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Structure based design of simplified analogues of insect kinins

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Abstract—Based on a receptor interaction model, simplified analogues of insect kinins were prepared. The compounds were templated on a conformationally restricted amino piperidinone carboxylate scaffold. The conformation of the analogues was studied by NMR and the biological activity of the compounds was tested in a bio-assay.

1. Introduction

Insect neuropeptides control essential physiological processes such as maturation, water balance, pheromone production and metamorphosis.[1] Natural insect neuropeptides are readily degraded by peptidases; however, neuropeptide analogues, which could resist peptidase degradation would be attractive leads in the development of new insect control agents.

Design of such analogues requires knowledge of the chemical and conformational features of the neuropeptides. Earlier structure-activity studies have shown that the generalised pentapeptide sequence Phe-Xaa-Xbb-Trp-Gly-NH_2 (Xaa = Tyr, Phe, His, Ser or Asn, and Xbb = Ala, Ser or Pro) is present at the C-terminus of a number of insect neuropeptides, which are controlling diuretic and myotropic activity in cockroaches and crickets.[2] Within this active core pentapeptide, the aromatic residues Phe[1] and Trp[4] are both critical for myotropic and diuretic activity as was revealed by an alanine replacement scan. Moreover, by means of NMR and molecular dynamics analysis, a cis-Pro type VI β-turn has been shown to be involved in an active *cyclo*(Ala-Phe-Phe-Pro-Trp-Gly) insect kinin peptide analog centered around the Phe-Phe-Pro-Trp moiety. The possible presence of this turn conformation in the receptor bound state was further corroborated via the synthesis and biological screening of tetrazole containing analogues of these kinins which are known to mimic cis-peptide bonds. Similar, conclusions were obtained using a 4-aminopyroglutamate as a cis-peptide bond inducer.[3]

A receptor interaction model was presented in which it was assumed that the type VI β-turn over residues 1–4 places the side-chains of Phe and Trp on the same side of the structure where they can interact as a continuous aromatic surface with the Malpighian tubule receptor. On the other hand, the side-chains of Phe and Pro lie on the opposite face away from the receptor explaining why these positions are tolerant of considerable modification in the unconstrained structures described above.

Based on this structural information, a small library of 'pseudotetrapeptides' was designed containing the basic features assumed to be important for receptor binding (minimalistic approach): these are the cis-peptide bonds of the type VI β-turn and the side-chains of the Phe and Trp amino acids important for myotropic and/or diuretic activity (Fig. 1), the N-terminal amine function and the C-terminal carboxylate (as well as the glycine amide) were omitted in this approach. We assumed the beta turn properties of the systems could be imposed by means of a turn mimicking amino piperidinone carboxylate system (APC).[4] The side-chains of this APC system (which replaces the residues 2 and 3 in the parent peptide) are of no major importance since according to the receptor model these are diverted away from the receptor. Hence we chose one of the most simple APC systems available (Fig. 1). Phe and Trp side-chains were introduced as simple phenylalkylamides and indolylalkyl amides. Different tether lengths were chosen and also

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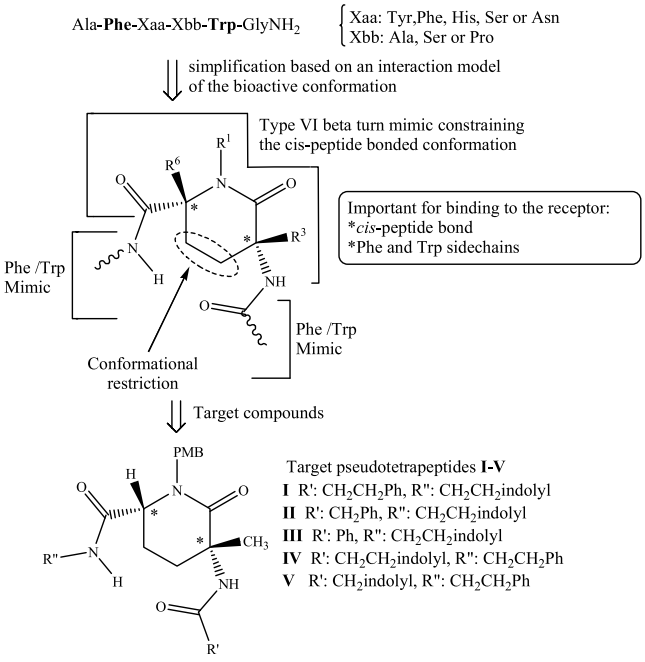


Figure 1. Design approach towards simplified insect kinin analogues.

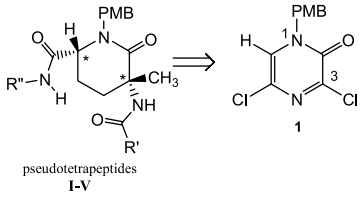
the position of the side-chains (N- or C-terminus) was varied. Compounds of this type, incorporating a β-turn mimic, are of course also interesting candidates for more general broad screening, especially in view of the major importance of the Phe and Trp amino acids in biological processes.

2. Results and discussion

In the following subsections, we will first describe our synthetic approach towards the target analogues **I–V** and discuss structural features of models **I** and **IV** by analysis of the NMR spectra. Finally, a bioassay performed on some of the analogues will be presented.

2.1. Insect kinin analogue synthesis

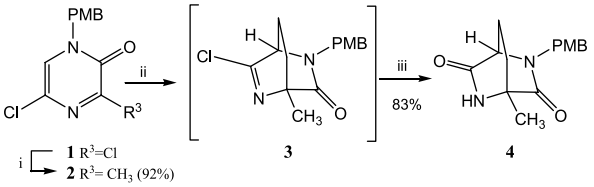
The target compounds **I–V** can be brought back to a 3,5-dichloropyrazinone **1** using chemistry previously developed in our laboratory (Scheme 1).[4] Key steps in the synthesis are a Diels–Alder reaction and a selective methanolysis as will be discussed further.



Scheme 1. Pyrazinone **1** as a precursor for compounds **I–V**.

2.1.1. Synthesis of the APC precursor.

2.1.1.1. Diels–Alder reaction and hydrolysis of the adduct: introduction of the conformational restriction of the APC system. The initially required *N*-(*p*-methoxybenzyl) pyrazinone **1** (Scheme 2) was generated using our



Scheme 2. Generation of bislactam **4**. Reagents and conditions: (i) $(CH_3)_4Sn$, toluene, 110 °C, tetrakis; (ii) ethene (35 atm), 145 °C, toluene, 4 h; (iii) air moisture.

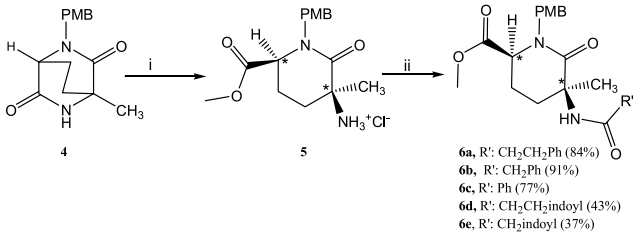
established procedure via cyclisation of 2-[*N*-(4-methoxybenzyl) amino] acetonitrile (in the form of an HCl salt) with oxalyl chloride.[5] The PMB group by now has become the standard protective group for the lactam function in this type of precursors. According to our design approach, the substituent R^3 is believed to be of minor importance for activity, so we have chosen to introduce the easily accessible methyl group at this position.

The 3-methyl analogue **2** was obtained through catalytic cross-coupling of 3,5-dichloro-2(1*H*)-pyrazinone **1** with tetramethyltin using the procedure described before.[6]

The 2(1*H*)-pyrazinone **2** underwent Diels–Alder addition with ethene[7] to produce the imidoyl chloride **3**, which was converted into the bis (lactam) **4** via hydrolysis (Scheme 2). In this reaction step, both the conformational restriction and the cis-relationship of the amine and the carboxylate of the target APC system are imposed.

