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In silico Enhancement of VEGFR-2 Binding Properties of 5-Amino-2-(3,4-Dimethoxyphenylsulfonamido)-5-Oxopentanoic Acid (SM-1) for Improving its Anticancer Activity

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Abstract

Previously, 5-Amino-2-(3,4-dimethoxyphenylsulfonamido)-5-oxopentanoic acid (SM-1) was established by the authors as a potent antiangiogenic and anticancer agent in Multiple Myeloma with no toxic effect on normal cells. The primary antiangiogenic activity and anticancer activity were checked using HUVEC and RPMI-8226 cell lines respectively. Further, the antiangiogenic activity of SM-1 was confirmed, observing inhibition of phosphorylation at tyrosine 1175 residue of VEGFR-2, with the help of western blot technique. It was also found to be non-toxic to normal epithelial cells, observing the cytotoxic effect on VERO cell line. In the present investigation, SM-1 was taken as a lead molecule with an aim to design more prospective drug candidates. It was modified by docking at the active site of VEGFR-2 using drug designing software, Discovery Studio. Several grow points and scaffolds were strategically identified, and grow scaffold and scaffold hopping techniques were applied. Out of 13,650 compounds generated by the software, 5 hit compounds were identified and selected based on the docking results. The docking results were compared with SM-1 and sorafenib as reference compounds and it was observed that the five-hit compounds have better interaction with the critical amino acid residues at the catalytic site of VEGFR-2 than SM-1. The compounds may be synthesized and tested for antiangiogenic and anticancer activity in Multiple Myeloma in the future investigations.

Keywords: Antiangiogenic, Multiple Myeloma, VEGFR-2, Docking, Scaffold hopping.

1. Introduction

Lead optimization is a stage in drug discovery where the lead compound is modified to improve potency, selectivity, safety profiles, or pharmacokinetic features to bring the candidate drug into the preclinical phase. [1,2,3] The lead compounds, often found in high-throughput screening as hits, usually lack drug-like properties. [4] However, they can be employed as starting points or scaffolds to explore more chemical space to improve relevant aspects like synthesizability, affinity, and ADMET. [5] In-silico 3D methods and virtual ADMET prediction approaches help to decide what to synthesize and what not to. [6,7] There are various ways to perform lead optimization. Computational tools for lead optimization can propose synthetically feasible candidates from a reagent library representing accessible chemistry focused on the protein target. [8] In an earlier report, we described the discovery of a lead compound *5-amino-2-(3,4-dimethoxyphenylsulfonamido)-5-oxopentanoic acid* (SM-1) as biologically active against Ehrlich Ascites Carcinoma (EAC) and bear antiangiogenic property. The primary antiangiogenic property was evaluated on the HUVEC cell line, and its selective cytotoxicity towards cancer cells was established by checking its action on the VERO cell line. [9] With the help of western blot technique, it was further

confirmed by the authors, that, the antiangiogenic activity of SM-1was due to inhibition of phosphorylation at the tyrosine 1175 residue of VEGFR-2. [10] Abiding the rationale explained in the research, [10] the compound has been tested on a human Multiple Myeloma (MM) cell line, RPMI8226 and was found to be active. MM is a type of blood cancer of the plasma cells. [11] The malignant plasma cells accumulate in the bone marrow and outnumber the healthy plasma cells, thereby causing a drastic change in the microenvironment of the bone marrow and cause a bone tumor, kidney damage, and hijack the immune system. [12,13] Bone marrow angiogenesis is a hallmark of MM and helps in the progression of the disease. [14] Angiogenesis is a physiological process of the formation of new blood vessels from the pre-existing vasculature. [15] Tumours are most vulnerable at the level of their blood supply for their growth and proliferation. Several positive and negative angiogenic growth factors and inhibitors regulate the process of angiogenesis by balancing between them. [16,17] Antiangiogenic compounds are reliable anticancer agents. Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) is a crucial cell type marker that primarily responds to Vascular Endothelial Growth Factor (VEGF) signal and is responsible for endothelial cell migration and proliferation. [18,19,20] Upon binding of VEGF, VEGFR-2 goes to an activated state, and autophosphorylation of the intracellular *Tyrosine1175* residue leads to a cascade of downstream signalling. [21,22] The result is endothelial cell production, proliferation, and migration. In typical cancers, VEGFR-2 expression is enormous. Inhibiting VEGFR-2 expression has therapeutic implications in cancer treatment.

