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INSILICO STUDIES OF SOME NOVEL HETEROCYCLIC DERIVATIVES AS ANTICANCER AGENTS

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ABSTRACT

A set of eight substituted amino derivatives of 4-[(substituted amino)methyl]-*N*-(4-methyl-3-{[4-(1*H*-pyrrol-2-yl) pyrimidin-2-yl]amino}phenyl)benzamide were designed and screened for their physiochemical properties and druglikeliness score using Molinspiration software. All the newly designed compounds 13(a-h) were found in compliance with Lipinski's rule of five recommendations for new chemical entities to have good oral bioavailability with no violations. All the compounds from 13(a-h) were further evaluated for their binding affinity towards Vascular Endothelial Growth Factor Receptor-2 which comprises the family of protein kinase and plays an important role in vascularization, growth and metastasis of tumor. Among the eight compounds 13(a-h), compounds 13f (-9.39 kcal/mol) and 13h (-9.09 kcal/mol) showed a better binding affinity compared to the standard drug sunitinib (-8.97kcal/mol). All the other compounds showed lesser binding energies compared to the standard. In order to conclude, compounds 13f and 13h can be selected as an efficient lead compound for further anticancer studies targeting protein kinase receptor.

KEYWORDS: Pyrimidine, pyrrole, molinspiration, anticancer activity and docking.

INTRODUCTION

Cancer[1,2] is a leading cause of death in India and other countries. It is a diverse group of diseases characterized by uncontrolled growth of abnormal cells, which is becoming a major worldwide health problem. Although the cancer research has led to a number of new and

effective solutions, the medicines used as treatments have clear limitations. In the long history of medicinal chemistry, pyrimidine[3,4] based chemical architectures always finds a forefront place as a potential anticancer agent. Around the globe, various research activities are focused on this pyrimidine[5,6] based scaffolds. Currently there is a huge scientific and commercial interest in the discovery of potent, safe and selective anticancer drugs. This paved us a way to focus our research work in designing a potential anticancer lead targeting protein kinase receptors. For this purpose, pyrimidines[7] in fused scaffolds with pyrrole derivatives were designed and evaluated for their binding affinity towards the selected target. The aim of this present work was achieved by using various computational softwares like molinspiration[8-11] and Autodock 4.0.[12-14]

MATERIALS AND METHODS

Softwares and Data Sources

The software used in the present work was available on the Internet. The target protein, i.e. Vascular Endothelial Growth Factor Receptor–2(VEGFR-2) was downloaded from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB). The ligand structures were drawn in Advanced Chemistry Development (ACD)/Chemsketch software. CORINA Classic web server was used to convert the ligands from 2D to 3D format. The PRODRG2 web server[15] was used for the energy minimization of the ligands. Autodock 4.0 software was used for the docking studies. LIGSITE *csc* webserver[16] was used to find out the active site in the selected target. Accelrys Discovery Studio Visualizer is used for visualization of Protein – Ligand interaction.

METHODS

Selection of Drug target

VEGFR-2, which is a member of the family of Receptor Tyrosine Kinases, plays an important role in the cell signaling of Vascular Endothelial Growth Factor (VEGF) and tumor proliferation. Recent research has shown that the blockade of VEGFR-2 signaling by small molecular inhibitors to the kinases domain can inhibit the growth of solid tumors. Therefore, in the present study VEGFR-2 has been selected as a drug target.

Selection of Ligands

The structure of the ligands was sketched using the chemsketch software. These ligands were subjected to evaluation of its druglikeness properties and bioactivity spectra. Molinspiration online server was used for this purpose. Drug likeness was calculated as per the molecular

descriptors specified in Lipinski's "Rule-of-Five". Partition coefficient (LogP), Molecular weight (MW), hydrogen bonding acceptors (HBA) and Hydrogen bonding donors (HBD) were calculated along with Topological Polar Surface Area (TPSA) and Volume. All the newly designed compounds 13 (a-h) complies with the Lipinski's rule of five. These ligands were further subjected to docking studies using Autodock 4.0.

