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Designing of amino acids and their interactions in to unique tripeptide conformations for developing new peptide pharmaceuticals using Ramachandran plot

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Summary

The Ramachandran plot displays the main chain conformational angles (Phi and Psi) of the polypeptide chain of a protein molecule. The amino acid conformations of 13 tripeptide template analogue sets were analyzed with Ramchandran plots. Several of these amino acids are predicted to fold in to unique tripeptide conformations such as alpha helix, beta sheets and gamma turns. All the tripeptides (Thr-Phe-Arg; Thr-Trp-Lys; Thr-Phe-Lys; Thr-Tyr-His; Thr-His-Arg; Thr-Arg-Asn; Phe-Trp-Lys; Phe-Lys-Gln; Phe-His-Arg; Phe-Arg-Asn; Glu-Trp-Lys; Glu-His-Arg and Thr-Lys-Gln) showed most favourable allowed regions of alpha helix in lower left quadrant of plot thereby satisfied with ideal phi values (–57°) and psi values (–47°). Similarly, the above all templates have shown most favourable allowed regions of beta sheets in upper left quadrant by satisfying the ideal phi values (–80%) and psi values (+150°). In addition, all the subjected templates have shown less favourable generously allowed regions of gamma turns in upper left quadrant by fulfilling the phi and psi values (–80°; +80°). Among all the inbuilt templates tested for conformational analysis, we found that Phe-Lys-Gln; Phe-Arg-Asn; Glu-Trp-Lys and Glu-His-Arg have shown maximum percentage of low energy allowed regions (15.5%; 16%; 16%; 15.5%) as the most stable conformations in developing a new peptide based pharmaceuticals.

Key words: Phi values, Psi values, Tripeptides, Ramachandran plot, Conformations, Gamma turns

Introduction

The description of conformations of amino acids in peptides and proteins by two torsion angles, Φ and Ψ, was proposed by Ramachandran and Sasisekharan[1]. Ramachandran plot was first used to predict the possible conformations of the main chain based entirely on model building of shorter chain peptides, taking steric hindrance in to account especially, Ramachandran predicted with a remarkable precision of the conformations that could occur in proteins.

As proteins structures became known, it was verified experimentally that Ramachandran had correctly predicted the number of allowed areas and also the approximate numerical values of the Φ,Ψ torsion angles. Today, the Ramachandran plot is not only a basic diagram in textbooks on protein structures, but also a useful tool for assessing the correctness of a protein structure determination.[2-3]

In the first protein structures, determined to low resolution and without refinement, the , values were rather widely scattered throughout the Ramachandran plot. However, with the advent of refined high resolution structures, the conformation of amino acids clustered more and more near the allowed areas as predicted by Ramachandran. The conformational differences between the two conformations of the peptides can be clarified with a Ramachandran plot.[4]

Different secondary structure elements have their characteristic torsion angles, which can be visualized using the Ramachandran plot. Each dot on the Ramachandran plot shows the phi and psi values for an amino acid in the protein in question. The horizontal axis are phi value while the vertical shows psi values. The regions on the plot with the highest density of dots are the so called allowed regions of the Ramachandran plot, also called low-energy regions. Some values of Phi and Psi can be forbidden for an amino acid to adopt. The reason is that for some torsion angles the atoms within the polypeptide chain will come too close to each other, a so-called "steric clash", which would result in very high energy of the system. On the Ramachandran plot such regions can be easily distinguished; For a high-quality experimental structure, these regions are simply empty or almost empty. Very few amino acid residues in a protein have their torsion angles within these regions. But there are exclusions from this rule. Sometimes such values can be found and they most probably will result in some strain in the polypeptide chain. In such cases additional interactions must be present to stabilize such structures. They may have functional significance and may be conserved within a protein family, which may be related to possible conformational dynamics of the structure.[5]

Theoretically, the average Phi and Psi values for alpha-helices and -sheets should be clustered around −57, −47 and −80, +150, respectively.[6] Thus, the Ramachandran plot provides valuable information on the quality of a protein structure and gives some additional insights in to the relationships between the amino acid sequence and the three-dimensional protein structure.

Today, more than 40 years after the introduction of the Ramachandran plot, still this method is one such useful tool to study the interactions of amino acid combinations in tripeptides without having notable change in its conformation as compared to their plot of individual amino acids separately and thereby predicting their conformations of allowed regions in Ramachandran plot in order to develop a new peptide based pharmaceutical. That is the subject of this study.

Materials & Methods

Materials

For all the selected test analogues, we have performed conformational analysis using Ramachandran plot with Maestro, Schrodinger Suite 2011, Germany, supported by Windows 7, 32bit operating system with Interl (R) Core (TM) 2 Duo CPUE 4600 @ 2.40 GHZ and 1 GB RAM in HP 7540.

System Efficiency

We balance our design very differently from a

general–purpose supercomputer architecture. Relative to other high performance computing applications, Maestro uses only 1GB RAM and small L1 caches on our ASIC, with all code and data fitting on–chip in normal operation.

Methods

We selected 13 analogue tripeptide combinations, viz., Thr-Phe-Arg (TFR), Thr-Trp-Lys (TWK), Thr-Phe-Lys (TFK), Thr-Tyr-His (TYH), Thr-His-Arg (THR), Thr-Arg-Asn (TRN), Phe-Trp-Lys (FWK), Phe-Lys-Gln (FKQ), Phe-His-Arg (FHR), Phe-Arg-Asn (FRN), Glu-Trp-Lys (EWK), Glu-His-Arg (EHR) and Thr-Lys-Gln (TKQ) for this study plot of Ramachandran. All the 13 analogues set were made inbuilt chemical templates with 2D viewer in Schrodinger Suite 2011. From this plot, we could calculate torsion angles.

The Ramachandran plot was split in to four regions for detailed analysis of the conformations. Amino acids with torsion angles in the range –180 <Φ< 0°, –100 <Ψ< 45° were considered to be in the α-helical region. Amino acids with torsion angles in the range –180 <Φ< –45°, 45 <Ψ< 225° were considered to be in the β sheet region. The area 0 <Φ< 180°, –90 <Ψ< 90° is here called the turn region.[6]

The remaining area of the Ramachandran plot represents 36% of the area but contained only 1.9% of the amino acids and was not studied further. The bridging region between -helical and -sheet conformation is often given as –135 < < –45°, –25 < < 15°, but we found that this region should really be considered -helical and the bordering region should be moved upwards to –160 <Φ< –65°, 45 <Ψ< 90°.