Herein we describe the process of lead optimization of the compound, **SM-1** using Discovery Studio, [23] a standalone piece of software for computational chemistry and biology, utilising *Grow Scaffold* protocol and *Replace Fragment* protocol. *Grow Scaffold* protocol performs reaction-based ligand enumeration within a protein's active site, [24] and the *Replace Fragment* protocol allows us to generate novel compounds with different scaffolds by replacing one or more fragments in a lead structure. [25] These are the two methods for growing a scaffold and performing scaffold hopping of a lead compound for lead optimization. **SM-1** has been investigated for its ability to inhibit autophosphorylation of VEGFR-2 Tyr1175 using the western blot method, and inhibition of VEGFR-2 tyrosine kinase enzyme by inhibition assay. [10] **SM-1** was docked on the active site of the VEGFR-2 domain and the best-docked conformation was used as a scaffold for lead optimization. Docking is a molecular modelling technique that simulates and predicts how a molecule (ligand) gets attached and interacts with the active site of bio-macromolecules (enzyme, protein, and nucleic acid). [26]

2. Materials and Methods

For all simulation work, Discovery Studio (DS v.4.1) has been used. Research Collaboratory for Structural Bioinformatics-Protein Data Bank (RCSB-PDB) [www.rcsb.org] was used to retrieve VEGFR-2 protein (PDB accession code: **4ASD**) from the archive. ChemDraw (Chemical Structure Drawing Standard, Cambridge Soft Corporation, USA) was used to draw the 2D structure of the compounds.

2.1 *Retrieval of VEGFR-2 Protein from PDB and its Preparation for Docking*

A docking program was initiated to investigate the mode of interaction of SM-1 with the active site of VEGFR-2. The crystal structure of VEGFR-2 (Juxtamembrane and kinase domains) in complex with

Sorafenib (BAY 43-9006) was retrieved from the PDB (4ASD) website [27] The co-crystal structure was determined by the X-ray diffraction method at 2.03Å. The protocol prepares proteins for docking by removing any attached ligands, inserting missing atoms in incomplete residues, modeling missing loop regions, deleting alternate conformations (disorder), removing water molecules, standardizing atom names, and protonating titratable residues using predicted pKs, among other things.

2.2 ***Preparation of Ligand for Docking***

The prepare ligands protocol helps to prepare ligands for input into docking protocols, performing tasks such as set standard formal charges on common functional groups, kekulize molecules, fix bad valencies, removing duplicates or compounds with undesirable properties, enumerating isomers and canonical tautomers, generating 3D conformations using catalyst, enumerating valid ionization states at a given pH range. The 2D structure of the ligand **SM-1** was drawn in ChemDraw and imported into DS for preparation. A pH-based ionization approach with a pH range of 6.5-8.5 was used for dependable docking results. Tautomers are generated by default to predict correct binding mode through docking.

2.3 ***Define and Edit Binding Site***

A binding site is a set of points on a grid that lies in a cavity. The binding site tools allow us to identify, edit, and display the binding sites of a receptor. PDB files often have active sites defined using SITE records. When 4ASD was imported, groups were created for each SITE. The site sphere was defined as x, y, z, r, where x, y, z specifies the coordinates of the center and r is the radius of the sphere. The values are -24.7343, 0.359175, -10.9519, and 10.6 respectively. The 49 amino acid residues were identified to make up the active site of VEGFR-2, viz. LEU840, GLY841, GLN847, VAL848, ILE849, VAL865, ALA866, VAL867, LYS868, MET869, LEU882, GLU885, LEU886, ILE888, LEU889, ILE892, ASN897, VAL898, VAL899, ASN900, LEU901, LEU902, VAL914, ILE915, VAL916, GLU917, PHE918, CYS919, GLY922, ASN923, LEU924, LEU1019, HIS1026, ARG1032, ASN1033, ILE1034, LEU1035, LEU1036, SER1037, VAL1042, LYS1043, ILE1044, CYS1045, ASP1046, PHE1047, GLY1048, LEU1049, ALA1050, ARG1051.

2.4 Molecular ***Docking by LibDock Protocol***

LibDock uses protein site features referred to as HotSpots. [28] There are two sorts of hotspots: polar and apolar. A polar ligand atom prefers a polar HotSpot, while an apolar ligand atom prefers an apolar HotSpot. Before the docking method, the receptor HotSpot file was calculated. HotSpots were matched as triplets, and rigid ligand poses were fitted into the active site. Before scoring, the poses were clipped, and a final optimization step was conducted. The ligand poses were allocated hydrogens that were deleted during the docking process.

2.5 In Situ Ligand Minimization

This protocol minimizes a series of ligands. As the receptor molecule was specified, minimization was performed in the presence of the receptor (in situ). For this in situ minimizations, the receptor was held rigid. However, residues with atoms inside the specified sphere were allowed to move. The

CHARMm, cff, or MMFF forcefield were specified. [29] The ligands and receptors were typed on the server.

