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Molecular dynamics simulation of Tuftsin and its analogs in a receptor like environment.

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Abstract
Tuftsin known to be an effective immunomodulator also have several therapeutic applications. To obtain better potency and effectiveness of tuftsin, a varied range of analogs have been investigated for their biological and conformational properties by several research groups. Using theoretical molecular dynamics simulation and biophysical techniques like nuclear magnetic resonance, circular dichroism various researchers proposed a number of tuftsin conformations. Theoretical as well as experimental findings leads to the invention of super active analogs like c(Thr-Lys-Pro-Arg-Gly) and Thr-Lys-Pro-Pro-Arg and (Thr-Lys-Pro-Pro-Arg)$_4$.To arrive at a consensus regarding the linear tuftsin/analogs conformation, we used molecular dynamics simulations of 46 linear analogs known to have biological activity higher or close to the native peptide tuftsin, at a low dielectric 4 which is reflective of receptor like environment. By comparing each conformation in this environment, we find that all the super active analogs having more potency than the native peptide exert semi-extended conformation in the central part of the residues as observed in the crystal structure.

Keywords: Tuftsin, conformation, structure, function, simulations, NMR.

1. Introduction
A linear peptide tuftsin H-Thr-Lys-Pro-Arg-OH, which occurs in the CH_2 region of the Fc portion of human IgG Immunoglobin, located between residues 289 and 292 of the heavy chain of leukokinin stimulates the phagocytosis of polymorphonuclear granulocytes and phagocytes [1, 2]. This peptide has been found to stimulate all known functions of phagocytic cells, particularly of granulocytes and macrophage/monocytes. The potential clinical applications, chemistry and biology of tuftsin have been extensively reviewed [3-6]. Based on theoretical calculations many synthetic analog of tuftsin, i.e. quasi-cyclic analog of TKPR, Thr-Lys-Pro-Pro-Arg(TKPPR), c[Thr-Lys-Pro-Arg-Gly] and c[Thr-Lys-Pro-Arg-Asp] showed much higher activity than the native molecule potency were discovered [7, 8, 9, 10]. Von Wronski *et al.* unexpectedly found that TKPR and TKPPR bind selectively to neuropilin-1 which plays has an essential role in angiogenesis and axon guidance and this binding block the vascular endothelial growth factor (VEGF) binding to neuropilin-1 [11]. They also reported that the tetrameric forms of TKPPR have ~90times more affinity to neuropilin-1. First receptor bound crystal structure was discovered by Kooi *et al.* where in they reported that the tuftsin bind to its receptor neuropilin-1 between the interstand loop at the tip of the b1 domain and exert its activity [12].

1.1 Molecular Dynamic Simulation Conformations
Scheraga and coworkers using theoretical molecular dynamics simulations proposed that the minimum energy conformations of tuftsin were like a "hairpin with two split ends" [13-15]. Subsequently Nikiforovich *et al.* by employing build-up procedure, using Dashevsky 6-12 forcefield, proposed a quasi-cyclic structure [8]. To validate their rational approach these authors synthesized the quasi-cyclic analog Thr-c[Lys-Pro-Arg] which indeed showed biological activity comparable to the parent peptide consequently, propagate the quasi-cyclic hypothesis [16]. O'Connor and coworkers, using CHARMM package, employed high temperature quenched molecular dynamics (QMD) and continuum models at dielectric 45 and 80 to mimic the solvent effects of dimethyl sulfoxide and water respectively to predict the bioactive conformation of tuftsin [17]. In dielectric 45, the major family was proposed to possess a type IV β-turn conformation, stabilized by an *unusual* intramolecular hydrogen bond (*i.e.* Pro-N···H-N Arg; N_i···H-N_{i+1} type interaction) constituting a *pseudo*-C_5-conformation. In high dielectric continuum representing the aqueous environment, the backbone conformation

of the major family was almost comparable to the one observed in implicit DMSO except the ψ torsion angle of the Thr residue which is significantly folded (ψThr ~ 48°). O'Connor et *al.* theoretically investigated cyclic analogs c[Thr-Lys-Pro-Arg], c[Thr-Lys-Pro-Arg-Lys], c[Thr-Lys-Pro-Arg-Gly] and c[Thr-Lys-Pro-Arg-Asp] hoping that some of these would represent possible ring size(s) which may adopt a type IV β-turn conformation. Surprisingly, an evaluation of phagocytosis stimulating activity revealed that the analog c[Thr-Lys-Pro-Arg-Asp] was almost as active as the linear tuftsin, while the analog c[Thr-Lys-Pro-Arg-Gly] was 50 times more active [17]. The analog exhibiting comparable activity resulted in two families F1 (14 structures) and F2 (8 structures). While the former one was suggested to a type IV β-turn at Lys-Pro position, a type III β-turn across the Arg-Asp segment was proposed for the latter. On the other hand, the results of the QMD calculations for the highly active analog indicated the presence of a major family (22 structures) which adopted a type IV β-turn across Lys-Pro segment. In order to further clarify the conformational properties of tuftsin and its highly active cyclic analog c[Thr-Lys-Pro-Arg-Gly] Valdeavella and coworkers performed molecular dynamics studies in explicit water and 1.0M NaCl solution [18]. From the results of simulation studies in explicit water, on the *cis* and *trans* isomers of the tuftsin, the authors proposed that type VI β-turn conformation for the *cis* isomer whereas the absence of both β- and γ-turn structure was suggested for the *trans*-isomer. Kothekar*et al.* carried out a study on the conformational flexibility of tuftsin using all-atom based atom-atom potential and systematic search, simulated annealing molecular dynamics (SAMD) and molecular dynamics (MD) techniques [19]. Authors used 12.5Å as cut-off distance for non-bonded interaction and molecular dynamics was carried out for 650 picoseconds (ps) using AMBER 4.0 with explicit water in TIP3P model. They observed predominance of folded structure which was in agreement with data by Konoplinska *et al.* [20] NMR data by Blumenstein *et al.* [21] and D'Urs*iet al.* [22], Lys-2 interacts with Arg-4 COO- group giving rise to quasi-cyclic behavior and Arg-4 NH are turned inside pointing towards Lys-2 CO group therefore the conformation is neither β-turn nor γ-turn rather it is close to inverse γ-turn. They also observed that the structure was stabilized by strong electrostatic and weak hydrogen bonding interactions. Unrestrained molecular dynamics of tuftsin in DMSO revealed the occurrence of four different structural families for the major *trans* conformer. Although, the torsion angles of the central segment of tuftsin appears to be conformationally restricted in a type VII β-turn structure [23]. A survey of theoretical studies of tuftsin and its potent analogs indicates that there appears to be an overwhelming preference for the folded conformation which is neither a typical β-turn nor a typical γ-turn stabilized by 4→1 and 3→1 intramolecular hydrogen bonds, respectively. From these theoretical investigations, researchers could not able to give conclusive information about the conformation adopted by the native tuftsin and it's more potent analogs.

1.2 NMR conformations

Blumenstein *et al*. employed ^{13}C and ^{1}H NMR spectroscopy to investigate the preferred solution conformation inDMSO-d_6 of the native tuftsinand suggested that tuftsin in solution prefer a β-turn conformation presumably stabilized by 4→1(Arg NH···O=C Thr) intramolecular hydrogen bond [21]. To clarify the preferred solution conformation(s) of tuftsin, a combined application of NMR spectroscopy in DMSO-d_6 and molecular modeling were employed by D'Ursi *et.al* and suggested that the preferred conformation of tuftsin in DMSO is an inverse γ-turn structure stabilized by a 3→1 (Arg NH···O=C Lys) interaction for the trans isomer [24]. Ashish *et al*. observed a strong $d_{NN}(i, i+1)$ NOE connectivities, which is diagnostic of tightly folded β-turn structure, expected between Pro $C^{\delta}H_2$ and Arg NH groups is clearly absent suggesting it is neither a type I (III) nor a Type II β-turn structure [23]. From the observed ^{3}J values, dδ/dT of amide NHs and inter and intra residue NOE cross peaks suggest that the solution conformation of tuftsin in DMSO-d6 is predominantly a type VII β-turn not stabilized by 4→1 intramolecular hydrogen bond. D'Ursi et al. investigated the conformational properties of two cyclic analogs of tuftsin c[Thr-Lys-Pro-Arg-Gly] and c[Thr-Lys-Pro-Arg-β-Ala] by one and two-dimensional NMR spectroscopy in a cryoprotective DMSO/water mixture followed by molecular modeling [22]. Interestingly, these authors could experimentally confirm the existence of a cis peptide bond between Lys-Pro residues and classified the bioactive conformation as a type VIa β-turn based on the observation of characteristic strong Lys $C^{\alpha}H \leftrightarrow$Pro $C^{\alpha}H$ NOE.