Amino acids in Random coil were first subdivided in to the four areas of the Ramachandran plot: α-helical, β-sheets, turns and others. Within each of these areas, the stretches of random coil were sorted according to their lengths. Long stretches of amino acids in Random coil all in α-helical or all in β-sheet conformation were checked one by one.

The Ramachandra plots were produced in the following way. Firstly, all tripeptides used were sorted in to 90×90 squares, each covering an area of 4×4°. The number of amino acids in each such square was summed. The squares were sorted from the highest abundances to the lowest. Each square was colour-coded such that each colour represented allowed most favourable, less favourable and not favourable regions designated as red, yellow and white regions respectively. In order to make the plots easier to interpret, they were smoothed.

Results & Discussion

The conformations of all test sets subjected to Ramachandran plot are visualized from figure 1-13.

see Figs. 1 to 13

The results corresponding to all 13 test set analogues by Ramachandran plot are expressed in Table 1.

see Table 1.

Here, we will analyze the features of the different regions in the Ramachandran plot in greater detail. All the 13 tripeptides and α-helix region were observed in lower left quadrant and it obeys the ideal Phi and Psi values of –57° and –47° respectively, thereby significant in showing allowed regions and are considered most favourable.

Moreover, the β-sheet of all the 13 test analogues were observed in upper left quadrant, showing significance as most favourable by matching with the ideal Phi and Psi values (–80°, +150°).

In addition, the γ-turn region of all the 13 test analogues were observed in the upper left quadrant showing significance as less favourable or generously allowed regions by matching with the ideal Phi and Psi values (–80°, +80°).

All the torsion angles (Phi, Psi) of most favourable, less favourable and not favourable regions with their corresponding quadrants are given in Table 1 by tentative measurements with the Ramachandran plot.

The amino acid combinations of any of the one with Phenyl alanine, Tyrosine and Tryptophan in a tripeptide have quite similar distributions, which were observed with eight tripeptides, viz., TWK, TFK, TYH, FWK, FKQ, FHR, FRN and EWK respectively. Phe and especially Tyr also show two distinct maxima in the α-helical region. This was evidenced with the above mentioned tripeptides excluding two of the tripeptides TWK and EWK respectively.

The tripeptide containing Asn has quite similar Ramachandran plot, but differ from all amino acid combinations in a tripeptide in having a very complicated β-region and are more frequently found in the bridging region between α-helix and β-sheet as evidenced from 2 tripeptide combinations (TRN & FRN) observed in the plot.

The conformation of amino acids in all the thirteen tripeptide analogue set were surprisingly almost similar with respect to both the Φ and Ψ values with regard to α-helix, β-sheet and γ-turns. The spread of torsion angles for β-sheets in all the selected 13 tripeptide templates is much larger than for α-helices.

The last and least-populated regions of the Ramachandran plot, the turn region (also called the left handed α-helical region) around Φ = Ψ = 60°, is mainly populated by most of the amino acids except proline. All the tripeptides designed (except TRN & FRN) have shown their corresponding Φ + 45° to +70° and Ψ +40° to +55° as most favourable allowed γ-turns whereas for the tripeptide TRN, the most favourable γ-turns have +45° to +65° Phi values and +30° to +55° Psi values respectively. Similarly, the most favourable γ-turns of tripeptide FRN have shown the torsion angles Φ +55° to +72° and Ψ +30° to +55°.

From all the above results mentioned, it would be much better to call the three most populated areas of the Ramachandran plot are the α-helix, β-strand and γ-turn regions, with respect to individual amino acids and their interactions in to unique tripeptide combinations with their Φ/Ψ values observed.

The turn regions irrespective of their least population act as nucleation centres during protein folding, which reveals the therapeutic significance in protein misfolding diseases such as Alzheimers and Prions.

The average low energy allowed and generously allowed regions of all 13 tripeptide analogues in Ramachandran plot were 14.808% and 32.462%. The summation average corresponds to 47.27% for all tripeptides designed with this experiment.

In addition, the average high energy steric clash regions (empty regions) of all 13 tripeptide leads was found be 52.731%. Among all the 13 tripeptides subjected to Ramachandran plot of conformational analysis, we found that only four tripeptides, viz., FKQ, FRN, EWK and EHR have shown maximum low energy allowed regions as most favourable conformations with their corresponding values 15.5%, 16%, 16% and 15.5% respectively. These tripeptides could prove useful for developing new peptide based pharmaceuticals.

We have found several interesting and surprising features in short chain peptide inbuilts as a result of this extensive analysis of Ramachandran plots. The β-sheet region is especially interesting, with its complicated fine structure differing from one amino acid to another. Most amino acids of peptides have two distinct narrow regions of Φ, Ψ angles. Although many of the features can be discerned in the plots by Kleywegt and Jones[7] ,they are more evident here owing to our structure inbuilt in 2D viewer and the smoothing procedure. The Ramachandran plot is a very useful tool for checking tripeptide structure determinations. Our findings may also prove important for protein-folding prediction especially with γ-turn regions.

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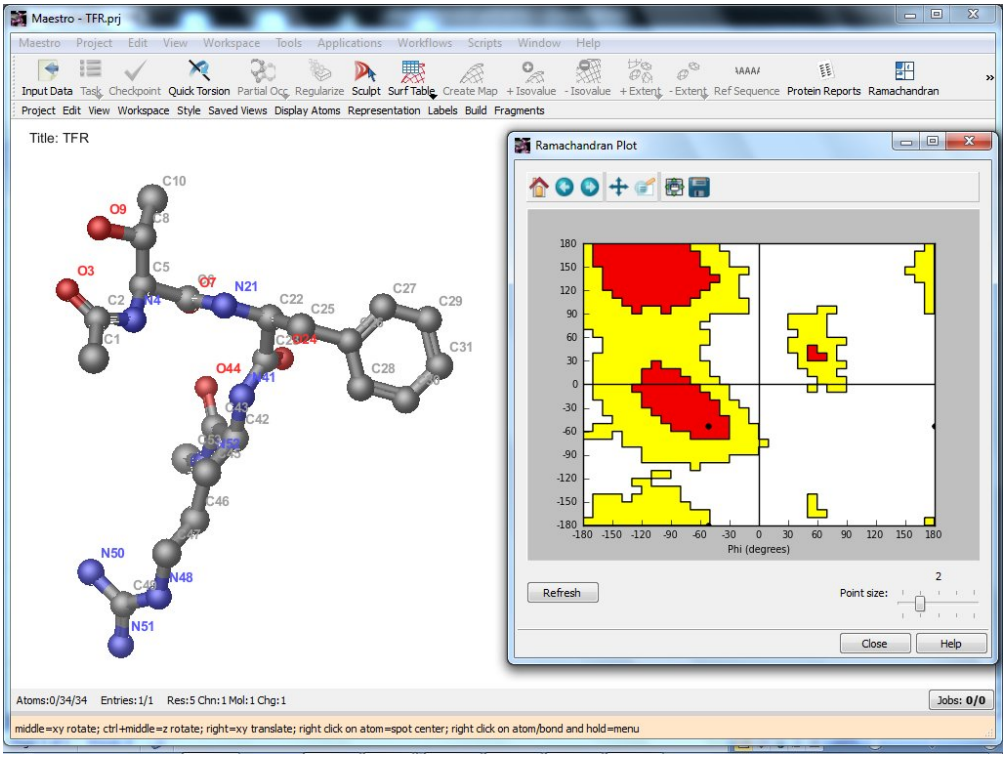

