Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2014; 6(3): 221-229

ISSN- 0975 1556

Research Article

Virtual Screening, Molecular Docking and Molecular Dynamics Studies For Discovery of Novel Vegfr-2 Inhibitors

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Available Online: 1st July 2014

ABSTRACT
VEGFR-2 is considered as potential target for cancer therapy. In this work, the stability, binding mode between the VEGFR-2 protein and its ligand have been evaluated using the pharmacophore guided virtual screening (VS), molecular docking, and molecular dynamics (MD) simulations. The small molecule VEGFR-2 inhibitors were identified through virtual screening of chemical databases based on pharmacophore guided VS approach, that searches multi-conformer representations efficiently using PHASE module of Schrödinger. In addition, the molecular docking, using GLIDE module of Schrödinger; and molecular dynamics simulation, using GROMACS software were performed to study the interaction between the protein and the ligand. Molecular docking enables the extraordinary structural diversity of synthetic products to be harnessed in an efficient manner. The best six ligands (ZINC01056202, ZINC06091460, ZINC06091450, ZINC04107510, ZINC04623218, and ZINC81582433) with different scaffolds are selected from docking studies. VEGFR-2 and ligand complex was found to be stable at room temperature demonstrated by 1000 ps molecular dynamic simulation study using water as a solvent. The predicted inhibitors are quite novel compared with the known VEGFR-2 inhibitors. The work provides insight for molecular understanding of VEGFR-2 and can be used for development of anticancer drugs.

Keywords: VEGFR-2, Virtual screening, Molecular docking, Molecular dynamics.

INTRODUCTION

Angiogenesis is important in the tumor development[1]. The term angiogenesis is first proposed by Judah Folkman in 1971 to define the hypothesis that tumor growth depended on the formation of new blood vessels from the pre-existing vascular bed[2]. The vascular endothelial growth factor (VEGF) is the most important pro-angiogenic factor. The VEGF gene is up-regulated by a host of stimuli, including estrogen, nitric oxide and a variety of growth factors, such as platelet derived growth factor, tumor necrosis factor alpha (TNF- α), fibroblast growth factor-4, keratinocyte growth factor, epidermal growth factor (EGF), interleukin (IL-6 and IL-1)[3]. The VEGF expression is sensitive to the presence of oxygen and is mediated by hypoxia that spreads most tumors, which is due to the aberrant nature of their vascular supply. The VEGF plays significant role in different cancers like colorectal cancer, breast cancer, non-small cell lung cancer, renal cell cancer, pancreatic cancer, prostate cancer, head and neck cancer, gynecological cancer, and hematological malignances[4]. The VEGF pathway is a good target for the anti-angiogenic therapy for various reasons like: it is produced in large quantities by growing primary tumors; VEGF pathway induces the production of sprouting blood vessels[5]; VEGF binds to endothelial cells involved in the formation of blood vessels, also endothelial cells are genetically stable and spontaneous mutations are rare when compared to unstable tumor cells; and VEGFR are expressed in low levels in normal cells, and extensively in tumor cells[6]. However, the drug resistance and low level selectivity is always a major concern in discovering potent novel inhibitors.

The most common methodologies used in discovery of small-molecule inhibitors are pharmacophore screening and molecular docking[7]. In modern *in silico* drug discovery, similarity based virtual screening acts as an integral part[8]. This approach allows us to identify the inhibitors quickly. Computational technologies are a practical solution to the horrific experimental costs associated with high-throughput screening of large compound libraries[9]. A variety of modeling techniques are available for today's medicinal chemists for the rapid and efficient discovery of lead molecules against biomolecular targets. Meanwhile, natural products are re-emerging as a valuable source of bioactive scaffolds that display remarkable chemical diversity in structure and function[10]. The use of virtual screening technologies ameliorates many of the problems associated with the incompatibility of natural products with high throughput screening. The combination of virtual screening and natural products allows the medicinal chemist to harness

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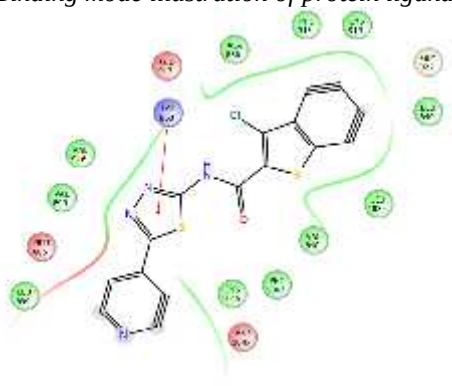
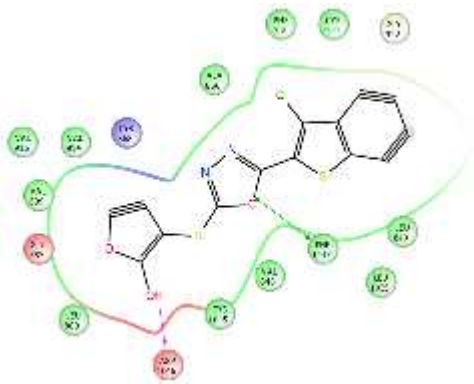
Fig. 1: Binding mode illustration of protein ligand complex

a)



