱ	-	46
Dhp-c ^{2,5} [DOrn-2-Nal-DPro-Gly] ⁱ	450 ± 77 ^a	109 ± 12 ⁱ	-	46
Tyr-c ^{2,5} (-NHCSNH-)[DDap-Gly-Phe-Dap]-NH ₂ ⁱ	0.4 ^a	5.4 ^e	-	64
Tyr-c ^{2,5} [DCys-Gly-Phe(4-NO ₂)-DCys]-NH ₂	0.443 ± 0.067 ^a	0.389 ± 0.051 ^j	1.53 ± 0.19 ^c	66
Dcp-c ^{2,5} [DCys-Gly-Phe(4-NO ₂)-DCys]-NH ₂	2.8 ± 0.3 ^a	26 ± 1 ^j	980 ± 170 ^c	59
Dhp-c ^{2,5} [DCys-Gly-Phe(4-NO ₂)-DCys]-NH ₂	4.8 ± 0.4 ^a	12 ± 1 ⁱ	300 ± 57 ^c	59
(3S)-Mdp-c ^{2,5} [DCys-Gly-Phe(4-NO ₂)-DCys]-NH ₂	2.1 ± 0.2 ^a	2.0 ± 0.1 ^j	50 ± 2 ^c	60
Tyr-c ^{2,5} (-CH ₂ CH ₂ -)[DAla-Gly-Phe-Ala]-NH ₂	2.3 ± 0.2 ^a	5.9 ± 0.8 ⁱ	309 ± 31 ^c	67
Tyr-c ^{2,5} (-CH ₂ CH ₂ -)[DAla-Gly-Phe-DAla]-NH ₂ ⁱ	1.2 ± 0.1 ^a	3.3 ± 0.5 ^j	72 ± 9 ^c	67
Tyr c ^{2,5} (-cisCH=CH-) [DAla-Gly-Phe-DAla]-OH ⁱ	1.4 ± 0.5 ^a	0.43 ± 0.16 ^h	580 ± 23 ^c	68
Tyr-c ^{2,5} (-transCH=CH-) [DAla-Gly-Phe-DAla]-OH ⁱ	1.3 ± 1.2 ^a	0.57 ± 0.21 ^h	75 ± 5 ^c	68
Tyr-c ^{2,5} (-S-)[DVal-Gly-Phe-DAla]-OH ⁱ	630 ^k	0.93 ^k	1600 ^k	69
Tyr-c ^{2,5} (-S-)[DVal-Gly-Phe-Ala]-OH ⁱ	130 ^k	0.79 ^k	>1000 ^g	69
Tyr-c ^{2,5} [DCys-Gly-Trp-Cys]-Asp-Phe-NH ₂	2.2 ^a	0.2 ^j	-	73
c ^{N,C} [DPro-Phe-Trp-Phe]	260 ^a	2600 ^h	47 ^k	83
c ^{N,5} [Trp ³ ,Trp ⁴ ,Glu ⁵]Dyn A (1-11)-NH ₂	330 ± 29 ^a	>8900 ^h	27 ± 3 ^g	80
c ^{2,5} [DAsp ² ,Dap ⁵]Dyn A (1-11)-NH ₂	0.52 ± 0.06 ^a	5.10 ± 0.50 ^h	0.46 ± 0.14 ^g	77
c ^{2,5} (-cisCH=CH-)[DAla ² ,Ala ⁵]Dyn A (1-11)-NH ₂ ⁱ	2.33 ± 0.20 ^a	9.30 ± 1.00 ^h	0.84 ± 0.10 ^g	79
c ^{2,5} [N-BzlTyr ¹ ,DAsp ⁵ ,Dap ⁸]Dyn A (1-11)-NH ₂ (Zyklophin)	5900 ± 1400 ^a	>10000 ^h	30 ± 2 ^g	81

^a[³H]DAMGO; ^b[³H]DLT; ^c[³H]U69,593; ^dIC₅₀; ^e[³H][Ile^{5,6}]DIDI; ^f[³H]nor-BNI; ^g[³H]diprenorphine; ^h[³H]DPDPE; ⁱStructure in Figure 3; ^j[³H]DSLET; ^k[³H]enadoline;

Figure 3. Structures of selected cyclic opioid ligands.

