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Opioid Activity of 4-Imidazolidinone Positional Analogues of Leu-Enkephalin

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Abstract—Modulation of opioid activity was accomplished for analogues of Leu-enkephalin through incorporation of a 4-imidazolidinone moiety. The peptide backbone was constrained via a methylene bridge between two neighboring amides within its regular peptide sequence, which was expected to disrupt the secondary structure of the original molecule. Five positional analogues of Leu-enkephalin based on the same sequence and different location of the imidazolidinone-constrict were designed, synthesized, and examined for their affinity to μ-, δ- and κ-opioid receptors.

Peptidomimetics[1] are generally designed to mimic the topology of peptides using a range of synthetic approaches to change typical amide backbone characteristics. Alteration of amide bonds using a variety of isosteres or insertion of unnatural amino acids into a peptide sequence has widely proven to enhance enzymatic stability of peptides.[2] Various approaches have also been used to rigidify an active structure by cyclization or by introduction of constraints into regular peptide molecules in order to amplify conformational and functional features beneficial to enzymatic stability and biological activity. Diverse peptidomimetics have found applications in immunology,[3] receptor binding interactions,[4] and in the design of enzyme inhibitors.[5] Our interest focused on the introduction of a simple heterocyclic constraint based upon the generation of an 4-imidazolidinone moiety at individual amide bonds while preserving the remaining amide backbone and side chain functionalities. 4-Imidazolidinone is a five-membered ring that can be formed directly on an N-terminus of a peptide backbone via a cyclic condensation with an aldehyde or ketone.[6,7] Traditionally, this cyclization mechanism has been used for the temporary protection of primary amines[7] or as a synthon for the preparation of unnatural amino acids.[8] Since the hydrolytic stability of N-terminal imidazolidinones is reported to be relatively low and C-2 substituent-dependent, its incorporation to 'temporarily' modify the peptide, has been attempted in order to create bioreversible prodrugs.[9] This concept was investigated by several groups on Leu-enkephalin analogues with diverse N-terminal tyrosine-based imidazolidinones.[10–13] The goal of those studies was to obtain an analogue having a slow rate of hydrolysis while providing complete recovery of the original active substance. Investigation of imidazolidinone derivatives formed from monosaccharide-modified enkephalins by intramolecular rearrangement[14,15] has also been carried out. To our knowledge, the incorporation of the 4-imidazolidinone group into specific sequences, in order to reduce the memory loss associated with aging,[16] is the only example of biological activity of imidazolidinone-modified peptides. 4-Imizolidinones, derived from single aromatic amino acids and acetone, have also been found to be moderate inhibitors of tyrosine and histidine decarboxylase.[17]

Recently, we developed a method for a solid-phase synthesis of 1,2,5-trisubstituted 4-imidazolidinones[18] based on Katritzky's benzotriazole-mediated reaction.[19] We have adopted this synthetic protocol in the solid-phase preparation of various imidazolidinone-based peptidomimetics.[20] This enables the incorporation of individual imidazolidinone constraints into each position of the Leu-enkephalin sequence and thus preparation of a set of peptidomimetic analogues (Scheme 1). This paper presents the relationship between the position of the imidazolidinone insert within a sequence and the opioid activity of such analogues. We have prepared

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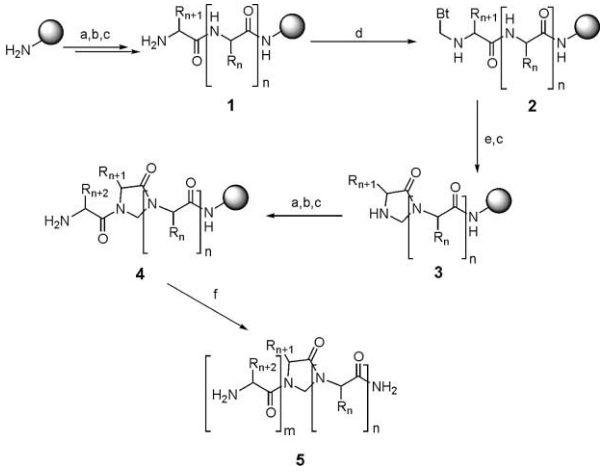
imidazolidinone-based Leu-enkephalin peptidomimetics **6**–**10** (Fig. 1) using a modification of a standard solid-phase peptide synthesis incorporating the formation of the imidazolidinone ring. All of the compounds were purified by RP HPLC and characterized by LC–MS (electrospray ionization), HRMS, and ^{13}C NMR. The peptidomimetics **6**–**10** were synthesized on *p*-methylbenzhydrylamine resin using the 'tea-bag' technique[21] and a synthetic protocol described elswhere.[20] Starting material **1** (see Scheme 1) was prepared by *tert*-butyloxycarbonyl (Boc) solid-phase peptide synthesis strategy



