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FULL PAPER

Syntheses and Conformational Analysis of *Aminopyrazolonyl* Amino Acid (APA)/Peptides

Amarnath Bollu,[a] and Nagendra K Sharma *[a]

Abstract: Pyrazole, pyrazolone and aminopyrazolone derived molecules are bioactive molecules and considered as potential therapeutic drug candidates because of their unique structural properties. These molecules have abilities to interact with several bio-macromolecules via non-covalent interactions such as hydrogen bonding and π-π interactions. In structural organization of dipeptides, pyrazole containing aromatic amino acid/dipeptides have been explored and considered as potential amino acid residue. In repertoire of unnatural aromatic amino acids, this report describes the syntheses of 4-aminopyrazolonyl containing amino acids and their crystal structures. The incorporation of 4-aminopyrazolonyl at N-terminal of native amino acid/dipeptides influences the conformational changes of respective peptide which induces the formation of distinctive supramolecular self-assembly structures such as β-sheet and α-helices in their solid state crystal. The structural conformation of those peptides, here, are also demonstrated in solution phase by ^{1}H-NMR (1D/2D) and DMSO-d_6 titration methods which support the formation of inter-/intramolecular hydrogen bonding in solution. Hence, these unnatural amino acid analogues are capable of tuning the secondary structure of natural amino acid/peptides by introducing at *N*-terminal via amide bond.

Introduction

Pyrazole, pyrazolone and aminopyrazolone are constituents of several natural products, synthetic drug molecules, agrochemical reagents, and metal chelating agents (Figure 1a).[1] For example, pyrazolone derivatives are well-known antipyretic and analgesic drugs for more than one century.[2] Many other pyrazolone derivatives are reportedly known as neuroprotective agents, HIV integrase inhibitors, phosphodiesterase inhibitors, and antibacterial agents.[3] Further the substituted pyrazolone derivative, 4-aminophenazone (Aminopyrene), and its metabolite (Ampyrone) have shown excellent analgesic, anti-inflammatory and antipyretic activities, though the some risk factors as agranulocytosis are alos associated (Figure 1a).[4] The substituted pyrazole and pyrazolone derivatives have shown strong DNA binding affinities, and considered as potential anti-cancer agents.[5] For an example, the amino substituted pyrazolone molecule, ampyrone, has been modified as Schiff base derivatives and employed for metal complexation with metal ions (Co^{2+}/Ni^{2+}/Fe^{3+}) which are considered as antimicrobial agents.[6] Further, ampyrone is explored as biochemical reagent for determination of phenol concentration including inside the cell.[7] Moreover, pyrazole and pyrazolone derivatives have abilities to form hydrogen bonding and have been explored in screening of their biological activities.[8] Thus pyrazole and pyrazolone could be considered as potential scaffold for syntheses of unnatural aromatic amino acids for tuning the peptide structures. In literature, Schrader and co-workers have reported that aminopyrazole containing amino acids, are the artificial templates for stabilization of β-sheet conformation (Figure 1a).[9] Recently, Sutherland and co-workers have reported the new class of conjugated unnatural α-amino acid containing 5-arylpyrazole residue as probe of serine proteases (Figure 1a).[10] In repertoire of unnatural aromatic amino acids, herein we report the syntheses of rationally designed aminopyrazolonyl amino acid (APA) derivatives (Figure 1b) and their roles in structural organization of native dipeptides.

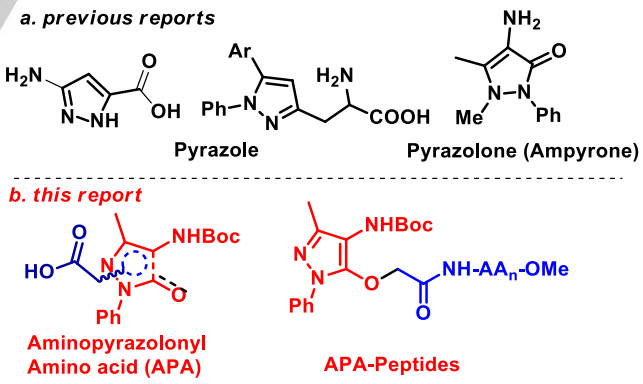

Figure 1. (a) Chemical structures of pyrazole/pyrazolone/aminopyrazolone; (b) rationally designed amino acids/peptides.

Results and Discussion

In Scheme 1, we began the syntheses of rationally designed unnatural aminopyrazolonyl amino acid (APA) from L-threonine. The carboxylic acid of threonine was protectected as methyl ester and its amino group as N-BOC. This protected threonine

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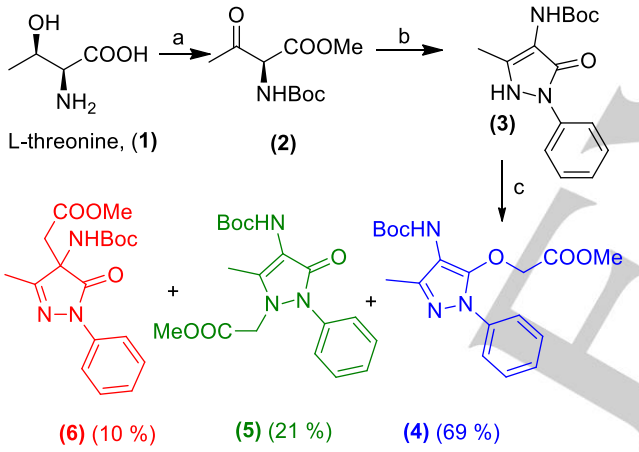
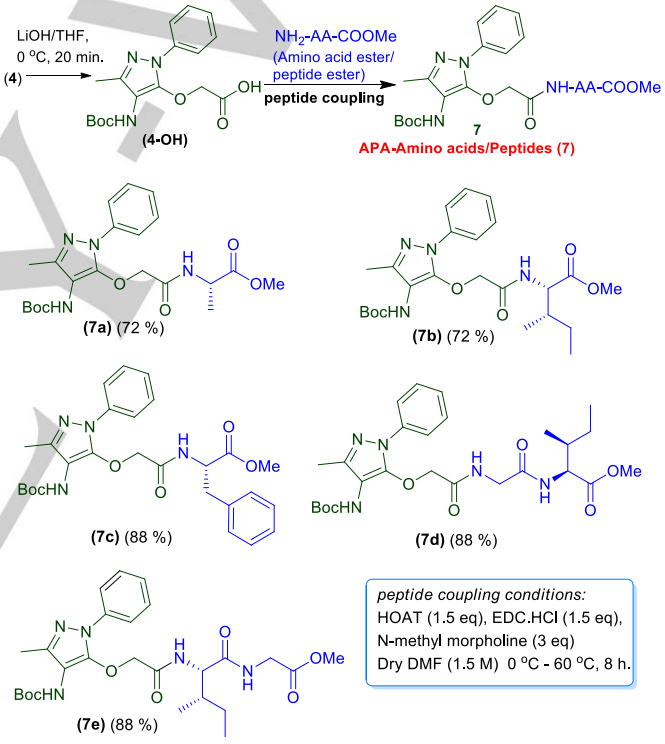
[^{1}H-/^{13}C-NMR and ESI-HRMS spectra of new compounds (**2**-**7**) are provided. The crystal parameters of compounds (**3**-**6**), (**7a**), and (**7e**) are tabulated as Table S2 and their cif files are provided separately. See DOI: 10.1039/x0xx00000x.