2.1.2. Conversion of the APC precursor into tetrapeptide analogues.

2.1.2.1. Methanolysis of the bicyclic lactam systems. Introduction of the side-chains of both Phe and Trp occurs in the next steps in the synthesis. Different combinations of alkylphenyl and alkylindolyl amides were envisaged both for the N- and the C-terminus of the APC scaffold. To do so, the bislactam system **4** firstly was selectively converted into the HCl salt **5** using a selective methanolysis reaction via treatment with an HCl–saturated methanol solution for 12 h (Scheme 3).[8] The ammonium chloride salt was trapped as the corresponding amide by dissolving the evaporated residue **5** from the methanolysis in dichloromethane and adding an acyl chloride R′COCl (3 equiv) and Et_3N (4 equiv) under an inert atmosphere. After stirring for 16 h at room temperature, the reaction mixture was purified by column chromatography (silica gel, 10% MeOH/CH_2Cl_2 as eluent) to yield the *N*-acylated products **6a–c** (Scheme 3).



Scheme 3. Reagents and conditions: (i) MeOH/HCl, rt, 16 h; (ii) R′COCl (3 equiv), Et_3N (4 equiv), DCM, rt, 16 h or $R'CO_2H$ (1 equiv), DIEA (5 equiv), CH_3CN, TBTU (1.2 equiv), rt, 4 h.

For the synthesis of compound **6d** with the Trp side-chain mimick, we first activated indol-3-yl propionic acid as its OBt ester by treating it with TBTU and DIEA in CH_3CN. To this solution, product **5** was added. Compound **6d** was isolated in a moderate yield of 43%. The lower yield is due to the recyclisation of the ammonium chloride salt **5** to the bicyclic **4** because the OBt ester in this case reacts slower than the corresponding acyl chlorides used for the preparation of **6a–c**. Using the same conditions as for **6d**, product **6e** was obtained in 37% yield.

2.1.2.2. Hydrolysis of the ester function and subsequent coupling reaction with amines: addition of the fourth amino acid mimicking residue. Direct conversion of the methyl ester **6a** to the target compound **I** with amines proved to be impossible (we tried to do the substitution with different amines under reflux conditions in methanol or pyridine). In order to further functionalise the C-terminus of these compounds, we first needed to convert them into the corresponding acids.

The conversion of **6** into the corresponding acids **7** was successfully effected using LiI in dry pyridine for 16 h at 115 °C (the more standard ester hydrolysis methods using KOH or NaOH in water/methanol mixtures failed in this case). Upon completion of the reaction, the pH of the reaction mixture was adjusted to 2 by adding a dilute HCl solution. After extraction, the acids **7** were further purified by chromatography on silica gel.

The obtained derivatives **7** were activated with TBTU in dry DMF and reacted with different amines again representing side-chains of Phe or Trp. After stirring these mixtures for 16 h at room temperature, the products were extracted several times with dichloromethane and purified by column chromatography to yield the final target compounds **I–V** (Scheme 4).

2.2. NMR analysis of model systems I and IV

NMR techniques were used to study the solution conformation of pseudotetrapeptides **I** and **IV** in $CDCl_3$. The analysis was performed using one dimensional NOE-diff experiments and two-dimensional COSY- and NOESY-techniques.

An APC system can in theory adopt two conformations A or B (Fig. 2). The actual conformation in solution depends on the substitution pattern of the APC system.

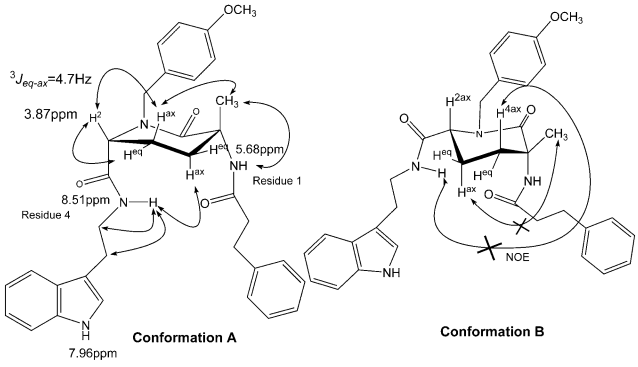


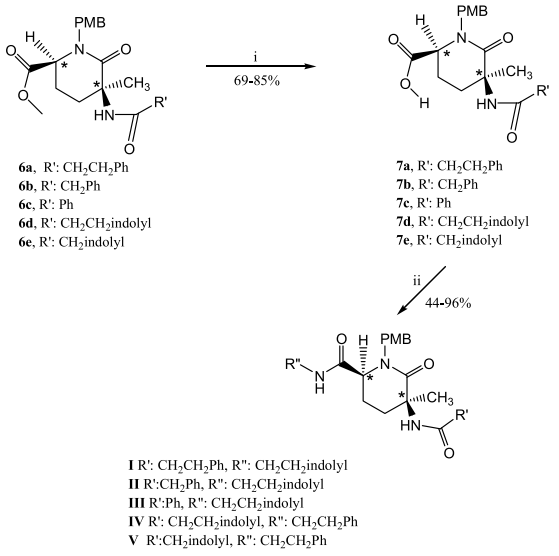
Figure 2. Observed through-space NOE's for pseudopeptide **I**.

In pseudopeptide **I**, the proton H^2 can be identified as the doublet at δ 3.87, which couples with both H^3 protons. One ^3J coupling constant is 4.7 Hz; the other H^3 coupling constant is not resolved in the 1D spectrum though it can be observed in the COSY spectrum. The axial protons H^3 and H^4 can be distinguished from the equatorial ones because they show broader multiplets due to the larger axial–axial coupling constants present in their spectrum. The axial H^3 proton shows a coupling in the NOESY spectrum with the methyl group and with H^2.

Based on the NOE-effect between the NH–amide proton at δ 8.51 and the axial proton H^4, it can be concluded that the conformation A of this pseudotetrapeptide prevails in solution and not conformation B (Fig. 2). The other NOE-effects in Figure 2 (as seen in the NOESY spectrum) are in agreement with the proposed conformation.

The chemical shift of the N*H*CH_2 amide proton shows a temperature dependence of −4.6 ppb/K indicating that it is not completely solvent exposed. This partial solvent shielding could be explained by the fact that a hydrogen bond is present in this structure as would be expected with a turn inducing APC scaffold.[4] However, the shielding is not complete and probably this hydrogen bonded conformation is in equilibrium with other non-hydrogen bonded conformations.

A similar analysis was performed for peptide **IV**. According to our NMR data the peptide **IV** adopts the same conformation as in the case of pseudopeptide **I**. Also in this case the temperature dependence of the chemical shift of the N*H*CH_2 amide proton of −4.7 ppb/K shows partial solvent shielding of this proton.



Scheme 4. Reagents and conditions: (i) LiI, dry pyridine, 115 °C, 16 h; (ii) TBTU, amine, dry DMF, rt.

2.3. Fluid secretion assay

In order to check whether the systems were suitable analogues (in structure and function) of the insect kinins,

biological testing of the products was performed. The exact details of the assay are discussed elsewhere.[1]

The most common way to analyse the rate of fluid secretion by Malpighian tubules (i.e., insect excretory organs) is the 'Ramsay assay'. Tubules are dissected and incubated in vitro in a physiological Ringer solution and the secretory tubule terminal is inserted in a drop of paraffin oil. A small droplet of liquid secretion is then easily visualised (appearing in the oil) and measured under a microscope (diameter can be measured and droplet volume can be calculated) after a given time interval (Schematic representation in Fig. 3).

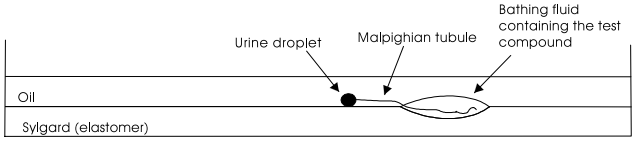


Figure 3. Schematic representation of the diuretic assay system.

All analogues were tested in the assay using 10 μM solutions (Fig. 4).[9] At first sight, all compounds seem to slightly increase the fluid secretion by the tubules compared to the secretion in the absence of analogue (basal level). A paired *t*-test however, reveals that the observed increase is only significant in the case of compounds **I**, **II** and **III**. In compound **IV** the side-chains of the N- and C-terminus were exchanged and, therefore, it shows less resemblance to the natural peptide than the other compounds.

