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# Computational studies of C60-derivatives against IspE of *Mycobacterium tuberculosis*

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***Abstract:*** **IspE is one the enzyme of non-mevalonate pathway in *Mycobacterium tuberculosis* and other pathogenic species. The non-mevalonate pathway synthesize isoprenoid precursor. The precursors of the isoprenoids are the isopentenyl diphosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). IspE catalyzes the transfer of γ-phosphoryl group from ATP to 4-diphosphocytidyl-2C-methyl-d-erythritol (CDP-ME) resulting in 4-diphosphocytidyl-2C-methyl-D-erythritol-2-phosphate (CDP-ME2P). Water soluble C60 derivatives were collected from the literature and were docked into the active site of IspE. The docking results of C60 derivatives and ATP analogues were compared. It was found that all the C60-deivatives have a very good docking than ATP analogues AMP-PNP.**

***Keywords:*** **IspE Inhibitors, Molecular docking, MD Simulation, ZINC database, MMPBSA analysis, *Mycobacterium tuberculosis.***

## I. INTRODUCTION

When the door to the nanoword opened, the nanotechnology and the nanoparticles have gained a significant attention for their potential application in medicle health [1-3]**.** The nanoparticles are the structures whose dimensions are1-100 nm in range [4,5]**.** Now the fast developing field of nanotechnology is in his full swing due to the cognition to control the properties of nanomaterials with greater exactitude [6-8][6-8] and much research has been focused on the characterization of carbon-based nanomaterials such as graphene surfaces, fullerenes and carbon nanotubes [9-11]**.**

The modern material nanoscience concerns carbon based materials, among which fullerenes take one of the first places. A fullerene is structure, composed of carbon atoms, in the form of hollow ellipsoid, sphere, tubes and many other structures. The first fullerene to be discovered was spherical fullerene called Buckminsterfullerene having molecular formula C60 in 1985, as a new type of carbon allotrope [12]**[**. Fullerene is a truncated icosahedron with 20 hexagons due to C5-C6 double bonds and with 12 pentagons due to C5-C5 single bonds [13,14]**.** In fullerene each carbon is in its *sp2* configuration and bound to other three carbons. There are two types of bond in fullerene, the double bond of 6:6 ring bonds and the shorter bond in 6:5 bonds. C60-fullerene reacts with electron rich species due to its electron-accepting nature and behaves like electron-deficient alkenes **.** The effect of nanomaterial on biological system is still little known regardless of remarkable developments in nanoscience [15]**.** There is an anxiety with the use of nanomaterials for medicle applications that it may induce cytotoxic effect but the inert behavior, small size, and stable structure account for the low toxicity of fullerenes, despite at relatively high concentrations [16]**.**

Since its discovery, a significant advances have been made to develop a variety of methods to incorporate C60-fullerene into organic and inorganic surfaces resulted from potential utility of surface-linked fullerene materials [17-20]**.** and different fullerene derivatives have designed by exohedral derivatization using additional polymers , proteins , chemicals, genetic vectors and antibodies [13,21-24]**.** Chemical addition of hydrophilic groups make the fullerene more water soluble and various pharmacological and biological properties are reported to possessed by water soluble modified fullerenes for example the pyrrolidinium fullerene derivative showed cytotoxicity in HL60 [25]**,**excellent antioxidant activity has shown by Malonic acid fullerene derivative [26]**,** inhibitory activity towards the human immunodeficiency

virus (HIV)-reverse transcriptase has shown by Proline modified fullerene derivative [27]. Although, Superamolecular assemblies of fullerene have been investigated for 15 years, the early work only concerned with the complexation of C60 with molecular building blocks to favor the formation of inclusion complexes [28]. Nowadays fullerene has attracted much attention in the field of medicinal chemistry arena due to its unique physical and chemical properties. Friedman et al. found that fullerene can easily cover the hydrophobic cavity of HIV protease and thus shield the catalytic site from substrate [29]. Some C60 derivatives have also antibacterial activity and even other target cancer cells [30]. Fullerene and its water soluble derivatives are known to possess many biological and pharmacological properties including neuroprotective [31]. The major contribution towards the oxidative injury, studied in cell culture of diseased model animal, was played by hydroxyl (OH) radicles, superoxide ($O^{2-}$), the non-radical molecules H2O2 and hypochlorous acid and a link was merged by free radicles and neurodegenerative conditions, such as Parkinson's disease and Alzheimer's dementia. Water soluble derivatives of C60 fullerene are excellent antioxidants endowed with a broad spectrum of neuroprotective abilities, reported by Dugan et al. [31,32]. DNA cleaving activity was also shown by water soluble covalently linked C60-porphyrin compound. Cuisong et al. reported that the credit of this extreme cleaving activity ascribed to the high water-solubility and affinity of $C_{60}$Por to DNA [33]. Several activities and uses are exhibited by fullerene and its derivatives including antimycobacterial [34], HIV protease inhibitors [35,36], drug delivery systems [37], potentiometric biosensing of glucose [38] and gene carrier [39]. Fullerene has now opened a new hope and a new window in the field of diagnosis and therapeutics due to its capability to cross cell membranes, this role has been attributed to its lipophilic carbon cage [40].

