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Identification of Effectual Inhibitors against Human Insulin like Growth Factor Binding Protein-2

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Abstract

Human Insulin-like Growth Factor Binding Protein-2 (IGFBP 2) is reported to be a modulator of the action of Insulin-like Growth Factors (IGFs), over expression of IGFBP 2 has been reported in cancers. Hence with an aim to design an inhibitor for selected drug target high-throughput virtual screening was implemented. The tertiary structure was retrieved from the protein databank. A 2D similarity search was performed using Ligand. Info for known inhibitor to acquire 778 structural analogs. The 3D structural conversion and multiple confirmations were generated using LigPrep. The docking and scoring calculations were performed using Glide v5.7. Five leads having better docking score compared to heparin were selected as potential IGFBP 2 inhibitors. Among them lead 1 with the highest docking score of -6.009 kcal/mol was proposed as a promising antagonist for IGFBP 2. Molecular dynamics simulations were performed using Desmond v3.6 to check the stability of docking complex.

Keywords: IGFBP 2; Cancer; Heparin; Ligan.info; LigPrep; Glidev5.7; Docking score; Lead 1; MD simulations

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Introduction

Cancers are the broad group of diseases involving unregulated cell growth was cells divide and grow uncontrollably to form malignant tumors. They invade nearby parts and also spread to more distant parts of the body through the lymphatic system or bloodstream [1,2]. By a statistical analysis 5-10% of cancers can be traced directly to inherited genetic defects. The chances of surviving the disease vary greatly by the type and location of the cancer and the extent of disease [3]. Cancer can affect people of all ages and a few types of cancers are more common in children, the risk of developing cancer generally increases with age. In 2007, cancer caused about 13% means 7.9 million of all human deaths worldwide. The percentage of deaths are rising as more people live to an old age and lifestyle changes occur in the developing world [4,5]. Hence, novel drugs for this life-threatening disease are more essential.

The Human Insulin like Growth Factor Binding Protein family includes six high affinity proteins (IGFBP-1 to 6) and several low affinity proteins (IGFBP related proteins) [6]. The IGFs are well known as key regulators of energy metabolism and growth of both normal and malignant cells [7,8]. The IGFs and IGFBPs that are produced locally regulate the tissue growth and differentiation. The IGFBPs control the action of IGFs in several ways; it includes an inhibitory model in which IGFBPs sequester IGFs from their receptors. Another way is an enhancing model in which IGFBPs transport IGFs to their site of action or by a receptor-independent model that may involve direct interaction of IGFBPs with IGFBP receptors. The degree of growth inhibition caused by the IGFBPs appears to be directly related to their concentrations relative to IGFs.

The variation of IGF levels by IGFBPs is further regulated by IGFBP proteases which cleave the high affinity IGFBPs into fragments with lower affinity for IGFs, thereby increasing free IGF bioavailability. The reduced inhibition of cell growth by IGFBPs finally occurs. IGFBP 2, located on chromosome 2 [9], having 325 amino acids with molecular weight of 34,814 Daltons. Human IGFBP 2 is present in many peripheral tissues and biological fluids. IGFBP 2 extends the half-life of circulating levels of IGFs [10]. Human IGFBP 2 binds to IGF and also regulates the action of insulin growth factors to control the growth, development, reproduction and also aging process [11,12]. IGFBP 2 was reported

as a modulator of the action of IGFs. The both N-terminal domains and C-terminal domains are required for the high-affinity binding of IGFs, because N-terminal domain and C-terminal domain fragments bind to IGFs with low affinities than full length IGFBPs. Deletion of C terminal domain residues from 222–284 of IGFBP 2 intensely reduces the binding affinity of IGFs [13]. The heparin binding site present on C-terminal domain of IGFBP 2 may have more implication for the development of cancers.

C-terminal domains of IGFBP contribute high-affinity binding likewise human IGFBP 2 shows high binding towards antagonists in the C-terminal domain. Elevated IGFBP 2 expression is found in many malignancies and can often serve as a prognostic factor. The role of IGFBP 2 in tumors has attracted as molecular target and increasing attention to design drugs. Hence, human IGFBP 2 was considered as one of the prime target in treating cancers. In the recent past, several studies reported heparin as inhibitor against human IGFBP 2. However the reported molecule showed adverse effects due to its poor pharmacological properties and it increases the bone mass leading to osteoporosis [14]. The existing inhibitor provide an ideal platform for applying virtual screening strategy to design novel and potent leads against human IGFBP 2 involved in cancers using *in silico* approach.

