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Molecular Interaction Studies of Chitosan Cross-linked Compounds as Drug Delivery Substrate for Anticancer Agents

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Abstract

Chitosan is known for its absorption and adhesion property and it is a non-toxic biodegradable hetero polymer. It has a strong affinity for water and high degree of solubility in acidic medium. In addition, chitosan hydrogels showed low mechanical strength and minimum ability to control the delivery of encapsulated compounds. And hence, in this investigation a set of compounds cross linked with Chitosan was screened for anticancer agent using computational technique (molecular docking) against NOS enzyme (PDB ID: 4NOS). The result was interesting as majority of the compounds screened turned up with favorable molecular interaction and binding affinity as evidenced from the docking score. Furthermore the molecular interaction analysis represents the cross linked compounds possessed heavy molecular interaction at the active site residue of the enzyme. Thus, Chitosan cross linked compounds target the specific active site residue and hence can be used in future for drug delivery.

Keywords: Chitosan; cross linked; NOS enzyme; anticancer

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Introduction

Hydrogels are cross-linked polymeric networks that trap water in the polymer matrices mainly by surface tension and are usually three dimensional while most of its properties can be altered by structural modification [1,2]. A kind of hydrogel called chitosan is finding wide application in many areas such as fuel cells, oil encapsulation, wound dressing and especially in drug delivery in recent years [3-6]. Chitosan [β-(1,4)-2-amino-2-deoxy-d-glucopyranose] is a non-toxic biodegradable hetero polymer, has a good absorption and adhesion property. It is a weak base obtained by deacetylation of chitin [3]. Because of their affinity for water and high degree of solubility in acidic medium, chitosan hydrogels comparatively shows low mechanical strength and minimum ability to control the delivery of encapsulated compounds [7], thereby facilitating chemical modification by its amino and hydroxyl groups. For a hydrogel to be introduced in a biological systems, its compatibility should be taken into account while it is seen that the pharmacy world will benefit from hydrogels like chitosan because of its hydrophilicity, flexibility, versatility, high water absorptivity, and greater compatibility with the biological system. It is also a choice for the pharmaceutical world because of its long life span in circulation and possibility of being actively or passively targeted to the known biophase like cancer cells [8]. Chitosan can be blended with different cross linking agent to produce a chemically active hydrogels for bioapplications. Like the amino group of chitosan can lead to ionic interaction between and anionic groups. These interactions can produce hydrogels with different material properties which depend upon the size of the anionic agents and charge density, also on degree of deacetylation and amount of chitosan polymer.

Chitosan cross linked with polyelectrolytes have been produced by water-soluble negatively charged molecules like DNA, alginate, hyaluronic acid, proteins like gelatin polyacrylic acid and mostly the stability of these compounds depends on solvent, charge density, temperature, ionic strength, and pH [9-12].

The NOS enzymes consist of oxygenase domain that binds arginine, tetrahydrobiopterin and heme and reductase domain with FAD and FMN prosthetic groups. They are complex, homodimeric heme enzyme which produces free radical nitric oxide that leads to variety of age-related diseases [13-15]. Nitric oxide synthase (NOS) in cells are of three isoforms. Neuronal/brain NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS). nNOS and eNOS belongs to constitutive NOS (cNOS) and NO produced from this type helps in maintaining normal vasoactivity through a Ca^{2+} -dependent pathway and also as a neurotransmitter for signal transmission. While NO produced from iNOS can trigger several disadvantage cellular responses and can cause some diseases including sepsis, stroke and inflammation, [13-19].

In the present investigation chitosan cross linked compounds were screened as inhibitors of iNOS revealing chitosan as a good carrier for delivering and unloading the drug at specific target.

Materials and Method

Chemical dataset

The 2D structure of Diethylsquarate, Glutaraldehyde, Formaldehyde, Ethyleneglycol diglycildylether, Blocked Diisocyanate, Phloretic and Activated Quinone cross-linked with Chitosan was generated with Chemoffice 2010. The energy of these cross-linked compounds were further optimized using MM2 force field method and save as sybyl mol2 (three dimensional) file format using ChemOffice 2010.

