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Bradykinin antagonists modified with dipeptide mimetic β-turn inducers

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Abstract—Bradykinin (BK) is involved in a wide variety of pathophysiological processes. Potent BK peptide antagonists can be developed introducing constrained unnatural amino acids, necessary to force the secondary structure of the molecule. In this paper, we report a structure–activity relationship study of two peptide analogues of the potent B2 antagonist HOE 140 by replacing the D-Tic-Oic dipeptide with conformationally constrained dipeptide mimetic β-turn inducers.

Bradykinin (**I**, BK, Table 1) is an endogenous linear nonapeptide generated by the proteolytic action of kallikreins on high molecular weight kininogen.[1] BK is involved in a wide variety of pathophysiological processes, such as pain, hyperalgesia, and inflammation.[2] Its biological activity is mediated by two subtypes of specific G-protein-coupled receptors (GPCRs) termed B1 and B2, which are expressed in many tissues. Because of its remarkable activities, BK has been deeply studied, and a lot of efforts were aimed at the development of therapeutic agents as competitive antagonists.

One of the most active peptide antagonists is HOE 140 (**II**, Icatibant, Table 1).[3] The C-terminal hydrophobic amino acids in HOE 140 play an important role for its binding interaction to the B2 receptor. Historically, more potent BK antagonists were developed by introducing bulky hydrophobic unnatural amino acids at the C-terminus, forcing the resulting peptide in a β-turn motif. In fact, it was hypothesized that the D-Tic^7-Oic^8 dipeptide at the C-terminus in HOE 140 is crucial for antagonist activity because of its β-turn conformation.[4]

Table 1. Peptide sequences of BK (**I**), HOE 140 (**II**), and of the HOE 140 analogues **III** and **IV**, respectively, containing at positions 7–8 the dipeptide isosters **1a** and **1b**

	Peptide sequence
I	H-Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9-OH
II	H-D-Arg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Thi^5-Ser^6-D-Tic^7-Oic^8-Arg^9-OH
III	H-D-Arg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Thi^5-Ser^6-**1a**$^{7-8}$-Arg^9-OH
IV	H-D-Arg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Thi^5-Ser^6-**1b**$^{7-8}$-Arg^9-OH

Essential for the activity of HOE 140 is a pseudocyclic conformation[5] involving a βII′-turn (positions 6–9) and a βII-turn (positions 2–5) cross-linked by an additional hydrogen bond between the amide hydrogen of Gly^4 and the carbonyl oxygen of D-Tic^7.

Martinez et al.[6] reported that substitution of the D-Tic^7-Oic^8 dipeptide in HOE 140 with constrained mimetics induced a potent B2 receptor agonist activity. Moreover, further modifications resulted in potent and selective B1 antagonist activity.[7]

In order to increase structure–activity relationship data of BK antagonists for the B2 receptor binding, we inves-

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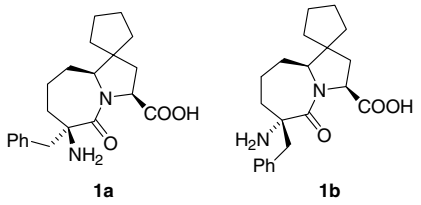


Figure 1. Structure of the dipeptide isosters **1a** and **1b** introduced at positions 7–8 in HOE 140 as β-turn inducers.

tigated the biological activity of the HOE 140 analogues **III** and **IV** (Table 1), in which the unnatural D-Tic[7]-Oic[8] dipeptide is replaced by the constrained mimetics **1a** and **1b**, respectively (Fig. 1).

The design of the proline-derived structures **1a** and **1b** was based on molecular mechanics calculations aimed at evaluating: (a) propensity to induce a reverse turn; (b) the effect of the configuration of the quaternary stereocenter adjacent to the carbonyl group of the 7-membered lactam ring; (c) the extent of similarity between the minimum energy conformations of **1a**,**b** and the D-Tic-Oic fragment of HOE 140. The 5-membered spirocycle linked to proline should mimic the hydrophobic cyclohexyl moiety present in Oic. Molecular mechanics calculations clearly indicated that the dipeptide mimetic **1a** could fulfill all the requirements described above.[8] The epimeric compound **1b** showed to be much less effective in inducing turn-like conformations.

