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Molecular Dynamics and Molecular Docking Studies on the Interaction between Four Tetrahydroxy Derivatives of Polyphenyls and Beta Amyloid

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ABSTRACT

Interactions of 3,3',4,4'-tetrahydroxybiphenyl (BPT) and three isomeric 3,3",4,4"-tetrahydroxyterphenyls (OTT, MTT, PTT) with Alzheimer's amyloid-β peptide (Aβ) were studied by molecular dynamics simulation and molecular docking. Structural parameters such as Root-mean-square derivations (RMSD), radial distribution function (RDF), helix percentage and other physical parameters were obtained. These inhibitors have been evaluated and compared for their activity against aggregation of Aβ. The results showed that all four compounds successfully inhibit association of Aβ and reduce aggregation of protein. For the tetrahydroxyterphenyls efficacy varies with linker geometry: the *ortho*-position affords the most successful inhibition and the *para*-geometry the least perhaps due to differing abilities of these inhibitors to bind amyloid-β peptide. Of the four small inhibitors studied 3,3',4,4'-tetrahydroxybiphenyl (BPT) is the most effective inhibitor. Molecular docking studies have been done to confirm the simulation results. Investigation of binding site and free energy confirmed that the efficiency of interaction with Aβ depends on differing abilities of these inhibitors to bind amyloid-β peptide. Binding energy of BPT is more negative than the other and it significantly decreases for PTT. Self-aggregation of this inhibitor decreases in comparison with BPT; therefore Aβ aggregation in the presence of biphenyl form is higher than terphenyls.

Keywords: Amyloid-β, Inhibitors, Molecular dynamics simulation, Molecular docking

INTRODUCTION

Alzheimer's disease (AD) is the leading cause of senile dementia. This disease is causally linked to the aggregation of amyloid-β peptide (Aβ). Hallmark symptoms of AD include memory loss general cognition impaired learning and dementia. In the absence of efficient drugs rate of occurrence of AD is expected to rise rapidly over the coming years. Currently several million people worldwide are believed to suffer from this disease.

The effects of a wide variety of compounds on amyloid-β peptide association have been investigated [1,2]. Among the many molecules found to prevent Aβ aggregation are aminonaphthalene sulfonates [3], benzofurans [4], coumarins [5], nicotine [6] and others [7,8]. Reinke *et al*. by studying on the effects of curcumin and related compounds on amyloid-β peptide aggregation understanding that the most successful inhibitors of this kind of possess terminal aromatic rings including hydrogen-bond donors and a relatively rigid central 'linker' region in length [9]. The effect of biphenyl-3,3",4,4"-tetrol (BPT) and tetrahydroxy-terphenyls with different geometries around the linker phenyl and terminal rings attached at the *para- meta-* and *ortho* positions ring (are called PTT, MTT and OTT, respectively) on the aggregation of Aβ monomers was investigated [10]. Their structural similarity to resveratrol which Reinke *et al*. stated exhibits good activity. Stevens *et al*. synthesized these compounds and evaluated them as Aβ aggregation inhibitors. They used Congo red spectral-shift test to evaluate the effectiveness of OTT, MTT, PTT and BPT as inhibitors of Aβ aggregation. These compounds are novel and effective drugs so in this study we want to investigate them as amyloid-β aggregation inhibitors by Molecular dynamics and molecular docking studies.

Molecular dynamics (MD) simulations have been successfully used to investigate interactions among proteins lipids and small molecules and provide atomic-level detail of many phenomena. Due to the inherent difficulty of obtaining high-resolution structural data of Aβ aggregates and their disassembly or assembly MD simulations is an ideal tool for studying these systems. Numerous MD studies have examined the basis for the stability of $A\beta_{40}$ fibril [11-

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13]. However, due to computer limitations, many studies focused on Aβ fragments, of which Aβ(16-22) was the most frequently studied [14,15]. Studies of Aβ(16-22) assembly are numerous because of the simplicity of the fragment (KLVFFAE), and more importantly, because this fragment comprises the central hydrophobic core (LVFFA) thought to be important in fibril formation of full-length Aβ.

On the other hand, molecular docking studies can be carried out to confirm the simulation results. Molecular dynamics and docking are widely used in the investigation of structure and activity of macromolecules and drug design [16-19]. In docking studies, different search algorithms, such as simulated annealing and genetic algorithm in combination with scoring function such as molecular mechanic calculations are used to study the binding of the candidate ligands to an protein with the known structure [20,21,19]. This methodology is both fast and efficient in providing potential inhibitors [22]. Its efficiency lies in the fact that it does not perform all possible combinations of mutations, therefore decreasing the computational time drastically. Through docking procedures, not only new biological active compound is introduced, but also the chemistry of the ligand-protein interaction is well recognized.

Since, experimental methods are more expensive or sometimes are not able to study aggregation in molecular level, the aim of this work is molecular investigation of the interaction between four different polyphenols and beta amyloid by molecular dynamics and molecular docking. As mentioned above, in the present work, we carried out the MD simulation analysis and molecular docking to investigate the interaction of BPT, PTT, MTT and OTT with Aβ as well as knowing changes in its structure in presence of these compounds. In this work two segments of Aβ(16-22) was used. The obtained data are in agreement with the Stevens's study results [10]. The results from this study should be useful to understand the ligand-protein interaction and design new inhibitors. We want to evaluate them as amyloid-β aggregation inhibitors.

METHODS

Molecular Dynamics Simulation

The structures of ligands (BPT, PTT, MTT and OTT) were drawn by Hyperchem 7 software (Scheme 1). The structures of these compounds were pre-optimized and final geometries were obtained with the semi-empirical AM1 method in this software. For this molecular dynamics simulation force field parameters and geometries were generated by PRODRG2 server (http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg_beta). A cubic simulation box of the volume 6.482 × 4.592 × 4.428 nm^3 was made. In each computation 10 ligands were placed randomly in this box. Then water molecules were randomly added into the simulation box and initial configurations were minimized. After 700 steps of energy minimization the system was equilibrated for 40000 ps (40 ns) at approximately body temperature (310 K) and constant pressure (1 atm) by the Nose-Hoover thermostat [23,24] and Parrinello-Rahman barostate [25] with coupling constants of 0.1 and 0.5, respectively. For all simulations the atomic coordinates were saved every 50 ps for analysis.

