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STRUCTURE BASED VIRTUAL IDENTIFICATION OF NOVEL HERBAL COMPOUNDS AGAINST TARGET PROTEINS IN HEPATOCELLULAR CARCINOMA

SHANDANA ALTAF
Assistant Professor, Khyber Medical College, Peshawar Pakistan and PhD Scholar Khyber Medical University, Peshawar, Pakistan. Corresponding author E-mail: shandanaaltaf@hotmail.com

SHAFIQ AHMAD TARIQ
Professor Pharmacology, Khyber Medical University, Peshawar, Pakistan.
E-mail: shafiqibms22861@gmail.com

SAMI SIRAJ
Director Institute of Pharmaceutical sciences, Khyber Medical University, Peshawar, Pakistan.
E-mail: samisiraj.ibms@kmu.edu.pk

JAMSHAID AHMAD
Assistant professor Center of Biotechnology and Microbiology, University of Peshawar, Pakistan.
E-mail: jamshaidbiotech@yahoo.com

SAIMA IKRAM
Center of Biotechnology and Microbiology, University of Peshawar, Pakistan.
E-mail: saimaikram290@gmail.com

SHEIKH ARSLAN SEHGAL
Department of bioinformatics Islamia University Bahawalpur, Pakistan. E-mail: arslansehgal@yahoo.com

Abstract

Growth factor receptors and enzymes are essential proteins that link extracellular signals with intracellular signaling pathways and support many phases of the development of cancer. Vascular endothelial growth factor receptor (VEGFR2) is a dominant player in the angiogenesis process of malignant cells and Sterol O-acyltransferase (SOAT1) is emerging as a new oncogenic enzyme in the development and progression of cancer. In this study we selected three plant-derived compounds *Indigoferin A (compound 1), Indigoferin B (compound 2)* and *Indigoferin C (compound 3)* as ligands and investigated their binding affinity and simulation energy with prospective targets VEGFR2 and SOAT1. *Indigofera Giardiana* is a shrub and is found in northern hilly areas of Pakistan and India. It has medicinal uses in conventional medicine for treating skin infections and stomach spasms. In the current work, ligand-receptor complexes were subjected to molecular dynamics and simulation analysis. Compounds Indigoferin *A, B* and *C* all have good binding affinity with the three selected targets (VEGFR2, SOAT1 and p53Mapkinase). In terms of metrics generated from molecular dynamics simulations, such as root mean square deviation and fluctuation, the ligand and receptor complexes were found stable. The molecules Indigoferin A, B, and C exhibit different protonation states that interact significantly with the target proteins' active site residues, as shown by the results of molecular dynamics and simulation analysis. The relative binding free energies have been precisely calculated using the MM-GBSA method. Preventing cell growth is an important strategy for treating cancer and these three compounds have the potential to inhibit the targeted enzyme (SOAT1) and receptor proteins (VEGFR2, p53 Map Kinase) thus helping to find novel compounds that could successfully restrain the growth of malignant cells.

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INDEX TERMS: Hepatocellular carcinoma, Indigofera giardiana, VEGFR2, SOAT1, p53mapkinase, molecular docking, molecular dynamic simulation, quantum polarized ligand docking

INTRODUCTION:

The growing incidence of hepatocellular carcinoma is affecting people all over the world. The pathogenesis is quite complex and involves a number of molecular signaling pathways. The management is effective in early stages of HCC and involves surgical resection and transplantation which is suitable for less than 25% of the patients (Bhayani et al., 2015). Molecular targeted therapy is a daunting challenge for researchers. It requires enough information about the background molecular structures and signaling connections that cause uncontrolled proliferation of hepatocytes. Recent studies have identified RAF/MEK/ERK protein signaling pathways and some tyrosine kinase receptors role in the growth, multiplication and angiogenesis of the liver cancer cells (Song et al., 2022) (Yang & Liu, 2017). For the past one decade, Sorafenib is the only FDA approved chemotherapy drug for the treatment of advanced unresectable HCC targeting RAF and tyrosine kinase receptors(Zhou et al., 2020) (Gao et al., 2015). Sudies have shown that overall Prognosis of Sorafenib is still inadequate and requires new drug exploration in pharmacological medicine (Marisi et al., 2018)(Leathers et al., 2019). VEGFR signaling pathway is known to have a significant role in angiogenesis which is a common finding in HCC (Moawad et al., 2020)(Choi et al., 2017). Sorafenib has been approved worldwide because of its antiangiogenic effect targeting VEGFR receptors and has shown better survival in advanced stage of HCC (Morse et al., 2019). VEGFR receptors expressed on endothelial cells have therefore been selected as potential target for the test compounds. The key residues of VEGFR2 that interact with Sorafenib through hydrogen bond are Lys 868, Glu 885, Cys 919 and Asp 1046. Asp 1046 is known to form the most stable hydrogen bond while Cys919 interact through weak hydrogen bond formation(Meng, 2013).

High expression of sterol O-acyltransferase 1) SOAT1 occurs in the early stages of HCC and catalyzes esterification of cholesterol with acyl-CoA within the endoplasmic reticulum membrane(Chen et al., n.d.). This esterification is responsible for increased neoplastic progression and poor prognosis (Khatib & Wang, 2019)(Jiang et al., 2019). Inhibition of this new therapeutic target is an important anti-cancer strategy as seen by marked reduction of tumor cell proliferation and tumor growth in mouse models (F et al., 2016). His460, N421 and W420 are the key active site residues that have been identified as SOAT1 targets (16).

P38 mitogen activated protein kinase (P38 MAP kinase) is a key molecule that transports extracellular signals from cell surface to the DNA in the nucleus and causes gene expression responsible for cell cycle, cell differentiation, migration, apoptosis and cell survival. Activation of P38MAPK occurs due to inflammatory cytokines (IL1, TNFα,) and growth factors (TGFβ) and has a role in cell differentiation and apoptosis. Overexpression seen in advanced stage of HCC makes it a good target for new drug development (Min et al., 2011). The common docking domain in p38MAPK include Asp313, Asp315 and Asp316 (Chang et al., 2002).

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A lot of *in silico* research has been done on medicinal plants to anticipate their anticancer properties through molecular docking and simulation studies by hitting several targets(Zhang et al., 2019). In this study we selected three novel herbal compounds *Indigoferin A, Indigoferin B, and Indigoferin C* derived from native shrub *Indigofera Giardiana* and docked against vascular endothelial growth factor 2 (VEGFR2), sterol O-acyltransferase1 (SOAT1) protein and human P38 mitogen activated protein kinase (P38MAPK), respectively(Tariq et al., 2011).

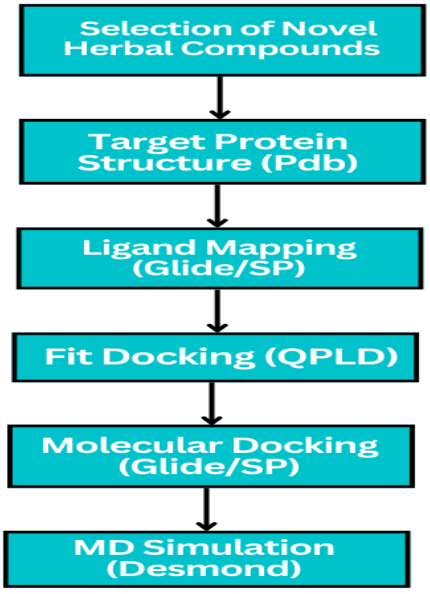
MATERIALS AND METHODS

Maestro/Schrodinger software was made available by the University of Peshawar's bioinformatics department for the analysis of protein-ligand interactions, molecular dynamics, and ligand binding site determination. The three compounds (*Indigoferin A, Indigoferin B, Indigoferin C*) with anti-inflammatory, antibacterial, and antifungal activities were selected from the PhD research study of Dr Shafiq Ahmad after he isolated them from a plant *Indigofera Giardiana.* Virtual docking of the ligand molecules against Hepatocellular carcinoma (HCC) specific kinase receptors was carried out and sorafenib, a multi-kinase inhibitor served as the reference medication.

