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Original Article

In-silico Screening of Gold-Based Compounds as Potential Non Competitive Inhibitors for Human Mitochondrial Thioredoxin Reductase

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ABSTRACT

Background: Thioredoxin reductase is an important enzyme in antioxidant defense and regulation of cell function, its inhibition has cytotoxic effects.
Aim: To predict if; there is correlation between the already *in vitro* cytotoxic studied Au (III) complexes & their docking studies
Material & Method: The enzyme in complex with a potential inhibitor will be downloaded from the Protein Data Bank (PDB). The inhibitory binding site of the enzyme will be defined as the 8 A°-sphere of residues surrounding the inhibitor.
Four gold-based compounds along with cisplatin and XAN were then docked into the defined site and their poses inside the active site were analyzed.
Results: These compounds have already shown *in vitro* activity against the Hep-2 cell line and this cytotoxicity compared with the results generated by the computer
Conclusion: Computer aided drug design is excellent & modern tool to correlate between the structures & inhibition properties of Au (III) complexes.

Keywords: Thioredoxin; Cytotoxic effects; XAN; Cisplatin.

INTRODUCTION

Cisplatin has an esteemed place in bioinorganic medicinal chemistry, since it is dominates as a “HERO “and a good representative for the most successful clinical applications of metallodrug in the war of combating various types of cancer; all over the world.

The continuous success of cisplatin in the clinic is striking and undeniable. It belongs to the most widely used anticancer

drugs, employed in the treatment of around 70% of all cancer patients[1].

Beside the effectiveness of cisplatin against cancer, it has encountered many drawbacks.

A high general toxicity (nephro-, neuro- and ototoxicity) leading to undesirable side-effects[2,3] has been reported. Also resistance of tumor cells to cisplatin treatment (either developed or intrinsic) has been reported in addition to its limited applicability to a relatively small range of cancer types[4].

Hence, several metal compounds (ruthenium, palladium, titanium, gold, tin etc.) were taken under consideration with distinctively different DNA binding modes from cisplatin, as promising alternative metal cores for cisplatin and in attempt to provide higher antitumor activity against cisplatin resistant cells.

Gold's application to treat a variety of ailments[5-7], and similar to Pt, auranofin and other Au complexes also possess anticancer activities[8-10].

The molecular mechanism of action of Au compounds in this regard is not clear, although studies previously identified auranofin and aurothioglucose, can inactivate the NADPH- reduced form of thioredoxin reductase (TrxR) in nanomolar concentration via binding to the Selenocystein (Sec) residue as a potent inhibitor of human TrxR[11].

The thioredoxin (Trx) system comprising Trx, thioredoxin reductase (TrxR), and NADPH participates in a broad range of cellular functions involved in cell survival and proliferation[12,13]. This thiol-disulfide exchange reaction is reversible and efficient for electron transport.

Trx-(SH) 2 + Protein-S2 ⟶ Trx-S2 + Protein - (SH) 2

Trx-S2 + NADPH + H^+ + TrxR ⟶ Trx-(SH) 2 + $NADP^+$

Accumulating evidence has indicated that the selenocysteine - containing mammalian TrxR is a valid molecular target for development of novel cancer therapeutics. On the other hand, in cancer, the properties and biological effects of the Trx system contribute to tumor growth and progression[14]. This indicates that the Trx system serves as a valid therapeutic target.

The present paper takes advantage of the availability of *in silico* docking capable of dealing with proteins as large as TrxR to study its affinity towards two good electrophile like both Au(III) metal core in assistance to thiol-containing ligand (thioglycolate [$–SCH_2COO^-$] & 2-mercaptoacetic acid [$SHCH_2COO^-$]), but with different distribution around central Au(III) metal. Due to the previous literature survey, it is suggested that the synthesized complexes C_1 - C_4 (figure 1) may be acting by virtue of their chelating properties at the cellular level thereby exerting their anticancer activity.

We , in this article addressed this hypothesis & the Hep-2 preliminary *in vitro* cytotoxicity by testing a four of Au (III) complexes to analyze possible *in silico* to find if any correlation of previously screened novel Au (III) with S/O – ligand donating complexes and the docking study using TrxR enzyme as a putative target for Au(III) compounds , also to facilitate the elucidation of the active site optimization and finally to build up a dependable structural activity relationship .Cisplatin and XAN (6-hydroxy-3-oxo-3H-xanthene-9-propionic acid) were also docked for compares.

