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Synthesis and conformational analysis of benzimidazole-based reverse turn mimics

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Abstract

New benzimidazole-based tetrapeptide mimics were synthesized and their conformational features were studied by NMR and molecular modeling techniques. All the analyses led to the conclusion that a β-turn is stabilized in both **2** and **3**. Since values of the torsion angles do not allow an univocal definition of the β-turn type, structures **2** and **3** could be assigned to the generic type IV classification.

1. Introduction

The design, synthesis and application of peptidomimetic compounds have been for many years a focal point of a research aimed to develop new therapeutics with peptide-like activity and enhanced resistance towards proteases.[1] Among the different strategies, of particular interest and success has been the replacement of a dipeptide substructure in a natural substrate with a constrained rigid analogue, capable to induce a folding in the peptide chain.[2] Reverse turns are structural motifs commonly found in bioactive peptides that, besides being fundamental in protein folding, play a central role as molecular recognition elements.[3] During the past decade, a large array of highly functionalized nitrogen heterocycles, mainly fused bicyclic lactams, have been synthesized and employed as reverse turn mimics.[4] In search of new, small peptidomimetics that combine the structural rigidity with the ease of synthetic access, we focused on the benzimidazole moiety. Benzimidazole is a very common aromatic pharmacophore, which is present as a rigid scaffold in many different biologically active molecules.[5] We envisaged that the amidine group of the benzimidazole ring could provide a rigid bioisostere of peptidic bond. In our strategy, the N-substituted benzimidazole **1** (Fig. 1) is designed as the dipeptide mimic of the $i+1-i+2$ residues core of β-turn motif. Herein, we report the synthesis of new benzimidazole-based β-turn mimics **2** and **3** and their conformational analysis by molecular modeling calculations[6] and ^{1}H NMR[7] spectroscopy. These structures have been prepared through a straightforward synthetic path, relying on the condensation of *ortho*-phenylenediamine with an aminoacid, thus making in principle the easy preparation of a library of derivatives possible. In this study, we employed L-alanine and L-proline as the amino acid counterpart of condensation with *ortho*-phenylenediamine, realizing the synthesis of **2** (Ac–AlaBidGly–NHMe, Bid = 1*H*-benzo[*d*]imidazole) and **3** (Ac–ProBidGly–NHMe) derivatives. These compounds were selected after preliminary investigation by computational methods (vide infra) for their good β-turn mimic

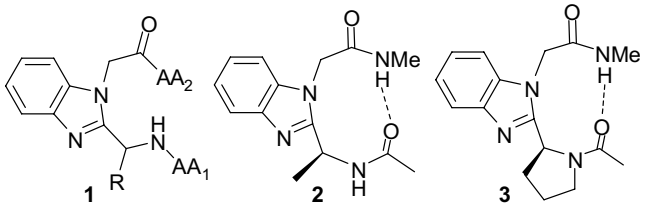

Fig. 1.

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propensity. Compound **3** was chosen to investigate the effect on the reverse turn propensity of an increased steric hindrance and conformational rigidity with respect to **2**.

2. Results and discussion

The synthesis of tetrapeptide mimics is outlined in Scheme 1. Reaction of *ortho*-phenylenediamine with *N*-acetyl-L-alanine produced intermediate **5** which was cyclized in acetic acid to yield the benzimidazole derivative **6**. Alkylation with bromoacetic acid methylester followed by treatment with methylamine allowed to obtain the desired product **2**.[8] The same synthetic strategy was used for the preparation of **3**. After condensation of *N*-acetyl-L-proline with *ortho*-phenylenediamine (DCC/HOBt), amide **7** was cyclized to compound **8**. Alkylation of **8** with bromoacetic acid methylester and subsequent treatment with methylamine afforded **3**.[9]

3. Conformational analysis: computational studies

Evaluation of the ability of **2** and **3** to induce reverse turn conformations was made by means of computational tools,[10] by computing and analyzing characteristic geometric parameters[3a] (Fig. 2).