In the present study, water soluble C60 derivatives were collected from literature and were docking into the active site of IspE. IspE is the fourth enzyme within the MEP pathway and is liable for catalyzing the ATP-dependent phosphorylation of 4-diphosphocytidyl-2-C-methyl-d-erythritol (CDP-ME) to grant 4-diphosphocytidyl-2-C-methyl-d-erythritol 2-phosphate (CDP-ME2P); it was recently identified to be essential in *Mycobacterium tuberculosis.*

## II. MATERIAL AND METHODS

***Molecular Dynamics Simulation:***

Molecular Dynamics Simulation of IspE was carried out using *pmemd.cuda* [41] module of Amber14 [42] . The protein was solvated in a rectangular box of TIP3P water molecules using tleap version of Amber14 [43] with 12 Å of buffer distance between the protein edge and box boundary in all direction. An appropriate number of NaCl salt molecules were added to form the system neutral. Amber ff14SB force field was used to generate coordinate and topology files for the protein [44]. In order to remove bad contacts between solvent and protein, energy minimization was carried out in two steps. First, the system was minimized keeping the protein fixed with harmonic constrain of a strength of 500 $kcal*mol^{-1}.Å^{-2}$. Secondly, the whole system was minimized without any constrain. The above each step was performed with the steepest descent minimization of 1000 steps followed by a conjugate gradient minimization of 1000 steps. The system was then heated to 300K during the 2000 steps. Finally the system was simulated for 20 ns and the trajectory was saved after each 2 fs. The SHAKE algorithm was used for the covalent bonds involving hydrogens [45]. The Particle Mesh Ewald (PME) method was adopted to treat the long-range electrostatic interactions [46].

***Molecular docking:***

MOE-Dock program implemented in MOE2014 was used for docking. The crystal structure of the target protein was used for the docking study. By applying torsion angles to all rotatable bonds in each ligand, multiple conformations for each ligand were generated. Ten conformations were generated for each ligand. The accepted conformations for each ligand against receptor were scored using of London dG scoring function which calculates the free energy for the binding of ligand from a given conformation. The final hits were docked using Autodock software to cross check and validate the results of MOE.

## III. RESULTS AND DISCUSSION

***Molecular Dynamic simulation:***

The molecular dynamic simulation was carried out in order to refine the structure. The final stable structure was used for the docking purpose. The stability of protein was checked in term of root mean square deviation (RMSD). The figure 1 shows the results of MD simulation. The figure shows that RMSD of the system reached the equilibrium after 2 ns and then fluctuate within a narrow range during the rest of rest simulation.

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***Validation of docking protocol:***

Prior to docking, the docking protocol discussed in material methods part was validated. The crystal structure of IspE complex with AMP-PNP was retrieved from the protein databank. The AMP-PNP was extracted and then re-docked. PISA (protein interfaces, surfaces and assemblies service) [47] was used to calculate the interface residues of each docked complex. The interface residues of resultant docked complex were compared to the interface residues of the crystal structure. The interface of complex (both crystal and docked) was studied through the PISA server. The interface study is summarized in the table 1. The number and type of interfacing residues of the crystal and docked complex are almost similar, suggesting the reliability of the protocol to reproduce the experimental binding mode