It has been found that a cavity at the interface of two thioredoxin reductase subunits can serve as a binding site for non-competitive inhibitors. This was first identified for glutathione reductse inhibited by XAN (6-hydroxy-3-oxo-3H-xanthene-9-

propionic acid)[22] and tricyclic compounds[21]. The inhibitor binding site was found to be away from the substrate binding site but when the inhibitor was bound, the binding was found to induce structural changes in the enzyme that prevent the binding of the macromolecule substrate[21,22.] Fritz-Wolf revealed in their published crystal structure of thioredoxin reductase that it has an intersubunit cavity that superimposed on the inhibitor binding site of glutathione reductase and concluded that this site can serve as inhibitor binding site for thioredoxin reductase. In the present study XAN was used as a reference compound[15].

EXPERIMENTAL

Methods & Materials

The four gold based compounds tested in this work were kindly taken by the researchers. Human mitochondrial thioredoxin reductase was downloaded from the Protein Data Bank (www.pdb.org) as the PDB entry 2J3N[15.] The programmes used included Scigress Explorer Ultra Version 7.7.0.47[16] which was used for the drawing and the minimisation of the compounds, for the molecular dynamics (MD) simulation applied to the enzyme and for docking study, PROCHECK Version 3.0[17] which was used to generate Ramachandran plots[18] for all the conformations from the MD simulation to check the validity of these conformations in terms of the phi and psi angles combinations of the residues, and Accelrys Discovery Studio Visulizer Version 2.5.1.9167[19] which was used for visualisation of the docked complexes and analysis of the interactions between the compounds and the active site. It was also used to generate all the 3-dimensional pictures presented in this work.

Preparation of the compounds

The gold compounds, cisplatin and XAN were drawn in Scigress Explorer Workspace, their valences beautified and an MM2 energy minimisation[20] was then applied to obtain a low energy conformation that is closest to the real compound as possible.

Preparation of the enzyme

The crystal structure of thioredoxin reductase used here (2J3N) contained six chains of the enzyme (A-F) in the form of three dimers. A molecule of flavin adenine dinucleotide (FAD) and a molecule of nicotinamide adenine dinucleotide phosphate (NADP+) are contained in each chain with 41 water molecules in the whole protein. There were also five molecules of 2-methyl-2, 4-pentandiol (MPD) distributed in between the chains[15.] The first dimmer (chains A and B) only was selected. The other chains were deleted. Hydrogen atoms were added to all the residues and were then allowed to relax using an MM2 energy minimisation technique[20]. The selected dimmer contained a single molecule of MPD at the interface between the two chains. The residues of the two chains lying within 8 Aº of MPD were selected to define the site to be used for docking. The remaining residues were locked and a molecular dynamic simulation was applied to the site after deleting MPD. The following specifications were used for the MD simulation:

Procedure: MD at constant energy (MM3) in water

Equilibration time: 0.5 ps (picosecond)

Simulation temperature: 300 K

Simulation duration: 100 ps

Simulation time step: 0.001 ps Output frequency: 200

Six conformations were taken from the MD trajectory to generate the models at 50, 60, 70, 80, 90 and 100 ps. A Ramachandran plot was generated for each conformation to check the phi and psi angles.

Docking study

Each of the four compounds, cisplatin and XAN was docked into the active site of the six conformations obtained from the MD trajectory using Scigress Project Leader. The following specifications were used during docking:

Type of docking: Flexible active site side chains an
Scoring Function: PMF
Calculation Type: Dock (Use Grids)
Use Amber van der Waals: Grid Spacing (Aº) 0.30000
Pop Size: 50
Crossover Rate: 0.80000
Elitism: 5
Maximum Gen: 3000
Mutation Rate: 0.20000
Convergence: 1.0000

Each compound was docked twice into each conformation to maximise the possibility of finding the correct binding mode. The docked complexes were then checked for the interactions between the compound and the inhibitory binding site using Accelrys Discovery Studio Visulizer.