Docking study

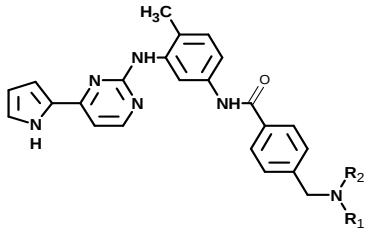
In order to evaluate the structural interactions' of the newly designed ligands 13 (a - h) with the VEGFR-2 receptor, molecular docking simulation was done by using Autodock 4.0. The crystal structure of VEGFR-2 (PDB entry: 1VR2) was retrieved from the Protein Data Bank (http://www.rcsb.org). Homology modeling of the target receptor was done using SWISS – MODEL webserver. The active sites in the target protein were identified using LIGSITE^{csc} webserver. The ligands were converted into their 3D format using CORINA classic. The ligands were energy minimized using PRODRG2 server. Thus prepared ligands and receptor were screened for their binding affinity by using Autodock 4.0.

RESULTS

Lipinski's rule of five

A series of eight newly designed compounds 13(a–h) were evaluated for their druglikeness and oral bioavailability based on Lipinski's rule of five which is tabulated in Table 1 and 2. 1. All the newly designed compounds 13 (a-h), met the criteria for Lipinski's rule with respect to Molecular weight, number of hydrogen bond donors, number of hydrogen bond acceptors and MilogP. It is to be noted that Topological Polar Surface Area (TPSA) is essential to predict the intestinal absorption and blood brain barrier penetration. Number of rotatable bonds has to be $\leq$ 10 to pass the oral bioavailability. The bioactivity scores of these compounds 13 (a-h) ranges from 0.90 to 0.71 indicate the probability of good binding affinity towards kinase inhibitors. Compounds 13 (a-h) thus evaluated were further subjected for docking simulation with the VEGFR-2.

Table 1: Calculation Of Bioactivity Score For Newly Designed Heterocyclic Compounds.



COMPOUND 13 (a-h)

Comp code	R_1	R_2	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
13a	$-CH_3$	$-CH_3$	0.16	-0.01	0.82	-0.33	-0.04	0.15
13b	-	-	0.14	-0.06	0.71	-0.27	-0.09	0.11
13c		-H	0.14	-0.07	0.79	-0.34	-0.00	0.14
13d	C_6H_5	-H	0.11	-0.06	0.72	-0.19	-0.08	0.15
13e	$-CH_3$	-H	0.16	-0.07	0.82	-0.32	-0.02	0.16
13f	-H	-H	0.18	0.03	0.90	-0.39	0.08	0.21
13g	-	-H	0.15	-0.04	0.76	-0.27	-0.05	0.14
13h	-	-H	0.17	-0.01	0.72	-0.26	-0.01	0.15
Sunitinib(standard)			-0.16	-0.62	0.51	-0.80	-0.51	-0.23