To look for the better potent analogs of tuftsin various researchers studied the tuftsin structure and function using different molecular dynamics simulations and diverse biophysical techniques and hypothesized varied conformations *i.e.*β-turn, γ-turn, inverse γ-turn etcof this molecule. Therefore, there is perplexity in tuftsin conformation. This work was undertaken to sample the conformational space of the all the 46 linear tuftsin analogs Table-1, known to have efficacy more or less than the native tuftsin to arrive at a consensus regarding the tuftsin conformation.

Table 1: Linear tuftsin and its analogs considered for conformational analysis in this study

Peptide	Peptide Analogs	Total charge	Average hydrophilicity	Molecular Weight	Isoelectric point	Relative Activity	Reference
1	Thr-Lys-Pro-Arg	2.0	1.4	500.6	11.5	1.0	27
2	Thr-Lys-Pro-**Lys**	2.0	1.4	472.6	10.6	0.70	28
3	Thr-Lys-Pro-Arg-NH_2~	3.0	1.4	499.6	14.0	0.60	29
4	Thr-Lys-Pro-Ala	1.0	0.5	415.5	10.1	0.65	30
5	Thr-Lys-Pro-Gly	1.0	0.7	401.5	10.1	0.45	30
6	Thr-Lys-Pro-Arg-Gly	2.0	1.1	557.7	11.5	0.42	31
7	Thr-Lys-Pro-D-Arg	2.0	1.4	500.6	11.5	0.35	28
8	Thr-Lys-Pro-His	1.1	0.5	481.6	10.1	0.34	30
9	Thr-Lys-Pro	1.0	0.9	344.4	10.1	<0.1	32

10	Thr-Lys-Ala-Arg	2.0	1.3	474.6	11.5	0.60	[30]
11	Thr-Lys-Gly-Arg	2.0	1.4	460.5	11.5	0.35	[9]
12	Thr-Lys-Thr-Arg	2.0	1.3	504.6	11.5	0.8	[30]
13	Thr-Lys-Leu-Arg	2.0	1.0	516.6	11.5	0.5	[30]
14	Thr-Lys-ILeu-Arg	2.0	1.0	516.6	11.5	0.4	[9]
15	Thr-Lys-Val-Arg	2.0	1.0	502.6	11.5	0.4	[9,30]
16	Thr-Lys-Phe-Arg	2.0	0.8	550.7	11.5	0.2	[9]
17	Thr-Lys-Tyr-Arg	2.0	0.8	566.7	10.4	0.35	[9]
18	Thr-Lys-His-Arg	2.1	1.3	540.6	11.5	0.15-0.2	[9]
19	Thr-Lys-Lys-Arg	3.0	2.2	531.7	11.6	0.45	[9]
20	Thr-Lys-Ser-Arg	2.0	1.5	490.6	11.5	0.1	[9]
21	Thr-Lys-Asp-Arg	1.0	2.2	518.6	10.1	0.15	[9]
22	Thr-Lys-Gln-Arg	2.0	1.5	531.6	11.5	0.45	[30]
23	Thr-Lys-Arg-Arg	3.0	2.2	559.7	12.4	0.85	[30]
24	Thr-Arg-Pro-Arg	2.0	1.4	528.6	12.4	1.0	[30,33]
25	Thr-Orn-Pro-Arg	1.0	-	486.6	11.04	0.45	[30]
26	Thr-Leu-Pro-Arg	1.0	0.2	485.6	11.0	0.25	[34]
27	Lys-Lys-Pro-Arg	3.0	2.3	527.7	11.6	0.30	[35,36]
28	Val-Lys-Pro-Arg	2.0	1.1	498.6	11.5	0	[35,36]
29	Leu-Lys-Pro-Arg	2.0	1.1	512.7	11.5	>1.0	[35]
30	Ser-Lys-Pro-Arg	2.0	1.6	486.6	11.5	0.20	[35]
31	Ala-Lys-Pro-Arg	2.0	1.4	470.6	11.5	0	[35,36]
32	Tyr-Lys-Pro-Arg	2.0	0.9	562.7	10.4	0.3(R)	[36,37]
33	Ac-Thr-Lys-Pro-Arg	1.0	1.4	542.6	11.5	0	[36]
34	Lys-Thr-Lys-Pro-Arg	3.0	1.7	628.8	11.6	0	[30]
35	Lys-Pro-Arg	2.0	2.0	399.5	11.5	0	[38]
36	Thr-Arg-Pro-Lys	2.0	1.4	500.6	11.5	1.0	[33]
37	Arg-Pro-Lys-Thr	2.0	1.4	500.6	11.5	1.0	[30]
38	Thr-Lys-Lys-Ala	2.0	1.3	446.5	10.6	N.D.	[30]
39	Thr-Lys-Ala-Ala	1.0	0.4	389.5	10.1	0.13	[30]
40	Thr-Gly-Gly-Lys	1.0	0.7	361.4	10.1	0-0.	[30]
41	Thr-Ala-Arg-Lys	2.0	1.3	474.6	11.5	0.70	[30]
42	Thr-Ala-Val-Arg	1.0	0.2	445.5	11.0	0.75	[30]
43	Thr-Orn-Pro-Ala	0.0	-	401.5	6.01	0.3	[30]
44	Thr-Pro-Lys-Ala	1.0	0.5	415.5	10.1	0.1	[30]
45	Thr-Lys-Pro-Pro-Arg	2.0	1.1	597.7	11.5	>1.0	[3,30]
46	Lys-Pro-Pro-Arg	2.0	1.5	496.6	11.5	0	[39]

Table 2: Conformational torsion angle (°) preferences of Tuftsin and its analogs in dielectric 4

Peptide	**ψ1**	**ϕ2**	**ψ2**	**ϕ3**	**ψ3**	**ϕ4**	**ψ4**	**ϕ5**	**ψ5**
1	±160±10	-97.6±10	120.7±10	-74.8±10	-35.8±10	-92.5±10	29±10		
2	±165±10	-97.0±10	122.2±10	-73.6±10	-25±10	-107.2±10	32.7±10		
3	±160±10	-98.4±10	128±10	-76.9±10	±160±10	-84.9±10	80.8±10		
4	±165±10	-84.0±10	132±10	-76±10	-26±10	-115±10	31±10		
5	±170±10	-75.0±10	147±10	-72±10	82±10	39±10	-41±10		
6	-35.6±10	-92.4±10	154±10	-58.2±10	-15.8±10	-108.5±10	103±10	64.3±10	49.5±10
7	±160±10	-94.3±10	125.6±10	-77.0±10	118.7±10	65.3±10	9.4±10		
8	±155±10	82.0±10	135.5±10	-74.3±10	-20.8±10	-96.2±10	120±10		
9	±160±10	-99.7±10	128.9±10	-77.1±10	-70 or +110±10	-84.9±10	80.8±10		
10	±167±10	-94.3±10	101.6±10	-98.9±10	-32.3±10	-95.1±10	-30.6±10		
11	±164±10	-102.6	-45 or +130±10	-90 or +100±10	-54.1±10	-130.9±10	-90±10		
12	±165±10	-97.7±10	124.1±10	-81.4±10	-40 or +130±10	+60 or-140±10	-34.4±10		
13	-160±10	-86.7±10	124.5±10	-114.7±10	-50.1±10	-110±10	-40 or +120±10		
14	163.4±10	-88.2±10	111±10	-103.7±10	-34±10	-134.1±10	-50 or +130±10		
15	±165±10	-89.8±10	123.1±10	-97.5	-45 or +140±10	-91.5±10	-60± or +120±10		
16	-160±10	-92.6±10	164.6±10	-92.9±10	-45 or +130±10	-130 or +50±10	-47.1±10		

