Garima Saxena et al /J. Pharm. Sci. & Res. Vol. 11(7), 2019, 2495-2503

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Molecular modeling and dynamics simulation studies of screened natural compounds: *1'-(4-(tert-butyl)benzoyl)spiro[chroman-2,4'-piperidin]-4-one* and *3-(4-methylthiazol-2-yl)-2-oxo-2h-chromen-7-yl pivalate* targeting VEGFR-2 protein as potential anti-angiogenic agents

Garima Saxena[a,b], Mala Sharma[b,c], Neha Sharma[a,b], M Kalim A Khan[a,b], M Haris Siddiqui[a,b], Salman Akhtar[a,b,d*]

[a]*Department of Bioengineering, Integral University, Lucknow, India*
[b]*Advanced Centre of Bioengineering and Bioinformatics, Integral Information and Research Centre, Integral University, Lucknow*
[c]*Department of Biosciences, Integral University, Lucknow, India*
[d]*Novel Global Community Educational Foundation7, Peterlee Place, Hebersham, NSW 2770, Australia*

Abstract

Natural compounds, *compound 'B'* and *compound 'C'* have been proposed as potential anti-angiogenic lead against target protein VEGFR-2 combating angiogenesis phenomenon. Through Virtual screening, Lipinski, ADME and toxicity filtering analysis, these two compounds were selected from a large dataset of 2029 compounds in comparison with standard control *compound 'A'*. Further they were subjected to molecular docking and simulation studies against protein VEGFR-2. Docking studies revealed its best pose of binding to form a complex with protein and simulation studies revealed its stability for a given period of time. Among in-depth molecular analysis and interactional contexts, both compounds in comparison to standard were found in better results with docking and molecular dynamics study and have been suggested as prominent anti-angiogenic leads for further analysis.

Abbreviation: compound B is *(1'-(4-(tert-butyl)benzoyl)spiro[chroman-2,4'-piperidin]-4-one*, compound C is *3-(4-methylthiazol-2-yl)-2 oxo-2h-chromen-7-yl pivalate* and compound A is *4,5,6,11-tetrahydro-1H-pyrazolo[4',3':6,7]cyclohepta[1,2-b]indole.*

Keywords: angiogenisis, anticancer lead compound, lipinski, molecular docking, simulation, toxicity prediction, VEGFR-2.

1. INTRODUCTION

Cancer is a miserable problem; spread incorrigibly with growth of anomalous cells by discounting the basic injunction of cellular division in human body. Cell's life, division and metamorphose in another cell depends on normal cells, because normal cells are responsible for creating contact to signals regularly while these signals also assist cancerous cells in originating a magnitude of sovereignty which ensures an unsuppressed thriving and escalation which if sustains can turn into malignant. It has been surveyed that approximately 90 % cases of deaths is caused by escalation of tumor i.e. metastasis [1]. Recent studies reveal the entanglement of angiogenesis (emergence of new capillaries from old ones) in the generation of metastasis and malignant tumors tremendously.

Angiogenesis is a very composite exercise, convoluted in different anatomical operations and compactly monitored at cellular level through the involvement of receptors, growth factors, extracellular matrix (ECM) etc. Angiogenesis originates primarily from the deficiency of oxygen in living environment i.e. hypoxia which results into the production and excretion of VEGF (vascular endothelial growth factor) [2]. VEGF is a key determinant which instigates angiogenesis [3] as it concludes multiple qualitative features to stimulate angiogenesis by its undeviating consequences.

There are different types of VEGF's *viz.* A, B, C, D as well as PIGF (placental growth factor) in VEGF family among which VEGF A plays prominent role in angiogenesis. Its characteristically functions across tyrosine kinase VEGFR-2 (VEGF receptor 2) while the functionality of VEGFR 1 (VEGF receptor 1) is comparatively indistinct [4].VEGFR-2 is comprised of *KDR* gene and is crucially responsible for its manifestation in vascular endothelial cells[5].This protein is called by different names suchlike Flk-1 (fatal liver kinase 1), CD309 and KDR (kinase insert domain receptor) in the aggregation of contrast nomenclature of haematogenic substance. From premature incurable growth to its manifestation in some of vascular endothelial cells, it is found to be very actively

manifested in new vascular tumor endothelial cells rather than in normal ones. [6, 7]

VEGFR-2 has been noticed as a promising target for anti-angiogenesis in recent researches. [8] Based on previous studies, multiple VEGFR-2 inhibitors including synthetic and natural compounds are subjected up to clinical trial levels and are found to lower anticancer activities with reduced toxicity [9]. It has also been observed, that thousands of natural compounds are best to focus in to find a suitable compound with enhanced anticancer activity and lowered toxicities.

Natural compounds have already been established to be used to clinically cure multiple diseases [10], as they are non-nutritious, multi-actionable, bio adjustable and are a known hub of inherent drug leads. [11, 12, 13] Natural compounds can be derived from varied resources like fungi, plants, marine environment, algae, sponges, and from other natural reservoirs. [10]

In the current study, *compound A* has been chosen as a standard control (mol weight 223.27 g/mol) [14] for comparative analysis with a database of 2029 natural compounds to search for a better lead compound by applying advanced drug discovery approaches. In this process *Compound B* and *compound C* retrieved from Natural Product-like Compounds database have been suggested to posses' optimum properties and stability against VEGFR-2 protein as compared to the standard drug and suggested for further analysis.

