

Synthesis and conformational analysis of benzimidazole-based reverse turn mimics

Giordano Lesma, Alessandro Sacchetti*, Alessandra Silvani

Dipartimento di Chimica Organica e Industriale, Università degli Studi di Milano, via G. Venezian 21, 20133 Milano, Italy

Received 21 November 2007; revised 12 December 2007; accepted 19 December 2007

Available online 25 December 2007

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. © 2007 Elsevier Ltd. All rights reserved.

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 calculations⁶ and ¹H NMR⁷ spectroscopy. These structures have been prepared through a straightforward synthetic path, relying on the condensation of *ortho*-phenylenediamine with an amino acid, 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

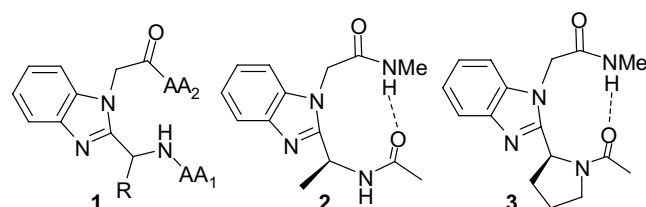


Fig. 1.

* Corresponding author. Tel.: +39 250314081; fax: +39 250314078.

E-mail address: alessandro.sacchetti@unimi.it (A. Sacchetti).

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 $d\alpha$ ($C_{\alpha_i}-C_{\alpha_{i+3}}$) 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 $|\beta| < 30^\circ$ is associated with a tight reverse turn, while $|\beta| < 60^\circ$ is usually referred to an open reverse turn.¹¹ Another feature of β -turns is the presence¹² 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 field¹³ 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

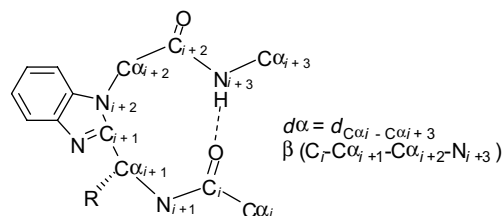


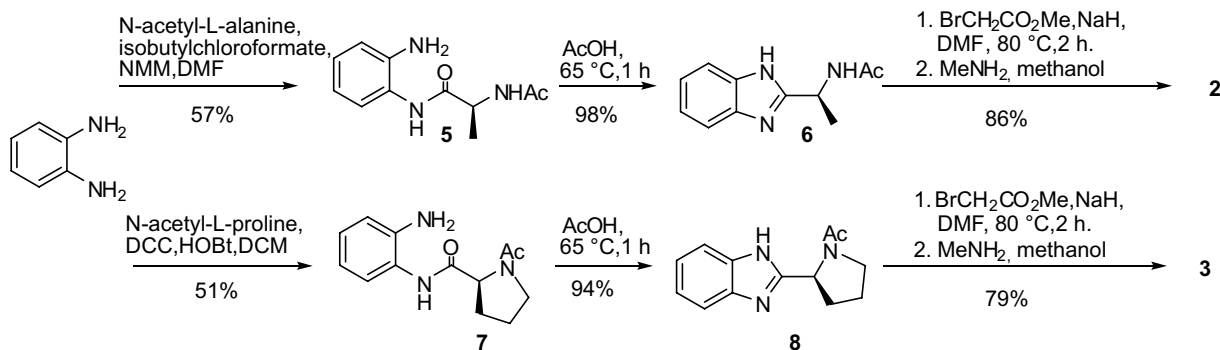
Fig. 2. Definition of parameters to characterize β -turn propensity of BID systems.

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

	No. of conf.	% $d\alpha < 7$ Å	% $ \beta < 30^\circ$	% $ \beta < 60^\circ$	% 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).

distance $d\alpha < 7$ Å and a torsion angle $|\beta| < 30^\circ$. 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 $d\alpha < 7$ Å and $|\beta| < 60^\circ$. 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 $d\alpha < 7$ Å and 43% have a torsion angle $|\beta| < 60^\circ$. 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.