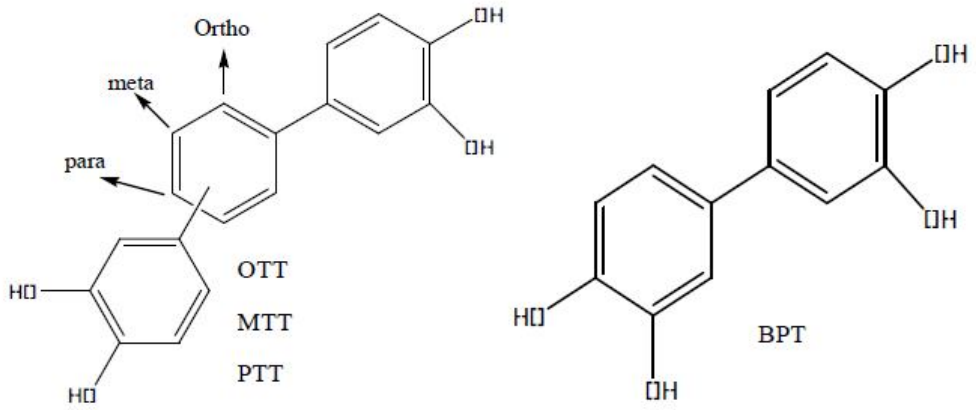
All MD simulations were carried out by the GROMACS 4.5.4 package [26] along with the GROMOS96 53a6 force field [27]. According to Gerben *et al*. research [28], we used from this force field. They compared atomistic molecular mechanics force fields (a case study on the Alzheimer's amyloid β-peptide) and observed some of force field such as GROMOS96 53A6 produce very similar results in terms of helical and β-strand content radii of gyration and calculated NMR shifts that agree well with experimental data. The simple point charge (SPC) model was used to describe water [29].

Molecular Dynamics Data Analyses

The simulation trajectories were analyzed using several auxiliary programs provided with the GROMACS 3.3 package. The percentages of helix, beta, coil and turn content can be calculated by web server VADAR [30]. The "g_rms" evaluates the deviation of the structure from the original starting structure over the course of the simulation. The conformational changes of each protein during MD simulations can be checked by RMSD with its X-ray structure as a reference.

The RMSD is defined as:

$$RMSD(t_1,t_2) = \left[\frac{1}{M}\sum_{i=1}^{N} m_i \|r_i(t_2) - r_i(t_1)\|^2\right]^{1/2} \quad (1)$$



Scheme 1. Chemical structure of terphenyl-3,3'',4,4''-tetrols (PTT, MTT, OTT) and biphenyl-3,3',4,4'-tetrol (BPT)

where m_i is the mass of atom *i* and r_i is the position of atom *i* with respect to the center of mass of the molecule. The RMSD can be computed of the backbone or of the whole protein. In this study each system underwent 20 ns MD runs until the RMSD fluctuated around a constant value to reach the equilibrium state. The "g_rdf" calculates radial distribution functions in different ways. The radial distribution function (RDF) between particles of type A and B is defined in the following term:

$$RDF = \frac{< \rho B(r) >}{< \rho B >_{local}} = \frac{1}{< \rho B >_{local}} \frac{1}{N_A} \sum_{i \in A}^{N_A} \sum_{j \in B}^{N_B} \frac{\delta(r_{ij} - r)}{4\pi r^2} \quad (2)$$

where $<\rho_B>_{local}$ is the particle density of type B averaged over all spheres around particles A with radius r_{max} and $<\rho_{B(r)}>$ is the particle density of type B at the distance *r* around particles A. Usually the value of r_{max} is considered as the half of the box length. The "g_hbond" are considered to be intact if the donor-to-acceptor distance is less than 0.35 nm and the donor-hydrogen-acceptor angle is within 30° of linearity. In fact Hydrogen bonds calculate the hydrogen bond interactions between hydrogen donors and acceptors through the course of the simulation. The "g_gyrate" measures the radius of gyration. This quantity gives a measure of the "compactness" of the structure. This gives a measure of the mass of the atom (s) relative to the center of the mass of the molecule and defined as:

$$R_{gyr} = \sqrt{\frac{\sum m_i r_i^2}{\sum m_i}} \quad (3)$$

Where r_i is the distance of atom *i* from the center of mass of the protein, and m_i is its mass. The "g_sas" computes hydrophobic hydrophilic and total solvent accessible surface area. g_sas computes hydrophobic, hydrophilic and total solvent accessible surface area.

Molecular Docking

To predict the binding energy of inhibitors to beta-amyloid peptide Autodock software was used. The PDB files of these inhibitors with the best geometries extracted from Hyperchem were loaded into Auto Dock Tool (ADT) to calculate free energy of interaction. After adding polar hydrogens Gasteiger charges were computed; the rigid root and the rotatable bonds were defined by the AutoTors tool of ADT. For the Aβ protein all water molecules were removed; Kollman charges and solvation parameters were added. Based on the atom types the suitable maps were calculated. Nonpolar hydrogens were merged for each atom. The grid module was employed to create grid maps with 100 × 100 × 100 points and a grid-point spacing of 0.375 nm. For each inhibitor 250 independent docking runs were conducted. The settings of parameters were as follows: population sizes of 50 a maximum number of 25 million

energy evaluations a maximum number of 27000 generations a crossover rate of 0.8 an elitism of 1and a mutation rate of 0.02 were set up. The docking conformation results were clustered using a root-mean-square deviation (RMSD) of 0.5 and the clusters were ranked in order of increasing energy. We want to investigate computationally whether inhibitors will interact or bind to beta-amyloid and if so we would like to compare the binding energy of them as well as the Gibbs energy of interaction and the affinity of the binding or interaction.

RESULTS AND DISCUSSIONS

Molecular Dynamics Simulation

Here, we describe simulation of Aβ in the presence of different inhibitors, focusing on the unfolding process of