ZINC01056202 - VEGFR-2 complex

b) ZINC06091460 - VEGFR-2 complex



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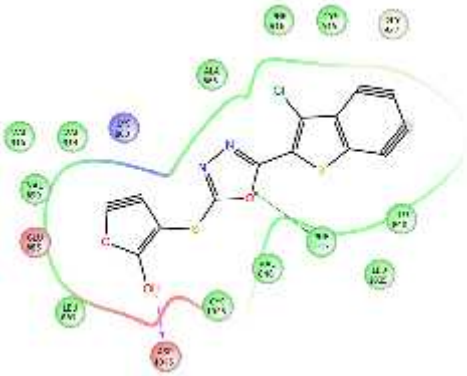
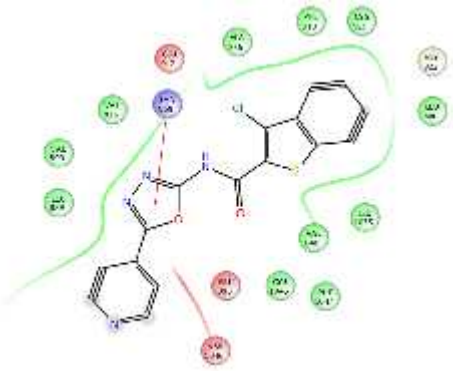
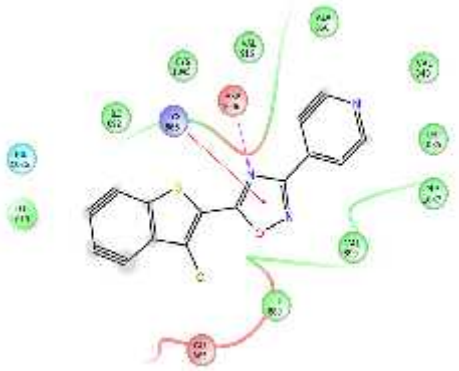


ZINC06091450 - VEGFR-2 complex

d) ZINC04107510 - VEGFR-2 complex

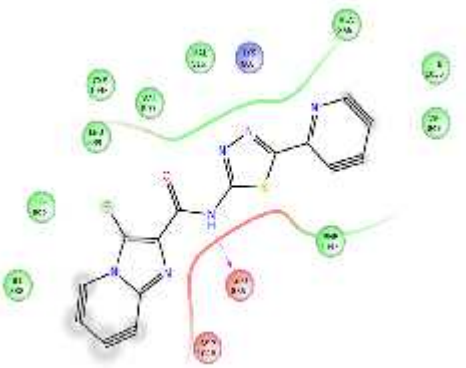


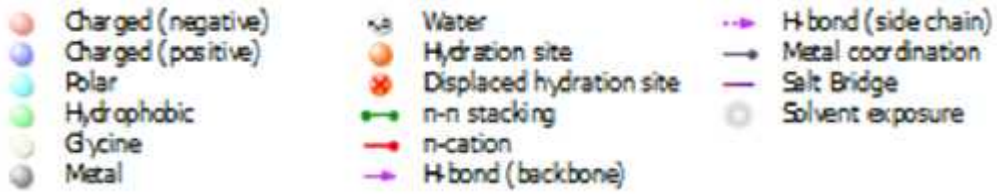
e)



ZINC04623218 - VEGFR-2 complex

f) ZINC81582433 - VEGFR-2 complex





the extraordinary potential of natural products in an efficient and inexpensive manner[11]. Molecular docking, while regarded as more complex and computationally demanding compared to pharmacophore modeling, has the potential to accurately predict binding affinities of screening hits as well as potentially reveal lead structures with novel modes of binding[12]. As scoring algorithms become more refined, together with the continuous improvement in computer processing power and capabilities, the molecular docking has great promise in virtual lead discovery.

The purpose of this study is to understand the stability, binding mode between VEGFR-2 and the ligand using pharmacophore guided virtual screening, molecular docking and molecular dynamics simulations.

MATERIALS AND METHODS

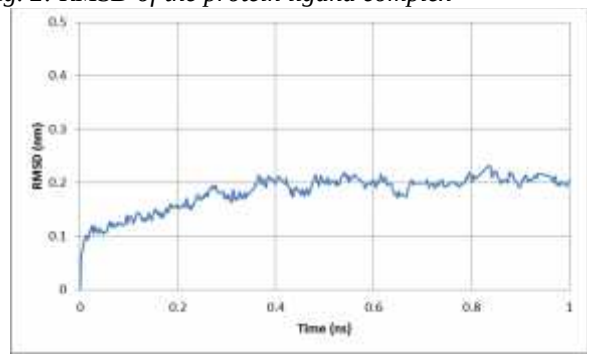
Pharmacophore guided virtual screening: Virtual screening has recently emerged as a powerful technique complementing traditional HTS technologies. Virtual screening can be broadly defined as the use of computational analysis of a database of chemical

structures to identify possible drug candidates for a specific pharmaceutical target, often a particular enzyme or receptor[13]. A fragment subset (1,389,525 compounds) and the lead like subset (6,687,370 compounds) of the ZINC small-molecule database are screened to best fit the pharmacophore model, which is constructed earlier for VEGFR-2[14]. This will reduce the time in screening the compounds which are more suitable for the active site binding. The number of compounds to be tested in lab can be decreased by eliminating the non-binding compounds *in silico*. Computer aided screening is thus a useful tool in identifying the potential compounds which can inhibit the target molecule[15]. Using this method, the collective ligand set has been brought down to 248 compounds, which have the best pharmacophoric properties with the selected hypothesis AAHRR192.