17	±160±10	-84.8±10	132.1±10	-104.6±10	+125 or -40±10	+50 or -125±10	65.8±10		
18	±150±10	-105.5±10	130.4±10	-132±10	-51.3±10	-103.4±10	102.4±10		
19	-170±10	-116.7±10	±165±10	-78.9±10	+130 or -50±10	-103.5±10	-60±10		
20	±170±10	-100.9±10	133±10	-117.7±10	-45 or +130±10	-125or 52±10	-30.3±10		
21	±155±10	-94.5±10	73.7±10	-97.4±10	-16.5±10	-130.7±10	-6.3±10		
22	±165±10	-96.9±10	140.1±10	-93.6±10	-50.3±10	-122.6±10	50.2±10		
23	±160±10	-109.9±10	136.2±10	-99.6±10	-28.3±10	-104.8±10	-40 or 130±10		
24	±160±10	-86±10	128.2±10	-71.5±10	-58.1±10	-129.3±10	-55 or 150±10		
25	±170±10	-78.8±10	141.8±10	-70.3±10	-48.5±10	-128.5±10	-60 or 120±10		
26	±160±10	-93.5±10	140.2±10	-73.3±10	87.7±10	-137±10	-12.2±10		
27	±157±10	-84.2±10	158.5±10	-69.7±10	128.4±10	61.9±10	-57±10		
28	±152±10	-100.4	125or 22.5±10	-71.8±10	-40or +130±10	-55.7±10	23.8±10		
29	137.7±10	-111.8±10	113±10	-75.6±10	-23.4±10	-113.8±10	66.7±10		
30	160±10	-102.3±10	132.6±10	-78.2±10	-36.8±10	-128.3±10	29.6±10		
31	160±10	-103.9±10	121.2±10	-76±10	-60 or +120±10	-66.5±10	±42.8±10		
32	153.8±10	-85±10	150.4±10	-72.9±10	134.5±10	50.3±10	-2.9±10		
33	146.7±10	-112.5±10	105.5±10	-78.2±10	-36.1±10	-136.5±10	-49.4±10		
34	145.7±10	-71.4±10	-46.8±10	-116.4±10	153±10	-66.3±10	-56.1±10	-98.5±10	-5.3±10
35	130.3±10	-73.3±10	-13.2±10	-99.1±10	-13±10				
36	160±10	-92.5±10	135.3±10	-72.5±10	-47.3±10	-134.4±10	-33.4±10		
37	130±10	-76.9±10	-71.1±10	-73.5±10	102.5±10	-95.1±10	32.2±10		
38	160±10	-101.6±10	137.8±10	-80.7±10	129.9±10	-56.9±10	50.7±10		
39	170±10	-83.4±10	128.5±10	-95.8±10	62.2±10	-6.96±10	-59.4±10		
40	140±10	92.5±10	-21±10	-75 or +110±10	-67.6±10	-118.5±10	22.2±10		
41	165±10	-98.8±10	96.8±10	-83±10	138.3±10	-120.7±10	-44.3±10		
42	150±10	60±10	-53.1±10	-119.4±10	101±10	64.5±10	60 or-100±10		
43	165.8±10	-86.1±10	147±10	-72.4±10	-33±10	-101.1±10	-48.8±10		
44	145.5±10	-76.4±10	-40 or +140±10	-124.4±10	135.9±10	-99.1±10	-60 or +105±10		
45	160±10	-87.7±10	152.5±10	-67.7±10	160.1±10	-72±10	36.2±10	-130±10	37.5±10
46	140±10	-70.8±10	161.9±10	-66.6±10	60±10	-114.7±10	40.1±10		

2. Materials and Methods

2.1 Molecular Dynamics simulation

Molecular Dynamics (MD) and energy minimization were carried out using the TINKER molecular modeling package v 4.2 [25, 26], a freely available package for molecular mechanics based potential energy calculations, geometry optimization, MD simulation, distance geometry and structural analysis. In order to map the available conformational space of peptides 1-46, a fully extended $\phi = \psi = 180°$ was considered as a starting structure and all atoms Amber99 force field parameters were employed. We employed dielectric constant of 4. Truncated Newton method was used to energy minimize the input structure having automated force field and automated precondition option with RMSD cut off of 0.01 kcal/mol/Å. Minimized coordinates were subjected to molecular dynamics with 5000000 number of steps, time step length in 1 fento second and snapshot in 5picoseconds at temperature in 300K. The resultant 1000 structures for each peptide were analyzed for their conformational preferences. MD simulation results were tabulated in Table-2. The chemical structure along with backbone torsion angles were depicted in Figure 1.

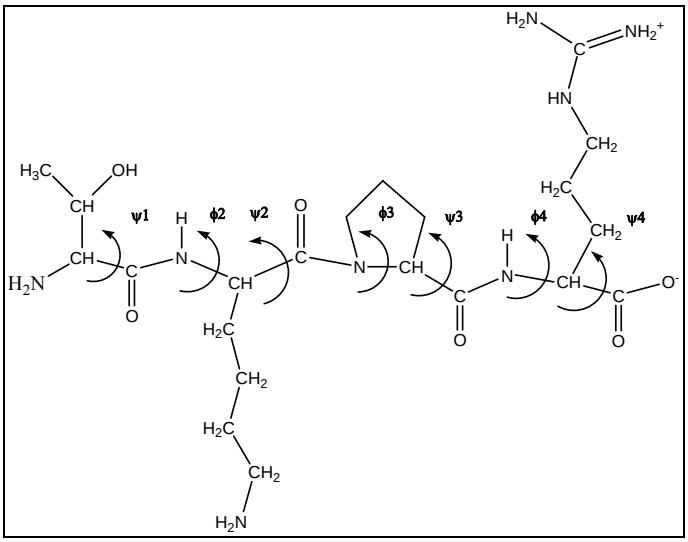


Fig 1: Chemical structure of native tuftsin and its representative backbone torsion angles

3. Results and Discussions

The average torsion angle preferences of the tuftsin and its analogs in dielectric 4 were tabulated in Table 2 and the lowest energy conformation along with possible intramolecular hydrogen bonding interactions were depicted in Figure 2(A-C). In receptor like environment, native peptide 1 adopts type IV β-turn like conformation in the central part of the molecule at Lys-Pro residues and close to type I β-turn conformation across Pro-Arg residues. The side chains of Lys and Arg residues orient in the same direction. The overall backbone conformation of the molecule looks like a spoon conformation. The molecule is stabilized by two hydrogen bonding interactions (Lys)$N^{\zeta}H\cdots O$=C(Arg) and (Arg)$N^{\varepsilon}H\cdots O$=C(Arg) with a distance 1.72Å and 1.94Å respectively. Peptide **2** in this environment has similar type IV β-turn conformation in central Lys-Pro residues but it differs in Pro-Lys residues in which distorted type III β-turn conformation is prevailed. The side chains of the Thr and Lys_1 residues directed exactly opposite to each other, whereas Lys1 side chain and Lys_3 main chain point towards each other keeping the side chain of Lys_3 to be right angle to its main chain. This molecule is also stabilized by one strong hydrogen bonding interaction between (Lys)$N^{\zeta}H\cdots O$=C(Lys) with a distance of 1.94Å and one weak hydrogen bonding interaction between Thr($O^{\gamma}H$)···HN(Lys) with a distance 2.41Å, which drastically different conformation as that of peptide 1 and the overall backbone adopts a chair like conformation . Peptide 3 which differs peptide 1 by the amide C-terminal has totally different conformation compared with peptide 1. The side chain of Lys directed right angled to side chains of Thr & Arg side chains. This peptide adopts semi-extended backbone conformation is stabilized by one weak intramolecular hydrogen bonding interaction between (Thr)$O^{\gamma}H\cdots$HN(Lys) with a distance of 2.49Å. In peptide 4, C-terminal Arg is replaced by Ala residue, in this case the peptide adopt a type VIa β-turn like conformation across Lys-Pro and a type VIII β-turn like conformation across Pro-Ala residues which is totally differ from the conformation adopted by the native peptide. The Thr and Pro side chains orient right angled to side chains of Lys residue. This conformation is stabilized by two weak intramolecular hydrogen bonding interaction (Thr)$O^{\gamma}H\cdots$HN(Lys) and (Lys)$N^{\zeta}H\cdots$O-C(Ala) with a distance of 2.41Å and 2.40Å respectively and one strong intramolecular hydrogen bonding interaction between (Lys)$N^{\zeta}H\cdots O$=C(Ala) with distance 1.78Å. In peptide 5, C-terminal Arg is replaced by Gly residue and this peptide adopts a type VIa β-turn like conformation across Lys-Pro and a type V β-turn like conformation across Pro-Gly residues. The Lys side chain and the Gly C-terminal point towards each other and is stabilized by three intramolecular hydrogen bond Thr(O^{γ})···HN(Lys), Lys(C=O)···HN(Gly) and (Lys)$N^{\zeta}H\cdots$O-C(Gly) with distances 2.15Å, 2.30 Å and 2.37 Å respectively. Peptide 6 adopt clearly adopts a type IV β-turn conformation across Lys-Pro, a distorted type I β-turn conformation across Pro-Arg residues and a distorted type V β-turn like conformation across Arg-Gly residues. The lys side chain experience a bent topology and pont towards Gly C-terminal while the overall peptide backbone adopts a turn conformation pushing the (Lys)C=O inside the turn. The conformation is stabilized by four intramolecular hydrogen bonds (Thr)$NH_1\cdots O$=C(Gly), (Thr)$NH_2\cdots O$=C(Gly), (Lys)NH···O=C(Gly) and (Lys)C=O···HN(Arg) with a distance of 2.24Å, 2.42Å, 2.36Å and 2.45Å respectively. Peptide **7** is an analog of native peptide having substitution at C-terminal Arg with (D)-Arg. This peptide adopts a distorted type IV β-turn like conformation across Lys-Pro and type IIβ-turn conformation across Pro-(D)Arg. Both Lys and (D)Arg side chains have propensities towards (D)Arg C-terminal. The molecule is stabilized by two intramolecular hydrogen bonding interactions Thr(O^{γ})···HN(Lys), (Arg)C=O···HN(Arg) with a distance of 2.21Å and 1.88Å respectively. Peptide 8 analog is the C-termini Arg residue is replaced with His. At low dielectric 4, this peptide adopts a type VIa β-turn like conformation across Lys-Pro and distorted type VIII β-turn like conformation across Pro-His residues. In the lowest energy conformation Lys and Pro side chains are positioned right angled to each other and Lys side chain shows its tendency towards His C-terminal. The molecule is stabilized by three intramolecular hydrogen bonding interactions between Thr(O^{γ})···HN(Lys), (Lys)$N^{\zeta}H\cdots O$=C(His) and (Lys)$N^{\zeta}H\cdots$O-C(His) with a distance of 2.28Å 1.88Å and 2.35Årespectively. Peptide 9 is the truncated tuftsin at position number 4 which adopts a distorted type IV β-turn like conformation across Lys-Pro. The (Lys)$N^{\zeta}H$ have a strong interaction with the C-terminal Pro and the molecule is stabilized by two intramolecular hydrogen bonding interactions Thr(O^{γ})···HN(Lys), (Lys)$N^{\zeta}H\cdots$O-C(Pro) with a distance of 2.49Å and 1.85Å respectively. In peptide 10, Pro residue is replaced with Ala. This peptide adopts a distorted type IV β-turn like conformation across Lys-Ala residue and type III β-turn like conformation across Ala-Arg residues. The side chains of Lys and Ala positioned opposite to each other with Ala residue backbone centering the turn conformation. This molecule is stabilized by three intramolecular hydrogen bonding interactions between Thr(O^{γ})···HN(Lys), (Lys)$N^{\zeta}H\cdots O$=C(Arg) and (Lys)$N^{\zeta}H\cdots$O-C(Arg) with a distance of 2.49Å2.20Å and 1.92Årespectively.