2. EXPERIMENTAL SECTION

2.1 Materials

Multiple online and offline software and tool have been used *viz.* Discovery Studio Visualizer, PreADMET, Chimera 1.12, AutoDock 4.2, PRODRG and Gromacs-4.0.5.

2.2 Method

2.2.1 Retrieval of Protein and Selection of Compounds

In this study, target protein VEGFR-2along with its standard inhibitor *(compound A)* was retrieved from RCSB PDB (3VID) having sequence length of 356 amino acid. The selection was made on the basis of properties such as resolution (2.3 Å) predicted by X-RAY diffraction method, R-Value free/work (0.281/0.245) and non-mutagenicity. To find a best lead (targeting VEGFR-2),a database of natural compounds named Natural Product-like Compounds database consisting 2029 natural compounds along with their SDF file and all other properties was downloaded for analysis.

2.2.2 Minimization of Complex structure

Before proceeding further for minimization of protein, its attached inhibitor was separated as necessary to make structure stable and for this Chimera 1.12 was used.[15]

2.2.3 Drug-Likeliness Prediction

Christopher A. Lipinski described Lipinski's rule (Rule of five) to determine drug likeliness of compounds by their pharmacokinetics based on physical and chemical properties to make compounds orally active. [16, 17] This rule outlines chief molecular features or properties to trace pharmacokinetics of compounds and also considers their absorption, distribution, metabolism, and excretion i.e. ADME narrating the configuration of compounds in human body. Though, this rule does not define whether compound is clinically active or not, Lipinski's rule affirms these criteria for compound's credibility:

Table 1 Lipinski's parameters.

Properties	Values
H-Bond Donors	<5
H-Bond Acceptors	<10
Molecular Weight	<500 Daltons
miLogP	<5

On the basis of these parameters of Lipinski, all compounds were filtered on the basis of molecular descriptor calculator program. All calculated compounds were applied to another module named ADME descriptor for prediction of ADME properties. The basic parameters of ADME [18] are mentioned in table 2:

Table 2 ADME Properties.

ADMET Descriptors	Values
ADMET_BBB_level (Blood Brain Barrier)	<=2
ADMET_Solubility_level	<=3
ADMET_Absorption_level	<=1
ADMET_CYP2D6	=0
ADMET_PPB_level (Plasma Protein Binding)	=0
ADMET_Hepatotoxicity	=0

2.2.4 Toxicity Prediction:

Prediction of a drug molecule proceeds through multiple processes, in which few approaches give positive results and exceptionally approx. half of drugs fails due to limitation of ADME prediction in developmental phase. Though, there are some *in vitro* techniques to overcome this failure during drug's development but it is a much known fact that they all are very time taking. Hence, an online tool named PreADMET was introduced to give a quick response for the prediction of drug. This tool includes some key feature to define toxicity of compounds for further analysis. [https://preadmet.bmdrc.kr/]

2.2.5 Molecular Docking: Prior to MD simulation, all filtered compounds were driven to molecular docking

analysis with target protein VEGFR-2 along with its standard inhibitor under an offline program AutoDock 4.2. Docking analysis defines most favorable conformation of a compound bound with protein to form a protein-ligand complex. [19] Basically, docking analysis involve four major steps, they are as follows:

- **Macromolecule Preparation:** In this step, minimized protein was prepared by adding polar hydrogen, removing redundant water molecules and creating protein.pdbqt file.
- **Ligand Preparation:** In this step, all compounds along with the standard one was prepared separately by setting their torsion range and saving them all in ligand.pdbqt file separately on AutoDock 4.2.
- **Grid box:** In this step, with respect to flexible docking a grid box was defined over the structure by setting their default values for flexible docking by generating grid.gpf file.
- **Docking Parameters:** In this step, genetic algorithm run was set to run docking by creating dock.dpf file.
- **Grid/Dock Run:** This final step involves, run analysis of grid.gpf and dock.dpf file one by one which results into grid.glg and dock.dlg file respectively.

2.2.6 Molecular Dynamics Simulation: MD simulation is a computational approach, used to predict variability of molecules by making trajectories. [20,21,22] In this study, best posed compounds on the basis of highest binding energy with target protein were retrieved separately after docking analysis and subjected to simulation analysis using GROMACS 4.0.5. Before proceeding to MD simulation, selected compound's topology and gro file were generated on PRODRG (an online server), then further moving to dynamicsGROMOS96 43a1 force field was applied to form protein ligand complex and after adding ions next subjected to energy minimization. Equilibration of system was completed by running NVT (volume regulation) and NPT (pressure regulation) respectively after energy minimization. Finally, 10 nanosecond MD run was applied with a leap-frog integrator of a step size of 2 fs during over all MD run and save result at the interval of every 2 picosecond for stability analysis.

