Bioorganic & Medicinal Chemistry 24 (2016) 1582–1588

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis, biological evaluation and structural analysis of novel peripherally active morphiceptin analogs

Anna Adamska [a], Alicja Kluczyk [b], Maria Camilla Cerlesi [c], Girolamo Calo [c], Anna Janecka [a], Attila Borics [d,*]

[a] Department of Biomolecular Chemistry, Faculty of Medicine, Medical University of Lodz, Mazowiecka 6/8, 92-215 Lodz, Poland
[b] Faculty of Chemistry, University of Wroclaw, F. Joliot-Curie 14, 50-383 Wroclaw, Poland
[c] Department of Medical Sciences, University of Ferrara, Via Fossato di Mortara 17/19, 44121 Ferrara, Italy
[d] Institute of Biochemistry, Biological Research Centre of Hungarian Academy of Sciences, Temesvári krt. 62, Szeged H-6726, Hungary

ARTICLE INFO

Article history:
Received 13 January 2016
Revised 23 February 2016
Accepted 24 February 2016
Available online 26 February 2016

Keywords:
Solid-phase peptide synthesis
Binding studies
Opioid receptors
Hot-plate test
Gastrointestinal transit
Molecular dynamics
Docking

ABSTRACT

Morphiceptin (Tyr-Pro-Phe-Pro-NH_2), a tetrapeptide amide, is a selective ligand of the μ-opioid receptor (MOR). This study reports the synthesis and biological evaluation of a series of novel morphiceptin analogs modified in positions 2 or/and 4 by introduction of 4,4-difluoroproline (F_2Pro) in L or D configuration. Depending on the fluorinated amino acid configuration and its position in the sequence, new analogs behaved as selective full MOR agonists showing high, moderate, or relatively low potency. The most potent analog, Tyr-F_2Pro-Phe-D-F_2Pro-NH_2, was also able to activate the κ-opioid receptor (KOR), although with low potency. Docking studies and the comparison of results with the high resolution crystallographic structure of a MOR-agonist complex revealed possible structure–activity relationships of this compound family.

1. Introduction

A number of milk protein fragments has been shown to behave as opioid receptor ligands, able to address opioidergic systems. One class of such opioid peptides that show some preference for the μ-opioid receptor (MOR)[1,2] is the group of β-casomorphins, originating from the milk protein, β-casein as a product of proteolytic fragmentation. A tetrapeptide amide Tyr-Pro-Phe-Pro-NH_2, known as morphiceptin, shows considerable opioid potency and MOR selectivity.[3] Morphiceptin, among other opioid peptides, elicits strong supraspinal antinociception when given intracerebroventricularly (i.c.v.), in other words directly to the central nervous system (CNS). This effect is not observed after peripheral administration, due to the relatively rapid degradation of morphiceptin, resulting in its short duration of action and limited delivery to the CNS. However, morphiceptin analogs have been proposed as peripheral agents for the treatment of diarrhea. Subcutaneous (s.c.) administration of a potent morphiceptin analog, Tyr-Pro-NMePhe-Pro-NH_2, inhibited diarrhea and decreased gastrointestinal transit in mice.[4] The advantage of such agents is the lack of the central effects, such as analgesia and sedation. Peripheral selectivity of morphiceptin and its analogs encourages further studies of this group of opioid peptides, whose therapeutic possibilities have not been fully appreciated yet.

Morphiceptin was one of the first opioid peptides, of which structure was investigated in detail to explain its affinity to the MOR. The L-configuration of Pro^2 was found vital for manifestation of opioid activity.[5] The phenolic OH and the protonated free amine group of Tyr^1 and the aromatic side chain of Phe^3 in a well-defined relative spatial arrangement were proposed to facilitate high affinity MOR binding, in which pose of the Pro^2 residue acts as a stereochemical spacer responsible for the correct orientation of pharmacophoric groups.[6] However, later studies comparing several MOR ligands of various affinities did not confirm the necessity of an intrinsic tendency of MOR ligands to adopt such spatial arrangement.[7] Instead, many parallel studies revealed that apart from possessing the necessary pharmacophores, the high propensity of a ligand to form bent backbone structure[8–12] or maintain high degree of conformational flexibility[7] may result in high affinity binding to the MOR. In the past, notable emphasis was put on the role of *cis–trans* isomerization of the peptide bond preceding Pro^2 in opioid peptides. As both exclusively *cis*[5] and *trans* peptide ligands[13] were shown to bind to the MOR with high affinity, this structural property was proved to be of little relevance with regard to MOR activity. The X-ray crystallographic structure of both, the

* Corresponding author.
E-mail address: borics.attila@brc.mta.hu (A. Borics).

http://dx.doi.org/10.1016/j.bmc.2016.02.034
0968-0896/

agonist-[14] and antagonist-bound MOR[15] has been published recently, which provided answer to numerous questions regarding ligand structure and activity. Nevertheless, the design of a MOR-agonist peptide with pharmaceutical properties appropriate for therapeutic application remains a challenge.