In peptide 11, Pro residue was replaced with Gly in the 3rd position. Due to the flexible nature of Gly residue, the peptide adopts multiple conformations across Ly-Gly and Gly-Arg. In the lowest energy conformation of this molecule is stabilized by two weak intramolecular hydrogen bonding interactions between Thr(O^{γ})···HN(Lys) and (Lys)$N^{\zeta}H\cdots O$=C(Arg)with a distance of 2.88Åand 2.67Å respectively. In peptide 12, Pro residue is replaced by Thr. This peptide also adopt multiple

conformations but major conformers are distorted type IV β-turn like conformation across Lys-Thr. The Lys side chain have high affinity towards Arg C-terminal and is stabilized by two intramolecular hydrogen bonding interactions between (Lys)$N^{\zeta}H\cdots O=C$(Arg) and (Lys)$N^{\zeta}H\cdots O$-C(Arg) with a distance of 1.92Åand 2.01Årespectively. Peptide 13 is Leu derivative of native peptide in 3rd position. This peptide adopts distorted type IV β-turn like conformation across Lys-Leu and is stabilized by one intramolecular hydrogen bonding interaction between (Lys)$N^{\zeta}H\cdots$O-C(Arg) with a distance of 2.05Å**.** Peptide 14 is an Ile analog of native tuftsin substitution at 3rd position. This peptide adopts distorted type IV β-turn like conformation across Lys-Ile. In this molecule both (Ile)NH and (Arg)NH lie inside the turn and is stabilized by (Lys)$N^{\zeta}H\cdots O=C$(Arg) and (Lys)$N^{\zeta}H\cdots O$-C(Arg) hydrogen bonding interactions with a distance of 2.20Åand 2.42Årespectively. Peptide 15 is an analog replacing Pro with Val at 3rd position. This peptide adopts distorted type IV β-turn like conformation across Lys-Val similar to peptide 13. In this molecule the Lys sidechain shows strong inclination towards Arg C-terminal and is stabilized by four intramolecular hydrogen bonding interactions between (Lys)$N^{\zeta}H\cdots O=C$(Arg), (Lys)$N^{\zeta}H\cdots$O-C(Arg), (Thr)$O^{\gamma}H\cdots N$(Lys) and Thr)$O^{\gamma}H\cdots O=C$(Lys)with a distance of 1.96Å, 2.28Å2.36Åand 2.28Årespectively. Peptide 16 is an analog replacing Pro with Phe residue at position 3. This molecule adopt distorted type IV β-turn like conformation across Lys-Phe similar to peptide 13. The Lys and Phe side chains position right angle to each other and is stabilized by one intramolecular hydrogen bond between (Lys)$N^{\zeta}H\cdots$O-C(Arg) with a distance of 2.10Å. A substitution at position 3 with Ala, Gly, Leu, Ile, Val, Phe results distorted type IV β-turn conformation across central i+1→i+2 position. In peptide 17, a substitution is done at position 3 replacing Pro with Tyr residue. Lys-Tyr sequence assumes a distorted type VIII β-turn like conformation. The side chains of Lys and Tyr orients in opposite directions and the Lys side chain oriented towards Arg C-terminal. This peptide is stabilized by one intramolecular hydrogen bond between (Lys)$N^{\zeta}H\cdots$O-C(Arg) with a distance of 1.97Å. In peptide 18, a substitution is done at position 3 replacing Pro with His residue. This peptide adopted type IV β-turn like conformation across Lys-His residue and type VIII β-turn like conformation across His-Arg residue. The Lys and His side chains were oriented in opposite directions while the His and Arg side chains positioned right angle to each other. The (His)NH and (Arg)NH lie inside the turn and is stabilized by two intramolecular hydrogen bonds between (Thr)$O^{\gamma}\cdots$HN(Lys) and (Lys)$N^{\zeta}H\cdots O=C$(Arg)with a distance of 2.48Åand 2.18Å respectively. Peptide 19 is a Lys derivative at position 3 replacing Pro. This molecule assumes type VIbβ-turn like structure across Lys-Lys though Lys ψ angle at position 3 is drastically different from the turn conformation and type IV β-turn like conformation across Lys-Arg residue. The Lys_1 side chain and Arg side chain oriented in same direction while Lys_3 side chain positioned right angled to Lys_1 side chain. This peptide is stabilized by two intramolecular hydrogen bonds between (Thr)$O^{\gamma}\cdots$HN(Lys) and (Lys)$N^{\zeta}H\cdots O=C$(Arg)with a distance of 2.28Åand 1.82Å respectively. Peptide 20 is a Ser derivative, replacing Pro at position 3. Peptide 20 adopts type IV β-turn like (major) conformation across Lys-Ser and Thr and Lys side chains positioned right angled to each other and this molecule is stabilized by one intramolecular hydrogen bond between (Thr)$O^{\gamma}\cdots$HN(Lys)with a distance of 2.24Å.