. RESULTS AND DISCUSSION

3.1Drug-likeliness study

By applying Lipinski's rule of five and ADME respectively on all 2029 compounds of selected database, 22 best compounds were filtered out by cross-checking their properties with standard compound on the basis of their pharmacological and pharmacokinetic study. As mentioned above, all standards of Lipinski and ADME are used to define the characteristics of compounds to select the drug-like compounds for further analysis. The list of filtered compounds from the database of 2029 compounds along with the standard one is given below in table 3:

The result shows best fit value of Lipinski and ADME of 22 filtered compounds comparable to standard compound in consensus with band limits for H-bond acceptor, donor, molecular weight, miLogP components etc. as mentioned in the table 1. In this table, *compound B* and *compound C* showed best values for miLogP, molecular weight, H-bond acceptor and H-bond donor against the standard *compound A*. Similarly, in ADME (absorption, distribution, metabolism and excretion) analysis these two compounds were found to be in better consensus than standard, describing the pharmacokinetic and dynamic configurations of compound into a human body. ADME's components blood-brain barrier, absorption level, solubility level, hepatotoxicity and CYP2D6 mentioned in table 2, are described as:

- **Blood-Brain Barrier**, regulates the transmission of matter and deliquescent macromolecule in blood and CSF (cerebrospinal fluid) respectively, and permits aqua phobic macromolecules and micro charged molecules. [23]
- **Absorption level**, unfavorably enumerates bio availability of compounds.
- **Solubility level**, one of the key features to clinch suitable accumulation of compound in regular motion for pharmacologically appropriate retaliation. [24]
- **Hepatotoxicity**,a stage of impairment of liver by toxic materials. [25]
- **CYP2D6**, (cytochrome P450 2D6) a key enzyme associates compound's bioactivity in human body, also involved in bioactivity and eradication of few old compounds. [26]
- **Plasma Protein Binding (PPB)**, influences compound's durability in human body and terminates compound's effectiveness too.

3.2 Toxicity Prediction

Toxicity of compounds was predicted by an online tool PreADMET, defining whether the compound is mutagenic/carcinogenic or not, by Ames test and hERG inhibition test values. All 22 best resulted compounds in previous analysis along with the standard inhibitor were subjected to toxicity profiling one by one on PreADMET, here the list of all compounds with their obtained values are mentioned in table 4:

Table 3 Compounds showing best results for Lipinski and ADME.

Compounds	Lipinski's Rule				ADME					
	miLogP	Mol_weight	H_Acceptors	H_Donors	BBB_Level	Absorption_ Level	Solubility_ Level	Hepatotoxicity	CYP2D6	PPB_Level
Compound A	**1.905**	**319.395**	**4**	**1**	**2**	**0**	**3**	**0**	**0**	**0**
(3,4-dimethoxyphenyl)(4a-hydroxyoctahydroisoquinolin-2(1H)-yl)methanone	2.758	337.454	5	1	2	0	3	0	0	0
1-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-(tert-pentyloxy)propan-2-ol hydrochloride	2.345	381.422	5	0	2	0	3	0	0	0
1'-(3,4-dimethoxybenzoyl)spiro[chroman-2,4'-piperidin]-4-one	2.96	301.38	3	1	2	0	2	0	0	0
3-(indoline-1-carbonyl)-1,2,2-trimethylcyclopentanecarboxylic acid	3.779	377.476	3	0	1	0	2	0	0	0
Compound B	**3.72**	**334.364**	**5**	**0**	**2**	**0**	**2**	**0**	**0**	**0**
ethyl 2-(tert-butyl)-5-(2-methoxy-2-oxoethoxy)benzofuran-3-carboxylate	3.64	409.356	6	1	2	0	2	0	0	0
8-((dimethylamino)methyl)-7-hydroxy-3-(3-methoxyphenoxy)-2-(trifluoromethyl)-4H-chromen-4-one	3.318	341.401	5	0	2	0	2	0	0	0
methyl 1-((2-oxo-2,6,7,8-tetrahydrocyclopenta[g]chromen-4-yl)methyl)piperidine-4-carboxylate	3.096	329.39	5	0	2	0	2	0	0	0
ethyl 1-((7-methyl-2-oxo-2H-chromen-4-yl)methyl)piperidine-4-carboxylate	3.666	355.427	5	0	1	0	2	0	0	0
ethyl 1-((2-oxo-2,6,7,8-tetrahydrocyclopenta[g]chromen-4-yl)methyl)piperidine-4-carboxylate	2.854	345.39	6	1	2	0	3	0	0	0
ethyl 1-((6-hydroxy-7-methyl-2-oxo-2H-chromen-4-yl)methyl)piperidine-4-carboxylate	2.814	283.322	4	0	2	0	2	0	0	0
10-acetyl-2,3,6,7-tetrahydro-1H,5H,11H-pyrano[2,3-f]pyrido[3,2,1-ij]quinolin-11-one	3.975	285.381	3	0	1	0	2	0	0	0
4-((3,5-dimethylpiperidin-1-yl)methyl)-7-methyl-2H-chromen-2-one	2.593	345.39	6	0	2	0	3	0	0	0
ethyl 1-((7-methoxy-2-oxo-2H-chromen-4-yl)methyl)piperidine-4-carboxylate	2.232	289.369	3	1	2	0	3	0	0	0
3-hydroxy-1-isopentyl-5-methyl-3-(2-oxopropyl)indolin-2-one	2.232	289.369	3	1	2	0	3	0	0	0
3-hydroxy-1-isopentyl-7-methyl-3-(2-oxopropyl)indolin-2-one	3.582	343.417	5	0	1	0	2	0	0	0
ethyl 1-((6,7-dimethyl-2-oxo-2H-chromen-4-yl)methyl)piperidine-4-carboxylate	2.854	345.39	6	1	2	0	3	0	0	0
ethyl 1-((7-hydroxy-8-methyl-2-oxo-2H-chromen-4-yl)methyl)piperidine-4-carboxylate	3.032	365.808	6	1	2	0	2	0	0	0
ethyl 1-((6-chloro-7-hydroxy-2-oxo-2H-chromen-4-yl)methyl)piperidine-4-carboxylate	3.837	343.397	5	0	2	0	2	0	0	0
Compound C	**3.284**	**288.295**	**5**	**1**	**2**	**0**	**2**	**0**	**0**	**0**
2-((4-methyl-6-oxo-7,8,9,10-tetrahydro-6H-benzo[c]chromen-3-yl)oxy)acetic acid	3.587	340.391	5	0	2	0	2	0	0	0
ethyl 2-(tert-butyl)-5-((methylsulfonyl)oxy)benzofuran-3-carboxylate	1.905	319.395	4	1	2	0	3	0	0	0