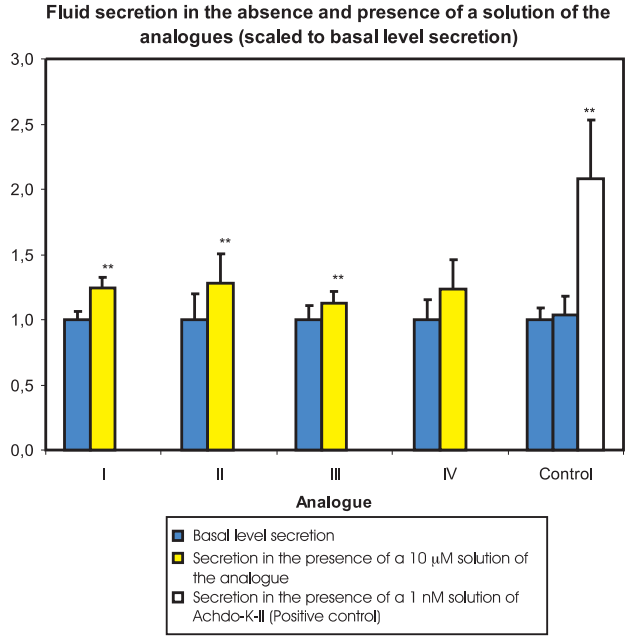


Figure 4. Diuretic assay: tubular fluid secretion in the absence (control; basal level) or presence of the analogues. Values marked with ** are significantly different from the basal level of secretion according to a paired *t*-test. As a positive test Achdo-K-II was added to the control sample.

The stimulation caused by compounds **I**, **II** and **III** should be considered very modest, this can be shown by adding a positive control (Achdo-K-II), which almost doubles the secretion. The fact however, that some stimulation is observed with the analogues is most probably due to meeting the minimal requirements for receptor-binding, which was the initial goal of this project.[10] Hence the APC system is an appropriate scaffold for stabilising the cis-peptide bond and for directing the side-chains of Phe and Trp towards the receptor surface as was stated in the receptor binding model. However, oversimplification of the parent system results in poor bioactivity.

3. Conclusion

In this paper, design and synthesis of simplified insect kinin analogues incorporating APC scaffolds was described. In the target pseudopeptides **I–V** the side-chains important for receptor binding were retained as well as the global beta turn conformation of the peptide backbone. We have shown that these simplified analogues are recognised by the receptor, for which they were developed. A similar minimalistic approach can be applied to other peptides, of which the bioactive conformation is established.

4. Experimental

4.1. General

All melting points are uncorrected, and were measured on an Electrothermal IA 9000 Digital melting point apparatus. Infrared spectra were recorded on a Perkin-Elmer 1720 Fourier transform spectrometer. Mass spectra were run using a Hewlett Packard MS-Engine 5989A apparatus for EI and CI spectra and a Kratos MS 50TC instrument using DS90 data system for exact mass measurements performed in the EI mode at a resolution of 10.000. APCI and ESI spectra were recorded on a Thermo Finnigan LCQ Advantage mass spectrometer. For NMR spectra (δ, ppm) a Bruker AMX 400 and a Bruker Avance 300 spectrometer were used. Analytical and preparative thin-layer chromatography; TLC plates were performed on Alugram Sil G/UV_{254}. Column chromatography was carried out using 70–230 mesh silica gel 60 (E. M. Merck) as the stationary phase.

4.2. Synthesis

4.2.1. Synthesis of 2-(1*H*)-pyrazinones 1 and 2. For the preparation of 3,5-dichloro-2(1*H*)-pyrazinone **1** and its functionalisation to pyrazinone **2**, see Refs. 4 and 7.

5-Chloro-1-(4-methoxybenzyl)-3-methyl-2(1H)-pyrazinone **2**. Yield: 92%; mp 92 °C (EtOH); IR (KBr) cm^{-1}: 1652 (s, CO), 1599 (s, C=N); ^1H NMR ($CDCl_3$, 400 MHz, ppm): 7.26 (d, $J=8$ Hz, 2H, H-2′+H-6′), 7.06 (s, 1H, H-6), 6.90 (d, $J=8$ Hz, 2H, H-3′+H-5′), 4.98 (s, 2H, N–CH_2 of PMB), 3.82 (s, 3H, O–CH_3), 2.49 (s, 3H, 3-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm):160.0 (CO), 158.7 (C–OCH_3 (PMB)), 155.4 (C-3), 130.7 (C-2′+C-6′), 126.6 (C-*ipso* PMB), 126.0 (C-5), 124.3 (C-6), 115.0 (C-3′+C-5′), 55.7 (O–CH_3), 52.3 (N–CH_2 of PMB), 21.3 (CH_3); EIMS *m/z* (%): 264 (M^+, 34), 121 ($CH_3OC_6H_4CH_2^+$, 100); HRMS: Calcd for $C_{13}H_{13}ClN_2O_2$: 264.0664; found: 264.0665.

4.2.2. Diels–Alder reaction and hydrolysis of the adduct: introduction of the conformational restriction in the APC system.

4.2.2.1. Synthesis of adduct 4. Pyrazinone **2** (1 mmol) is dissolved in 20 mL of toluene and the solution is transferred to a stainless steel bomb. The mixture is heated under ethene pressure (35 atm) at 145 °C for 4 h. Upon cooling and removal of ethene, the solvent is evaporated under reduced pressure. The imidoyl chloride intermediate is further hydrolised to the desired compound **4** by stirring it for 16 h in toluene open to air moisture. This compound is further purified by column chromatography (silica gel, MeOH–CH_2Cl_2 5/95).

5-(4-Methoxybenzyl)-1-methyl-2,5-diazabicyclo [2.2.2] octane-3,6-dione **4**. Yield: 83%; mp 156 °C (EtOH); IR (KBr) cm^{-1}: 3270 (w, NH), 1685 (s, CO); ^1H NMR ($CDCl_3$, 400 MHz, ppm): 7.18 (d, $J=8$ Hz, 2H, H-2′+H-6′), 7.17 (s, 1H, NH), 6.85 (d, $J=8$ Hz, 2H, H-3′+H-5′), 4.72 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 4.29 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 3.79 (s, 3H, O–CH_3), 3.87 (br s, 1H, H-4), 1.83–1.75 (m, 4H, CH_2CH_2), 1.51 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): 172.2 (CO), 171.2 (CO), 159.4 (C–OCH_3 (PMB)), 129.5 (C-2′+C-6′), 128.3 (C-*ipso* PMB), 114.2 (C-3′+C-5′), 59.0 (C-4), 58.1 (C-1), 55.2 (O–CH_3), 47.9 (N–CH_2 of PMB), 32.4 (CH_2–C1), 24.7 (CH_2–C4), 18.1 (CH_3); EIMS *m/z* (%): 274 ($M^{+\cdot}$, 92), 153 ($M^{+\cdot}-CH_3OPhCH_2$, 42), 121 ($CH_3OC_6H_4CH_2^+$, 100); HRMS: Calcd for $C_{15}H_{18}N_2O_3$: 274.1317; found: 274.1319.

4.2.2.2. Conversion of the bislactam into APC analogues. *a. Methanolysis reaction*. The bislactam **4** (0.5 mmol) was stirred in 10 mL of HCl–saturated methanol under an inert atmosphere for 16 h. Then methanol and HCl were evaporated to afford the amino piperidinone as the hydrochloride salt **5**. This crude product is used in step b without further purification.

b. Trapping the amine (Method A) (**6a–c**). The hydrochloride salt of the amino piperidinone **5** (0.7 mmol) was dissolved in CH_2Cl_2 and the acid chloride (2.1 mmol) was added followed by Et_3N (2.8 mmol). The reaction mixture was stirred for 4 h at room temperature. After evaporation of the solvent, the residue was subjected to column chromatography on silica gel using 5% EtOAc–CH_2Cl_2 as eluent to afford **6a–c**.

