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INSILICO MOLECULAR DOCKING OF SOME NOVEL HETEROCYCLIC COMPOUNDS TARGETING VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-2

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ABSTRACT

Molecular docking provides useful information about drug receptor interactions. It analyzes the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. In the present study eight Pyrimidine derivatives containing substituted Imidazole moiety 14(a–h) were subjected to molecular docking studies for the inhibition of the Vascular Endothelial Growth Factor Receptor-2 (VEGFR2) PDB ID 1VR2. The *insilico* molecular docking study results showed that, among the newly designed heterocyclic compounds, the compound 14h showed minimum binding energy and have good affinity towards the active pocket, thus, they may be considered as good inhibitor of Vascular Endothelial Growth Factor Receptor-2.

KEYWORDS: Docking, Vascular Endothelial Growth Factor Receptor-2, Pyrimidine, Autodock.

INTRODUCTION

Vascular Endothelial Growth Factor (VEGF) is an important signaling protein involved in both the growth of blood vessels from preexisting vasculature (angiogenesis) and the formation of the circulatory system (vasculogenesis). VEGF binding to tyrosine kinase receptors (VEGFR) can cause itself dimerization and become activated through transphosphorylation. There are three main subtypes of VEGFR: VEGFR-1, VEGFR-2, and VEGFR-3. Among which VEGFR2 appears to mediate almost all of the known cellular responses to VEGF. Inhibiting the tyrosine kinase VEGFR-2 signaling pathway[1-5] may disrupt the angiogenesis process of solid tumor, thus blocking tumor growth and spread. Therefore, the design of inhibitors targeting VEGFR-2 is an attractive approach for the development of new therapeutic agents. Molecular docking is a well established computational technique which predicts the interaction energy between two molecules. Molecular docking studies[6] are used to determine the interaction of two molecules and to find the best orientation of ligand which would form a complex with overall minimum energy. The small molecule, known as ligand usually fits within protein's cavity which is predicted by the search algorithm. These protein cavities become active when they come in contact with any external compounds and are thus called as active sites. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational drug design. Therefore, in the present study the series of newly designed heterocyclic compounds were selected based on their molecular properties and druglikeliness score and further investigated for its binding efficiency to evaluate their best fit using Auto Dock.

MATERIALS AND METHODS

Software required

Python 2.7 - language was downloaded from www.python.com, Cygwin was downloaded from www.cygwin.com, Molecular Graphics Laboratory(MGL) tools[7] and AutoDock 4.0 was downloaded from www.scripps.edu, SWISS-MODEL is a fully automated protein structure homology-modeling server, accessible via the ExPASy web server, or from the program Deep View (Swiss Pdb-Viewer). Chemsketch was downloaded from www.acdlabs.com, Discoverystudio visualizer 2.5.5 was downloaded from www.accelrys.com. Molecular properties and Bioactive Score were calculated using Molinspiration online Software. CORINA Classic (the classic command-line version of CORINA) was used to generate 3D structure for small and medium sized, typically drug-like molecules.

Insilico molecular docking studies

Preparation of Protein structure

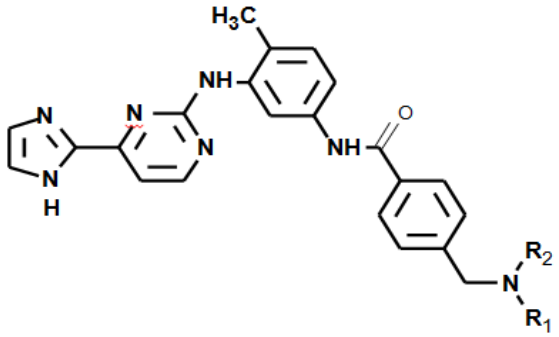
Protein target was downloaded from database[8] Protein Data Bank (PDB). 1VR2 is PDB id of the target protein Vascular Endothelial Growth Factor Receptor-2

(VEGFR-2). All water molecules were removed and on final stage hydrogen atoms were added to receptor molecule. Protein structure homology modeling was done using Swiss Model.[9-12]