Protein preparation

The 3D structure of human inducible nitric oxide synthase (PDB ID: 4NOS) was downloaded from the Protein Databank Bank (http://www.rcsb.org/). The coordinates of this enzyme is complexed with water molecules and iron protoporphyrin IX (heme) along with the Cofactors such as BH_4, Zn^{+2} atom. Moreover the 3D structure has a resolution of 2.25 Å. making it an excellent choice for molecular docking studies. All the water molecules were removed for the molecular docking simulation purpose since they are not taken into account during the scoring function [20].

Docking computation

The 3D structure of human inducible nitric oxide synthase (PDB ID: 4NOS) was then imported in Molegro Virtual Docker (MVD). The sidechains conformations of 4NOS were further minimized using PLP-potentials for steric and hydrogen bonding interactions, and the Coulomb potential for the electrostatic forces. And only the torsion angles are modified during the minimization which includes bond lengths and backbone atom positions are held fixed [20].

Further, the potential ligand binding site of the enzyme was predicted using MVD. The binding site have a volume of 470.02 A3 and 1158.84 A2. The binding site was set inside a restriction sphere of radius 15 A° (X 0.65, Y 99.58, Z 11.19) using MVD.

Then the Chitosan cross linked compounds were then imported in MVD. The Bond flexibility of the cross likned compounds was set as well as the side chain flexibility of residues near the potential ligand binding site was set with a tolerance of 1.10 and strength of 0.90 for docking simulations. The RMSD threshold was set at 2.00 Å for multiple cluster poses. The docking algorithm was set at 1,500 maximum iteration with simplex evolution size of 50 and a minimum of 20 runs were performed for each of the cross linked compound. The best pose was considered for subsequent protein-ligand interaction analysis.

Molecular docking was carried out using MVD which is based on a differential evolution algorithm. The algorithm of MVD considers the sum of the intermolecular interaction energy between the ligand and the protein and the intramolecular interaction energy of the ligand. The docking energy scoring function is based on the modified piecewise linear potential (PLP) with new hydrogen bonding and electrostatic terms included. Full description of the algorithm and its reliability compared to other common docking algorithm is described by Thomsen et al. [20].

Results and Discussion

The molecular docking simulation revealed the cross linked compounds bind at the active site of the NOS. From the docking score, it is revealed that the MolDock score holds favorable interaction for the Chitosan Cross linked compounds viz. Diethyl Squarate (-181.13 $kJmol^{-1}$), Formaldehyde (-179.522 $kJmol^{-1}$), Glutaraldehyde (-145.48 $kJmol^{-1}$), Blocked Diisocynate (-105.27 $kJmol^{-1}$), Activated Quinone (-104.72 $kJmol^{-1}$) in terms of negative energy **(Table 1)**. While Ethyleneglycol diglycildyl ether, Phloretic acid and Genepindo not possessed a favourable interaction.

Table 1 Molecular docking score of the Chitosan cross linked compounds

Chitosan Cross Linked Ligands	MolDock Score	Interaction	HBond	LE1	LE3
Diethyl Squarate	-181.13	-313.47	-7.75	-1.46	0.17
Formaldehyde	-179.52	-259.00	-14.09	-1.51	2.25
Glutaraldehyde	-145.48	-245.14	-12.19	-1.22	2.12
Blocked Diisocyanate	-105.27	-210.05	-10.23	-0.81	1.82
Activated Quinone	-104.73	-207.40	-8.29	-0.84	3.18
Ethyleneglycol diglycildyl ether	-35.45	-105.79	-8.16	-0.27	2.94
Phloretic acid	24.85	-124.02	-10.23	0.19	2.25
Genepin	90.39	20.50	-3.59	0.61	9.12