(Method B) (**6d–e**). A solution of indol-3-yl propionic acid (or indole-3-acetic acid) (0.48 mmol) in CH_3CN (3 mL) was treated with DIEA (0.72 mmol), ammonium chloride salt **5** (0.58 mmol) and TBTU (0.58 mmol). The reaction mixture was stirred at room temperature for 18 h. After filtration, the filtrate was evaporated and purified by preparative thin-layer chromatography (silica gel) using EtOAc as eluent to give **6d–e**.

(2S,5S*) Methyl 1-(4-methoxybenzyl)-5-methyl-6-oxo-5[(3-phenylpropanoyl) amino]-2-piperidinecarboxylate* **6a**. Yield: 84%; Method A; mp 103 °C (EtOH); IR (KBr) cm^{-1}: 3310 (w, NH), 1740 (s, CO), 1623 (s, CO); ^1H NMR ($CDCl_3$, 400 MHz, ppm): 7.29–7.16 (m, 5H, Ph-H), 7.09 (d, $J=8$ Hz, 2H, H-2′+H-6′), 6.84 (d, $J=8$ Hz, 2H, H-3′+H-5′), 6.57 (s, 1H, NH), 5.37 (d, $J=15$ Hz, 1H, N–CH_2 of PMB), 3.94 (br d, $J=4$ Hz, 1H, H-2), 3.79 (s, 3H, O–CH_3), 3.74 (s, 3H, CH_3 of ester), 3.63 (d, $J=15$ Hz, 1H, N–CH_2 of PMB), 2.94 (t, $J=7$ Hz, 2H, CH_2–CO), 2.68–2.64 (m, 1H, CH_2CH_2), 2.49–2.45 (m, 2H, CH_2-Ph), 2.08–2.02 (m, 3H, CH_2CH_2), 1.55 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): 173.7 (CO), 172.1 (CO), 172.0 (CO), 159.6 (C–OCH_3 (PMB)), 141.2 (C-*ipso* Ph), 129.9 (C-2′+C-6′), 128.5, 128.3, 126.5 (CH-Ph), 128.2 (C-*ipso* PMB), 114.5 (C-3′+C-5′), 58.4 (C-2), 56.8 (C-5), 55.2 (O–CH_3), 53.1 (CH_3 of ester), 49.8 (N–CH_2 of PMB), 39.3 (CH_2–CO), 32.0 (CH_2-Ph), 30.2 (C-4), 25.1 (CH_3), 23.4 (C-3); EIMS *m/z* (%): 438 ($M^{+\cdot}$, 30), 289 ($M^{+\cdot}-NHCOCH_2CH_2Ph$, 17), 261 ($M^{+\cdot}-CH_3$–CO–$NCH_2PhOCH_3$, 100); HRMS: Calcd for $C_{25}H_{30}N_2O_5$: 438.2147; found: 438.2154.

(2S,5S*) Methyl 1-(4-methoxybenzyl)-5-methyl-6-oxo-5-(phenacylamino)-2-piperidinecarboxylate* **6b**. Yield: 91%; Method A; mp oil; IR (NaCl) cm^{-1}: 3350 (w, NH), 1739 (s, CO), 1647 (s, CO); ^1H NMR ($CDCl_3$, 400 MHz, ppm): 7.36–7.25 (m, 5H, Ph-H), 7.07 (d, $J=8$ Hz, 2H, H-2′+H-6′), 6.83 (d, $J=8$ Hz, 2H, H-3′+H-5′), 6.59 (s, 1H, NH), 5.36 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 3.93 (br d, $J=5$ Hz, 1H, H-2), 3.78 (s, 3H, O–CH_3), 3.74 (s, 3H, CH_3 of ester), 3.61 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 3.52 (s, 2H, CH_2-Ph), 2.64 (br d, $J=14$ Hz, 1H, H-4eq), 2.16–1.99 (m, 3H, CH_2CH_2), 1.54 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm):173.1 (CO), 171.6 (CO), 170.4 (CO), 159.2 (C–OCH_3 (PMB)), 135.0 (C-*ipso* Ph), 129.5 (C-2′+C-6′), 129.2, 128.8, 127.0 (CH-Ph), 128.1 (C-*ipso* PMB), 114.1 (C-3′+C-5′), 58.0 (C-2), 56.8 (C-5), 55.2 (O–CH_3), 52.6 (CH_3 of ester), 49.4 (N–CH_2 of PMB), 44.4 (CH_2-Ph), 29.8 (C-4), 24.7 (CH_3), 23.0 (C-3); EIMS *m/z* (%): 424 ($M^{+\cdot}$, 35), 289 ($M^{+\cdot}-CH_3OC_6H_4CH_2$–$CH_3$, 30), 261 ($M^{+\cdot}-CH_3OPhCH_2$–$CO_2CH_3$, 100); HRMS: Calcd for $C_{24}H_{28}N_2O_5$: 424.1994; found: 424.1998.

(2S,5S*) Methyl 5-benzoylamino-1-(4-methoxybenzyl)-5-methyl-6-oxo-2-piperidinecarboxylate* **6c**. Yield: 77%; Method A; mp oil; IR (NaCl) cm^{-1}: 3393 (w, NH), 1739 (s, CO), 1646 (s, CO); ^1H NMR ($CDCl_3$, 400 MHz, ppm): 7.94 (s, 1H, NH), 7.75 (d, $J=7$ Hz, 2H, *ortho* Ph), 7.43 (t, $J=7$ Hz, 1H, *para* Ph), 7.33 (t, $J=7$ Hz, 2H, *meta* Ph), 7.09 (d, $J=8$ Hz, 2H, H-2′+H-6′), 6.81 (d, $J=8$ Hz, 2H, H-3′+H-5′), 5.39 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 3.97 (br d, $J=4$ Hz, 1H, H-2), 3.72 (s, 3H, O–CH_3), 3.70 (s, H, CH_3 of ester), 3.66 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 2.77 (br d, $J=13$ Hz, 1H, CH_2CH_2), 2.23 (td, $J=13$, 5 Hz, 1H, CH_2CH_2), 2.08–1.86 (m, 2H, CH_2CH_2), 1.68 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): 173.5 (CO), 171.5 (CO), 166.7 (CO), 159.3 (C–OCH_3 (PMB)), 134.9 (C-*ipso* Ph), 131.3, 128.5, 127.0 (CH-Ph), 129.5 (C-2′+C-6′), 127.9 (C-*ipso* PMB), 114.2 (C-3′+C-5′), 58.2 (C-2), 57.1 (C-5), 55.2 (O–CH_3), 52.6 (CH_3 of ester), 49.5 (CH_2 of PMB), 29.8 (C-4), 24.7 (CH_3), 23.0 (C-3); EIMS *m/z* (%): 410 ($M^{+\cdot}$, 25), 289 ($M^{+\cdot}-CH_3OPhCH_2$, 23), 121 ($CH_3OC_6H_4CH_2^+$, 86); HRMS: Calcd for $C_{23}H_{26}N_2O_5$: 410.1827; found: 410.1841.

