In-Silico Study Of Water Soluble C60-Fullerene Derivatives And Different Drug Targets

Mohammad Teimouri, Hamidreza Kamrani, Kiana oskouei, Junaid Muhammad

Abstract: Fullerene (C60) is a unique carbon molecule that adopts a sphere shape. It has been proved that fullerene and some of its derivatives several disease targets. Fullerene itself is insoluble in water. So, fullerene application is hindered in medical field. In this study, a literature search was performed and all derivatives were collected. The fullerene binding protein, previously reported in literature were also retrieved from protein databank. The docking study were performed with fullerene derivatives and its binding proteins. The selected proteins include Voltage-Gated Potassium Channel, estrogenic 17beta-hydroxysteroid dehydrogenase, and monoclonal anti-progesterone antibody. The binding affinity and binding free energy were computed for these proteins and fullerene derivatives complexes. The binding affinity and binding free energy calculation of the co-crystal ligands were also carried out. The results show the good fitting of fullerene derivatives in the active site of different proteins. The binding affinities and binding free energies of fullerene derivatives are better. The present study gives a detail information about the binding mode of C60 derivatives. The finding will be helpful in fullerene-based drug discovery and facilitate the efforts of fighting many diseases.

Index Terms: Fullerene, C60-derivatives, molecular docking, Binding affinity, Voltage-Gated Potassium Channel, Monoclonal anti-progesterone antibody, Estrogenic 17beta-hydroxysteroid dehydrogenase.

1 INTRODUCTION

Among carbon based materials, fullerene C60 takes the first place and has concerned by an important area of research in modern material nanoscience. C60 molecule (fullerene) was discovered by using carbon vaporization method [1] and then produced in macroscopic quantities with resistible heat by graphite vaporization. It has gained the important role in scientific scene when the Noble prize of 1996 for the chemistry was awarded by the Kroto, Curl and Smalley for the discovery [2]. After the discovery, the research activity on fullerene has gain much attention and their chemical and physical properties along with their applications in several areas has determined [2, 3]. Only physical properties were studied in the early research but later the synthetic chemists have become interested in their functionalization [4] such as halogenation [5], hydrogenation [6], epoxidation [7] and alkylation [8]. The interesting properties of fullerene result in synthesis of huge number of fullerene based compounds attaining promising effects on different targets [9, 10]. The main hindrance, to use C60 in medicinal chemistry, is its insolubility in the water. In 1992, water soluble C60 derivatives, a polyhydroxylated C60, was synthesized[11, 12]. The conjugation with cyclodextrin and calixarenes can enhance its solubity [13]. The first conjugation complex of cyclodextrin and fullerene with improved water solubility was shown by Shashadhar Samal et,al.[14] .They covalently bind the fullerene to the cyclodextrin, it results in high water solubility and biocompatibility. Taking advantage of centered flexible hydroxyl group in calixarenes, Jerry L.Atwood et,al. docked the fullerene with the calixarene [15].

The landmark for the application of fullerene in medical uses was led by Friedman et al [16] . Friedman et al. theorized that the nature and radius of C60 and HIV protease are almost the same so, an opportunity exists for the hydrophobic interactions between C60 and HIV protease active site. To further strengthen this interaction, R. Sijbesma et,al. synthesized a derivatives, bis(phenethy1amincuccinate)C60 [17]. Its pharmacological evaluation was done by research groups at University of California, San Francisco and Emory University [16]. The EC50 was found to be 7μM against HIV protease. It is considered a good inhibitory activity. It inhibitory activity was found to be due to the substitution of cationic moieties. Schinazi, RF et, al found that the derivatives of fullerene have a good inhibitory activity in micro molar concentration range against HIV- 1 and HIV-2 reverse transcriptase [18]. The biological activity of many synthesized C60 derivatives were found to be very good against different targets [19],[20], [21], [22], [23],[24]. It is fair to say that the derivatives of fullerene have good effect on different targets. Until now the understanding of fullerene and protein interactions has only been partly achieved and much remains to be done. The pharmacological activity of C60 and its derivative can be explained in term of its interactions with proteins. It also provides indications about its potential toxicity, a topic still under debate [25, 26]. The development of fullerene derivatives was campaign for the advancement in nanoscience, material chemistry [27] and biochemistry [28]. In the present study the binding affinity of fullerene derivatives with different proteins was investigated using computational approaches. We show here that the derivatives of fullerene have a good binding affinity with different target proteins. And these derivatives may be lead chemicals for drug design.