Table 4 Toxicity profiling

Compound	Ames_test	Carcino_Mouse	Carcino_Rat	hERG_Inhibition
Compound A	**Mutagen**	**Positive**	**Negative**	**Medium_risk**
(3,4-dimethoxyphenyl)(4a-hydroxyoctahydroisoquinolin-2(1H)-yl)methanone	Mutagen	Negative	Negative	Low_risk
1-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-(tert-pentyloxy)propan-2-ol hydrochloride	Non-mutagen	Negative	Negative	Low_risk
1'-(3,4-dimethoxybenzoyl)spiro[chroman-2,4'-piperidin]-4-one	Mutagen	Negative	Positive	Medium_risk
3-(indoline-1-carbonyl)-1,2,2-trimethylcyclopentanecarboxylic acid	Mutagen	Negative	Negative	Low_risk
Compound B	**Non-mutagen**	**Negative**	**Negative**	**Medium_risk**
ethyl 2-(tert-butyl)-5-(2-methoxy-2-oxoethoxy)benzofuran-3-carboxylate	Mutagen	Negative	Negative	Low_risk
8-((dimethylamino)methyl)-7-hydroxy-3-(3-methoxyphenoxy)-2-(trifluoromethyl)-4H-chromen-4-one	Mutagen	Positive	Negative	Medium_risk
methyl 1-((2-oxo-2,6,7,8-tetrahydrocyclopenta[g]chromen-4-yl)methyl)piperidine-4-carboxylate	Mutagen	Negative	Negative	Low_risk
ethyl 1-((7-methyl-2-oxo-2H-chromen-4-yl)methyl)piperidine-4-carboxylate	Mutagen	Negative	Negative	Low_risk
ethyl 1-((2-oxo-2,6,7,8-tetrahydrocyclopenta[g]chromen-4-yl)methyl)piperidine-4-carboxylate	Mutagen	Negative	Negative	Low_risk
ethyl 1-((6-hydroxy-7-methyl-2-oxo-2H-chromen-4-yl)methyl)piperidine-4-carboxylate	Mutagen	Negative	Negative	Low_risk
10-acetyl-2,3,6,7-tetrahydro-1H,5H,11H-pyrano[2,3-f]pyrido[3,2,1-ij]quinolin-11-one	Mutagen	Negative	Negative	Medium_risk
4-((3,5-dimethylpiperidin-1-yl)methyl)-7-methyl-2H-chromen-2-one	Mutagen	Negative	Negative	Medium_risk
ethyl 1-((7-methoxy-2-oxo-2H-chromen-4-yl)methyl)piperidine-4-carboxylate	Mutagen	Negative	Negative	Low_risk
3-hydroxy-1-isopentyl-5-methyl-3-(2-oxopropyl)indolin-2-one	Non-mutagen	Negative	Negative	Low_risk
3-hydroxy-1-isopentyl-7-methyl-3-(2-oxopropyl)indolin-2-one	Non-mutagen	Negative	Negative	Low_risk
ethyl 1-((6,7-dimethyl-2-oxo-2H-chromen-4-yl)methyl)piperidine-4-carboxylate	Mutagen	Negative	Negative	Low_risk
ethyl 1-((7-hydroxy-8-methyl-2-oxo-2H-chromen-4-yl)methyl)piperidine-4-carboxylate	Mutagen	Negative	Negative	Low_risk
ethyl 1-((6-chloro-7-hydroxy-2-oxo-2H-chromen-4-yl)methyl)piperidine-4-carboxylate	Mutagen	Negative	Negative	Low_risk
Compound C	**Non-mutagen**	**Negative**	**Negative**	**Medium_risk**
2-((4-methyl-6-oxo-7,8,9,10-tetrahydro-6H-benzo[c]chromen-3-yl)oxy)acetic acid	Mutagen	Negative	Negative	Low_risk
ethyl 2-(tert-butyl)-5-((methylsulfonyl)oxy)benzofuran-3-carboxylate	Mutagen	Negative	Negative	Low_risk