(2S,5S*) Methyl 5-(3-1H-indol-3-yl-propionylamino)-1-(4-methoxybenzyl)-5-methyl-6-oxo-2-piperidine-carboxylate* **6d**. Yield: 43%; Method B; mp 90 °C (EtOH); IR (KBr) cm^{-1}: 3301 (w, NH), 1740 (s, CO), 1640 (s, CO); ^1H NMR

($CDCl_3$, 400 MHz, ppm): 8.54 (br s, 1H, NH-ind), 7.58 (d, $J=7$ Hz, 1H, H-4″i), 7.32 (d, $J=7$ Hz, 1H, H-7″i), 7.15 (t, $J=7$ Hz, 1H, H-6″i), 7.08 (d, $J=8$ Hz, 2H, H-2′+H-6′), 7.07 (t, $J=7$ Hz, 1H, H-5″i), 6.98 (s, 1H, H-2″i), 6.83 (d, $J=8$ Hz, 2H, H-3′+H-5′), 6.56 (s, 1H, NH), 5.39 (d, $J=15$ Hz, 1H, N–CH_2 of PMB), 3.95 (br d, $J=4$ Hz, 1H, H-2), 3.77 (s, 3H, O–CH_3), 3.73 (s, 3H, CH_3 of ester), 3.66 (d, $J=15$ Hz, 1H, N–CH_2 of PMB), 3.07 (t, $J=7$ Hz, 2H, CH_2-ind), 2.57–2.55 (m, 3H, H-4eq+CH_2–CO), 2.12–2.00 (m, 3H, CH_2CH_2), 1.53 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): 173.1 (CO), 172.2 (CO), 171.5 (CO), 159.1 (C–OCH_3 (PMB)), 136.3 (C-7a″i), 129.4 (C-2′+C-6′), 128.1 (C-3a″i), 127.1 (C-*ipso* PMB), 121.7, 121.6 (C-2″i+C-6″i), 118.9 (C-5″i), 118.6 (C-4″i), 114.7 (C-3″i), 114.1 (C-3′+C-5′), 111.0 (C-7″i), 57.9 (C-2), 56.5 (C-5), 55.1 (O–CH_3), 52.5 (CH_3 of ester), 49.3 (N–CH_2 of PMB), 37.7 (CH_2–CO), 29.9 (C-4), 24.7 (CH_3), 22.9 (C-3), 21.0 (CH_2-ind); EIMS *m/z* (%): 477 ($M^{+\cdot}$, 57), 445 ($M^{+\cdot}-OCH_3$, 16), 261 ($M^{+\cdot}-OCH_3$–$NHCH_2CH_2$indole, 58); HRMS: Calcd for $C_{27}H_{31}N_3O_5$: 477.2244; found: 477.2263.

(2S,5S*) Methyl 5-(2-1H-indol-3-yl-acetylamino)-1-(4-methoxybenzyl)-5-methyl-6-oxo-2-piperidine carboxylate* **6e**. Yield: 37%; Method B; mp oil; IR (NaCl) cm^{-1}: 3311 (w, NH), 1741 (s, CO), 1642 (s, CO); ^1H NMR (C_6D_6, 400 MHz, ppm): 9.27 (s, 1H, NH-ind), 7.74 (dd, $J=6$, 2 Hz, 1H, H-4″i), 7.38 (dd, $J=6$, 2 Hz, 1H, H-7″i), 7.21–7.17 (m, 2H, H-5″i+H-6″i), 6.99 (s, 1H, NH), 6.97 (d, $J=8$ Hz, 2H, H-2′+H-6′), 6.81 (br d, $J=2$ Hz, 1H, H-2″i), 6.70 (d, $J=8$ Hz, 2H, H-3′+H-5′), 5.55 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 3.75 (br d, $J=5$ Hz, 1H, H-2), 3.67 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 3.62 (s, 2H, CH_2–CO), 3.37 (s, 3H, CH_3 of ester), 3.30 (s, 3H, O–CH_3), 2.44 (td(ddd), $J=12$ Hz, 1H, H-4ax), 2.36 (br d, $J=14$ Hz, 1H, H-4eq), 1.82 (br d, $J=14$ Hz, 1H, H-3eq), 1.53–1.51 (m, 1H, H-3ax), 1.40 (s, 3H, CH_3); ^{13}C NMR (C_6D_6, 100 MHz, ppm): 173.9 (CO), 172.0 (CO), 171.9 (CO), 160.2 (C–OCH_3 (PMB)), 137.7 (C-7a″i), 130.2 (C-3a″i), 129.5 (C-2′+C-6′), 128.3 (C-*ipso* PMB), 125.0 (C-2″i), 122.5, 120.1 (C-6″i+C-5″i), 119.6 (C-4″i), 114.9 (C-3′+C-5′), 112.5 (C-7″i), 109.5 (C-3″i), 58.9 (C-2), 57.1 (C-5), 55.3 (O–CH_3), 52.6 (CH_3 of ester), 50.4 (N–CH_2 of PMB), 34.9 (CH_2–CO), 31.1 (C-4), 25.4 (CH_3), 23.7 (C-3); EIMS *m/z* (%): 463 ($M^{+\cdot}$, 34), 307 ($M^{+\cdot}-COCH_2$indol, 18); 261 ($M^{+\cdot}-NHCOCH_2$indol–OCH_3, 18); HRMS: Calcd for $C_{26}H_{29}N_3O_5$: 463.2096; found: 463.2107.

4.3. General procedure for the deprotection of the ester compounds

Ester compound **6** (0.63 mmol) and anhydrous lithium iodide (1.9 mmol) in 10 mL of dry pyridine was refluxed for 16 h under argon. After cooling to room temperature the solution was adjusted to pH=2 by slowly adding dilute HCl solution. The residue was extracted with EtOAc (3×). The combined organics layers were dried over magnesium sulphate and evaporated under reduced pressure. The crude product was subjected to column chromatography on silica gel using 5% MeOH/CH_2Cl_2 as eluent to give the corresponding acid compound **7**.

(2S,5S*) 1-(4-Methoxybenzyl)-5-methyl-6-oxo 5 [(3-phenyl-propanoyl) amino]- piperidine-2-carboxylic acid* **7a**. Yield: 72%; mp 78 °C (EtOH); IR (KBr) cm^{-1}: 3288 (w, NH), 1648 (s, CO); ^1H NMR ($CDCl_3$, 400 MHz, ppm, 55 °C): 8.66[11] (br s, 1H, OH), 7.14–7.06 (m, 7H, Ph-H+ (H-2′+H-6′)), 6.70 (d, $J=7$ Hz, 2H, H-3′+H-5′), 6.20 (br s, 1H, NH), 5.50 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 3.85 (br d, $J=4$ Hz, 1H, H-2), 3.69 (s, 3H, O–CH_3), 3.60 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 2.89–2.88 (m, 2H, CH_2-Ph), 2.55–2.30 (m, 2H, CH_2–CO), 2.42–2.32 (m, 1H, CH_2CH_2), 2.19–2.15 (m, 1H, CH_2CH_2), 1.99–1.89 (m, 1H, CH_2CH_2), 1.81–1.76 (m, 1H, CH_2CH_2), 1.36 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm, 55 °C): 172.5 (CO), 171.7 (CO), 158.7 (C–OCH_3 (PMB)), 140.7 (C-*ipso* Ph), 129.2 (C-2′+C-6′), 128.3 (C-*ipso* PMB), 129.2, 128.3, 126.0 (CH-Ph), 113.8 (C-3′+C-5′), 59.5 (C-2), 56.0 (C-5), 55.1 (O–CH_3), 49.3 (N–CH_2 of PMB), 38.0 (CH_2–CO), 31.3 (CH_2-Ph), 30.3 (C-4), 25.3 (CH_3), 23.3 (C-3); EIMS *m/z* (%): 424 ($M^{+\cdot}$, 3), 406 ($M^{+\cdot}-H_2O$, 6), 380 ($M^{+\cdot}-CO_2H$, 2); HRMS: Calcd for $C_{24}H_{28}N_2O_5$: 424.1993; found: 424.1998.

(2S,5S*) 1-(4-Methoxybenzyl)-5-methyl-6-oxo-5-(phen-acylamino) piperidine-2-carboxylic acid* **7b**. Yield: 82%; mp 113 °C (EtOH); IR (KBr) cm^{-1}: 3259 (w, NH), 1725 (s, CO), 1649 (s, CO); ^1H NMR ($CDCl_3$, 400 MHz, ppm): 7.32–7.19 (m, 5H, Ph-H), 7.14 (d, $J=8$ Hz, 2H, H-2′+H-6′), 7.06 (br s, 1H, NH), 6.83 (d, $J=8$ Hz, 2H, H-3′+H-5′), 5.52 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 3.98 (br d, $J=5$ Hz, 1H, H-2), 3.82 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 3.78 (s, 3H, O–CH_3), 3.50 (s, 2H, CH_2-Ph), 2.45 (td(ddd), $J=14$ Hz, 1H, CH_2CH_2), 2.24 (br d, $J=14$ Hz, 1H, CH_2CH_2), 1.87 (tdd(dddd), $J=14$, 4 Hz, 1H, CH_2CH_2), 1.65 (br d, $J=14$ Hz, 1H, CH_2CH_2), 1.35 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): 172.9 (CO), 172.2 (CO), 171.8 (CO), 159.3 (C–OCH_3 (PMB)), 134.6 (C-*ipso* Ph), 129.7 (C-2′+C-6′), 129.3, 128.8, 127.1 (CH-Ph), 128.2 (C-*ipso* PMB), 114.6 (C-3′+C-5′), 60.3 (C-2), 58.7 (C-5), 55.2 (O–CH_3), 49.6 (N–CH_2 of PMB), 42.7 (CH_2-Ph), 30.8 (C-4), 25.1 (CH_3), 23.0 (C-3); EIMS *m/z* (%): 410 ($M^{+\cdot}$), 396 ($M^{+\cdot}-CH_3$, 18), 121 ($CH_3OC_6H_4CH_2^+$, 100); HRMS: Calcd for $C_{23}H_{26}N_2O_5$: 410.1841; found: 410.1841.