Unique physico-chemical properties of fluorine, such as small size and high electronegativity, seem to be of special advantage in drug design. Fluorinated compounds show higher bioavailability and metabolic stability. One of the major effects of fluorination is a modulation of acidity of a parent compound which can strongly influence binding affinity and pharmacokinetic properties of an analog. While substitution of fluorine for a hydrogen atom results in minor steric alterations, electrostatic interactions in a molecule may lead to significant conformational changes.[16] So far, only a few fluorinated amino acids have been introduced into the analogs of opioid peptides.[17–19]

In the search for novel morphiceptin analogs with improved pharmacological profile we have synthesized a series of analogs modified in positions 2 or/and 4 by introduction of 4,4-difluoro-Pro (F_2Pro) in L or D configuration (Fig. 1). Novel analogs of morphiceptin were tested in vitro in radioligand receptor binding assays and calcium mobilization-based functional tests. Conformational preferences of the new ligands were studied by performing molecular dynamics (MD) simulations. In order to reveal atomistic details of specific interactions between the new ligands and the MOR, docking studies were performed.

2. Materials and methods

2.1. Peptide synthesis

Most of the chemicals were purchased from Sigma Aldrich. Protected amino acids were purchased from NovaBiochem or Bachem. MBHA Rink-Amide peptide resin (100–200 mesh, 0.6 mmol/g) was obtained from NovaBiochem. Fmoc-protected L- and D-F_2Pro was purchased from TriMen Chemicals (Lodz, Poland). Peptides were synthesized by a standard solid-phase procedure, using technique for Fmoc-protected amino acids on MBHA Rink-Amide resin as described earlier.[20] 20% piperidine in DMF was used for the deprotection of Fmoc groups and TBTU was employed as a coupling

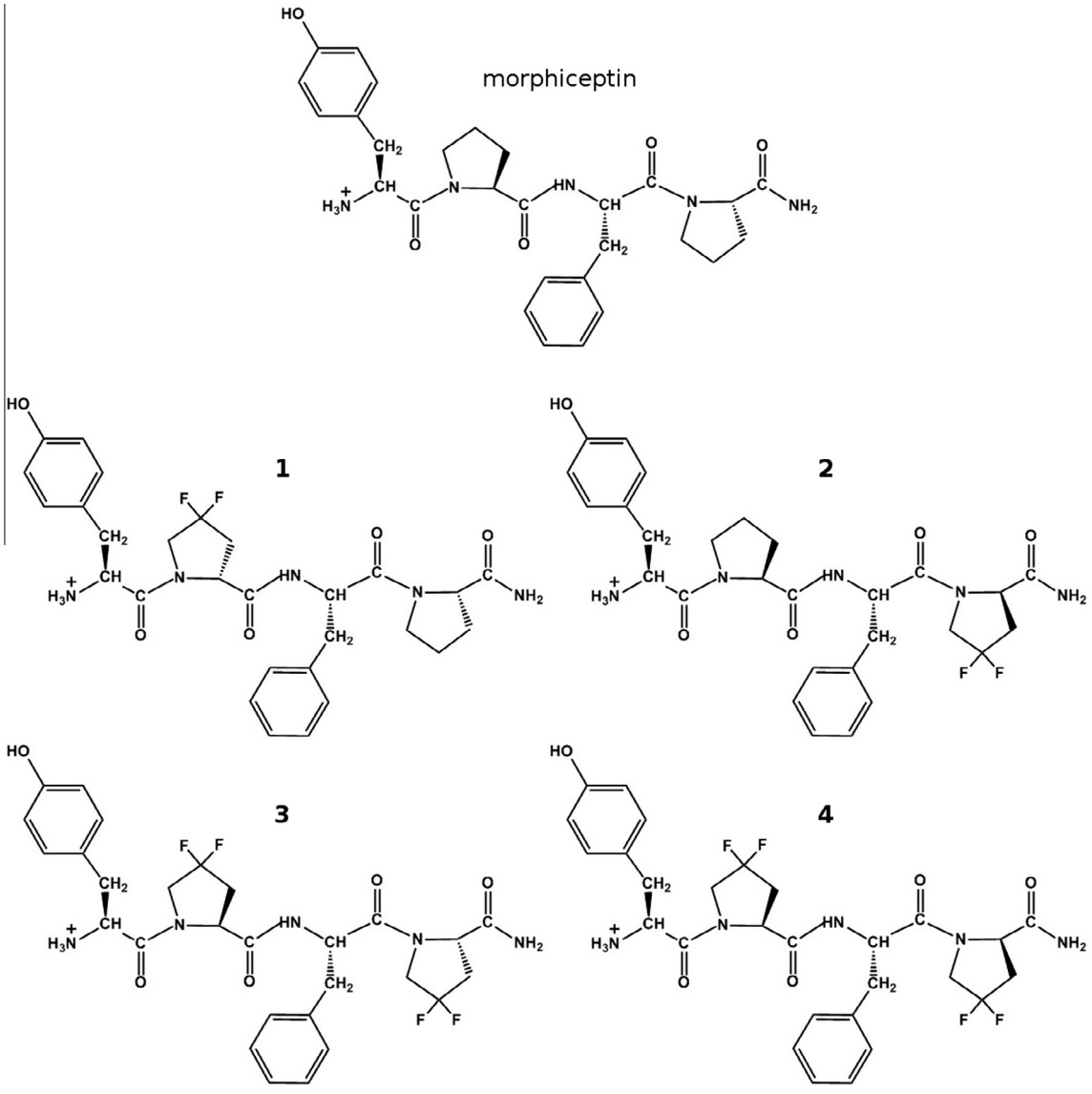

Figure 1. Structure of the studied fluorinated derivatives **1–4** and their parent compound, morphiceptin.