The 3D structure of human IGFBP 2 was retrieved from the PDB (2H7T). In the present study ligand binding site was retrieved from literature databases, PubMed. Structural analogs were chosen from an in-house library of databases such as ChemBank, ChemPDB, KEGG, NCI, e-molecules, annotated NCI, AKos GmbH, Asinex Ltd., and TimTec [15] and their docking interactions at the ligand binding site of human IGFBP 2 were studied. Molecular docking study was performed using Schrodinger software suite to accomplish the challenge of identifying novel inhibitors against human IGFBP 2 [13]. Three tier GLIDE docking protocol (HTVS, SP and XP) [16,17] was implemented to identify better leads than the known inhibitor. Molecular dynamics simulations were also implemented in the study to know the stability of the protein-ligand docked complex using Desmond v3.6. In these studies, over expression of IGFBP 2 C-terminal domain was concentrated, molecular docking and molecular dynamics simulations study were applied to propose potential inhibitors and to check the stability of IGFBP 2-lead 1 complex at an atomic level. Considering the molecular docking and molecular dynamics simulations study, the proposed leads were suggested as a novel antagonist against human IGFBP 2, which could inhibit human IGFBP 2 which decreases the cell proliferation in cancers.

Materials and Methods

Hardware and software

The work was carried out in a HP Z800 workstation with six-core Intel® Xeon® processors, 12 GB RAM, one TB Hard drive, NVIDIA Quadro FX 5800 3 GB graphics card running in Linux operating system and connected to dedicated 4 Mbps Broadband internet connection. For the current study biological databases like PubChem, drug bank, protein data bank and software's like Chemdraw, Schrodinger software suite were employed [20].

Protein preparation

The structure of human C-terminal domain of IGFBP 2 (2H7T) was retrieved from the protein databank. Hydrogen atoms and charges were added to the protein structure was submitted to a series of restrained, partial minimizations using the optimized potential for liquid simulations-all atom (OPLS-2005) force field [21]. The 3D structure was then processed by using the 'protein preparation module' with the 'preparation and refinement' option. The heparin binding site residues for human IGFBP 2 were selected from literature database, PubMed [13].

Ligand preparation

The Ligand.Info meta database is a compilation of various publicly available databases of small molecules such as ChemBank, ChemPDB, KEGG, NCI, e-molecules, annotated NCI, AKOs, GmBH, AsineX Ltd and Time Tec, with a total size of about one million compounds. The known inhibitor of human IGFBP 2, heparin was used as a base for structural analogue search using Ligand.Info meta database [15,16]. Ligand dataset was prepared by choosing top 50 structural analogs form each sub database.

Docking studies using Schrodinger

The common problems such as missing hydrogen atoms, incomplete side chains and loops, ambiguous protonation states and flipped residues can be corrected by using Schrödinger's. OPLS2005 force field was used for the assignment of charges to all atoms of the 3D structure. Energy minimization and refinement was done by running the protein preparation wizard module of Schrödinger's maestro [22]. Minimization of the protein was performed with the average root mean square deviation of non-hydrogen atoms reached 0.30 Å [23].

Glide v5.7 (Grid-based ligand docking with energetics) from Schrodinger 2011 [24] was implemented to select the best leads. A 10 x 10 x 10 Å grid [25] was generated around the centroid of the heparin binding site of human IGFBP 2.The 2D molecular structures were converted to 3D molecular structures using LigPrep. Lipinski's filters and reactive filters were applied to refine the generated tautomers. Glide v5.7 protocol was implemented which includes three modes of docking such as HTVS (High Throughput Virtual Screening), SP (Standard Precision) and XP (Extra Precision) docking methods. The docking methods screen all the compounds with a high accuracy by eliminating false positives [26].

Glide XP docking method determines all reasonable confirmations for each low-energy conformer in the designated binding site. Glide scoring function (G-score) was used to select the best leads. Comparing the XP Gscore of known inhibitor with obtained leads, the best leads were proposed and further evaluated for drug like properties.

Drug-like properties

High oral availability is often an important consideration for the development of bioactive molecules as therapeutic agents [27]. The selected optimized molecules were studied for their drug-like properties based on Lipinski's parameters using QikProp for rapid ADMET (Absorption Distribution Metabolism Excretion Toxicity)

predictions of drug candidates. QikProp efficiently evaluates pharmaceutically relevant properties and is an indispensable lead generation and lead optimization tool. The best selected leads using docking studies were analyzed using QikProp 3.2 to know the probability of drug likeness [25,26].

Molecular dynamics simulations

Molecular dynamics simulations have been extensively applied to investigate the conformational changes of a molecule induced by protein-protein interactions and protein-ligand interactions, including peptide and small molecular inhibitor [28]. MD simulations for the lead 1-IGFBP 2 docking complex was performed to evaluate the stability and conformational changes using Desmond v3.6. Implemented in Maestro v9.2 with 10 ns simulations. The initial system was embedded with Simple Point Charge (SPC) water model and neutralized by replacing solvent molecules with counter ions using OPLS-AA 2005 force field. The full system of 43,465 atoms was simulated through the multistep MD protocols devised in Maestro v9.2.