(2S,5S*) 5-Benzoylamino-1-(4-methoxybenzyl)-5-methyl-6-oxo-piperidine-2-carboxylic acid* **7c**. Yield: 69%; mp 84 °C (EtOH); IR (KBr) cm^{-1}: 1730 (s, CO), 1635 (s, CO); ^1H NMR ($CDCl_3$, 400 MHz, ppm): 7.80 (d, $J=7$ Hz, 2H, *ortho* Ph), 7.50 (t, $J=7$ Hz, 1H, *para* Ph), 7.43 (t, $J=7$ Hz, 2H, *meta* Ph), 7.17 (d, $J=8$ Hz, 2H, H-2′+H-6′), 7.14 (br s, 1H, NH), 6.86 (d, $J=8$ Hz, 2H, H-3′+H-5′), 5.46 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 4.10 (br d, $J=5$ Hz, 1H, H-2), 3.79 (s, 3H, O–CH_3), 3.72 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 2.55 (br t(dd), $J=16$ Hz,1H, H-4ax), 2.22 (br d, $J=16$ Hz,1H, H-3eq), 2.06–1.94 (m, 2H, H-4eq+H-3ax), 1.65 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm):172.9 (CO), 171.3 (CO), 167.8 (CO–NH), 159.4 (C–OCH_3 (PMB)), 132.9 (C-*ipso* Ph), 132.3, 128.6, 127.3 (CH-Ph), 129.8 (C-2′+C-6′), 127.9 (C-*ipso* PMB), 114.2 (C-3′+C-5′), 59.6 (C-2), 56.2 (C-5), 55.2 (O–CH_3), 49.5 (N–CH_2 of PMB), 30.9 (C-4), 25.5 (CH_3), 23.1 (C-3); EIMS *m/z* (%): 396 ($M^{+\cdot}$, 4), 352 ($M^{+\cdot}-CO_2H$, 2), 121 ($CH_3OC_6H_4CH_2^+$, 100); HRMS: Calcd for $C_{22}H_{24}N_2O_5$: 396.1685; found: 396.1700.

(2S,5S*) 5-(3-1H-Indol-3-yl-propionylamino)-1-(4-meth-oxybenzyl)-5-methyl-6-oxo-piperidine-2-carboxylic acid*

7d. Yield: 85%; mp 64 °C; IR (KBr) cm^{-1}: 3350 (w, NH), 1634 (s, CO); ^1H NMR ($CDCl_3$, 400 MHz, ppm): 8.04 (br s, 1H, NH-ind), 7.57 (d, $J=7$ Hz, 1H, H-4″i), 7.34 (d, $J=7$ Hz, 1H, H-7″i), 7.19 (t, $J=7$ Hz, 2H, H-5″i+H-6″i), 7.13 (d, $J=8$ Hz, 2H, H-2′+H-6′), 7.06 (s, 1H, H-2″i), 6.83 (d, $J=8$ Hz, 2H, H-3′+H-5′), 6.18 (s, 1H, NH), 5.39 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 4.07 (br d, $J=5$ Hz, 1H, H-2), 3.78 (s, 3H, O–CH_3), 3.73 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 3.12–3.08 (m, 2H, CH_2-ind), 2.64–2.58 (m, 2H, CH_2–CO), 2.34–1.61 (m, 4H, CH_2CH_2), 1.36 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): 173.1 (CO), 172.2 (CO), 171.5 (CO), 159.1 (C–OCH_3 (PMB)), 134.6 (C-7a″i), 129.4 (C-2′+C-6′), 127.8, 127.1 (C-3a″i+C-*ipso* PMB), 121.7, 121.6 (C-6″i+C-2″i), 119.1 (C-5″i), 118.6 (C-4″i), 114.2 (C-3′+C-5′), 111.2 (C-7″i), 109.1 (C-3″i), 57.9 (C-2), 56.5 (C-5), 55.2 (O–CH_3), 49.4 (N–CH_2 of PMB), 37.7 (CH_2–CO), 29.9 (C-4), 24.7 (CH_3), 22.9 (C-3), 21.0 (CH_2-ind); EIMS *m/z* (%): 463 ($M^{+\cdot}$, 10), 445 ($M^{+\cdot}-H_2O$, 11), 130 (indolCH_2^+, 49); HRMS: Calcd for $C_{26}H_{29}N_3O_5$: 463.2107; found: 463.2116.

(2S,5S*) 5-(2-1H-Indol-3-yl-acetylamino)-1-(4-methoxybenzyl)-5-methyl-6-oxo-piperidine-2-carboxylic acid* **7e**. Yield: 75%; mp 104 °C (EtOH); IR (KBr) cm^{-1}: 3389 (w, NH), 1730 (s, CO), 1638 (s, CO); ^1H NMR ($CDCl_3$, 300 MHz, ppm): 9.60 (br s, 1H, OH), 8.73 (s, 1H, NH-ind), 7.56 (d, $J=7$ Hz, 1H, H-4″i), 7.49 (d, $J=7$ Hz, 1H, H-7″i), 7.29 (d, $J=7$ Hz, 2H, H-5″i+H-6″i), 7.09 (d, $J=8$ Hz, 1H, H-2′+H-6′), 7.01 (s, 1H, NH), 6.92 (s, 1H, H-2″i), 6.80 (d, $J=8$ Hz, 1H, H-3′+H-5′), 5.47 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 3.98 (br s, 1H, H-2), 3.82 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 3.74 (s, 3H, O–CH_3), 3.57 (s, 2H, CH_2–CO), 2.41 (br t(dd), $J=17$ Hz, 1H, CH_2CH_2), 2.18 (br d, $J=17$ Hz, 1H, CH_2CH_2), 1.87 (br t(dd), $J=16$ Hz, 1H, CH_2CH_2), 1.53 (br d, $J=17$ Hz, 1H, CH_2CH_2), 1.24 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): 173.1 (CO), 172.8 (CO), 159.7 (C–OCH_3 (PMB)), 137.4 (C-7a″i), 130.1 (C-3a″i), 128.2 (C-2′+C-6′), 124.6 (C-2″i), 122.9, 120.4 (C-6″i+C-5″i), 119.0 (C-4″i), 114.6 (C-3′+C-5′), 111.9 (C-7″i), 108.3 (C-3″i), 59.8 (C-2), 56.0 (C-5), 55.6 (O–CH_3), 50.0 (N–CH_2 of PMB), 33.3 (CH_2–CO), 31.3 (C-4), 25.5 (CH_3), 23.4 (C-3); CIMS *m/z* (%): 450 (MH^+), 432 (MH^+-H_2O, 9), 404 (MH^+-CO_2H, 4), 275 (MH^+-COCH_2indol–H_2O, 100).

4.4. Amide formation: addition of the fourth amino acid mimicking residue

General procedure: to a solution of acid compound **7** (0.35 mmol) in 10 mL of dry DMF at room temperature, TBTU (0.14 mmol) was added followed by the corresponding amine (1.05 mmol). The reaction mixture was stirred for 16 h at room temperature. After extraction with ethyl acetate (3×25 mL), the combined organic layers were dried over magnesium sulphate and evaporated under reduced pressure. Chromatographic separation of the residue on a silica gel column eluting with a dichloromethane/ethyl acetate mixture gave the target compounds **I**–**V**.