Figure 1. Ramachandran plot representation of tripeptide *Thr-Phe-Arg (TFR)* in the four Quadrant Square with its chemical inbuilt showing Carbon, Oxygen and Nitrogen atoms at respective positions.

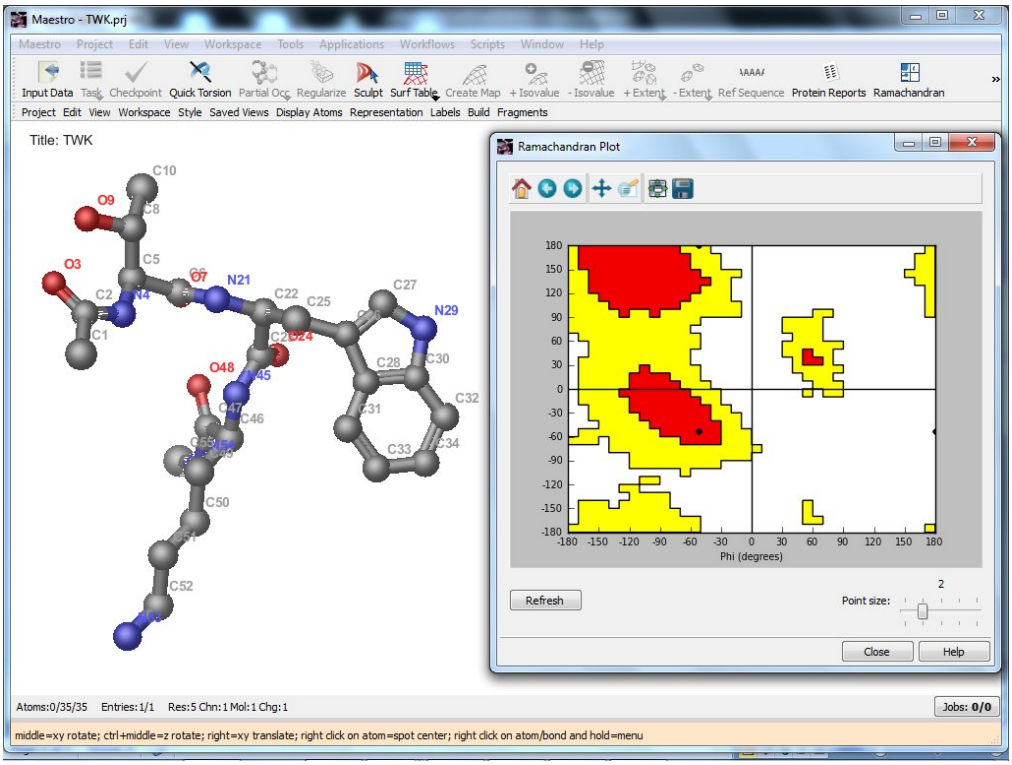

Figure 2. Ramachandran plot representation of tripeptide *Thr-Trp-Lys (TWK)* in the four Quadrant Square with its chemical inbuilt showing Carbon, Oxygen and Nitrogen atoms at respective positions.

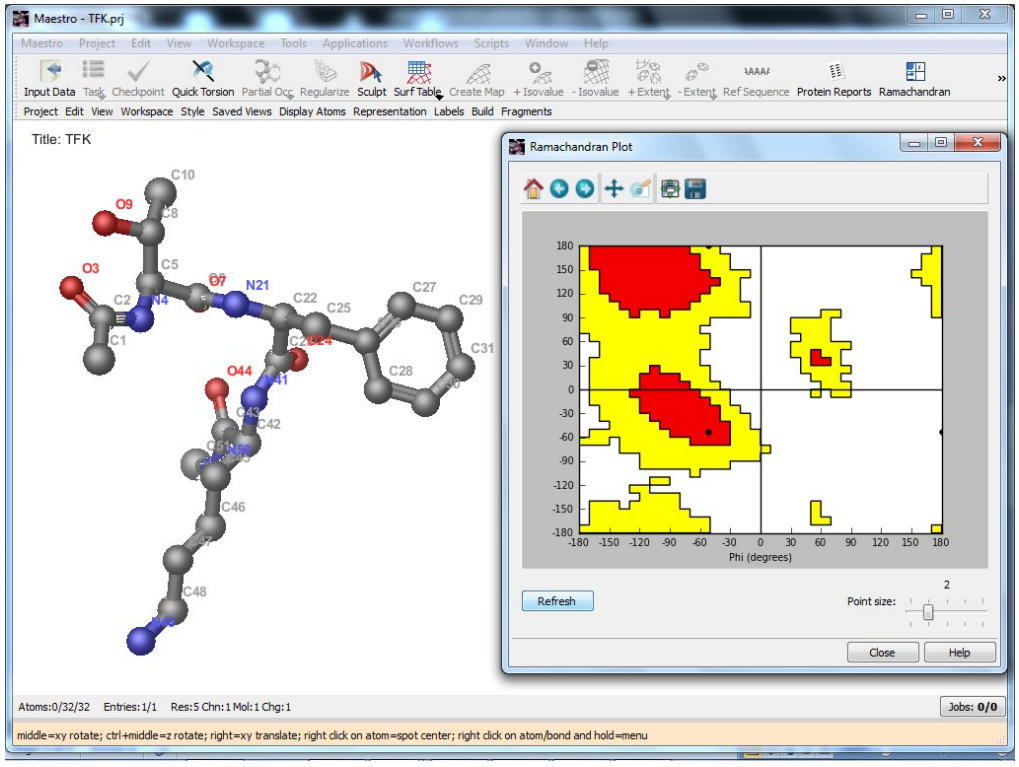

Figure 3. Ramachandran plot representation of tripeptide *Thr-Phe-Lys (TFK)* in the four Quadrant Square with its chemical inbuilt showing Carbon, Oxygen and Nitrogen atoms at respective positions.