The full system minimization with restraints was performed for maximum 2000 iterations of a hybrid of the steepest descent and the limited memory Broyden Fletcher Gold farb Shanno (LBFGS) algorithms. The system was minimized for 2000 iterations without restraints [29]. The minimized system was relaxed with two subsequent short span simulations with NVT ensemble (constant number of atoms N, volume V and temperature T) for a simulation time of 12 picoseconds (ps) containing all non-hydrogen solute atoms with a temperature constant of 10 K. Further NPT (constant number of atoms N, pressure P and temperature T) ensemble was simulated for 12 ps containing all non-hydrogen solute atoms with a temperature constant of 300 K. Further NPT (constant number of atoms N, pressure P and temperature T) ensemble was simulated for 24 ps restraining all non-hydrogen solute atoms with restrains and NPT ensemble without restraints were simulated for a time of 24 ps [30,31]. The relaxed system was finally simulated for 10 ns and examined for stability of lead 1-human IGFBP 2 docking complex. Energy fluctuations and inter molecular interactions of lead 1-IGFBP 2 docking complex in each trajectory were examined with respect to simulation time.

Results and Discussion

Active site determination

The structure of human C terminal domain of IGFBP 2 was retrieved from the PDB (2H7T). The heparin binding site residues namely Lys-227, His-228, Asn-232, Leu-233, Lys-234 and His-271 were selected from literature [13]. These residues are located mainly at the β-turn connecting the first and second strands, part of the third strand and the beginning of the C-terminal tail.

Ligand preparation

The SMILE (Simplified Molecular Input line Entry) format of known inhibitor heparin (**Table 1**) was submitted to Ligand. Info meta database and a dataset of 778 analogs were obtained. **LigPrep** software was used to generate 10,351 multiple conformations.

Docking analysis through Schrödinger's

The LigPrep-treated and energy minimized ligands were docked into the prepared receptor grid. The ligands with suitable ADMET properties and zero reactive functional groups were subjected to Schrödinger Glide software by following a systematic approach (HTVS, SP and XP docking) of docking. The binding affinity was evaluated from the Glide Score parameter. 4279 confirmations were generated from 778 structural analogs in LigPrep. 4073 confirmations showed drug-like propertieswhich were predicted using QikProp. Lipinski's filter and reactive filter were applied subsequently to the prepared structural analogs to avoid false positive molecules and 1254 conformations passed through Lipinski's filters. 1040 compounds filtered through reactive filters were further selected for Glide protocol wherein high throughput virtual screening method generated 125 compounds. Standard precision (SP) docking method was used to minimize the false positives and 30 leads were generated. The extra precision (XP) docking, a physically accurate docking method was finally applied to generate 15 leads. Among the 15 leads five leads with better XP Gscores than heparin were selected. The SMILES format and XPG score of five leads were listed in **Table 1**.

Correlation between the leads and heparin

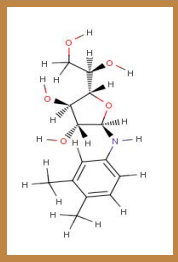
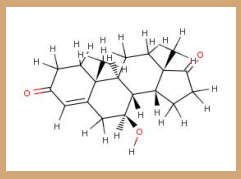
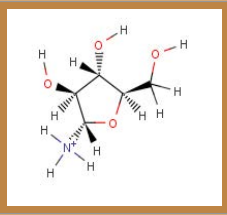
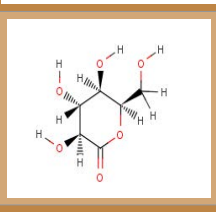
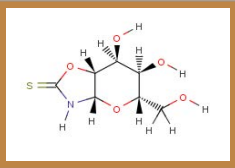
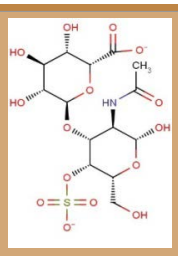
Correlating the XP Glide scores of leads and existing inhibitors, brings out a good approximation to carry off unnecessary compounds. Keeping on specific criteria like XP GScore, pharmacological and ADMET properties, Heparin was considered as the reference inhibitor for the present work for human IGFBP 2. Inspite of its ADMET violations, docking was done without filters (Lipinski's and reactive filters) to know its binding affinity towards human IGFBP 2. The XP GScore of heparin was -2.726 kcal/mol. By comparing with the XP GScore of heparin five leads with best ranked poses were proposed as potent inhibitors against human IGFBP 2. The 2D structures of five proposed leads along with heparin and XP Gscore were mentioned in **Table 1**.