(2S,5S*) 1-(4-Methoxybenzyl)-5-methyl-6-oxo-5-[(3-phenylpropionyl) amino]-piperidine-2-carboxylic acid [-2(1H-indol-3-yl) ethyl]-amide* **I**. Yield: 96%; mp 120 °C (EtOH); IR (KBr) cm^{-1}: 3276 (w, NH), 1639 (s, CO); ^1H NMR ($CDCl_3$, 400 MHz, ppm): 8.52 (t, $J=5$ Hz, 1H, NH–CO–C2), 7.95 (s, 1H, NH-ind), 7.69 (d, $J=8$ Hz, 1H, H-4″i), 7.32 (d, $J=8$ Hz, 1H, H-7″i), 7.24–7.18 (m, 5H, Ph-H), 7.14 (t, $J=7$ Hz, 1H, H-6″i or H-5″i), 7.10–7.06 (m, 2H, H-2″i+H-6″i or H-5″i), 7.05 (d, $J=8$ Hz, 2H, H-2′+H-6′), 6.79 (d, $J=8$ Hz, 2H, H-3′+H-5′), 5.68 (s, 1H, NH-C5), 5.29 (d, $J=15$ Hz, 1H, N–CH_2 of PMB), 3.86 (br d, $J=6$ Hz, 1H, H-2), 3.76 (s, 3H, O–CH_3), 3.71–3.64 (m, 2H, CH_2–NH), 3.30 (d, $J=15$ Hz, 1H, N–CH_2 of PMB), 3.11 (t, $J=2$ Hz, 2H, CH_2-ind), 2.96 (t, $J=2$ Hz, 2H, CH_2-Ph), 2.62–2.54 (m, 1H, CH_2–CO), 2.48–2.41 (m, 1H, CH_2–CO), 2.35 (td(ddd), $J=14$, 4 Hz, 1H, H-4ax), 2.03 (br d, $J=14$ Hz, 1H, H-3eq), 1.87 (tdd(dddd), $J=14$, 6, 4 Hz, 1H, H-3ax), 1.44 (br d, $J=14$ Hz, 1H, H-4eq), 1.38 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): 171.7 (CO), 171.1 (CO), 170.6 (CO), 159.1 (C–OCH_3 (PMB)), 140.5 (C-*ipso* Ph), 136.2 (C-7a″i), 129.7 (C-2′+C-6′), 128.6 (C-3a″i), 127.6 (C-*ipso* PMB), 128.5, 128.3, 126.3 (CH-Ph), 122.0 (C-6i″), 121.0 (C-2″i), 119.3, 119.0 (C-4i″+C-5i″), 114.8 (C-3′+C-5′), 113.5 (C-7″i), 110.9 (C-3″i), 60.9 (C-2), 55.3 (C-5), 55.2 (O–CH_3), 48.9 (N–CH_2 of PMB), 40.0 (CH_2–NH), 37.8 (CH_2–CO), 31.3 (CH_2-Ph), 30.4 (C-4), 25.4 (CH_3), 24.8 (CH_2-ind), 23.1 (C-3); EIMS *m/z* (%): 566 ($M^{+\cdot}$, 20), 424 ($M^{+\cdot}-CH_2CH_2$indol, 58), 143 (CH_2CH_2indole, 55); HRMS: Calcd for $C_{34}H_{38}N_4O_4$: 566.2893; found: 566.2910.

(2S,5S*) 1-(4-Methoxybenzyl)-5-methyl-6-oxo-5-(phenacylamino) piperidine-2-carboxylic acid {2-(1H-indol-3-yl)-ethyl}-amide* **II**. Yield: 44%; mp 213 °C (EtOH); IR (KBr, cm^{-1}): 3439 (w, NH), 1634 (s, CO); ^1H NMR ($CDCl_3$, 400 MHz, ppm): 8.45 (t, $J=5$ Hz, 1H, NH–CH_2), 8.10 (s, 1H, NH-ind), 7.62 (d, $J=7$ Hz, 1H, H-4″i), 7.31 (d, $J=7$ Hz, 1H, H-7″i), 7.28–7.24 (m, 5H, Ph-H), 7.13 (t, $J=7$ Hz, 1H, H-6″i), 7.09 (t, $J=7$ Hz, 1H, H-5″i), 7.01 (d, $J=9$ Hz, 2H, H-2′+H-6′), 7.00 (br s, 1H, H-2″i), 6.76 (d, $J=9$ Hz, 2H, H-3′+H-5′), 5.82 (s, 1H, NH-C5), 5.26 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 3.85 (br d, $J=6$ Hz, 1H, H-2), 3.73 (s, 3H, O–CH_3), 3.65 (m, 2H, CH_2–NH), 3.52 (s, 2H, CH_2-Ph), 3.30 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 3.04 (t, $J=8$ Hz, 2H, CH_2-ind), 2.31 (td(ddd), $J=14$, 3 Hz, 1H, H-4ax), 1.99 (br d, $J=14$ Hz, 1H, H-3eq), 1.83 (tdd(dddd), $J=14$, 6, 3 Hz, 1H, H-3ax), 1.41 (br d, $J=14$ Hz, 1H, H-4eq), 1.31 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): 171.0 (CO), 170.7 (CO), 170.6 (CO), 159.1 (C–OCH_3 (PMB)), 136.3 (C-*ipso* Ph), 134.5 (C-7a″i), 129.5 (C-2′+C-6′), 129.4, 128.9, 127.3 (CH-Ph), 128.5 (C-*ipso* PMB), 127.6 (C-3a″i), 122.1 (C-6″i), 121.7 (C-2″i), 119.1 (C-5″i), 118.9 (C-4″i), 114.0 (C-3′+C-5′), 113.1 (C-3″i), 110.9 (C-7″i), 60.3 (C-2), 55.3 (C-5), 55.0 (O–CH_3), 49.0 (N–CH_2 of PMB), 43.2 (CH_2–NH), 30.2 (CH_2-Ph), 25.2 (C-4), 24.6 (CH_3); 23.0 (C-3), 20.9 (CH_2-ind); EIMS *m/z* (%): 553 ($M^{+\cdot}$, 3), 410 ($M^{+\cdot}-CH_2CH_2$indol, 31), 365 ($M^{+\cdot}-NHCH_2CH_2$indol–OCH_3, 5), 121 ($CH_3OPhCH_2^+$, 100); HRMS: Calcd for $C_{33}H_{36}N_4O_4$: 552.2736; found: 552.2746.

(2S,5S*) 5-Benzoylamino-1-(4-methoxybenzyl)-5-methyl-6-oxo-piperidine-2-carboxylic acid {2-(1H-indol-3-yl)-ethyl}-amide* **III**. Yield: 58%; mp 215 °C (EtOH); IR (KBr) cm^{-1}: 3286 (w, NH), 1638 (s, CO); ^1H NMR ($CDCl_3$, 400 MHz, ppm): 8.45 (t, $J=5$ Hz, 1H, NH–CH_2), 8.20 (s, 1H, NH-ind), 7.76 (d, $J=7$ Hz, 2H, *ortho* Ph), 7.63 (d, $J=7$ Hz, 1H, H-4″i), 7.50 (t, $J=7$ Hz, 1H, *para* Ph),