TABLE 1: The key phases of docking and MD simulation studies

Docking	Protein ligand structure	docking Glide /SP
MD simulation	MM/ GBSA analysis	QPLD analysis

TABLE 2: flowchart outlining the methodology steps



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Protein Preparation

Crystal structure of the proteins were downloaded from protein databank (PDB) and prepared using the Maestro protein preparation tool by adding hydrogen atoms and fixing side chains. Following cross validation, the amino acid sequence was confirmed. Using the protein preparation wizard from the Schrodinger module, disulphide bond loops were created (Hussan et al., 2020). The Glide module's Receptor grid creation application was used to create an active-site grid as well as to pinpoint the ligand's location in the receptor's active-site region (Placeholder2) (Placeholder3). The grid of 20 Å was generated over ligand molecule and the functional sites of the targeted proteins and sorafenib was explored. Using pyMOL, the docked complexes were overlaid onto the original crystal structure to determine the root mean square deviation (RMSD). For structural optimization of protein, we used OPLS 2005 force field and PROPKA adjusted the protonation statuses of the amino acids at pH 7.4.

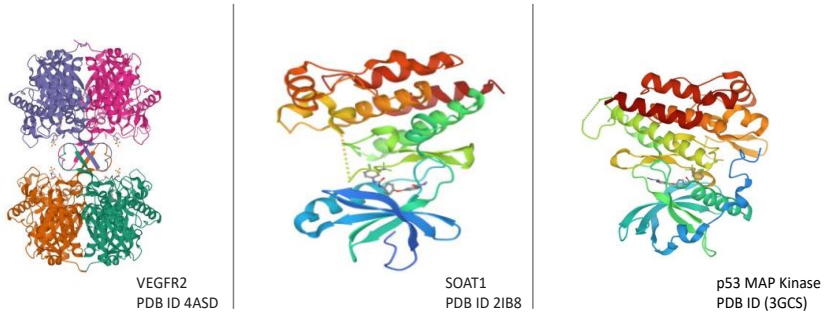

Fig. 1; PDB structures of target proteins

Ligand preparation

a. Compound 1: *Indigoferin A* 6(-hydroxy-1-(2, 4, 6-trihydroxyphenyl) heptan-1-one

b. Compound 2: *Indigoferin B* 6-methyl-1-(4-((2S, 3S, 4S, 5S, 6R)-3, 4, 5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yloxy) phenyl) heptan-1-one

c. Compound 3: Indigoferin C (2R,3R,4R,5R,6S)-2-(hydroxymethyl)-6-(4-(5-methylhexyl) phenoxy) tetrahydro2H-pyran-3,4,5-triol

We prepared the above three novel herbal compounds as ligand molecules against three main protein targets (VEGFR, SOAT1, P38MAP kinase). 2D structures were drawn by using 2D sketcher, which were then converted to 3D by adding hydrogen atoms for further use. For compound preparation, the LigPrep module of the Maestro molecular modelling software was combined with the OPLS2005 forcefield. The protonation conditions of the ligand at neutral pH of 7.0 were determined using Epik, a software tool that predicts pKa and generates protonation states.