The solid-phase synthesis of peptides **III** and **IV**, following the Fmoc/*t*-Bu strategy, required orthogonal protection of the dipeptide isomers **1a** and **1b** (Scheme 1). The preparation of the methyl ester of the two dipeptide mimetics **2a** and **2b** was performed as previously described.[8] After saponification with LiOH of the methyl esters **2a** or **2b**,[9] we obtained **3a** (90%) and **3b** (96%), respectively. Protection of the amino function with Fmoc-OSu in dioxane yielded **4a** (74%) and **4b** (82%).[10]

The Fmoc-protected dipeptide mimetics **4a** and **4b** were then used in the solid-phase peptide synthesis (SPPS) of the two HOE 140 peptide analogues **III** and **IV**.[11] The Fmoc/*t*-Bu SPPSs were performed on the functionalized Fmoc-Arg(Pbf)-Wang resin in a manual batch synthesizer. Fmoc deprotection was obtained using 20% piperidine in DMF (2× 15 min). For each coupling step, the resin was treated for 1 h with a solution of the Fmoc-protected amino acid (4 equiv) in the presence of TBTU (4 equiv), HOBt (4 equiv), and NMM (8 equiv) in DMF. Couplings were checked by the Kaiser test[12] and a double treatment of the amino acid was performed after a positive test. The two dipeptide isomers **3a** and **3b** were coupled as standard Fmoc-amino acids. After acidic cleavage from the resin, the crude peptides **III** and **IV** were purified by RP-HPLC and then characterized by LC-ESIMS (Table 2).

Table 2. HPLC and ESIMS data for peptides **III** and **IV**

Peptide	HPLC[a] (R_t)	ESIMS[b](m/z)
III	7.7 min (25–65% B in 15 min)	1332.6 (1332.4)
IV	10.6 min (20–60% B in 20 min)	1332.6 (1332.4)

[a] RP-HPLC: Phenomenex Aqua C18 column (150 × 2.0 mm), flow rate 200 μL/min, solvent system A: 0.1% TFA in H_2O, B: 0.1% TFA in CH_3CN.

[b] Found and determined as $[M+H]^+$.

Homologous and heterologous competition binding curves[13] for BK and for the peptides **III** and **IV** were performed in membrane preparations from CHO cells stably transfected with the human kinin B2 receptor (Fig. 2, Table 3).

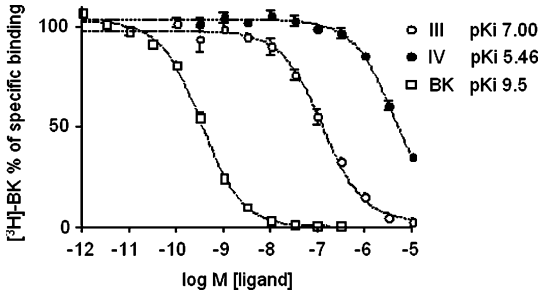
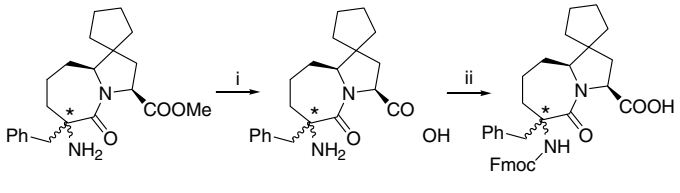


Figure 2. Competition binding curves for BK, peptide **III**, and peptide **IV**.

Table 3. B2 receptor binding affinity and antagonist activity data for the HOE 140 peptide analogues **III** and **IV**

Peptide	hB2 (pK_i)	B2 bioassay (pA2)
III	7.0	5.6
IV	5.5	<5
HOE 140	10.6[a]	9.55

[a] As previously determined in Ref. 14.



Scheme 1. Synthesis of the dipeptide mimetics **4a** and **4b** protected for the Fmoc/*t*-Bu SPPS. Reagents and conditions: (i) LiOH, 56 °C; (ii) Fmoc-OSu, 25 °C.