Two main geometric features are used to characterize a β-turn: a value of the interatomic distance dα ($C\alpha_i$–$C\alpha_{i+3}$) less than 7 Å and the measure of the virtual torsion angle β, defined by C_i–$C\alpha_{i+1}$–$C\alpha_{i+2}$–N_{i+3}, for which $|\beta| < 30°$ is associated with a tight reverse turn, while $|\beta| < 60°$ is usually referred to an open reverse turn.[11] Another feature of β-turns is the presence[12] of a 10-membered ring hydrogen bond between the carbonyl oxygen at C_i and the hydrogen on N_{i+3}. The computational procedure consisted of an unconstrained Monte Carlo/energy minimization conformational search using the molecular mechanics MMFF94 force field[13] both in water and in vacuo. Only conformations within 6 kcal/mol of the global minimum were kept. Results are reported in Table 1 as percentage of conformers which meet the cited requirements. Compound **2** shows moderate percentage of conformers satisfying the β-turn geometry. In vacuo, only three conformers (27%) have a distance dα < 7 Å and a torsion angle $|\beta| < 30°$. The first two lowest energy conformers are β-turn mimics and the next conformer (conf. no. 3), which is not predicted to be a β-turn, is 1.08 Kcal/mol above the minimum. In water, a more favourable situation is revealed: 42% of conformers have dα < 7 Å and $|\beta| < 60°$. In this case, the two first conformers are β-turn mimics as well. Conformer no. 3 is not a β-turn mimic and its energy is 1.56 Kcal/mol above the minimum. A better situation is observed for compound **3**. In this case, no difference between in vacuo and in water simulations is observed. A good percentage (57%) of conformers have a distance dα < 7 Å and 43% have a torsion angle $|\beta| < 60$. Again, the first two lowest energy conformers are good β-turn mimics and the next conformer (conf. no. 3, $\Delta E = 2.88$ Kcal/mol in vacuo, $\Delta E = 2.57$ Kcal/mol in water) is not a β-turn.

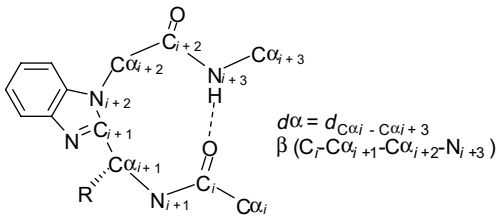
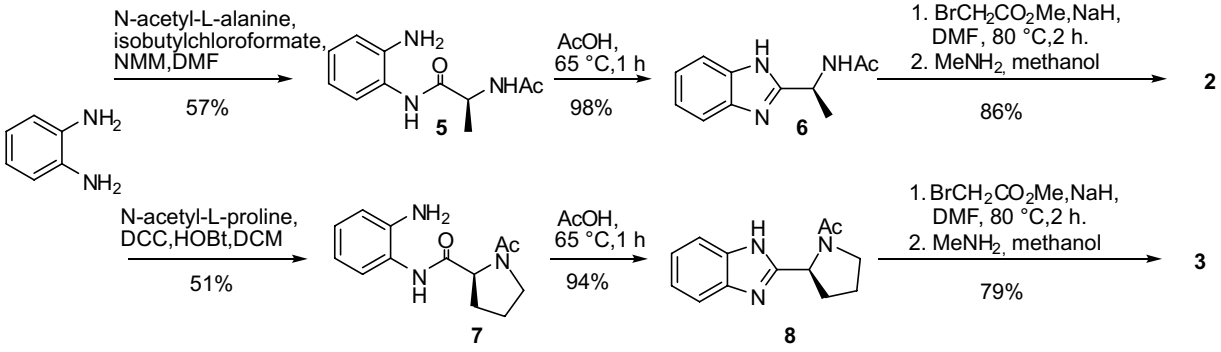


Fig. 2. Definition of parameters to characterize β-turn propensity of BID systems.

