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N-alkylated dipeptide amides and related structures as imitations of the melanocortins' active core

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Abstract

Thirty-three low molecular mass structures combining both peptide and peptoid features were prepared and tested on human melanocortin receptors $MC_{1,3–5}R$. Most of them displayed low micromolar activity with preference for diamines, guanidino and 2-naphthyl derivatives compared to monoacetylated, amino and 3-indolyl counterparts. Some contained L- or D-histidine residues, but the change did not influence affinity. QSAR modelling yielded excellent models for the $MC_{3–5}$ receptors explaining $R^2Y = 0.89–0.91$ and predicting $Q^2 = 0.77–0.80$ of the affinity variations. One compound (**12c**) displayed MC_1R selectivity (13-fold and more). An NMR study of **12c** showed that it exists as a mixture of four rotamers at its tertiary amide bonds. Comparisons with earlier data for melanocortin core tetrapeptide analogues indicate that the novel peptide–peptoids interact with the melanocortin receptors in a different way.

Keywords: Melanocortins; Peptidomimetics; Peptoid–peptide hybrids; Solid phase organic synthesis; Structure–activity; QSAR; NMR

1. Introduction

To improve proteolytic stability and oral bio-availability of peptide hormones efforts directed to reduce both molecule size and peptide character is a common approach [24]. An important aspect of this process is the imitation of the 'biologically active' conformation of the natural ligands [11]. Rather than using the extended natural sequences only few amino acid residues long 'active core' is often a starting point for modifications. Active cores of neuropeptides cholecystokinin [22], substance P [2] and bombesin [6] were subjected to changes called 'peptoid design strategy', which resulted in highly active and selective compounds [23]. Active core modifications were applied also in the development of ligands for melanocortin receptors. Thus, macro-heterocyclic β-turn mimetics containing pharmacophoric groups from the melanocortins' active core were prepared [13]. Recently, another study described analogous structures that also possessed melanocortinomimetic activity [3]. We previously reported *N*-alkylaminoacids and their derivatives [30], and reductive amination products [29], which all contained features of the active core of the melanocortins. All these substances showed a moderate activity on melanocortin receptors [18].

Replacement of amino acid residues with *N*-substituted glycines results in so called 'peptoids', which are protease resistant [27], and therefore attractive for pharmaceutical use. However, the peptoid backbone differs from the peptide one, as it lacks both asymmetrical carbons and hydrogen-bond donors [35].

The split-and-mix approach was used to obtain a tripeptoid library related to the active core of the melanocortins. Estimation of binding affinity and deconvolution of the library identified new ligands for the MC_1 receptor and the GRP-preferring bombesin receptor [14]. In a recent study, it

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was found that a tri-peptoid based on agouti-related protein AGRP 111-113 inhibited α-MSH stimulated cAMP generation [36]. Peptide–peptoid hybrids were used to evaluate the importance of the amino acid residues within the active core tetrapeptide of the melanocortins. Systematic replacements with corresponding peptoid residues (including preparation of substances consisting of peptoid units only) were performed and structure–functional activity relationships investigated [17].

Calculation of Ramachandran-type plots suggests that peptoids exist in more conformational states than peptides [35]. Theoretical conformation analysis of a model peptoid using molecular dynamics simulations implied that the α_D form (derived from the fully extended peptide conformation) is the preferred secondary structure both for *cis* and *trans* amide bond orientations in solution [28]. Investigation of the conformations of high affinity tripeptoids by NMR showed that in aqueous solution a 'hydrophobic collapse' (i.e., clustering of side chains) and preferred *trans* geometry of amide bonds takes place. Changing the solvent to DMSO resulted in a more equal distribution of rotamers of the main chain [5].

The present study was devoted to the development of new peptidomimetics in a different way, by combining features of peptides and peptoids. The most active compound was subjected to extensive NMR studies to get insight into its conformations.