Preparation of Ligands

Review of Literature show that Pyrimidine[13-16] and Imidazole[17-22] contains wide spectrum of activity. Hence it was decided to design a newer heterocyclic compound of series 14(a–h) containing Pyrimidine fused with Imidazole. The ligands were drawn in Chemsketch freeware assigned with proper 2D orientation and they are converted in to Three – Dimensional structure using CORINA Classic. All the compounds from 14(a-h) were subjected to evaluate their compliance for Lipinski's rule of five. All the newly designed compounds were found in compliance with Lipinski's rule of five recommendations for new chemical entity to have good oral bioavailability with no violations. The miLogP value of all compounds were found below five, suggesting that the molecules have good permeability across the cell membrane which in turn is needed for generation of bioactivity. Number of violations for all the compounds is zero; it means all newly designed compounds will easily bind to receptors. All the compounds 14(a-h) are within the limit, that is, 160^0A in respect of Topological Polar Surface Area (TPSA), which showed that molecules are fulfilling the optimal requirement for drug absorption. The values are tabulated in the (Table. 1 and 2) given below. Hence, all the newly designed heterocylic compounds which satisfy Lipinski's rule and druglikeness property has been taken as a lead for anti cancer drug targeting protein kinase receptor. Energy of the molecules was minimized using Dundee PRODRG2 server. The energy minimized compounds were then read as input for AutoDock 4.0, in order to carry out the docking simulation.

TABLE 1: CALCULATION OF BIOACTIVITY SCORE FOR NEWLY DESIGNED HETEROCYCLIC COMPOUNDS



COMPOUND 14 (a-h)

Comp code	R_1	R_2	GPCR ligand	Ion channelmodulator	Kinase inhibitor	Nuclear Receptor ligand	Protease inhibitor	Enzyme inhibitor
14a	-CH3	-CH3	0.26	0.01	0.78	-0.58	0.06	0.21
14b	-C2H5	-C2H5	0.23	0.03	0.68	-0.51	0.07	0.17
14c		-H	0.24	0.02	0.75	-0.58	0.10	0.20
14d	C6H5	-H	0.20	0.02	0.68	-0.41	0.01	0.20
14e	-CH3	-H	0.27	0.02	0.78	-0.58	0.09	0.22
14f	-H	-H	0.29	0.12	0.86	-0.66	0.19	0.28
14g	-C2H5	-H	0.25	0.05	0.73	-0.53	0.06	0.20
14h	-C3H7	-H	0.27	0.08	0.69	-0.51	0.09	0.21
Sunitinib(standard)			-0.16	-0.62	0.51	-0.80	-0.51	-0.23