1.4. Bioavailability of cyclic peptides

A major drawback for the use of peptides as CNS drugs is attributed to their lack of biodistribution to the brain due to poor metabolic stability and an inability to cross the BBB [87,88]. Diffusion across the BBB is often based upon a compound's lipid solubility, molecular size, and charge, although specific transporters are present at the BBB that allow nutrients to enter the brain and waste products to exit [89,90]. The BBB has a high concentration of efflux-transporters that do away with a vast range of compounds from the cytoplasm of endothelial cells prior to crossing into the brain parenchyma. BBB capillary endothelia lack openings and are sealed due to tight junctions which in turn prevents any substantial paracellular transport [91]. The BBB endothelial cells contain a reduced number of vesicles which provide less vesicular transport, and in addition, the BBB contains various enzymes known to breakdown peptides such as aminopeptidases A and M and angiotensin converting enzyme [92]. Methods have been developed to circumvent these issues such as increasing a compound's lipophilicity, glycosylation, targeting transporters, inclusion of charged moieties in peptides, terminal modifications, cyclizations, and the incorporation of D-amino acid residues. Discussed here are recent investigations looking into increasing the biodistribution of peptides through the use of cyclization combined with other approaches.

Two series of EM-1 analogues, c^{N,C}[Cys-Tyr-L/DPro-L/DTrp-Phe-Cys] and Tyr-c^{2,5}[Cys-L/DTrp-Phe-Cys], cyclized via a disulfide bond were synthesized and tested for their stability in human plasma and were subjected to lipidation and glycosylation [93]. (Figure 4) The first series conserved the EM-1 structure with two Cys residues on the outside resulting in a 20-membered ring cycle. The second series had Pro³ substituted with DCys which was cyclized to Cys at the C-terminus

affording a 14-membered ring. All tested cyclic EM-1 analogues exhibited an increased half-life up to 27-fold in human plasma relative to EM-1 ($t_{1/2} = 5.6$ min), showing that cyclization improves metabolic stability compared to the parent peptide.

DADLE is resistant to peptidase, but has meager BBB and intestinal mucosa permeation. A cyclic prodrug of DADLE was synthesized by conjoining the N- and C-termini through a coumarinic acid (CA)-linker, yielding a lipophilic and uncharged cyclic peptide [94,95]. Through cell culture models, CA-DADLE was determined to be a substrate for apically polarized efflux transporters within the intestinal mucosa and falls victim to oxidative metabolism at two major positions through liver microsomes and human recombinant cytochrome P450 3A4 (hCYP3A4), Tyr¹ and Phe⁴ [96-100]. In lieu of this, additional cyclic prodrugs of DADLE, CA-[Cha⁴,DLeu⁵]-ENK and CA-[Cha⁴,DAla⁵]-ENK, were synthesized to investigate what effect modification of the 4th position has on oxidative metabolism and cell permeation [100]. Cha-substituted analogues did not exhibit greater metabolic stability relative to CA-DADLE. This may be due to the Tyr¹ residue still being present, which is crucial for the opioid receptor interaction. The analogues were found to be more stable than CA-DADLE when incubated with hCYP3A4 pointing to other CYP450 isozymes being accountable for oxidative metabolism as was shown when analogues were incubated with both animal and human liver microsomes. These results are significant considering hCYP3A4 is highly expressed in intestinal mucosal cells and have been demonstrated to decrease the bioavailability of drugs when administered orally [101,102]. CA-[Cha⁴,DLeu⁵]-ENK and CA-[Cha⁴,DAla⁵]-ENK showed favorable cell membrane permeation, but exhibited poor *in vitro* Caco-2 cell permeation due to preserving their affinity for efflux transporters [103].