Table 2a Molecular interaction analysis of Chitosan cross linked Diethyl squarate

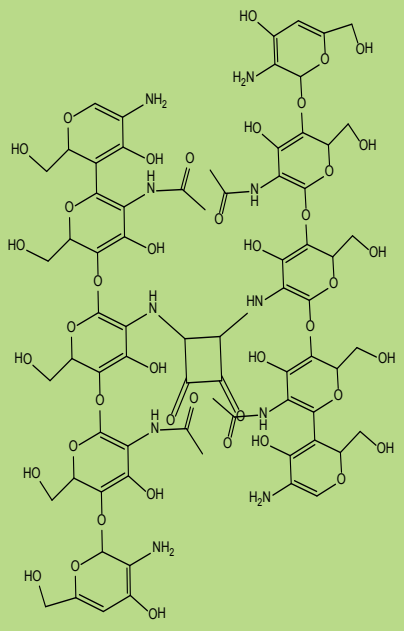
Chitosan Cross Linked Ligands	Ligand--- Protein Interaction	Interaction energy	Interaction Distance
Diethyl squarate 	N(54)---Glu494(OE1)	-2.5	2.87 Å
	O(97)---Arg388(NH2)	-0.28	2.73 Å
	O(65)---Gln263(NE2)	-2.5	2.90 Å
	O(101)---Asn283(OD1)	-2.44	2.90 Å
	O(106)---Ala282(O)	-1.82	3.23 Å
	O(28)---Val386(N)	-1.88	2.85 Å
	O(26)---Thr121(OG1)	-0.59	3.48 Å
	O(36)---Asp385(OD2)	-2.02	3.20 Å
	O(95)---Tyr373(OH)	-0.67	3.47 Å
	O(101)---Agr266(NH1)	-2.5	2.93 Å
	O(77) ---Agr266(NH1)	-0.38	3.52 Å
	N(112)--- Ala282(O)	-0.65	2.82 Å
	O(70)--- Gln263(NE2)	-0.19	3.56 Å
	O(68)--- Gln263(NE2)	-0.01	3.60 Å
	O(97)---Asp383(OD1)	-1.78	2.51 Å
	O(93)---Trp372(O)	-0.96	2.42 Å
	O(93)---Glu377(OE2)	-0.92	2.41 Å
	O(103)---Glu377(OE1)	-1.46	3.31 Å
	O(93)---Met374(N)	-0.60	3.40 Å
	O(102)---N(16) HEM Cofactor	-1.26	3.35 Å
	O(102)---N(24) HEM Cofactor	-2.19	3.16 Å
	N(107)---N(24) HEM Cofactor	-1.60	3.28 Å
	N(107)---N(32) HEM Cofactor	-2.5	3.01 Å
	N(108)---O(14) HEM Cofactor	-1.16	3.37 Å
	O(103) ---O(14) HEM Cofactor	-0.93	3.41 Å