derivative (*BocNH-Thr-OMe*) was converted into β-keto ester (**2**) by oxidation of its hydroxyl group with Dess-Martin oxidizing agent. This β-keto ester (**2**) was treated with phenylhydrazine under mild acidic conditions for preparation of aminopyrazolone deivative (**3**). For preparation of amino acid derive, the introduction of carboxylate groups was required. To introduce carboxylate group at pyrazolone ring, compound (**3**) was treated with methyl bromoacetate under basic conditions. Pleasently, we isolated not only *O*-alkylated APA derivatives (**4**) in 81% yield but also *N*-alkylated (**5**), and *C*-alkylated (**6**) APA derivatives with regioselectivity as 69:21:10 (4:5:6). All compounds (**3-6**) were characterized by NMR ($^{1}H/^{13}C$) and ESI-HRMS (See Electronic Supporting Information, ESI). Importantly, we obtained single crystals of APA derivatives (**3**/**4**/**5**/**6**) and studied by single crystal X-ray differatometer which confirmed their chemical structurs in solid sate crystald. Their ORTEP diagrams are provided in ESI (Figure S43-S46), and cif files are deposited to the Cambridge Crystallographic Data Centre (CCDC) with number CCDC 1831988 for compound (**3**), CCDC 1831989 for amino acid ester (**4**), CCDC 1831990 for amino acid ester (**5**), and CCDC 1831993 for amino acid ester (**6**).



Scheme 1. Synthesis of 4-aminopyrazolone amino acids. *reagents and reaction conditions*: (a) (i) $SOCl_2$, MeOH, 0 °C then reflux, 12 h, (ii) CH_3CN:H_2O (85:15), Et_3N, $(Boc)_2O$, 0 °C-rt, 13 h, 85 % (iii) Dess-Martin periodinane, DCM, 2.5 h, rt, 85 % (b) phenyl hydrazine, CH_3COOH, MeOH, Reflux, 16 h, 86 %; (c) methylbromo acetate ($BrCH_2COOMe$), K_2CO_3, CH_3CN, 3 h, rt.

To explore the role of pyrazolonyl amino acid residue in conformational changes of peptides, we planned to incorporate *O*-alkylated amino acid at *N*-terminal of natural amino acids and dipeptides. We chose neutral hydrophobic chiral amino acids with increasing the bulkiness such as *Ala*, *Ile* and *Phe*. Moreover *Phe* could involve in conformational changes in peptides by π-π stacking with APA aromatic ring moiety. Additionally we planned to couple APA with dipeptides to switch the steric hindrance between APA and *Ile* with a flexible *Gly* residue. In Scheme 2, the ester of compound (**4**) was hydrolysed with LiOH (1 M) and then neutralized with HCl (1 M) solution to accomplish the desired *N*-Boc-Aminopyrozolonyl amino acid (**4-OH**) which was coupled with neutral natural amino acids/peptides (*Ala, Ile, Phe, Gly-Ile,* and *Ile-Gly*) to yield respective APA-peptide derivatives such as *BocNH-APA-Ala-OMe* (**7a**), *BocNH-APA-Ile-OMe* (**7b**), *BocNH-APA-Phe-OMe* (**7c**), *BocNH-APA-Gly-Ile-OMe* (**7d**) and *BocNH-APA-Ile-Gly-OMe* (**7e**). All these peptides were well characterized by $^{1}H/^{13}C$-NMR and ESI-HRMS (See ESI). In next, we attempted to crystalize APA containing peptides (**7a-7e**) under various conditions, though only dipeptide (**7a**)/tripeptide (**7e**) crystalized under MeOH:H_2O (1:1) conditions. The single crystal structures of both peptides (**7a** & **7e**) were analysed by X-ray studies which confirmed the structure of respective peptides (**7a** & **7e**). The ORTEP diagrams of compounds (**7a** & **7e**) are depicted in ESI (Figure S47-S48) and other crystal parameters are given in Table S2. Their cif-files has deposited to the Cambridge Crystallographic Data Centre (CCDC) with following CCDC Numbers: CCDC 1831991 for peptide (**7a**) and CCDC 1831992 for peptide (**7e**).



Scheme 2. Synthesis of 4-aminopyrazolonyl amino acids/peptides (**7a**-**7e**).

X-Ray studies. To examine the role of *N*-terminal APA residue in peptide conformation in their solid states, we studied the crystal packing arrangements of aminopyrazolone derivative (**3**), APA esters (**4-6**), APA peptides (**7a**/**7e**) using crystal visualizing software, Diamond version 3.2k. The crystal packing arrangement of amino pyrazolone compound (**3**) exhibits a non-linear type of sheets with multiple intermolecular hydrogen bonds among three molecules, one from pyrC=O----H-NBoc, and other two from pyrC=O----H-Npyr (Figure 2). Importantly, the *O*-atom of pyrazolonyl ring carbonyl (pyrC=O) exhibits bifurcated hydrogen bonding with BocN-H and pyrazolone ring N-H, which generated

unique type of supramolecular self-assembly with marginal difference in their hydrogen bond length.