TABLE 2: CALCULATION OF PHYSIOCHEMICAL PROPERTIES FOR NEWLY DESIGNED HETEROCYCLIC COMPOUNDS

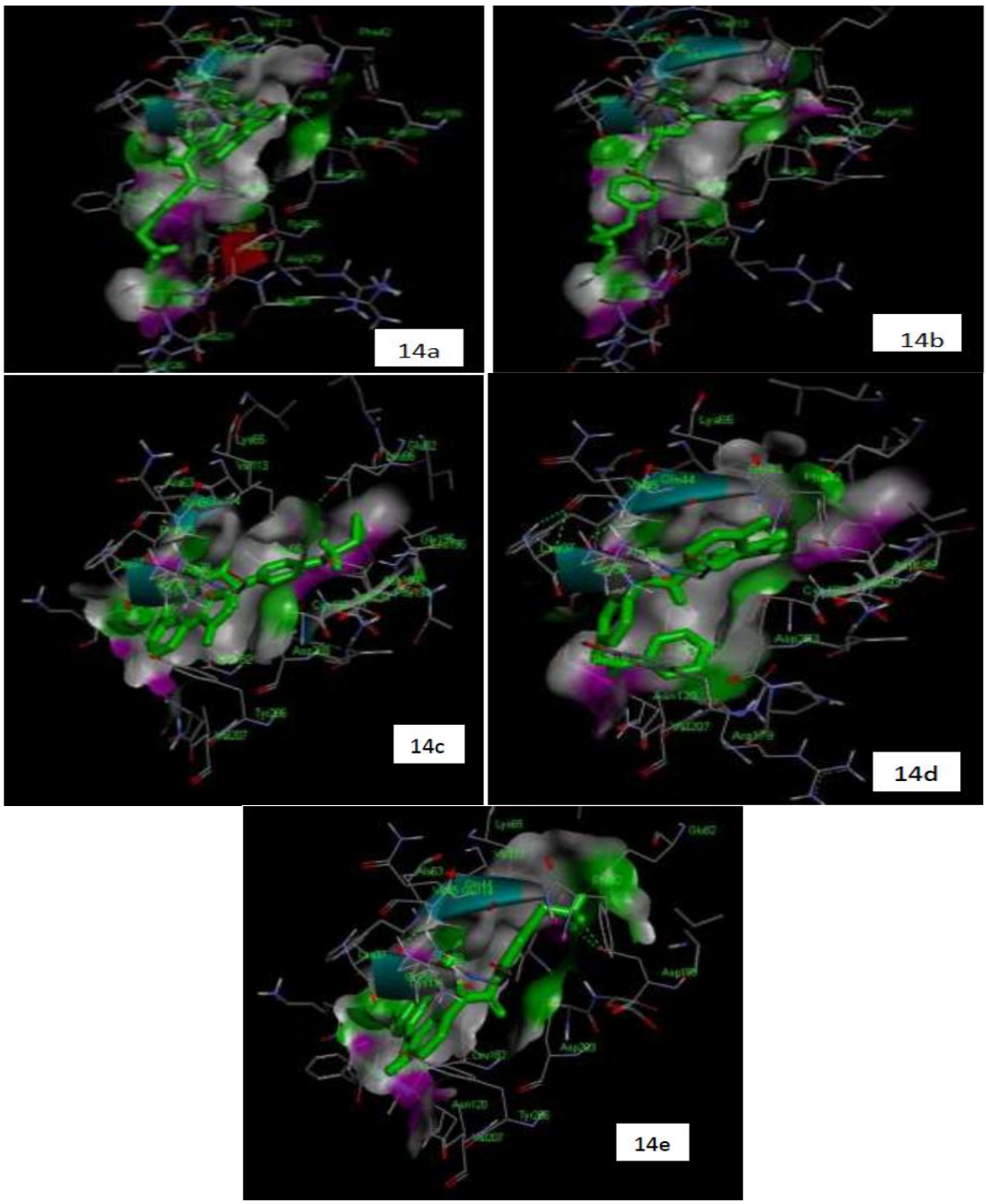
Comp code	miLog P	TPSA	MW	nON	nOHNH	nviola	nrot	Volu
14a	3.38	98.83	427.51	8	3	0	7	393.84
14b	4.13	98.83	455.57	8	3	0	9	427.45
14c	3.50	107.62	439.52	8	4	0	8	399.93
14d	4.83	107.62	475.56	8	4	0	8	431.75
14e	3.13	107.62	413.49	8	4	0	7	376.90
14f	2.76	121.61	399.46	8	5	0	6	359.23
14g	3.51	107.62	427.51	8	4	0	8	393.70
14h	4.01	107.62	441.54	8	4	0	9	410.50
Sunitinib	1.95	80.99	398.48	6	3	0	7	370.95

Docking Studies

The Graphical User Interface program ‘‘AutoDock Tools’’ was used to prepare, run, and analyze the docking simulations. Kollman united atom charges, salvation parameters and polar hydrogen’s were added to the receptor for the preparation of protein in docking simulation. Since ligands are not peptides, Gasteiger charge was assigned and then non-polar hydrogens were merged. AutoDock requires pre-calculated grid maps, one for each atom type, present in the ligand being docked as it stores the potential energy arising from the interaction with macromolecule. The following docking factors were chosen for the Lamarckian genetic algorithm as follows: population size of 150 individuals, 2.5 million energy evaluations, maximum of 27000 generations, and number of top individuals to automatically survive to next generation of 1, mutation rate of 0.02, crossover rate of 0.8 and 10 docking runs. Auto Dock was run various times to obtain various docked conformations, and used to calculate the predicted binding energy.

RESULTS AND DISCUSSION

The docking poses were obtained according to their docking parameters and their corresponding binding pockets. This evaluation of the newly designed compounds 14(a-h) were based upon their binding parameters with the target Vascular Endothelial Growth Factor Receptor-2. In **Fig. 1**, docked pose of VEGFR-2 with the ligands 14(a-h) clearly demonstrate the binding positions of the ligand with the target. The potential binding sites of the compound 14(ah) was tabulated in the **Table 3** given below.