RESULTS AND DISCUSSION

Validity of the models

All the conformations taken from the MD simulation were checked by generating Ramchandran plots. All the models produced results within acceptable range for the residues in the allowed and disallowed regions as can be seen in Table 1 and Figure 2.

Docking results

The docking conformation of each compound in each model was checked using Accelrys Discovery Studio Visulizer[19] for its pose in the active site as well as the interactions with active site residues. C1 was found to enter the active site in 50 ps and 70 ps models. C2 entered the active site of 60 ps, 70 ps and 100 ps models C3 docked well in the active site of models 60 ps, 70 ps and 80 ps. Finally, C4 was found to dock well in the active site of models 60 ps, 70 ps, 90 ps and 100 ps. It can be clearly seen that all the compounds docked well in the active site of the model 70 ps. Therefore this model was taken as the best model for the enzyme. Table 2 represents the docking score of each of the four compounds in 70 ps along with the residues of the active site that formed hydrogen bonds with the compound.

Figure 3 shows the poses of the four compounds in 70 ps and the residues forming hydrogen bonds with the compounds. It stated that a cavity located at the interface of two enzyme chains of thioredoxin reductase can serve as a binding site for non-competitive inhibitors[15,21]. Furthermore, it has also been found that GLN72 forms hydrogen bond with the inhibitor as revealed by crystallographic structure[15.]

When cisplatin was tried against thioredoxin reductase as a reference for the gold based compounds, it was found to enter the active site in all the models but the scores were rather bad; ranging from (-197)-(-107) kcal/mol with the highest score (-107) being in 70 ps model without forming hydrogen bonds (Figure 4).

The potency parameter (IC_{50})of the tested complexes (C_1-C_4), although not significant, it represent high potency compared to the reference compound (cisplatin) (Table3), the lack of cross-resistance suggests that gold (III) induce cytotoxicity through different mechanism[22], This agreement was generally found between the inhibitory potency of Au complexes and the computed affinity & stability compound/ TrxR adduct inhibitory active site.

Inhibitor binding site

XAN docked well in 70ps without forming any hydrogen bonds (Figure 4). Its score was -205.825 kcal/mol. When this core

is compared to the scores of the gold compounds, it can be concluded that our compounds may have higher affinity to bind to the inhibitor binding site of thioredoxin reductase than XAN.

Since the mode of inhibition suggests that XAN binds reversibly both to the enzyme and to the enzyme substrate complexes at a site distinct from that of the natural substrate(s)[23,] while the lower score associated with the tested compounds revealed that Au (III) -tested may have higher affinity (irreversible**)** to bind to the inhibitor binding site of TrxR than XAN.

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Table 1. Residue ratios in different regions of Ramachandran plots for conformations obtained from MD simuation

Conformation	Residues in most favoured regions	Residuse in additional allowed regions	Residues in generously allowed regions	Residuses in disallowed regions
50 ps	80.3%	19.2%	0.5%	0%
60 ps	80.3%	19.2%	0.5%	0%
70 ps	80.3%	19.2%	0.5%	0%
80 ps	80.3%	19.2%	0.5%	0%
90 ps	80.3%	19.2%	0.5%	0%
100 ps	80.1%	19.4%	0.5%	0%

Table 2. Docking scores and interacting residues of the four compounds in 70 ps

Compound	Score (Kcal/mol)	Residues forming H-bonds	Chain
C1	-229.385	GLN72, ARG416	B, B
C2	-240.336	GLN72, ARG416	B, B
C3	-249.115	ARG416	B
C4	-305.557	GLN72	B