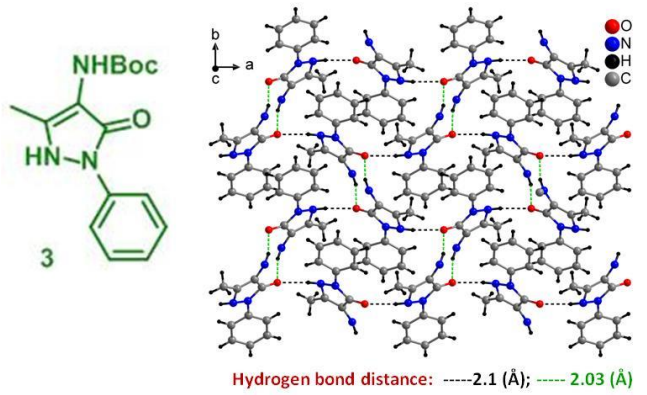


Figure 2. Crystal packing arrangement of 4-aminopyrazolone (**3**) (Boc group has omitted for representation).

In case amino acid derivative, the crystal packing arrangement of *O*-alkylated ester derivative (**4**) exhibits repeated intermolecular hydrogen bonding among pyrN----H-NBoc with a distance of 2.1 Å (Figure 3). The packing arrangement of ester (**4**), along the b-axis forms helical structure with the pitch value of ~10.8 Å.

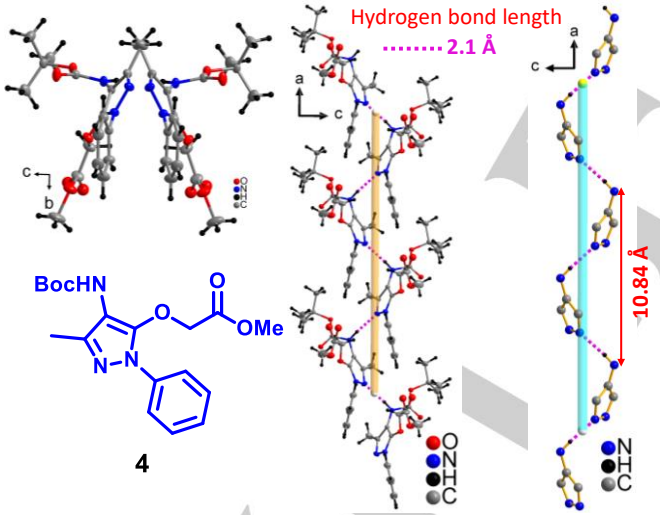


Figure 3. Crystal packing pattern of APA ester derivative (**4**) with intermolecular hydrogen bonding (pink dotted line) in different axis (few atoms are ommited for representation in middle and right).

However, the packing arrangement of *N*-alkylated amino acid ester derivative (**5**) shows a linear supramolecular *β*-sheet type of structures with repeated anti-parallel dimers which are formed *via* intermolecular hydrogen bonds between pyrC=O---H-NBoc with distance of ~2.0 Å (Figure 4, top). Further, the crystal packing arrangement of *C*-alkylated, *spiro* amino acid ester (**6**) exhibits a non-linear supramolecular self-assembled structure with the help of classical and non-classical intermolecular hydrogen bonding (CH-π interactions). The classical intermolecular hydrogen bonding forms dimer in anti-parallel orientation (Figure. 4, bottom), while the non-classical intermolecular hydrogen bonding, between π-C of phenyl group with BocCH_3, involves in the formation of unique three dimensional structure, supramolecular self-assembly structures (ESI, Figure S46). Overall, these structural studies results suggest that pyrazolone ring *N*-atom (pyrN), pyrazolone ring carbonyl (pyrC=O) and BocN-H of APA esters (**4**/**5**/**6**) are involved in intermolecular hydrogen bonding, and play important role for the formation of helical and antiparallel-β-sheet types of supramolecular self-assembled structures. In recent literature, native dipeptides derivatives (BocNH-Val-Val-OMe & BocNH-Ile-Ala-OMe) are also known to form parallel β-sheet type of structure.[11].

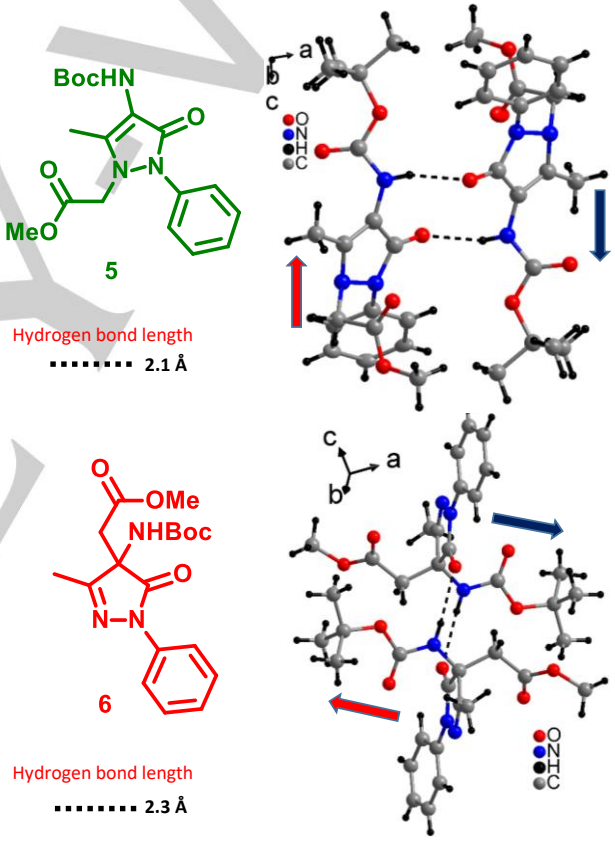


Figure 4. Intermolecular hydrogen bonding in APA ester derivatives, (**5**) top and (**6**) bottom.