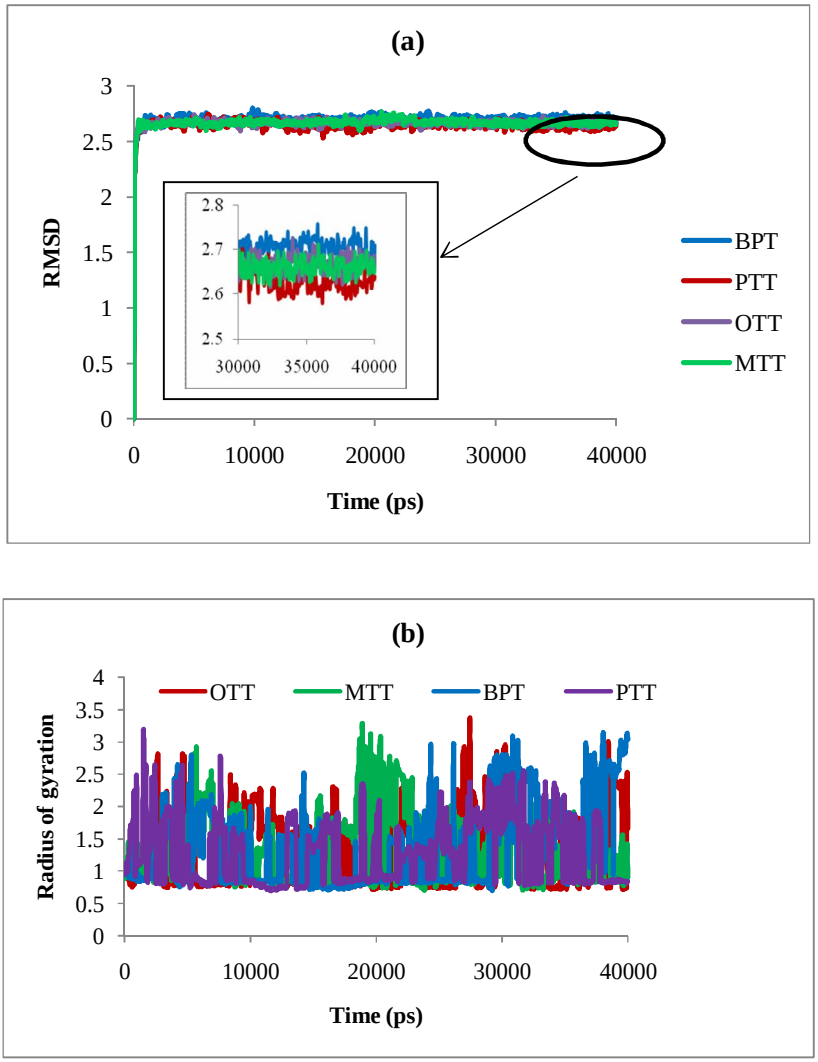


Fig. 1. Calculated (a) RMSD (b) radius of gyration (c) solvent accessible surface area (d) hydrogen bond (p-p) (e) Hydrogen Bond(p-d) by molecular dynamics for Aβ in the presence of 8 molecules of BPT (blue) OTT (violet) MTT (green) and PTT (red).

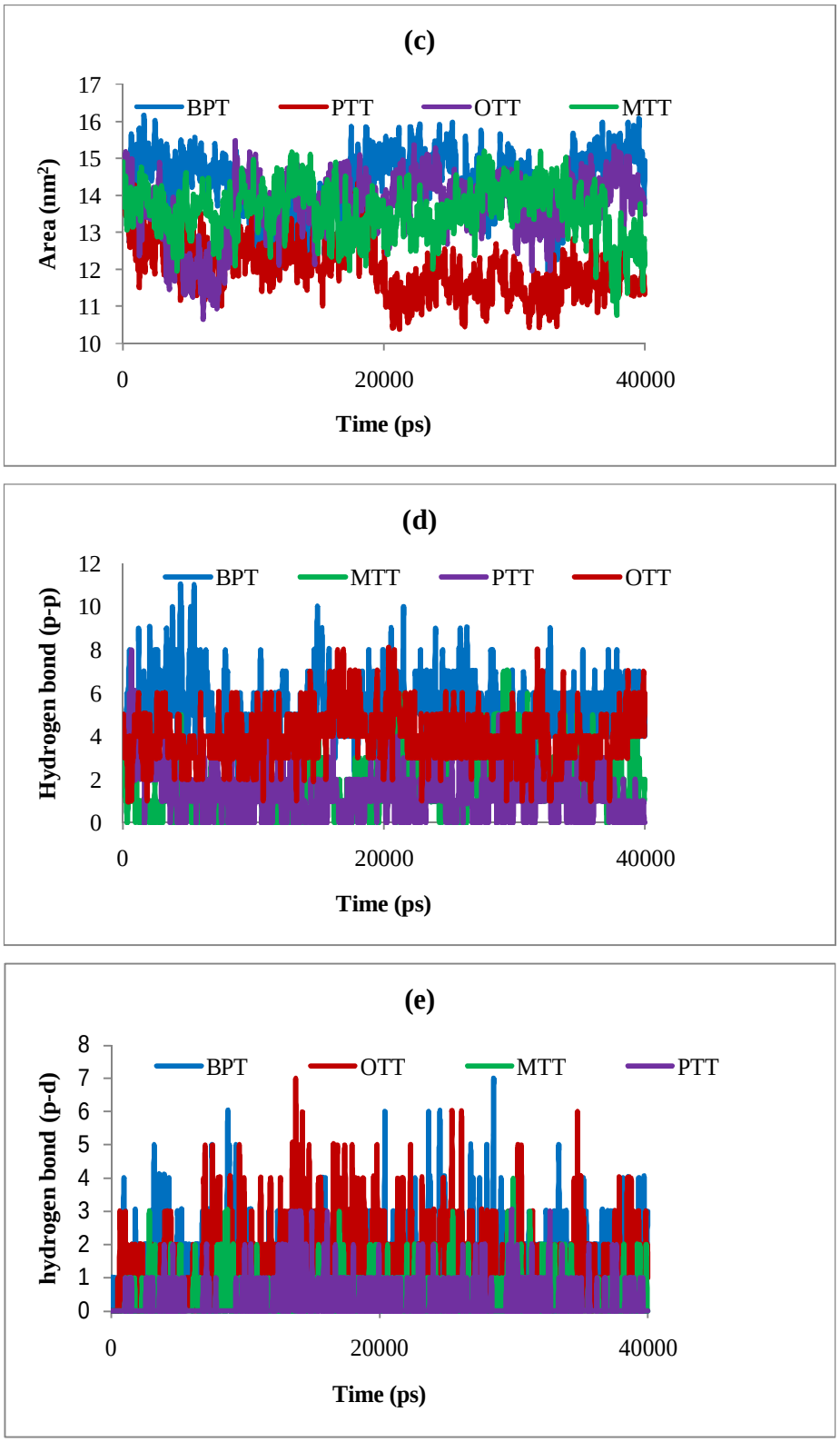


Fig. 1. Continued

protein. For this aim, MD calculations were performed on the Aβ peptides by GROMACS software. These inhibitors believed to alter the structure of the network of hydrogen bonds for protein in water and increase the SAS and protein size as well as decrease the intermolecular hydrogen bond, electrostatic and hydrophobic interactions of proteins. Structure parameters were obtained from MD simulation for each inhibitor and results were analyzed for comparison. The structure information such as intermolecular hydrogen bonding (HB), root-mean-square derivations (RMSD) were obtained and averaged in the presence of each inhibitor.