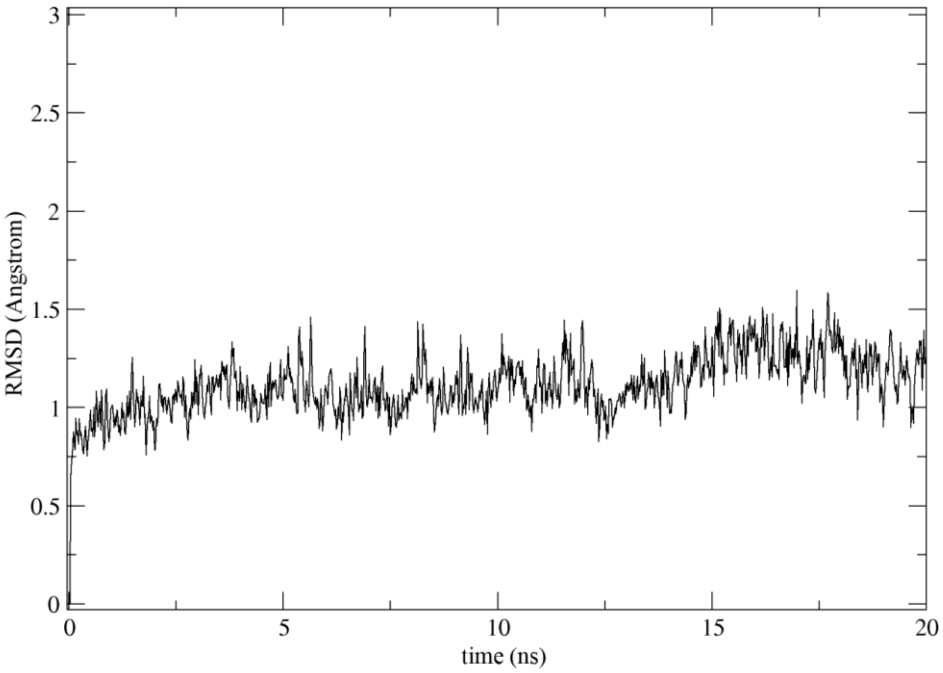


**Fig.1: The molecular dynamics simulation of the IspE complex: the complex is simulated over a period of 20 ns. The RMSD of the simulation was plotted against time, according to the simulation trajectories**

**Table 1: The interface study of IspE complex with AMP-PNP. The interface of X ray crystal structure and re-dock complex**

| Complexes | Number of residues at interface | Interface residues |
|---|---|---|
| **IspE/AMP-PNP (x-ray structure)** | 23 | LYS13, ASN15, LEU56, LEU64, ARG69, ASN70, LEU71, LYS95, VAL99, ALA100, GLY101, GLY102, MET103, ALA104, GLY105, GLY106, SER107, ASP109, ASP140, THR181, SER255, GLY256, SER257, GLY258 |
| **IspE/AMP-PNP (re-dock complex)** | 24 | LYS13, LEU64, ASN70, LEU71, LYS95, ILE97, PRO98, VAL99, ALA100, GLY101, GLY102, MET103, ALA104, GLY105, GLY106, SER107, ASP140, GLY178, LEU179, LEU180, THR181, SER257, GLY258, PRO259 |

***Molecular docking:***

The crystal structure of the IspE complex with AMP-PNP was retrieved from the PDB databank with accession number 3PYF. All the crystal water molecules were removed from the crystal structure. The structural preparation program embedded in MOE was used to add any missing hydrogen atom, correct the charges and assign correct hybridization state of each residue. The protonate 3D module embedded in MOE was used to assign the correct protonation state using Generalized Born/Volume Integral (GB/VI) electrostatic function. Multiple conformations were generated for each ligand by applying a preferred torsion angles to all rotatable bonds in each ligand. The Amber12: EHT force field was used for all the computational procedures. The energy of the system was minimized upto RMS gradient of 0.05 Kcal/mol.Å$^2$. The

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interactions of AMP-PNP with the active site of IspE were observed. The AMP-PNP binding site residues were defined as those within the 4 Å of AMP-PNP using Pymol software (Fig 2).