Scheme 1. Solid-phase synthesis of 4-imidazolidinone analogues of peptides: (a) Boc-Aaa, DIPCI, HOBt, DMF; (b) TFA in DCM; (c) DIEA in DCM; (d) benzotriazole, formaldehyde, DMF, 85 °C; (e) $BF_3{\cdot}Et_2O$, DCM; (f) HF, 0 °C.

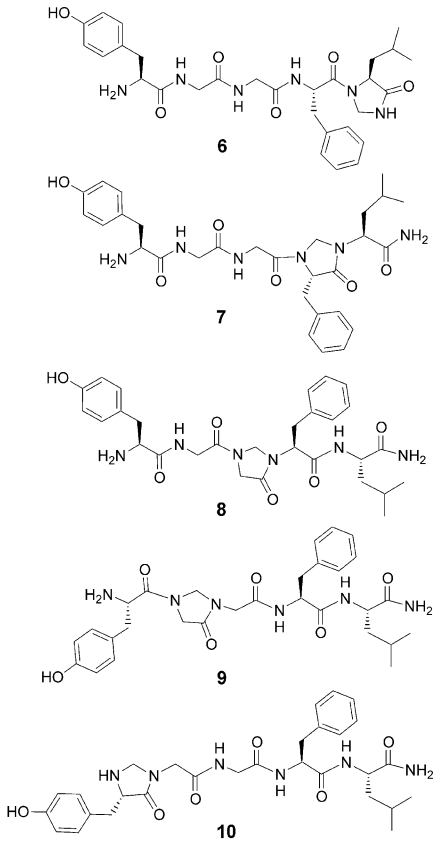


Figure 1. 4-Imidazolidinone-based Leu-enkephalin analogues.

using activation with diisopropylcarbodiimide (DIPCI) and 1-hydroxybenzotriazole (HOBt) in dimethylformamide (DMF). Following Boc-deprotection and neutralization, amine **1** was reacted with formaldehyde and benzotriazole to form adduct **2**. The imidazolidinone **3** was formed by spontaneous nucleophilic substitution of the benzotriazole (Bt) group by the amide nitrogen of the neighboring moiety in the presence of a Lewis acid ($BF_3{\cdot}Et_2O$). The remainder of the synthetic steps for analogues **6**–**9** was continued by classical Boc peptide synthesis generating the resin-bound peptidomimetic **4**. The coupling of Boc-amino acids on the secondary amine of intermediate **3** was performed after additional neutralization with 5% diisopropylethylamine (DIEA) in dichloromethane (DCM). The final step involved HF treatment of resin **4** at 0 °C (1.5 h) to yield product **5**. The synthesis of analogue **10** was stopped at resin-bound intermediate **3** and the resin treated with HF to obtain the desired product. Following extraction (AcOH), lyophilization and RP HPLC purification, all products were obtained as white solids in yields greater than 70%.[22] Despite the expected partial decomposition[20] of analogue **10**, all analogues were stable under physiological conditions (phosphate buffer pH 7.4, 24 h, 37 °C).

Purified compounds were tested in μ-, δ-, and κ-opioid receptor binding assays as described elsewhere. Each sample contained 0.5 mL of membrane suspension, 3 nM of a tritiated competitor of binding ([D-Ala^2, MePhe^4, Gly^5-ol]enkephalin (DAMGO) for the μ-receptor, [D-Ser^2, Leu^5, Thr^6]enkephalin (DSLET) for the δ-receptor, and U69,593 for the κ-receptor.[23] Each imidazolidinone analogue was initially tested at a concentration of 0.1 mg/mL, and 50 mM Tris–HCl in a final total volume of 0.65 mL. Unlabeled DAMGO, unlabeled DSLET and unlabeled U50,488, respectively, were used to generate standard curves and determine non-specific binding. The results, shown in Table 1, indicate that the presence of the imidazolidinone ring at the C-terminus yields enhanced affinity for binding to the μ-receptor (analogue **6**). This analogue shows good affinity for the δ-receptor and poor affinity for the κ-receptor. Dramatic loss of activity was observed for the analogue in which the ring was shifted by one position to the N-terminus (analogue **7**). This analogue was also totally inactive at the κ- and δ-receptors. Analogues