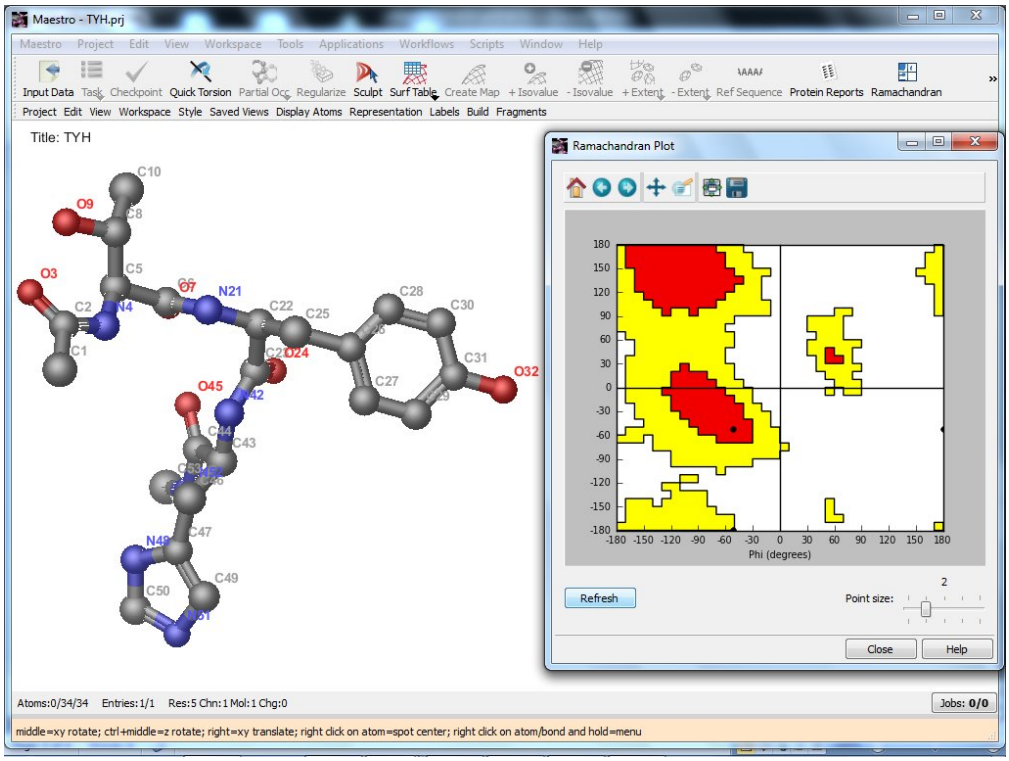

Figure 4. Ramachandran plot representation of tripeptide *Thr-Tyr-His (TYH)* in the four Quadrant Square with its chemical inbuilt showing Carbon, Oxygen and Nitrogen atoms at respective positions.

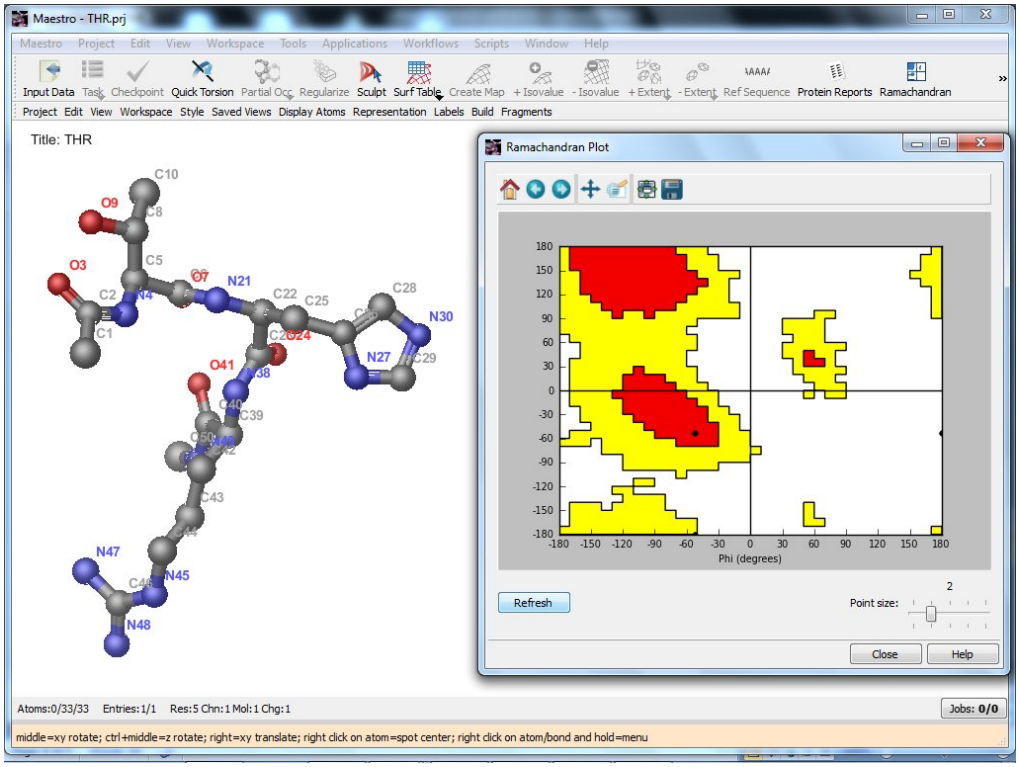

Figure 5. Ramachandran plot representation of tripeptide *Thr-His-Arg (THR)* in the four Quadrant Square with its chemical inbuilt showing Carbon, Oxygen and Nitrogen atoms at respective positions.