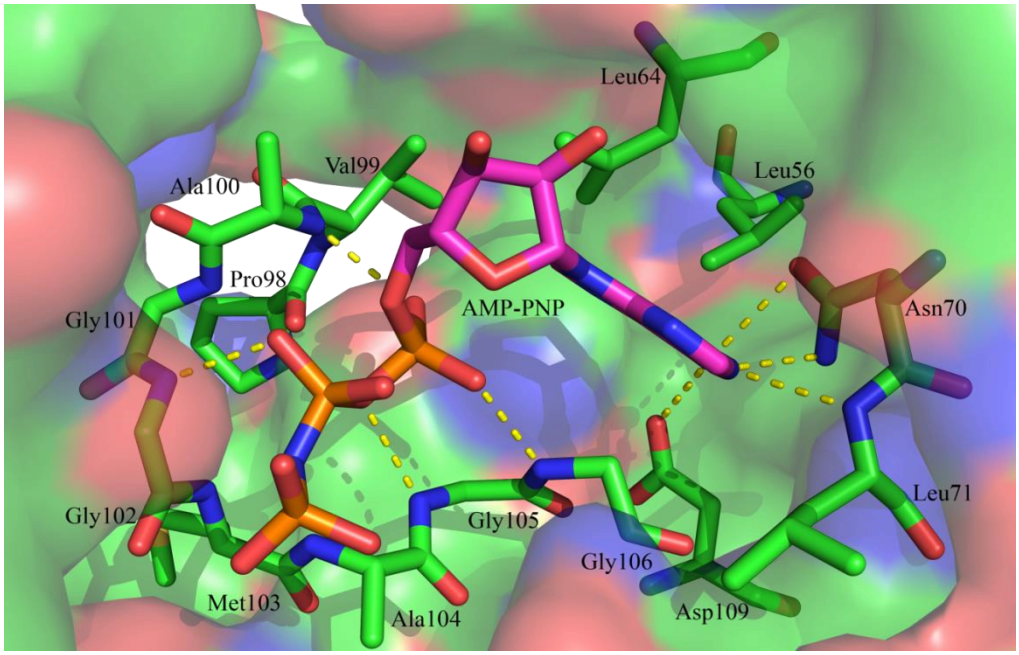


**Fig.2: Binding mode of AMP-PNP: The AMP-PNP binding site residues were defined as those within the 4 Å of AMP-PNP using Pymol software. The yellow dotted lines show the hydrogen bonds. The AMP-PNP is shown in stick model**

Analysis of the IspE complex structure shows important residues that help in binding of AMP-PNP. The adeninyl moiety makes several hydrogen bonds with Asn70, Leu71 and Asp109. In addition the phosphate moiety makes hydrogen bonds with Ala100, Gly102, Gly105 and Gly106. The other residues such as Leu56, Leu64, Pro98, Val99, Gly101, Met103 and Ala104 are involved in hydrophobic interaction. The AMP-PNP was rescored in the active site of IspE and the score was found to be -4.83. The docking score of AMP-PNP was used as a cut off value to further screen C60 derivatives. Interestingly, all the C60 derivatives have a very good docking score as mentioned in the figure 3. The docking results of all the C60 derivatives were summarized in the table 1.

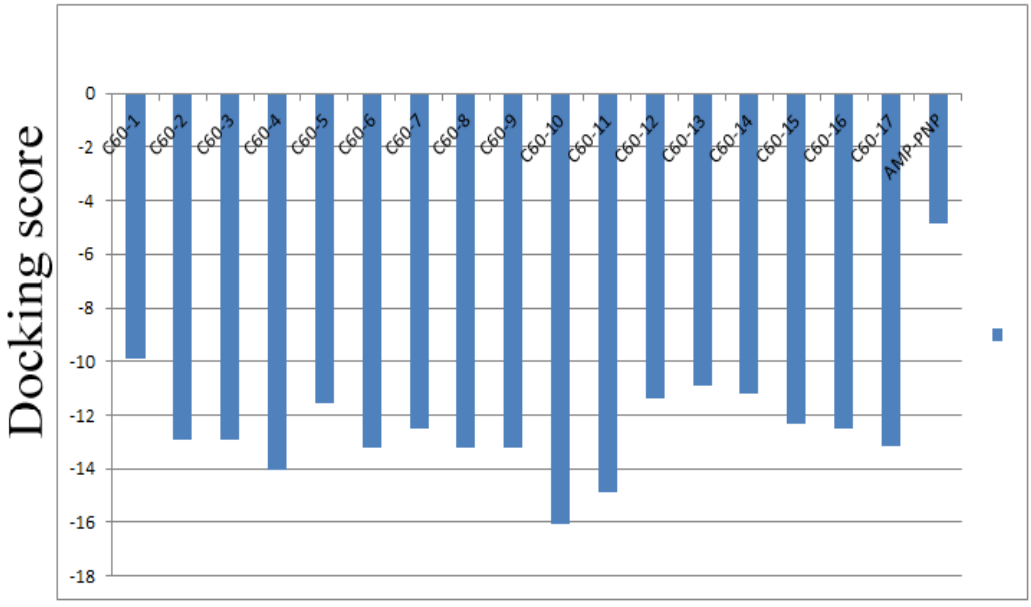


**Fig.3 Docking score of the C60 derivatives: the results shows that all the C60 derivatives have a very good docking score than AMP-PNP**