Table 1. Affinity of Leu-enkephalins **6**–**13** analogues for opioid receptors (μ, κ, δ)[a]

Compd	μ-Receptor K_i (nM)	κ-Receptor K_i (nM)	δ-Receptor K_i (nM)
6	4	2892	109
7	2555	>10,000	>10,000
8	236	>10,000	141
9	92	>10,000	229
10	547	>10,000	>10,000
11	46	2226	67
12	145	1368	95
13	28	1691	209
Leu-enkephalin	24	>10,000	31

[a]Values are the mean of three experiments; the variation between experiments was less than 10%.

8 and **9** have moderate binding to μ- and δ-receptors and no affinity to the κ-receptor. Analogue **10** shows low binding to the μ-receptor. None of the compounds had high receptor selectivity. When compared to Leu-enkephalin only analogue **6** has showed higher affinity at μ- and κ-receptors. As expected, all the modifications located toward the N-terminus lowered affinity. High affinity and selective peptide opioids are frequently based on constrained cyclic analogues of enkephalin, β-casomorphin, demorphin, or deltorphin I.[24–26] The tertiary amide bonds formed would be expected to have clear conformational preferences due to *cis*/*trans* behavior. This type of modification was found in an array of morphiceptin[27] analogues with characteristics ranging from high affinity and selectivity to total inactivity. Our compounds (except analogue **10**) exhibit *cis*/*trans* conformational equilibrium both on RP HPLC (ranging from broad to split peaks) and ^{13}C NMR. Comparison of the NMR spectra at rt and 80 °C showed that atoms neighboring the tertiary amide bond had double signals at rt, while when heated to 80 °C these signals converged to single lines.[22] We were interested in determining if there was any similarity in opioid activity between an imidazolidinone-based analogue **6** and its counterpart with N-methyl group(s) in the same position(s). We prepared three analogues **11**–**13** (Fig. 2) varying the location of *N*-methyl groups on the backbone toward the C-terminus. These were prepared by a combination of classical solid-phase peptide synthesis using *N*-methylated Boc protected amino acids and solid-phase *N*-methylation described elsewhere.[28] The compounds were purified, analyzed, and tested as described above. These three compounds show good binding affinity for the μ- and δ-receptors and low affinity to the κ-receptor. Their affinities for the μ-receptor were poorer than that of analogue **6** but the similarity to this analogue is clear.

The five-membered, ring-based moiety employed here was partly inspired by the pseudo-proline concept originally established by Mutter et al.[29] in order to disrupt the secondary structure of peptides, prevent peptide aggregation, and improve solubility. Our main goal was to influence bioactivity. The highest μ-receptor affinity was found for analogue **6** ($K_i = 4$ nM) which is higher than that of regular Leu-enkephalin ($K_i = 24$ nM). In this case, the C-terminal backbone constriction potentially yields a bioactive conformation which improves binding. We have also found comparable activity features for its *N*-methylated counterparts which suggest a similarity of both modifications. Analogues with an imidazolidinone ring in other positions (**8**–**10**) retained some opioid activity for the μ- and δ-receptors. All weak κ-active compounds (**6**, **11**–**13**) were modified on their C-terminus. In contrast, imidazolidinone modification of analogue **7** completely changes the original opioid character of the molecule, resulting in loss of activity. In summary, we have tested the imidazolidinone constraint for modulation of opioid activity on five analogues of Leu-enkephalin. The synthetic method presented enables the preparation of peptidomimetics for any peptide sequence by straightforward solid-phase techniques. The whole concept enables modification of physicochemical properties of peptides, alteration of their activity, and is a useful tool for structure–activity studies.