Table 3. IC_{50} of $C_1 - C_4$ compared to cisplatin

C_1	C_2	C_3	C_4	D (cisplatin)
0.39	0.45	0. 45	0.325	0.36

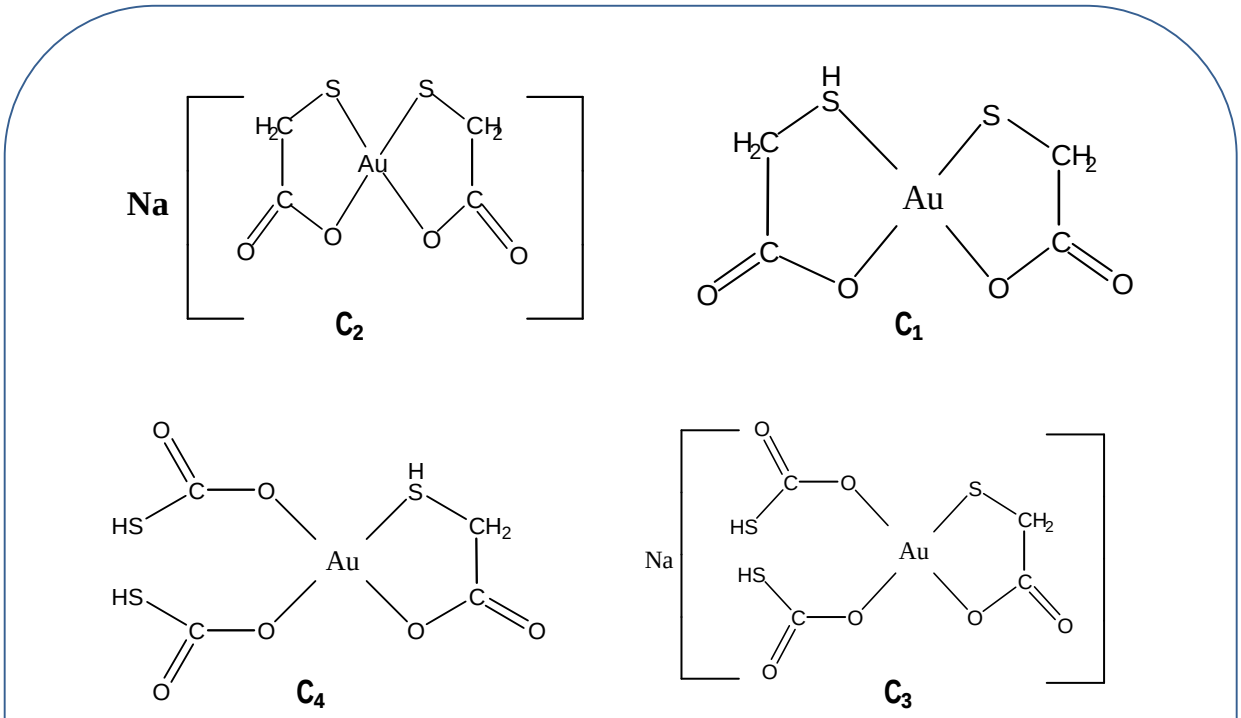


Figure 1. The Chemical structure of the previously synthesized & Hep-2 *in vitro* cytotoxic screened complexes