In case of peptides, the crystal packing arrangement of dipeptide**,** *BocNH-APA-Ala-OMe* (**7a**) exhibits supramolecular self-assembly structure with semi-cylindrical channel (Figure 5). This distinctive structure is formed with sequential hydrogen bonding such as (a) amide N-H with adjacent amide C=O (N-H----O=C), and (b) BocN-H with adjacent BocC=O (N-H----O=C). We have extracted the radius of supramolecular channel as 2.0 Å. In literature, the formation of similar type of self-assembly structures

are reported and considered as parallel β-sheet type of structure.[11] Herein, the self-assembly of dipeptide (**7a**) is further stabilized by phenyl residues with non-classical T-shaped multiple CH-π interactions with bond length ranging from 2.9-3.7 Å (∠140° to ∠180°), shown in (Figure 5) at the bottom with purple dotted line.

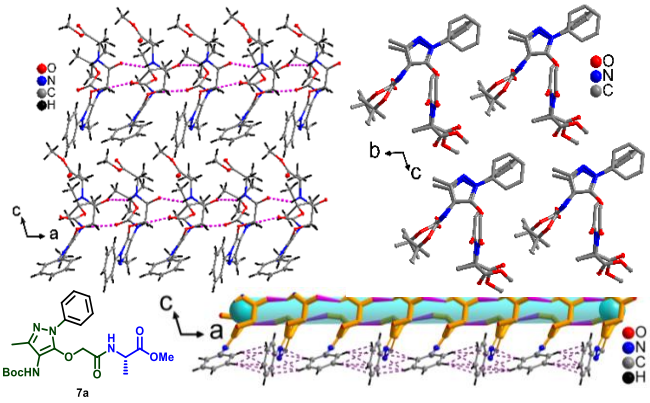


Figure 5. Crystal packing pattern of APA peptide (**7a)** with intermolecular hydrogen bonding. CH-π interactions with bond length ranging from 2.9-3.7 Å (∠140° to ∠180°), shown at the bottom in purple dotted line along with classical hydrogen bonding (pink) (few atoms are ommited for representation in middle and right).

In case of tripeptide, the crystal packing arrangement *O*-alkylated APA tripeptide, *BocNH-APA-Ile-Gly-OMe*, (**7e**) are depicted in (Figure 6). The packing arrangement of that crystal (**7a)** exhibits two types of intermolecular hydrogen bonding: (a) Boc carbonyl (-C=O) with *Ile* amide N-H as BocC=O-----H-N*Ile*; (b) pyrazolone amino acid carbonyl with *Gly* amide N-H (*Pyr*-C=O----H-N-*Gly*) (Figure 6). As resultant, peptide (**7e**) has formed new type of helical structure with the pitch ~8.9 Å. Thus these analyses confirm the role of pyrazolonyl ring in APA-peptides for the formation of unique conformations as *β*-sheet and helices type of supramolecular structures via intermolecular hydrogen bonding in tripeptide **7e**.

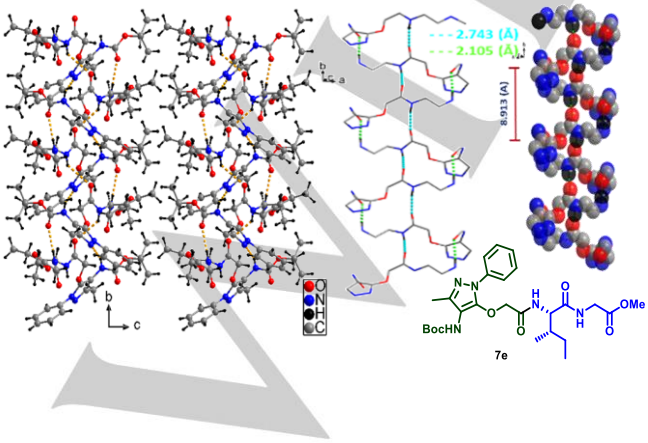


Figure 6. Crystal packing pattern of APA peptide in (**7e)** with intermolecular hydrogen bonding (few atoms are ommited for representation in middle and right).

Herein, It is important to note that the crystal structures of *O*-alkylated ester (**4**) and their peptides (**7a** & **7e**) attains an unusual *syn* conformation between -***O***-***C***H$_2$–***C***O-***N***H- and also exhibit a weak intramolecular hydrogen bonding between *H*-atom (-CO-NH-) and *O*-atom (–***O***-*C*H$_2$-). For examples, we noticed the hydrogen bond length of 2.25 Å (with ∠108°) and 2.30 Å (with ∠106°) in peptide (**7a** & **7e**) respectively, ESI (Figure S44 and S47-S48).

CD-Studies. Circular Dichroism (CD) study of peptides predicts their secondary structures. However, nature of solvent environment has critical role in the conformational changes of peptide structures.[12] The solvent dependent CD spectra of peptides have significant role in finding the defined structure and conformation of peptides.[13] To examine the role of APA residue in the conformational changes in their peptides, we recorded the CD spectra of APA peptides (**7a**-**7e**) in different solvent systems AcCN, MeOH, $CHCl_3$ and TFE, and their CD spectra are provided in ESI (Figure S50-S53). The CD spectra of APA di-/tri-peptides (**7a**-**7e**) are similar in AcCN and MeOH, which exhibit Cotton effect with distinctive CD signals as the maxima at wavelength (λ) ~200 nm/220 nm and the minima at λ ~260 nm, though the maxima at λ220 nm is more prominent in MeOH. In solvent $CHCl_3$, however, the CD signal of peptides (**7a**-**7e**) exhibit poorly resolved maxima and minima. In solvent TFE, the CD spectra of peptides (**7a**-**7e**) exhibit only maxima at λ200 nm & λ220 nm. These CD signals of peptides (**7a**-**7e**) are possibly due to electronic transition of amide carbonyl group (π-π*/n-π*) and pyrazolonyl/phenyl aromatic rings (π-π*). The CD spectra of peptides (**7a**-**7e**) are also solvent dependent which further supports that intermolecular hydrogen bonding are involved in conformational cahnges of peptides in AcCN/MeOH.

