FENTEPHALINES: DIMERIC BIOACTIVE PEPTIDES BASED ON AMINO ACIDS COUPLED TO 4-ANILINO-N-PHENETHYL-PIPERIDINE

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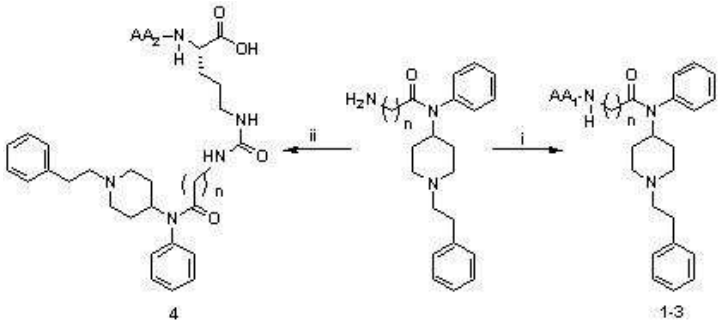
Introduction

4-Anilidopiperidines represent the most powerful synthetic analgesics, which include fentanyl and related compounds. Fentanyl is a μ-selective synthetic analgesic which is 50 - 100 times more potent than morphine, has a short duration of action and an onset of action almost immediately after *iv* administration. Fentanyl, sufentanyl, and alfentanyl are used for analgesia in clinical practice despite the side effects such as respiratory depression, physical dependence, and rapid tolerance. Our work constitutes the synthesis of chimeric opiates which contain the N-terminal tetrapeptide (Tyr-D-Ala-Gly-Phe) as the message moiety and a 4-anilinopiperidine moiety as the address portion. The 4-anilinopiperidine part is expected to assist transport and binding of the peptide portion to the opiate receptors. In electrically stimulated preparations of ileum and vas deferens, the inhibitory effect of our previously synthesized opioid agonist, H-Tyr-D-Ala-Gly-Phe-β-Ala-ANPP, showed nanomolar binding affinity [1] and a rapid onset of analgesia, even when low concentrations were used, which suggests that the novel class of opioids merits further development.



Scheme 1. (i) Stepwise chain elongation (n=1, AA_1: H-Tyr-X-Gly-Phe, X: *D*-Ala, *D*-Phe, *D*-Abu); (ii) n=2, AA_2: H-Tyr-*D*-Ala-Gly-Phe-Leu-Arg, SPPS using Fmoc-AA-OH on loaded BocLys(Fmoc) Merrifield resin.

Results

We previously reported that coupling to 4-anilino-N-phenethyl-piperidine (ANPP) can be efficiently mediated by using phthaloyl amino acids in the presence of DIPEA, followed by cleavage with ethanolamine (n = 1) or hydrazine hydrate (n = 2). The designed analogues (Scheme 1) were prepared in good yields by solution phase synthesis using Pht/Boc chemistry and tested for *in vitro* opioid activity. One of the analogues was synthesized on a solid support using Boc-Lys(Fmoc)OH loaded Merrifield resin. Interestingly, in this case coupling of Leu^5 did not lead to sequence deletion or diketopiperazine formation and the target peptide was obtained in quantative yield.

Table 1. Functional analysis & affinity for opioid receptors.

	Competitive Binding		GTP Binding			
	NG108-10DkDOR	*CHOrMOR*	*NG108-10DkDOR*		*CHOrMOR*	
Drug	[^{3}H] DPDPE	[^{3}H] DAMGO	EC_{50} (nM)	Emax (%)	EC_{50} (nM)	Emax (%)
(1) H-Tyr-*D*-Ala-Gly-Phe-Leu-Gly-ANPP	26.6	13.3	n.d.	n.d.	n.d.	n.d.
(2) H-Tyr-*D*-Abu-Gly-Phe-Leu-Gly-ANPP	2.8	0.43	5.8	22	35	77
(3) H-Tyr-*D*-Phe-Gly-Phe-Leu-Gly-ANPP	11.9	0.09	36.5	49	44.6	43
(4) H-Tyr-*D*-Ala-Gly-Phe-Leu-Arg-Lys(β-Ala-ANPP)-OH	11.5	0.35	n.d.	n.d.	n.d.	n.d.
(5) H-Tyr-*D*-Ala-Gly-Phe-Leu-Arg-OH*	4.79	0.63				
(6) H-Tyr-*D*-Ala-Gly-Phe-β-Ala-ANPP	1.1	0.9	62	141	60	142

** Data according to reference [2]; n.d. not determined.*

Compounds **1**, **2**, **3** and **4** were evaluated for their binding affinities at the μ, and δ opioid receptors following reported procedures. The results of our biological tests show that substitution of the propionyl moiety of fentanyl with opioid peptide ligands leads to compounds possessing high affinity for opioid receptors.

Discussion

The inherent μ-opioid receptor selectivity of fentanyl and its analogues allowed introduction of μ-selectivity into corresponding opioid peptide analogues. Incorporation of D-Phe in to the opioid peptide sequence (entry **3**), produced an analogue with a very high μ-opioid receptor affinity but low potency in GPI (K_i = 952.0 ± 134.8 nM), and MVD (K_i = 177.0 ± 17.8 nM) bioassays, which suggests that this peptide acts as an antagonist at the μ-opioid receptor. Incorporation of D-Abu (entry **2**) into the novel peptides proved to be more advantageous compared to D-Ala (entry **1**). Attachment of the 4-anilinopiperidine moiety to the side chain of lysine via urea bond preserved the free carboxy terminus of the peptide, and had a positive effect on the potency: compound 4 showed K_i = 8.557 +/- 1.710 nM in MVD compared to K_i = 273 +/- 35 nM of the parent compound, dalargin [3].

Acknowledgments

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References

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