Table 2b Molecular interaction analysis of Chitosan cross linked Formaldehyde

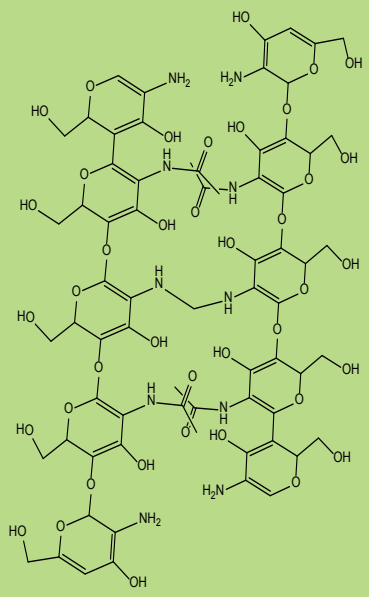
Formaldehyde 	O(93)---Lys497(NZ)	-2.5	3.06 Å
	O(93)---Glu494(OE1)	-2.5	2.98 Å
	O(51)---Gln492(N)	-1.35	3.33 Å
	O(34)---Glu285(OE1)	-2.02	3.20 Å
	O(95)---Thr121(OG1)	-2.5	2.92 Å
	O(87)--- Thr121(OG1)	-0.56	3.49 Å
	N(54)---Pro350(O)	-2.5	2.96 Å
	O(47)--- Phe369(O)	-0.82	3.44 Å
	O(40)---Ala262(O)	-2.5	2.73 Å
	O(38)--- Ala262(O)	-1.24	3.35 Å
	O(38)---Asn354(ND2)	-2.5	2.73 Å
	O(97)---Asp385(OD2)	-1.74	3.25 Å
	O(70)---Arg381(NH2)	-0.96	3.39 Å
	O(72)--- Arg381(NH2)	-0.57	3.45 Å
	O(72)--- Arg381(NH1)	-1.24	3.35 Å
	O(105)--- Arg381(NH1)	-0.67	2.56 Å
	O(42)---Glu377(OE1)	-2.5	3.08 Å
	O(101)---Ile119(O)	-2.38	3.00 Å
	O(56)---Tyr373(OH)	-0.69	2.78 Å
	O(101)--- Arg199(NH2)	-0.17	3.36 Å
	O(45)---O(41) HEM Cofactor	-0.48	3.50 Å
	N(52)---O(14) HEM Cofactor	-1.08	3.38 Å
	N(111)---O(14) HEM Cofactor	-1.45	3.31 Å

Table 2c Molecular interaction analysis of Chitosan cross linked Glutaraldehyde

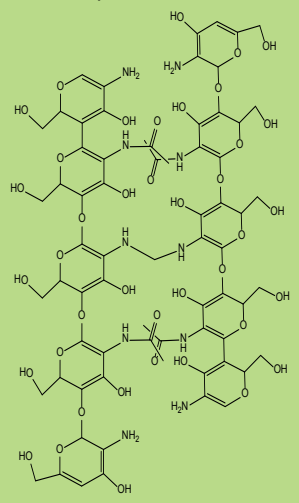
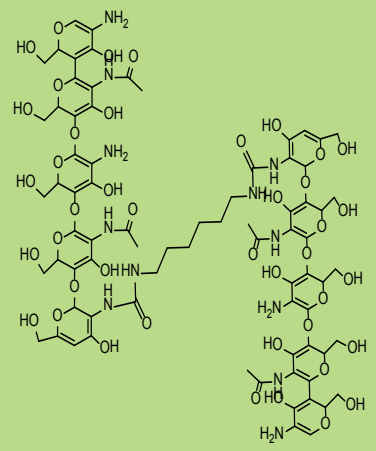
Glutaraldehyde			
	O(46)---Arg381(NH1)	-2.5	2.92 Å
	O(46)--- Arg381(NH2)	-1.61	2.61 Å
	O(110)---Tyr347(OH)	-2.5	2.68 Å
	O(65)---Gln263(NE2)	-2.5	2.74 Å
	O(101)---Glu494(OE1)	-0.63	3.47 Å
	H(180)---Gln492(O)	-2.5	1.76 Å
	N(113)---Asn354(O)	-2.5	2.75 Å
	O(106)---Asn354(OD1)	-2.5	2.94 Å
	O(99)---Tyr491(OH)	-1.95	3.21 Å
	O(56)---Trp463(NE1)	-0.38	2.94 Å
	O(42)---Met120(N)	-0.52	3.05 Å
	O(102)---Trp372(O)	-2.5	2.92 Å
	N(107)---Glu377(OE1)	-0.07	2.31 Å
	O(95)---O(41) HEM Cofactor	-2.5	2.82 Å
	O(102)---N(16) HEM Cofactor	-2.5	3.09 Å
	O(99)---O(42) HEM Cofactor	-0.20	3.56 Å
	H(177)---O(41) HEM Cofactor	-2.5	1.82 Å