This study reflected specially the filtered compound's *B* and *C* showing no toxicity as compared to standard *compound A*. In this analysis, *compound A* is showing mutagenicity with medium risk of hERG inhibition along with carcinogenicity and both filtered compounds are having non-mutagenicity and non-carcinogenicity with medium risk.

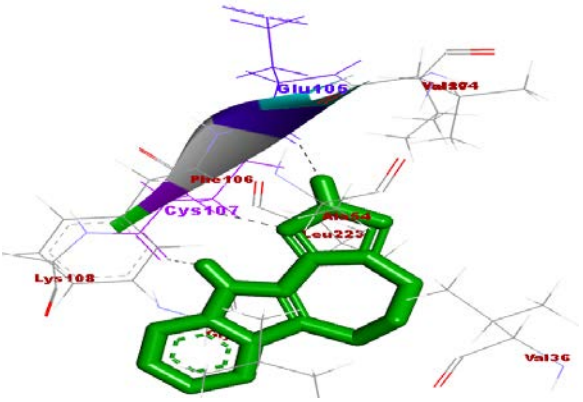
Table 5 Docking result: showing binding energy and inhibition constant of best compounds in comparison with standard

Compounds	Binding Energy (kcal/mol)	Inhibition Constant (uM/nM)
Compound A	-7.45	3.45 uM
Compound B	-9.87	58.22 nM
Compound C	-8.28	854.63 nM

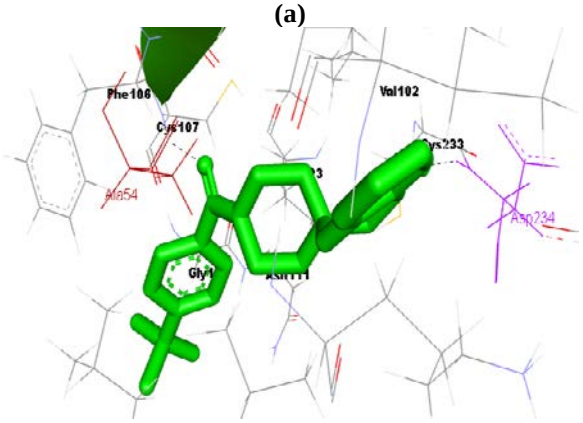
3.3 Docking Analysis

In this step, docking analysis based on flexible grid was performed for selected 2 compounds *B* and *C* from the above study along with standard *compound A*with VEGFR-2 protein using AutoDock 4.2 program. The retrieved binding energy and inhibition constant of standard compound as well as both filtered compound with VEGFR-2 after 25 genetic algorithm runs is given below in table 5:

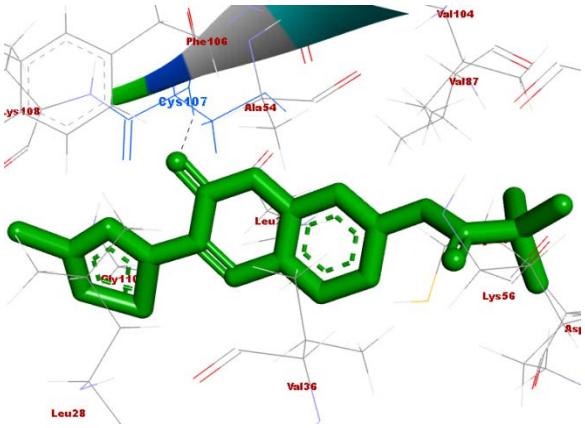
This result shows the comparative analysis of binding energies and inhibition constant of filtered best two *compounds B* and *C* from the database and the standard *compound A* docked with VEGFR-2. Both compound *B* and *C* are shown to outperform the standard on the set docking parameters. Further hydrogen bond interaction studies under docking analysis are given below-



(a)



(b)



(c)

Figure 1: Hydrogen bond interaction in docked complex with VEGFR2.
(a) Compound A, (b) Compound B, (c) Compound C

Figure 1 is showing the H-bond interaction highlighted particularly in each image for all the three compounds *A*, *B* and *C* along with their distances. Discussing about the number of H-bonds found along with distance, here is a tabular representation of all three compounds-

