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Formylated polyamines as peptidomimetics

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ABSTRACT

A new construct for imitating a natural peptide ligand using a modified retro-inverso sequence is described. It is demonstrated through the synthesis of a peptidomimetic derived from the endogenous sequence of leucine enkephalin. The product was active at 400 nM and selective for μ-opioid receptors.

Peptides mediate a wide variety of biological processes and represent a major source of inspiration in medicinal chemistry.[1] The specific sequences involved in many relevant biochemical pathways are known[2] and enormous libraries are readily available through combinatorial synthesis. However, their limited stability in vivo and unfavorable pharmacokinetics resulting from their high polarity, preclude peptides as medicinal candidates per se.[3] Rather, small hydrophobic ligands, often with seemingly unrelated chemical structures, are generally preferred medicines.[4] The retro-inverso (RI) strategy[5]—mimicking the natural L-peptide by using the D-peptide of the reverse sequence—leads to a structurally related peptidomimetic with increased metabolic stability and the correct presentation of side chains (Fig 1). Even so, these mimetics do not show improved cell membrane penetration. They also fail to present the correct hydrogen bond donor (HBD) and acceptor (HBA) pattern of the peptide backbone, which can be involved in recognition by the target receptor or enzyme (Fig. 1). We report here modified structures that overcome these limitations.

The new peptidomimetics are formylated polyamines (FPA). FPAs are structurally related to RI peptides but feature backbones that resemble those of the original peptide (Fig. 1). They present the correct HBA–HBD polyamide pattern by incorporating formamides as homologous carbonyl groups and methylenes to replace the N–H groups. In peptides, such N→C substitutions lead to ketomethylene peptide isosteres and have been used in numerous peptidomimetic applications.[6] The replacement of secondary peptide bonds by the more lipophilic tertiary amides decreases the polarity[7] and improves the pharmacokinetic properties. The reduced number of HBD's[8] compared with the parent peptide is expected to improve membrane permeability.[9]

To illustrate, we perpared the FPA (Scheme 1) of the sequence for leucine enkephalin (LE).[10] LE is a potent endogenous opioid receptor agonist[11,12] involved in the modulation of the perception of pain and of considerable medicinal interest. The poor cell penetration and high susceptibility to enzyme degradation render exogenous LE essentially devoid of practical biological activity in vivo.[13]

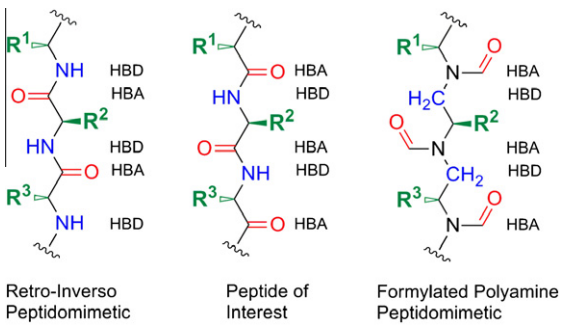

Figure 1. Structural features of peptides, retro-inverso mimics and the new formylated polyamines.

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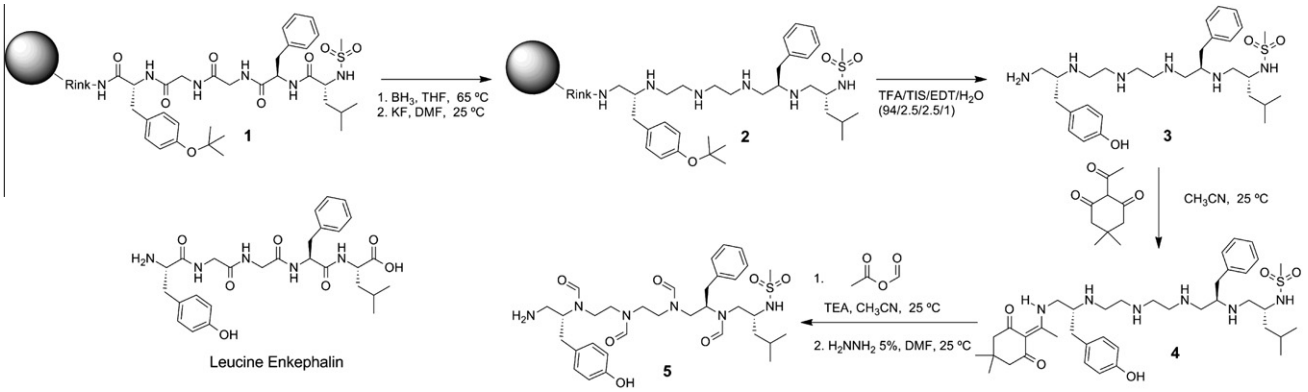