Figure 1a shows the RMSD of Aβ during 40 ns in the presence of eight molecules of BPT, OTT, MTT and PTT. It shows that protein gets a flat curve and reaches to a stable state after this time. Structural change of system in the presence of BPT is more than other inhibitors.

Figure 1b shows radius gyration (*Rg*) of Aβ in the presence of mentioned inhibitor molecules. This figure shows decrease of *Rg* for Aβ in the presence of tetrahydroxyterphenyls. This proves the protein has been unfolded more in the presence of tetrahydroxybiphenyl and therefore radius of gyration of Aβ in system with BPT is more than other.

Accessible surface area of Aβ in the 40 ns time evolution in the presence of mentioned inhibitors was computed. Figure 1c shows the accessible surface area of Aβ in the presence of BPT, OTT, MTT and PTT. For the tetrahydroxyterphenyls, efficacy varies with linker geometry: the *ortho*-arrangement (OTT) affords the most successful inhibition and the *para*-geometry (PTT) the least, perhaps due to differing abilities of these compounds to bind Aβ. Of the four small molecules studied, 3,3′,4,4′-tetrahydroxybiphenyl (BPT) is the most effective inhibitor.

Figure 1d displays the variation of hydrogen bond of beta-amyloid in the presence of mentioned inhibitors that reveals the most increase of Aβ hydrogen bond in the presence of BPT. Therefore, the effect of BPT on unfolding of Aβ is higher than OTT, MTT and PTT. This result is in good accordance with increase of hydrogen bond between protein and inhibitor in the presence of BPT.

Figures 2a and 2c show the starting point of the MD simulation for BPT and PTT, respectively which are selected as two samples (the most and the least effective inhibitors between four mentioned compounds). The software automatically distributes ligands around the Aβ. The final structures, after 40 ns, are depicted in Figs. 2b and 2d. It shows that after simulation Aβ has been unfolded, especially in presence of BPT (Fig. 2b). It shows that this inhibitor tends to locate to the specific site.

Radial distribution function (RDF) is a criterion for the distribution of atoms, molecules or other species around a target species. Figures 3a and 3b shows RDF diagram of protein-inhibitor and inhibitor-inhibitor, respectively. Figure 3a denotes that the existence probability of BPT around the protein is more than PTT. Figure 3b also confirms this result, because according to this figure, in the presence of BPT, the presence of inhibitor around other inhibitors enhances.

Different 3D-structures of Aβ from initial to final time of simulation were extracted from the trajectory file and entered into VADAR. This web server calculates helix, coil, turn and beta percentage for each sample. Figures 4a and 4b show the variation of helix and coil for Aβ in the presence of BPT (the most successful inhibition) and PTT (the lowest successful inhibition). It has been shown that both inhibitors increase the coil and decrease the helix. These variations are higher in the presence of BPT. This proves that the protein structure has been slightly unfolded and its amino acids have become more accessible to the solvent. On the other hand the decrease of the secondary structure is higher in the presence of BPT.

DOCKING RESULTS

The interaction of BPT (the most successful inhibition) and the lowest successful inhibition (PTT) PTT (the lowest successful inhibition) with amyloid-β peptide has been investigated by AutoDock software. Also calculations of binding energy of these ligands to Aβ have been done by this software. AutoDock program reports 250 sites which some of these sites have equal energy and so form a cluster. Table 1 shows the results of free energy of docking for the

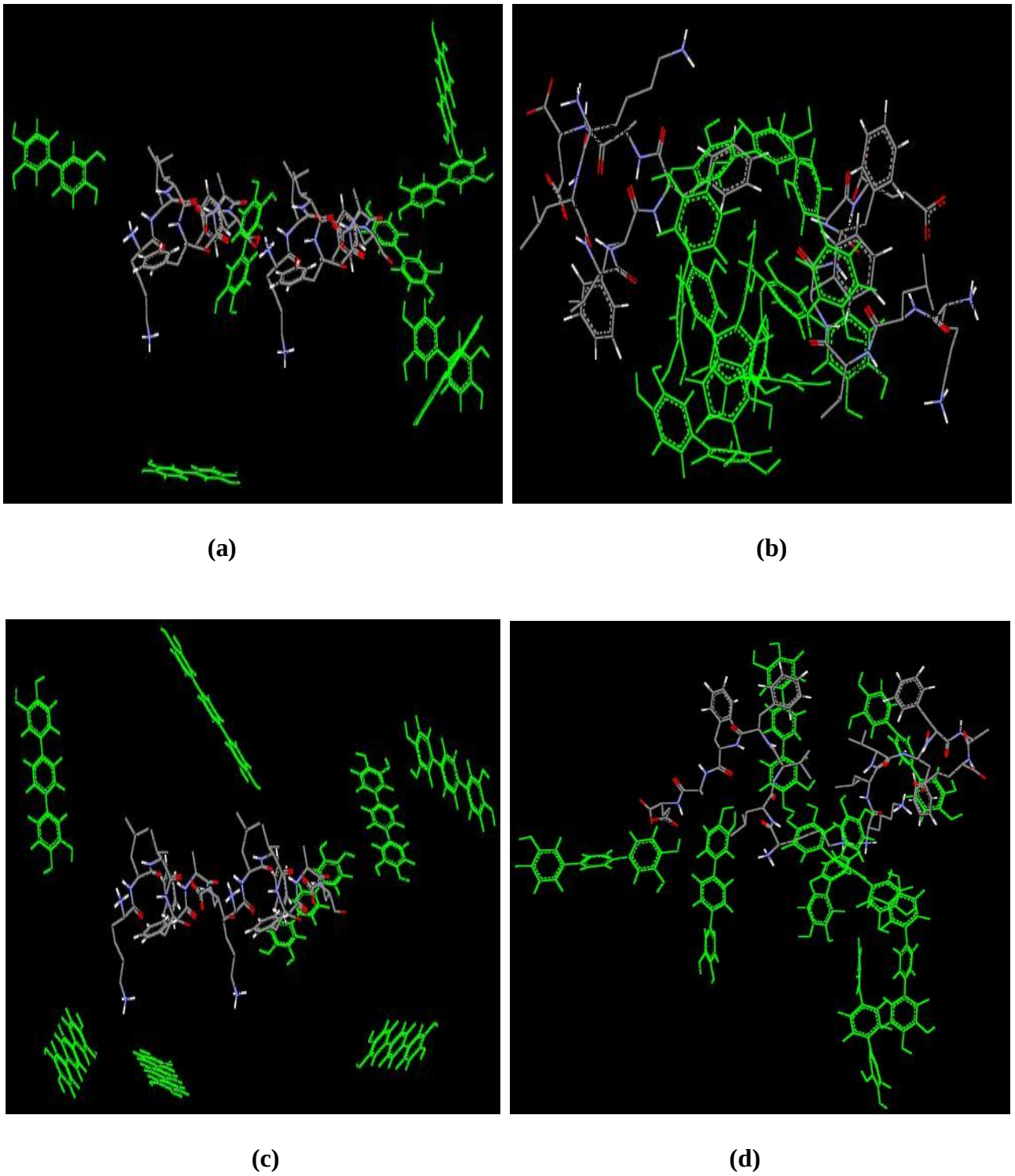
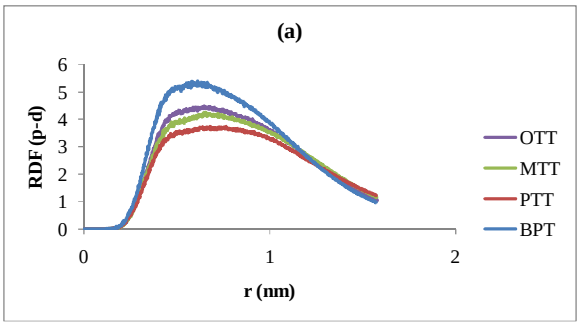