7.43 (t, $J=7$ Hz, 2H, *meta* Ph), 7.25 (d, $J=7$ Hz, 1H, H-7″i), 7.14 (t, $J=7$ Hz, 1H, H-6″i), 7.09–7.04 (m, 4H, H-2′+H-6′+H-5″i+H-2″i), 6.79 (d, $J=8$ Hz, 2H, H-3′+H-5′), 6.49 (s, 1H, NH-C5), 5.25 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 3.85 (br d, $J=6$ Hz, 1H, H-2), 3.73 (s, 3H, O–CH_3), 3.70 (q, $J=7$ Hz, 2H, CH_2–NH), 3.31 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 3.04 (td, $J=7$ Hz, 2H, CH_2-ind), 2.32 (td(ddd), $J=14$ Hz, 3 Hz, 1H, H-4ax), 1.97 (br d, $J=14$ Hz, 1H, H-3eq), 1.88 (tdd(dddd), $J=14$ Hz, 3 Hz, 1H, H-3ax), 1.41 (br d, $J=14$ Hz, 1H, H-4eq), 1.31 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): 171.1 (CO), 170.7 (CO), 170.6 (CO), 159.1 (C–OCH_3 (PMB)), 136.3, 133.5 (C-*ipso* Ph +C-7a″i), 131.9, 128.5, 127.1 (CH-Ph), 129.6 (C-2′+C-6′), 128.7 (C-3a″i), 127.7 (C-*ipso* PMB), 121.8 (C-2″i), 121.1 (C-6″i), 119.8 (C-4″i), 118.6 (C-5″i), 114.2 (C-3′+C-5′), 113.4 (C-3″i), 111.0 (C-7″i), 61.0 (C-2), 55.7 (C-5), 55.2 (O–CH_3), 49.0 (N–CH_2 of PMB), 40.1 (CH_2–NH), 30.3 (C-4), 25.6 (CH_3), 24.7 (CH_2-ind), 23.1 (C-3); EIMS *m/z* (%): 538 ($M^{+\cdot}$, 4), 396 ($M^{+\cdot}-CH_2CH_2$indole, 31), 351 ($M^{+\cdot}-NHCOCH_2CH_2$indole, 5); HRMS: Calcd for $C_{32}H_{34}N_4O_4$: 538.2580; found: 538.2585.

(2S,5S*) 5-(3-1H-Indol-3-yl-propionylamino)-1-(4-methoxybenzyl)-5-methyl-6-oxo-piperidine-2-carboxylic acid phenethyl-amide* **IV**. Yield: 50%; mp 130 °C (EtOH); IR (KBr) cm^{-}: 3280 (w, NH), 1640 (s, CO); ^{1}H NMR ($CDCl_3$, 400 MHz, ppm, 328 K): 8.48 (t, $J=5$ Hz, 1H, NH–CO–C2), 8.31 (br s, 1H, NH-ind), 7.56 (d, $J=7$ Hz, 1H, H-4i″), 7.36 (d, $J=7$ Hz, 1H, H-7″i), 7.26–7.23 (m, 5H, Ph-H), 7.16 (t, $J=7$ Hz, 1H, H-6″i), 7.08 (t, $J=7$ Hz, 1H, H-5″i), 7.05 (d, $J=8$ Hz, 2H, H-2′+H-6′), 6.95 (br d, $J=2$ Hz, 1H, H-2″i), 6.78 (d, $J=8$ Hz, 2H, H-3′+H-5′), 6.08 (s, 1H, NH-C5), 5.28 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 3.84 (br d, $J=6$ Hz, 1H, H-2), 3.74 (s, 3H, O–CH_3), 3.60 (q, $J=13$ Hz, 2H, CH_2–NH), 3.31 (d, $J=14$ Hz, 1H, N–CH_2 of PMB), 3.05 (t, $J=7$ Hz, 2H, CH_2-ind), 2.95 (q, $J=13$ Hz, CH_2-Ph), 2.48 (dt, $J=7$, 2 Hz, 2H, CH_2–CO), 2.25 (td(ddd), $J=14$, 3 Hz, 1H, H-4ax), 1.92 (br d, $J=14$ Hz, 1H, H-3eq), 1.79 (tdd(dddd), $J=14$, 6, 3 Hz, 1H, H-3ax), 1.32 (br d, $J=14$ Hz, 1H, H-4eq), 1.28 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm, 328 K): 172.2 (CO), 171.1 (CO), 170.9 (CO), 159.1 (C–OCH_3 (PMB)), 139.1 (C-7a″i), 135.3 (C-*ipso* Ph), 129.4 (C-2′+C-6′), 128.5 (C-3a″i), 128.8, 128.3, 126.1 (CH-Ph), 127.0 (C-*ipso* PMB), 121.9 (C-6″i), 121.8 (C-2″i), 119.1 (C-5″i), 118.4 (C-4″i), 114.4 (C-3″i), 114.0 (C-3′+C-5′), 111.3 (C-7″i), 61.0 (C-2), 55.1 (C-5), 55.0 (O–CH_3), 49.0 (N–CH_2 of PMB), 40.7 (CH_2–NH), 36.5 (CH_2–CO), 34.9 (CH_2-Ph), 30.3 (C-4), 25.1 (CH_3), 22.9 (C-3), 20.6 (CH_2-ind); EIMS *m/z* (%): 566 ($M^{+\cdot}$, 20), 418 ($M^{+\cdot}-NHCOCH_2CH_2Ph$, 19); HRMS: Calcd for $C_{34}H_{38}N_4O_4$: 566.2893; found: 566.2921.

(2S,5S*) 5-(2-1H-Indol-3-yl-acetylamino)-1-(4-methoxybenzyl)-5-methyl-6-oxo-piperidine-2-carboxylic acid phenethyl-amide* **V**. Yield: 43%; mp 201 °C (EtOH); IR (KBr) cm^{-1}: 3280 (w, NH), 1640 (s, CO); ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): 8.53 (t, $J=5$ Hz, 1H, NH–CH_2), 8.35 (s, 1H, NH-ind), 7.65 (d, $J=7$ Hz, 1H, H-4″i), 7.39 (d, $J=7$ Hz, 1H, H-7″i), 7.29–7.15 (m, 8H, Ph-H+H-6″i+H-5″i+H-2″i), 7.09 (d, $J=8$ Hz, 2H, H-2′+H-6′), 6.80 (d, $J=8$ Hz, 2H, H-3′+H-5′), 6.04 (s, 1H, NH-C5), 5.33 (d, $J=13$ Hz, 1H, N–CH_2 of PMB), 3.85 (br d, $J=6$ Hz, 1H, H-2), 3.75 (s, 3H, O–CH_3), 3.73 (2×d, $J=13$ Hz, 2H, CH_2–CO), 3.67–3.54 (m, 2H, CH_2–NH), 3.36 (d, $J=13$ Hz, 1H, N–CH_2 of PMB), 2.94 (dt, $J=7$ Hz, 2H, CH_2-Ph), 2.35 (td(ddd), $J=14$, 3 Hz, 1H, H-4ax), 1.95 (br d, $J=14$ Hz, 1H, H-3eq), 1.83 (tdd(dddd), $J=14$, 6, 3 Hz, 1H, H-3ax), 1.42 (br d, $J=14$ Hz, 1H, H-4eq), 1.22 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): 171.2 (CO), 171.1 (CO), 170.7 (CO), 159.1 (C–OCH_3 (PMB)), 139.2 (C-*ipso* Ph), 136.4 (C-7a″i), 129.7 (C-2′+C-6′), 128.9, 128.3, 126.1 (CH-Ph), 128.6 (C-3a″i), 126.9 (C-*ipso* PMB), 123.8, 122.6, 120.1, 118.7 (CH-ind), 114.0 (C-3′+C-5′), 111.0 (C-7″i), 108.6 (C-3″i), 61.0 (C-2), 55.3 (C-5), 55.2 (O–CH_3), 49.0 (N–CH_2 of PMB), 40.7 (CH_2-Ph), 35.0 (CH_2–NH), 33.3 (CH_2–CO), 30.3 (C-4), 25.2 (CH_3), 23.0 (C-3); EIMS *m/z* (%): 552 ($M^{+\cdot}$, 20), 396 ($M^{+\cdot}-COCH_2$indole, 28), 121 ($CH_3OPhCH_2^+$, 100); HRMS: Calcd for $C_{33}H_{36}N_4O_4$: 552.2736; found: 552.2742.

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9. Actual concentrations: 8571, 9.05 μM; 8587, 9.29 μM; 8588, 8.83 μM; 8514, 9.71 μM. All compounds were tested as racemates.
10. We assume the diuretic effect is due to stimulation of the same receptors the natural peptides bind to.
11. Broad signals probably due to aggregation effects.