2 MATERIAL AND METHODS

2.1 Preparation of fullerene derivatives

All the fullerene derivatives were collected from the literature (Fig. 1). Their chemical structures were drawn with the help of MOE-builder program. Merck Molecular Force Field 94X (MMF94X) was used to assign to the all the molecules [29]. Subsequently, the energy of all fullerene derivatives was minimized with a convergence criterion = 0.05 kcal/mol Å2 using the conjugated gradient Newton optimization algorithm.

- *Author name is Mohammad Teimouri is currently pursuing P.H.D degree program in biochemistry in Huazhong University of Science and Technology, China, PH-13297904075. E-mail: mmohteimouri@hust.edu.cn*

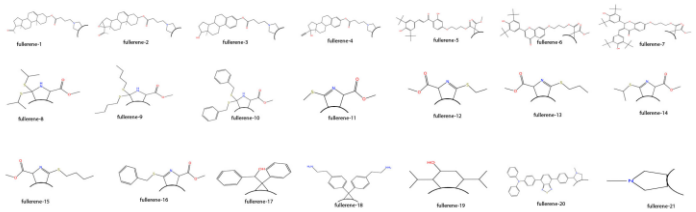

Figure 1*. The chemical structures of fullerene derivatives*

2.2 Molecular docking

MOE2014 was used as docking program. Three dimensional structure of each target were retrieved from the protein data bank. The crystal water were removed. The C60 derivatives contain rotatable bonds. So, all the rotatable bonds were kept flexible and multiple conformation were generated. The accepted poses for each ligand against each receptor were scored on the basis of London dG scoring function. All the accepted conformations were submitted to a further refinement step, based on the force field. The residues were ignored that are away from the pre-refined pose with a cutoff distance of 6 Å to speed up the calculation during refinement and final energy evaluation. During the process of refinement, the reaction field functional form for the electrostatic energy term was used to calculate the solvation effect. To calculate the docking score and final energy, GBVI/WSA dG function was used [30].

2.3 Binding Energy and Binding Affinity Calculations

Generalized Born / volume integral (GB / VI) implicit solvent method was used to further screen the C6- derivatives [39]. Generalized Born interaction energy comprise of non-bonded interaction energy between the receptor and the ligand. It is the sum of Vander Waals, Coulomb electrostatic interaction, and solvent interaction energies [31]. Solvent molecules were ignored during calculation. GBVI/WSA dG scoring function was used to estimate binding affinity. The bindig affinity was reported in unit of Kcal/Mol. All the atoms that exist beyond the 8 Å from the ligand were kept fixed. The atoms were kept flexible that were exist in the vicinity (8 Å) of the ligand. Before calculating binding affinity, energy minimization was carried out for active site. The binding affinity calculation in unit of Kcal/Mol was followed by energy minimization.

3 Results and Discussion

Twisted arrangements of Carbons form different nanostructure. For example, a graphene with diameter 1nm is a single walled nanotube (SWNT) can be formed from Carbons. Fullerenes are the other structures can be formed from Carbon arrangements as it is the most stable molecule. Among fullerene, C60 molecule is the most stable and readily available with a diameter of 0.72 nm. Large number of water soluble fullerene derivatives have also been synthesized nowadays. To confirm whether the C60 show any interact with different targets, a molecular docking study was performed. The effect of each derivative against each target was measured in term of its affinity. Surprisingly, many of water soluble fullerene derivatives have good binding free energy and binding affinity.