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Molecular docking

For docking investigations of the three compounds (F1, F2, F3) derived from Indigofera giardiana, a variety of methods from Maestro molecular modeling programmer were utilised for both flexible and rigid ligand docking, including Glide/ Standard Precision (SP) and Quantum Mechanics- Polarized Ligand Docking (QPLD) method(Kumar et al., n.d.). We docked each of the ligand compounds in the binding pockets of VEGFR2, and SOAT1 proteins to analyze ligand- receptor interactions. Twenty postures were constructed for each compound using Glide /SP mode and identified main catalytic residues (L840-868, G885, C 919, Asp 868-1046, D1046, E1075) of VEGFR2 , (L184, C126,) for SOAT1as the basis for the docking simulation boxes. Around catalytic residues, a receptor grid box was created, and certain amino acids were let to spin their side chains to increase the target's flexibility. To make protein and ligand complexes, the GOLD algorithm (5.3.0 software) was employed (Stoilov et al., n.d.). The catalytic residues were considered flexible and used to generate docking poses with realistic docking score and interaction with other active side residues were analyzed by pose viewer. QM calculations were carried out to overcome charge polarisation brought on by the protein environment and geometric bond angles (degrees) and lengths (angstrom) between donor and acceptor were computed using B3LYP/6-31G considering hydrogen polarization at the active site of the protein.

Molecular Dynamic Simulations Desmond was used to run 10-100ns molecular dynamics (MD) simulation on ligand-protein complexes. Free binding energy ΔG_{bind} throughout the simulation was computed using OPLS2005 (Placeholder4)(Shivakumar et al., 2012). SPC water model produced a rhombohedral stacked simulation box. The requisite amount of Na or Cl ions were added to the system to neutralize the complex and balance the charges. The simulation maintained the system's temperature (310 K) and pressure (1.01 bar). To determine the stability of protein ligand complexes, a quick MD simulation of 100 trajectory frames were captured at intervals of 0.5ns. To find compounds of high expected affinity to the target structures, Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) were used(Wang et al., 2019).

RESULTS AND DISCUSSION

The reported binding pockets of VEGFR2 (Pdb 4ASD), SOAT1 (Pdb 2IB8), and MAP Kinase (Pdb 3GCS) were all completely docked by the three ligand molecules (Pdb 3GCS). According to published data, binding pocket (ASP1046, CYS919, and GLU885). The reported glide energy 61.19 kJ/mol and docking score 7.36 Kcal/mol of Sorafenib were used as reference values. The interaction of Sorafenib with water contains the following amino acid residues (ALA866, ILE888, LEU889, 840, 1035, PHE918, THR916, and VAL848) and are responsible for stability of VEGFR and sorafenib complex.

Protein receptor VEGFR2 (PDB ID: 4ASD) and binding ligands Delta binding energy **(**ΔG) of the compound 1,2 and 3*,* was calculated as -42.09Kcal/mol, -68.56Kcal/mol and -68.38 Kcal/mol, respectively. The benzene ring of the compound 1 forms hydrogen bond

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with ILE 1025 and Pi Pi stacking interaction with HIE 1026 (Fig 3.1a). Compound 2 had its major interaction at ASP 1046 like reported for sorafenib while Pi cation interaction at LYS 868 (Fig 3.1b). Similarly, compound 3 also interacts specifically with negative charged residue ASP 1046 and positively charged LYS 868 (Fig 3.1c).