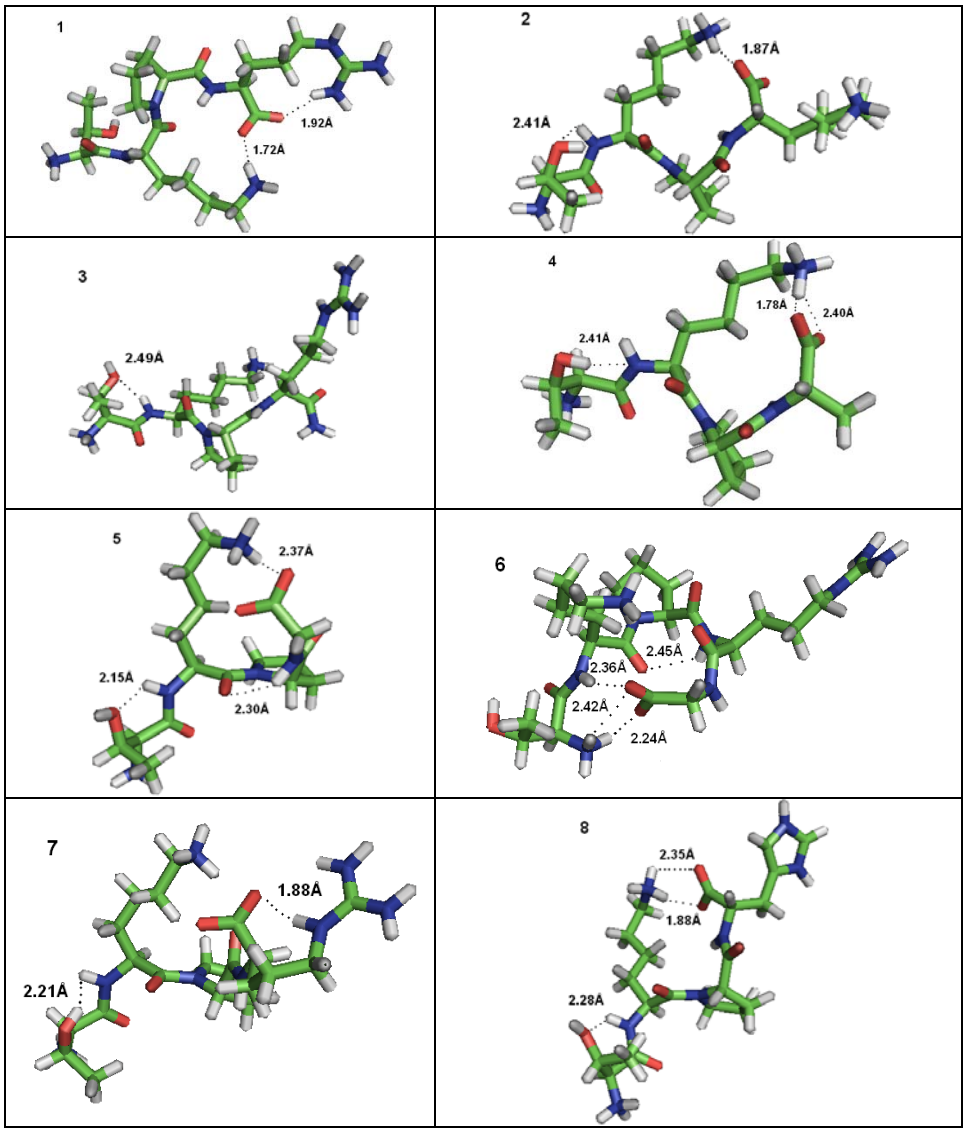
In peptide 21, Pro residue is replaced by Asp. This molecule adopts type IV β-turn like conformation across Lys-Asp and type I β-turn like conformation across Asp-Arg and the Lys side chain oriented in opposite directions to Asp and Arg side chains. This ordered conformation is stabilized by five intramolecular hydrogen bonding interactions between (Thr)$O^{\gamma}\cdots$HN(Lys), (Thr)NH$\cdots$O-C^{γ}(Asp), (Arg)$N^{\varepsilon}H\cdots$O-C^{γ}(Asp), (Arg)$N_{\eta}H\cdots$O-C^{γ}(Asp) and (Lys)$N^{\zeta}H\cdots O=C$(Arg) with a distance of 2.10Å, 2.14Å, 2.23Å, 1.96Å and 1.91Å respectively. In peptide 22, Pro residue is replaced by Gln residue. This molecule adopts type IV β-turn like structure across Lys-Gln and VIII β-turn like conformation across Gln-Arg sequence and the Lys side chain directed towards Arg C-terminal and Gln side chain positioned exactly opposite to the Lys side chain. This conformation is stabilized by four intramolecular hydrogen bonding interactions between (Thr)$O^{\gamma}\cdots$HN(Lys), (Lys)$N^{\zeta}H\cdots O=C$(Arg), (Lys)$N^{\zeta}H\cdots$O-C(Arg) and (Arg)$N^{\varepsilon}H\cdots$O-C(Arg) with a distance of 2.41Å, 1.93Å, 2.31Å and 2.28Å respectively. Peptide 23 is an Arg derivative, replacing Pro at position 3. This peptide adopts type IV β-turn conformation across Lys-Arg residue and type III β-turn like conformation across Arg-Arg sequence. The Thr and Lys side chains oriented in opposite directions while the Arg_3 and Arg_4 side chain located right angled to each other. The conformation is stabilized by one intramolecular hydrogen bonding interaction between (Lys)$N^{\zeta}H\cdots O=C(Arg_4)$ with a distance of 1.85Å. Peptide 24 is an Arg derivative, replacing Lys at position 2. This peptide assumes type IV β-turn conformation across Arg-Pro residue and type III β-turn like conformation across Pro-Arg sequence with distinct difference in $Arg_4\phi$ torsion angle. The $(Arg_2)N^{\varepsilon}H$ and (Arg)$N_{\eta}H$ directed towards Arg_4 C-terminal and is stabilized by three intramolecular hydrogen bonding interactions between (Thr)$O^{\gamma}\cdots$HN(Arg),$(Arg_2)N^{\varepsilon}H\cdots O=C(Arg_4)$ and (Arg)$N_{\eta}H\cdots O=C(Arg_4)$ with a distance of 2.84Å, 2.55Å and 2.55Å respectively. Peptide 25 is an Orn derivative, replacing Lys at position 2. This peptide also adopts type IV β-turn conformation across Orn-Pro similar to peptide 24 and distorted type III β-turn like conformation across Pro-Arg sequence. The Orn side chain oriented towards Arg C-terminal and this conformation is stabilized by four intramolecular hydrogen bonding interactions between (Thr)$O^{\gamma}\cdots$HN(Orn), (Orn)$N^{\varepsilon}H\cdots O=C$(Arg), (Orn)$N^{\varepsilon}H\cdots$O-C(Arg) and (Arg)$N^{\varepsilon}H\cdots$O-C(Arg) with a distance of 2.28Å, 2.45Å, 2.42Å and 2.23Å respectively. Peptide 26 is a Leu derivative, replacing Lys at position 2. Peptide assumes type VIa β-turn like conformation across Leu-Pro and type IV β-turn like conformation across Pro-Arg sequence. The Leu side chain and Pro ring oriented in same direction while Arg side chain inclined towards Arg C-terminal and this conformation is stabilized by two weak intramolecular hydrogen bonding interaction between (Thr)$O^{\gamma}\cdots$HN(Leu) and (Arg)$N_{\eta}H\cdots O=C$(Arg) with a distance of 2.62Å and 2.77Å respectively. Peptide **27** is a Lys derivative, replacing Thr at position 1. Peptide assumes semi extended like conformation across Lys-Pro and type V β-turn like conformation across Pro-Arg sequence. The Lys1 and Arg4 side chains positioned in opposite direction and this peptide is stabilized by four intramolecular hydrogen bonding interaction between $(Lys_1)N^{\zeta}H\cdots O=C$(Arg), $(Lys_2)N^{\zeta}H_1\cdots$O-C(Arg), (Lys_2) $N^{\zeta}H_2\cdots$ O-C(Arg) and $Lys_2)N^{\zeta}H_1\cdots O=C$(Pro) with a distance of 2.48Å,

2.33Å, 2.44Å and 2.18Å respectively. Peptide 28 is a Val derivative, replacing Thr at position 1. This peptide adopts multiple conformations due to the branching of the side chain of Val. And the Lys side chain and Pro ring located right angle to each other and this conformation is stabilized by one strong and one weak intramolecular hydrogen bonding interaction between (Lys)$N^{\zeta}H\cdots O$=C(Arg), (Lys)$N^{\zeta}H\cdots$O-C(Arg) with a distance of 2.08Å and 2.62Å respectively. Peptide 29 is an important analog of tuftsin which shows better relative activity than the native peptide. This is a Leu derivative, replacing Thr at position 1. This peptide adopts type IV β-turn like conformation across Lys-Pro residue and type VIII β-turn like conformation across Pro-Arg residue. The Leu and Lys side chains oriented trans to each other and the Lys side chain shown to be twisted towards the C-terminal Arg. The conformation is stabilized by one intramolecular hydrogen bonding interaction between (Lys)$N^{\zeta}H\cdots$O=C(Pro) with a distance of 2.42Å. Peptide 30 is a Ser derivative replacing Thr at position 1. This peptide adopts type IV β-turn like conformation across Lys-Pro residue and type VIII β-turn like conformation across Pro-Arg residue.The Lys side chain and the Pro ring positioned trans to each other and (Lys)$N^{\zeta}H$ inclined towards the C-terminal Arg residue. This molecule is stabilized by three intramolecular hydrogen bonding interactions between (Ser)$O^{\gamma}\cdots$HN(Lys), (Lys)$N^{\zeta}H\cdots$O=C(Arg), (Lys)$N^{\zeta}H\cdots$O-C(Arg) with a distance of 2.27Å, 2.40Å and 2.17Å respectively.

