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Hybrid peptidomimetics for the use in neuropathic pain

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Introduction

Neuropathic pain is a type of chronic pain that results from the damage to the nerve tissue. The difficulty of treatment rely on the lack of suitably potent and long-acting drugs, because opioids successfully used in acute pain lose their analgesic efficacy in neuropathic pain. This effect is explained as a result of excessive pathological damage inducing activation of endogenous systems which cause pain intensification. The action of these systems is opposed to the analgesic effect of opioids, so the effect of these drugs is weakened. A number of such endogenous pain-enhancing systems have been described [1], among them the melanocortin system (MC), particularly the melanocortin 4 (MC4) receptor, is of great interest. Published data suggest that melanocortins are considered endogenous functional antagonists of opioids [2, 3]. Moreover, it was found that the administration of antagonists of MC4 receptor resulted in analgesia and intensify the effect of morphine [4]. Based on this and other studies on the MC4 receptor available in the literature [5], it has been hypothesized that the activation of opioid receptors while blocking MC4 receptors will improve the efficacy of opioids in the treatment of neuropathic pain. The simultaneous effect on both systems (opioid activation and melanocortin inhibition) in the same part (fragment) of nociceptive pathways may provide bifunctional hybrid compounds containing two components: the opioid receptor agonist (OP) and the MC4 receptor antagonist.

The purpose of this work was to design and synthesize hybrid peptidomimetics containing an opioid agonist and a melanocortin antagonist linked by various spacers, and to examine the analgesic activity of these compounds in neuropathic pain.

Results and Discussion

Nine new hybrid peptidomimetics containing two ligands: an enkephalin analogue (opioid agonist) [6] and SHU 9119 (an MC4 receptor antagonist) [4] linked by various spacers have been designed. Spacers in designed peptidomimetics can be classified as short (residues of D-Ala, β-Ala), flexible (residue of 6-aminohexanoic acid (Ahx)), rigid (residues of 4-aminomethylbenzoic acid (4AMB), 4-aminophenylacetic acid (4APhAc) and semi-rigid (dipeptidyl fragment of Pro-Gly [7]).

Tyr[1]-D-Ala[2]-Gly[3]-Phe[4] —-X—–Nle [6]-c[Asp[7]-His[8]-D-Nal(2')[9]-Arg[10]-Trp[11]-Lys[12]]-NH_2 X= spacer

All hybrid peptides were synthesized on a MBHA resin (Bachem, 0.27 mmol/g) using the standard Boc strategy and carbodiimide (DIC) as the coupling reagent.

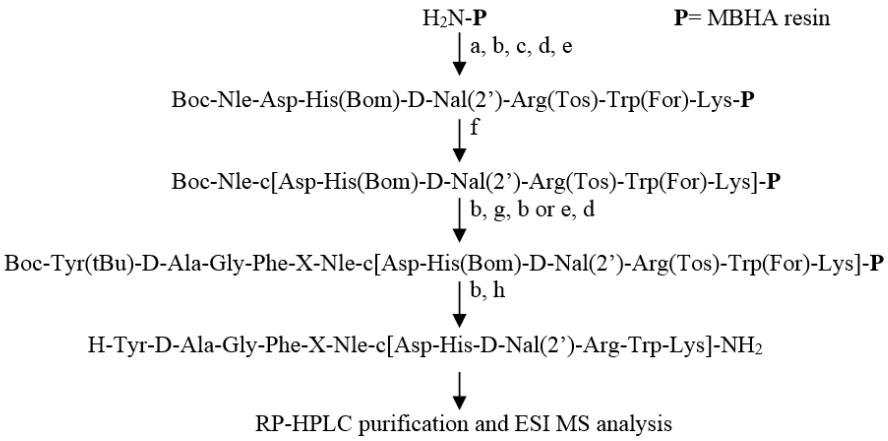

Figure 1: Synthesis of hybrid peptidomimetics (a) Boc-AA, DIC, HOBt, (b) TFA, (c) DIEA, (d) steps a-c (e) piperidine, (f) DIC, HOBt, (g) Protected spacer: Boc-D-Ala or Boc-β -Ala or Fmoc-Ahx or Fmoc-4-AMB or Fmoc-4-APhAc or Boc-Gly, Boc-Pro, (h) HF

Table 1: Calculated ED50 values for effect of hybrids 1-9 and reference compounds: enkephalin analogue and SHU9119, in acute pain (tail-flick test) and neuropathic pain in CCI-exposed mice (von Frey and cold plate test). The experiments were performed on naive mice or 7-14 days after CCI procedure, all compounds were administered intrathecally (i.t.).

Compound	ED_{50} (μg)		
	Naive mice	Mice subjected to CCI	
	Tail-flick	Von Frey (allodynia)	Cold plate (hyperalgesia)
Tyr-D-Ala-Gly-Phe-NH_2 (parent opioid agonist)	0.03 (0.02 – 0.05)	0.16 (0.1 – 0.25)	9.3 (2.7 – 32)
SHU9119 (parent antagonist of MC4 receptor)	*	3.58 (0.01 – 803)	#
1 (X=D-Ala)	#	#	#
2 (X=Ahx)	#	0.0004 (0.0001 – 0.002)	0.008 (0.005 – 0.01)
3 (X= β-Ala)	104 (48 – 226)	0.08 (0.5 – 1.2)	251 (55 – 1157)
4 (X=Ahx-Ahx)	*	0.02 (0.00003 – 15)	0.2 (0.008 – 6.5)
5 (X=4AMB)	#	0.03 (0.01 – 0.1)	0.3 (0.2 – 0.4)
6 (X=4APhAc)	42 (21 – 85)	0,03 (0,0004 – 1,9)	0,04 (0,0009 – 2,2)
7 (X= Pro-Gly)	#	0,7 (0,5 – 1,1)	0,29 (0,13 – 0,63)
8 (X=Pro-Gly-Pro-Gly)	10 (0.8 – 121)	*	*
9[a] (X=Ahx)	#	0,005 (0,000005 – 6,35)	0,016 (0,01 – 0,02)

a- the residue of tyrosine was replaced by 2,6-dimethyl-L-tyrosine (DMT) residue, *- it was impossible to calculate ED50 due to lack of analgesic effect, # - it was impossible to calculate ED50 due to weak dose-related effect

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