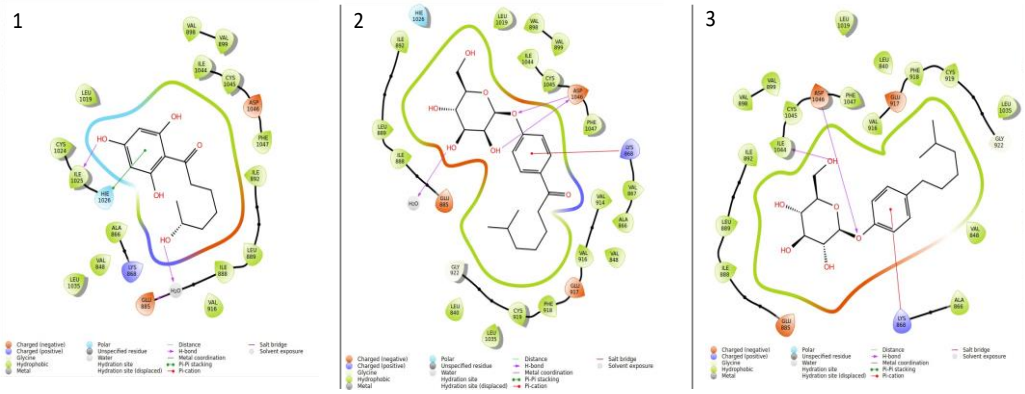

Fig. 2. Docking pose of compounds1, 2 and 3 with 4ASD complex

Protein receptor SOAT1 (PDB ID:2IB8) and binding ligands After placing the compound 1 into the binding pocket, the free binding energy score (ΔG) was calculated as– 5.3Kcal/mol. In the binding pocket of SOAT1, the phenol moiety of the chemical makes hydrogen bonds with the amino acid residues THR 279, LEU286, TYR C:170, CYS A:1, and SER 284. (Fig 3.2 A). Compound 2 exhibits a hydrogen bond interaction with SER 284, ALA 280, and GLU285. Its benzene ring forms hydrogen bond interaction with the following residues: CYS 413, CYS 126 and THR 285 (Fig 3.2 C).

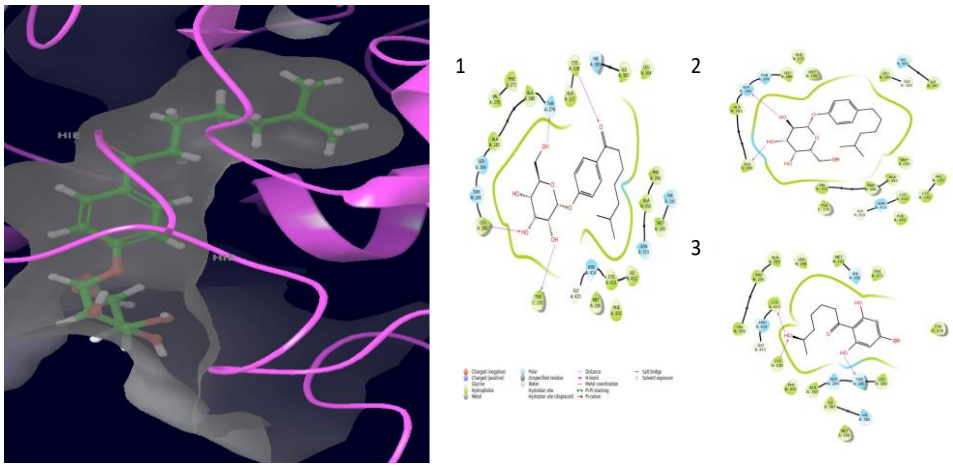

Fig. 3: Docking pose of compounds1,2 and 3 with 2IB8 complex

Protein receptor p53 MAP Kinase (PDB ID:3GCS) and binding ligands: The carboxylic moiety of the compound 1 has hydrogen bond interaction with negatively

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charged ASP168 while the benzene ring interacts at GLU71 maintaining stability through hydrophobic amino acid residues (Fig 3.3 a). The compounds 2 and 3 besides forementioned interactions also establishes pi pi stacking at PHE169 at the binding site (Fig 3.3 b, c).