Table 6 Compounds showing their H-bond interactions with distance

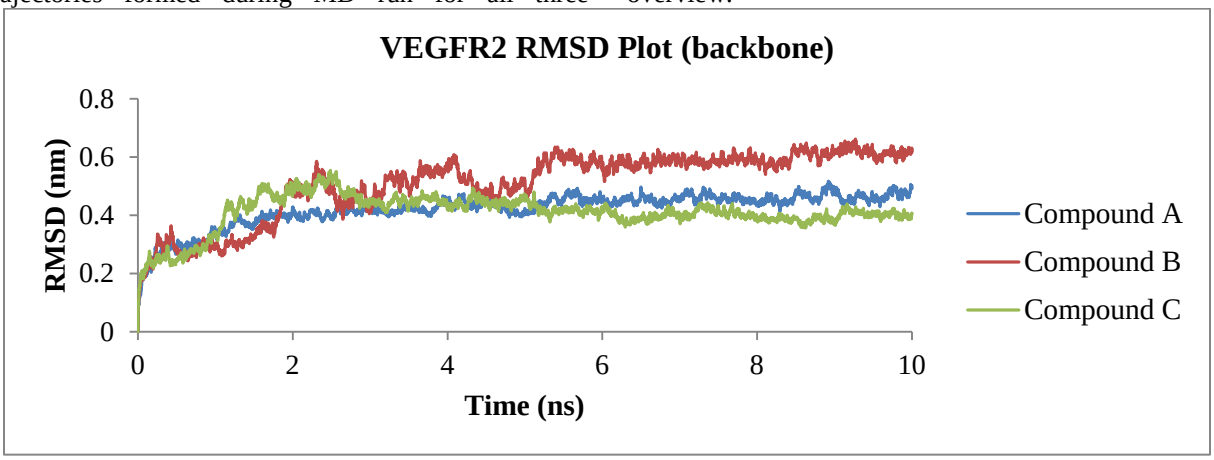
Compound	H-Bond Interaction	Distance (Å)
Compound A	CYS107:H – UNK2001:N8 UNK2001:H4 - :CYS107:O UNK2001:H14 - :GLU105:O	1.964 2.215 1.777
Compound B	CYS107:H - :UNL1:O ASP234:H - :UNL1:O	1.654 1.627
Compound C	CYS107:H- :UNL1:O	1.568

Similarly based on the previous studies, the intermolecular distances are sought to be always be less than 3 Å. [27] Both the compounds *B* and *C* along with the standard compound *A*are set to having intermolecular distances (H-bond)less than 3 Å. The selected compounds along with standard compound were subjected to stability check under simulation studies for further analysis.

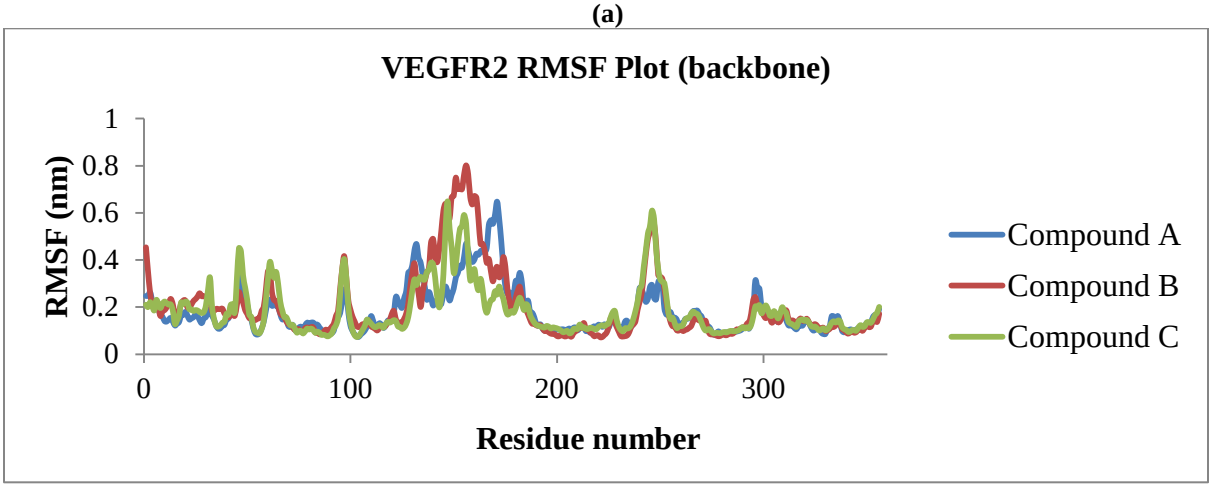
3.4 Simulation Study

MD simulation study was performed for 10 nanoseconds on all three compounds with VEGFR-2 to check complex stability on GROMACS-4.0.5. Root-