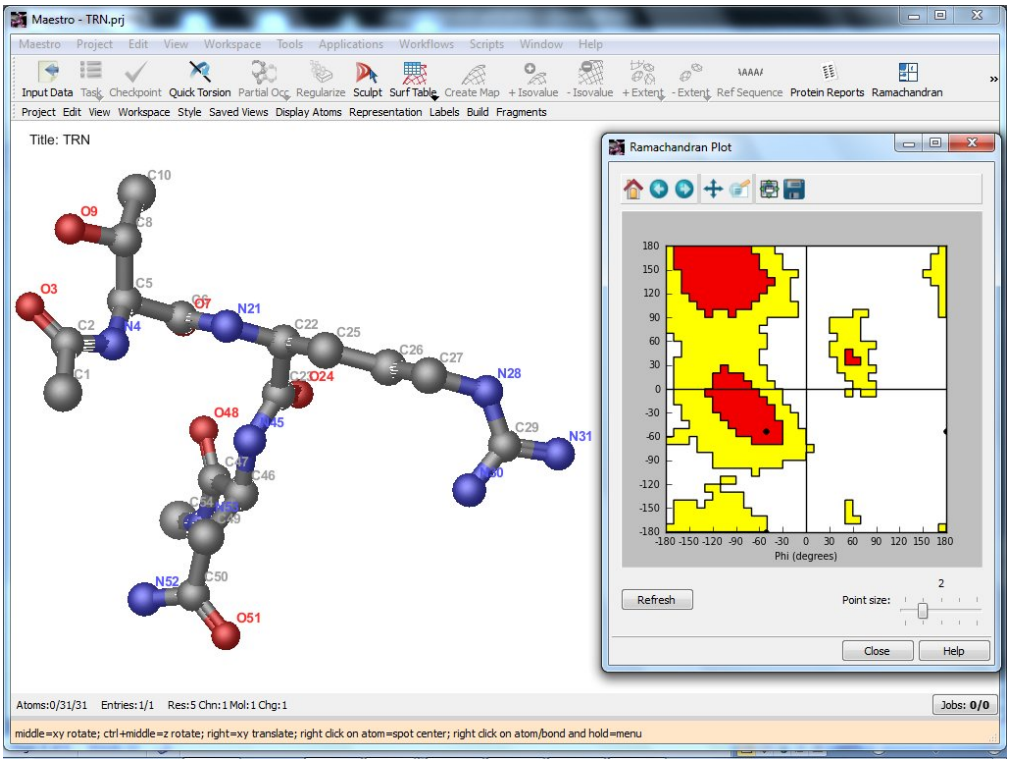

Figure 6. Ramachandran plot representation of tripeptide *Thr-Arg-Asn (TRN)* in the four Quadrant Square with its chemical inbuilt showing Carbon, Oxygen and Nitrogen atoms at respective positions.

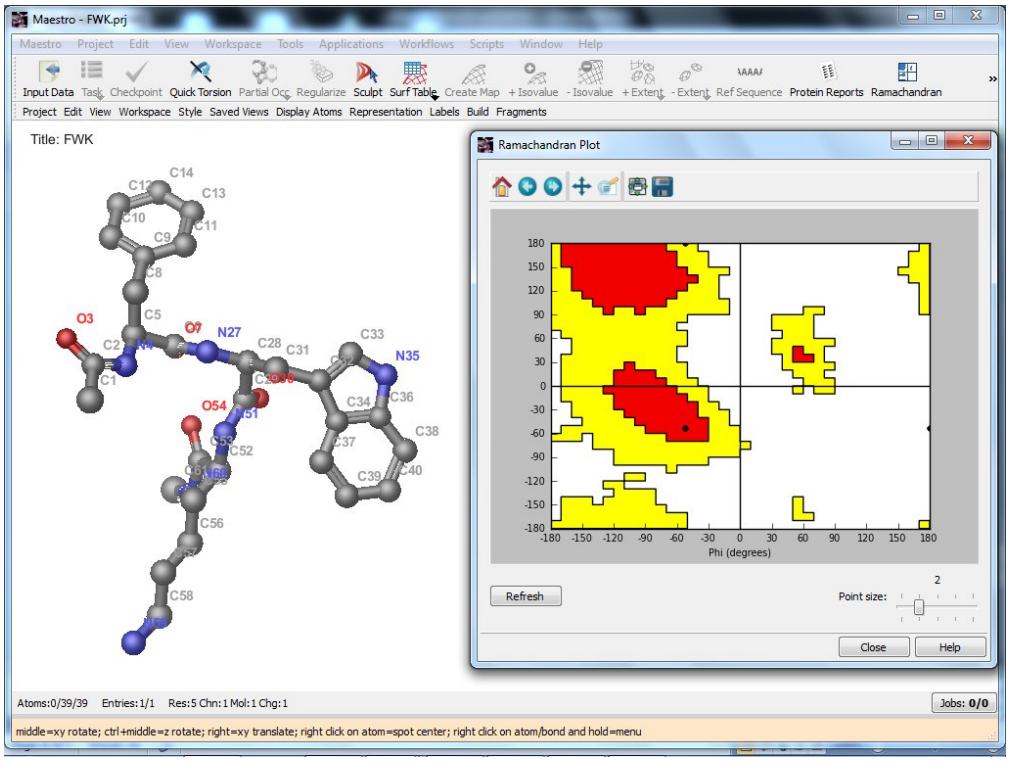

Figure 7. Ramachandran plot representation of tripeptide *Phe-Trp-Lys (FWK)* in the four Quadrant Square with its chemical inbuilt showing Carbon, Oxygen and Nitrogen atoms at respective positions.

