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Truncation of the peptide sequence in bifunctional ligands with mu and delta opioid receptor agonist and neurokinin 1 receptor antagonist activities

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Abstract

The optimization and truncation of our lead peptide-derived ligand TY005 possessing eight amino-acid residues was performed. Among the synthesized derivatives, NP30 (Tyr[1]-*D*Ala[2]-Gly[3]-Phe[4]-Gly[5]-Trp[6]-O-[3′,5′-Bzl(CF_3)$_2$]) showed balanced and potent opioid agonist as well as substance P antagonist activities in isolated tissue-based assays, together with significant antinociceptive and antiallodynic activities *in vivo*.

Keywords

bifunctional compounds; opioid receptor agonists; neutokinin-1 receptor antagonists; Truncation of peptide sequence; NMR structure

The clinical treatment of pain, especially prolonged and neuropathic pain is still a major challenge. Current analgesic drugs, such as opioid drugs are widely used following major surgery and controlling the pain of terminal diseases such as cancer, but their use is limited by several undesired side effects, including the development of tolerance and physical dependence. The mechanisms for these side effects are still largely unclear, but it is clear that prolonged pain as well as sustained opioid administration develops neuroplastic changes in the central nerve system (CNS) in which pain-enhancing neurotransmitters, such as substance P, and their corresponding receptors are up-regulated to lead to more pain and tolerance[1-3]. Current treatment of prolonged and/or neuropathic pain generally can only modulate pain, and cannot counteract against these induced neuroplastic changes. Thus, it is

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not surprising that current analgesic drugs do not work well in these pathological conditions.[1]

In order to address these problems, we are working at a new approach in which the opioid agonist and neurokinin 1 (NK1) antagonist activities were combined into one ligand, to neutralize the induced neuroplastic changes. The desirable pharmacological activities of our ligand would include potent analgesic affects in both acute pain and in neuropathic pain states without the development of tolerance. In fact, our lead compound TY005 (**1**: Tyr^{1}-*D*Ala^{2}-Gly^{3}-Phe^{4}-Met^{5}-Pro^{6}-Leu^{7}-Trp^{8}-O-[3′,5′-Bzl$(CF_3)_2$]) has been shown to reverse neuropathic pain in a rodent model, no sign of opioid-induce tolerance, and no development of reward liability, validating our hypothesis that a single compound possessing opioid agonist/NK1 antagonist activities could be an effective treatment against neuropathic pain.[2a] The designed multivalent chimeric molecules also have simple metabolic and pharmacokinetic properties compared to a cocktail of individual drugs for easy administration, a simple ADME property and no drug-drug interaction.

As previously reported, our drug-design strategy is based on the overlapping pharmacophores concept, in which the opioid agonist pharmacophore is incorporated at the N-terminus and the NK1 antagonist pharmacophore locates at the C-terminus of a single peptide-derived molecule (Fig 1). [3] The opioid pharmacophore of these chimeric peptides were designed based on the sequence of biphalin and DADLE,[3,4] while the structures from 3′,5′-(bistrifluoromethyl)-benzyl ester of N-acylated tryptophans was modified into the NK1 antagonist pharmacophore[5]. These two pharmacophores could be divided into “address” and “message” regions. Based on the previous structure-activity relationship (SAR) studies in our bifunctional ligands,[3] opioid agonist pharmacophore works as a message region for NK1 antagonist activity, and vise versa, implying that **1** has four-residues (Met-Pro-Leu-Trp-O-[3′,5′-Bzl$(CF_3)_2$]) message region for opioid activity and seven-residues (Tyr-*D*Ala-Gly-Phe-Met-Pro-Leu) message sequence for NK1 pharmacophore with Met^{5}-Pro^{6}-Leu^{7} overlapping for both of them (Fig. 1).

In this article, the contraction of the peptide sequence of **1** in its address regions was examined. The changes in their activities and selectivities were observed and discussed, and analgesic activity of a contracted peptide derivative was confirmed *in vivo*. It should be stressed that the contracted peptides have several advantages over longer peptides for easier synthesis, lower preparative cost, and being a better template to be orally-available as small molecule peptide mimetics.[3e,6]

We initiated this research with the optimization of **1** in the Met^{5}-Pro^{6}-Leu^{7} sequence, which is the address region for both pharmacophores. Since the importance of Met^{5} and Leu^{7} were already validated especially for opioid activities,[3] the Pro^{6} was modified into Ala (**2**) (Table 1). The ligand **2** showed reduced affinities at both human delta opioid receptor (DOR) and rat mu opioid receptor (MOR), and its functional activities as a mu opioid agonist were also reduced compared to those of **1** (EC_{50} in GTPγS binding assay, 72 nM; IC_{50} in GPI assay, 1200 nM). The binding affinity of **2** at the rat NK1 receptor (rNK1) was also reduced. **3, 4** and **5**, possessing CLeu, Aib and *D* Pro at the sixth position, respectively, have the same trend in the binding affinities and functional activities: lower binding affinities at DOR, MOR and rNK1 receptors, and decreased functional activity in MVD and GPI assays, compared to those in **1**. These results combined with our previous SAR clearly suggested that the Met^{5}-Pro^{6}-Leu^{7} is a crucial sequence for both opioid and NK1 activities in **1**. In fact, ligand **6**, which possesses a seven aminoacid sequence with only a missing Met^{5} compared to the sequence of **1**, displayed reduced binding affinities for DOR and MOR, as well as for all the functional activities in the GTPγS binding and the isolated tissue-based assays.

However, the opioid affinities and activities were rather improved in ligands with six amino-acid residues. Interestingly, compound **7** with Met in between the two message regions showed higher affinities for both DOR and MOR than those in **1** (IC_{50} = 2.9 and 23, respectively). The EC_{50} values of **7** in the GTPγS binding assays were 4.8 and 14 nM at DOR and MOR, respectively, consistent with its binding affinities. The delta opioid agonist activity in the MVD assay was at the similar level compared to **1** (26 nM), while its mu opioid agonist activity was improved (IC_{50} value in GPI assay was 45 nM). The binding affinities of **7** at the human and rNK1 receptors were nearly equivalent to those of **1**. **8** has Leu in its fifth position and showed superior affinities in the radioligand binding assays and functional activities in the GTPγS binding assays at both DOR and MOR. **8** also showed two-fold higher IC_{50} value in the MVD assay, but the IC_{50} value in the GPI assay for mu opioid agonist activity was comparable to that of **1**. The binding affinities and substance P antagonist activity in the GPI assay were improved from those of **1**. While ligand **9** possessing Pro^6 showed lower binding affinities and functional activities for delta opioid agonist (IC_{50} = 5.7 nM, EC_{50} = 76 nM, IC_{50} = 85 nM for radioligand binding assay, GTPγS binding assay and MVD assay, respectively). The IC_{50} value of **9** at hNK1 was nearly equivalent to that of **1**, but its K_i value for rNK1 was 5-fold decreased from **1**.

It should be noted that these three contracted chimeric peptides **7**-**9** displayed delta selectivity (2.6 to 55-fold) in their opioid agonist activities, but compound **10** with Gly^6 showed 16 fold selectivity for the mu receptor opioid. This mu-selectivity of **10** was maintained in the GTPγS binding assays, but the isolated tissue-based assays displayed negligible selectivity. The higher E_{max} value at DOR (87%) than at MOR (36%) could have some effect on this shift in the delta/mu opioid selectivity. **10** showed 14-fold higher K_i value at hNK1 receptor than that of **1**, but its K_i value at the rNK1 was decreased. The IC_{50} value in the GPI assay against substance P stimulation was 59 nM, which is within three-fold difference from the IC_{50} values in MVD and GPI assays for opioid agonist activities. These results in the isolated tissue-based assays imply that ligand **10** could work as a well-balanced and potent delta and mu opioid agonist as well as a substance P antagonist *in vivo*. This potent and balanced trio of activities motivated us to perform *in vivo* animal studies using compound **10**, to confirm its *in vivo* analgesic efficacy.

Compound **10** was evaluated for *in vivo* antinociceptive activity in non-injured rats following spinal administration. Rats treated with **10** (10μg/5μL**)** withdrew the hind paw from a radiant heat source (Hargreaves) at significantly longer latencies than those treated with vehicle 15 min after compound injection ($p<0.05$) (Fig. 2). Vehicle was 10% dimethyl sulfoxide (DMSO) in 90% distilled water. Next, **10** was evaluated for antiallodynic activity (von Frey) after spinal administration in spinal nerve ligated (SNL) rats. Following intrathecal injection, **10** induced significant attenuation of SNL induced tactile allodynia at 3, 30, and 30μg/5μL when compared to vehicle ($p < 0.05$). The duration of action of compound **10** was dose-dependent. However, no significant reversal of SNL induced tactile or thermal hypersensitivity was observed following oral administration of **10** compared to vehicle, indicating its limited oral bioavailability (data not shown). These *in vivo* experiments were performed as previously reported.[2a]

In this report, we performed optimization for truncation of our lead peptide-derived ligand **1** in Met^5-Pro^6-Leu^7, which is a address sequence for both opioid agonist and NK1 antagonist pharmacophores. Among the synthesized derivatives, **10**, contracted to six amino acid residues, showed mu-selective opioid and effective NK1 antagonist affinities, together with balanced and potent opioid agonist as well as substance P antagonist activities in isolated tissue-based assays. Moreover, **10** showed significant antinociceptive and antiallodynic activities *in vivo*. Since the truncated peptide is a good template to design novel peptide-mimetic small molecules, these results indicate that compound **10** could be considered as an important research tool to develop a novel analgesic drugs, possibly with oral bioavailability.

Acknowledgments

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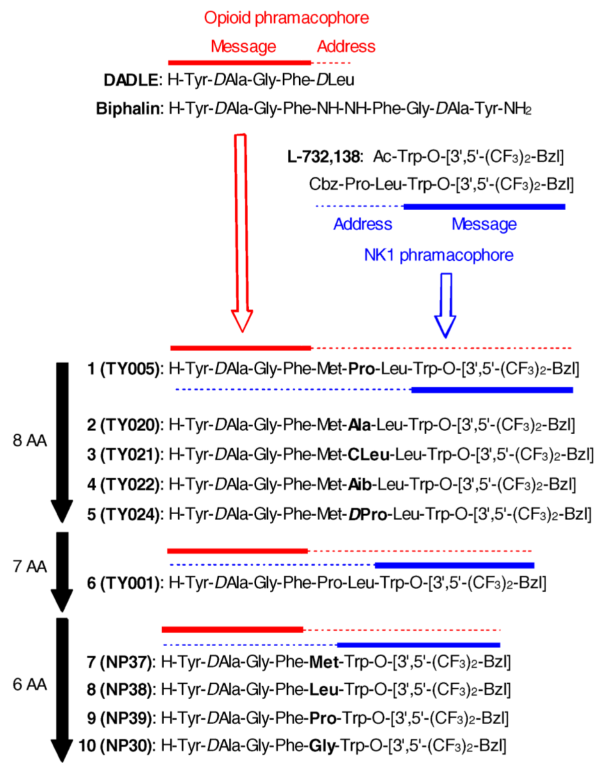

Figure 1.
Sequences of bifunctional ligands **1-10**.

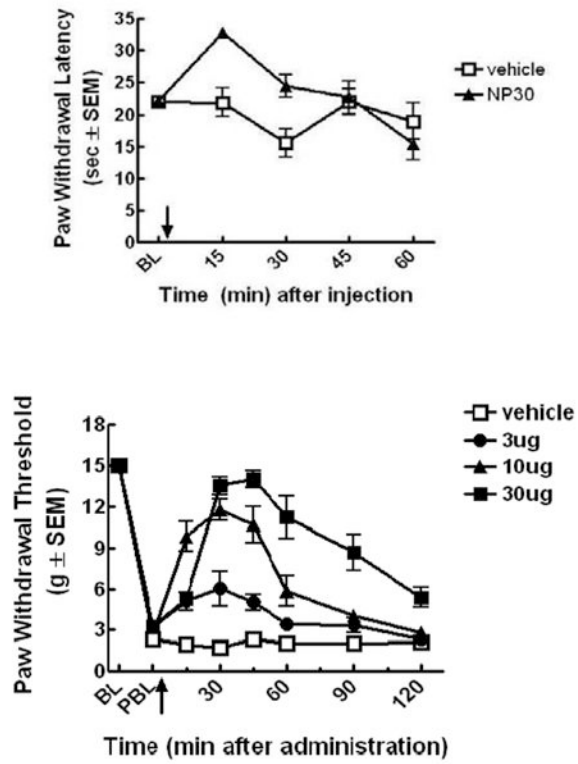

Figure 2.

Antinociceptive (top) and antiallodynic (bottom) effect of bifunctional peptide derivative **10** after intrathecal injection. Arrow indicated the time of injection. PBL means post-spinal nerve ligated baseline.

Table 1

Binding affinities and functional activities of bifunctional peptide derivatives at δ/μ opioid receptors and NK1 receptors

	Opioid agonist activities									NK1 antagonist activities			
	radioligand binding assays			$[^{35}S]$ GTPγS binding assays				MVD (δ)	GPI (μ)	radioligand binding assays			GPI
	hDOR[a,b]	rMOR[a,c]		hDOR[a]		rMOR[a]		Opioid agonist		hNK1[d,e]	rNK1[d,f]		Substance P antagonist
no	K_i (nM)[g]	K_i (nM)[g]	$K_i(\mu)/K_i(\delta)$	EC_{50} (nM)[h]	E_{max} (%)[i]	EC_{50} (nM)[h]	E_{max} (%)[i]	IC_{50} (nM)[j]	IC_{50} (nM)[j]	K_i (nM)[g]	K_i (nM)[g]	$K_i(hNK1)/K_i(rNK1)$	Ke (nM)[k]
1[l]	2.8	36	13	2.9	45	32	42	22	360	0.082	0.29	3.5	25
2	25	150	6.0	2.5	26	72	51	12	1200	0.034	1.1	32	380
3	5.0	110	22	0.26	41	54	52	5.1	6.8%@1μM	0.066	7.8	118	2.0
4	3.1	63	20	5.5	28	100	27	6.9	42%@1μM	0.12	9.8	82	12
5	13	76	5.8	190	25	120	30	8.5	25%@1μM	0.88	3.6	4.1	29
6[m]	50	180	3.6	35	16	140	26	400	520	0.0023	1.6	696	3.6
7	2.9	23	7.9	4.8	60	14	54	26	45	0.09	0.29	3.2	Not tested
8	0.6	33	55	1.1	36	0.80	51	9.1	390	0.0090	0.089	9.9	2.0
9	5.7	15	2.6	76	56	37	47	85	260	0.097	1.6	16	1.8
10	4.7	0.29	0.062	27	87	10	36	21	26	0.0057	4.2	737	59
Biphalin[n]	2.6	1.4	0.54	1.1	83			2.7	8.8				
DAMGO						37	150						
L-732,138										0.73	130	180	250

[a]Competition analyses were carried out using membrane preparations from transfected HN9.10 cells that constitutively expressed the DOR and MOR. See reference 3 for detailed procedures.

[b]$[^3H]$DPDPE; $K_d = 0.45 \pm 0.1$ nM.

[c]$[^3H]$DAMGO; $K_d = 0.50 \pm 0.1$ nM.

[d]Competition analyses were carried out using membrane preparations from transfected CHO cells that constitutively expressed rat or human NK1 receptors. See reference 3 for detailed procedures.

[e]$[^3H]$Substance P; $K_d = 0.16 \pm 0.03$ nM.

[f]$[^3H]$ Substance P; $K_d = 0.40 \pm 0.17$ nM.

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[g] The data were collected from at least two independent experiments performed in duplicate. The K_i values are calculated using the Cheng and Prusoff equation to correct for the concentration of the radioligand used in the assay. See reference 3 for detailed procedures.

[h] The EC_{50} values were determined from the non-linear regression analysis of data collected from at least two independent experiments performed in duplicate. See reference 3 for detailed procedures.

[i] [Total bound − Basal]/[Basal − Non-specific] × 100.

[j] Concentration at 50% inhibition of muscle contraction at electrically stimulated isolated tissues (n = 4).

[k] Inhibitory activity against the Substance P induced muscle contraction in the presence of 1 μM naloxone, Ke: concentration of antagonist needed to inhibit Substance P to half its activity (n = 4). See reference 3 for detailed procedures.

[l] Reference 3a.

[m] Reference 3b.

[n] Reference 7.