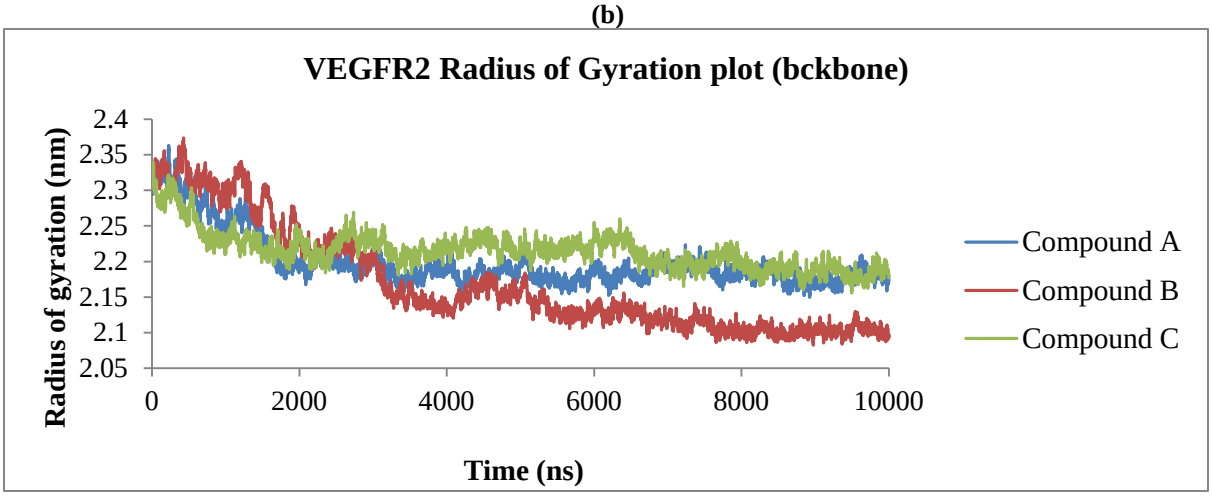
Mean Square Deviation (RMSD), Root-Mean Square Fluctuation (RMSF) and Radius of gyration was calculated to study the time dependent behavior of trajectories formed during MD run for all three compounds. The RMSD, Radius of gyration and RMSF graph plot (backbone) representation of all three compounds with VEGFR-2 is given below for detailed overview.



(a)



(b)



(c)

Figure 2: Evaluation of backbone studies.
(a) RMSD plot of protein-ligand complexes.
(b) Radius of Gyration plot of protein-ligand complexes.
(c) RMSF plot of protein-ligand complexes.

In figure 2, all three compounds *A*, *B* and *C* are showing their best region of stability in all aspects like RMSD, RMSF and Radius of gyration on the basis of their backbone molecules. Plot (a) is representing RMSD values in nanometer to calculate the equilibration of generated trajectories for all three compounds and showing the comparison of their longest stability among them, all three compounds (*A*, *B* and *C*) are equilibrated from 0.4 to 0.6 nm for 5 to 10 nanosecond. These compounds are showing high stability to protein at same point and remain equilibrated. To check the flexibility of all three compounds, RMSF was calculated (plot (b)) which indicates whether the compound is highly flexible by its high peak fluctuation or showing inadequate activity by its low peak during MD run. Though compound B and compound C are showing high flexibility of about 0.6 to 0.8 nm on residue number 150, and again both are showing a high peak of 0.6 nm on residue number 250 while they are also showed fluctuation multiple times in comparison to compound *A*. Radius of gyration was calculated to check how dense the protein is by its folding and unfolding. Stable folding defined by a constant R*g* value shows if protein get unfold then the R*g* value will alter repeatedly. Thus in plot (c) protein remains stable for some time of about 0 to 2000 nanosecond with 2.3 to 2.5 nm R*g* for all three compounds, then it is showing longest stability on 2.2 to 2.3 nm R*g* from 3000 to 10000 nanosecond. Finally, it was forecasted that both selected compounds are comparatively showing best binding in all terms to the target protein VEGFR-2.

4. CONCLUSION

This computational study based on docking and simulation concludes that both compounds *B* and *C*, retrieved from the database of natural compounds promises to be a better target leads than the standard compound *A* against VEGFR-2. Both of them are in best results with toxicity prediction, docking and molecular dynamics study against standard compounds. Thereby the current study proposes them as a prominent anticancer compound prior to *in vivo* and *in vitro* analysis as for advance computational ability in finding better and novel anticancer leads for the welfare of humanity and sciences.

ACKNOWLEDGEMENTS

The authors are heartily thankful to the Council of Science and Technology - Uttar Pradesh (CST-UP), Lucknow, India for funding this work under Young scientist project (CST/224) at Department of Bioengineering, Integral University, Lucknow, India.