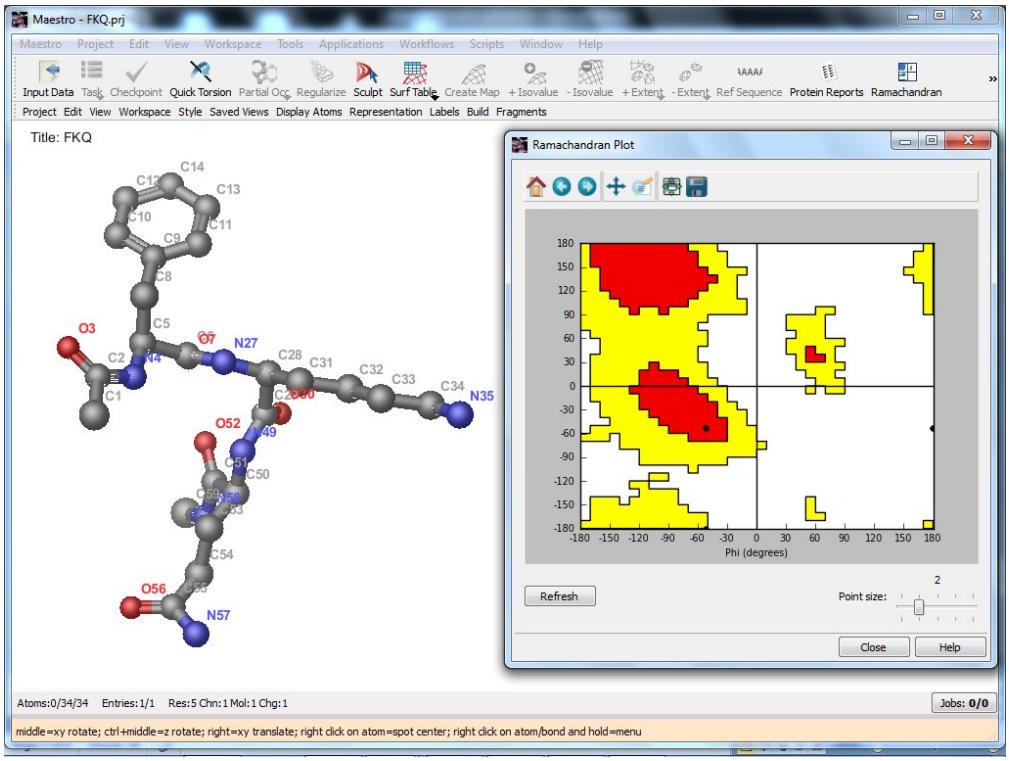

Figure 8. Ramachandran plot representation of tripeptide *Phe-Lys-Gln (FKQ)* in the four Quadrant Square with its chemical inbuilt showing Carbon, Oxygen and Nitrogen atoms at respective positions.

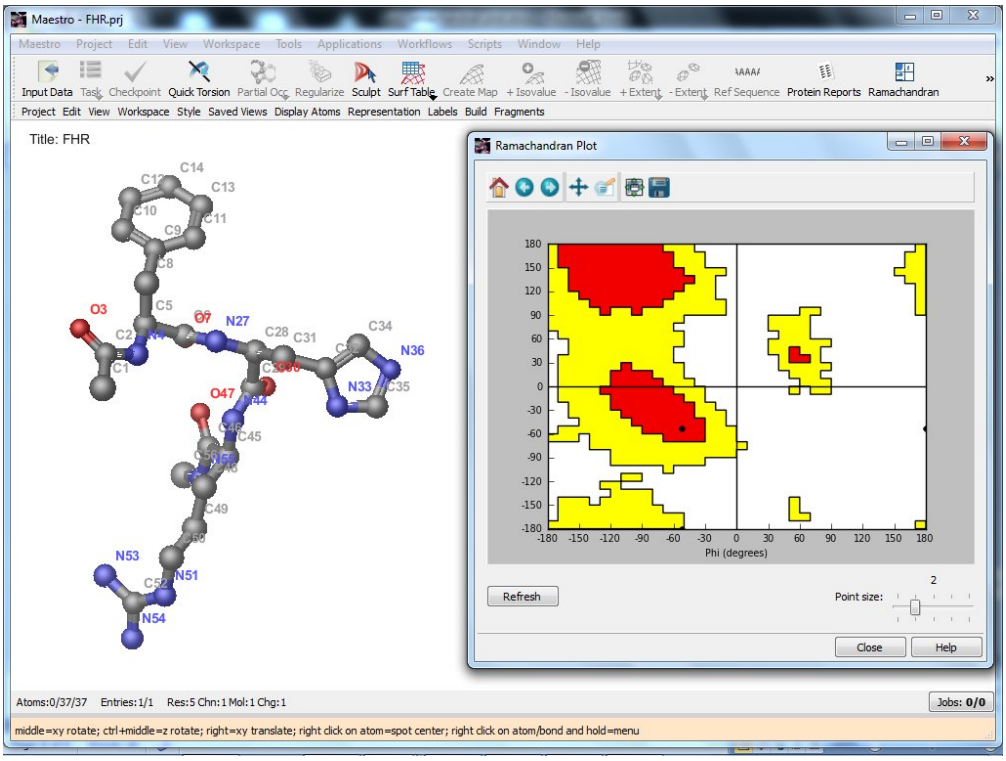

Figure 9. Ramachandran plot representation of tripeptide *Phe-His-Arg (FHR)* in the four Quadrant Square with its chemical inbuilt showing Carbon, Oxygen and Nitrogen atoms at respective positions.

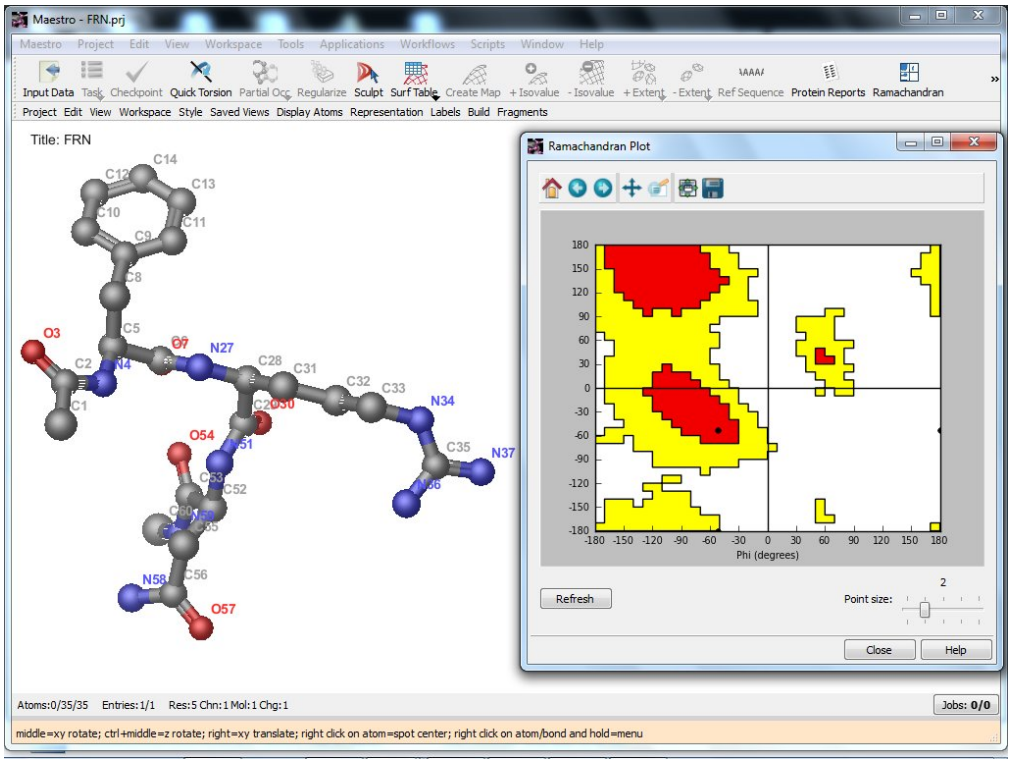

Figure 10. Ramachandran plot representation of tripeptide *Phe-Arg-Asn (FRN)* in the four Quadrant Square with its chemical inbuilt showing Carbon, Oxygen and Nitrogen atoms at respective positions.

