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Bifunctional Opioid-Neuropeptide FF Ligands as Analgesics with Reduced Side Effects

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Introduction

The development of safer analgesics remains one of the main challenges in contemporary medicine. The "gold standard" opioids, used to treat moderate to severe pain, are unavoidably linked to adverse side effects, such as tolerance, respiratory depression, constipation, and physical dependence [1]. All of these side effects have an inevitable impact on the quality of life. To overcome this burden, the development of dual ligands, also called hybrids or bifunctional ligands, has increased over the past decade, more specifically by co-targeting opioid and non-opioid receptors involved in nocicaption [2,3]. Herein, the aim was to synthesize opioid-neuropeptide FF hybrids with selective G protein signaling over β-arrestin recruitment at the μ-opioid receptor (MOR). G protein signaling is responsible for pain relief, while the β-arrestin recruitment is linked to receptor internalization and, hence, desensitization, and flanked by opioid-induced side effects [3,4,5]. As non-opioid receptors, the neuropeptide FF 1 and 2 receptors (NPFF1/2R) are known to be involved in opioid-induced analgesic tolerance, among other side effects [3]. Interestingly, antagonism of the NPFF1/NPFF2 receptors prevents the increase of analgesic tolerance and opioid-induced hyperalgesia [3,7,8]. After screening a first set of OPFF hybrids [3], a subsequent series based on lead sequence **1** (Figure 1), was developed and underwent biological *in vitro* evaluation. Subsequently, only the best analogues were selected to be tested *in vivo* for an assessment of common opioid side effects.

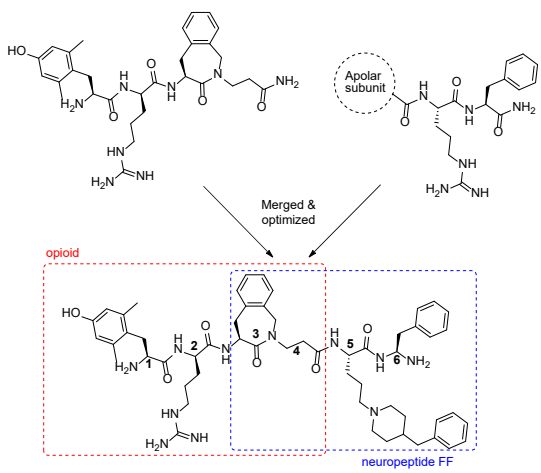

*Fig. 1. Fusion of the opioid peptide **KGOP01** and minimal NPFF pharmacophores led after optimization to the merged lead peptide **1**.*

Results and Discussion [9]

In this study, a new series of opioid-neuropeptide FF (OPFF) hybrids was synthesized, based on the lead peptide **1** (Figure 1). Peptide **1** consists of an opioid pharmacophore: H-Dmt-D-Arg-AbaβAla-, and a NPFF pharmacophore (here Bpa-Phe-NH_2). The opioid pharmacophore constituted a previously optimized opioid peptide **KGOP01**, while the standard NPFF pharmacophore consists of RF-NH_2 like moieties. This latter one was optimized to provide the Bpa-Phe-NH_2 moiety.

All hybrids were synthesized by solid phase peptide synthesis (SPPS) using the classical Fmoc/*t*Bu strategy with the Rink Amide AM resin as solid support. Coupling conditions were HBTU/DIPEA, DIC/Oxyma Pure or DIC/HOBt. The synthesis of Fmoc-Bpa-OH was performed, as previously described [10]. In addition, all constrained dipeptides, incorporated in position 3 & 4, were manually synthesized in our lab, according to known procedures [3,11,12].

Table 1. In vitro data of the most promising compounds.

Code	Sequence	MOR		NPFF1	NPFF2
		cAMP EC_{50} /E_{max}	*β-arrestin-2* EC_{50}/E_{max}	IC_{50} *antagonist* IC_{50} *binding*	IC_{50} *antagonist* IC_{50} *binding*
1	H-Dmt-D-Arg-Aba-βAla-Bpa-Phe-NH_2	13 ± 3 nM 90 ± 3 %	133 ± 18 nM 43 ± 2 %	≈ 5 µM K_i = 228 ± 58 nM	5 µM K_i = 7.1 ± 3.6 nM
2	H-Dmt-D-Pro-Aba-βAla-Bpa-Phe-NH_2	11 ± 1 nM 53 ± 1 %	No recruitment	≈ 5 µM K_i = 1.1 ± 0.2 µM	≈ 5 µM K_i = 27 ± 8 nM
3	H-Dmt-*N*(Me)-D-Ala-1Ana-Gly-Bpa-Phe-NH_2	4.9 ± 0.5 nM 76 ± 0.1 %	No recruitment	> 5 µM K_i = 1.2 ± 0.01 µM	≈ 5 µM K_i = 58 ± 6 nM
4	Guanidyl-Dmt-D-Arg-Aba-βAla-Bpa-Phe-NH_2	14 ± 2 nM 58 ± 5 %	119 ± 97 nM 15 ± 8 %	< 5 µM K_i = 85 ± 0.4 nM	< 5 µM K_i = 5.1 ± 0.9 nM
5	H-Dmt-*N*(Me)-D-Ala-1Ana-βAla-Bpa-Phe-NH_2	4.8 ± 1.8 nM 77 ± 5 %	No recruitment	> 5 µM K_i = 1.5 ± 0.2 µM	> 5 µM K_i = 38 ± 3 nM

Subsequently, the *in vitro* profiles (Table 1) of the OPFF hybrid peptides were determined at the human MOR, by examining the cAMP inhibition and the β-arrestin-2 recruitment. In addition, affinity and activity at the NPFF receptors were tested on the most favorable peptides.

Conclusions

As shown in Table 1, peptides **2-5** presented lowered β-arrestin-2 recruitment, together with a selectivity of NPFF1/NPFF2. Consequently, these peptides were tested *in vivo*. Upon investigation of acute analgesia, all peptides showed a prolonged duration of action, as compared to morphine. Gratifyingly, peptide **5** showed an enhanced analgesic activity, in comparison to the previous lead peptide **1**. In contrast to morphine, preliminary data also indicated that peptide analogues **4** and **5** induce no hyperalgesia. Altogether, the hybrids **4** and **5** were selected as lead compounds to be further evaluated *in vivo* during future endeavors.

Acknowledgments

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