[Ala²,DLeu⁵]-ENK, [DAla²,Leu⁵]-ENK, and [Ala²,Leu⁵]-ENK prodrugs were cyclized from the N- and C-termini using oxymethyl coumarinic acid (OMCA) as a linker to determine the impact of Ala² and Leu⁵ chirality on peptide conformation, physiochemical properties, and cell penetration [104]. These cyclic analogues interacted with efflux transporters unlike a series of linear variants which did not contain OMCA, but were instead N-acetylated and C-amidated. This pointed towards the linker moiety having influence on efflux transporter recognition either through making the prodrug lipophilic enough to access the transporters or help create a structure that better complements the efflux transporters. Stability and pharmacokinetic studies showed that acyloxyalkoxy-DADLE, and CA-DADLE cyclic prodrugs convert to DADLE by esterase *in vitro* and *in vivo* and enhance stability relative to DADLE [105]. However, these prodrugs were unsuccessful due to an inability to deliver appreciable amounts of DADLE to the brain, stemming from rapid biliary excretion, poor BBB permeation, and slow conversion from the prodrug to the active form. A modified CA-based cyclic prodrug, OMCA-DADLE, incorporating an oxymethyl sequestered between the phenolic and carboxylate moieties of CA, could accelerate the bioconversion of prodrugs by reducing steric hindrance around the ester bond [106]. The OMCA derivative was highly susceptible to esterase activity, but was found to interact with efflux transporters.

A series of substituted cyclic Leu-ENK and CA-DADLE prodrugs with various alkyl substituents on the phenolic ring were synthesized and their release rates were evaluated to elucidate the effect of substitution [107]. Release rates were slower when the phenolic ring was substituted compared to the non-substituted prodrug. Methyl substituents that are ortho to the alkenyl or the phenolic carboxyl moieties show greater stability relative to their unsubstituted derivatives. In an effort to increase the nucleophilicity of the hydroxyl group to escalate the cyclization rate of CA, a methyl substituent was incorporated to the para position of the hydroxyl group, but no enhanced rate was observed. No defined pattern could be deduced from the impact of substitution of the phenolic ring on release rates of the series of cyclic prodrugs.

Figure 4. Structures of cyclic opioid ligands with improved bioavailability.

1.5. Polycyclic peptides

Polycyclic peptides have numerous benefits and should be considered for future designs in opioid receptor ligands. Implementation of additional cycles further constrains a given peptide and decreases the number of conformations it can take on, ultimately leading to high specificity and selectivity to their target receptors. An example of this can be seen with ω -conotoxin MVIIA, a natural polycyclic peptide isolated from cone snails that inhibits N-type voltage-sensitive calcium channels with high selectivity and picomolar affinity [108]. It is bridged by 3 disulfide bonds that form a cystine knot [109]. In addition to this attenuated flexibility, electrostatic/steric interactions between amino acid side chains, and intramolecular hydrogen bonding can guide the peptide to conform to an explicit structure in solution, an example being the natural polycyclic peptide phalloidin [110].

Polycyclic peptides have an enhanced resistance to proteolytic degradation. If the N- and C-termini are involved in cyclizations, then these ends are not available to exopeptidases. The increased constraint on the peptide backbone also enables it to be inaccessible to endopeptidases attributing to their inability to optimally conform to the enzyme's active site. SFTI-1 exposed backbone and disulfide bridge-free analogues were investigated and shown to break down in the presence of trypsin faster than that of SFTI-1 [111]. In another study, bridged bicyclic norbornane-like peptides were subjected to human serum [112]. Both bicyclic and monocyclic variants of the peptides showed high resistance to proteolytic degradation after 24 hours as opposed to their linear variants which were rapidly degraded.

Although proposed ligands for the opioid receptors are relatively few, there has been success with small polycyclic peptides in the therapeutic realm. Romidepsin, a prodrug histone deacetylase inhibitor, is a bicyclic peptide composed of natural and unnatural amino acids with a molecular weight of 541 Da [113]. Reduction of its disulfide bond via glutathione yields the active drug. The polycyclic structure of romidepsin is stable to proteolytic digestion, but its reduced form is quickly inactivated. This drug is cell permeable as it acts on intracellular targets.