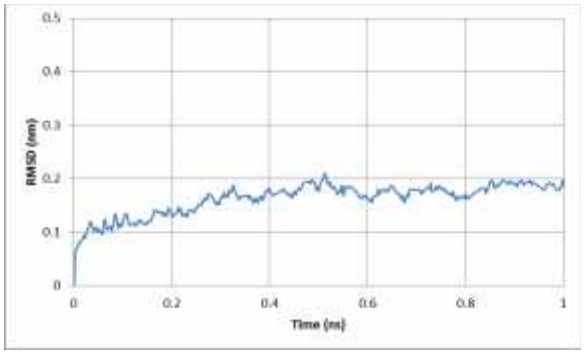
Molecular docking: Docking is a popular structure based method to study the binding mode of small molecules into protein pockets[16,17]. Molecular docking requires knowledge of the 3D structure of the bio-molecular target with or without a bound ligand, at atomic resolution. The crystal structure VEGFR-2 (PDB ID: 3VHE) is chosen for molecular docking analysis because of its highest resolution and relatively intact structure. The final subset of 248 compounds of ZINC small molecule database are docked into the active site of VEGFR-2 structure using GLIDE[18,19]. It used a hierarchical filter to rapidly score hydrophobic and polar contacts, followed by Monte Carlo sampling with the ChemScore scoring function. The protein flexibility can be incorporated into docking algorithms in various ways, including through induced fit docking, ensemble, soft docking or side chain rotamer libraries. The major challenge of docking methods is the scoring of protein ligand complexes[20,21]. Scoring functions used in docking have to compromise between complexity and simplicity, on the one hand estimating the free energy of binding as accurately as possible, on the other hand allowing efficient calculations. Most scoring functions used today show little correlation with the actual ligand binding affinity and their results are highly target-dependent[22,23,24,25].

Energy minimization and molecular dynamics: The

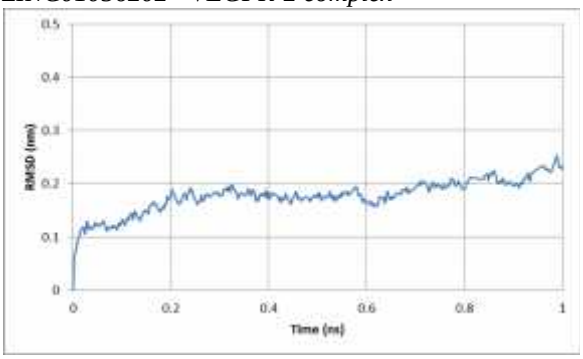
Fig. 2: RMSD of the protein ligand complex