Moreover, the peptides **III** and **IV** were tested as antagonists in comparison with HOE 140, blocking the BK induced concentration-dependent contractions of the longitudinal smooth muscle of guinea pig ileum[14] (Table 3).

None of the peptides produced agonist effect, up to 10 μM concentration. Concentration–response curve to BK could be significantly antagonized (rightward shifted) only at the highest antagonist concentration tested (10 μM), and the antagonist affinity was calculated as pA2 value of 5.6 and $\leqslant 5$ for **III** and **IV**, respectively (Table 3). As expected by the molecular modeling studies, **III** gave better results than **IV** in terms of binding affinity to the B2 receptor, but both peptides showed a decreased antagonist activity and binding affinity for the B2 receptor, compared to HOE 140.

A detailed NMR investigation was undertaken in order to correlate the biological activity profile of peptides **III** and **IV** with their structural features. The solution conformational analysis was done in SDS micellar environment recording 1D and 2D ^{1}H NMR spectra. Chemical shift assignments[15] of proton spectra were achieved via the standard systematic application of DQF-COSY,[16] TOCSY,[17] and NOESY[18] experiments, using the SPARKY[19] software package according to Wüthrich's procedure.[20] The NOESY spectra revealed the presence of a consistent number of sequential and medium range NOE effects that are suggestive of the presence of folded ordered conformers. In particular, sequential NH-NH effects are diffusively observable together with crucial α-NH(i,i + 2) or α-NH(i,i + 3) which are diagnostic of turn folded structures.

On the basis of the reported NOE data, 3D structures of **III** and **IV** were calculated by simulated annealing in torsion angle space using DYANA software package.[21] The models of peptides **III** and **IV** demonstrated the presence of the β-turn structure involving 2–5 and 6–9 residues. The main difference between the two analogues **III** and **IV** concerns the NOE effects of the phenyl ring of the dipeptide mimetics **1a** and **1b**. NOE data of **III** suggest that the phenyl group is oriented toward the structure of the dipeptide mimetic **1a**, while in peptide **IV** the different stereochemistry of **1b** forces the phenyl ring in a different conformational space.

Comparison of peptides **III** and **IV** with the HOE 140 structure, previously published by Kessler et al.,[4a] allows a structure–activity relationship analysis. Indeed, the most active peptide **III** shows a better overlapping at C-terminal level with HOE 140 (Fig. 3A).

On the other hand, peptide **IV** is forced to extend the dipeptide mimetic **1b** in a region significantly different than that occupied by the Oic residue of HOE 140 (Fig. 3B). Therefore, the activity is strictly related to the secondary structure adopted by the HOE 140 analogues **III** and **IV**.

In conclusion, the constrained dipeptide mimetics **4a** and **4b**, introduced at positions 7–8 of the potent B2 antagonist HOE 140, induced β-turn structures in the two resulting peptides **III** and **IV**. The structure–activity relationship study performed for **III** and **IV** further demonstrated how critical is conformation in the selective interaction of antagonists with BK receptors.

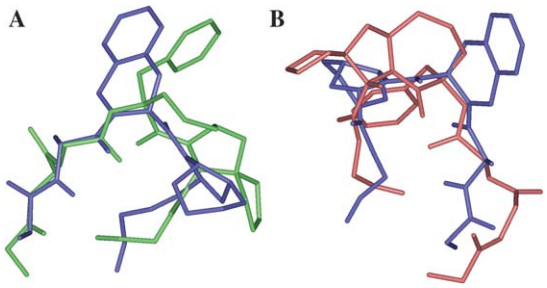


Figure 3. Low energy conformer of peptide **III** (green, panel A) and of peptide **IV** (red, panel B) overlapped with HOE 140[4a] (blue), C-terminus in the βII′-turn conformation.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.01.125.