Analysis of docking

The amino acid residues Asn-224, Asp-226, Lys-227, Leu-230, Asn-232, and Leu-233 were seen in the heparin-IGFBP 2 docking complex. Among them Lys-227 Asn-232 and Leu-233 were well matched with heparin binding site residues shown in the results of Kaung et al., 2006. [13] and the residue Lys-227 showed one hydrogen bond in **Figure 1**.

The docking analysis of C-terminal domain of human IGFBP 2 revealed that all five leads were having better XP Gscores than the known inhibitor, heparin in **Table 1**. Among all the five leads lead 1 exhibited better XP Gscore (-6.009 kcal/mol). In the docking complex Asp-226, Leu-230, Asn-232, Lys-234, Leu-233, Try-231, Lys-227, Val-250, Asn-251, Pro-252 and Asn-224 were involved in vander Waals interactions with lead 1. It was found that lead 1 bound strongly into the heparin binding pocket of human IGFBP 2 forming six hydrogen bonds with Asp-226, Tyr-231, Asn-232 and Leu-233. The residue Leu-233 formed single hydrogen bond, Tyr-231 formed single hydrogen bond whereas Asp-226 formed two hydrogen bonds and Asp-226 formed two hydrogen bonds in **Figure 2A**. Among them the amino acid residues Lys-227, Asn-

Table 1: Structure of heparin and five proposed leads of human IGFBP 2.

Lead	IUPAC names	SMILES format of leads	XP G score
	(2S,3R,4R,5R)-2-[(1R)-1,2-dihydroxyethyl]-5-[(3,4-dimethylphenyl)amino]oxolane-3,4-diol.	[H]OC([H])([H])[C@@]([H])(O[H])[C@]1([H])O[C@@]([H])(N([H])C2=C([H])C([H])=C(C(=C2[H])C([H])([H])[H])C([H])([H])[H])[C@]([H])(O[H])[C@@]1([H])O[H]	-6.009 kcal/mol
	(1S,2R,9S,10R,11R,15S)-9-hydroxy-2,15 dimethyltetracyclo[8.7.0.0^{2,7}.0^{11,15}] heptadec-6-ene-5,14-dione.	[H]O[C@@]1([H])C([H])([H])C2=C([H])C(=O)C([H])([H])C([H])([H])[C@]2(C([H])([H])[H])[C@@]2([H])C([H])([H])C([H])([H])[C@@]3(C(=O)C([H])([H])C([H])([H])[C@]3([H])[C@]12[H])C([H])([H])[H]	-4.754 kcal/mol
	(2S,3S,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-aminium.	[H]OC([H])([H])[C@@]1([H])O[C@]([H])([N+]([H])([H])[H])[C@@]([H])(O[H])[C@]1([H])O[H]	-4.506 kcal/mol
	(3S,4R,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-one	[H]OC([H])([H])[C@@]1([H])OC(=O)[C@@]([H])(O[H])[C@]([H])(O[H])[C@@]1([H])O[H]	-4.370 kcal/mol
	(3aS,5S,6R,7S,7aR)-6,7-dihydroxy-5-(hydroxymethyl)-hexahydro-2H-pyrano[2,3-d][1,3] oxazole-2-thione.	[H]OC([H])([H])[C@]1([H])O[C@]2([H])N([H])C(=S)O[C@]2([H])[C@@]([H])(O[H])[C@@]1([H])O[H]	-3.951 kcal/mol
 Existing inhibitor	Heparin	C(=O)N[C@@H]1[C@H]([C@H]([C@H](O[C@H]1O)CO)OS(=O)(=O)[O])O[C@H]2[C@@H]([C@H]([C@@H]([C@@H](O2)C(=O)[O-])O)O)O	-2.762 kcal/mol

232, Leu-233 and Lys-234 were well matched with the heparin binding site residues of human IGFBP 2. Hydrogen bonds and van der Waals interactions formed between the lead 1 (2S,3R,4R,5R)-2-[(1R)-1,2-dihydroxyethyl]-5-[(3,4-dimethylphenyl)amino] oxolane-3,4-diol and human IGFBP 2 were highly important for stabilizing the protein-inhibitor complex.