3.1 Voltage-Gated Potassium Channel

Membrane proteins account for approximately 30% of the organism's total proteome. The voltage gated potassium channel (Kv) exhibit the most widespread and diverse class of membrane proteins [32]. Other classes are also defined, based on the primary structure of channel-forming sub-unit, such as Ca2+-activated channels, two-pore domain and inwardly rectifying channels [33]. Many cellular process are regulated by the Kv channels such as the regulation of apoptosis [34] , controlling the function of excitable cells [35-37] and differentiation and growth of cell [38]. The normal function of Kv channel is important for the release of neurotransmitters [39] , hormones [40, 41] and for the maintaining of cardiac activity [42]. The role of Kv in T-cell-mediated autoimmune diseases making it an attractive pharmacological drug target. Our docking results show that the derivatives of fullerene have good binding affinity with potassium ion channel. The X-ray crystal structure of Voltage-Gated Potassium Channels (PDB ID 1JVM) complex with tetrabutylammonium was used for the docking purpose. The structure consists of four α-subunits. Unlike the typical voltage gated channel (that has six transmembrane helices), each subunit consists of two transmembrane helices, one pore helix and one selectivity filter loop. The MOE_Dock program was allowed to use the whole protein as a receptor to find the potential binding pocket for the fullerene derivatives. Our docking approach identified the binding pocket in the chamber and not at the entry region. This result recapitulated the published finding of the Matteo Calvaresi and Francesco Zerbetto [43]. The recently published Kv channel blocker, Nifedipine [44] has reduced Kv2.1 currents with the IC50 value of 37.5 ± 5.7 μM, was docked using the whole protein as a receptor. The Nifedipine was docked by the Moe_dock program in the same binding pocket as fullerene derivatives (Fig. 2).

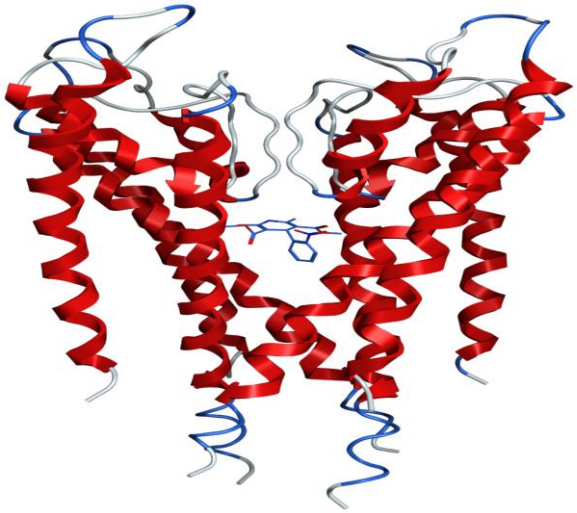

Figure 2. *3D representing of Nifedipine in the active site*

The effectiveness of Nifedipine was measured in term of binding free energy, binding affinity and MOE docking score. The binding free energy, binding affinity and docking score for the Nifedipine are -12.01 kcal/mol, -7.35 kcal/mol and -6.53 respectively. These values have been used as cut off values to select the best fullerene derivative. Out of 62 derivatives, fullerene-21 has good binding affinity -16.98kcal/mol as compared to the binding affinity of the Nifedipine -7.35kcal/mol. The calculation of binding free energies also supports our results and showed that fullerene-21 has a good binding free energy -46.98kcal/mol among all derivatives. The side chain of fullerene-21 makes two hydrogen bonds with Thr74 and Thr75 of chain A. The fullerene cage is

involved in arene-hydrogen bond with Ile100 of chain A, C and D, Thr107 of chain A and C and Gly104 of chain C (Fig. 3).