NMR Studies: Generally amide bonds of peptides are involved in formation of peptides' secondary structure mainly by inter/intra-molecular hydrogen bonding. Herein, APA *di/tri*-peptides (**7a**-**7e**) have two-three amide bonds which are capable for hydrogen bonding. We recorded 2D-NMR (^{1}H-COSY) spectra of those peptides (**7a**-**7e**) and assigned their N-H protons (see in ESI Figure S21-S32). We assigned the chemical shift (δ) of BocNH as NH(1), and amide NH's as NH(2)/NH(3) (Table 1). Herein, the amide N-H (NH(2)), close to APA residue, appear at higher chemical shift (downfield) as compared to NH(1)/NH(3) in all those peptides in solvent $CDCl_3$. Thus NH(2) of APA peptides is more deshielded than their BocNH and other amide N-H.

Table 1. NMR assignment of N-H of APA-Peptides (**7a**-**7e**) in $CDCl_3$.

	Chemical shift, (δ, in ppm)		
	NH(1)	NH(2)	NH(3)
APA-Ala-OMe (**7a**)	6.072	6.87	-
APA-Ile-OMe (**7b**)	5.936	6.851	-
APA-Phe-OMe (**7c**)	6.092	6.855	-
APA-Gly-Ile-OMe (**7d**)	6.302	7.118	6.507
APA-Ile-Gly-OMe (**7e**)	6.568	6.957	6.275

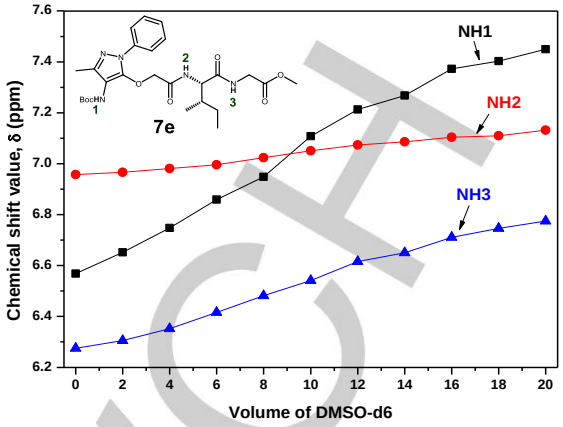


Figure 7. Changes in δ (in ppm) with DMSO-d6 (in µL) titration of peptide **7e** in $CDCl_3$ (~40 mM).

To examine the involvement of *amide*/*Boc*NH of peptides (**7a**-**7e**) as intra-/intermolecular hydrogen bonding in $CDCl_3$ solution, we performed DMSO-d_6 titration ^{1}H-NMR experiment with synthesized *di*/*tri*-peptides (**7a**-**7e**). Since DMSO-d_6 is strong hydrogen bond acceptor and perturb only intermolecular hydrogen bonding. In this titration experiment, the downfield shift of N-H protons occur with increasing the DMSO-d_6 concentration, while the little shift or no shifts occur with intramolecular hydrogen bonded N-H.[14] For APA-peptides, the NMR titration profile (chemical shift vs DMSO concentration plot) of N-H protons of tri-peptides (**7e**) is depicted in (Figure 7), while for other APA-peptides (**7a**-**7e**) are provided in ESI (Figure S33-S42). Our NMR analyses suggest that the chemical shift of BocN-H(1) of peptides (**7a**-**7e**) are gradually shifting toward downfield with increasing the concentration of DMSO-d_6 in their respective ^{1}H-NMR. The amide N-H(2) (adjacent to APA residue) of peptide (**7a**-**7e**) show a marginal downfield chemical shift in their respective ^{1}H-NMR with increasing the concentration of DMSO-d_6. The amide N-H(3) of peptide (**7a**-**7e**) show significant downfield shift but less than BocN-H(1). It seems BocN-H and amide N-H are involved in intermolecular hydrogen bonding for the formation of secondary structure in peptides though BocN-H is comparatively weak in comparison to amide N-H (NH(2)/NH(3)). Our single crystal studies of two APA-peptides (**7a**/**7e**) also confirm the BocNH and amide N-H are participated in supramolecular self-assembly structure *via* intermolecular hydrogen bonding.

Further it is noticed that the increasing bulkiness of amino acid side chain, from *Gly* to *Ile*/*Phe,* in peptides (**7a**-**7e**) decrease the difference in chemical shift values (dδ) of amide NH(2) (ESI Table. S1). This supports, the steric hindrance at that amide position has important role in the formation of rigid structure via intramolecular hydrogen bonding as compared to other N-H. As evidently, we also notice a weak hydrogen bonding in crystal structures of APA-peptides (**7a**) and (**7e**).These results support that there are no strong intramolecular hydrogen bonging in APA-peptides (**7a**-**7e**). To understand the structure and conformations of APA peptides in solution state, we recorded ^{1}H-NOESY (2D-NMR) of peptides (**7a**) and (**7c**-**7e**) in $CDCl_3$ (ESI, Figure S21-S32). A portion of NOE spectra of APA peptides (**7a**) and (**7e**) is depicted in (Figure 8). The amide NH, NH(2), of peptide (**7a**) shows three cross peaks (cross peak 2, 6 & 7) which show respective NOE with methylene protons (–*O*-C***H***$_2$-), alanyl residue (-CH_3) and APA *N*-phenyl (*ortho*-protons) residues. Interestingly, the methylene protons (–*O*-C***H***$_2$-) also shows NOE (cross peak 1) with *N*-phenyl (ortho-protons) group. While the BocNH, NH(1) shows three weak NOE's (cross peaks 3, 4 & 5) with respective methylene protons (–*O*-C***H***$_2$-), *tert*-butyl group of BocNH and methyl group on pyrazole ring. We also notice the similar NOE's in all other APA peptides (**7c**-**7e**).