Table 2d Molecular interaction analysis of Chitosan cross linked Blocked diisocyanate

Blocked diisocyanate			
	N(113)---Thr121(O)	-2.5	2.99 Å
	O(106)---Thr121(N)	-1.99	3.20 Å
	O(36)---Glu494I(OE2)	-2.32	2.58 Å
	H(152)---Ala282(O)	-2.5	1.95 Å
	O(45)---Thr121(OG1)	-0.74	3.45 Å
	N(52)--- Thr121(OG1)	-0.02	2.30 Å
	O(127)---Arg266(NH1)	-2.01	2.83 Å
	N(48)---Ala282(O)	-0.95	3.12 Å
	O(34)---Trp496(NE1)	-0.68	2.64 Å
	O(44)---Asn354(ND2)	-2.5	3.09 Å
	O(129)---Arg381(NH2)	-1.97	2.66 Å
	O(87)--- Arg381(NH1)	-2.5	2.64 Å
	O(79)--- Arg381(NH1)	-2.28	3.14 Å
	O(79)--- Arg381(NH2)	-0.15	3.56 Å
	O(102)---O(15) HEM Cofactor	-2.5	2.61 Å
	O(102)---O(14) HEM Cofactor	-1.23	2.45 Å
	O(46)---O(41) HEM Cofactor	-1.28	3.35 Å
	N(53)---O(41) HEM Cofactor	-1.88	3.22 Å

The MolDock scoring function (MolDock Score) which determined the binding affinity in the present investigation is derived from the Piecewise Least Potential (PLP) scoring functions. The MolDock scoring function further improves the PLP scoring functions with new hydrogen bonding term and new charge schemes. The docking scoring function, E_{Score}, is defined by the following energy terms:

$$E_{Score} = E_{inter} + E_{intra}$$

Where,

E_{inter} is the ligand-protein interaction energy

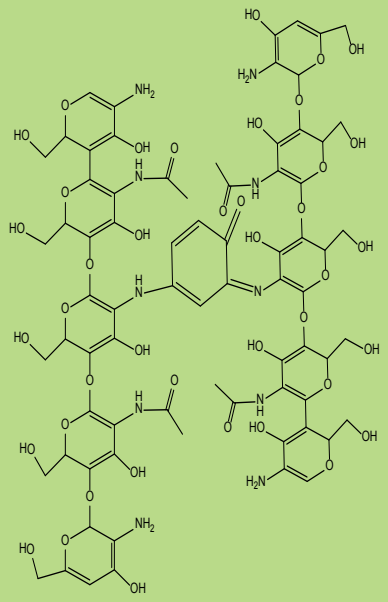
E_{intra} is the internal energy of the ligand

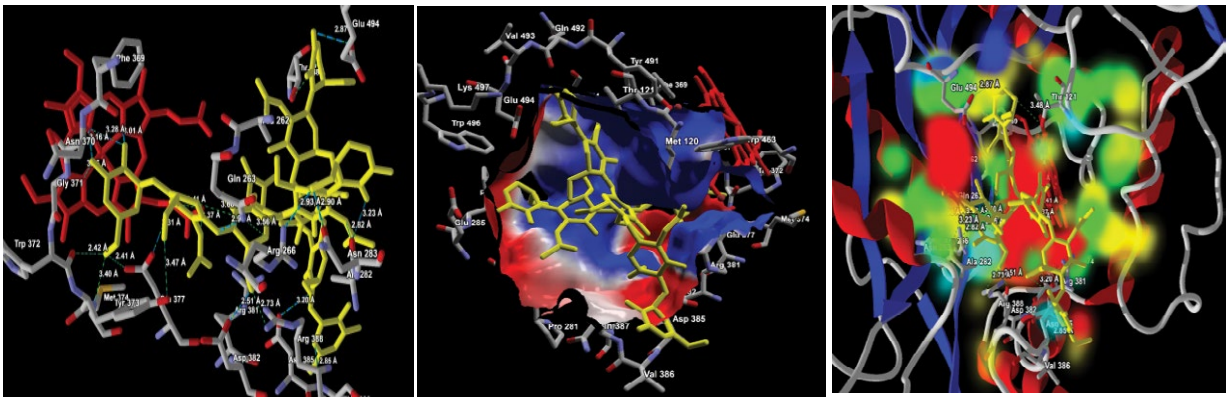
Further, the ligand-protein interaction calculation using ligand energy inspector for the Chitosan cross linked compounds is shown in **Table 2** which indicates the ligand-protein interaction energy and its molecular interaction distances along with the interacting atoms. It is observed that the compounds also exhibit molecular interaction with the Cofactor HEM molecule. Thus, indicating a strong binding affinity towards the active site of NOS. The snap shots illustrating the protein-ligand interaction of Chitosan cross linked with Diethyl Squarate, Formaldehyde, Glutaraldehyde, Blocked Diisocyanate and Activated Quinone is shown in **Figures 1-5** respectively. Hence, from the figures the plausibility of Chitosan cross linked compounds delivering ligands at a given specific target is viable.