A starting point for designing polycyclic peptide ligands for the opioid receptors may reside with the endogenous ligand Dyn containing 17 amino acids. Due to its longer length and unique biological profile [114], it will be worthwhile to design a

variety of polycyclic ligands based on the Dyn A structure. Prominent positions for cyclization have been at positions 2 and 5 of enkephalin and thus may serve as one cyclic moiety. The additional points for cyclization may either be overlapped in the enkephalin pentapeptide or be fully encompassed in the C-terminal domain. It has been shown that the Arg residue at position 7 is not essential for opioid and non-opioid receptor recognition in linear analogues of Dyn A and thus may be substituted with a residue to achieve an additional cyclization through various linkages with the side chain or C-terminal acid. Synthesis of polycyclic peptides is more arduous than that of monocyclic and linear variants as the design typically has to incorporate the previous cyclization's influence on the ligand's ability to endure a next cyclization. Similarly to cyclic peptides, the same cyclization strategies are utilized (i.e. side-chain to side-chain, side-chain to terminus, terminus to terminus) along with a variety of covalent bridging such as disulfides, lactones, lactams, lanthionines, thioethers, and olefins (Figure 2). SPPS and/or LPPS coupled with an orthogonal protection strategy are common approaches for the synthesis of polycyclic peptides.

CONCLUSION

Historical peptide ligands for the MOR, KOR, and DOR have stood the test of time as many are still currently used as an agonist/antagonist in *in vitro* binding models. The cyclic peptides that have been discussed herein demonstrate the therapeutic potential they have over conventional linear ligands due to their increased receptor selectivity, metabolic stability, and cell penetration characteristics. Numerous different scaffolds/approaches have been successfully used that highlight the benefits of cyclization and have provided many promising leads for novel therapeutic ligands. Synthesis of polycyclic ligands for the opioid receptors should be evaluated as the positive attributes of a single cyclization moiety in a compound can be enhanced. The increased rigidity/topological geometry of polycyclic peptides further attenuates the dynamic nature of the compound promoting greater affinities and selectivities at target receptors as well as increased *in vivo* stability. The future of providing relief from disease states at the opioid receptors is encouraging as recent developments in cyclic opioid peptide ligands are augmenting our capabilities of using biologically derived molecules as strong therapeutic agents.

LIST OF ABBREVIATIONS

cAmp: cis- γ -amino-L-proline; BBB: blood brain barrier; Bcp: 4'-[N-((4'-phenyl)-phenethyl) carboxamido]phenylalanine; Bzl: benzyl; CA: coumarinic acid; cAmp: cis- γ -amino proline; CCK: cholecystokinin; CNS: central nervous system; Dab: 2,4-diaminobutyric acid; D-ACAla: 4-aminocyclohexyl-D-alanine; DADLE: [DAla²,Dleu⁵]enkephalin; DALCE: [DAla², Leu⁵, Cys⁶]enkephalin; DALDA: [DAla², Lys⁴]dermorphin(1-4) amide; Dap: 2,3,-diaminopropionic acid; Dcp: 3-(2,6-dimethyl-4-carbamoylphenyl)propanoic acid; DLT: deltorphin; DER: dermorphin; Dhp: 3-(2,6-dimethyl-4-hydroxyphenyl)propionic acid; DIDI: [Ile^{5,6}]deltorphin; Dmt: 2,6,-dimethyl tyrosine; DOR: delta opioid receptor; DPDPE: c[DPen²,DPen⁵]enkephalin; DPLPE: c[DPen²,Pen⁵]enkephalin; DREK: dermenkephalin; DSLET: [DSer², Leu⁵]enkephalin; DYN: dynorphin; END: endorphin; ENK: enkephalin; EM: endomorphin; GPI: guinea pig ileum; Hmc: (S)- α -hydroxymethylcysteine; KOR: kappa opioid receptor; LPPS: liquid phase peptide synthesis; Mdp: des-amino-2,6-dimethyl tyrosine; MOR: mu opioid receptor; MVD: mouse vas deferens; Nal: naphthylalanine; NEO: neoendorphin; NK-1: neurokinin-1; OM: oxymethyl; OMCA: oxymethyl coumarinic acid; Orn: ornithine; SFTI: sunflower trypsin inhibitor; SMS: somatostatin; SPPS: solid phase peptide synthesis; Tmt: β -methyl-2',6'-dimethyl tyrosine

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Received:

Revised:

Accepted: