

Effects of Methyl and Halogens in Analgesic Peptide Molecules for Their Potent Agonist Activities at MOR/DOR and Antagonist Activity at NK1R

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Introduction

Treating pain has been always challenging, especially when it becomes chronic in nature. Current drugs (e.g. opioid drugs) cannot treat this problem effectively. In addition, constant use of these drugs has deadly side effects including drowsiness and mental clouding, nausea and emesis, and constipation [1]. Development of analgesic tolerance and hyperalgesia in many patients as the results of taking these drugs are also a serious concern. Overexpression of substance P and its receptor has been observed during prolonged pain states. Studies revealed that co-administration of cocktails of drugs containing μ/δ opioid agonist and NK1 antagonist can provide better analgesic effects in rat model while reducing side effects [2-4]. Herein, we report design, synthesis and SARs of few multifunctional ligands having μ/δ opioid agonist (μ -preferring) and NK1 antagonist activities. The detail of this study has already been communicated to *J. Med. Chem.* for publication [5]. We anticipate that these ligands will show better analgesia while reducing the adverse side effects. Our drug design principle is based on overlapping and adjacent pharmacophores (Figure 1) and we considered our previously published μ -selective (binding) ligand **TY012** (H-Tyr-D-Ala-Gly-Phe-Pro-Leu-Trp-NH-Bn(3',5'-(CF₃)₂)) [6].

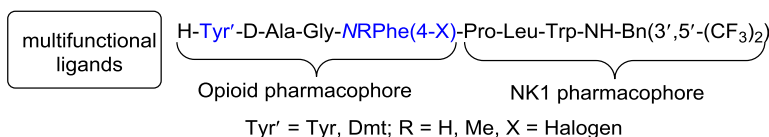


Fig. 1. Design of multifunctional ligands.

Synthesis of the ligands

Linear peptides [Boc-Tyr(^tBu)-(AA)_n-Pro-Leu-Trp(Boc)-OH] were synthesized using Fmoc/^tBu solid phase chemistry on a 2-chlorotrityl resin. The C-terminal carboxylic acid was amidated in solution phase. Removal of all protecting groups followed by purification afforded the pure ligands required for biological study. The ligands we designed and synthesized are given in Figure 2.

AKG117: H-Tyr-D-Ala-Gly-NMePhe-Pro-Leu-Trp-NH-Bn(3',5'-(CF₃)₂)
AKG115: H-Dmt-D-Ala-Gly-NMePhe-Pro-Leu-Trp-NH-Bn(3',5'-(CF₃)₂)
AKG116: H-Dmt-D-Ala-Gly-Phe-Pro-Leu-Trp-NH-Bn(3',5'-(CF₃)₂)
AKG127: H-Dmt-D-Ala-Gly-Phe(4-F)-Pro-Leu-Trp-NH-Bn(3',5'-(CF₃)₂)
AKG128: H-Dmt-D-Ala-Gly-NMePhe(4-F)-Pro-Leu-Trp-NH-Bn(3',5'-(CF₃)₂)
AKG190: H-Tyr-D-Ala-Gly-Phe(4-F)-Pro-Leu-Trp-NH-Bn(3',5'-(CF₃)₂)

Fig. 2. Structures of our multifunctional ligands.

Results and Discussion

The present study was designed to synthesize μ -selective ligands while maintaining the antagonist activity at NK1 receptor. To achieve our goal we considered our previously published μ -selective (binding) ligand **TY012** [6] and structural modification was conducted in the opioid pharmacophore moiety. We introduced Dmt at the 1st position, and NMe-Phe or halogenated-Phe at the 4th position

(Figure 2) to achieve our desired selectivity with high potency at all receptors. **AKG117** having NMe-Phe at 4th position showed a small increase in μ -agonist activity (Table 1). **AKG115** having Dmt at the 1st position and NMe-Phe at the 4th position showed selectivity (both binding and functional) for MOR over DOR (Table 1) while **AKG116** containing Dmt at the 1st position and Phe at the 4th position lacked selectivity in binding assay and displayed higher agonist activity at DOR compared to that at MOR (Table 1). **AKG127** containing Dmt at the 1st position and Phe(4-F) at the 4th position showed no selectivity in binding assay. However, it showed higher agonist activity for DOR compared to that at MOR (Table 1). **AKG128** containing Dmt at the 1st position and NMe-Phe(4-F) at the 4th position showed no improvement in selectivity. Replacement of Dmt with Tyr at the 1st position of **AKG127** produced the ligand **AKG190**, which became selective for DOR over MOR in functional assay (Table 1).

Table 1. Binding affinities of the multivalent ligands at MOR, DOR, and NK1R.

Ligand ID	K_i^μ (nM)	K_i^δ (nM)	K_i^{hNK1} (nM)	IC_{50}^μ (nM) (Agonist)	IC_{50}^δ (nM) (Agonist)	IC_{50}^{NK1} (nM) (Antagonist)
TY012	9.5	72	0.6	350	45	8.5
AKG117	27	260	3.4	230	102	21
AKG115	1	5	2.2	21	31	9.7
AKG116	3	1	1.4	81	3.1	25
AKG127	1	1	0.9	42	2	5.3
AKG128	0.4	4	2.6	77	11	11
AKG190	4	7	5.6	65	12	5.8

For binding studies, each sample was run three times at each receptor except **AKG190** which was run for twice at MOR. For functional activity studies, each sample was run four times.

We have designed and synthesized novel multifunctional ligands, which are highly potent at the receptors of our interest with desired activities. Ligand **AKG115** has shown selectivity at MOR over DOR in binding as well as in functional assays. *In vivo* study with this ligand is now in progress to evaluate its analgesic activity.

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