2.6 *Scoring Ligand Poses*

Ligand scoring is a method to rapidly estimate the binding affinity based on candidate ligand pose geometry docked into a target receptor structure. Scoring methods typically use *empirical functions* developed by fitting various functional forms, which characterize various aspects of the receptor-ligand interactions against binding affinity data, or *a knowledge-based approach* that uses statistical analysis of known ligand-receptor structures and the frequency occurrence of specific receptor-ligand interactions without requiring any information about binding affinities. Some scoring functions are Jain, LigScore1, LigScore2, Piecewise Linear Potential (PLP), and Potential of Mean Force (PMF). The first four of the above methods were developed using the *empirical fitting approach*. The PMF function was developed using the knowledge-based statistical approach. The PLP function was initially developed as a docking function but has been shown to correlate well with binding affinities [30] The correct docking and scoring of candidate control ligands help to confirm that the docking and scoring methodologies and parameters are appropriate for the target receptor. Identification of hit ligands among the (random) input ligands was aided by the ranking of suitable control ligands.

2.7 *Calculating Binding Energies*

The binding energy and the average binding energy across a set of related poses were estimated between a receptor and a ligand. Additionally, the loss of conformational entropy and energy of a bound ligand was also estimated. [31] The binding energy was calculated using the following equation:

EnergyBinding = EnergyComplex - EnergyLigand - EnergyReceptor

2.8 *Studying Docking Interactions*

Non-bond interactions are interactions between two molecules or close contacts within macromolecules. Identifying and optimizing these interactions between a ligand and a protein is often a goal in structure-based drug design. The different types of interaction vary in strength, but the cumulative effect of even the weaker types can be significant. Several types of interactions were monitored, e.g., Hydrogen Bonds, Electrostatic, Hydrophobic, Halogen, and Unfavourable. [32] Many interactions were between single atom pairs. However, some interactions were based on groups of atoms (e.g., π-π, π-alkyl, etc.).

2.9 *Growing Scaffold*

After selecting the appropriate grow-points in SM-1, **(Figure 1)** the protocol grew the input ligand scaffold at the scaffold grow points based on the selected reactions and reagents. The enumerated ligands fit the binding site of the input receptor.

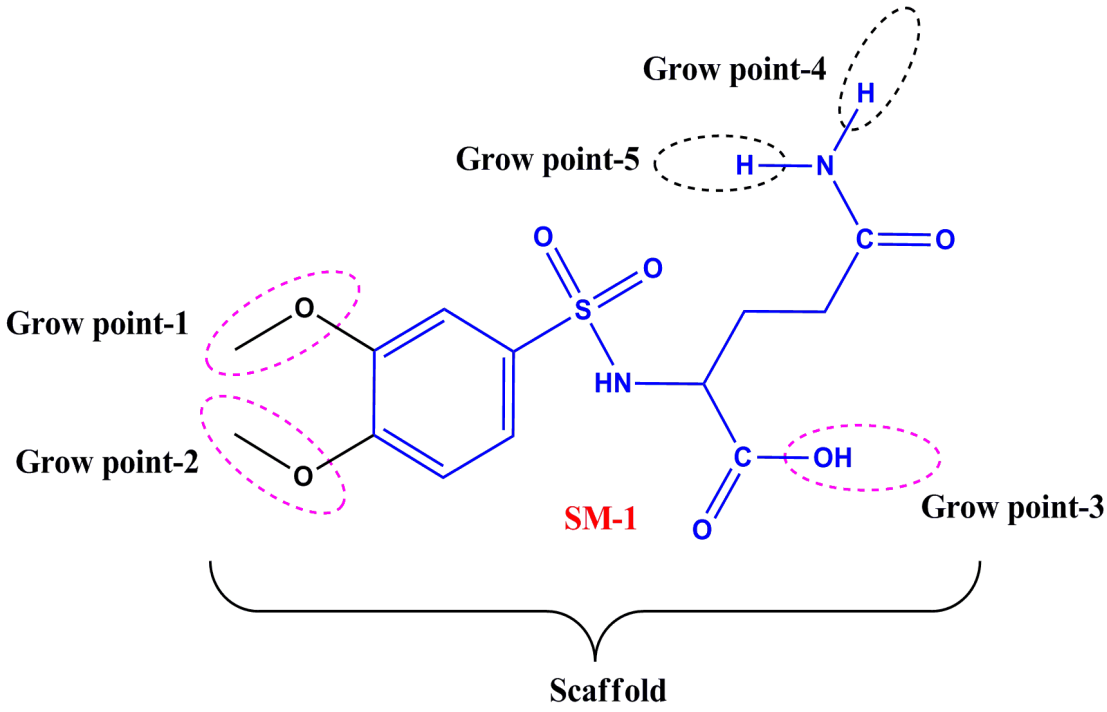

Figure 1: Five different selected grow points shown in the scaffold of SM-1 from which novel ligands will be designed.