REFERENCES

1. Hejmadi, M., *Introduction to Cancer Biology*, 2009.
2. Dimova, I., Popivanov, G., Djonov, V., *Angiogenesis in cancer-general pathways and their therapeutic implications*. JBUON, 2014, *19*, 1107-0625.
3. Neufeld, G., Cohen, T., Gengrinovitch, S., Poltorak, Z., *Vascular endothelial growth factor (VEGF) and its receptors*. FASEBJ. 1999, *13*, 0892-6638.
4. Ferrara, N., Gerber, H, P., LeCouter, J., *The biology of VEGF and its receptors*. Nat Med, 2003, *9*, 669-676.
5. Shibuya, M., *Vascular endothelial growth factor (VEGF)-receptor 2: its biological functions, major signaling pathway, and specific ligand VEGF-E*. Endothelium, 2006, *13*. 63-69.
6. Cortés, F., Debacker, C., Peault, B., Labastie, M, C., *Differential expression of KDR/VEGFR-2 and CD34 during mesoderm development of the early human embryo*. Mech Dev., 1999, *83*, 161–164.
7. Smith, N, R., Baker, D., James, N, H., Ratcliffe, K., Jenkins, M., Ashton, S, E., Sproat, G., Swann, R., Gray, N., Ryan, A., *Vascular endothelial growth factor receptors VEGFR2 and VEGFR-3 are localized primarily to the vasculature in human primary solid cancers*. Clin Cancer Res, 2010, *16*, 3548–3561.
8. Li, Jing., Zhou, N., Luo, K., Zhang, W., Li, X., Wu, C., Bao, J., *In Silico Discovery of Potential VEGFR-2 Inhibitors from Natural Derivatives for Anti-Angiogenesis Therapy*. International journal of molecular sciences, 2014, *15*, 15994-16011.
9. Dev, I, K., Dornsife, R, E., Hopper, T, M., Onori, J, A., Miller, C, G., Harrington, L, E., Dold, K, M., Mullin, R, J., Johnson, J, H., Crosby, R, M., *Antitumour efficacy of VEGFR2 tyrosine kinase inhibitor correlates with expression of VEGF and its receptor VEGFR2 in tumour models*. British journal of cancer, 2004, *91*, 1532-1827.
10. Dias, D, A., Urban, S., Roessner, U., *A Historical Overview of Natural Products in Drug Discovery*. Metabolites, 2012, *2*,303-336.
11. Mishra, B, B., Tiwari, V, K., *Natural products: An evolving role in future drug discovery*. Eur. J. Med. Chem., 2011, *46*,4769–4807.
12. Rey-Ladino, J., Ross, A, G., Cripps, A,W., McManus, D, P., Quinn, R., *Natural products and the search for novel vaccine adjuvants*. Vaccine, 2011, *29*, 6464–6471.
13. Cragg, G, M., Newman, D, J., *Biodiversity: A continuing source of novel drug leads*. Pure Appl. Chem., 2005, *77*, 7–24.
14. Iwata, H., Oki, H., Okada, K., Takagi, T., Tawada, M., Miyazaki, Y., Imamura, S., Hori, A., Lawson, J, D., Hixon, M, S., Kimura, H., Miki, H., *A Back-to-Front Fragment-Based Drug Design Search Strategy Targeting the DFG-Out Pocket of Protein Tyrosine Kinases*. ACS Med ChemLett, 2012, *3*, 342-346.
15. Pettersen, E, F., Goddard, T, D., Huang, C., Greenblatt, D, M., Meng, E., Ferrin, T, E., *UCSF Chimera – A Visualization System for Exploratory Research and Analysis*. Article in Journal of Computational Chemistry, 2004, *25*, 1605-12.
16. Lipinski, C, A., Lombardo, F., Dominy, B, W., Feeney, P, J., *Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings*. Adv. Drug Deliv. Rev, 1997, *23*, 3-25.
17. Lipinski, C, A., *Lead-and drug-like compounds: the rule-of-five revolution*. Drug Discovery Today: Technologies, 2004, *1*, 337-341.
18. Studio, Accelrys, Discovery.,*2.1; Accelrys: San Diego, CA*. 2009.
19. Lengauer, T., Rarey, M., *Computational methods for biomolecular docking". Current Opinion in Structural Biology*. Current Opinion in Structural Biology, 1996, *6*, 402-406.
20. Alder, B, J., Wainwright, T, E., *Studies in molecular dynamics. I. General method*. J. Chem. Phys., 1959, *31*, 459-466.

21. Rahman, A., *Correlations in the motion of atoms in liquid argon*. Physical Review, 1964, *136*, 405-411.
22. Johansen, A., Hansen, H, D., Svarer, C., Szabolcs, L., Leth-Petersen, S., Kristensen, J, L., Gillings, N., Knudsen, G, M., *The importance of small polar radiometabolites in molecular neuroimaging: A PET study with [11C] Cimbi-36 labeled in two positions*. Journal of Cerebral Blood Flow & Metabolism, 2018, *38*, 659-668.
23. Fermi, E., *Los Alamos Report LA-1940, published later in Fermi E, Pasta J and Ulam S 1965 Collected Papers of Enrico Fermi ed E Segré*. 1995.
24. Savjani, K, T., Gajjar, A, K., Savjani, J, K., *Drug solubility: importance and enhancement techniques*. ISRN pharmaceutics, 2012, *2012*.
25. Friedman, S, L., Grendell, J, H., *Current diagnosis & treatment in gastroenterology*. ISBN, 2003, *127*, 241-247.
26. Wang, B., Yang, L, P., Zhang, X, Z., Huang, S, Q., Bartlam, M., Zhou, S, F., *New insights into the structural characteristics and functional relevance of the human cytochrome P450 2D6 enzyme*. Drug metabolism reviews, 2009, *41*, 573-643.
27. Sharma, N., Akhtar, S., Jamal, Q, M, S., Kamal, M, A., Khan, M, K, A., Siddiqui, M, H., Sayeed, U., *Elucidation of antiangiogenic potential of Vitexin obtained from Cucumissativus targeting Hsp90 protein: a novel multipathway targeted approach to restrain angiogenic phenomena*. Medicinal Chemistry, 2017, *13*, 282-291.