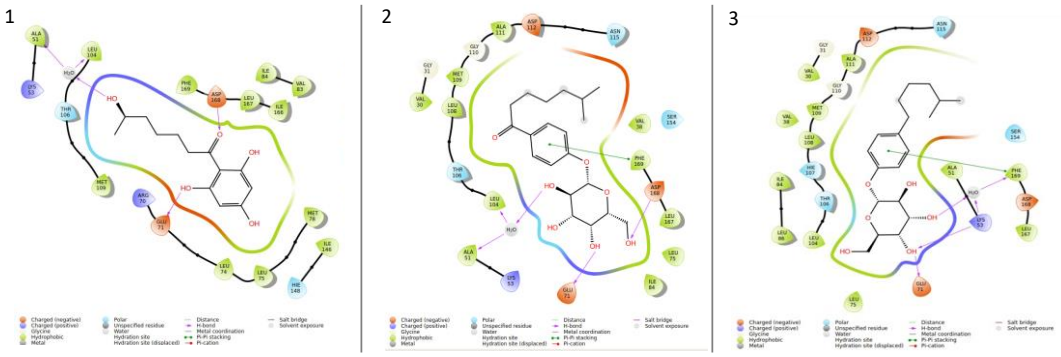

Fig. 4: Docking pose of compound 1, 2, 3 with 3GCS complex

TABLE 3: Docking scores of Compounds 1, 2, and 3

No.	Ligands	Chemical Name	Molecular Formula	Atomic Weight	Docking Score
1	Compound 1	6(-hydroxy-1-(2, 4, 6-trihydroxyphenyl) heptan-1-one	$C_{20}H_{30}O_7$	382.4481	-7.52 Kcal/mol
2	Compound 2	*B* 6-methyl-1-(4-((2S, 3S, 4S, 5S, 6R)-3, 4, 5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yloxy) phenyl) heptan-1-one	$C_{14}H_{20}O_5$	268.3061	-5.30 Kcal/mol
3	Compound 3	(2R,3R,4R,5R,6S)-2-(hydroxymethyl)-6-(4-(5-methylhexyl) phenoxy) tetrahydro2H-pyran-3,4,5-triol	$C_{19}H_{30}O_6$	354.4380	-6.81 Kcal/mol

TABLE 4: Binding energy of ligand receptor complexes

Ligands	VEGFR2	SOAT1	p53MAPKinase
Compound 1	-42.09 kcal/mol	-33.69 kcal/mol	-32.64 kcal/mol
Compound 2	-68.56 kcal/mol	-63.77 kcal/mol	-54.40 kcal/mol
Compound 3	-68.38 kcal/mol	-62.61 kcal/mol	-56.39 kcal/mol

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Simulation study using molecular dynamics (MD simulation studies) In this study, MD simulations were carried out to better understand the confirmative stability of the receptors. The MD trajectory analysis of Desmond was used to determine the time-dependent variations in the ligand stability in the protein binding pocket by computing "root mean square deviation," "root mean square fluctuation," and "protein-ligand interactions". Figure 3 depicts how the RMSD values for the backbone atoms of the ligand-bound protein changed over time. Utilizing ligand RMSD (lig-fit-prot plots), the molecular dynamics determined the RMSD values of the compound 1, 2 and 3 for active site of VEGFR2 and found they maintained structural stability within range of 2.0A° throughout simulation (Fig 3.2). Free binding energies (G) of compounds 1, 2, and 3 for VEGFR were determined to be -42.09 Kcal/mol, -68.56 Kcal/mol, and -68.38 Kcal/mol, respectively. For SOAT1 the compound 1has RMSD of 2.0°A and stability lasted for 4ns from 6 to 10 ns. The compound 2 with RMSD 2.8°A maintained stability for 2ns and then deviated from the binding protein pocket achieving stability for 8ns. Comparatively, compound 3 had a high RMSD more than 5°A. ΔG binding energy of the compound 1, 2 and 3 is -33.69, - 63.77 and -62.61 Kcal/mol. Residue interactions of compound 1revealed hydrogen bonding with GLU885 and ASP1046. For p53 MAP Kinase, the RMSD value of compound 1 was computed as 2.6 °A and stability of 4ns. The compound 2 with RMSD value of 2.8°A remained stable in the binding pocket for 5ns with little change in protein fluctuation. Compound 3 had an average RMSD value less than 2.5°A. The following equation was used to calculate RMSD values.

$$RMSD_x = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(r_i'(t_x)) - r_i(t_{ref}))^2}$$