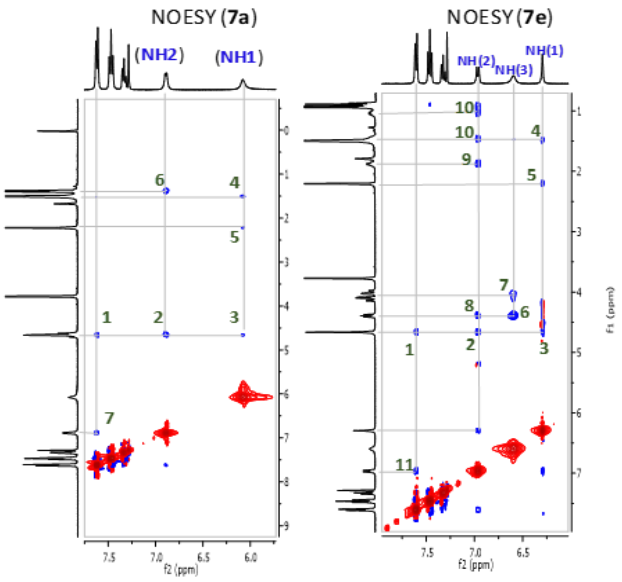


Figure 8. ^{1}H-NOESY spectra of APA peptides **7a** & **7e** in $CDCl_3$. (Showing only amide region).

All these NOE interactions of APA-peptides are cautiously analyzed and demonstrated in (Figure 9). Importantly, in (Figure 9), we notice NOE's between methylene protons (*–O-CH$_2$-*) of APA residue with *N*-phenyl group (*ortho*-protons), amide NH (NH(2)) and BocNH (NH(1)). This support that one of the methylene proton's NOE with N-phenyl group and the other one with BocNH (NH(1)). Additionally amide NH (NH(2)) also shows NOE with *N*-phenyl group (ortho-protons).

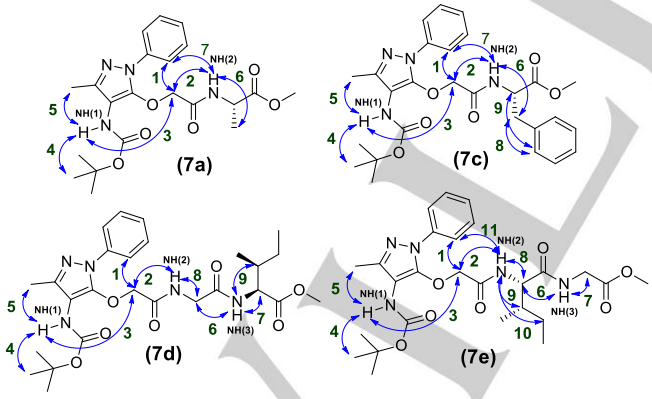


Figure 9. NOE interactions observed in APA peptides in $CDCl_3$ (**7a, 7c-7e**).

Our NMR assignments strongly suggest that NH(2) (amide NH) is most deshielded in solution ($CDCl_3$) as compared to NH(1)/NH(3). Further DMSO titration NMR experiments confirm that NH(2) is involved in an intramolecular H-bonding while NH(1)/NH(3) are involved in intermolecular hydrogen bonding in aprotic polar solvents. Importantly, NOESY results show that NOE interactions of APA phenyl (*ortho* C-H) residue with -O-CH$_2$- and amide NH(2) are consistent in all APA-peptides. Herein, we propose the preferably conformation of APA-peptides in solution as (Figure 10a) which have acquired *syn* conformation between O-CH$_2$-and amide NH(2) and oriented appropriately for intramolecular hydrogen bonding as N-H-----O-CH$_2$ as (Figure 10b). Further, crystal structure of two APA-peptides (**7a**/**7e**) have also acquired the similar type of *syn* conformation by O-CH$_2$-and amide NH(2) in solid state (Figure 10c). From NMR and X-ray correlation studies, we propose structure of APA peptides as (Figure 10d) which show inter-/intramolecular hydrogen bonding in solution state.

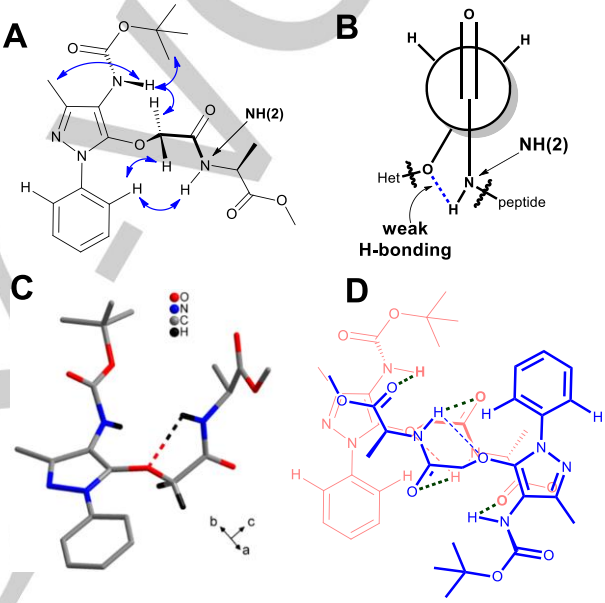


Figure 10. Proposed structures and conformations in solution state and observed single crystal X-ray structure of (**7a**). A. possible structure of (**7a**) from NOESY NMR; B. *syn* conformation with weak intramolecular H-bonding; C. single crystal X-ray structure of (**7a**) with intramolecular H-bonding; D. Possible intermolecular H-bonding in solution for (**7a**).

Conclusions

In summary, three new 4-aminopyrazolonyl amino acid (APA) derivatives, unnatural aromatic amino acids, are successfully synthesized from the same precursor and their crystal structures are confirmed by single crystal X-ray analyses. One of those amino acid, *O*-alkylalted APA derivative, is linked at *N*-terminal native amino acid/dipeptides. Importantly the crystal structure of two peptides (**7a**/**7e**) are analysed which confirmed the distinctive type of supramolecular self-assembled structures in solid state due to involvement of pyrazolonyl residue in intermolecular hydrogen bonding. The NMR studies have also supported the formation of inter-/intramolecular hydrogen bonding in APA peptides in solution state with distinctive conformations. So far no report is available about 4-aminopyrazolone linked amino acids. Hence our results reveal the role of aminopyrzolone in conformational changes of amino acid/dipeptides which lead to

distinctive secondary structures of native dipeptides by acylation at *N*-terminal of amino acid and peptides.