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9. A solution of LiOH (151 mg, 3.6 mmol) in H_2O (4.5 mL) was added to a solution of **2a** (676 mg, 1.8 mmol) in MeOH/THF (1:1, 9 mL) and the reaction mixture was refluxed for 2 h. The solvent was evaporated and the residue after lyophilization was extracted with DCM at pH 6, affording 576 mg of **3a** (90%) as a white powder. The same procedure, applied to **2b** (450 mg, 1.2 mmol), afforded 412 mg of **3b** (96%) as a white powder. Compound **3a**: IR (DMSO-d_6): 3448.0, 2250.7, 1653.9, 1028.7 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 0.72–0.81 (m, 1H), 1.16–1.47 (m, 4H), 1.46–1.64 (m, 7H), 1.73 (dd, 1H, J = 11.7, 8.8 Hz), 1.77–1.86 (m, 2H), 1.95 (dd, 1H, J = 11.7, 6.8 Hz), 2.85 (d, 1H, J = 13.7 Hz), 3.02 (d, 1H, J = 13.7 Hz), 3.45 (dd, 1H, J = 2.9, 8.8 Hz), 4.14 (dd, 1H, J = 7.8, 8.8 Hz), 5.54 (br s, exchanges with D_2O, 3H), 7.17 (d, 2H, J = 6.8 Hz), 7.20–7.30 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, mixture of conformers): 23.4 (CH_2), 23.6 (CH_2), 28.2 (CH_2), 32.3 (CH_2), 32.5 (CH_2), 37.6 (CH_2), 38.3 (CH_2), 38.8 (CH_2), 46.9 (CH_2), 52.8 (CH), 53.4 (CH), 55.1 (C), 55.7 (C), 56.7 (C), 62.9 (CH), 64.4 (CH), 65.1 (CH), 66.1 (C), 67.0 (C), 67.7 (C), 67.9 (C), 127.3 (CH_{arom}), 128.2 (CH_{arom}), 128.7 (CH_{arom}), 130.3 (CH_{arom}), 130.6 (CH_{arom}), 130.8 (CH_{arom}), 133.7 (C_{arom}), 168.6 (CON), 176.2 (COOH); ESIMS (*m*/*z*): calcd for $[M+H]^+$ 357.23, found 357.21; Anal. Calcd for $C_{21}H_{28}N_2O_3$: C, 70.76; H, 7.92; N, 7.86. Found: C, 70.64; H, 8.03; N, 7.71. Compound **3b**: IR (DMSO-d_6): 3439.5, 2251.4, 2125.8, 1661.3, 1057.0, 1028.6, 1008.4 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 0.98–1.69 (m, 17H, 14 after exchange with D_2O), 1.73 (dd, 1H, J = 8.8, 12.7 Hz), 1.94 (dd, 1H, J = 7.8, 11.7 Hz), 2.94 (d, 1H, J = 13.6 Hz), 3.01 (d, 1H, J = 13.6 Hz), 4.22 (dd, 1H, J = 7.8, 8.8 Hz), 4.33 (d, 1H, J = 10.7 Hz), 7.08–7.33 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): 24.2 (CH_2), 25.3 (CH_2), 25.9 (CH_2), 30.1 (CH_2), 32.3 (CH_2), 34.2 (CH_2), 38.1 (CH_2), 39.8 (CH_2), 49.6 (CH_2), 51.3 (CH), 53.6 (C), 61.9 (C), 65.4 (CH), 127.6 (CH_{arom}), 129.1 (CH_{arom}), 132.0 (CH_{arom}), 135.5 (C_{arom}), 171.9(CON), 176.3 (COOH); ESIMS (*m*/*z*): calcd for 357.23 $[M+H]^+$, found 357.21; Anal. Calcd for $C_{21}H_{28}N_2O_3$: C, 70.76; H, 7.92; N, 7.86. Found: C, 70.61; H, 8.08; N, 7.79.