Scheme 1. Synthesis of the FPA peptidomimetic **5** from the resin-bound RI peptide **1** and the structure of leucine enkephalin peptide.

The D-peptide precursor for the synthesis was the RI peptide **1**, obtained by classical solid-phase peptide synthesis on Rink-amide resin followed by N-terminus modification using methanesulfonyl chloride (Scheme 1). The terminal sulfonamide represents the *C*-terminal carboxylic acid of LE as a bioisostere.[14] The wholesale reduction of the backbone amides on-bead[15] produced the polyamine **2**. A similar approach was successfully applied to the construction and decoding of a small on-bead library.[16] Structurally related polyamines showed in vitro activity for opioid receptors[17] and more recently, they were also considered for DNA and RNA gene delivery systems.[18] Compound **2** was cleaved from the resin with acidic treatment in 42% overall yield.[19] A terminal amine is an essential pharmacophore in LE.[20] Accordingly, the primary amine was selectively protected using 2-acetyldimedone.[21] Next, formylation of the remaining secondary amines of **4** was accomplished with distilled acetic formic anhydride.[22] The FPA **5** was obtained after primary amine deprotection using hydrazine and purified by RP-HPLC.

Compound **5** was found reasonably stable to degradation in human serum, showing a half-life of 41 h. Compound **5** also inhibited radioligand ([3H]DAMGO) binding to recombinant human μ-opioid receptors with a K_i value of about 400 nM (Fig. 2).[23] However, the K_i values for displacement of radioligands from the recombinant human κ ([3H]DPDPE) and δ ([3H]U69,593) opioid receptors were greater than 5 μM. In addition, Compound **5** did not alter [^{35}S]GTPγS binding, indicating that it is not an agonist at μ-opioid receptors. Recently, an analogous acylation of secondary amines was used as a convenient means of introducing additional side chains into the structure of a peptidomimetic that was found to inhibit protein-protein interactions.[24] However, in the case of FPAs, the formamides are only responsible for restoring the hydrogen bond features and improve the cell permeation. The side chains come from the RI peptide allowing for a more straightforward structure-derived approach.

In conclusion, the RI concept was modified to improve bioavailability and enhance molecular recognition features[25] of a leucine enkephalin mimic. While the amino acid side chains involved in this case were tolerant of amide reduction, the ready availability of amino alcohols as starting materials opens possibilities for synthesis of formylated polyamines corresponding to most naturally-occurring sequences.

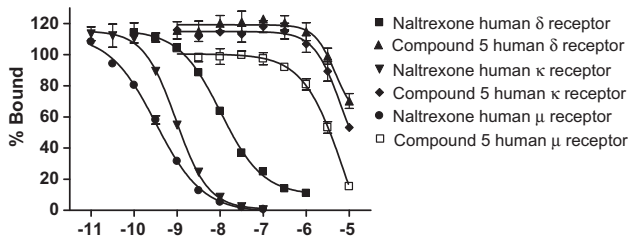

Figure 2. Biological activities with opioid receptors. The average IC_{50} measured for **5** with the human μ-opioid receptor was 3.2 μM corresponding to a K_i of 400 nM.[23]

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.09.008.

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