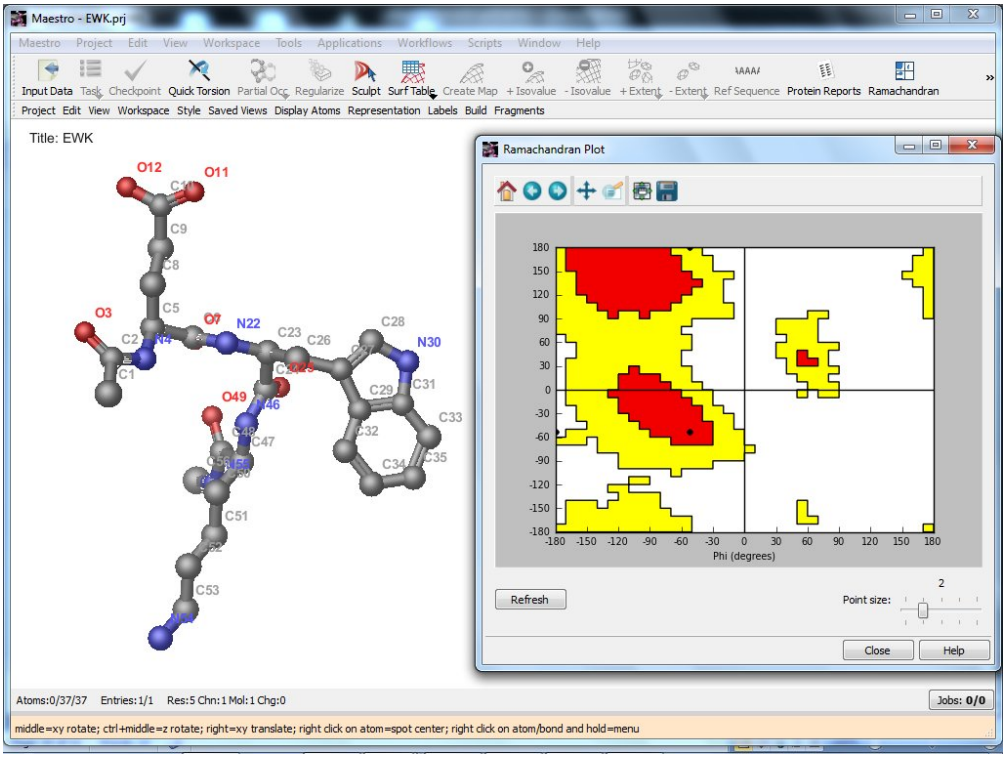

Figure 11. Ramachandran plot representation of tripeptide *Glu-Trp-Lys (EWK)* in the four Quadrant Square with its chemical inbuilt showing Carbon, Oxygen and Nitrogen atoms at respective positions.

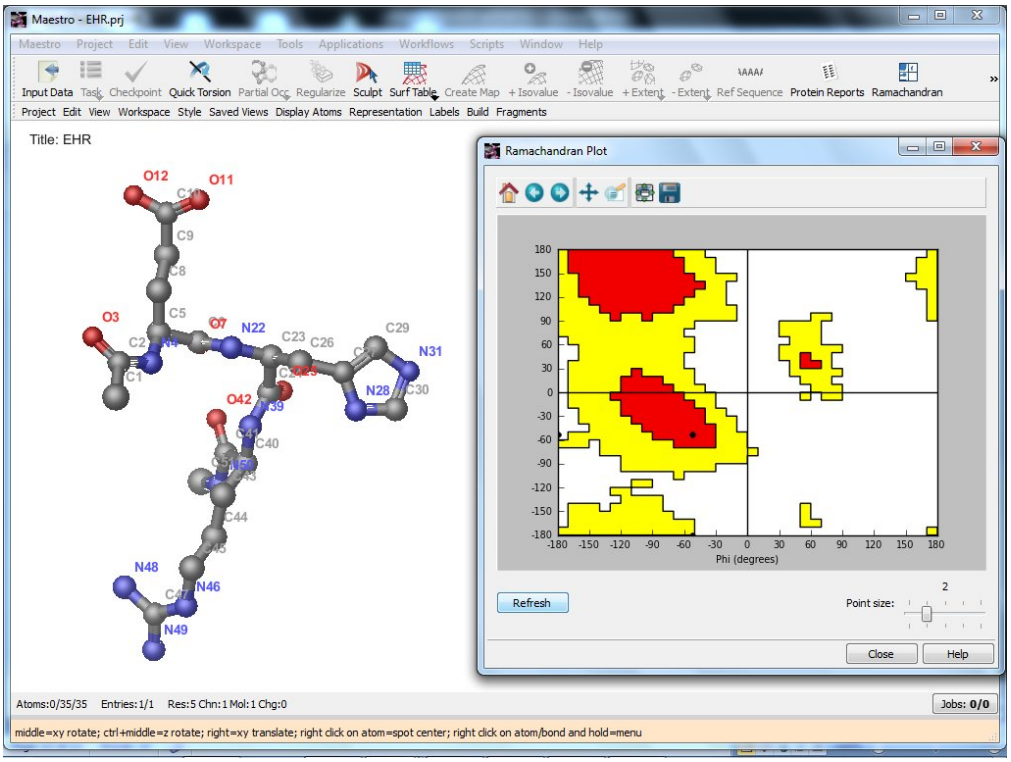

Figure 12. Ramachandran plot representation of tripeptide *Glu-His-Arg (EHR)* in the four Quadrant Square with its chemical inbuilt showing Carbon, Oxygen and Nitrogen atoms at respective positions.