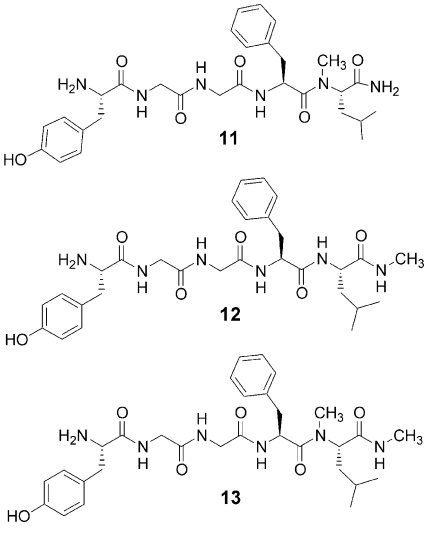


Figure 2. *N*-Methylated analogues of Leu-enkephalin.

Acknowledgements

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References and Notes

1. Adang, A. E. P.; Hermkens, P. H. H.; Linders, J. T. M.; Ottenheijm, H. C. J.; van Staveren, C. J. *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 63.
2. Pauletti, G. M.; Gangwar, S.; Siahaan, T. J.; Aube, J.; Borchardt, R. T. *Adv. Drug. Del. Rev.* **1997**, *27*, 235.
3. Falcioni, F.; Ito, K.; Vidovic, D.; Belunis, C.; Campbell, R.; Berthel, S. J.; Bolin, D. R.; Gillespie, P. B.; Huby, N.; Olson, G. L.; Sarabu, R.; Guenot, J.; Madison, V.; Hammer, J.; Sinigaglia, F.; Steinmetz, M.; Nagy, Z. A. *Nat. Biotechnol.* **1999**, *17*, 562.
4. Dooley, C. T.; Houghten, R. A. *Biopolymers (Peptide Sci.)* **1999**, *51*, 379.
5. Rinnova, M.; Hradilek, M.; Barinka, C.; Weber, J.; Soucek, M.; Vondrasek, J.; Klimkait, T.; Konvalinka, J. *Arch. Biochem. Biophys.* **2000**, *382*, 22.
6. Panetta, C. A.; Pesh-Imam, M. *J. Org. Chem.* **1972**, *37*, 302.
7. Hardy, P. M.; Samworth, D. J. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1954.
8. Polt, R.; Seebach, D. *Helv. Chim. Acta* **1987**, *70*, 1930.
9. Oliyai, R. *Adv. Drug Del. Rev.* **1996**, *19*, 275.
10. Bundgaard, H.; Johansen, M. *J. Pharm. Sci.* **1980**, *69*, 44.
11. Rasmussen, G. J.; Bundgaard, H. *Int. J. Pharm.* **1991**, *76*, 11.
12. Bak, A.; Fich, M.; Larsen, B. D.; Frokjaer, S.; Friis, G. J. *Eur. J. Pharm. Sci.* **1999**, *7*, 317.
13. Summers, M. C.; Lightman, S. L. *Biochem. Pharmacol.* **1981**, *30*, 1621.
14. Horvat, S.; Varga-Defterdarovic, L.; Roscic, M.; Horvat, J. *Chem. Commun.* **1998**, 1663.
15. Varga-Defterdarovic, L.; Vikic-Topic, D.; Horvat, S. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2829.