Conclusion

To conclude, molecular docking simulation was carried out against NOS enzyme with a set of Chitosan cross linked compounds. The molecular docking results showed favourable binding affinity and docking score of the majority of the compounds at the active site of NOS enzyme. The molecular interaction analysis also revealed heavy molecular interaction with the active site residues. Thus,

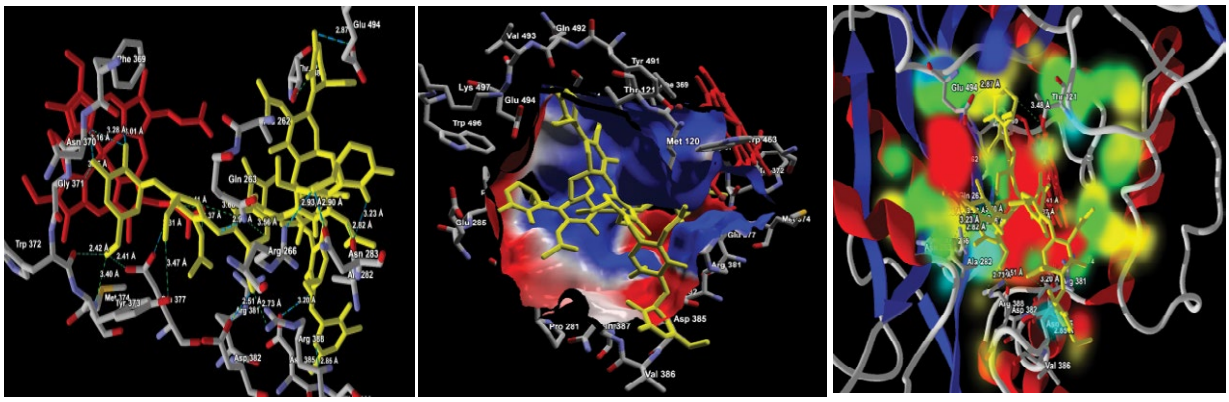
Table 2e Molecular interaction analysis of Chitosan cross linked Activated Quinone

