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Triazolo-β-Aza-ε-Amino Acid and Its Aromatic Analogue as Novel Scaffolds for β-turn Peptidomimetics

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Introduction of a conformationally constrained nonpeptide isostere into peptide backbones in order to achieve desirable secondary structures along with pharmacologically viable peptide-based drug candidates is of great interest in recent time. Among the various secondary structures,2-3 β-turns3 are important targets for mimicry, both because they serve as recognition sites in peptides and proteins as well as they allow a protein chain to fold back upon itself to form a compact structure.4 Considerable efforts have thus been invested in delineating the impact of appended molecular scaffolds in one hand and nucleating turn mimics on the other hand, on the conformational preferences of proteins and peptides in solution.5 Despite an exponential growth on the development of constrained non-peptidic molecular scaffolds, very few peptidomimetic drugs have been developed, necessitating an overhaul in the existing design principles.1-3

As a part of our ongoing research efforts on the design of unnatural biomolecular building blocks4 via click chemistry and β-turn peptidomimetics,4c we report herein on the simple synthesis and application of triazolo-β-aza-ε-amino acid (1, \textsuperscript{Alt}TAA) and its aromatic analogue (2, \textsuperscript{ArTAA}) as new and novel constrained molecular scaffolds (Fig. 1). Similar to the sugar amino acids,3\textsuperscript{1-3} the rigid frameworks of pseudo-aromatic triazole units prompted us to use triazolo amino acids as novel molecular scaffolds in peptidomimetic studies.3 These two molecules with constrained backbone angles, \(\omega(i)\) and \(\phi(i+1)\), are expected to induce folded conformations in linear peptides. The triazolo amino acids are advantageous with respect to their metabolically inertness, easy associability with biological targets and tolerance to various reaction conditions used in peptide synthesis. Moreover, the triazolo unit acts as trans-amide mimetic which makes the scaffolds more prone to nucleate β-turn structure while present in a short peptide backbone. Though the click chemistry has been utilised in mimicking the protein’s secondary structures, to the best of our knowledge, the triazolo amino acids as scaffold has not been explored.5 We envisioned that upon incorporation of \textsuperscript{Alt}TAA/\textsuperscript{ArTAA} into backbone a linear peptide, such as, Leu-enkephalin analogue, might adopt β-turn conformation.3,7
The synthesis of the aliphatic triazolo amino acid, $^{14}$TAA (1, Fig. 1) proceeded through a novel click reaction path followed by hydrolysis of azido ester (11) and then reduction of the azide (12) (Scheme 1). The aromatic triazolo amino acid (2, $^{14}$TAA, Fig. 1) was synthesized in a similar way via a click chemistry protocol (ESI†, Scheme S2). The amino acid scaffolds were characterised by NMR, mass spectrometry, and single crystal X-ray diffraction analysis for scaffold 2. 

The crystal structure analysis of scaffold 2 (mp 168 °C; chiral space group P2$_1$2$_1$2$_1$) revealed that the aminophenyl unit was 25.1° out of plane with respect to triazole unit. Overall the scaffold adopted a hairpin shape wherein the two hairpins were linked with a signature of turn conformation (Fig. 2C). Therefore, the origin of chirality was the restricted rotation of out-of-plane aminophenyl unit about C$_1$-C$_2$ (characteristic of tyrosine) indicating a β-sheet like structure with 20% turn conformation (Fig. 3A). 

Next, the secondary structure of peptide 3, a Leu-enkephalin analogue, was estimated by recording its CD spectrum in methanol which showed a strong positive band at 206 nm and a negative band at 191 nm indicating a type II β-turn conformation (Fig. 3A). The peptide secondary structure estimation using CD estima program$^{38}$ showed 100% β-turn structure in peptide 3 the existence of which implied the possible presence of intramolecular H-bonds between the peptide strands. CD spectrum also indicated a predominantly type II β-turn conformation in fluorescent pentapeptide 5 (Fig. 3A). Moreover, the signature of aromatic π-π stacking interaction between Phe and Tyr in peptide 3 was also evident from the appearance of a positive band at 217 nm, 8a,c The tripeptide 4 containing the aromatic scaffold in the backbone showed a broad negative peak at 223 nm and a positive peak at 283 (characteristic of tyrosine) indicating a β-sheet like structure with 20% turn conformation (Fig. 3A). The less propensity of the tripeptide 4 for adopting a fully turn structure might be because of short peptide chain length and high rigidity of the scaffold 2 which in turn conformation might be acting as a β-turn mimic like β-sheet folding nucleator. 

To probe the intramolecular H-bonding in all peptides we used IR and variable temperature NMR (VT NMR)
spectroscopy. Thus, the presence of intramolecular H-bonded and free amide -NH stretching absorptions at 3281-3313 and 3409-3440 cm\(^{-1}\), respectively, in the IR spectra of all the peptides also supported the turn structures (ESI†, Section 8).\(^9\)

From the VT-NMR experiment, strong intramolecular H-bonding involving the amide NH (of Phe) at i+3 was observed in peptide 3 supporting the type II β-turn structure in peptide 3 (ESI†, Section 8).\(^10\) The amide NHs of tripeptide 4 containing the aromatic amino acid scaffold 2 showed medium to weak intramolecular H-bonding ability. The amide NH (of scaffold) and Leu-NH of both the termini in peptide 5 showed strong intramolecular H-bonding supporting the β-turn structure.