2. Materials and methods

2.1. Chemical synthesis

2.1.1. General information

Automated peptide synthesis was made on a *Pioneer* system (Applied Biosystems). Solid phase syntheses were also made on a Nautilus 2400 synthesizer (Argonaut Technologies). Reagents were used without purification and were unless otherwise stated obtained from Aldrich or Fluka. DMF and *N*,*N*-diisopropylethylamine were from Applied Biosystems. PyBroP was from Novabiochem. Exact molecular masses were determined on a Micromass Q-Tof 2 mass spectrometer equipped with an electrospray ion source. LC/MS was performed on a Perkin-Elmer PE SCIEX API 150EX instrument with a Turboionspray Ion Source and Dr. Maisch Reprosil-Pur C18-AQ, 5 μ, 150 mm × 3 mm HPLC column using a gradient formed from water and acetonitrile with 5 mM ammonium acetate additive. Analytical HPLC was performed on a Waters system equipped with Millenium 32 Workstation, 2690 Separation Module, 996 Photodiode Array Detector. Small scale preparative HPLC was carried out on a LKB system consisting of a 2150 HPLC Pump, 2152 LC Controller, 2151 Variable Wavelength Monitor and Vydac RP C_{18} column (10 mm × 250 mm, 90 Å, 201HS1010), the eluent being an appropriate concentration of MeCN in water + 0.1% TFA, flow rate 5 ml/min, detection at 280 nm. Evaporations of solvents were made on a vacuum rotary evaporator at 30 °C and 20 mbar. Freeze-drying was carried out at 0.01 bar on a Lyovac GT2 Freeze-Dryer (Finn-Aqua) equipped with a Trivac D4B (Leybold Vacuum) vacuum pump and a liquid nitrogen trap.

2.1.2. Method 1: 4-nitrophenylcarbonate Wang resin (**1**)

To a suspension of Wang resin (10 g, 11 mmol) in 50 ml CH_2Cl_2, a solution of 4-nitrophenylchloroformate (11 g, 57 mmol) in 150 ml CH_2Cl_2 was added. The suspension was cooled to 0 °C and *N*-methylmorpholine (12.1 ml, 110 mmol) was added in several portions with Vortex-shaking. Shaking and cooling were continued for 30 min. The suspension was then allowed to warm up to room temperature and shaken for further 3 h. The mixture was filtered through a porous glass filter and the resin on the filter was washed with methanol–CH_2Cl_2 (1:1, 5 × 40 ml) followed by CH_2Cl_2 (5 × 40 ml) where after it was dried in vacuo over P_2O_5.

2.1.3. Method 2: N-(Wang resin-oxycarbonyl)-pentamethylenediamine (**2a**)

To a suspension of resin **1** (1.2 g, 1.1 mmol) in 20 ml DMF a solution of 1,5-diaminopentane (0.39 ml, 3.3 mmol) in DMF (5 ml) was added with shaking. The mixture was allowed to stand at room temperature for 20 h with periodical shaking and then filtered, where after the resin on the filter was washed with DMF (5 × 10 ml), methanol (3 × 10 ml) and CH_2Cl_2 (3 × 10 ml). Finally, the resin was dried in vacuo over P_2O_5.

2.1.4. Method 3: N-(Wang resin-oxycarbonyl)-N′-(N-tert-butyloxycarbonyl-3-indolylmethyl)-pentamethylenediamine (**3a**)

Resin **2a** (0.6 mmol) was placed into a Nautilus reaction vessel and a solution of *N-tert*-butyloxycarbonyl-3-indolaldehyde [34] (0.74 g, 3.0 mmol) in 5 ml trimethyl orthoformate was added and the vessel was agitated for 20 h at room temperature. The suspension was then filtered, the resin washed with methanol (3 × 4 ml) and CH_2Cl_2 (3 × 4 ml) and dried in vacuo. Sodium cyanoborohydride (189 mg, 3.0 mmol) was added and carefully mixed with the resin using a spatula. The reaction vessel was attached to the Nautilus system and 5 ml of 2% acetic acid in trimethyl orthoformate was added (in one portion) with immediate intense shaking for 5 min. The suspension was then filtered, the resin washed with methanol, water, methanol and CH_2Cl_2 (in sequence, each solvent 3 × 5 ml) and finally dried in vacuo.

2.1.5. Method 4: N-(Wang resin-oxycarbonyl)-N′-(N-tert-butyloxycarbonyl-3-indolylmethyl)-N′-bromoacetyl-pentamethylenediamine (**4a**)

To resin **3a** (0.6 mmol) a solution of bromoacetic acid (363 mg, 2.4 mmol) and diisopropylcarbodiimide (375 μl, 2.4 mmol) in 4 ml DMF was added. The suspension was agitated for 34 h, filtered, the resin washed with DMF, methanol and CH_2Cl_2 (each solvent 3 × 5 ml) and dried in vacuo.