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**Table 2: Docking score of C60 derivatives along with the interacting residues**

| Compounds | Docking Score | Interacting Residues | |
|---|---|---|---|
| | | Hydrophobic interaction | Hydrogen bond interaction |
| C60-1 | -9.8877 | LEU64, LEU71, VAL99 | |
| C60-2 | -12.9196 | LEU64,VAL99 | ASN70, GLY102, ALA104, GLY106 |
| C60-3 | -12.9282 | LEU71 | LYS13, GLY102, LEU179, SER257, GLY258 |
| C60-4 | -14.0482 | LEU64, LEU71, VAL99 | LYS13, GLY102, GLY102, ALA104, GLY106 |
| C60-5 | -11.5662 | LEU64 , LEU71 , VAL99 | PRO98, GLY105, GLY102, ALA104, GLY106 |
| C60-6 | -13.1922 | LEU180 | SER107, SER139, GLY102, ALA104, GLY106 |
| C60-7 | -12.5172 | LEU64, LEU71 | GLY102, GLY105, ARG69, ASN70, PRO98, GLY106 |
| C60-8 | -13.2068 | LEU64 1, LEU71, LEU180 | GLY101, GLY105, SER107, SER139, ASP140 |
| C60-9 | -13.1925 | LEU64 | PRO98, GLY105, ARG69, ASN70, PRO98, GLY106 |
| C60-10 | -16.0672 | LEU64, LEU71, VAL99 | LYS13, ASP140. |
| C60-11 | -14.8989 | LEU64, LEU71 | ARG69, ASN70, PRO98, GLY106 |
| C60-12 | -11.352 | LEU71, LEU180 | SER107, SER139, ASP140 |
| C60-13 | -10.9167 | LEU31 | ASP140, THR181 |
| C60-14 | -11.1872 | LEU64, LEU71, ILE97 | SER107, SER139, ASP140 |
| C60-15 | -12.3384 | LEU64, VAL99 | PRO98, GLY101 |
| C60-16 | -12.4921 | LEU64, LEU71, ILE97 | ASN70, ALA100, GLY102, ALA104, GLY106, ASP140, THR181 |
| C60-17 | -13.1556 | GLY258, VAL99 | LYS13, ASP140, THR18 |
| AMP-PNP | -4.83 | LEU64, LEU71 | ASN70, ALA100, GLY102, ALA104, GLY106 |

## IV. CONCLUSION

The non-mevalonate pathway uses seven enzymes in the synthesis of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) from the starting material pyruvate and glyceraldehyde-3-phosphate. In the present study, the binding site of AMP-PNP, a stable analog of ATP, was used. The molecular docking shows that C60-derivatives may serve as good inhibitors for the inhibition of IspE.

## REFERENCES

[1] Gerber C, Lang HP (2006) How the doors to the nanoworld were opened. Nature nanotechnology 1: 3-5.

[2] Singh N, Manshian B, Jenkins GJ, Griffiths SM, Williams PM, et al. (2009) NanoGenotoxicology: the DNA damaging potential of engineered nanomaterials. Biomaterials 30: 3891-3914.

[3] Ahamed M, AlSalhi MS, Siddiqui M (2010) Silver nanoparticle applications and human health. Clinica chimica acta 411: 1841-1848.

[4] Kim S, Choi JE, Choi J, Chung K-H, Park K, et al. (2009) Oxidative stress-dependent toxicity of silver nanoparticles in human hepatoma cells. Toxicology in vitro 23: 1076-1084.

[5] Chaloupka K, Malam Y, Seifalian AM (2010) Nanosilver as a new generation of nanoproduct in biomedical applications. Trends in biotechnology 28: 580-588.

[6] Mout R, Moyano DF, Rana S, Rotello VM (2012) Surface functionalization of nanoparticles for nanomedicine. Chemical Society Reviews 41: 2539-2544.

[7] Lee J, Mahendra S, Alvarez PJ (2010) Nanomaterials in the construction industry: a review of their applications and environmental health and safety considerations. Acs Nano 4: 3580-3590.

[8] de la Rica R, Matsui H (2010) Applications of peptide and protein-based materials in bionanotechnology. Chemical Society Reviews 39: 3499-3509.