Peptide 31 is a Ala derivative replacing Thr at position 1. This peptide adopts type IV β-turn like conformation across Lys-Pro residue and close to type I β-turn like conformation across Pro-Arg residue. The Lys and Arg side chains directed parallel to each other while Lys side chain and Pro ring located trans to each other and the (Lys)$N^{\zeta}H$ spotted close to C-terminal Arg. This peptide is stabilized by one strong and one weak intramolecular hydrogen bonding interactions between (Lys)$N^{\zeta}H\cdots$O-C(Arg) and (Lys)$N^{\zeta}H\cdots O$=C(Arg), with a distance of 2.04Å and 2.73Å respectively. In peptide 32, Thr is replaced by Tyr at position 1. This peptide adopts semi-extended conformation across Lys-Pro residues and type II β-turn like conformation across Pro-Arg residue. The Tyr and Pro ring situated cis to each other and also Lys and Arg side chains positioned in the same direction. The (Lys)$N^{\zeta}H$ group shown to be very close to C-terminal Arg and the conformation is stabilized by two strong intramolecular hydrogen bonding interactions between (Lys)$N^{\zeta}H\cdots$O-C(Arg) and (Arg)$N_{\eta}H_1\cdots O$=C(Arg) with a distance of 2.12Å and 2.11Å respectively. Peptide 33 is an N-terminal acetylated derivative of native tuftsin. This peptide adopts semi-extended conformation across Thr-Lys residue, a type IV β-turn like conformation across Lys-Pro sequence and type III β-turn like conformation across Pro-Arg residue. The Thr and Lys side chains located trans to each other and the molecule is stabilized by two intramolecular hydrogen bonding interactions between (Lys)$N^{\zeta}H\cdots O$=C(Arg) and (Arg)$N_{\eta}H_1\cdots O$=C(Pro) with a distance of 2.05Å and 2.49Å respectively. In peptide 34, another amino acid Lys was attached at the C-terminal tuftsin. This peptide adopts a type VIII β-turn across Thr-Lys, IV β-turn like conformation across Lys-Pro sequence and Type I β-turn like conformation across Pro-Arg sequence. The Lys1 and Lys3 side chain amino group were twisted towards C-terminal Arg residue and there are three hydrogen bonding interactions were observed between (Lys_1)$N^{\zeta}H\cdots$O-C(Arg), (Lys_1)$N^{\zeta}H\cdots O$=C(Pro) and (Lys_3)$N^{\zeta}H\cdots O$=C(Arg)) with a distance of 1.99Å, 2.28Å and 1.98Å respectively. In peptide 35, the first amino Thr is deleted from native peptide sequence. This peptide formed a type III β-turn across Pro-Arg sequence and the Lys and Arg side chain oriented in cis to each other and Pro ring positioned right angled to both Lys and Arg side chains. This peptide is stabilized by one intramolecular hydrogen bond between (Lys)$N^{\zeta}H\cdots$O-C(Arg) with a distance of 2.41Å. Peptide 36 is a scrambled analog, Thr-Arg-Pro-Lys. This peptide adopts type IV β-turn like conformation across Arg-Pro sequence and type III β-turn like conformation across Pro-Lys residues. The Thr and Arg side chain oriented trans to each other and (Arg)$N^{\varepsilon}H$ group oriented towards the C-terminal Lys and the molecule is stabilized by four intramolecular hydrogen bonding interaction between (Thr)$O^{\gamma}\cdots$HN(Arg), (Arg)$N^{\varepsilon}H\cdots$O=C(Lys), (Arg)$N^{\varepsilon}H\cdots$O-C(Lys) and (Arg)$N_{\eta}H_1\cdots$O-C(Lys) with a distance of 2.21Å, 2.07Å, 2.18Å and 1.98Å respectively. Peptide 37 is a reverse analog of tuftsin, Arg-Pro-Lys-Thr. This peptide adopts type VIII β-turn like conformation across Pro-Lys sequence and type VIa β-turn like conformation across Lys-Thr residues. The Arg and Lys side chains were positioned in the same direction and located near to C-terminal Thr and the molecule is stabilized by three intramolecular hydrogen bonding interactions between (Arg)$N^{\varepsilon}H\cdots O$=C(Lys), (Arg)$N_{\eta}H_1\cdots O$=C(Thr) and (Lys)$N^{\zeta}H\cdots$O-C(Thr) with a distance of 1.99Å, 1.86Å and 2.19Å respectively. In peptide 38, Pro residue was replaced by Lys and Arg was replaced by Ala residue. The central Lys-Lys sequence adopts semi-extended conformation and The Thr and Lys2 side chain positioned right angled to each other while Lys2 and Lys3 side chain were traced closed to C-terminal Ala. The molecule is stabilized by two intramolecular hydrogen bonding interactions between (Thr)$O^{\gamma}\cdots$HN(Lys) and (Lys)$N^{\zeta}H\cdots$O-C(Thr) with a distance of 2.09Å and 2.10Å respectively. In peptide 39, the 3rd and fourth positions were replaced by Ala residue. The Lys-Ala sequence adopts distorted semi-extended conformation and type IV β-turn like conformation across Ala-Ala residue. The Lys side chain shown to twisted towards C-terminal Ala and this conformation is stabilized by one intramolecular hydrogen bonding interaction between (Lys)$N^{\zeta}H\cdots O$=C(Ala) with a distance of 2.01Å. In peptide 40, the Lys and pro residues were replaced by Gly residue and the Arg is replaced by Lys residue. This peptide adopts multiple conformations across Gly-Gly sequence showing its flexible nature. The Thr N-terminal positioned towards C-terminal Lys with $i\rightarrow i+3$ interaction showing a prefect turn structure with both main chain (Gly)NHs pointed inside the turn. This molecule is stabilized by two intramolecular hydrogen bonding interactions between (Thr)NH$\cdots$O=C(Lys) and (Lys)$N^{\zeta}H\cdots$O-C(Lys) with a distance of 1.74Å and 2.01Å respectively.

In peptide 41, the 2nd 3rdand 4th amino acids of the native tuftsin were replaced by Ala, Arg and Lys residue respectively. This peptide adopts a disordered conformation across Ala-Arg sequence and type IV β-turn like conformation across Arg-Lys residue. The Arg and Lys side chains were pointed towards C-terminal Lys and the overall conformation of the molecule is shown to be extended conformation. This peptide is stabilized by three intramolecular hydrogen bonding interactions between (Thr)$O^{\gamma}\cdots$HN(Ala), (Arg)$N_{\eta}H_1\cdots O$=C(Lys) and (Lys)$N^{\zeta}H\cdots$O-C(Lys) with a distance of 2.28Å, 2.09Å and 2.42Å respectively. In peptide 42, the 2nd and 3rd position of the tuftsin were replaced by Ala and Val residues. This molecule also shows some flexibility at the central Ala-Val

sequence and adopts multiple conformation with type VIII β-turn like conformation across Ala-Val sequence and the Ala and Val side chains positioned in the same direction and (Thr)NH and (Arg)$N^{\varepsilon}H$ groups pointed towards the C-terminal Arg. This molecule is stabilized by three intramolecular hydrogen bonding interactions between (Thr)$O^{\gamma}\cdots$HN(Ala), (Thr)NH···O-C(Arg) and (Arg)$N^{\varepsilon}H\cdots$O=C(Arg) with a distance of 2.10Å, 2.35Å and 1.89Å respectively. In peptide 43, the 2nd and 4th positions were replaced by Orn and Ala residues respectively. The Orn-Pro sequence adopts type IV β-turn like conformation and type III β-turn like conformation across Pro-Ala sequence. The Thr and Orn side chain positioned in the same direction while the Pro ring situated opposite to Orn side chain and (Orn)$N^{\varepsilon}H$placed very close to C-terminal Ala. The Lys side chain situated very close to C-terminal Ala and the molecule is stabilized by two intramolecular hydrogen bonding interaction between (Orn)$N^{\varepsilon}H_1\cdots$O=C(Ala) and (Orn)$N^{\varepsilon}H_1\cdots$O-C(Ala) with a distance of 1.91Å and 2.43Å respectively. In peptide 44, the 2nd, 3rd and 4th positions of the tuftsin were replaced by Pro, Lys and Ala residues. The Pro-Lys sequence adopts a type VIII β-turn like conformation and Lys-Ala sequence prefers type IV β-turn like conformation. This conformation is stabilized by three intramolecular hydrogen bonding interactions between (Thr)$O^{\gamma}\cdots$HN(Lys), (Lys) $N^{\zeta}H_1$ ···O=C(Ala) and (Lys)$N^{\zeta}H_2\cdots$O-C(Ala) with a distance of 2.22Å, 2.34Å 2.47Å respectively. In peptide 45, the 4th amino acid of tuftsin was replaced with two residues Pro-Arg. The peptide backbone assumes semi-extended conformation across Lys-Pro sequence, a distorted type VIa β-turn like conformation across Pro-Pro residues. The Thr and Lys side chains oriented in opposite directions and also both the Pro rings placed in opposite to each other. The (Lys)$N^{\zeta}H$ and (Arg)$N^{\varepsilon}H$ placed very close to C-terminal Arg and the peptide is stabilized by four intramolecular hydrogen bonding interactions between (Thr)$O^{\gamma}\cdots$HN(Lys), (Lys)$N^{\zeta}H\cdots$O=C(Arg), (Pro_1)C=O···HN(Arg) and (Arg)$N^{\varepsilon}H\cdots$O-C(Arg) with a distance of 2.31Å, 1.94Å, 2.08Å and 1.98Å respectively. In peptide 46, the 1st and 2nd amino acid of the tuftsin were replaced by Lys and Pro residues. This Pro-Pro sequence assumes distorted type VIa β-turn conformations and type VIII β-turn like conformation across Pro-Arg sequence. The (Lys)$N^{\zeta}H$ and (Arg)$N^{\varepsilon}H$ groups were positioned very close to C-terminal Arg and both the Pro rings were placed right angles to each other. The molecule is stabilized by four intramolecular hydrogen bonding interactions between (Lys)$N^{\zeta}H\cdots$O=C(Arg), (Lys)$N^{\zeta}H\cdots$O-C(Arg), (Arg)$N^{\varepsilon}H\cdots$O=C(Arg) and (Pro_1)C=O···HN(Arg) with a distance of 2.41Å, 2.29Å, 2.01Å and 2.20Å respectively.