Fig. 2. Snapshot molecular picture obtained by DS Visualizer program at the initial (a and c) and final time of simulation (b and d) for amyloid-β in the presence of BPT (a,b) and PTT (c,d) respectively. Water molecules were removed.



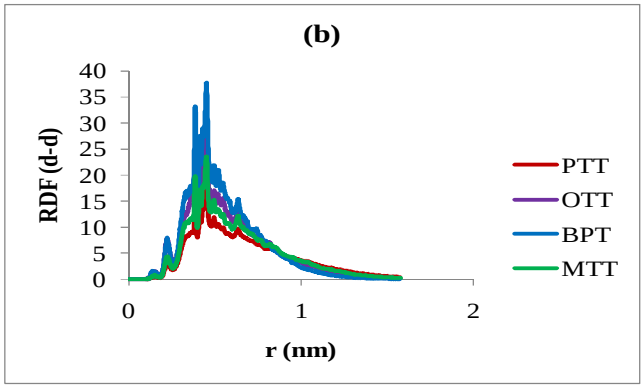
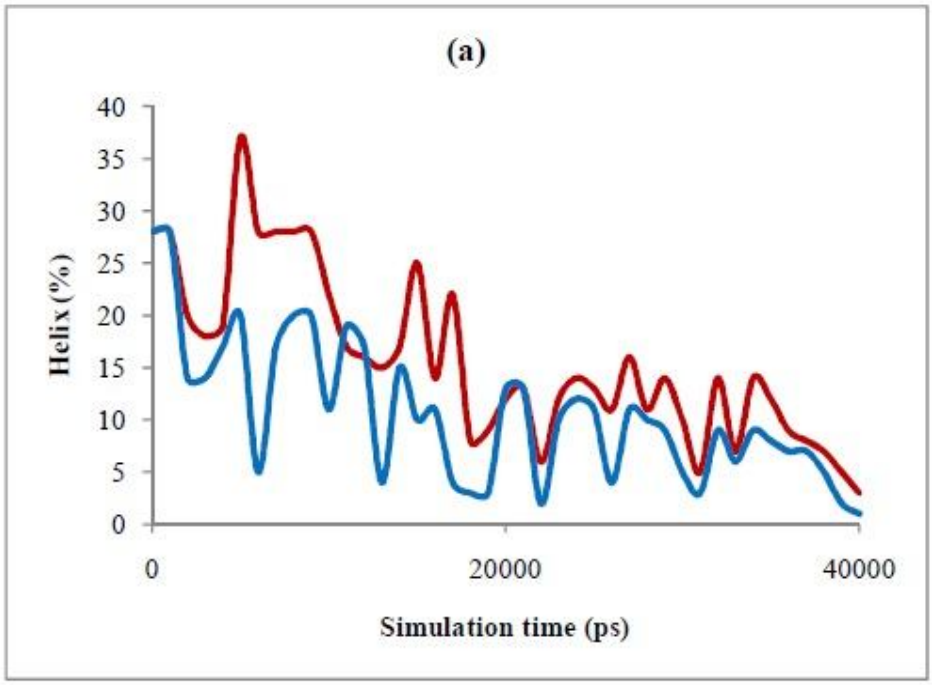


Fig. 3. Calculated (a) RDF for protein-inhibitor and (b) inhibitor-inhibitor by molecular dynamics in the presence of 8 molecules of BPT (blue) OTT (violet) MTT (green) and PTT (red).

first 10 ranks belong to BPT and PTT (as two samples). These tables show that free energy of binding for BPT is more negative than PTT; the first rank is the most probable docking site because of their higher cluster rank.

We can see in second column of table the free energy of docking and negative value find at higher position. Third column in each calculation run shows number of sites with similar energy that are in one cluster it means the number of randomly occupied sites which are selected was repeated three times.

Docking results show that each ligand binds to different site, having different free energies of docking. The box surrounded the entire protein. The free energy of docking was calculated and sorted so the highest negative free energy appeared in the first rank. The results for the protein in the presence of both inhibitors in same states indicate that the docking free energy for BPT is lower than PTT. Since this value is negative in all conditions; docking results are