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

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

	d α (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 α _{i+1}–C_{i+1} torsion angle.

^b N_{i+1}–C α _{i+1}–C_{i+1}–N_{i+2} torsion angle.

^c C_{i+1}–N_{i+2}–C α _{i+2}–C_{i+2} torsion angle.

^d N_{i+2}–C α _{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 IV¹⁴ 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: ¹H 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.

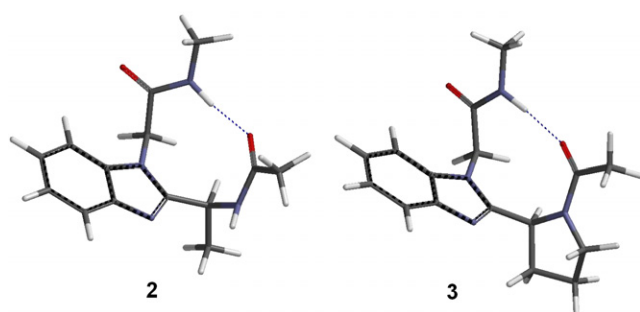


Fig. 3. Lowest energy conformers obtained by MM/MC calculations.

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

		δ DMSO- <i>d</i> ₆ (ppm)	$\Delta\delta/\Delta T$ (ppb/K)
2	NHMe	8.14	−3.2
	NHAc	7.40	−6.3
3	NHMe	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 ¹H NMR experiments. The temperature dependence ($\Delta\delta/\Delta T$) of the ¹H 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 NH amide proton, a value of $\Delta\delta/\Delta T$ 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 ¹H NMR spectra between 298 and 343 K. Results are summarized in Table 4. For compound **2** the NHMe 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 NHAc hydrogen is not involved in H-bond ($\Delta\delta/\Delta T = -6.3$ ppb/K). The ¹H 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 NHMe hydrogen of both conformers of compound **3** ($\Delta\delta/\Delta T = -4.0$ 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](https://doi.org/10.1016/j.tetlet.2007.12.110).

References and notes

- For reviews, see: (a) Gentilucci, L.; Tolomelli, A.; Squassabia, F. *Curr. Med. Chem.* **2006**, *13*, 2449–2466; (b) Perez, J. J.; Corcho, F.; Llorens, O. *Curr. Med. Chem.* **2002**, *9*, 2209–2229; (c) Bursavich, M. G.; Rich,

