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Research Paper

Synthesis and activity of opioid peptidomimetics with β^2- and β^3-amino acids

Katarzyna Gach-Janczak[a], Justyna Piekielna-Ciesielska[a], Anna Adamska-Bartłomiejczyk[a], Renata Perlikowska[a], Rafał Kruszyński[b], Alicja Kluczyk[c], Julia Krzywik[d], Jarosław Sukiennik[d], Maria Camilla Cerlesi[e], Girolamo Calo[e], Andrzej Wasilewski[f], Marta Zielińska[f], Anna Janecka[a,*]

[a] Department of Biomolecular Chemistry, Medical University, Lodz, Poland
[b] Department of X-ray Crystallography and Crystal Chemistry, Institute of General and Ecological Chemistry, Lodz University of Technology, Lodz, Poland
[c] Faculty of Chemistry, University of Wroclaw, F. Joliot-Curie 14, 50-383 Wroclaw, Poland
[d] TriMen Chemicals Ltd., 92-318 Lodz, Poland
[e] Department of Medical Sciences, Section of Pharmacology and Italian Institute of Neuroscience, University of Ferrara, 44121 Ferrara, Italy
[f] Department of Biochemistry, Faculty of Medicine, Medical University of Lodz, Poland

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ABSTRACT

Morphiceptin (Tyr-Pro-Phe-Pro-NH_2) is a selective ligand of the mu opioid receptor, an important target in pain regulation. In this study, morphiceptin was modified at positions 2 or 3 by introduction of β^2- or β^3-amino acids and additionally in position 1 by replacing Tyr by Dmt (2′,6′-dimethyltyrosine), which resulted in obtaining enzymatically stable analogs with mixed opioid receptor affinity profiles. An analog of the sequence Dmt-D-Ala-(*R*)-β^2-1-Nal-Pro-NH_2 [Nal = 3-(1-naphthyl)-alanine] showed very high activity at the mu and delta receptors in the calcium mobilization functional test but did not cross the artificial membrane imitating the blood-brain barrier. In the in vivo test this analog induced strong antinociceptive effect in the writhing test in mice after intraperitoneal but also oral administration and inhibited diarrhea similarly to loperamide. Therefore, it may become an interesting lead compound in the development of peripherally restricted drugs for the treatment of gastrointestinal disorders.

1. Introduction

Opioid peptidomimetics, which arise from modification of existing peptides, are designed to improve their stability and biological activity, having an important role in the development of new drug candidates. One approach to obtain opioid peptidomimetics is the use of β-amino acids [1,2]. In α-amino acids carboxy and amino groups are separated by only one carbon atom, while in β-amino acids two carbon atoms separate these functional groups. β-Amino acids with specific side chains (other than H), can exist as *R* or *S* isomers at either the α (C2) carbon or the β (C3) carbon, producing β^2- or β^3-amino acids, respectively (Fig. 1). There are numerous examples showing that replacing α- by β-amino acids was successful in creating peptidomimetics with potent biological activities and greatly increased stability [3–5].

It was also shown that in β-amino acids introduction of an additional methylene group did not cause steric hindrance and did not confine orientation of the side chain to regions of space different from those permitted in the α-amino acid [6].

Opioid receptors (mu, delta, kappa) and opioid peptides play an essential role in pain perception and modulation and therefore are important targets in medicinal chemistry. Extensive structure-activity relationship studies of opioid receptor ligands are still in progress to provide specific information about their structure-activity relationship and to obtain new potential drug candidates [7–9].

An opioid peptide, morphiceptin (Tyr-Pro-Phe-Pro-NH_2), a tetrapeptide amide present in the enzymatic digest of bovine β-casein, is a selective ligand of the mu opioid receptor. In the past, we synthesized a morphiceptin analog, Dmt-D-Ala-D-1-Nal-Pro-NH_2 [Dmt = 2′,6′-dimethyltyrosine and 1-Nal = 3-(1-naphthyl)-alanine] which displayed very high mu receptor affinity, resistance to enzymatic degradation and in the in vivo studies inhibited the gastrointestinal transit in mice [10].

Here, we report further modifications of this analog which include the incorporation of β^2- and β^3-amino acids (β-Ala and β-1-Nal). (*R*)- and (*S*)-β^2-1-Nal were not available commercially, so their synthesis and determination of the absolute configuration were performed. The obtained peptide analogs were tested in vitro in the receptor binding

* Corresponding author at: Department of Biomolecular Chemistry, Medical University of Lodz, Mazowiecka 6/8, 92 – 215 Lodz, Poland.
E-mail address: anna.janecka@umed.lodz.pl (A. Janecka).