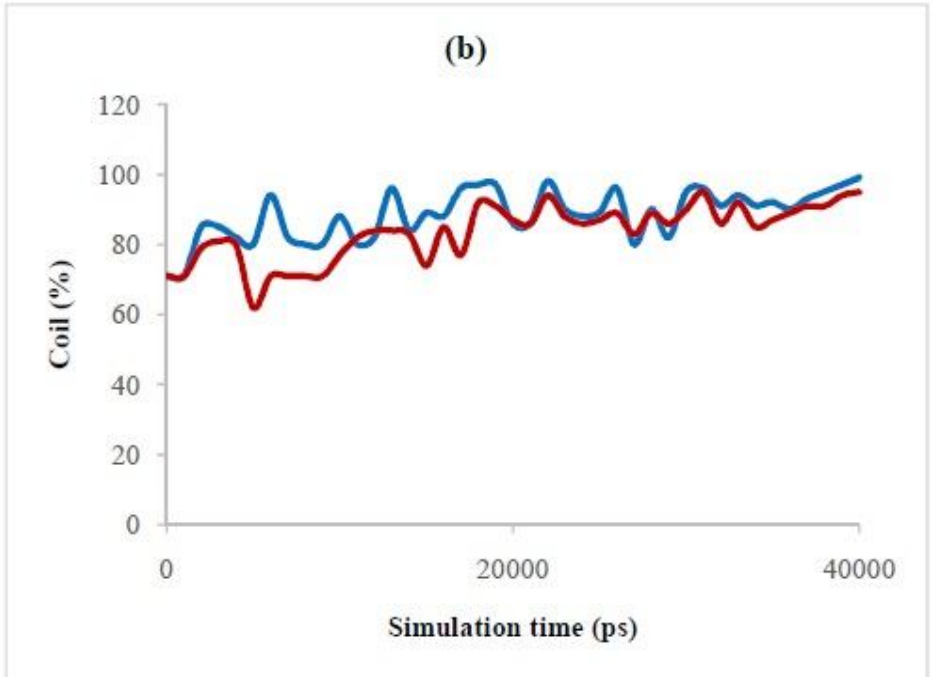


Fig. 4. Calculated (a) helix and (b) coil percentages for Aβ in the presence of 8 molecules of inhibitors. BPT (blue), PTT (red).

Table 1. Binding Free Energy (ΔG_{bind}) in kcal mol^{-1} for Biphenyl-3,3",4,4"-tetrol (BPT) and Terphenyl-3,3",4,4"-tetrol (PTT) Calculated by AutoDock

BPT			PTT		
Cluster rank	Lowest docked energy	Number of runs in a cluster	Cluster rank	Lowest docked energy	Number of runs in a cluster
1	-8.10	8	1	-6.73	3
2	-8.09	3	2	-6.58	9
3	-8.00	1	3	-6.58	5
4	-7.98	2	4	-6.54	9
5	-7.97	2	5	-6.50	2
6	-7.92	3	6	-6.44	3
7	-7.90	1	7	-6.43	14
8	-7.81	2	8	-6.40	3
9	-7.76	1	9	-6.32	2
10	-7.73	6	10	-6.30	2

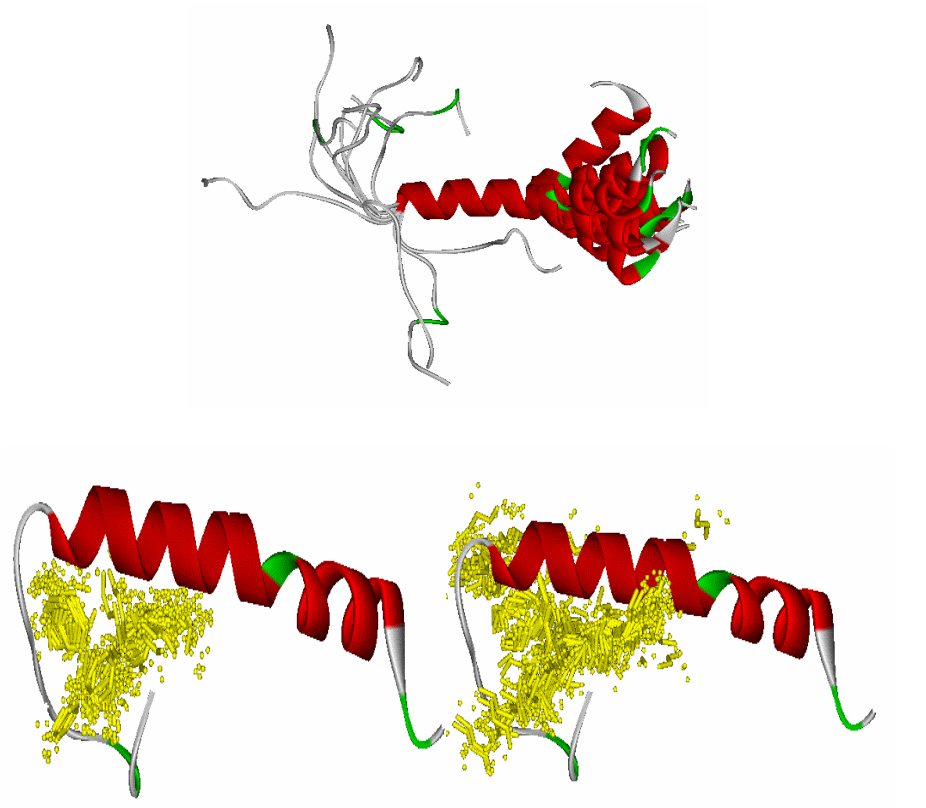


Fig. 5. a) Protein structure (1BA4) taken from protein data bank www.RCSB.org; b) the binding sites for the all ranks negative clusters of BPT and (c) PTT near the Aβ.

now compatible with simulation data.

Values of lowest docking energy for interaction of BPT and PTT for whole of Aβ was obtained -8.10 and -6.37 kcal mol^{-1}, respectively. Comparison of Table 1 shows more negative binding energy of BPT with Aβ which is in good agreement with simulation results. As a result BPT can stabilize protein structure.

Figure 5a shows the structure of Aβ (1BA4) taken from protein data bank www.RCSB.org. Figures 5b and 5c Show all distributions for the 250 runs corresponding to BPT and PTT around the Aβ respectively as two sample figures.

Figure 6a shows the binding sites for a few of the most negative clusters for BPT near the important Aβ amino acids. These clusters are including Lys16, Phe19, Phe20 and Glu22. According to Fig. 6b, the binding sites for a few of the most negative clusters for PTT are not located exactly near the important amino acids (16-22). Figures 6c and 6d show expended of part a and b which depicts the BPT and PTT approximately located near important Aβ amino acids.

On the other hand, electrostatic surfaces correspond to the most negative docking sites (first negative rank) for interaction of BPT and PTT with Aβ were shown in Fig. 7. Amino acid residues were shown for these inhibitors (Fig. 6) so that the blue color represents the positive charges and red color represents the negative charge. As we see in this figure red color surfaces (representative of negative charges) are located around of BPT (Fig. 7a) while there isn't this position in PTT (Fig. 7b).

