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Synthesis, characterization and nociceptive screening of new VV-hemorphin-5 analogues

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ABSTRACT

In the present study, some new analogues of VV-hemorphin-5, modified at position 1 and 7 by the non-proteinogenic and/or natural amino acids followed the structures Xxx-Val-Val-Tyr-Pro-Trp-Thr-Gln-NH_2 and Val-Val-Tyr-Pro-Trp-Thr-Yyy-NH_2, where Xxx is Ile or Aib and Yyy is Lys/Orn/Dap/Dab were synthesized to investigate their potential antinociceptive activities. We report also the redox potentials and the acid/base properties as pKa values of these peptide analogues which were compared toward electrochemical behaviour of tryptophan containing peptides. All analogues showed a short lasting initial antinociceptive effect, however H2 hemorphin analogue is characterized with prolong and strong antinociceptive effect, while the other peptide analogues exerted more variable effects on the visceral nociception depending on the dose or time after the intracerebral injection.

During the last five years the number of peptide and protein drugs drastically increase because of advances in recombinant DNA and hybridoma techniques. Peptides are increasingly used in the prevention or treatment of various diseases such as hypertension, epilepsy, diabetes, chronic pain, cancer, and other diseases with social significance. Chronic pain is very widespread and difficult to treat with available therapies; therefore, the development of new analgesics is of interest and is of major importance for pharmaceuticals.[1–5]

Endogenous hemorphin-related peptides belonging to the family of "atypical" opioid peptides released from sequentially hydrolyzed hemoglobin with affinity for opioid receptors and morphinomimetic properties.[6–8] Hemorphins exhibit the activity typical for opioid receptor agonists in standard guinea pig ileum (GPI) contraction[9] and naloxone-dependent experimental models of analgesia.[10] Analysis of activity dependence on peptide sequence in GPI contraction test allowed identification of the YPW amino acid sequence as essential for binding to opioid receptors; it has to be note that binding also depends on flanking amino acid sequences.[8] A sequential degradation of hemoglobin generates a series of structural overlapped bioactive peptides with N- and/or C-terminal extensions of Tyr-Pro-Trp. Hemorphin-3 to -7 represent the C-terminal truncations. The hemorphin subfamilies involve N-terminal sequences and identify as hemorphins, V-hemorphins, VV-hemorphins and LVV-hemorphins.[11,12] VV-hemorphin-5 (VVYPWTQ) is a short-chain μ-selective opioid peptide found in serum, and released by the action of pancreatic elastase from human haemoglobin.[7,8] This heptapeptide, also known as valorphin, is an opioid ligand that also binds the δ- and k- opioid receptors and plays a role of endogenous opioid peptide *in vivo*.[11] V-hemorphin-5 (VYPWTQ) could be converted to hemorphin-5 (YPWTQ) by leucine aminopeptidase, and released in larger amount parallel to above mentioned peptide at the same time.[13] LVV-hemorphin-7 is a specific agonist of angiotensin IV (AT4) receptor that belongs to the class of insulin-regulated aminopeptidases (IRAP).[14,15] The AT4 receptor is composed by a catalytic domain with aminopeptidases activity,[16,17] which is capable of degrading several neuropeptides such as vasopressin and oxytocin.[18] In addition, LVV-hemorphin-7 modulates some important physiological processes not only by blocking IRAP activity but also by additional mechanisms including binding to distinct receptor types.[19]

Based on these findings, we report in the present study the synthesis, characterization and the nociceptive screening of new analogues of VV-hemorphin-5, modified at position 1 and 7 by the non-proteinogenic and natural amino acids followed the structures Xxx-Val-Val-Tyr-Pro-Trp-Thr-Gln-NH_2 and Val-Val-Tyr-Pro-Trp-Thr-Yyy-NH_2, where Xxx is Ile or Aib (α-aminoisobutyric acid) and Yyy is Lys/Orn/Dap (2,3-diaminopropanoic acid)/Dab (2,4-diaminobutanoic acid) (see Fig. 1).