Experimental Section

General procedure of alkylation:

O-alkylated (**4**)*, N-alkylated* (***5***) *and C-alkylated* (***6***) *products.* To a stirred solution of **3** (1.0 g, 3.46 mmol) in AcCN (35 mL) was added anhydrous K_2CO_3 (1.43 g, 10.3 mmol) and stirred for 30 min. Then methyl bromoacetate (0.393 mL, 4.15 mmol) was added dropwise and stirred an additional 3h. The crude reaction mixture was filtered and concentrated to dryness under vacuum. The residual yellow oil was purified via chromatography with 0.5-5 % MeOH in CH_2Cl_2 to yield 0.749 g (59 %) of *O*-alkylated (**4**), 0.229 g (18.4 %) of *N*-alkylated (**5**) and 0.072 g (6 %) of *C*-alkylated (**6**).

O-alkylated (**4**). R_f = 0.26 (0.1:19.9 MeOH/CH_2Cl_2); FT-IR ($\tilde{\nu}$, cm^{-1}), $CHCl_3$: 3320, 2975, 2932, 1766, 1718, 1604, 1512, 1447, 1378, 1248, 1162, 1081, 1048, 762. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.66 (d, J = 7.8 Hz, 2H), 7.41 (dd, J = 10.8, 5.1 Hz, 2H), 7.33 – 7.21 (m, 1H), 5.89 (s, 1H), 4.69 (s, 2H), 3.75 (s, 3H), 2.18 (s, 3H), 1.49 (s, 9H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 169.03 (s), 154.96 (s), 147.34 (s), 146.73 (s), 138.40 (s), 128.93 (s), 126.65 (s), 122.48 (s), 102.70 (s), 80.54 (s), 68.09 (s), 52.31 (s), 28.23 (s), 12.02 (s). HRMS (ESI-TOF) m/z: calcd. for $C_{18}H_{24}N_3O_5^+$ $[M+H]^+$: 362.1710, found 362.1751. mp: 148-149 °C.

N-alkylated (**5**). R_f = 0.05 (0.1:19.9 MeOH/CH_2Cl_2); FT-IR ($\tilde{\nu}$, cm^{-1}), $CHCl_3$: 3250, 2976, 2927, 2851, 1744 1707, 1674, 1637, 1593, 1491, 1366, 1248, 1210, 1162, 1043, 757. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.25 (m, 5H), 6.04 (s, 1H), 4.21 (s, 2H), 3.66 (s, 3H), 2.26 (s, 3H), 1.48 (s, 9H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 167.07 (s), 162.64 (s), 153.81 (s), 148.49 (s), 134.45 (s), 129.41 (s), 127.31 (s), 124.54 (s), 110.04 (s), 80.46 (s), 52.46 (s), 48.40 (s), 28.23 (s), 11.60 (s). HRMS (ESI-TOF) m/z: calcd. for $C_{18}H_{24}N_3O_5^+$ $[M+H]^+$: 362.1710, found 362.1694. mp: 180-182 °C.

C-alkylated (**6**). R_f = 0.39 (0.1:19.9 MeOH/CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.89 (d, J = 7.9 Hz, 2H), 7.39 (t, J = 7.9 Hz, 2H), 7.18 (t, J = 7.4 Hz, 1H), 6.22 (s, 1H), 3.75 (s, 3H), 2.66 (d, J = 9.1 Hz, 2H), 2.09 (s, 3H), 1.36 (s, 9H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.04 (s), 169.11 (s), 159.32 (s), 138.03 (s), 128.83 (s), 125.12 (s), 118.80 (s), 63.29 (s), 37.85 (s), 29.70 (s), 28.06 (s), 13.37 (s). HRMS (ESI-TOF) m/z: calcd. for $C_{18}H_{24}N_3O_5^+$ $[M+H]^+$: 362.1710, found 362.1732. mp: 118-120 °C.

General experimental procedure for compounds, **7a**-**7e**:

Compound **4a**, corresponding Amine (TFA or HCl salt form) (1.5 equivalent), HOAT (1.5 equivalent) was dissolved in Dry DMF (1.5 M). After stirring for 10 min, N-methyl morpholine (3 equivalent) was added drop wise and cooled the temperature to 0 °C and added EDC.HCl. After 20 min allowed to warm to rt followed by heating at 60 °C for 8h. The crude reaction mixture was evaporated under reduced pressure. The resultant crude was purified by column chromatography with MeOH in CH_2Cl_2 (1-3 %).

APA-Ala-OMe (**7a**).R_f = 0.30 (0.3:9.7 MeOH/CH_2Cl_2); yield 72 %; FT-IR ($\tilde{\nu}$, cm^{-1}), $CHCl_3$: 3315, 2981, 1744, 1691, 1513, 1454, 1254, 1173, 1059, 762. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 7.7 Hz, 2H), 7.44 (t, J = 7.9 Hz, 2H), 7.31 (t, J = 7.4 Hz, 1H), 6.87 (d, J = 7.5 Hz, 1H), 6.07 (s, 1H), 4.72 – 4.55 (m, 3H), 3.76 (s, 3H), 2.19 (s, 3H), 1.48 (s, 9H), 1.36 (d, J = 7.2 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 173.00, 166.99, 155.00, 147.06, 138.11, 129.18, 126.96, 122.65, 102.73, 80.57, 70.74, 52.59, 47.49, 28.20, 17.94, 11.87. HRMS (ESI-TOF) m/z: calcd. for $C_{21}H_{29}N_4O_6^+$ $[M+H]^+$ 433.2104, found 433.2082.