[9] D'Rozario RS, Wee CL, Wallace EJ, Sansom MS (2009) The interaction of C60 and its derivatives with a lipid bilayer via molecular dynamics simulations. Nanotechnology 20: 115102.

[10] Sanchez VC, Jachak A, Hurt RH, Kane AB (2011) Biological interactions of graphene-family nanomaterials: an interdisciplinary review. Chemical research in toxicology 25: 15-34.

[11] Zuo G, Zhou X, Huang Q, Fang H, Zhou R (2011) Adsorption of villin headpiece onto graphene, carbon nanotube, and C60: effect of contacting surface curvatures on binding affinity. The Journal of Physical Chemistry C 115: 23323-23328.

[12] Kroto HW, Heath JR, O'Brien SC, Curl RF, Smalley RE (1985) C 60: buckminsterfullerene. Nature 318: 162-163.

[13] Bosi S, Da Ros T, Spalluto G, Prato M (2003) Fullerene derivatives: an attractive tool for biological applications. European journal of medicinal chemistry 38: 913-923.

[14] Mintmire J (1996) Fullerene formation and annealing. Science 272: 45-46.

[15] Mahmoudi M, Lynch I, Ejtehadi MR, Monopoli MP, Bombelli FB, et al. (2011) Protein− nanoparticle interactions: opportunities and challenges. Chemical Reviews 111: 5610-5637.

[16] Love SA, Maurer-Jones MA, Thompson JW, Lin Y-S, Haynes CL (2012) Assessing nanoparticle toxicity. Annual Review of Analytical Chemistry 5: 181-205.

[17] Mirkin CA, Brett Caldwell W (1996) Thin film, fullerene-based materials. Tetrahedron 52: 5113-5130.

[18] Saito Y, Ohta H, Terasaki H, Katoh Y, Nagashima H, et al. (1996) Separation of calixarenes with a chemically bonded C60 silica stationary phase in microcolumn liquid chromatography. Journal of High Resolution Chromatography 19: 475-477.

[19] Stalling D, Guo C, Saim S (1993) Surface-linked C60/70-polystyrene divinylbenzene beads as a new chromatographic material for enrichment of coplanar PCBs. Journal of chromatographic science 31: 265-278.

[20] Stalling DL, Guo C, Kuo KC, Saim S (1993) Fullerene-linked particles as LC chromatographic media and modification of their electron donor/acceptor properties by secondary chemical reactions. Journal of Microcolumn Separations 5: 223-235.

[21] Wei P, Zhang L, Lu Y, Man N, Wen L (2010) C60 (Nd) nanoparticles enhance chemotherapeutic susceptibility of cancer cells by modulation of autophagy. Nanotechnology 21: 495101.

[22] Zhu J, Ji Z, Wang J, Sun R, Zhang X, et al. (2008) Tumor-Inhibitory Effect and Immunomodulatory Activity of Fullerol C60 (OH) x. Small 4: 1168-1175.

[23] Hahn U, Gégout A, Duhayon C, Coppel Y, Saquet A, et al. (2007) Self-assembly of fullerene-rich nanostructures with a stannoxane core. Chemical Communications: 516-518.

[24] Nakamura E, Isobe H (2003) Functionalized fullerenes in water. The first 10 years of their chemistry, biology, and nanoscience. Accounts of chemical research 36: 807-815.

[25] Nishizawa C, Hashimoto N, Yokoo S, Funakoshi-Tago M, Kasahara T, et al. (2009) Pyrrolidinium-type fullerene derivative-induced apoptosis by the generation of reactive oxygen species in HL-60 cells. Free radical research 43: 1240-1247.

[26] Okuda K, Mashino T, Hirobe M (1996) Superoxide radical quenching and cytochrome< i> C</i> peroxidase-like activity of C< sub> 60</sub>-dimalonic acid, C< sub> 62</sub>(COOH)< sub> 4</sub>. Bioorganic & Medicinal Chemistry Letters 6: 539-542.

[27] Mashino T, Shimotohno K, Ikegami N, Nishikawa D, Okuda K, et al. (2005) Human immunodeficiency virus-reverse transcriptase inhibition and hepatitis C virus RNA-dependent RNA polymerase inhibition activities of fullerene derivatives. Bioorganic & medicinal chemistry letters 15: 1107-1109.