ADME/T properties

An *in silico* study was carried out for the prediction of pharmacokinetic properties. The properties like log P, log S, molecular weight, hydrogen bond acceptor and donar etc., were used to judge the compound's overall potentiality as a drug molecule. All the five leads have suitable logP (octanol/water) value for biological efficacy. Each of them had zero Lipinski's rule violation and satisfying pharmacological properties of 95% available drugs with high to medium predicted oral absorption availability. Molecular weight of each ligand falls within the range of 200-480 Daltons. The leads have no toxic functional groups. Log S values of these ligands were within the range (95%) to exist as drugs. The overall pharmacological properties of the leads justify that the molecules were biologically active without any toxic functional groups. Poor oral availability and permeability

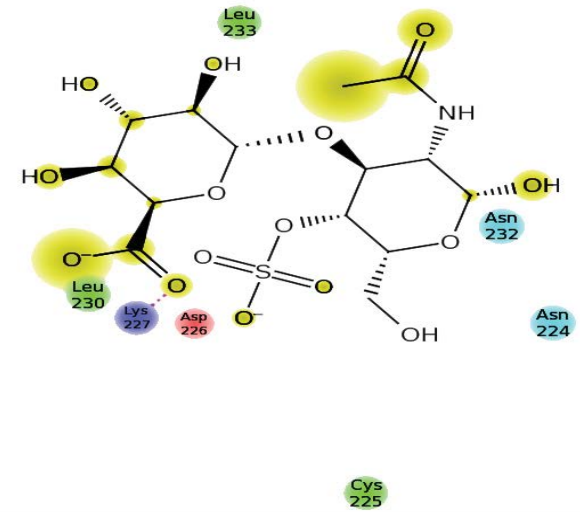


Figure 1 Docking interactions between IGFBP 2 and heparin.

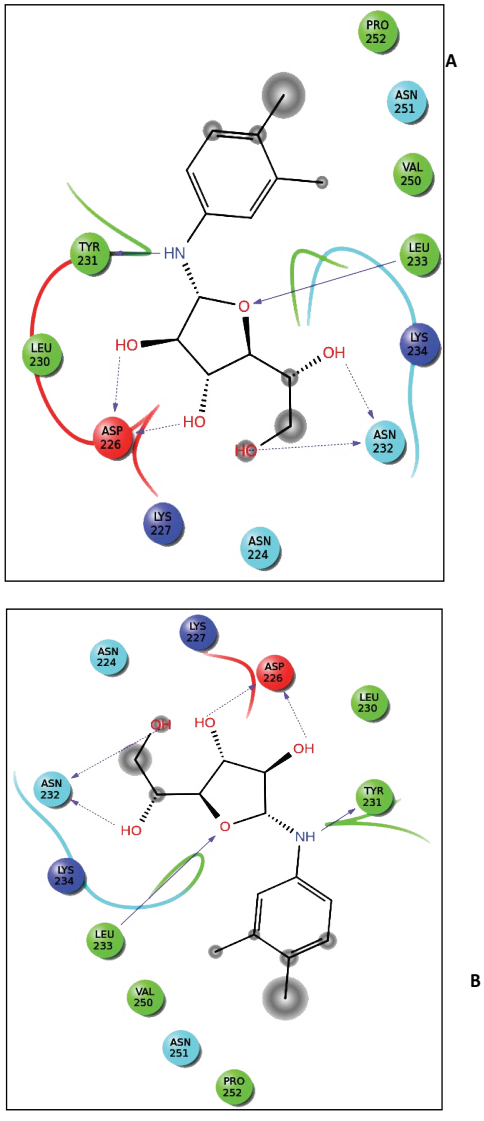


Figure 2 Docking interactions of lead 1-IGFBP 2. (A) Molecular docking, (B) Molecular dynamics simulations.

may lead to drug failure. The overall ADMET properties of all five leads were well with in the normal ranges without any violations and biologically active with out any toxic functional groups. The most significant properties of druglikeness for the five proposed leads were mentioned in **Table 2**.

MD simulations analysis

The conformations obtained after simulations were more stable and credible than the docked conformations. However, MD simulations are carried out closer to the physiological environmental condition. Therefore, the binding orientations of lead molecules predicted through MD simulations show better correlation to their biologically active states [32]. The influence of lead 1 and IGFBP 2 on the structural and dynamical properties have been clarified by analyzing the trajectory data obtained from the MD simulations **(Figures 2A and 2B)**. The energy plot analysis revealed that the lead 1-human IGFBP 2 docking complex was stable throughout 10000 ps MD simulation time in **Figure 3**. Interactions of the IGFBP 2-lead 1 docking complex produced in the MD simulations were analyzed in all 2084 trajectories.

The trajectory data of lead 1-IGFBP 2 docking complex was plotted for energy. The energy minimization plot analysis of lead 1-IGFBP 2 docked complex revealed a consistent stability during 10000 ps of MD simulation time in **Figure 3**. The analysis of the RMSD plot for backbone and lead 1 remained within the limit of 2 Å. The RMSD during MD simulations for lead 1-IGFBP 2 showed consistent nature of docking conformations in all 1046 trajectoreis in **Figure 4**. The RMSF of a given residue in theMD trajectories were calculated by averaging over all the atoms of the given residues in **Figure 5**. The average RMSF of backbone and heavy atoms for the lead 1-IGFBP 2 docking complex during 10000 ps simulation time revealed most of the residues were within the limit of 3 Å. The energy plot, RMSD plot and RSMF plot analysis revealed that both the lead 1-IGFBP 2 docked complex was stable during 10000 ps MD simulation time.