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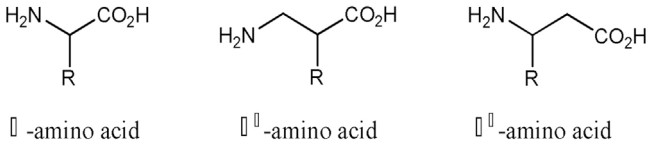

Fig. 1. The structure of α- and β-amino acids.

assay and in calcium mobilization functional assay, and their enzymatic stability was evaluated using dipeptidyl peptidase IV (DPP IV). The in vivo antinociceptive and anti-diarrheal activities of the selected analogs were assessed in mice.

2. Materials and methods

2.1. Materials

All protected amino acids, with the exception of (*R*)- and (*S*)-β^2-1-Nal, were purchased from Bachem AG (Bubendorf, Switzerland). Opioid radioligands, [^{3}H]DAMGO, [^{3}H]deltorphin-2 and [^{3}H]U-69593, and human recombinant opioid receptors were purchased from PerkinElmer (Krakow, Poland). Analytical and semi-preparative RP-HPLC was performed using Waters Breeze instrument (Milford, MA, USA) with dual absorbance detector (Waters 2487) on a Vydac C_{18} column (5 μm, 4.6 × 250 mm) and a Vydac C_{18} column (10 μm, 22 × 250 mm), respectively. High resolution mass spectra were recorded using Bruker micrOTOF-Q mass spectrometer (Bruker Daltonics, Bremen, Germany) with electrospray ionization (ESI–MS).

2.2. Synthesis of fmoc protected (R)- and (S)-β^2-1-Nal

2.2.1. Synthesis of ethyl 2-cyano-3-(1-naphthalenyl)acrylate (1)

A mixture of 1-naphthaldehyde (10 g; 64 mmol), ethyl cyanoacetate (7 mL; 64 mmol), DABCO catalyst (10% mol) and distilled water (20 mL) was placed in a 100 mL round bottom flask [11]. The reaction mixture was stirred at room temperature until yellow solid precipitated. It was filtered off, washed with water to remove the catalyst and dried to give the crude product as yellow solid, still contaminated with naphthyl aldehyde. The product was purified by crystallization from methanol to give yellow needles (12.1 g; 75% yield).

2.2.2. Synthesis of racemic 3-[(tert-butoxycarbonyl)amino]-2-(1-naphthylmethyl)propanoic acid (2)

A suspension of ethyl 2-cyano-3-(1-naphthalenyl)acrylate (**1**) (10 g; 39.8 mmol) in ethanol (250 mL) was hydrogenated in the presence of PtO_2 (200 mg) and concentrated sulfuric acid (3 mL) at 50 °C overnight [12]. The catalyst was removed by filtration and the solution was concentrated to the volume of 150 mL. Water (120 mL) and LiOH monohydrate (7.5 g; 170 mmol) were added. The mixture was heated to reflux for 3 h, then Boc_2O (11 g; 50 mmol) was added and the reaction was stirred overnight. Ethanol was evaporated under reduced pressure and the aqueous residue was washed with diethyl ether. Aqueous layer was acidified with 2 M HCl and extracted with ethyl acetate. The organic layer was concentrated and purified by column chromatography in hexane/ethyl acetate solvent system to give 10 g (75% yield) of the product. TLC (dichloromethane/methanol, 50:1 v/v) R_f = 0.28.

2.2.3. Synthesis of (R)- and (S)-3-Boc-amino-2-(1-naphthylmethyl) propanoic acid (S)-α-methyl-benzylamide (3)

Racemic 3-[(*tert*-butoxycarbonyl)amino]-2-(1-naphthylmethyl)propanoic acid (**2**) (10 mmol; 3.29 g) was dissolved in dichloromethane (30 mL). Then (*S*)-α-methylbenzylamine (11 mmol; 1.33 g), triethylamine (12 mmol; 1.65 mL) and TBTU (10 mmol; 3.21 g) were added. The reaction was stirred overnight, then washed twice with 2 M HCl, twice with 5% $NaHCO_3$ and dried over $MgSO_4$. After filtration the solvent was removed in vacuo and the residue was subjected to flash chromatography in hexane/ethyl acetate (10:1) solvent system to give separated diastereoisomers, 2 g of each.

Isomer (*R*,*S*): TLC (ethyl acetate/hexane, 1:5 v/v) R_f = 0.36; ^{1}H NMR (700 MHz, $CHCl_3$-d) δ: 1.35 (d, *J* = 7.0 Hz, 3H), 1.47 (s, 9H), 2.91–2.96 (m, 1H), 3.24–3.31 (m, 2H), 3.45–3.50 (m, 2H), 4.96 (p, *J* = 7.0 Hz, 1H), 5.13 (bs, 1H), 5.44 (bs, 1H), 6.77 (d, *J* = 6.3 Hz), 7.15–7.24 (m, 5H), 7.45–7.51 (m, 2H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.84 (d, *J* = 8.0 Hz, 1H), 8.00 (d, *J* = 8.3 Hz, 1H); ESI–MS (calcd. for $C_{27}H_{32}N_2O_3$: 432.56): *m/z* = 455.10 [M + Na^+].