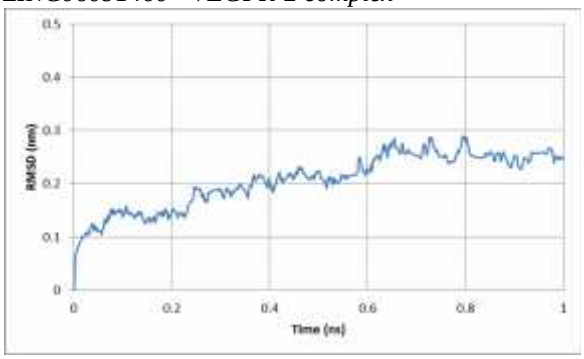
a) ZINC01056202 - VEGFR-2 complex



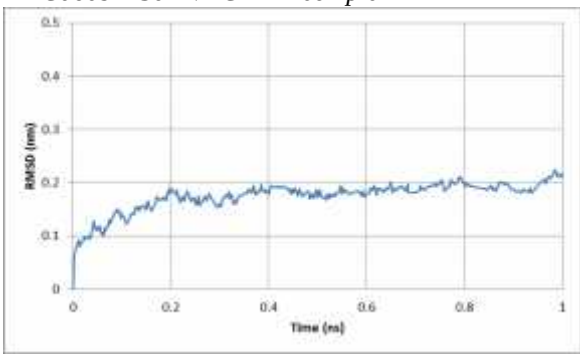
b) ZINC06091460 - VEGFR-2 complex



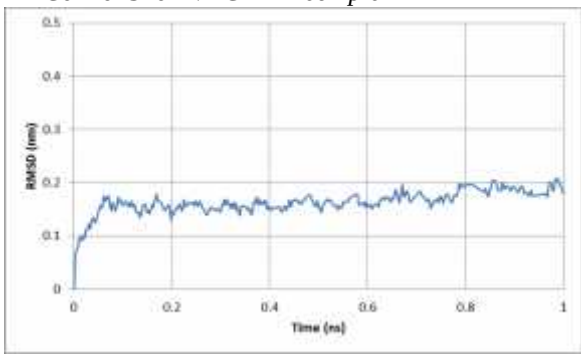
c) ZINC06091450 - VEGFR-2 complex



d) ZINC04107510 - VEGFR-2 complex



e) ZINC04623218 - VEGFR-2 complex



f) ZINC81582433 - VEGFR-2 complex

Table 1: Molecular docking scores

Ligand Name	GScore	LipophilicEvdW	PhobEn	PhobEnHB	PhobEnPairHB	HBond
ZINC01056202	-9.80	-6.26	-1.00	-1.5	0	-0.66
ZINC06091460	-9.50	-4.79	-1.53	-1.0	0	-1.83
ZINC06091450	-9.41	-4.81	-1.55	-1.0	0	-1.83
ZINC04107510	-9.23	-6.10	-1.05	-1.0	0	-0.66
ZINC04623218	-9.17	-5.20	-1.05	-1.5	0	-0.67
ZINC81582433	-9.10	-4.01	-1.20	-1.5	0	-0.95
Ligand Name	Electro	Sitemap	PiCat	ClBr	LowMW	
ZINC01056202	0.06	-0.40	0	0	-0.28	
ZINC06091460	-0.20	0.00	0	0	-0.35	
ZINC06091450	-0.07	0.00	0	0	-0.35	
ZINC04107510	0.04	-0.40	0	0	-0.34	
ZINC04623218	-0.18	-0.26	0	0	-0.48	
ZINC81582433	-0.34	-1.00	0	0	-0.39	
Ligand Name	Penalties	HBPenal	ExposPenal	RotPenal		
ZINC01056202	0	0	0	0.25		
ZINC06091460	0	0	0	0.21		
ZINC06091450	0	0	0	0.21		
ZINC04107510	0	0	0	0.27		
ZINC04623218	0	0	0	0.17		
ZINC81582433	0	0	0	0.29		

GScore - Total Glide Score; sum of XP terms. LipophilicEvdW - Lipophilic term derived from hydrophobic grid potential at the hydrophobic ligand atoms. PhobEn - Hydrophobic enclosure reward. PhobEnHB - Reward for hydrophobically packed H-bond. PhobEnPairHB - Reward for hydrophobically packed correlated H-bonds. HBond - ChemScore H-bond pair term. Electro - Electrostatic rewards; includes Coulomb and metal terms. Sitemap - Site Map ligand-receptor non-H bonding polar-hydrophobic terms. π Stack - Pi-pi stacking reward. π Cat - Reward for pi-cation interactions. ClBr - Reward for Cl or Br in a hydrophobic environment that pack against Asp or Glu. LowMW - Reward for ligands with low molecular weight. Penalties - polar atom burial and desolvation penalties and penalty for intra-ligand contacts. HBPenal - Penalty for ligands with large hydrophobic contacts and low H-bond scores. ExposPenal - Penalty for solvent-exposed ligand groups; cancels van der Waals terms. RotPenal - Rotatable bond penalty.

biological functions of a protein and ligand complex can be revealed by studying their internal motions. To completely understand the complex, not only the static structures to be used but also the dynamical information generated by simulating their internal motions or dynamic process. One of the feasible tools available in market to carry out this experiment is the GROMACS 4.6.5 molecular dynamic (MD) simulation software[26]. PODRG is used to generate the topology for ligand molecules[27]. The complex reached its lowest energy conformation within 900 ps using the united-atom GROMOS96 43A1 force field and steepest descent minimization method. The complex is equilibrated at constant temperature (300 K) and constant pressure (1 bar) using the leap-frog integrator algorithm. The Linear Constraint solver (LINCS) method is used to constrain bond lengths[28]. The MD simulations are performed for 5, 00,000 steps with time step 2 fs. The complete process is executed on Ubuntu 11.04 machine, typically taking 10 days of CPU time on a dual core 2.65 GHz processor[29,30].

RESULTS AND DISCUSSION

Pharmacophore guided virtual screening: The Nature Products and Asinex database downloaded from Zinc database are used for pharmacophore based virtual screening in the study. The database is completed using the pharmacophore AAHRR192 and the 248 best fit compounds are further used for molecular docking studies.

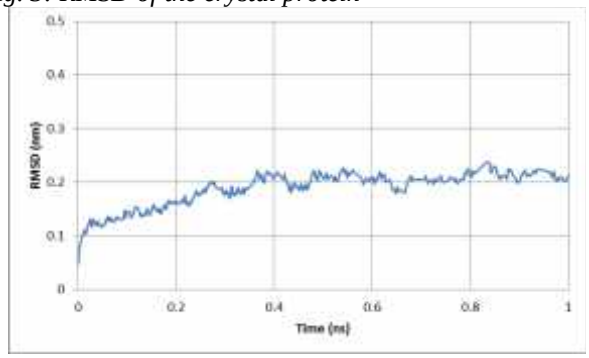
Molecular docking: Before docking analysis, to establish a good docking method and docking accuracy, it is really important to understand the rationality of docking parameters. The re-docking of the ligand into the binding pocket of receptor is the simple method to assess the performance of molecular docking. In order to study the binding mode, the 248 best fit compounds in pharmacophore guided virtual screening are docked to the active site of VEGFR-2 crystal protein. Finally, the following six compounds (ZINC01056202, ZINC06091460, ZINC06091450, ZINC04107510, ZINC04623218, and ZINC81582433), which has high glide score (Table 1) are selected for further exploratory studies. The binding mode and pharmacophore mapping of most active ligands with the VEGFR-2 protein are illustrated in figure 1.