According to all results of molecular dynamics simulations and docking study that explained above, OTT

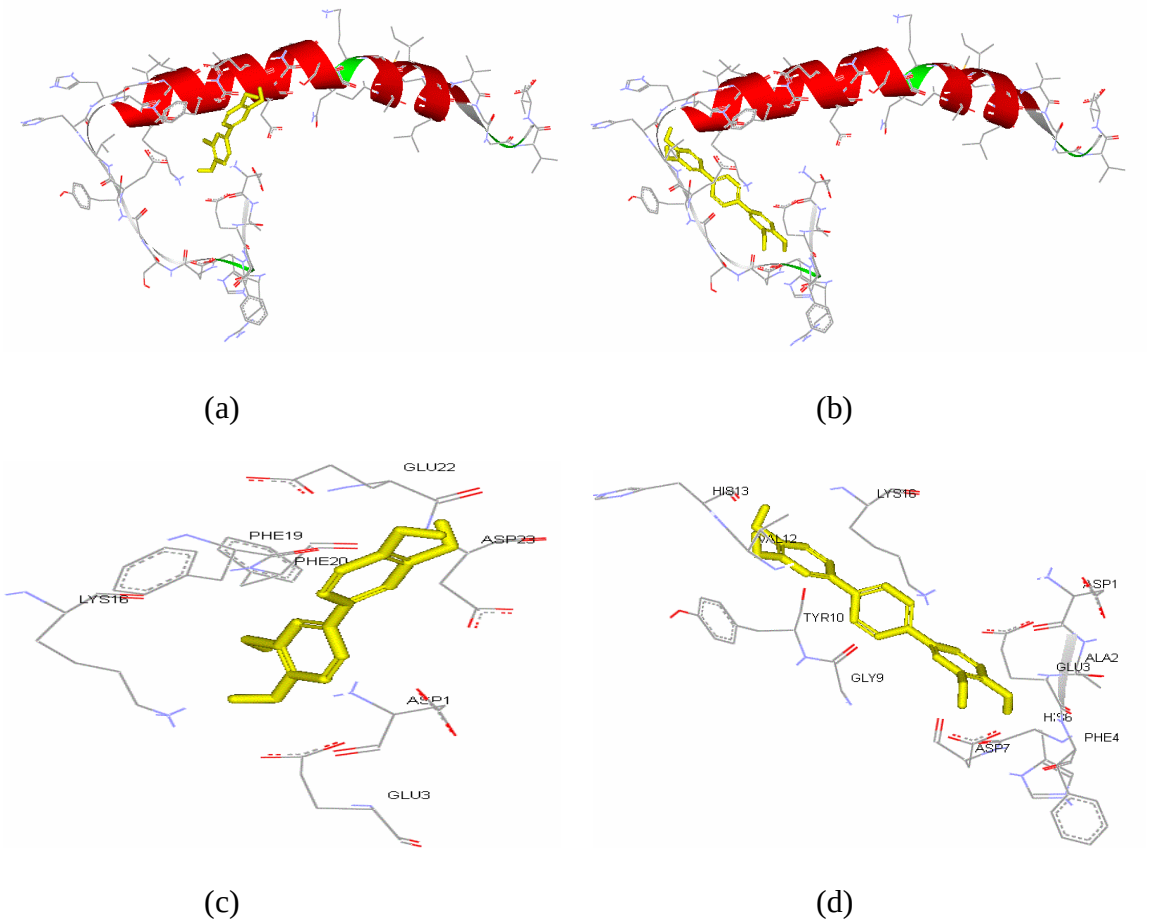


Fig. 6. a) The binding sites for the most negative clusters of (a) BPT and b) PTT near the Aβ; c) and d) Expanded part a, b, respectively.

affords the most successful inhibition and PTT the least and of the four considered inhibitions BPT is the most effective inhibitor. The inhibition effects of these inhibitors can relate to structural properties of them. For increasing of validation of model, the surface area for mentioned inhibitors was computed by Hyperchem. According to these results, surface area of BPT (318.95) is more than OTT (380.17), MTT (388.07) and PTT (408.93). It is clear that surface area among of tetrahydroxyterphenyls with different linker geometry, OTT (380.17) have higher unfolding effect relative to MTT (388.07) and PTT (408.93). as follow: OTT > MTT > PTT.

CONCLUSIONS

For the tetrahydroxyterphenyls efficacy varies with linker geometry: the *ortho*-position affords the most successful inhibition and the *para*-geometry the least perhaps due to differing abilities of these inhibitors to bind amyloid-β peptide. Of the four small inhibitors studied 3,3",4,4"-tetrahydroxybiphenyl is the most effective inhibitor. Binding energy of BPT to Aβ is more negative than the other, Therefore interaction of BPT with Aβ is more than OTT, MTT and PTT. Protein unfolding is more in the presence of BPT. Collectively, the results described herein indicate that the geometry around the linker phenyl ring significantly influences inhibitory efficacy in the terphenyltetrols OTT, MTT and PTT perhaps because it impacts inhibitor binding to Aβ assemblies. Surprisingly the biphenyltetrol BPT which lacks the linker phenyl ring is the most effective inhibitor of beta-amyloid aggregation. As mentioned above in order to confirm simulation results. We