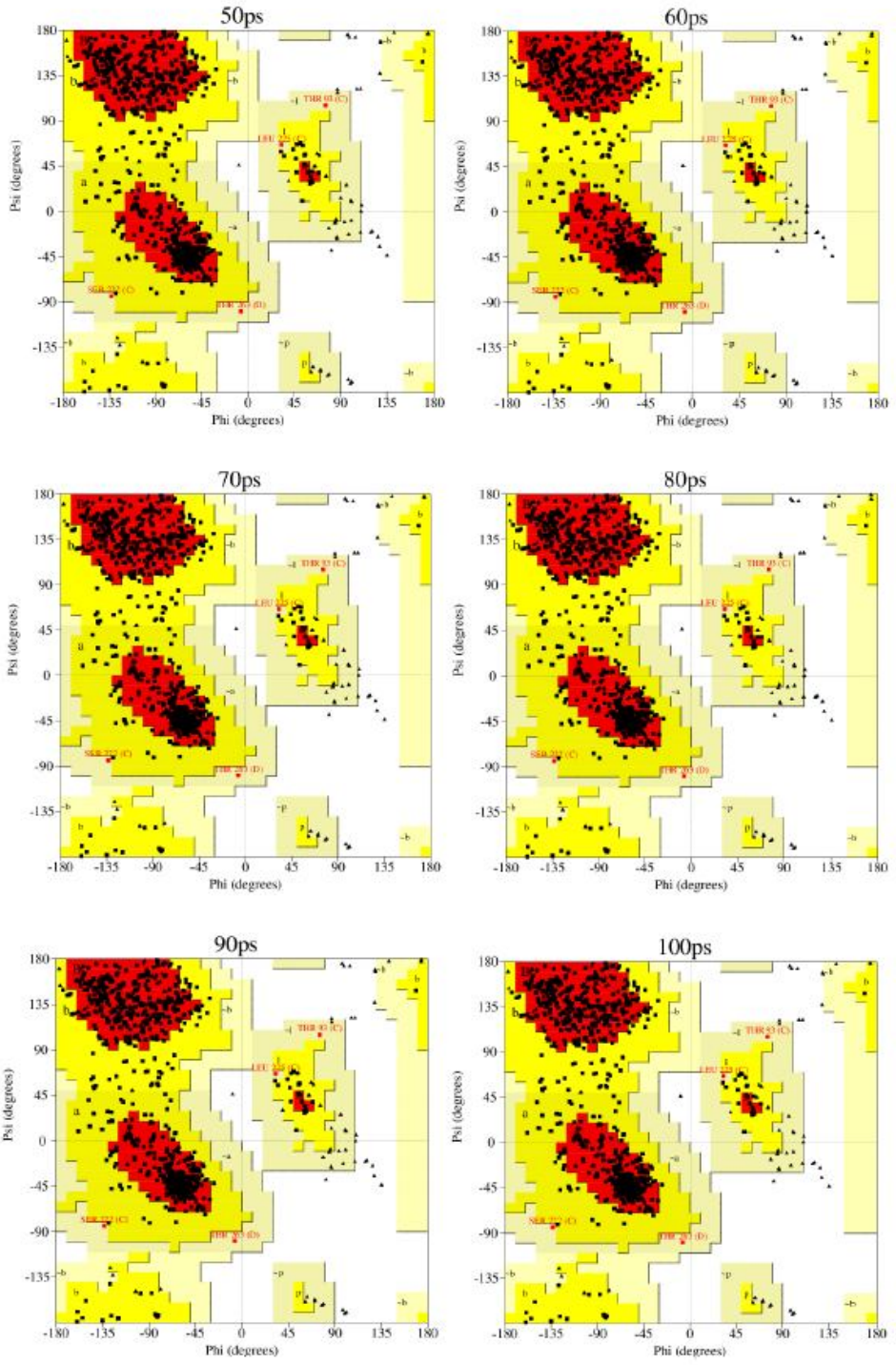
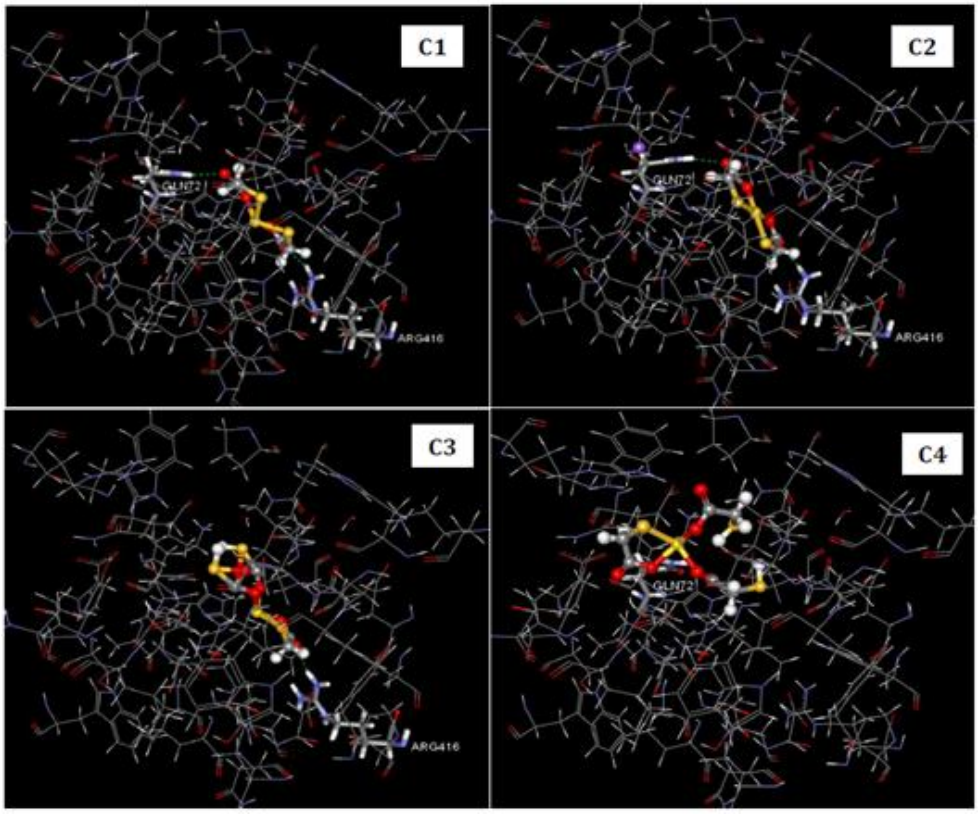
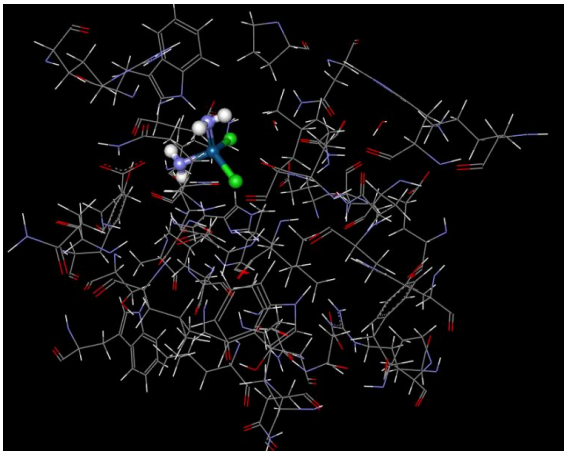