The hydroxyl groups in some of the ligand form hydrogen group with negatively charged ASP1046 amino acid. On other hand, the there is a clear evidence for the formation cation- π interaction with aromatic rings in the ligand. In addition, the hydrophobic and hydrophilic interaction also plays an important role in binding. The benzene ring of the compounds is a hydrophobic group; it formed hydrophobic interaction with residues around it, such as ALA866, PHE1047, CYS919, PHE918, VAL848, and CYS1045. There are also other forms of hydrophobic interactions seen around the residues like; LEU889, ALA866; with the nitrogen containing six-membered ring. In most of the scenarios, the hydrophobic

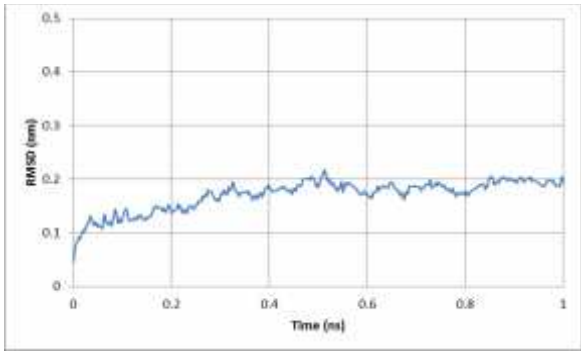
interactions favor the strong affinity of the ligands with the protein moiety. There are also instances that there is slight occurrence of metal interaction in the complex. It also has been observed that the hydrogen bonds strengthen the interaction of protein and the ligand. It is of particular interest that the above six ligands showed the most potent anticancer effect by blocking the activity of VEGFR-2 signals.

Molecular dynamics: The root-mean-square deviation (RMSD) is the measure of the average distance between the atoms (usually the backbone atoms) of superimposed proteins. To examine the conformational stability of the protein ligand complex within a solvent system, the RMSD of is calculated after 1 ns (Fig. 2). It can be clearly