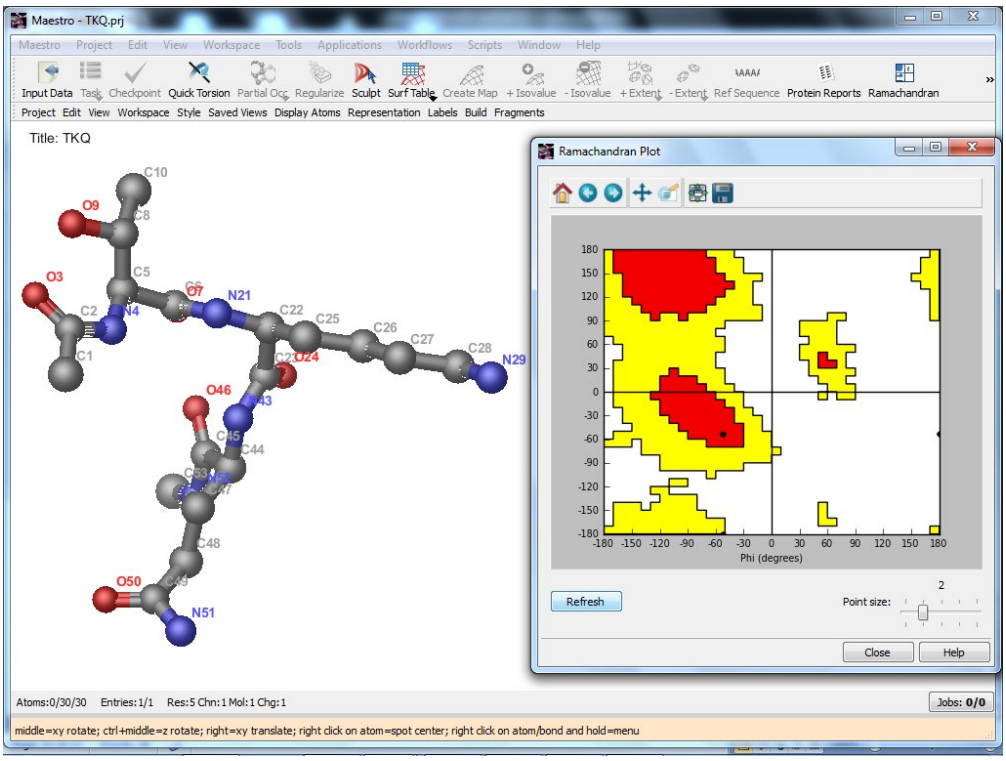

Figure 13. Ramachandran plot representation of tripeptide *Thr-Lys-Gln (TKQ)* in the four Quadrant Square with its chemical inbuilt showing Carbon, Oxygen and Nitrogen atoms at respective positions.

S.No.	Test Set	α-Helix				β-Sheet				γ-Turn			
		Phi value (Ideal)	Psi value (Ideal)	Region Observed (Quadrant)	Significance	Phi value (Ideal)	Psi value (Ideal)	Region Observed (Quadrant)	Significance	Phi value (Ideal)	Psi value (Ideal)	Region Observed (Quadrant)	Significance
1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.	TFR	−57°	−47°	L.L	Most Favourable	−80°	+150°	U. L	Most Favourable	−80°	+80°	U. L	Less Favourable
2.	TWK			L.L	Most Favourable			U. L	Most Favourable			U. L	Less Favourable
3.	TFK			L.L	Most Favourable			U. L	Most Favourable			U. L	Less Favourable
4.	TYH			L.L	Most Favourable			U. L	Most Favourable			U. L	Less Favourable
5.	THR			L.L	Most Favourable			U. L	Most Favourable			U. L	Less Favourable
6.	TRN			L.L	Most Favourable			U. L	Most Favourable			U. L	Less Favourable
7.	FWK			L.L	Most Favourable			U. L	Most Favourable			U. L	Less Favourable
8.	FKQ			L.L	Most Favourable			U. L	Most Favourable			U. L	Less Favourable
9.	FHR			L.L	Most Favourable			U. L	Most Favourable			U. L	Less Favourable
10.	FRN			L.L	Most Favourable			U. L	Most Favourable			U. L	Less Favourable
11.	EWK			L.L	Most Favourable			U. L	Most Favourable			U. L	Less Favourable
12.	EHR			L.L	Most Favourable			U. L	Most Favourable			U. L	Less Favourable
13.	TKQ			L.L	Most Favourable			U. L	Most Favourable			U. L	Less Favourable

S. No.	Most Favourable Quadrant Regions			Less Favourable Quadrant Regions				Not Favourable Quadrant regions		Low Energy Regions (%)		High Energy Steric Clash Regions (%)
	U.L (approx)	L.L (approx)	U.R (approx)	U.L (approx)	L.L (approx)	U.R (approx)	L.R (approx)	L.R (approx)	U.R (approx)	Allowed (%)	Generously allowed (%)	
	15	16	17	18	19	20	21	22	23	24	25	26
1.	ϕ = −55° to −150° ψ = +100° to +180° ϕ = -70° to -120° ψ = +0° to +25°	ϕ = −45° to −115° ψ = +0° to −60°	ϕ = +45° to +70° ψ = +30° to +55°	ϕ = −55° to −170° ψ = +30° to +90°	ϕ = −66° to −170° ψ = −140° to −180° ϕ = −122° to −160° ψ = +0° to −70° ϕ = +0° to −120° ψ = −70° to −95°	ϕ = +30° to +90° ψ = +0° to +105° ϕ = +165° to +180° ψ = +90° to +180°	ϕ = +50° to +70° ψ = −145° to −170° ϕ = +170° to +180° ψ = −170° to −180°	ϕ = +10° to +170° ψ = −10° to −140° ϕ = +0° to +50° ψ = −140° to −180° ϕ = +70° to +175° ψ = −140° to −175°	ϕ = +30° to +150° ψ = +100° to +180° ϕ = +0° to +30° ψ = +0° to +105° ϕ = +92° to +180° ψ = +0° to +105°	15	32.5	52.5
2.										13	33	54
3.										15	31	54
4.										14	33	53
5.										14.5	32	53.5
6.										13.5	32.5	54
7.										14.5	32.5	53
8.										15.5	32.5	52
9.										15	31.5	53.5
10.										16	33.5	50.5
11.										16	34	50
12.										15.5	33	51.5
13.										15	31	54

Table 1. Various Notation values and their significance of Ramachandran plot for the designed set of tripeptide analogues.
U.L. Upper Left; U.R. Upper Right; L.L. Lower Left; L.R. Lower Right