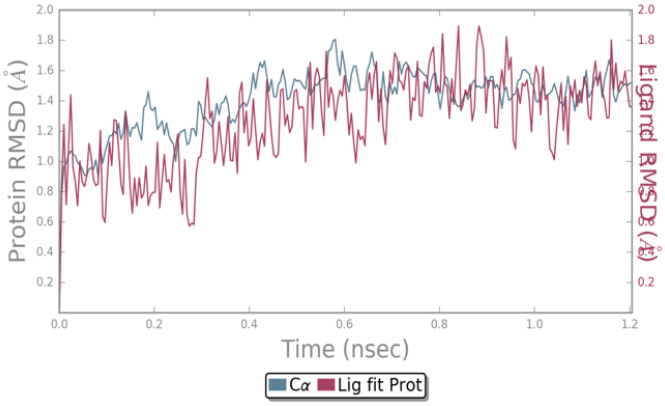

Fig. 5. RMSD for Compound2 with VEGFR2

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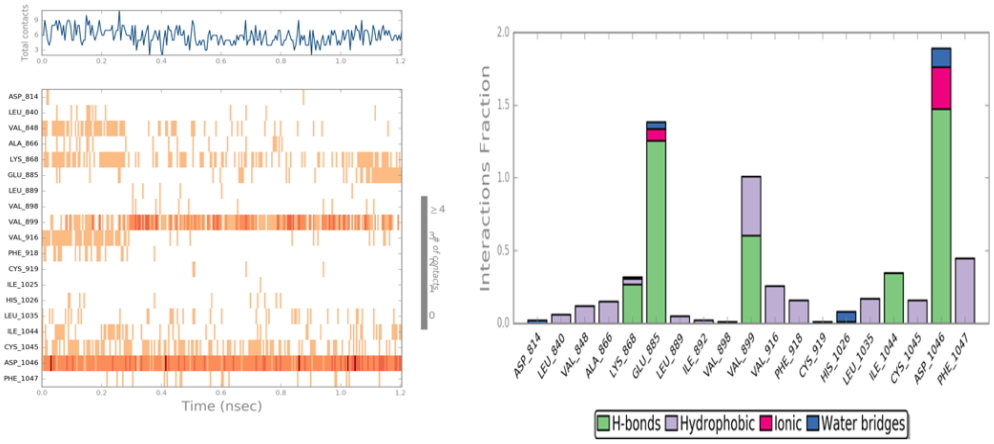

Fig. 6: Residue interaction diagrams for compound 2 with VEGFR 2 during simulation studies

To characterize local variations in a protein chain, the Root Mean Square Fluctuations (RMSF) method was employed. For SOAT1 and compound 1complex, residues in the binding area fluctuate less than those in the rest of the protein. Compounds 2 and 3 had multiple fluctuations mostly in the loop regions. Structural components like alpha helices and beta strands are more rigid than loop regions in general pattern of fluctuation and remain stable throughout simulation. Hydrogen bonds and hydrophobic interactions of all the three ligands at the protein's binding pocket were analyzed throughout the simulation time. Residue interactions of compound 1 with VEGFR2 had many hydrogen bonds at ASN923, ARG1032, GLU917 and LEU840. Compound 2 showed hydrogen bonding with CYS1045 and VAL899. For SOAT1, compound 1 showed hydrogen bond interaction with ASP1046, GLU885 and VAL899. Compound 2 had significant hydrogen bond formation with A-GLU255 and A-PHE55. Compound 3 had a significant hydrogen bond with A-HIS192. When residue interactions for MAP kinase were analyzed with compound 1, it showed significant hydrogen bonding with GLU71, LYS53, HIS148. Compound 2 had hydrogen bond formation with ASP168, GLY110, MET109 and GLU71. Compound 3 had significant hydrogen bonding with GLU71, ASP168 and Lys53.

CONCLUSION

This computational study identified three new inhibitor molecules that possess effective interaction with the ATP binding sites of the receptor proteins and possess potential to modulate the target function. In order to decrease the target activity, the compound *Indigoferin B* (compound 2) has significant interaction with both biological targets VEGFR2 and SOAT1and best binding energy score compared to *Indigoferin A* (compound 1) and *Indigoferin C* (compound 3). We predicted that the results of these compounds will be beneficial in the future research studies of drug development for hepatocellular carcinoma.

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