The NOESY and ROESY spectra of peptide 4 revealed that the scaffold itself remained in a hairpin shape with interactions among the Triazole-CH/Aromatic-H; PheNH/ Aromatic-H; Aromatic-H/Tyr-OH leading to overall turn like shape. The NOESY and ROESY spectra of peptide 5 showed the interaction between H\(_2\) (of TPh\(_2\)Ala\(_{34}\))/OMe-H (of TPh\(_2\)Ala\(_{34}\)); TPyAr-H (of TPyAla\(_{34}\))/CH\(_2\) (N-terminal) of scaffold, triazole-CH, LeuNH (C-terminal), CH\(_2\) (N-terminal) of scaffold/LeuNH (C-terminal) which supported the turn structure with the pyrene inside the loop closer to the triazole unit of the scaffold (ESI†, Section 8).

To gain insight into the conformation observed in peptide 3-5 molecular dynamics (MD) simulations were carried out using Schrodinger Macromodel (Maestro vs. 9.0) software package with OPLS 2005 force field.\(^3, 11\) The superimposition of eight minimum energy conformations out of 20 generated structures revealed that the peptide 3 took type II β-turn structure stabilized by backbone H-bonding involving the carbamate >CO at i and the amide NH (of Phe) at i+3 and aromatic–aromatic stacking which was also supported by CD, IR and VT-NMR study (Fig. 4A).\(^3\) The folded conformation with close proximity of the parallelly oriented Phe and Tyr suggested that the peptide 3 might serve as a potential ligand for the δ-receptor.\(^12\) The short chain tripeptide 4 showed some turn structure having backbone H-bond between carbamate >C=O at i and amide NH (of scaffold) at i + 2 which is also possible as was revealed from a VT-NMR study reflecting turn structure. The existence of 20% turn structure in peptide 4 revealed from the CD spectroscopic study was thus supported by macromodel study. Moreover, it was stabilized by a second side chain H-bonding involving –OH of Tyr at N-terminus and amide NH of Phe at C-terminus (ESI†, Section 10). The type II β-turn structure as was evident from spectroscopic studies was also supported by a MD simulation for peptide 5 (Fig. 4B).

Finally, we examined the possibility of photophysical interaction between the terminal triazolyl unnatural amino acids in fluorescent pentapeptide 5 in methanol. The UV-visible and fluorescence spectra of the TPh\(_2\)Ala\(_{34}\) containing dipeptide (34) and TPyAla\(_{34}\) containing dipeptide (40) revealed that the fluorescence spectrum of former overlapped significantly with the absorption spectrum of the latter (ESI†, Section 12). Moreover, the peptide 5 containing these two amino acids could selectively be excited at 300 nm (λ\(_{\text{max}}\)\text{abs} of TPh\(_2\)Ala\(_{34}\)) where
there was very low absorbance of TPhenAlaDo. Therefore, these two triazolyl amino acids should form a FRET pair in our designed pentapeptide 5 where the conceptual donor amino acid TPhenAlaDo and TPyAlaDo acted as FRET donor and acceptor, respectively. With this observation we turned our attention to study the FRET process in detail. Thus, when excited at absorption maximum of the donor, TPhenAlaDo, increased from that of the free acceptor emission by almost four-five times in presence of donor, TPyAlaDo, in peptide 5 decreased almost three times of that of the free donor fluorescence in presence of acceptor, TPyAlaDo. This ratiometric change in fluorescence intensity of donor/acceptor revealed the visual evidence of FRET process from TPhenAlaDo to TPyAlaDo in peptide 5 (Fig. 5A). The calculated Förster radius (R0), the efficiency of energy transfer (E) and the donor-acceptor distance (r) were found to be 31 Å, 85% and 28 Å, respectively. The occurrence of FRET process was also evident from a time resolved fluorescence study wherein we observed a decrease in donor life time (TPhenAlaDo; λem = 293 nm, λexc = 370 nm) from 13.7 ns to 2.0 ns. More interestingly, the lifetime of acceptor (TPyAlaDo; λexc = 293 nm, λem = 400 nm) in presence of donor was found to increase from 18.2 ns (in absence of donor) to 19.0 ns (in presence of donor) evidencing the FRET process (Fig. 5B, and ESI†, Section 13).

Conclusions

In conclusion, the easily accessible aliphatic and aromatic triazolo amino acids 1 and 2, respectively, were introduced for the first time, as β-turn-mimetic constrained molecular scaffolds. The structural and conformational analysis of Leu-enkephalin analogue peptide 3 and fluorescent peptide 5 by various spectroscopic techniques and MD simulation studies established well-defined type II β-turn structure induced by the novel β-turn-mimetic constrained molecular scaffold, the triazolo amino acid 1. Moreover, we established the FRET process in peptide 5 containing a new class of fluorescent unnatural triazolyl amino acids at the two termini. Under study are the explorations of turn mimetic amides of these molecular scaffolds which might lead to the generation of a new family of distamycin analogues.

This work was funded to S. S. Bag by the CSIR [01(2330)/09/EMR-II], Govt. of India. SJ and AY are thankful to CSIR for their fellowships. We are thankful to Professor S. Ghosh and Mr. Mihitoshi Dey, Department of Biotechnology, for using the CD spectropolarimeter facility.

Notes and references

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Electronic Supplementary Information (ESI) available: [Synthesis, characterisation data, spectroscopic data, macromodel study and 1H and 13C NMR spectra]. See DOI: 10.1039/b000000x/