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DESIGN OF A HIGH AFFINITY PEPTIDOMIMETIC OPIOID AGONIST FROM PEPTIDE PHARMACOPHORE MODELS

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Abstract: A pair of diastereomeric peptidomimetics based upon opioid receptor-binding pharmacophore models derived for a series of opioid tetrapeptides was synthesized. Both analogues display high opioid receptor affinity, moderate selectivity for the μ opioid receptor, and are potent, full agonists.

We have previously proposed models for the δ and μ opioid receptor binding conformations of the high affinity tetrapeptide Tyr-c[*D*-Cys-Phe-*D*-Pen]OH (JOM-13, Pen, penicillamine is β,β-dimethylcysteine)[1] and related analogues based on experimental and theoretical conformational analysis of these peptides and correlations of conformational preferences of further conformationally restricted analogues in this series with their receptor binding affinities.[2-7] In these pharmacophore models the relative orientation of the exocyclic tyrosine residue and the phenylalanine side chain, both of which are key binding elements, is critical for high affinity binding and distinguishes analogues with binding selectivity for μ vs. δ opioid receptors. We describe here the design, synthesis, and in vitro pharmacological assessment of a high affinity/high potency peptidomimetic based upon these pharmacophore models.

The goal of our peptidomimetic design was a simplified, synthetically straightforward structure that maintains the key opioid binding elements (i.e. a tyramine group and a benzyl side chain) in an orientation similar to that in JOM-13, but which eliminates the 11-membered, disulfide-containing scaffold found in the peptide. This goal was accomplished via the synthesis, outlined in Scheme 1, of 4-amino-6-benzyl-1, 2, 3, 4-tetrahydroquinoline, **6**, which functions as a dipeptide mimic of the *D*-Cys-Phe fragment of JOM-13. Unlike most dipeptide mimics, **6** does not contain both amine and carboxylic acid functions and thus does not serve as an "interior" dipeptide mimic, but rather replaces the entire C-terminal tripeptide fragment of JOM-13, including the *D*-Pen residue, a component of the peptide scaffold. Most importantly, the dipeptide mimic **6** is designed to retain the critical benzyl side chain of the Phe[3] residue of JOM-13, rather than serving as merely a peptide backbone replacement. Dipeptide mimic **6** was synthesized as a racemic mixture and coupled with 2',6'-dimethyl-*L*-tyrosine (DMT) to yield the diasteromeric peptidomimetic compounds **7a** and **7b**. DMT was chosen, rather than *L*-tyrosine, because of its demonstrated ability to improve binding affinity/pharmacological potency in opioid peptides.[8] As shown in Figure 1, both peptidomimetic diastereomers allow excellent superpositioning of the tyramine moiety and benzyl aromatic ring with the corresponding components of JOM-13, however the 4*R* analogue, which is the stereochemical equivalent of the *D*-Cys[2] of JOM-13, allows a better overlap of the corresponding scaffolds of the peptidomimetic and the peptide.

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Scheme 1. Synthesis of Tyrosylamido-6-benzyl-1, 2, 3, 4-tetrahydroquinoline

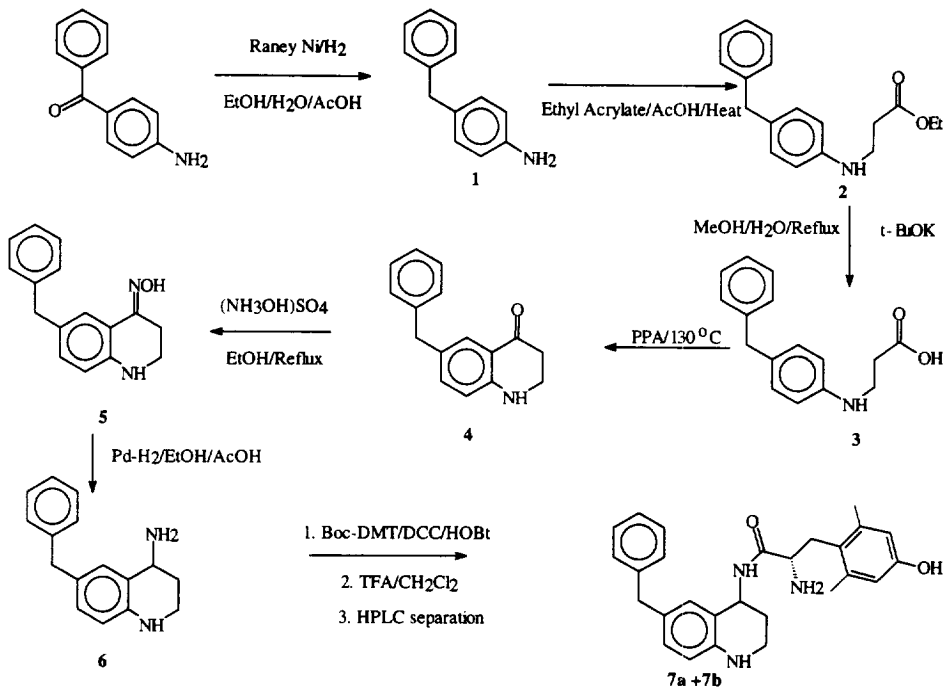
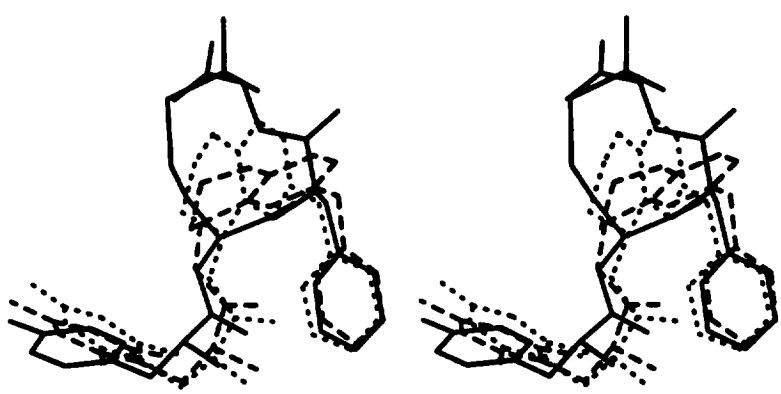