In docking studies human IGFBP 2-lead 1 interactions showed six hydrogen bonds were formed with the residues Asp-226, Tyr-231, Asn-232 and Leu-233, were Asp-226 formed two hydrogen bonds and Asn-232 formed two hydrogen bonds whereas the residues formed in molecular docking studies reproduced in MD simulations results showing six hydrogen bonds with the same residues Asp-226, Tyr-231, Asn-232, and Leu-233, were the residue Asp-226 formed two bonds, Asn-232 formed two bonds, Try-231 formed one bond and Leu-233 formed one bond with lead 1 which remained stable throughout the simulation period in **Figure 6**.

During MD simulations the hydrogen bond interactions formed between IGFBP 2-lead 1 were monitored in **Figure 6**, which revealed the stability of docking complex. The hydrogen bonds formed by amino acid residue, Tyr-231 were >98% which were conserved in all the trajectories in **Figure 6A**. The amino acid residue, Asp-226 formed >71.3% of highly conserved hydrogen bonds in **Figure 6B**. The residue Asn-232 formed >21.3% of highly conserved hydrogen bonds stable in all 2084 trajectories in **Figure**

Table 2: ADME properties of five proposed leads.

Lead	Mol. Wt.	Rotor	tLog P o/w	Log S	Log BB	Log Kp	DonorHB	Accept HB	% Human oral absorption	Globularity	Rule of three	Rule of Five
1	283.32	8	-0.1	-1.98	-1.53	-3.7	5.00	9.5	66.9	0.86	1	0
2	149.14	5	-2.2	-0.4	-0.77	-6.6	5.00	7.8	41.8	0.94	0	0
3	178.14	6	-0.1	-2.09	-1.23	-3.6	4.00	7.8	71.7	0.87	1	0
4	178.14	5	-2.2	-0.89	-1.53	-5.3	4.00	9.8	45.9	0.93	0	0
5	221.22	4	-1.2	-1.58	-0.83	-4.3	4.00	9.8	61.9	0.91	0	0

Parameter:	Normal range:
Molecular weight	(MW=130.0/725.0)
Number of rotatable bonds	(Rotor=0.0/15.0)
Log P for octanol/water	(LogP o/w=-2.0/6.5)
Log S for aqueous solubility	(LogS=-6.5 /0.5)
Log BB for brain/blood	(LogBB=-3.0/1.2)
Log KP for skin permeability	(Log KP=KP in cm/h)
Donor-hydrogen Bonds	(DonorHB=0.0/6.0)
Acceptor-hydrogen Bonds	(AccptHB=2.0 /20.0)
% Human oral absorption in GI	(<25% is poor)
Globularity	(Glob=0.75/0.95)
Lipinski rule of 5 violations	(Maximum is 4)
Jorgensen rule of 3 violations	(Maximum is 3)

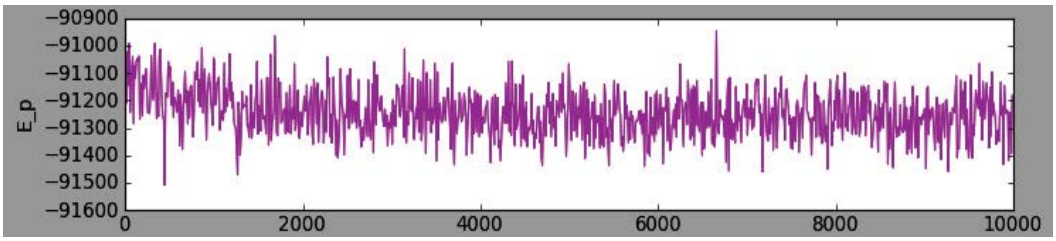


Figure 3 Energy plot of IGFBP 2-lead 1 docking complex during MD simulations.