Figure 2. Ramachandran plots for the conformations detailed in Table 1



(A)



(B)

Figure 3. Poses of the four compounds in 70 ps. The compounds (A) and cisplatin (B) are shown as ball and stick and colored by element while interacting residues are shown as stick and colored by element

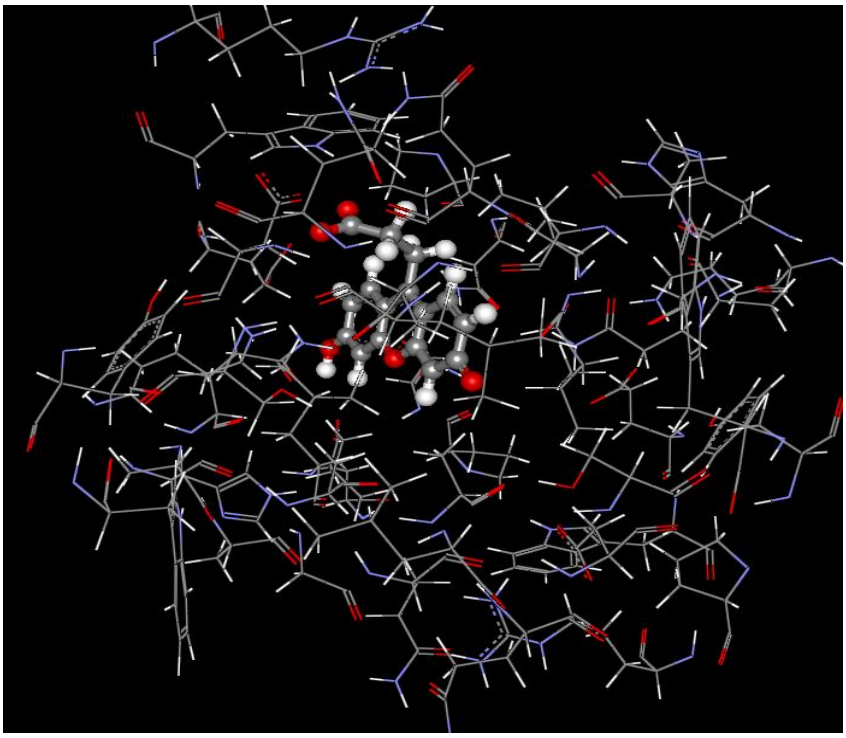


Figure 4. XAN docked well in 70ps without forming any hydrogen bonds