Table 1
MC/EM conformational analysis for **2** and **3**

	No. of conf. <6 kcal/mol	% dα < 7 Å	% $\|\beta\| < 30°$	% $\|\beta\| < 60°$	% H bond (10 membered ring)
Compound **2**					
In vacuo	11	27 (3)	27 (3)	36 (4)	27 (3)
In water	12	42 (5)	33 (4)	42 (5)	27 (3)
Compound **3**					
In vacuo	7	57 (4)	43 (3)	43 (3)	43 (3)
In water	7	57 (4)	43 (3)	43 (3)	43 (3)

Results are reported as percentage of conformers which meet the requirements (the occurrence numbers are given in parentheses).



Scheme 1. Synthesis of tetrapeptide mimics **2** and **3**.

Table 2
Characteristics of low-energy conformers calculated for **2** and **3**

	dα (Å)	β (°)	φ_2[a] (°)	ψ_2[b] (°)	φ_3[c] (°)	ψ_3[d] (°)
2	5.38	11.04	−84.40	123.65	−115.26	81.00
3	5.57	−8.72	−68.32	113.98	−127.66	63.79

[a] C_i–N_{i+1}–$C\alpha_{i+1}$–C_{i+1} torsion angle.
[b] N_{i+1}–$C\alpha_{i+1}$–C_{i+1}–N_{i+2} torsion angle.
[c] C_{i+1}–N_{i+2}–$C\alpha_{i+2}$–C_{i+2} torsion angle.
[d] N_{i+2}–$C\alpha_{i+2}$–C_{i+2}–N_{i+3} torsion angle.

Computational studies support the prediction that molecules **2** and **3** would easily achieve a β-turn conformation, with the observation that the presence of a proline residue in **3** is a favourable condition. The calculated backbone geometries for **2** and **3** are reported in Table 2. The φ and ψ backbone torsion angles in residue i+1 and i+2 define the specific β-turn type.[3a] Since analysis of the torsion angles does not allow an univocal classification of the β-turn type, structures **2** and **3** are assigned to the generic type IV[14] classification.

An analysis to evaluate and quantify the similarity of **2** and **3** to standard type β-turns was performed by superimposing the atoms of the amide backbone. Results are reported as scores,[15] for which a value of 1 means a perfect similarity (Table 3). This analysis confirms the impossibility to unambiguously assign a β-turn type, even if a slight preference for a type II β-turn geometry is revealed for both **2** (score 0.87) and **3** (score 0.89). The lowest energy conformers for **2** and **3** are shown in Figure 3.

4. Conformational analysis: ^{1}H NMR studies

Complete characterization of compounds **2** and **3** was realized through mono- and bidimensional NMR spectroscopy. A well established procedure to confirm the presence

Table 3
Similarity analysis of **2** and **3** with standard type β-turns

	I	I′	II	II′	III	III′	V	V′
2	0.80	0.83	0.87	0.80	0.79	0.80	0.65	0.53
3	0.84	0.80	0.89	0.78	0.82	0.78	0.67	0.50

Results are reported as scores, see Ref. 15.

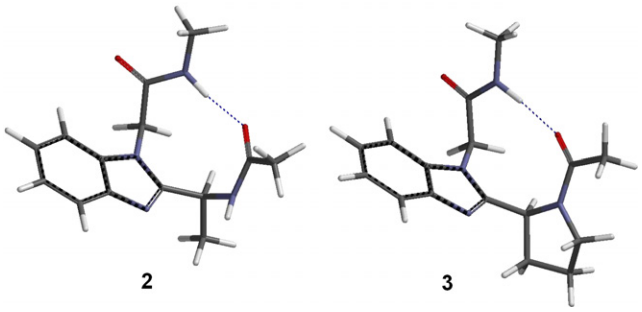


Fig. 3. Lowest energy conformers obtained by MM/MC calculations.