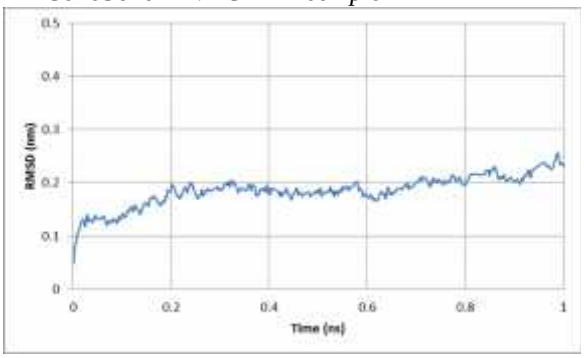
Fig. 3: RMSD of the crystal protein



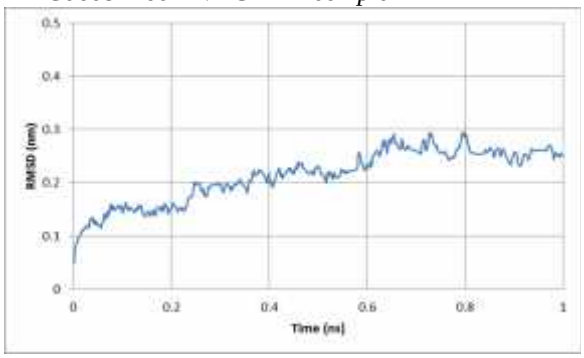
a) ZINC01056202 - VEGFR-2 complex



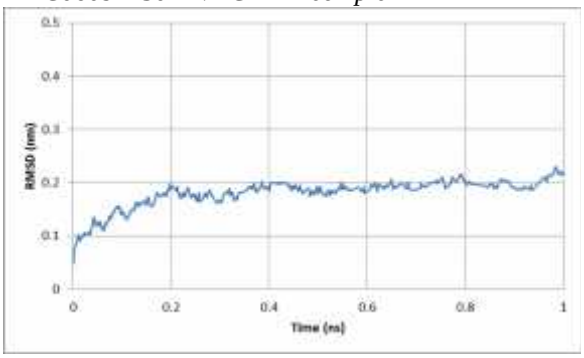
b) ZINC06091460 - VEGFR-2 complex



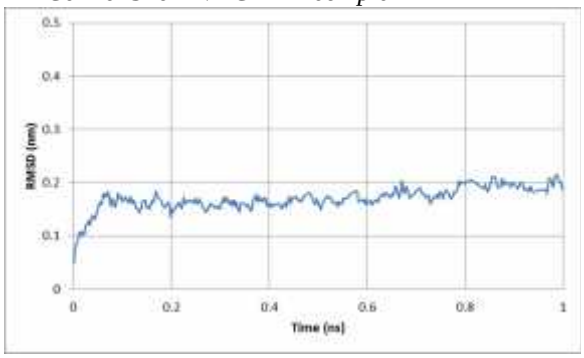
c) ZINC06091450 - VEGFR-2 complex



d) ZINC04107510 - VEGFR-2 complex

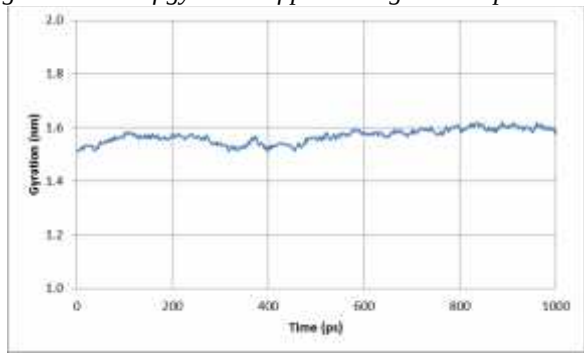


e) ZINC04623218 - VEGFR-2 complex

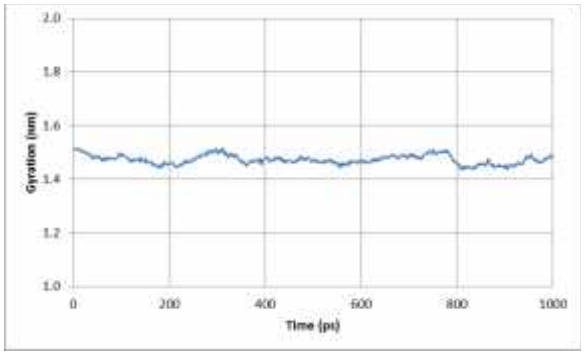


f) ZINC81582433 - VEGFR-2 complex

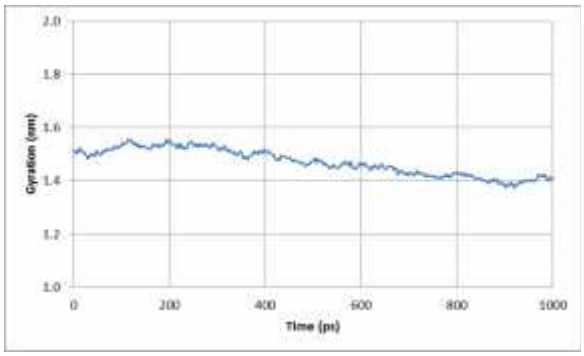
Fig. 4: Radius of gyration of protein ligand complex



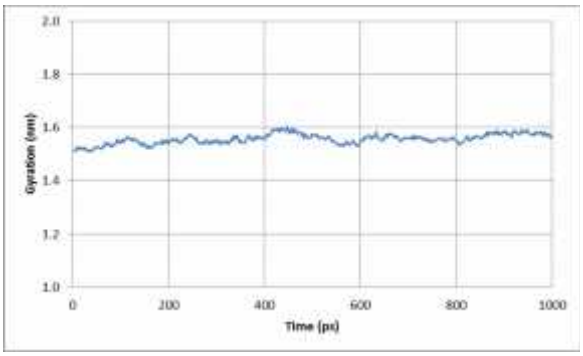
a) ZINC01056202 - VEGFR-2 complex



b) ZINC06091460 - VEGFR-2 complex



c) ZINC06091450 - VEGFR-2 complex



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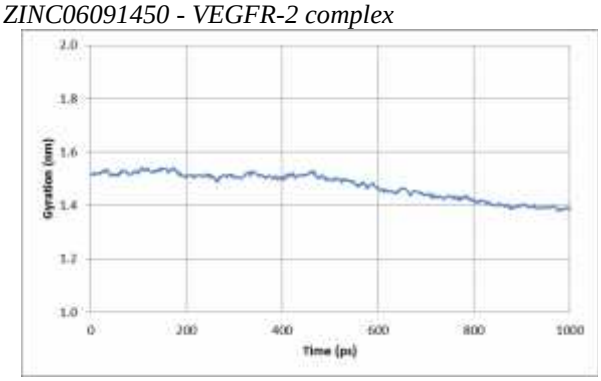
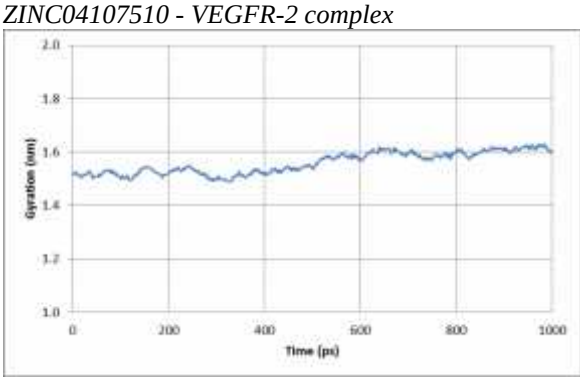
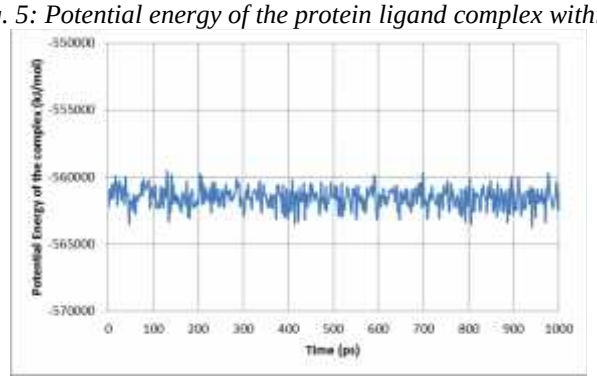