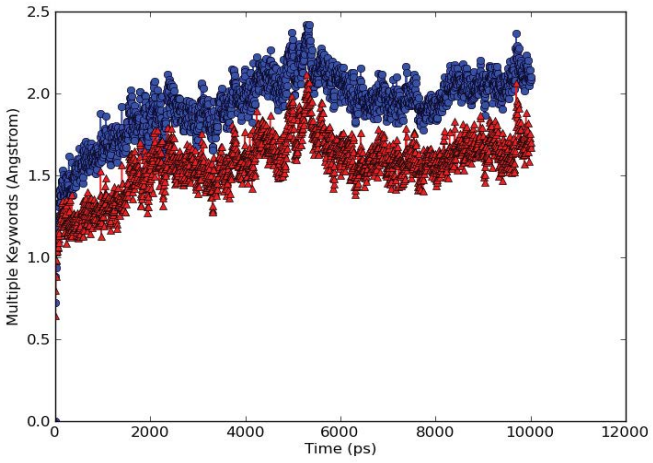


Figure 4 RMSD of IGFBP 2-lead 1 docking complex.

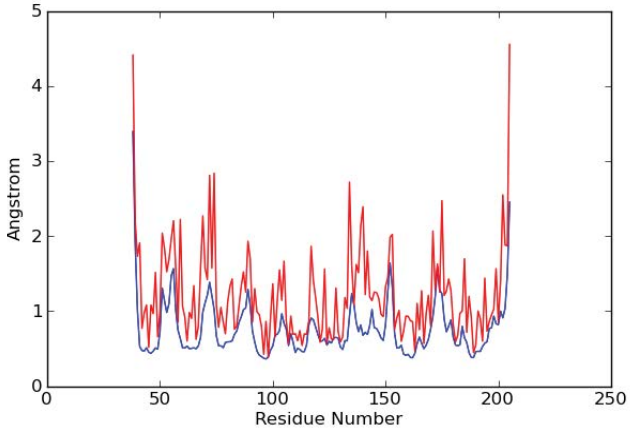


Figure 5 RMSF plot of IGFBP 2-lead 1 docking complex.

6C. Leu-233 in all 2084 trajectories revealed >15.8% hydrogen bonds which were stable and highly conserved in **Figure 6D**.

The amino acid residues such as Asn-232 and Leu-233 were well correlated with the heparin binding site residues, heparin-IGFBP 2 docking complex in **Figure 1**, docking studies. In **Figure 2A** and also MD simulations in **Figure 2B**. The analysis of results revealed that lead 1 had better binding orientation, good pharmacological properties and also showed good stability with IGFBP 2 than the heparin. The molecular docking and MD simulation results such as energy analysis, RMSF, RMSD and formation of hydrogen bond between C-terminal domains of IGFBP 2-lead 1 docking complex proved that the binding was highly stable. The IGFBP 2-lead