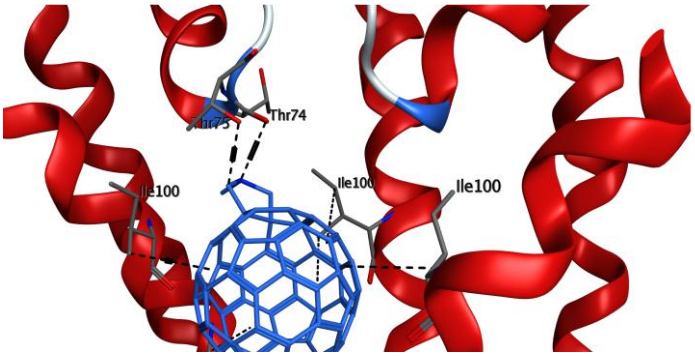

Figure 3. *3D representing of fullerene-21 in the active site*

3.2 Monoclonal anti-progesterone antibody

In case of monoclonal anti-progesterone antibody, the x-ray crystal structure with PDB ID 1DBB was used for docking. The crystal structure contains a progesterone molecule and its binding affinity and binding energy were calculated as -7.23kcal/mol and -25.68kcal/mol respectively. The fullerene-7 and 20 have a good binding free energy -34.55kcal/mol, -29.74kcal/mol and binding affinity -9.11kcal/mol, -8.65kcal/mol respectively as compared to the progesterone. The fullerene cage of fullerene-7 make arene-hydrogen bond with the His93 and Val94 while the side chain make one hydrogen bond with the Ser27E and two arene-hydrogen bonds with the Trp50 and Tyr97 (Fig. 4a). In case of fullerene-53 only the fullerene cage is involved in the binding with the Ser27A and His93 (Fig. 4b).

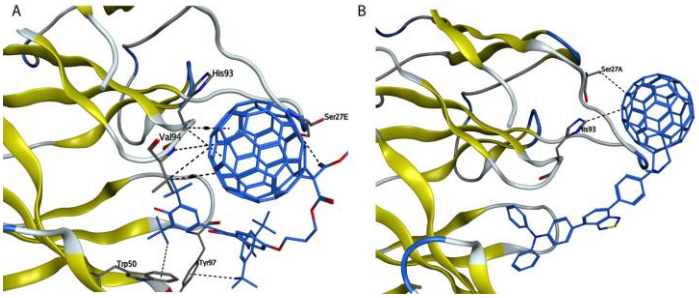

Figure 4*. (A) 3D representing of fullerene-7 in the active site (B) 3D representing of fullerene-53 in the active site*

3.3 Estrogenic 17beta-hydroxysteroid dehydrogenase

In case of human estrogenic 17beta-hydroxysteroid dehydrogenase (PDB ID 1DHT), the fullerene-4 fit well in the active pocket with binding affinity and binding free energy -11.08kcal/mol and -17.65kcal/mol respectively. One hydrogen bond is formed by the Met193 and the side chain of the fullerene. The residues Phe226, Thr190, Val188, Ile14, Thr140 and Lys159 are involved in the arene-hydrogen bond with the fullerene cage (Fig. 5a). The fullerene-6 fit well in the active site with binding affinity and binding free energy -10.67kcal/mol and -29.53kcal/mol respectively. It makes two hydrogen bonds with the Arg258 and one arene-hydrogen bond with Tyr155 (Fig. 5b). The fullerene-5 has a good binding affinity -9.99kcal/mol and good binding free energy -31.37kcal/mol with best fitting in the active pocket. The side chain of fullerene-5 makes one hydrogen bond and one arene-hydrogen bond with Arg258 and His221 respectively. The fullerene cage is also involved in the arene-hydrogen bond with Arg258 and Pro261 (Fig. 5c).

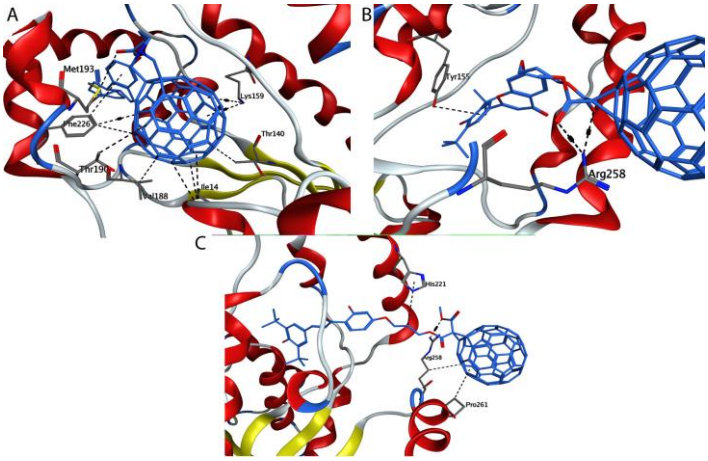

Figure 5*. 3D representing in the active site of (A) fullerene-4 (B) fullerene-6 (C) fullerene-5*

4 Conclusion

In present study, the docking study of water soluble C60-deriviatives were performed with different potential targets. The interaction of each derivative were characterized with each targets. Few fullerene derivatives were found to have a good binding affinity and binding energy against these targets. The present study gives a detail information about the binding mode of C60 derivatives. These results will be contributory in the progress of C60-based medicines against many diseases.

5 References

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