16. Pinza, M.; Farina, C.; Banfi, S.; Pfeiffer, U. US Patent 4,703,054, 1987. CAN 106; 138445.
17. Smissman, E. E.; Inloes, R. L.; El-Antably, S. *J. Med. Chem.* **1976**, *19*, 161.
18. Rinnova, M.; Vidal, A.; Nefzi, A.; Houghten, R. A. *J. Comb. Chem.* **2002**, *4*, 209.
19. Katritzky, A. R.; Lan, X.; Yang, J. Z.; Denisko, O. V. *Chem. Rev.* **1998**, *98*, 409.
20. Rinnova, M.; Nefzi, A.; Houghten, R. A. *Tetrahedron Lett.* **2002**, *43*, 2343.
21. Houghten, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 5131.
22. Analogue **6**: yield 72%; HR MS calcd 567.2931, found 567.2924; ^{13}C NMR (125 MHz, DMSO-d_6, value with prime is for the other *cis*/*trans* rotamer): δ 21.46, 22.41′, 23.33, 23.72, 36.19, 37.13, 38.43, 41.14, 41.36′, 41.90, 51.18, 51.57′, 53.73, 55.07, 56.78, 115.33, 124.77, 126.45, 126.72′, 128.13, 128.43′, 129.25, 129.30′, 130.44, 137.02, 128.43′, 129.25, 129.30′, 130.44, 137.02, 137.30′, 156.54, 168.12, 168.23′, 168.48, 168.54, 169.22, 171.96, 172.25 (aromatics at 80 °C: 115.05, 124.61, 126.06, 127.77, 128.73, 129.88, 136.77, 156.17). Analogue **7**: yield 74%; HR MS calcd. 567.2931, found 567.2915; ^{13}C NMR (125 MHz, DMSO-d_6, value with prime is for the other cis/*trans* rotamer): δ 21.23, 21.42′, 22.73, 22.88′, 23.23, 23.42′, 33.70, 36.25, 36.75, 37.14′, 40.80, 40.94′, 41.72, 51.16, 51.25′, 53.70, 58.96, 59.11′, 59.42, 115.36, 124.79, 126.58, 126.86′, 128.19, 128.23, 129.72, 129.83′, 130.50, 135.11, 135.60′, 156.56, 166.65, 167.41′, 168,20, 168.47, 168.57, 168.66′, 171.59 (aromatics at 80 °C: 115.05, 124.56, 126.63, 127.67, 129.29, 129.92, 156.17). Analogue **8**: yield 81%; HR MS calcd 567.2931, found 567.2946; ^{13}C NMR (125 MHz, DMSO-d_6, value with prime is for the other *cis*/*trans* rotamer): δ 21.49, 22.99, 24.27, 34.41, 36.34, 40.73, 40.98′, 42.66, 46.81, 51.07, 53.52, 53.88, 54.30, 60.35, 115.33, 124.68, 124.79′, 126.25, 126.61′, 128.02, 128.37′, 128.52, 128.73′, 129.21, 130.33, 130.51′, 137.08, 156.56, 166.08, 167.47, 168.47, 169.08, 173.67. Analogue **9**: yield 89%; HR MS calcd 567.2931, found 567.2959; ^{13}C NMR (125 MHz, DMSO-d_6, value with prime is for the other *cis*/*trans* rotamer): δ 21.57, 22.99, 24.20, 35.38, 36.23′, 37.46, 38.12′, 40.95, 42.78, 43.00, 46.96, 50.96, 52.12, 53.76, 53.89′, 115.35, 115.42′, 124.01, 124.15′, 126.26, 128.03, 129.20, 130.46, 130.53′, 137.69, 156.55, 156.74′, 166.42, 166.50′, 168.27, 168.42, 168.58′, 170.50, 170.72′, 173.84. Analogue **10**: yield 70%; HR MS calcd 567.2931, found 567.2943; ^{13}C NMR (125 MHz, DMSO-d_6): δ 21.59, 23.00, 24.19, 37.41, 40.88, 42.30, 43.52, 50.95, 53.97, 115.19, 126.25, 128.02, 129.16, 130.07, 137.72, 156.20, 167.14, 168.39, 170.64, 173.86.
23. Dooley, C. T.; Ny, P.; Bidlack, J. M.; Houghten, R. A. *J. Biol. Chem.* **1998**, *273*, 18848.
24. Hruby, V. J.; Bartosz-Bechowski, H.; Davis, P.; Slaninova, J.; Zalewska, T.; Stropova, D.; Porreca, F.; Yamamura, H. I. *J. Med. Chem.* **1997**, *40*, 3957.
25. Shreder, K.; Zhang, L.; Dang, T.; Yaksh, T. L.; Umeno, H.; DeHaven, R.; Daubert, J.; Goodman, M. *J. Med. Chem.* **1998**, *41*, 2631.
26. Alfaro-Lopez, J.; Okayama, T.; Hosohata, K.; Davis, P.; Porreca, F.; Yamamura, H. I.; Hruby, V. J. *J. Med. Chem.* **1999**, *42*, 5359.
27. Yamazaki, T.; Ro, S.; Goodman, M.; Chung, N. N.; Schiller, P. W. *J. Med. Chem.* **1993**, *36*, 708.
28. Dorner, B.; Husar, G. M.; Ostresh, J. M.; Houghten, R. A. *Bioorg. Med. Chem.* **1996**, *4*, 709.
29. Wohr, T.; Wahl, F.; Nefzi, A.; Rohwedder, B.; Sato, T.; Sun, X.; Mutter, M. *J. Am. Chem. Soc.* **1996**, *118*, 9218.