- D. H. *J. Med. Chem.* **2002**, *45*, 541–558; (d) Haubner, R.; Finsinger, D.; Kessler, H. *Angew. Chem., Int. Ed.* **1997**, *36*, 1374–1389.
2. For reviews, see: (a) Ohfuné, Y.; Shinada, T. *Eur. J. Org. Chem.* **2005**, *24*, 5127–5143; (b) Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219–3232; (c) Gibson, S. E.; Guillo, N.; Tozer, M. J. *Tetrahedron* **1999**, *55*, 585–615; (d) Hanessian, S.; McNaughton-Smith, G.; Lombart, H.-G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789–12854.
3. (a) Ball, J. B.; Hughes, R. A.; Alewood, P. F.; Andrews, P. R. *Tetrahedron* **1993**, *49*, 3467–3478; (b) Grison, C.; Coutrot, P.; Genève, S.; Didierjean, C.; Marraud, M. *J. Org. Chem.* **2005**, *70*, 10753–10764; (c) Trabocchi, A.; Occhiato, E. G.; Potenza, D.; Guarna, A. *J. Org. Chem.* **2002**, *67*, 7483–7492; (d) Chakraborty, T. K.; Srinivasu, P.; Vengal Rao, R.; Kiran Kumar, S.; Kunwar, A. C. *J. Org. Chem.* **2004**, *69*, 7399–7402.
4. (a) Perdih, A.; Kikelj, D. *Curr. Med. Chem.* **2006**, *13*, 1525–1556 and references cited therein; (b) Freidinger, R. M. *J. Med. Chem.* **2003**, *46*, 5553–5566; (c) Cordero, F. M.; Pisaneschi, F.; Meschini Batista, K.; Valenza, S.; Machetti, F.; Brandi, A. *J. Org. Chem.* **2005**, *70*, 856–867; (e) Bittermann, H.; Gmeiner, P. *J. Org. Chem.* **2006**, *71*, 97–102; (f) Andreu, D.; Ruiz, S.; Carreño, C.; Alsina, J.; Albericio, F.; Jiménez, M. A.; de la Figuera, N.; Herranz, R.; García-López, M. T.; González-Muñiz, R. *J. Am. Chem. Soc.* **1997**, *119*, 10579–10586.
5. (a) Balboni, G.; Guerrini, R.; Salvadori, S.; Negri, L.; Giannini, E.; Bryant, S. D.; Jinsmaa, Y.; Lazarus, L. H. *J. Med. Chem.* **2005**, *48*, 8112–8114; (b) Lopez-Rodriguez, M. L.; Benhamu, B.; Morcillo, M. J.; Tejada, I.; Avila, D.; Marco, I.; Schiapparelli, L.; Frechilla, D.; Del Rio, J. *Bioorg. Med. Chem.* **2004**, *12*, 5181–5191; (c) Sawada, Y.; Kayakiri, H.; Abe, Y.; Mizutani, T.; Inamura, N.; Asano, M.; Hatori, C.; Aramori, I.; Oku, T.; Tanaka, H. *J. Med. Chem.* **2004**, *47*, 2853–2863; (d) Balboni, G.; Salvadori, S.; Guerrini, R.; Negri, L.; Giannini, E.; Jinsmaa, Y.; Bryant, S. D.; Lazarus, L. H. *J. Med. Chem.* **2002**, *45*, 5556–5563; (e) Pascual, D.; Giron, R.; Alsasua, A.; Benhamu, B.; Lopez-Rodriguez, M. L.; Martin, M. I. *Eur. J. Pharm.* **2003**, *462*, 99–107; (f) Balboni, G.; Guerrini, R.; Salvadori, S.; Bianchi, C.; Rizzi, D.; Bryant, S. D.; Lazarus, L. H. *J. Med. Chem.* **2002**, *45*, 713–720.
6. (a) Leach, A. R. *Molecular Modelling: Principles and Applications*; Prentice-Hall, 2001; (b) Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 512–523.
7. Gellman, S. H.; Dado, G. P.; Liang, G.; Adams, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 1164–1173. and references cited therein.
8. ¹H 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). ¹³C NMR (100 MHz, CDCl₃): 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. ¹H 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). ¹³C 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](#).
10. *Spartan'06*, Wavefunction, Inc. Irvine, CA.
11. Belvisi, L.; Bernardi, A.; Manzoni, L.; Potenza, D.; Scolastico, C. *Eur. J. Org. Chem.* **2000**, 2563–2569.
12. This was evaluated by means of the ‘hydrogen bonds’ function implemented in the software, according to which hydrogen bonds are defined as non-bonded contacts between nitrogen or oxygen and a hydrogen attached to nitrogen or oxygen, separated by a distance ranging from 1.6 Å to 2.1 Å and making an X–H–Y (X, Y = N, O) angle >120°.
13. Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490–519.
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.
16. (a) Chakraborty, T. K.; Uday Kumar, S.; Mohan Krishna, B.; Sarma Dattatreya, G.; Kiran Udaya, M.; Jagadeesh, B. *Tetrahedron Lett.* **2007**, *48*, 6945–6950; (b) Crisatu, P.; Martin, M. T.; Tran Huu Dau, M. E.; Vors, J. P.; Zhu, J. *Org. Lett.* **2004**, *6*, 3183–3186.