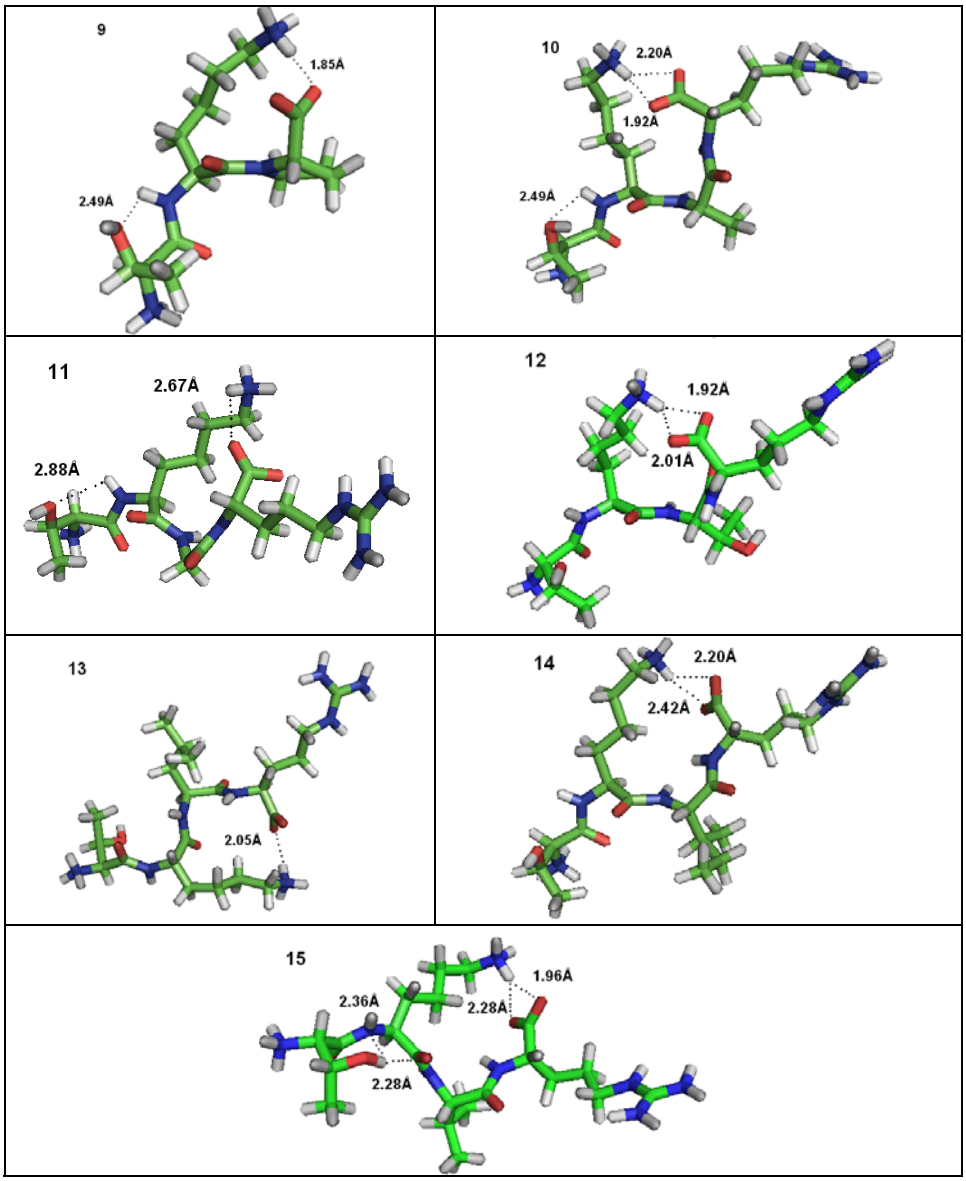
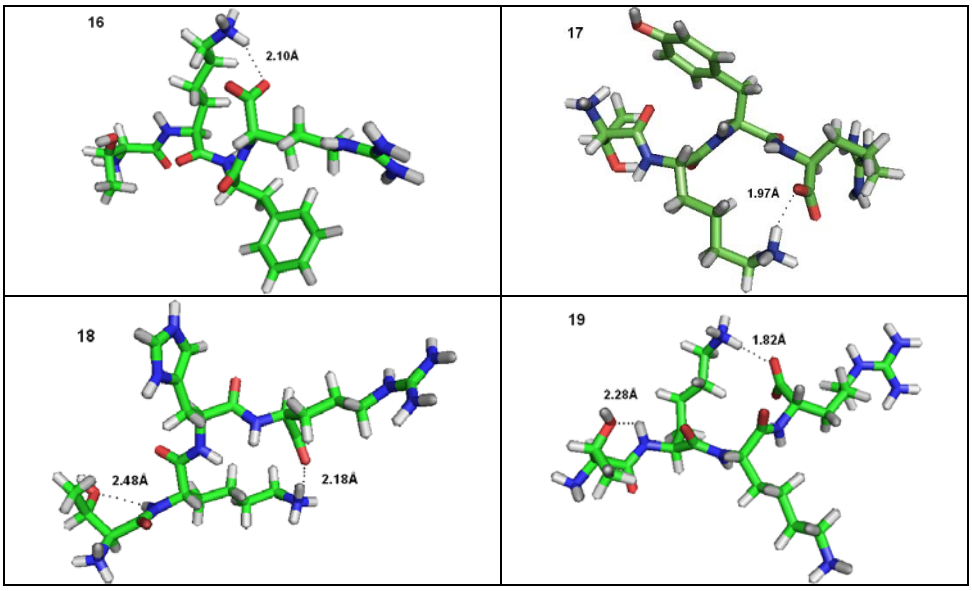


Fig 2A: Lowest energy conformation of Tuftsin and its analogs peptide **1-15** with hydrogen bonding interaction shown in dotted line.