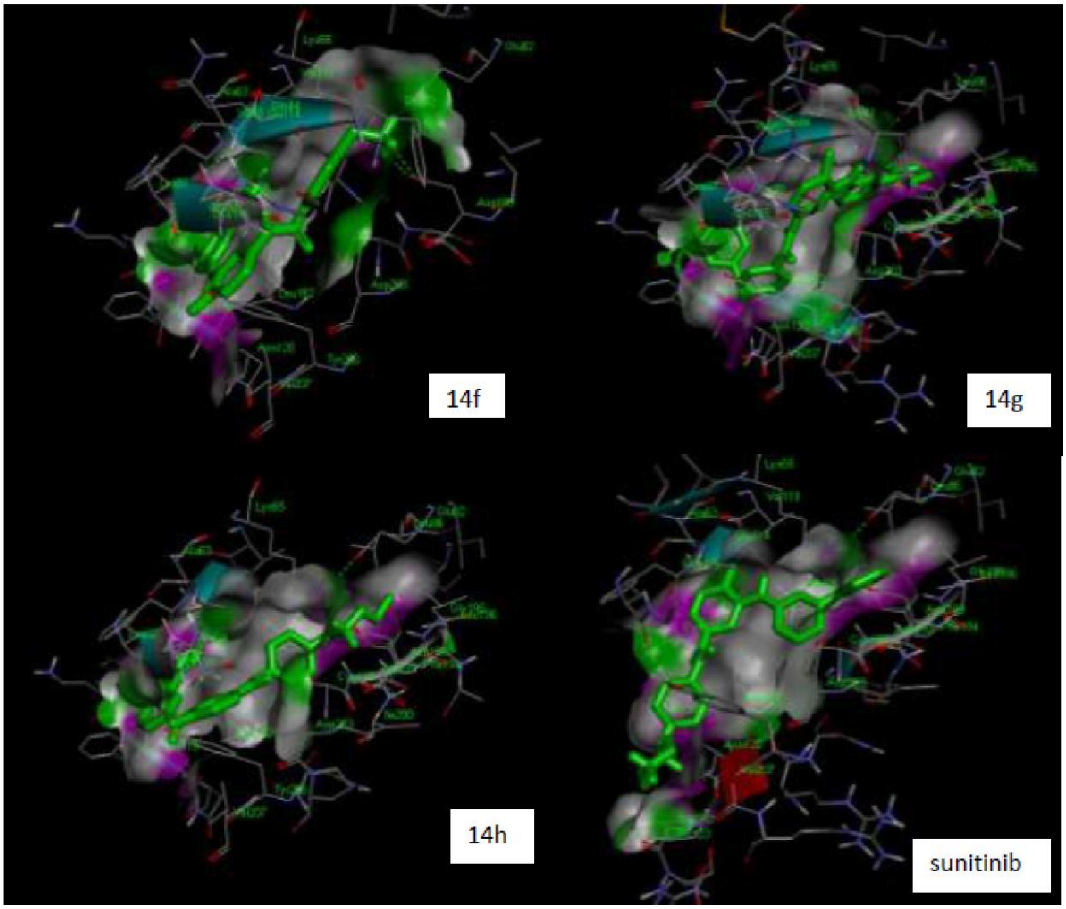


Table 3: Potential binding sites of the compound 14(a-h) in VEGFR-2

S. No	Compound Code	Potential binding sites
1.	14a	Leu37,Gly38,Arg39,Val45,Ala63,Val96,Val113,Glu114,Cys116,Gly119,Asn120,Thr123,Leu 182,Asp203,Tyr206,Val207
2.	14b	Leu37,Val45,Ala63,Lys65,Val96,Val113,Glu114,Cys116,Gly119,Asn12, Thr123,Leu182,Cys192,Asp193,Asp199,Asp203,Val207
3.	14c	Leu37,Gly38,Arg39,Val45,Ala63,Lys65,Glu82,Leu86,Val96,Val113 Glu114,Phe115,Cys116,Leu182,Cys192,Asp193,Phe194,Gly195,Leu196 Asp199,Asp203,Tyr206
4.	14d	Leu37,Gly38,Arg39,Phe42,Gly43,Gln44,Val45,Lys65,Val96,Gly119,Asn120,A rg179,Leu182,Cys192,Asp193,Asp199,Asp203,Val207
5.	14e	Leu37,Gly38,Val45,Ala63,Lys65,Glu82,Leu86,Phe115,Cys116,Gly119 Asn120,Leu182,Cys192,Asp193,Phe194,Gly195,Leu196,Asp199,Asp203, Val207
6.	14f	Leu37,Gly38,Arg39,Phe42,Gln44,Val45,Ala63,Lys65,Glu82,Val113,Glu114, Cys116,Gly11 9,Asn120,Leu182,Asp199,Asp203,Tyr206,Val207
7.	14g	Leu37,Gly38,Arg39,Val45,Ala63,Lys65,Glu82,Val113,Phe115,Cys116, Leu182,Cys192,Asp193,Phe194,Gly195,Leu196,Asp199,Asp203,Tyr206
8.	14h	Leu37,Gly38,Arg39,Ala63,Lys65,Glu82,Leu86,Cys116,Gly119,Leu182 Cys192,Asp193,Phe194,Gly195,Leu196,Asp199,Ile200,Asp203,Tyr20 Val207
9.	Sunitinib	Leu37,Gly38,Arg39,Val45,Ala63,Lys65,Val113,Cys116,Gly119,Asn120, Leu182,Cys192,Asp193,Asp203,Val207

This proves that the effective binding sites are present in the newly designed compounds when compared with the standard. It proves that the ability of inhibiting the VEGFR-2 by the newly designed compounds.

TABLE 4: BINDING ENERGIES OF THE COMPOUNDS 14 (a-h)

S. No	Compound Code	Binding Energy (-Ve) (Kcal/Mol)
1.	14a	-7.50
2.	14b	-6.61
3.	14c	-8.44
4.	14d	-7.18
5.	14e	-8.63
6.	14f	-8.92
7.	14g	-8.66
8.	14h	**-9.15**
9.	Sunitinib (Standard)	-8.97

In Table 4, compounds 14h (-9.15Kcal/mol) showed better binding energy when compared to that of standard Sunitinib (-8.97 Kcal/mol). This proves that compound 14h contain potential VEGFR-2 inhibitory binding sites. All the other compounds showed binding energy in the range of -8.92 to -6.61Kcal/mol. It is to be noted that compounds containing Alkyl substitution at R_1 and R_2 position showed very less binding affinity compared with the compounds containing alkyl substitution only in R_1 position.