Activated Quinone			
	N(107)---Ser118(O)	-2.45	3.11 Å
	O(93)---Arg381(NH1)	-2.5	2.91 Å
	O(65)--- Arg381(NH1)	-2.5	2.60 Å
	O(63)--- Arg381(NH1)	-0.08	3.58 Å
	O(65)--- Arg381(NH2)	-0.22	3.31 Å
	O(63)--- Arg381(NH2)	-0.03	3.59 Å
	O(43)---Arg388(NH2)	-0.65	3.20 Å
	O(43)---Asp382(OD1)	-1.84	3.23 Å
	O(43)---Gln263(NE2)	-0.12	3.56 Å
	O(45)--- Gln263(NE2)	-2.37	3.13 Å
	O(26)---Asn354(ND2)	-0.97	3.41 Å
	O(18)--- Asn354(ND2)	-1.57	3.29 Å
	O(42)--- Asn354(ND2)	-2.06	3.07 Å
	O(42)---Asn354(O)	-1.48	2.48 Å
	O(40)--- Asn354(OD1)	-2.37	2.58 Å
	O(47)---Thr121(OG1)	-2.5	2.90 Å
	N(54)---Glu494(OE1)	-2.5	2.88 Å
	O(28)---Tyr373(OH)	-1.14	3.37 Å
	O(20)---Tyr347(OH)	-2.5	2.97 Å
	N(48)--- Tyr347(OH)	-2.5	2.84 Å
	O(36)--- Tyr347(OH)	-2.5	2.94 Å
	O(43)---Arg388(NH1)	-2.5	2.96 Å
	O(79)---Trp463(NE1)	-0.15	2.95 Å
	O(44)---Tyr373(OH)	-0.35	3.32 Å
	O(44)---Glu377(OE1)	-1.89	2.53 Å
	N(49)---Glu377(OE1)	-1.27	3.16 Å
	O(34)---O(15) HEM Cofactor	-1.48	3.30 Å
	N(108)---O(15) HEM Cofactor	-1.86	2.52 Å
	N(108)---O(14) HEM Cofactor	-0.97	3.41 Å
	O(104)---O(14) HEM Cofactor	-1.77	3.25 Å
	O(34)---O(14) HEM Cofactor	-0.84	3.43 Å
	O(46)---O(41) HEM Cofactor	-0.07	3.59 Å



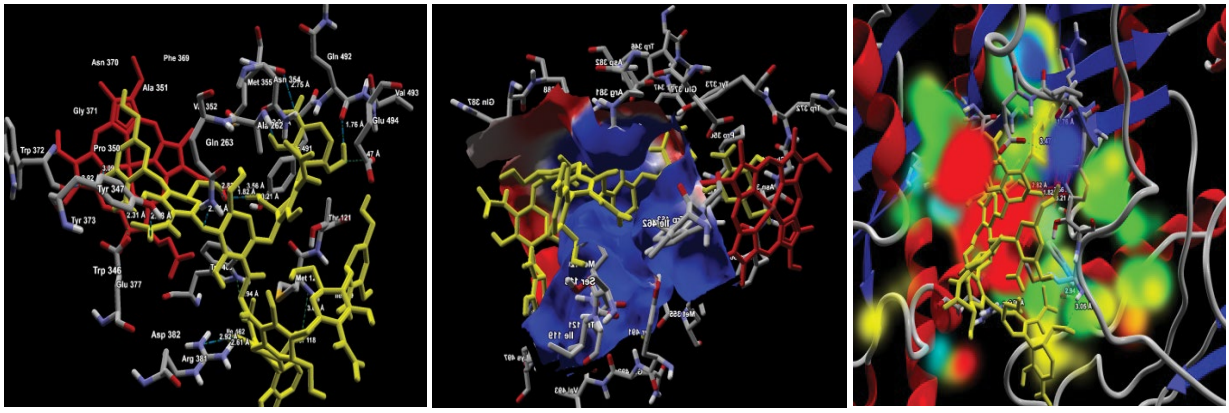
A B C

Figure 1 (A) Binding mode (B) Electrostatic interaction and (C) Energy map of Chitosan cross linked Diethyl Squarate at the active site of NOS enzyme