e) ZINC04623218 - VEGFR-2 complex

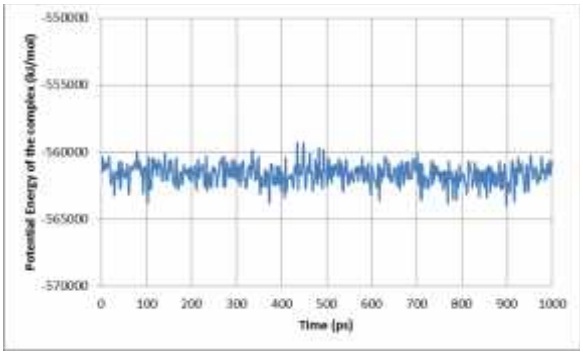


f) ZINC81582433 - VEGFR-2 complex

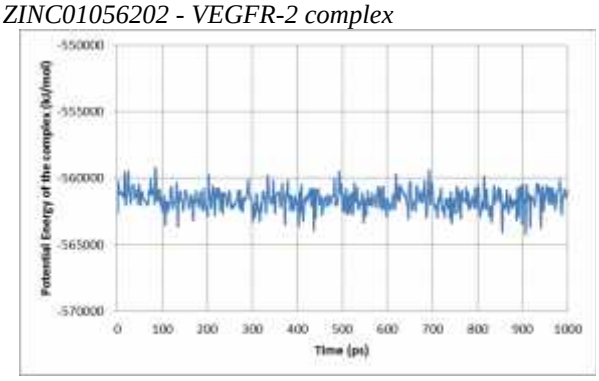
Fig. 5: Potential energy of the protein ligand complex within a solvent



a) ZINC01056202 - VEGFR-2 complex



b) ZINC06091460 - VEGFR-2 complex



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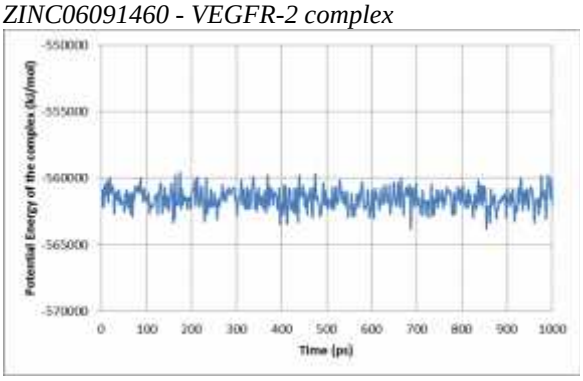
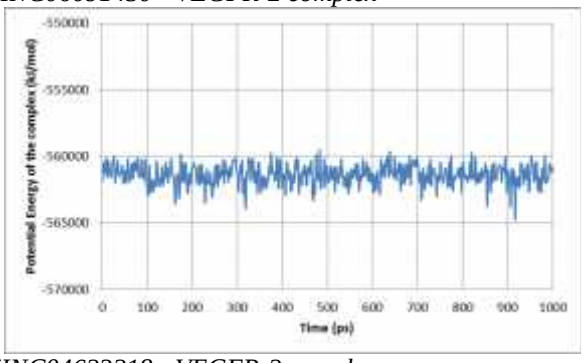


d) ZINC04107510 - VEGFR-2 complex



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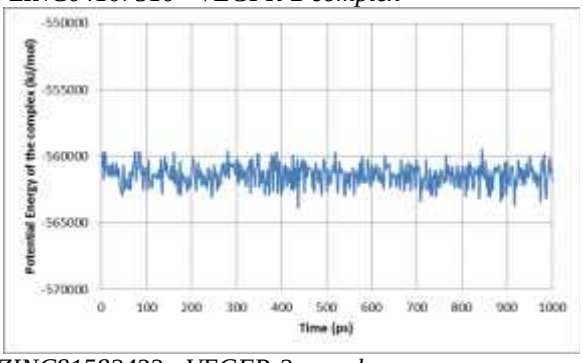


f) ZINC81582433 - VEGFR-2 complex

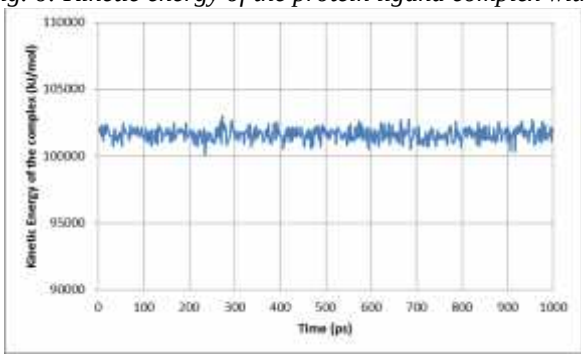
seen that the complex unfolds itself after on an average of 15 ps to attain its conformational stability. In most cases, the final stability is after 200 ps. In order to double check the stability of protein itself in each interaction, the RMSD is calculated for the crystal protein (Fig. 3) as well. It also confirms the stability of complex as the RMSD of the crystal protein is almost in correlation with the RMSD of the complex. The subtle difference between the plots in Fig. 2 and Fig. 3 are slightly different as it is expected since it has energy minimized and the position restraints are not 100% perfect. Though there are minor differences in the RMSD, the overall stability of the complex is not compromised during the simulation.

Radius of gyration or gyradius is the name of several related measures of the size of an object, a surface, or an ensemble of points. It is calculated as the root mean square distance of the objects' parts from either its center of gravity or a given axis. It also tells about the compactness of the protein. As illustrated in Fig. 4, the protein is stably folded over time indicating a positive effect that the complex is stable within a solvent. The plot of potential and kinetic energy (Fig. 5 and 6) of the complex indicates that protein is stable during the simulation period of 1 ns. All the above measures give strong evidence that those six ligands are more intact and stable within a solvent.