It is also observed that the increase in the no. of. carbon chain in R_1 position increases the binding affinity of compound. Presence of bulky groups like phenyl and cyclopropyl group decreases the binding affinity. Presence of hydrogen at both R_1 and R_2 position shows binding affinity slightly similar to that of the standard.

Table 5: Inhibition Constant of the newly designed compounds

S. No	Compound Code	Inhibition Constant Ki (micromolar/nanomolar)	Intermolecular Energy (kcal/mol)
1.	14a	3.16 uM (micromolar)	-8.97
2.	14b	14.20 uM (micromolar)	-8.63
3.	14c	652.24 nM (nanomolar)	-9.81
4.	14d	5.46 uM (micromolar)	-8.54
5.	14e	475.93 nM (nanomolar)	-9.77
6.	14f	289.10 nM (nanomolar)	-10.23
7.	14g	445.44 nM (nanomolar)	-10.13
8.	14h	197.70 nM (nanomolar)	-10.75
9.	Sunitinib (Standard)	264.68 nM (nanomolar)	-10.30

In addition, two other parameters like inhibition constant (Ki) and intermolecular energy were also determined. As shown in **Table 5**, Compounds showed inhibition constant ranging from 197.70nM to 3.16µM. The compound 14h (197.70nM) showed the lowest inhibition constant when compared to the standard (264.68nM). Inhibition constant is directly proportionalto binding energy. Thus, the VEGFR-2 inhibitory activity of the compounds was proved using molecular simulations. As shown in **Table 5**, the compounds 14h showed lesser intermolecular energy compared to the standard (-10.30kcal/mol). This result further indicates that compound 14h have better and stronger VEGFR-2 inhibitory activity.

CONCLUSION

In conclusion, the results of the present study clearly demonstrated that, among the newly designed leads, 14h showed better binding sites and strong interactions with VEGFR-2 compared to the standard. Further investigations on the above compounds and *in vitro* studies are necessary to develop potential chemical entities for the treatment of Cancer.

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