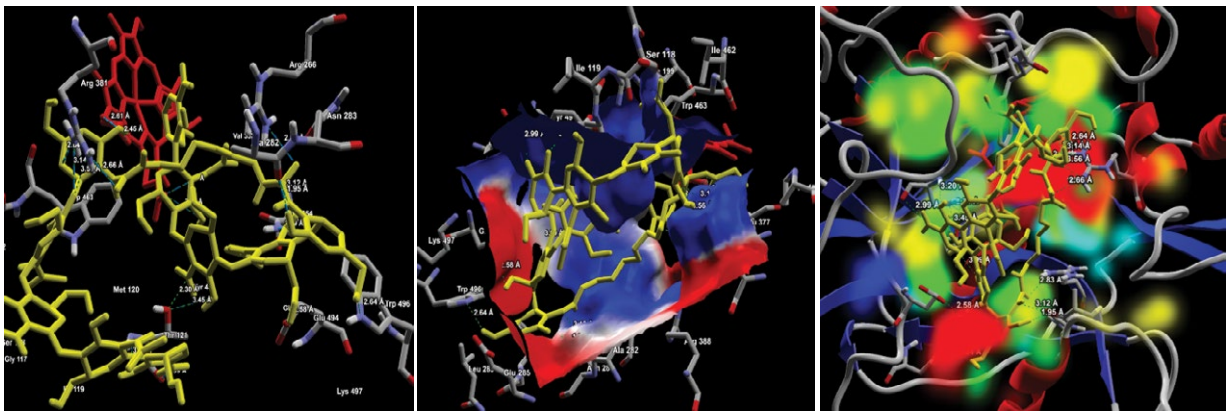
A B C

Figure 2 (A) Binding mode (B) Electrostatic interaction and (C) Energy map of Chitosan cross linked Formaldehyde at the active site of NOS enzyme



A B C

Figure 3 (A) Binding mode (B) Electrostatic interaction and (C) Energy map of Chitosan cross linked Glutaraldehyde at the active site of NOS enzyme



A B C

Figure 4 (A) Binding mode (B) Electrostatic interaction and (C) Energy map of Chitosan cross linked Blocked Diisocyanate at the active site of NOS enzyme

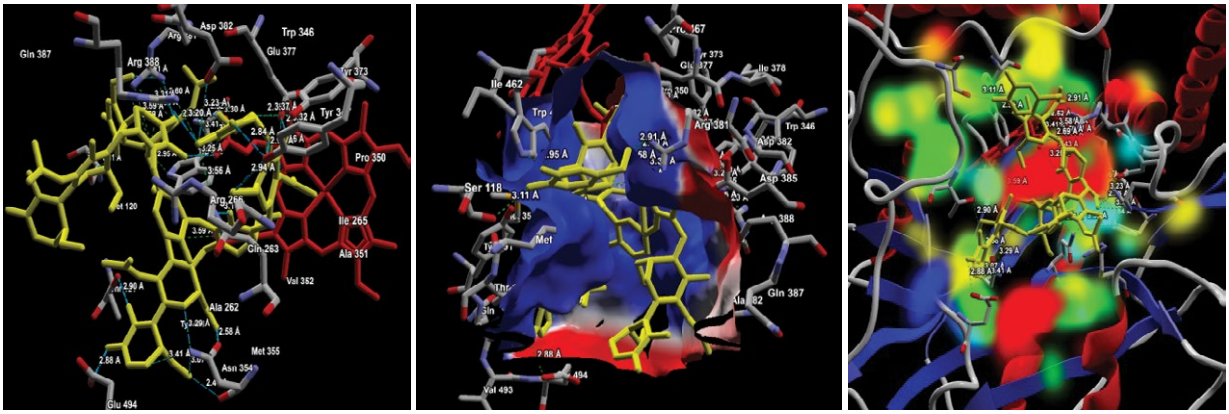


Figure 5 (A) Binding mode (B) Electrostatic interaction and (C) Energy map of Chitosan cross linked Activated Quinone at the active site of NOS enzyme

we bring to a cause that Chitosan cross linked compounds is a good inhibitor of NOS enzyme as anticancer agents. Moreover, Chitosan is successful in delivering the compounds at the specific active site of the target enzyme.

Conflict of interest

The authors declare no conflict of interest exist

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