

Addressing the Problem of Prolonged and Neuropathic Pain with Multivalent Ligands That Treat the Disease State

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Introduction

The treatment of acute pain with opiates, nonsteroidal anti-inflammatory drugs (NSAIDs), COX inhibitors, etc. often works quite well despite the inherent toxicities for each of these treatment modalities. On the other hand, the treatment of prolonged pain and especially neuropathic pain is much more problematic due to numerous "complications." Indeed, current treatments generally only modulate the pain, and generally tolerance occurs [1] requiring increased doses leading to severe toxicities. In the case of opioids prolonged treatment often results in more pain. The mechanisms for these effects are still unclear. Nonetheless it is clear that prolonged pain states lead to neuroplastic changes in both ascending and descending pathways in the spinal column in which there is increased release of neurotransmitters (e.g. substance P) that enhance pain and increased expression of receptors for those new released pain-causing neurotransmitters. Not surprising current treatments often do not work. To address these problems we have taken a new approach to drug design for prolonged pain and neuropathic pain [2,3]. In this approach we are designing multivalent ligands (ligand specifically designed to have multiple biological activities) that act as agonists at mu and delta opioid receptors and as antagonists at biological receptors to block one of

these newly expressed pain-causing ligands. These new ligands will be expected to have synergistic biological activities and possible overlapping pharmacophores.

Results and Discussion

In this study we have designed ligands that have potent "balanced" mu and delta opioid agonist activity (which greatly reduces toxicities associated with mu opioid ligands) and selective neurokinin-1 (NK-1) receptor antagonist activity. Our basic design is shown in Figure 1.

Neurokinin-1 pharmacophore
H-Tyr-D-Ala-Gly-Phe-Xaa-Pro-Leu-Trp-O-3',5'-(CF₃)₂-Bn Opioid pharmacophore

Fig. 1. Structure designed to be a delta/mu agonist and NK-1 antagonist.

A list of several of the peptides synthesized is given in Table 1 along with some of their measured *in vitro* biological activities. Additional *in vivo* assays need to be run in order to examine selectivity and specificity of action including efficacy.

Table 1. Structure and selected *in vitro* binding affinity and bioassays for bivalent ligands

Structure	Affinity (nM)			NK1 GPI (nM)	
	rNK1	hDOR	rMOR	Agonist	Antagonist
1. H-Tyr-D-Ala-Gly-Phe-Phe-Pro-Leu-Xaa	0.88	15	28	0% ^a	14
2. H-Tyr-D-Ala-Gly-Phe-Met-Pro-Leu-Xaa	29	2.8	36	0% ^a	25
3. H-Tyr-D-Ala-Gly-Phe-Met-Pro-Leu-Yaa	7.3	0.64	16	0%	10
4. H-Tyr-D-Ala-Gly-Phe-Met-Pro-Leu-Zaa	700	0.44	1.8	0%	9.9
5. H-Tyr-c[D-Cys-Gly-Phe-DCys]-Pro-Leu-Yaa	45	7.8	52	0%	4.7

Xaa=Trp-0.3',5'-Bn(CF₃)₂; ^a at 1 μM; Yaa=Trp-NH-3',5'-Bn(CF₃)₂; Zaa=Trp-NH-Bn

The results obtained are very promising. For the opioid pharmacophore we chose an enkephalin related fragment which was incorporated into the bivalent structure biphalin, which has been shown to be a mixed mu/delta agonist ligand and more importantly to have high synergistic antinociceptive potency [4], to be metabolically quite stable [5], and to show only very minor amounts or none of the toxic side effects of opioid mu ligands and to show minimal tolerance [4]. For the NK-1 receptor antagonist pharmacophore we chose the tripeptide peptidomimetic $-\text{Pro-Leu-Trp-O-3',5'-Bn}(\text{CF}_3)_2$ [5] which has been shown to be a potent and selective neurokinin-1 (NK-1) receptor antagonist [6]. In addition an amino acid residue has added between the two pharmacophores which is added as an address moiety to enhance the potency at all three receptor (mu, delta, and NK-1) without affecting the overall biological activities. All of the compounds were synthesized by a combination of solid phase and solution phase synthesis, using appropriate orthogonal protecting group schemes for the synthesis [7].

Examination of the *in vitro* binding and biological activity assays demonstrate that our designed ligands have mixed and potent mu and delta opioid receptor affinity generally with weak selectivity for the delta opioid receptor. All were demonstrated to be agonists at both the mu and delta opioid receptors (data not shown) using the GTP(γ)S assay for measuring agonist activity. The ester compound **2** was shown to have very short half lives in serum so we converted **2** into the 3',5'-Bn(CF₃)₂ amides in **3** or to the simple benzyl amide **4** (Table 1). Interestingly these amides were even more potent than the esters in binding to the mu and delta opioid receptors and **4** was a picomolar binder at the delta opioid receptor. Though **4** lost considerable binding affinity for the rat NK-1 receptor it showed good antagonist potency in the guinea pig ileum. Subsequently we have shown that **4** has over 100 fold greater binding affinity for the human NK-1 receptor (data not shown). Finally we also have made cyclic analogues (**5**, Table 1) of our lead compounds and it also was shown to have high potency at all three receptors with agonist activity at delta and mu opioid receptors and antagonist activity at neurokinin-1 receptors. Stability studies indicated that the amides have $t_{1/2S}$ in serum of 5 to 6 hours whereas the cyclic amides have serum half lives greater than 20 hours (data not shown).

Finally, though these compounds have only modest antinociceptive activities in acute models of pain, in preliminary studies we have shown these compounds have potent bioactivity in models of prolonged and neuropathic pain consistent with our design

hypothesis. Further *in vivo* studies are needed to examine the level of potency and the effect of prolonged stability in serum on the duration of the antinociception activity of these compounds on neuropathic pain.

In summary, we have designed, synthesized, and evaluated *in vitro* and *in vivo* short multivalent peptidomimetics that have agonist activities at mu and delta opioid receptors and antagonist activities at neurokinin-1 receptors, and examined their binding affinities and *in vitro* and *in vivo* biological activities. Ligands with nanomolar and even picomolar binding affinities, and potent *in vitro* and *in vivo* biological activities with the proposed biological activity profiles have been obtained. These compounds have great potential for the treatment prolonged and neuropathic pain which currently have no effective treatment modalities.

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