[28] Diederich F, Gómez-López M (1999) Supramolecular fullerene chemistry. Chemical Society Reviews 28: 263-277.

[29] Friedman SH, DeCamp DL, Sijbesma RP, Srdanov G, Wudl F, et al. (1993) Inhibition of the HIV-1 protease by fullerene derivatives: model building studies and experimental verification. Journal of the American Chemical Society 115: 6506-6509.

[30] Tsao N, Kanakamma PP, Luh T-Y, Chou C-K, Lei H-Y (1999) Inhibition of Escherichia coli-induced meningitis by carboxyfullerence. Antimicrobial agents and chemotherapy 43: 2273-2277.

[31] Dugan LL, Turetsky DM, Du C, Lobner D, Wheeler M, et al. (1997) Carboxyfullerenes as neuroprotective agents. Proceedings of the National Academy of Sciences 94: 9434-9439.

[32] Dugan LL, Gabrielsen JK, Yu SP, Lin T-S, Choi DW (1996) Buckminsterfullerenol free radical scavengers reduce excitotoxic and apoptotic death of cultured cortical neurons. Neurobiology of disease 3: 129-135.

[33] Zhou C, Liu Q, Xu W, Wang C, Fang X (2011) A water-soluble C60-porphyrin compound for highly efficient DNA photocleavage. Chemical Communications 47: 2982-2984.

[34] Kumar A, Patel G, Menon SK (2009) Fullerene isoniazid conjugate–a tuberculostat with increased lipophilicity: synthesis and evaluation of antimycobacterial activity. Chemical biology & drug design 73: 553-557.

[35] Durdagi S, Supuran CT, Strom TA, Doostdar N, Kumar MK, et al. (2009) In silico drug screening approach for the design of magic bullets: a successful example with anti-HIV fullerene derivatized amino acids. J Chem Inf Model 49: 1139-1143.

[36] Marcorin GL, Da Ros T, Castellano S, Stefancich G, Bonin I, et al. (2000) Design and synthesis of novel [60]fullerene derivatives as potential HIV aspartic protease inhibitors. Org Lett 2: 3955-3958.

[37] Zakharian TY, Seryshev A, Sitharaman B, Gilbert BE, Knight V, et al. (2005) A fullerene-paclitaxel chemotherapeutic: synthesis, characterization, and study of biological activity in tissue culture. J Am Chem Soc 127: 12508-12509.

[38] Kumar A, Patel B, Patel G, Menon SK (2010) Potentiometric biosensing of glucose by fullerene-based silver selective electrodes. Fullerenes, Nanotubes, and Carbon Nanostructures 18: 186-197.

[39] Maeda-Mamiya R, Noiri E, Isobe H, Nakanishi W, Okamoto K, et al. (2010) In vivo gene delivery by cationic tetraamino fullerene. Proceedings of the National Academy of Sciences 107: 5339-5344.

[40] Foley S, Crowley C, Smaihi M, Bonfils C, Erlanger BF, et al. (2002) Cellular localisation of a water-soluble fullerene derivative. Biochem Biophys Res Commun 294: 116-119.

[41] Gotz AW, Williamson MJ, Xu D, Poole D, Le Grand S, et al. (2012) Routine Microsecond Molecular Dynamics Simulations with AMBER on GPUs. 1. Generalized Born. J Chem Theory Comput 8: 1542-1555.

[42] Case DA, Cheatham TE, 3rd, Darden T, Gohlke H, Luo R, et al. (2005) The Amber biomolecular simulation programs. J Comput Chem 26: 1668-1688.

[43] Jorgensen WL, Chandrasekhar J, Madura JD, Impey RW, Klein ML (1983) Comparison of simple potential functions for simulating liquid water. The Journal of chemical physics 79: 926-935.

[44] Hornak V, Abel R, Okur A, Strockbine B, Roitberg A, et al. (2006) Comparison of multiple Amber force fields and development of improved protein backbone parameters. Proteins: Structure, Function, and Bioinformatics 65: 712-725.

[45] Ryckaert J-P, Ciccotti G, Berendsen HJ (1977) Numerical integration of the cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes. Journal of Computational Physics 23: 327-341.

[46] Darden T, York D, Pedersen L (1993) Particle mesh Ewald: An N· log (N) method for Ewald sums in large systems. The Journal of chemical physics 98: 10089-10092.

[47] Krissinel E, Henrick K (2007) Inference of macromolecular assemblies from crystalline state. J Mol Biol 372: 774-797.