10. To a stirred solution of **3a** (360 mg, 1 mmol) in dioxane (3 mL) was added a 10% aqueous solution of Na_2CO_3 up to pH 9. The reaction mixture was cooled at 0 °C and Fmoc-OSu (534 mg, 1.6 mmol) was added in small portions, and the whole mixture was stirred at rt for 8 h. The solution was then concentrated to dryness and the resulting oily residue was dissolved in EtOAc (10 mL), washed with 3% NH_4Cl (2× 5 mL), H_2O (5 mL), and brine (5 mL), and dried over Na_2SO_4. Recrystallization from EtOAc/hexane yielded the product **4a** (470 mg, 82%) as a white powder. The same procedure, applied to **3b** (480 mg, 1.35 mmol), afforded **4b** (580 mg, 74%) as a white powder. Compound **4a**: IR ($CDCl_3$): 3371.4, 3066.4, 3030.4, 1715.0, 1631.8 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 1.40–1.53 (m, 3H), 1.60–1.81 (m, 6H), 1.84–2.02 (m, 3H), 2.41 (m, 2H), 3.37 (d, 1H, J = 13.7 Hz), 3.67 (d, 1H, J = 13.7 Hz), 3.88 (d, 1H, J = 8.0 Hz), 4.04–4.30 (m, 3H), 4.15 (dd, 1H, J = 13.7, 6.8 Hz), 4.23 (dd, 1H, J = 13.7, 6.8 Hz), 4.33–4.45 (m, 1H), 4.48–4.62 (m, 1H), 6.62 (br s, exchanges with D_2O, 1H), 6.93–7.06 (m, 2H), 7.10–7.34 (m, 5H), 7.40 (t, 2H, J = 13.7, 6.8 Hz), 7.54 (d, 1H, J = 6.8 Hz), 7.62 (d, 1H, J = 6.8 Hz), 7.77 (d, 2H, J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 21.5 (CH), 24.0 (CH_2), 24.4 (CH_2), 29.5 (CH_2), 33.3 (CH_2), 37.8 (CH_2), 39.4 (CH_2), 47.7 (CH), 54.3 (C), 61.3 (CH), 65.1 (CH), 66.9 (CH_2), 120.3 (CH_{arom}), 125.8 (CH_{arom}), 127.3 (CH_{arom}), 127.5 (CH_{arom}), 128.0 (CH_{arom}), 128.8 (CH_{arom}), 130.1 (CH_{arom}), 136.5 (C_{arom}), 141.7 (C_{arom}), 144.5 (C_{arom}), 154.9 (OCON), 171.8 (CON), 177.1 (COOH); ESIMS (*m*/*z*): calcd for 579.3 $[M+H]^+$, found 579.4. **4b**: IR ($CDCl_3$): 3338.4, 3066.1, 2952.6, 1740.8, 1638.7 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 1.57–1.70 (m, 6 H), 1.78–1.87 (m, 4H), 1.89–1.98 (m, 2H), 2.52–2.62 (m, 2H), 3.53 (d, 2H, J = 13.7 Hz), 3.86 (bd, 1H, J = 6.8 Hz), 4.06 (d, 1H, J = 5.9 Hz), 4.24 (pt, 1H, J = 6.8 Hz), 4.35 (d, 1H, J = 7.8 Hz), 4.39 (d, 1H, J = 9.0 Hz), 4.59 (d, 1H, J = 6.8 Hz), 5.14 (br s, exchanges with D_2O, 1H), 6.90–6.96 (m, 2H), 7.19–7.26 (m, 3H), 7.30–7.49 (m, 4H), 7.60 (d, 2H, J = 6.8 Hz), 7.64 (d, 2H,J = 6.8 Hz), 7.73–7.86 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): 22.5 (CH), 23.7 (CH_2), 24.6 (CH_2), 28.7 (CH_2), 33.1 (CH_2), 37.9 (CH_2), 39.2 (CH_2), 46.8 (CH), 53.8 (C), 60.6 (CH), 65.5 (CH), 66.4 (CH_2), 120.6 (CH_{arom}), 125.2 (CH_{arom}), 125.6 (CH_{arom}), 127.5 (CH_{arom}), 127.9 (CH_{arom}), 128.6 (CH_{arom}), 132.0 (CH_{arom}), 137.1 (C_{arom}), 141.7 (C_{arom}), 142.8 (C_{arom}), 154.7 (OCON), 169.0 (CON), 176.1 (COOH); ESIMS (*m*/*z*): calcd for 579.3 $[M+H]^+$, found 579.5.
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