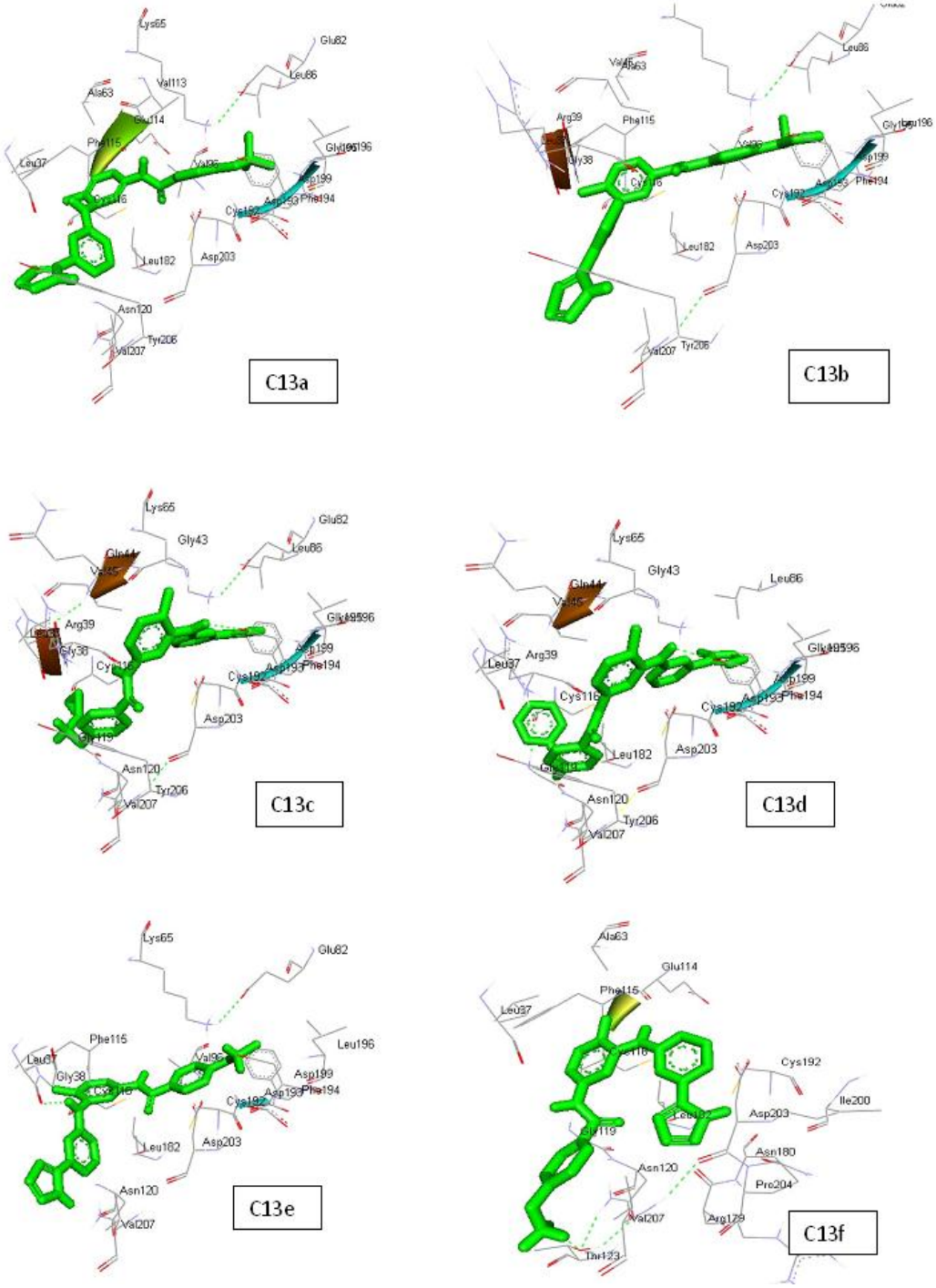
GPCR – G Protein Coupled Receptor.

Table 2: Calculation Of Physiochemical Properties For Newly Designed Heterocyclic Compounds.

Comp code	miLogP	TPSA	Mol.Wt	nON	nOHNH	No. of. violation	nrot	Volume
13a	3.86	85.94	426.52	7	3	0	7	398.00
13b	4.61	85.94	454.57	7	3	0	9	431.60
13c	3.98	94.73	438.54	7	4	0	8	404.08
13d	5.31	94.73	474.57	7	4	1	8	435.90
13e	3.62	94.73	412.50	7	4	0	7	381.06
13f	3.24	108.72	398.47	7	5	0	6	363.38
13g	3.99	94.73	426.52	7	4	0	8	397.86
13h	4.50	94.73	440.55	7	4	0	9	414.66
Sunitinib	1.95	80.99	398.48	6	3	0	7	370.95

TPSA – Topological Polar Surface Area, **nrot** – Number of rotatable bonds, **Mol.wt** – Molecular weight, **nON –** No. of. Hydrogen bond donors**, nOHNH –** No. of. Hydrogen bond acceptors.

The potential lead molecules 13 (a-h) and drug target were prepared by adding Kollman and Gasteiger charges. The docking poses (**fig. 1**) were obtained according to their docking parameters and their corresponding binding pockets.