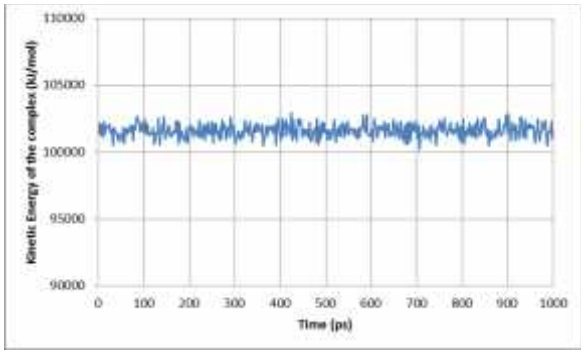
CONCLUSION

Drug resistance is always a major concern in the development of targeted agents. The effective treatment may be produced in the combination of different targeted agents, chemotherapies in cancer treatment. Stepwise pharmacophore based virtual screening of databases such as ZINC has resulted in new scaffolds for developing VEGFR-2 kinase inhibitors. The Protein-Ligand interaction plays an important role in structural based drug designing. In the present work, the receptor VEGFR-2 and the identified ligands have a strong interaction; and therefore can be used in development of anti-cancer treatments. When the VEGFR-2 receptor is docked with the six ligands (ZINC01056202, ZINC06091460, ZINC06091450, ZINC04107510,

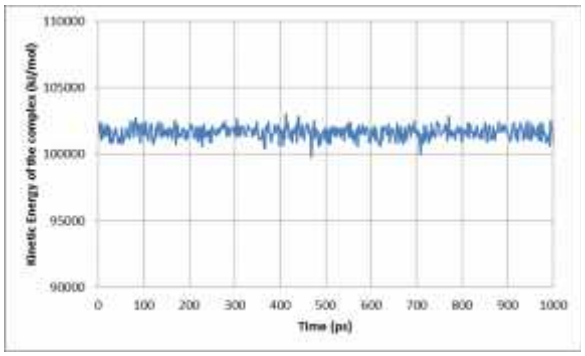
Fig. 6: Kinetic energy of the protein ligand complex within a solvent



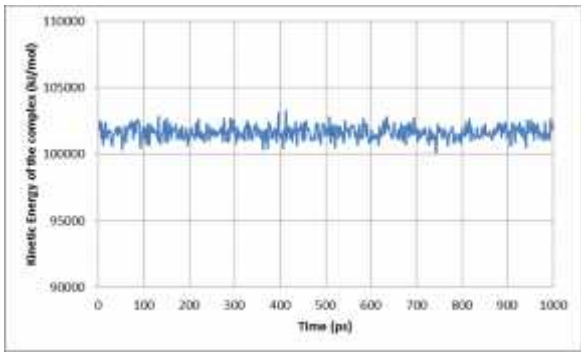
a) ZINC01056202 - VEGFR-2 complex



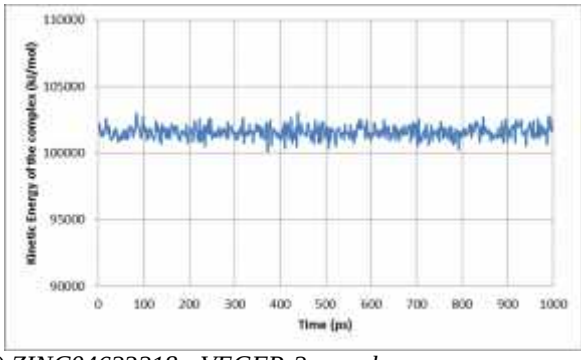
b) ZINC06091460 - VEGFR-2 complex



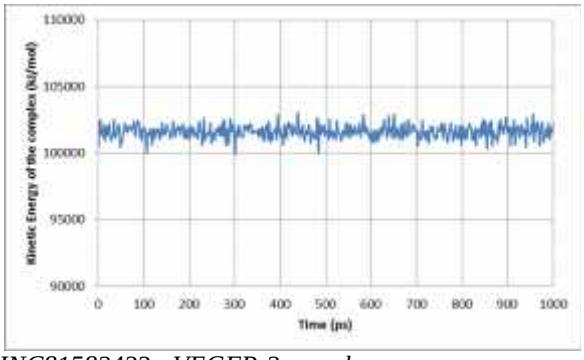
c) ZINC06091450 - VEGFR-2 complex



d) ZINC04107510 - VEGFR-2 complex



e) ZINC04623218 - VEGFR-2 complex



f) ZINC81582433 - VEGFR-2 complex

ZINC04623218, and ZINC81582433); the scores indicated them that they are more potent in blocking the VEGFR-2 signals. The molecular dynamics study also favored the stability of the complex within a solvent. From this, it has been concluded that some of the modified drugs are better than the commercial drugs available in the market. In future research work, the free energy of those ligands will be carried out to study the free energies of solvation/hydration and free energy of binding for a small molecule to larger VEGFR-2 receptor biomolecule.

ACKNOWLEDGEMENT

The authors would like to thank the Chancellor, Chief Executive Officer, Vice Chancellor, and Registrar of Karpagam University for providing facilities and encouragement; and Dr. Raghu, EO, Schrödinger for providing software support.

DECLARATION OF INTEREST

The authors report no declaration of interest.

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