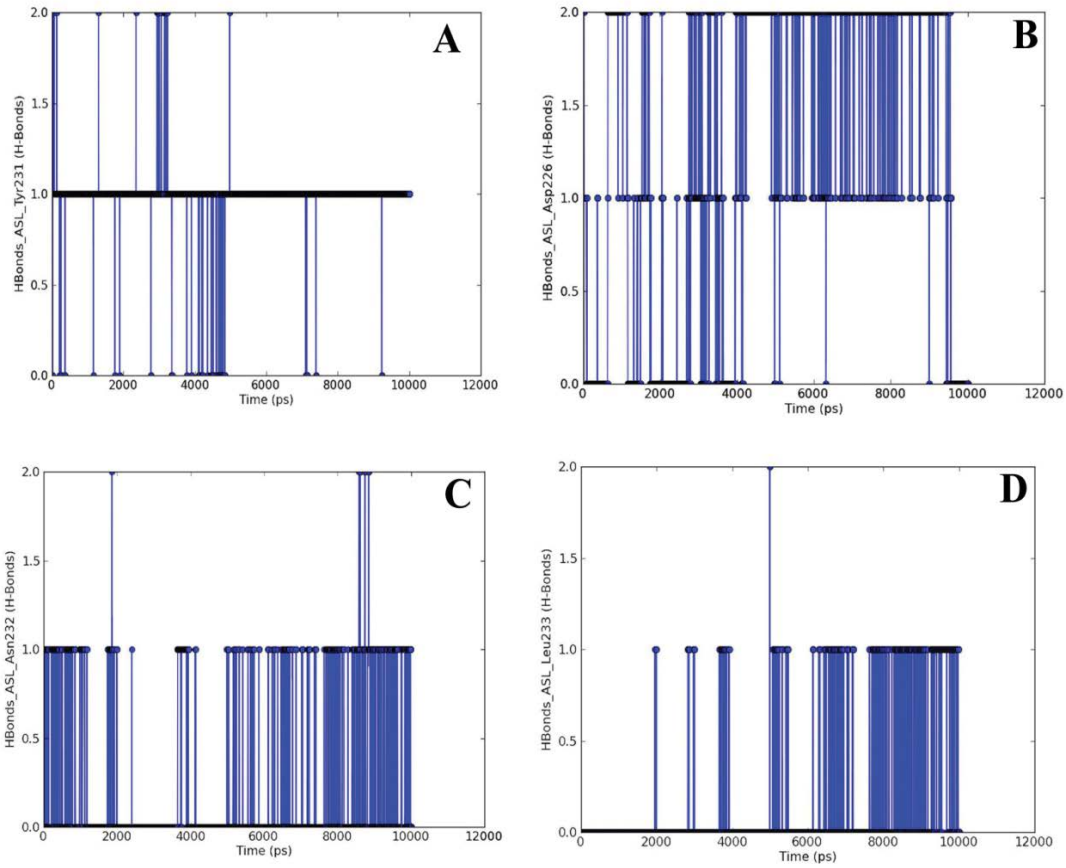


Figure 6 Monitoring of hydrogen bonds between IGFBP 2-lead 1 during MD simulations. (A) Try-231, (B) Asp-226, (C) Asn-232, and (D) Leu-233.

1 complex was consistent throughout the run time of 10 ns simulations. Therefore, the study showed novel insight into the natural dynamics by revealing presence of hydrogen bonds on different timescales of lead 1-human IGFBP 2 docking complex in solution. Hence, lead 1 is having good pharmacological properties and also with high stability can act as potential competitive inhibitor of IGFBP 2 to blocks its activity and would be useful for developing new therapeutic on cancers.

Over expression of human IGFBP 2 plays a critical role in causing cancers. Structural analog search was carried out using Ligand. Info, based on the heparin having poor pharmacokinetics. Various modules (LigPrep, Protein preparation wizard, Glide, QikProp) of Schrödinger were implemented successfully to generate interesting new lead compounds. Five best ranked leads with good binding affinity and pharmacokinetic properties towards human IGFBP 2 were proposed. Lead 1 has better XP GScore (-6.009 kcal/mol) among the proposed leads can be suggested as therapeutic antagonist for human IGFBP 2 in the treatment of various cancers.

Discussion

The experimental approaches are time consuming, more in cost and also obtain very few results whereas computational approaches have been widely used to search for novel inhibitors as they are fast, efficient and cost effective and get more desired results. These approaches are also helpful in reducing the problem of searching novel inhibitors from a large pool of small molecule databases using various methods [33,34]. The availability of existing inhibitors in combination with Bioinformatics tools and databases are of great assistance in reducing the problem of searching for potential inhibitors in a large pool of gene/protein polls. IGFBP 2 is a potential marker selected in the present study affirmed to play critical role in over expression of cancers. As effective inhibitor of IGFBP 2 is not available till date, an attempt was made to identify novel antagonist against human IGFBP 2. Several studies reported heparin as one of the crucial inhibitor against human IGFBP 2 [33]. Heparin provided an ideal platform to design leads against human IGFBP 2 involved in cancers.

Human IGFBP 2 binds to IGF and regulates insulin growth factor actions in controlling growth, development, reproduction and aging. The C-terminal domains of IGFBPs contribute to high-affinity IGF binding, in the over expression of human IGFBP 2 therefore, IGFBP 2 was considered in the present study (Kuang 2006). Protein preparation and ligand preparations were done and XP GScore of heparin was compared with ligand molecules of human IGFBP 2. The results revealed that heparin showed XPG

score of -2.726 kcal/mol whereas five lead molecules showed XPG scores of -6.009 kcal/mol, -4.754 kcal/mol, -4.506 kcal/mol, -4.370 kcal/mol, and -3.951 kcal/mol respectively. The lead 1-IGFBP 2 docked complex with highest negative docking score was also compared with heparin-IGFBP 2 docking complex. Lead formed six hydrogen bonds were formed with the residues Asp-226, Tyr-231, Asn-232 and Leu-233, were Asp-226 formed two hydrogen bonds and Asn-232 formed two hydrogen bonds were well correlated with the heparin binding site residues of human IGFBP 2 revealing the good binding orientation of lead 1. To check the stability, further lead 1-IGFBP 2 docking complex was analyzed through molecular dynamic simulations using Desmond. The amino acid residues such as Asp-226, Try-231, Asn-232 and Leu-233 formed six hydrogen bonds were formed with the residues Asp-226, Tyr-231, Asn-232 and Leu-233, were Asp-226 formed two hydrogen bonds and Asn-232 formed two hydrogen bonds which were well matched with the docking complex. The molecular dynamics simulations revealed the hydrogen bonds formed, energy plot, RMSD, RMSF of lead 1-IGFBP 2 docked complex was more stable in the physiological environmental conditions.

The proposed lead 1 molecule is showing good pharmacological properties, binding affinity, binding orientation, docking scores and hydrogen bonds interactions. The identified lead molecules in the present study would bring new possibility in developing powerful drug molecules for the treatment regimen in cancers.

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