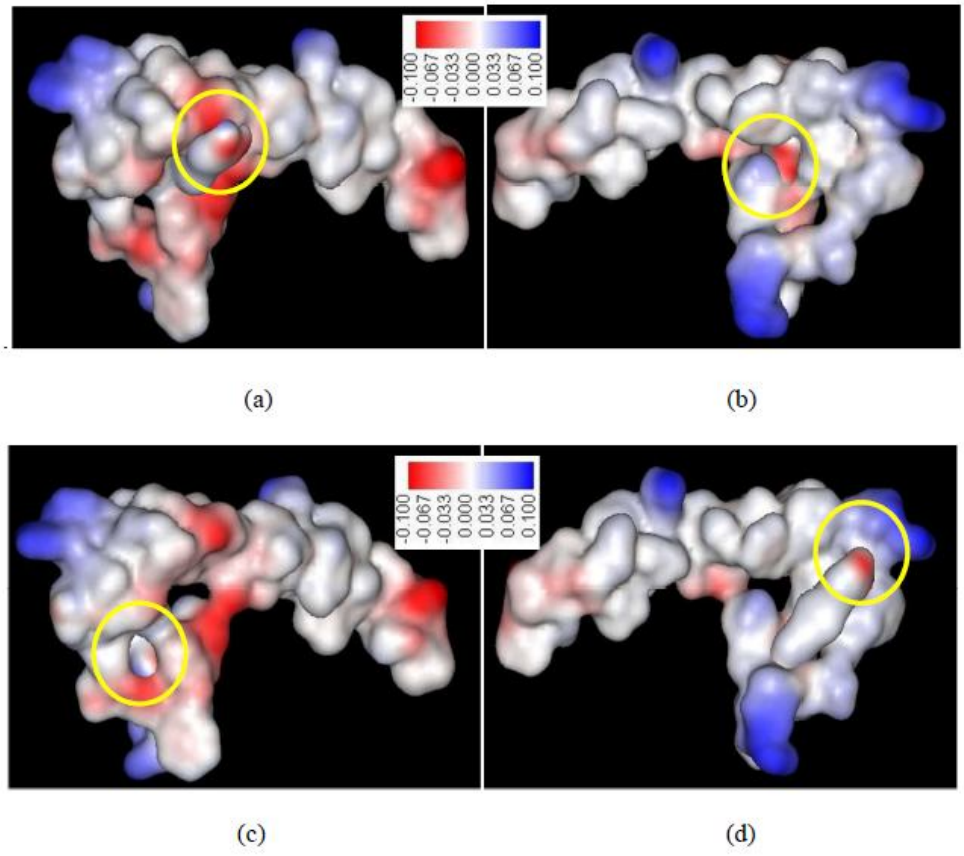


Fig. 7. The electrostatic surface potential obtained from docking of BPT (a,b) and PTT (c,d) to Aβ from two aspect. Blue red and white colors are representative of positive negative and neutral charges respectively.

have compared docking energy between each inhibitor and Aβ by auto dock tool. To better understand which ligand unfolds more or which of them has more effect on Aβ stability we have calculated their free energies.

According to Table 1 docking results, BPT has the most interaction with Aβ. In conclusion BPT bind better to Aβ. This calculation is carried out without substrate and is in good agreement with simulation results. The obtained results can support the future design of newer compounds with better amyloid-β aggregation inhibitor activity.

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REFERENCES

[1] C.A. Hawkes, V. Ng, J. Drug Dev. Res. 70 (2009) 111.

[2] F. Re, C. Airoldi, C. Zona, M. Masserini, B. La Ferla, N. Quattrocchi, F. Nicotra, Curr. Med. Chem. 17 (2010) 2990.

[3] A.D. Ferrão-Gonzales, B.K. Robbs, V.H. Moreau, A. Ferreira, L. Juliano, A.P. Valente, F.C.L. Almeida, Biol. Chem. 280 (2005) 34747.

[4] D.R. Howlett, A. Perry, F. Godfrey, J.E. Swatton, K.H. Jennings, C. Spitzfaden, H. Wadsworth, S.J. Wood, R.E. Markwell, Biochem. J. 340 (1999) 283.

[5] D.D. Soto-Ortega, B.P. Murphy, F.J. Gonzalez-Velasquez, K.A. Wilson, F. Xie, Q. Wang, M.A. Moss, Bioorg. Med. Chem. 19 (2011) 2596.

[6] S.A. Moore, T.N. Huckerby, G.L. Gibson, N.J. Fullwood, S. Turnbull, B.J. Tabner, O.M. El-Agnaf, D. Allsop, Biochemistry 43 (2004) 819.

[7] S.M. Johnson, S. Connelly, I.A. Wilson, J.W. Kelly, J. Med. Chem. 51 (2008) 6348.

[8] P.A. Novick, D.H. Lopes, K.M.A. Branson Esteras-Chopo, I.A. Graef, G. Bitan, V.S. Pande, J. Med. Chem. 55 (2012) 3002.

[9] A.A. Reinke, J.E. Gestwicki, Chem. Biol. Drug Des. 70 (2007) 206.

[10] C.B. Stevens, J.M.J.R. Hanna, R.K. Lammi, Bioorg. Med. Chem. Lett. 23 (2013) 1703.

[11] N.V. Buchete, R. Tycko, G. Hummer, J. Mol. Biol. 353 (2005) 804.

[12] T. Takeda, D.K. Klimov, Biophys. J. 96 (2009) 442.

[13] T. Takeda, D.K. Klimov, Proteins 77 (2009) 1.

[14] G. Favrin, A. Irback, S. Mohanty, Biophys. J. 87 (2004) 3657.

[15] D.K. Klimov, J.E. Straub, D. Thirumalai, Proc. Natl. Acad. Sci. USA 101 (2004) 14760.

[16] D. Ajloo, M. Sangian, M. Ghadamghahi, M. Evini, A.A. Saboury, Int. J. Biol. Macromol. 55 (2013) 47.

[17] D. Ajloo, S. Ghalehaghababaie, N. Mahmoodabadi, Phys. Chem. Res. 1 (2013) 152.

[18] S. Nandi, M.C. Bagchi, Mol. Divers. 14 (2010) 27.

[19] L. Ye, J. Wu, J. Yang, W. Chen, Y. Luo, Y. Zhang, Med. Chem. Res. (2015).

[20] M. Amiri, D. Ajloo, Chem. Med. Res. 23 (2014) 969.

[21] X. Liu, X. Chen, L. Zhang, P. Zhan, X. Liu, Med. Chem. Res. (2015).

[22] G. Eskici, M. Gur, Plos one 8 (2013) e66178.

[23] W.G. Hoover, Phys. Rev. 31 (1985) 1695.

[24] S.A. Nosé, Mol. Phys. 52 (1984) 255.

[25] M. Parrinello, A. Rahman, Phys. Rev. Lett. 45 (1980) 1196.

[26] E. van Der Spoel, B. Lindahl, G. Hess, A.E. Groenhof, H.J. Mark, H.J.C. Berendsen, J. Comput. Chem. 26 (2005) 1701.

[27] W.F. Van Gunsteren, S.R. Billeter, A.A. Eising, P.H. Hunenberger, P. Kruger, A.E. Mark, W.R.P. Scott, I.G. Tironi, Verlag der Fachvereine, Zürich 1 (1996) 1024.

[28] S.R. Gerben, J.A. Lemlul, A.M. Brown, D.R. Bevan, J. Biomol. Struct. Dyn. 32 (2014) 1817.

[29] H.J.C. Berendsen, J.P.M. Postma, W.F. van Gunsteren, J. Hermans, Interaction Models for Water in Relation to Protein Hydration, in: B. Pullman (Ed.), Intermolecular Forces, Reidel: Dordecht Holland, 1981, pp. 331.

[30] L. Willard, A. Ranjan, H. Zhang, H. Monzavi, R.F. Boyko, B.D. Sykes, D.S. Wishart, Nucleic Acids Res. 31 (2003) 3316.