These novel analogues of VV-hemorphin-5 have been synthesized by

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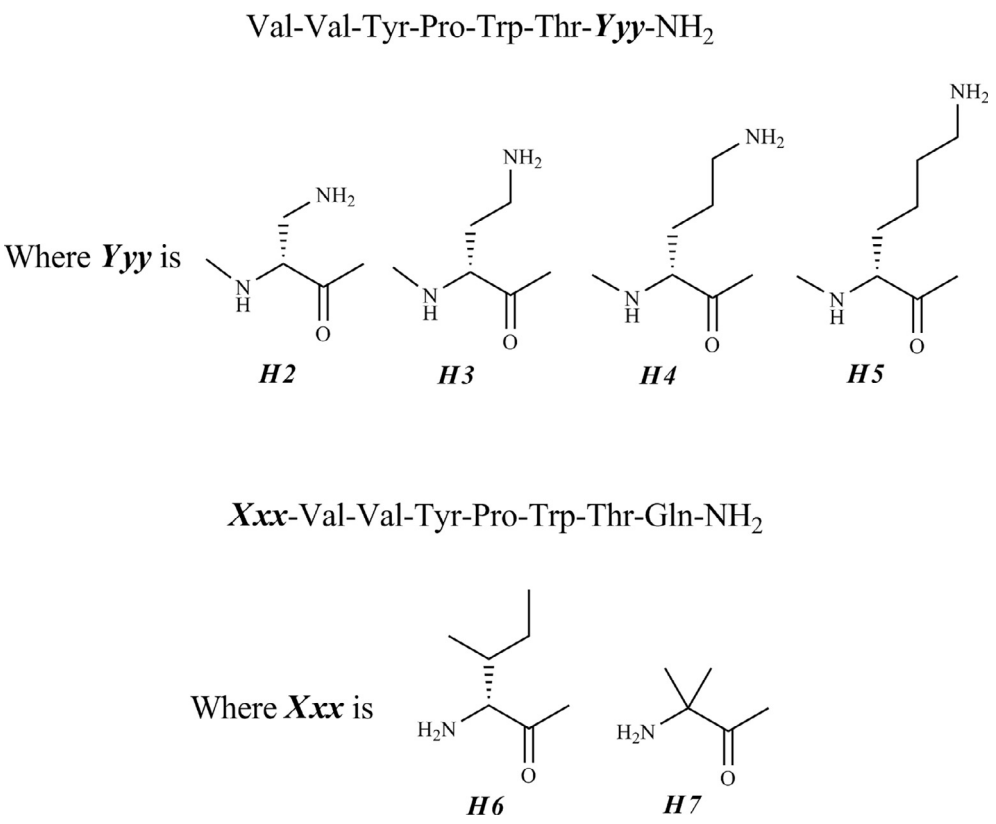
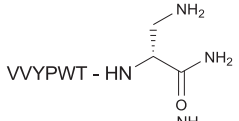
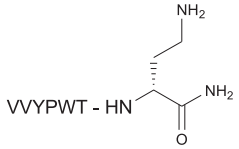
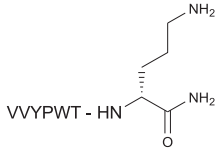
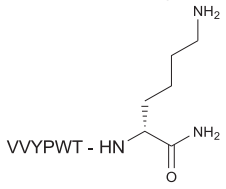


Fig. 1. Chemical strucrures of new analogues of VV-hemorphin-5.

including un/natural amino acids at position 1 and 7, respectively, using solid-phase peptide synthesis (SPPS) by Fmoc (9-fluorenylmethoxycarbonyl) chemistry (see Supplementary data). The analytical data of the synthetic peptides are shown in Table 1.

Indeed, the handling of the amino acidic scaffold can be regarded as a potentially powerful tool in both bioorganic and biomedical investigations and the development of new drugs. Among the increasing number of publications on this area, a few general reviews addressing

Table 1
Analytical data of synthetic peptides.

№	Peptides	[a] $t_{R,\ min}$	[b] ESI MS: $(MH)^+$		pK_a constant
			Calculated	Found	
H1	VVYPWTQ – NH_2	16.72	891.0	891.4	7.13
H2		15.48	848.9	849.4	9.23
H3		15.22	863.0	863.4	8.12
H4		15.07	877.0	877.4	7.83
H5		15.28	890.9	891.5	8.24

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