Isomer (*S*,*S*): TLC (ethyl acetate/hexane, 1:5 v/v) R_f = 0.42; ^{1}H NMR (700 MHz, $CHCl_3$-d) δ: 0.91 (d, *J* = 7.0 Hz), 1.43 (s, 9H), 2.83–2.87 (m, 1H), 3.22–3.28 (m, 2H), 3.40–3.47 (m, 2H), 4.85 (d, *J* = 7.0 Hz, 1H), 5.04 (bs, 1H), 5.20 (bs, 1H), 7.05–7.07 (m, 2H), 7.16–7.19 (m, 1H), 7.22–7.25 (m, 2H), 7.35–7.37 (m, 1H), 7.39–7.42 (m, 1H), 7.48–7.51 (m, 1H), 7.53–7.56 (m, 1H), 7.76 (d, *J* = 8.1 Hz, 1H), 7.87 (m, 1H), 8.04 (d, *J* = 8.4 Hz, 1H); ESI–MS (calcd. for $C_{27}H_{32}N_2O_3$: 432.56): *m/z* = 455.00 [M + Na^+].

2.2.4. Determination of the crystal structure of one diastereoisomer

The colorless needle crystal of one diastereoisomer was mounted on a Rigaku Synergy Dualflex automatic diffractometer equipped with Pilatus 300 K detector and used for the data collection. X-ray intensity data were collected with mirror monochromated CuK_α (λ = 1.54184 A, micro-focus sealed PhotonJet X-ray tube) radiation at temperature of 100.0(1) K, with ω scan mode. The shuterless mode was used, and reflections inside Ewald sphere were collected up to θ = 78.98°. The unit cell parameters were determined from 11104 strongest reflections. Examination of the same reference reflections, measured before and after measurement, showed no loss of the intensity during measurement. Lorentz, polarization and empirical absorption (using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm) corrections were applied during the data reduction. The structure was solved by partial structure expansion procedure. All the non-hydrogen atoms were refined anisotropically using full-matrix, least-squares technique on F^2. All the hydrogen atoms were found from difference Fourier synthesis after four cycles of anisotropic refinement, and refined as "riding" on the adjacent atom with geometric idealisation after each cycle of refinement and individual isotropic displacement factors equal 1.2 times the value of equivalent displacement factors of the parent non-methyl carbon and nitrogen atoms, and 1.5 times of parent methyl carbon atoms. The methyl groups were allowed to rotate about their local three-fold axes. The SHELXS, SHELXL and SHELXTL [13–15] programs were used for all the calculations. Atomic scattering factors were taken from International Tables for Crystallography [16].

Tables of crystal data and structure refinement, anisotropic displacement coefficients, atomic coordinates and equivalent isotropic displacement parameters for non-hydrogen atoms, H-atom coordinates and isotropic displacement parameters, bond lengths and interbond angles have been deposited with the Cambridge Crystallographic Data Centre under No. CCDC 1530132.

2.2.5. Synthesis of (R)- and (S)-3-[(9-fluorenylmethoxycarbonyl)amino]-2-(1-naphthylmethyl)propanoic acid [Fmoc-(R)- and (S)-β^2-1-Nal]

Each chiral amide (100 mg; 0.23 mmol), obtained in the previous step, was refluxed in concentrated hydrochloric acid (10 mL) until completion of the hydrolysis (based on LC–MS). Then the solution was concentrated, diluted with water (10 mL) and pH was adjusted to 9–10 with 2 M aqueous potassium carbonate. Acetone (10 mL) was added followed by 9-fluorenylmethyl N-succinimidyl carbonate (168 mg; 0.5 mmol). After 1 h acetone was evaporated and the aqueous residue washed twice with diethyl ether. Aqueous layer was acidified with 2 M HCl, extracted with ethyl acetate, dried over $MgSO_4$ and evaporated to give 100 mg of the final product.

^{1}H NMR (500 MHz, DMSO-*d*) δ: 2.87 (p, *J* = 7.0 Hz, 1H), 3.13–3.24 (m, 4H), 4.17 (t, *J* = 7.0 Hz, 1H), 4.20–4.26 (m, 2H), 7.23–7.28 (m, 2H), 7.29–7.32 (m, 1H), 7.33–7.39 (m, 3H), 7.48 (dd, *J* = 6.5 Hz),