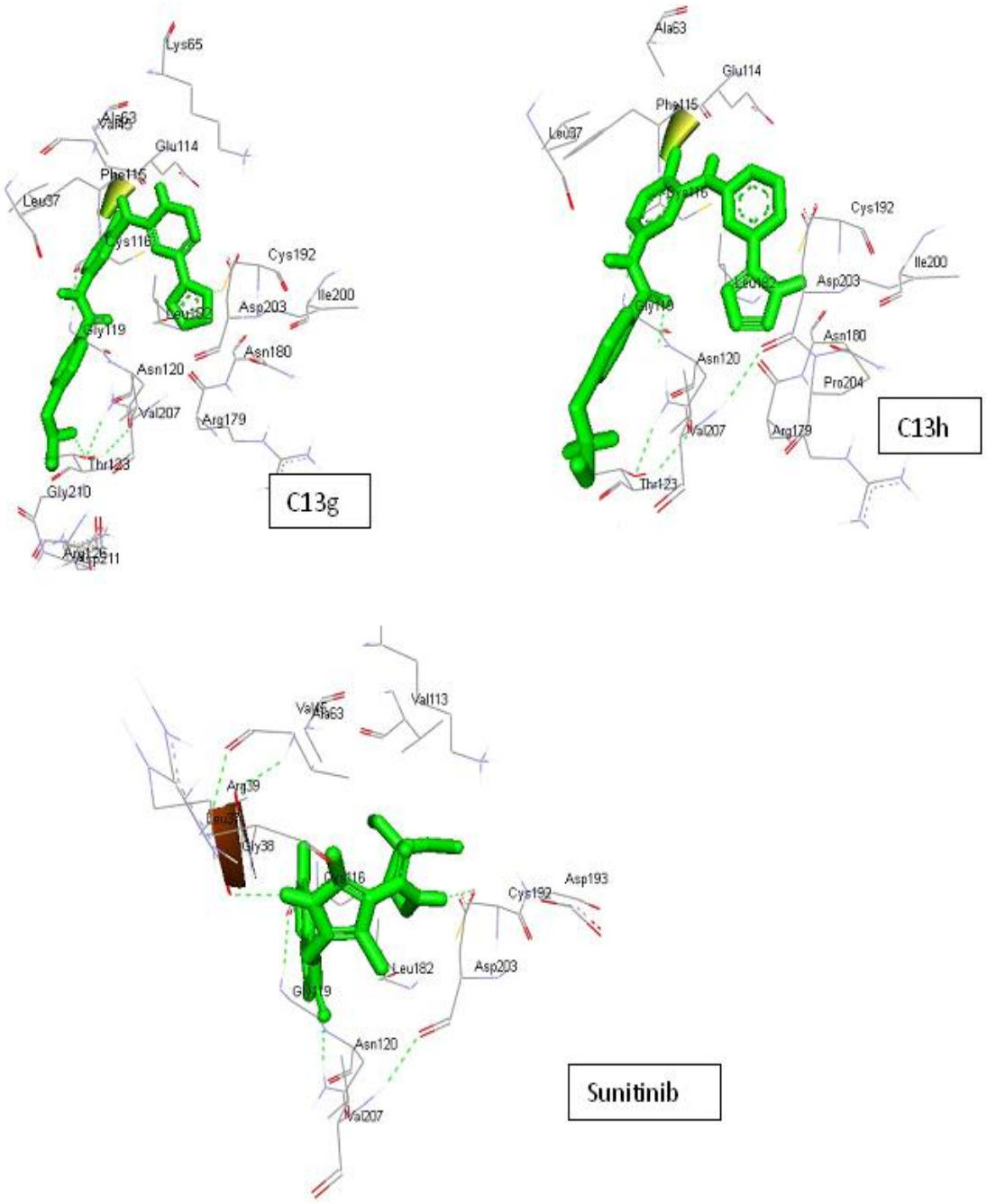


Fig 1: Showing the docking poses of compound 13(a-h).

This evaluation of the newly designed compounds of series 13(a-h) was based upon their binding parameters with the target Vascular Endothelial Growth Factor Receptor-2. The potential binding sites of the compounds were tabulated in the **Table 3** given below.

Table: 3 Potential binding sites of the compound 13(a-h) in VEGF Receptor-2.