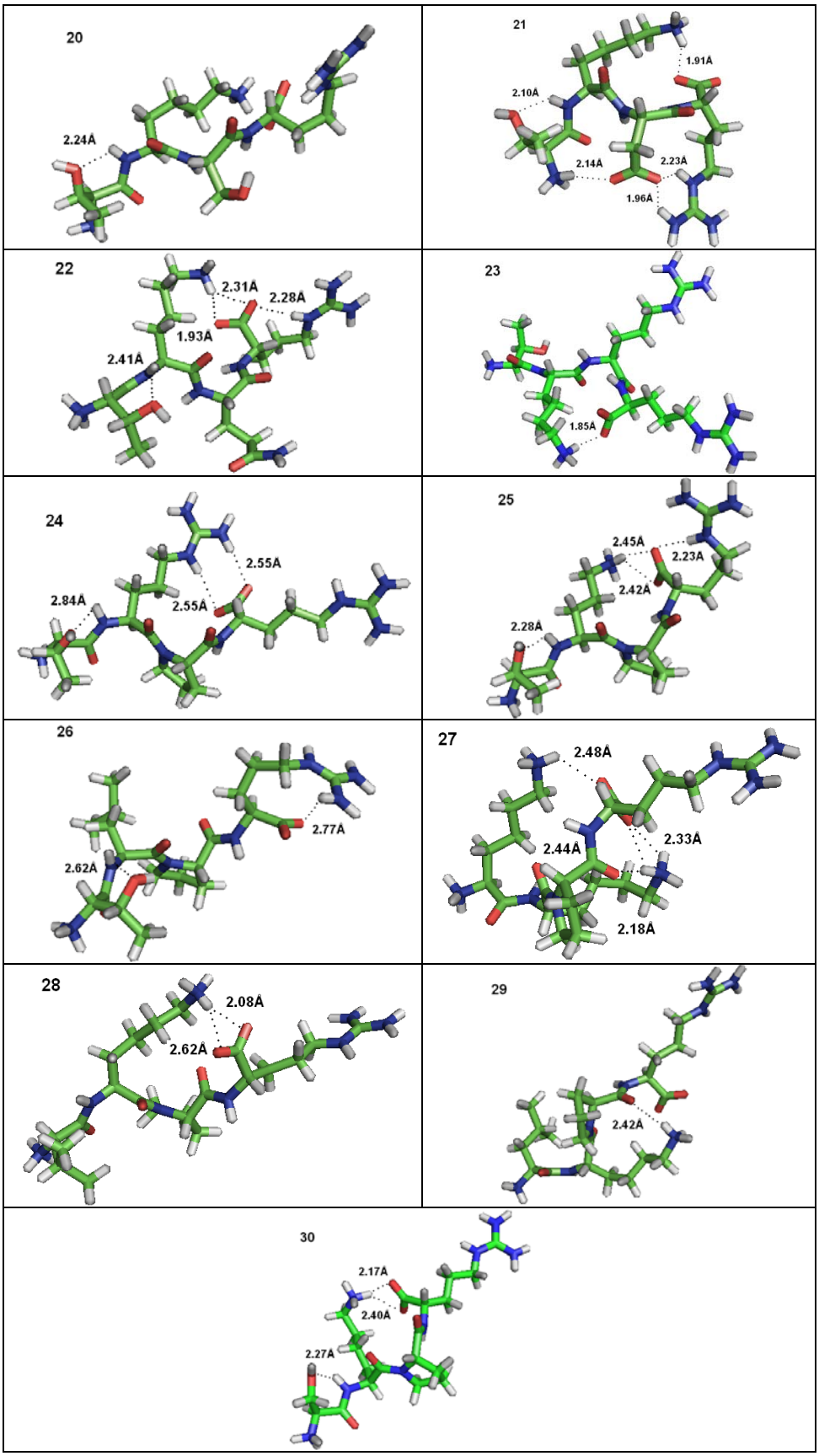
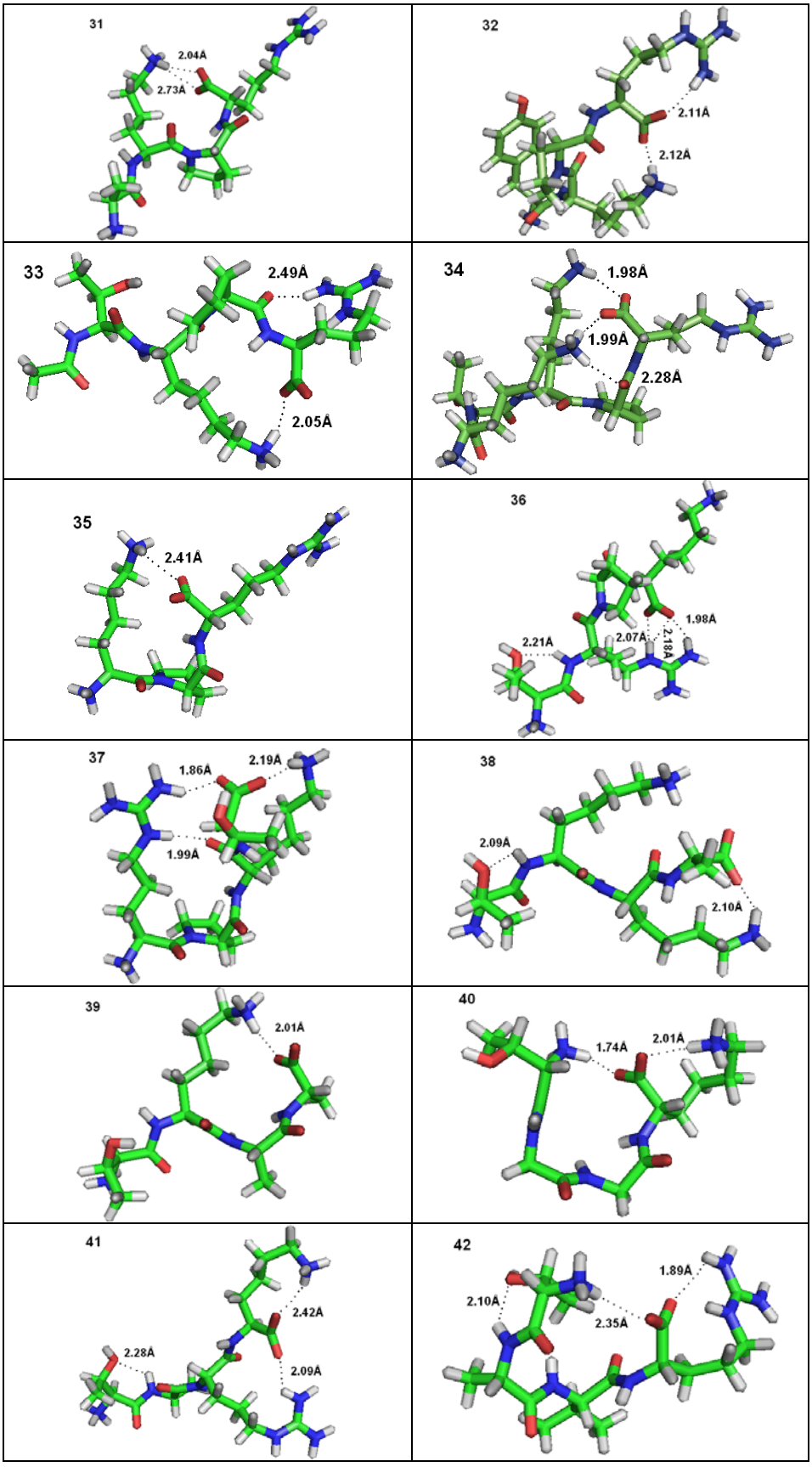


Fig 2B: Lowest energy conformation of Tuftsin and its analogs peptide **16-30** with hydrogen bonding interaction shown in dotted line.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2.07Å
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1.86Å
2.19Å
1.99Å
38
2.09Å
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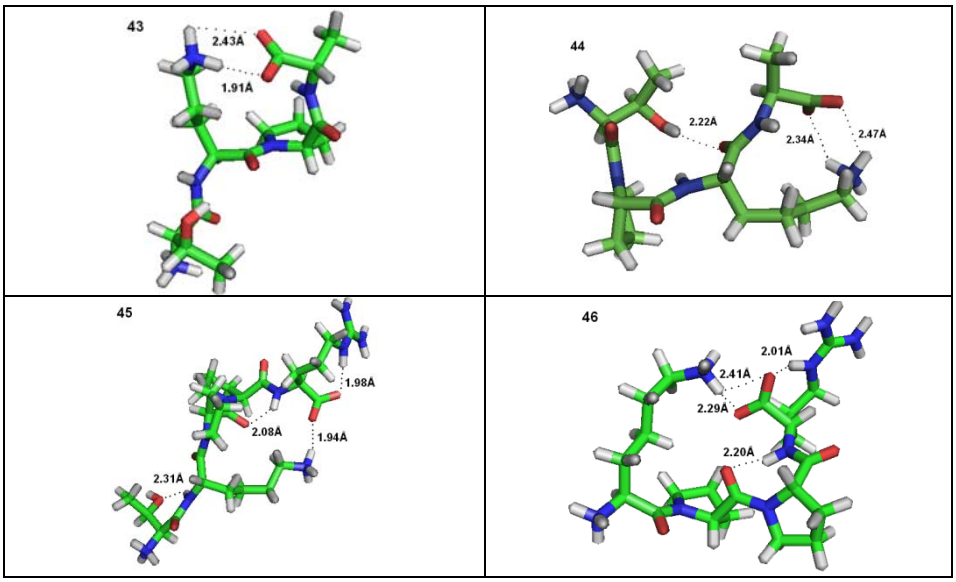

Fig 2C: Lowest energy conformation of Tuftsin and its analogs peptide **30-46** with hydrogen bonding interaction shown in dotted line.

4. Conclusions

In receptor like environment the native peptide 1 adopts IV β-turn like conformation across Lys-Pro (i+1 and i+2) and type I β-turn conformation across Pro-Arg (i+2 and i+3). Substitution at position 1 with Leu, Ser, Ala (peptides 29, 30, 31), replacement position 2 and 4 with Arg and Lys (peptide 36) or Orn and Ala (peptide 43), substitution at position 3 with His, Ser, Asp, Gln (peptides 18, 20, 21,22), substitution at position 4 with Lys (peptide 2) and acetylating N-terminal of the native peptide (peptide 33) does not affect the conformation between i+1 and i+2 residues. They have a common type IV β-turn like conformation across i+1 and i+2 but these substitution does have an impact on the conformation across i+2 and i+3 residues showing diverse conformations. Substitution at position 4 with D-Arg (peptide **7**), substitution at position 3 with Ala, Leu, Ile, Val, Phe (peptides 10, 13, 14, 15, 16) have also common conformational preferences *i.e.* distorted type IV β-turn conformation across the central i+1 and i+2 residues. Replacing Pro with Arg at position 3 (peptide 23), substituting Lys with Arg or Orn (peptide 24, 25) yielded type IV β-turn conformation across i+1 and i+2 residues and type III β-turn like conformation across i+2 and i+3 residues. In peptides 11, and 40 where the flexible Gly residue occupies at i+2 or i+1 and i+2 positions and Val at position 1 (peptide 28) the peptide backbone adopted multiple conformations. In peptides 34, 37, 42, 44, the central residues adopt type VIII β-turn like conformations. The most interesting finding of this study was the observation of semi-extended conformation of the central residues as obtained from the crystal structure of neuropilin-1-tuftsin when we change the C-terminal COOH to $CONH_2$ (peptide 3), substituting 1 and 2 position with Lys (peptide 27), substituting position 3 and 4 with Lys and Ala (peptide 38) and substituting C-terminal Arg with Pro-Arg (peptide 45**)**. All these peptides show much higher efficacy than the native tuftsin. Therefore, from this study we conclude that the various amino acids at various positions of the tuftsin poised diverse back bone conformations not a static conformation in the low dielectric environment and the super active analogs demonstrated a static conformation i.e. semi-extended back bone conformation as observed in the receptor bound neuropilin-1-tuftsin crystal structure.

5. Acknowledgements

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