Table 4
Spectroscopic data of tetrapeptide mimics **2** and **3**

		δ DMSO-d_6 (ppm)	Δδ/ΔT (ppb/K)
2	N*H*Me	8.14	−3.2
	N*H*Ac	7.40	−6.3
3	N*H*Me	8.35, 8.24	−4.0

of a β-turn conformation is to investigate the presence of the intramolecular hydrogen bond (C_iO–H–N_{i+3}) by means of variable temperature ^{1}H NMR experiments. The temperature dependence (Δδ/ΔT) of the ^{1}H NMR chemical shifts of the amide protons for **2** and **3** was evaluated in DMSO-d_6. The use of this solvent has been previously reported in the literature.[16] In this solvent, intermolecular hydrogen bonds and those to the solvent are readily cleaved by increasing temperature. In general, for the chemical shift of the N*H* amide proton, a value of Δδ/ΔT below −4.0 ppb/K is considered as an evidence of the absence of intramolecular hydrogen bonding.[6b] All analyses were performed on 3.0 mM solutions to assure the absence of significant molecular aggregation. Variable temperature NMR coefficients were measured by recording four ^{1}H NMR spectra between 298 and 343 K. Results are summarized in Table 4. For compound **2** the N*H*Me amide hydrogen appeared downfield at 8.14 ppm, with a coefficient of −3.2 ppb/K, according to an H-bonded conformation. As expected, the N*H*Ac hydrogen is not involved in H-bond (Δδ/ΔT = −6.3 ppb/K). The ^{1}H NMR spectrum of **3** revealed the presence of two sets of signals in a 3:1 ratio (coalescence was observed at 410 K) for the presence of two conformers. The temperature coefficient values for the N*H*Me hydrogen of both conformers of compound **3** (Δδ/ΔT = −4.0 for both conformers) suggested that these protons were involved in an equilibrium between a hydrogen-bonded and a non-hydrogen-bonded state.

In summary, in this Letter, we have described the synthesis of new tetrapeptide mimics. Both MM calculations and spectroscopic NMR investigations support the conclusion that the Bid-based scaffolds **2** and **3** are good reverse turns. In particular, VT NMR studies indicated the presence of an equilibrium between a hydrogen-bonded state and a non-hydrogen-bonded state. A type IV β-turn can be postulated for both **2** and **3** in their lowest energy state, based on the analysis of the torsion angles.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.12.110.

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8. ^{1}H NMR (400 MHz, DMSO-d_6,): δ 8.14 (br s, 1H), 7.61–7.38 (m, 3H), 7.23–7.21 (m, 2H), 5.00–4.89 (m, 3H), 2.63 (d, $J = 4.5$ Hz, 3H), 2.02 (s, 3H), 1.50 (d, $J = 6.8$ Hz, 3H, CH3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 167.5, 142.3, 137.1, 134.2, 123.1, 122.5, 119.7, 109.1, 52.7, 43.4, 25.6, 22.2, 20.1. HRMS *m*/*z* calcd 274.1430, found 274.1427. Experimental procedure for the preparation of **2** is reported in the Supplementary data.
9. ^{1}H NMR (400 MHz, DMSO-d_6, 410 K): δ 7.88 (br s, 1H), 7.58 (d, $J = 7.2$ Hz, 1H), 7.41 (d, $J = 7.2$ Hz, 1H), 7.19 (m, 2H), 5.25 (m, 1H), 3.85–3.65 (m, 2H), 2.63 (d, $J = 4.5$ Hz, 3H), 2.45–2.10 (m, 2H), 2.02 (s, 3H), 1.95–1.50 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6, 410 K): δ 168.8, 167.6, 156.7, 142.4, 136.0, 122.4, 122.1, 119.1, 110.6, 54.9, 48.0, 46.3, 33.8, 26.1, 24.8, 22.8. HRMS *m*/*z* calcd 300.1586, found 300.1588. Experimental procedure for the preparation of **3** is reported in the Supplementary data.
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14. Type IV β-turns are defined as those having two or more angles which differ by at least 40° from the definitions of β-turns types I, I′, II, II′, III and III′.
15. Scores are reported as obtained by the similarity analysis function implemented in the Spartan'06 software. The score is defined as $[(1 - R^2)/N]$, where R^2 is the rms distance between template and molecule centers and N is the number of similarity centers.
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