S. No	Comp Code	Potential binding sites
1.	13a	Leu37, Phe42, Val45, Ala63, Lys65, Glu82, Val96, Glu114, Cys116, Gly119, Asn120, Arg179, Leu182, Asp193
2.	13b	Leu37, Arg39, Val45, Ala63, Lys65, Val96, Val113, Glu114, Cys116, Gly119, Asn120, Arg179, Leu182, Cys192, Asp193, Asp199, Asp203
3.	13c	Leu37,Gly38,Arg39,Val45,Lys65,Val96,Val113,Glu114,Phe115,Cys116,Gly11, ,Asn120,Arg179,Leu182,Asp203,Tyr206
4.	13d	Leu37,Gly38,Val45,Lys65,Val96,Glu114,Cys116,Arg179,Asn180, Leu182, Cys192,Asp199,Asp203,Pro204,Val207
5.	13e	Leu37,Gly38,Val45,Lys65,Val96,Glu114,Cys116,Arg179,Asn180, Leu182, Cys192,Asp199,Asp203,Pro204,Val207
6.	13f	Leu37,Gly38,Gln44,Val45,Ala63,Lys65,Glu82,Val113,Glu114,Cys116,Gly119, Arg179,Asn180,Leu182,Cys192,Asp199
7.	13g	Leu37,Val45,Ala63,Lys65,Glu114,Phe115,Cys116,Gly119,Asn120,Thr123,Arg 126,Arg179,Asn180,Leu182,Cys192,Ile200,Asp203,Val207,Gly210,Asp211
8.	13h	Leu37,Ala63,Glu114,Phe115,Cys116,Gly119,Asn120,Thr123,Arg179 Asn180,Leu182,Cys192,Ile200,Asp203,Pro204,Val207,Gly210
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 13(a-h).

S. No	Compound Code	Binding Energy (-Ve) (Kcal/Mol)
1.	13a	-6.04
2.	13b	-1.07
3.	13c	-6.96
4.	13d	-8.45
5.	13e	-6.72
6.	13f	-9.39
7.	13g	-8.21
8.	13h	-9.09
9.	Sunitinib (Standard)	-8.97

The newly designed compounds 13f and 13h showed better affinity towards the receptor when compared to that of standard Sunitinib (-8.97 kcal/mol). The results are summarized in the Table 4. This proves that compounds contain potential VEGFR-2 inhibitory binding effect.

Table 5: Inhibition Constant of the compounds.

S. No	Compound Code	Inhibition Constant (µM/mM/nM) Ki	Intermolecular Energy (kcal/mol)
1.	13a	37.19 µM	-7.24
2.	13b	164.91mM	-2.45
3.	13c	7.88 µM	-9.34
4.	13d	639.20 nM	-11.44
5.	13e	11.88 µM	-7.84
6.	13f	131.74 nM	-10.98
7.	13g	958.84 nM	-9.51
8.	13h	215.61 nM	-10.97
9.	Sunitinib (Standard)	264.68 nM	-10.30

µM (micromolar), mM (millimolar), nM (nanomolar)

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 131.74 nM to 164.91 mM. The compounds 13f and 13h had lesser inhibition constant when compared to the standard (264.48 nM). Inhibition constant is directly proportional to binding energy. Thus, the VEGFR-2 inhibitory activity of the compounds was proved using molecular simulations.

As shown in Table 5, Compounds 13d,13f and 13h showed intermolecular energy -11.44 kcal/mol,-10.98 kcal/mol and -10.97 kcal/mol respectively, which was lesser when compared to the standard (-10.30 kcal/mol). These results further proved these compounds have better VEGFR -2 inhibitory activity.

CONCLUSION

In conclusion, the docking study revealed that the compounds 13f and 13h showed better alignment at active site by interacting with all crucial aminoacid residues present in the target receptor VEGFR-2.The compounds 13f and 13h may exert significant anticancer activity. Further investigations with the above compounds and *in vitro* studies are necessary to develop potential chemical entities for the treatment of Cancer. The present study will be extremely beneficial to the medicinal chemists working on designing and synthesis of anticancer drug targeting protein kinase receptor.

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