Figure 1. Superposition (stereo) of JOM-5 (solid line) with the 4*R* (dotted line) and 4*S* (dashed line) isomers of **7**



Opioid receptor binding data[9] for the peptidomimetics **7a** and **7b** are shown (± standard error of the mean) in Table 1 and are compared with the corresponding data for JOM-13 and for the related peptide JOM-5 (Tyr-c[*D*-Cys-Phe-*D*-Pen]NH_2), which differs from JOM-13 only in its C-terminus, JOM-5 substituting a carboxamide for the carboxylic acid of JOM-13. Both **7a** and **7b** display high affinity and modest selectivity

Table 1. Opioid receptor binding affinities and agonist potencies of peptidomimetics

Compound	K_i (δ) (nM)	K_i (μ) (nM)	K_i (κ) (nM)	[^{35}S]GTPγS stimulation EC_{50} (nM)
7a	9.35 ± 0.75	0.22 ± 0.02	68.0 ± 1.9	0.18 ± 0.04
7b	56.0 ± 4.5	2.62 ± 0.31	222 ± 48	16.4 ± 1.1
JOM-13	0.74 ± 0.08	51.5 ± 4.4	>10,000	-----
JOM-5	57.7 ± 4.7	7.01 ± 0.45	>10,000	18.2 ± 3.0
morphine	710 ± 116	6.51 ± 1.85	110 ± 22	21.3 ± 1.9
fentanyl	679 ± 25.5	8.45 ± 1.05	1130 ± 470	32.4 ± 2.8

for the μ type of opioid receptor, with **7a** exhibiting approximately an order of magnitude higher affinity than **7b**. By contrast, JOM-13 binds preferentially to δ opioid receptors. This selectivity difference can be attributed to the effect of the C-terminal carboxylic acid moiety, which generally enhances δ and diminishes μ receptor binding affinity in opioid peptides.[10] This is clearly seen in the corresponding data in Table 1 for JOM-5, which displays a similar μ vs. δ receptor binding selectivity profile as **7a** and **7b**. As shown in Table 1, binding to κ opioid receptors was weak relative to the preferred receptor for each ligand.

Opioid receptors are members of the G protein-coupled receptor family and a hallmark property of agonist ligands of receptors in this family is the ability to stimulate the binding of GTP to the receptor-linked G protein. Consequently, the peptidomimetics **7a** and **7b** as well as the peptide JOM-5 were examined for the ability to stimulate [^{35}S]GTPγS (a nonhydrolyzable GTP analogue) binding to membranes from C6 glioma cells stably expressing rat μ opioid receptors.[11] All three ligands effected stimulation of [^{35}S]GTPγS binding equivalent to the maximal stimulation caused by the standard μ agonist fentanyl and hence are full agonists. Ligand potencies in this assay are reported in Table 1 as EC_{50} values (± s.e.m.). As can be seen from the Table, agonist potencies of the ligands are consistent with the observed binding affinities of the ligands for μ receptors. The very high potency of **7a**, which is approximately two orders of magnitude higher than **7b**, JOM-5, or the prototypical μ ligands morphine and fentanyl, is particularly noteworthy. The high binding affinity and high potency of **7a** suggest that **7a** incorporates the 4*R* isomer of **6**, which, as noted above, corresponds to the stereochemistry found in the parent tetrapeptide series. This assignment, of course, is only tentative.

The agonist behavior displayed by **7a** and **7b** is an important criterion for judging the success of the peptidomimetic design since the molecular recognition requirements of an agonist, which must bind to the receptor in a manner similar to the native ligand for the receptor, are more stringent than those for an antagonist, which need only serve as a competitive inhibitor of agonist binding. The results described here can also be taken as evidence in support of the pharmacophore models proposed for the tetrapeptides, themselves, and represent a successful demonstration of rational ligand design.

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