APA-Ile-OMe (**7b**). R_f = 0.30 (0.3:9.7 MeOH/CH_2Cl_2); yield 72 %; FT-IR ($\tilde{\nu}$, cm^{-1}), $CHCl_3$: 3417, 3310, 2970, 2937, 1723, 1691, 1518, 1442, 1243, 1168, 1048, 757. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 7.8 Hz, 2H), 7.44 (t, J = 7.8 Hz, 2H), 7.30 (t, J = 7.4 Hz, 1H), 6.87 (d, J = 8.9 Hz, 1H), 6.12 (s, 1H), 4.68 (d, J = 4.0 Hz, 2H), 4.61 (dd, J = 9.0, 4.9 Hz, 1H), 3.74 (s, 3H), 2.19 (s, 3H), 1.93 – 1.79 (m, 1H), 1.49 (s, 9H), 1.32 (m, J = 21.3, 13.0, 9.4 Hz, 1H), 1.08 – 0.95 (m, 1H), 0.94 – 0.82 (m, 6H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 172.10, 167.06, 154.97, 147.11, 138.13, 129.26, 127.01, 122.75, 102.66, 80.76, 70.68, 55.98, 52.29, 37.60, 28.21, 24.92, 15.47, 11.91, 11.50. HRMS (ESI-TOF) m/z: calcd. for $C_{24}H_{34}N_4O_6Na^+$ $[M+Na]^+$ 497.2371, found 497.2376.

APA-Phe-OMe (**7c**). R_f = 0.35 (0.3:9.7 MeOH/CH_2Cl_2); yield 88 %; FT-IR ($\tilde{\nu}$, cm^{-1}), $CHCl_3$: 3412, 3310, 2981, 2932, 2856, 1744, 1723, 1691, 1604, 1518, 1442, 1366, 1248, 1162, 1051, 762. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.51 (d, J = 7.7 Hz, 2H), 7.37 (t, J = 7.7 Hz, 2H), 7.32 – 7.21 (m, 4H), 7.09 – 7.01 (m, 2H), 6.80 (d, J = 8.0 Hz, 1H), 5.87 (s, 1H), 4.89 (dd, J = 13.8, 6.7 Hz, 1H), 4.62 (s, 2H), 3.72 (s, 3H), 3.08 (ddd, J = 20.8, 13.9, 6.2 Hz, 2H), 2.18 (s, 3H), 1.48 (s, 9H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.58, 166.99, 155.03, 147.10, 138.09, 135.50, 129.16, 129.04, 128.70, 127.31, 126.96, 122.59, 102.39, 80.65, 70.39, 52.71, 52.50, 37.72, 28.23, 11.88. HRMS (ESI-TOF) m/z: calcd. for $C_{27}H_{33}N_4O_6^+$ $[M+H]^+$ 509.2395, found 509.2438.

APA-Gly-Ile-OMe (**7d**). R_f = 0.36 (0.3:9.7 MeOH/CH_2Cl_2); yield 88 %; FT-IR ($\tilde{\nu}$, cm^{-1}), $CHCl_3$: 3304, 2970, 2932, 2878, 1664, 1523, 1453, 1378, 1259, 1162, 1054, 757. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.57 (d, J = 7.9 Hz, 2H), 7.41 (t, J = 7.8 Hz, 2H), 7.34-7.18 (m, 2H), 6.75 (s, 1H), 6.56 (d, J = 17.0 Hz, 1H), 4.69 – 4.56 (m, 3H), 3.99 (s, 2H), 3.72 (s, 3H), 2.17 (s, 3H), 1.89 (ddd, J = 7.4, 6.9, 5.2 Hz, 1H), 1.54 – 1.34 (m, 10H), 1.33-1.05 (m, 1H), 0.95 – 0.84 (m, 6H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 172.47, 168.62, 168.24, 162.65, 155.34, 147.23, 138.13, 129.10, 126.80, 122.49, 102.79, 80.29, 70.67, 56.66, 52.13, 42.02, 37.63, 28.18, 25.05, 15.44, 11.87, 11.48. HRMS (ESI-TOF) m/z: calcd. for $C_{26}H_{38}N_5O_7^+$ $[M+H]^+$ 532.2766, found 532.2796.

APA-Ile-Gly-OMe (**7e**). R_f = 0.35 (0.3:9.7 MeOH/CH_2Cl_2); yield 88 %; FT-IR ($\tilde{\nu}$, cm^{-1}), $CHCl_3$: 3412, 3304, 3072, 2981, 2937, 2884, 1750, 1691, 1658, 1604, 1518, 1447, 1383, 1248, 1210, 1168, 1059, 762. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J = 7.8 Hz, 2H), 7.43 (t, J = 7.8 Hz, 2H), 7.29 (t, J = 7.4 Hz, 1H), 6.95 (d, J = 8.8 Hz, 1H), 6.65 (s, 1H), 6.32 (s, 1H), 4.63 (s, 2H), 4.45 – 4.32 (m, 1H), 4.02 (ddd, J = 50.5, 18.2, 5.2 Hz, 2H), 3.74 (s, 3H), 2.17 (s, 3H), 1.46 (s, 10H), 1.00 (dd, J = 14.2, 7.9 Hz, 1H), 0.95 – 0.78 (m, 6H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.47, 169.98, 167.53, 155.22, 147.20, 138.06, 138.06, 129.20, 126.90, 122.56, 102.91, 80.40, 70.54, 57.02, 52.19, 41.04, 37.12, 28.17, 24.57, 15.27, 11.86, 11.15. HRMS (ESI-TOF) m/z: calcd. for $C_{26}H_{37}N_5O_7Na^+$ $[M+Na]^+$ 554.2585, found 554.2567.

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Keywords: Peptides 1 • Amino Acids 2 • 4-Aminopyrazoles 3 • Synthetic methods 4 • Conformational analysis 5

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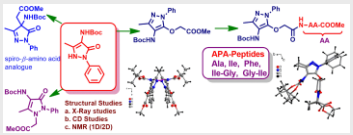
Syntheses of three new 4-aminopyrazolone based amino acid derivatives from 4-aminopyrazolone and demonstration of their solid state structures by single crystal X-ray studies. One of amino acid, *O*-isomer, is coupled at *N*-terminal of amino acid/dipeptides and explored its role in secondary structure formation by X-ray, CD and NMR (1D/2D) studies.



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*Amarnath Bollu,[a] and Nagendra K Sharma *[a]*

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