The sequential steps that were performed in this protocol; creation of a reagent library, calculation of a protein grid, enumeration of scaffold link points, filtering ligands by residue interactions, minimization of ligand energy, and prioritization of ligands (Pareto sort). In the Pareto sort, ligands were prioritized to optimize receptor interactions, minimize receptor-bumps, minimize Lipinski's rule of five (RO5) violations, and optimize ligand novelty.

2.10 *Replace Fragment (Scaffold Hopping)*

The Replace Fragment protocol performs **scaffold hopping** by replacing part of the scaffold structure **(Figure 2)** while maintaining the favourable binding between the receptor and the ligand. It first searches the fragment libraries to identify isofunctional chemotypes (isosteric fragments) and then uses the isosteric fragments to replace the original fragment to generate novel compounds with different scaffolds by replacing one or more fragments in a lead structure. To refine the generated novel ligands, the protocol allowed the use of steric restraint of the protein active site and the ligands that interact with any residues defined in the interacting residues group. Ligand "novelty" was calculated by ranking and sorting the ligands by, number of chain assemblies, number of double and aromatic bonds, and N, S, O atom count.

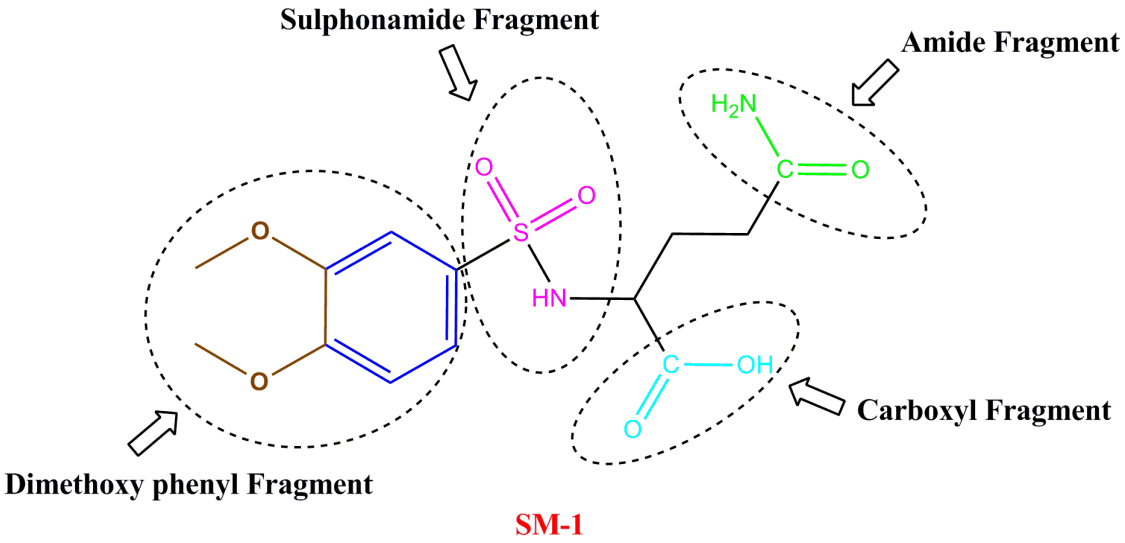

Figure 2: Substructures of SM-1 are highlighted for scaffold hopping (replace fragment) in a step-wise manner.

2.11 *Re-Docking of the Novel Ligands*

The Pareto sorted novel ligands were re-docked following the protocol laid in DS into the active site of VEGFR-2. This re-docking helps us compare the designed ligands' docking parameters with the control ligand Sorafenib.

3. Results and Discussion

The protocol computed the docking parameters for the active compound SM-1 and the control ligand Sorafenib at the active site of VEGFR-2. The docked pose of Sorafenib was found to be comparable to its crystal structure in 4ASD, with an RMSD of 0.20, indicating minimal variance in dock pose prediction. The protocol enumerated 13,650 novel ligands (*hits*) with different features. Some filters were applied to reduce the number of hits to a rational number promising, e.g., Lipinski's rule of five (RO5), setting the threshold for the LibDock score as 103.023 and binding energy -29.3578 kcal/mol. The protein's active site was used to refine the suggested fragments, and the novel ligands generated better reflect the environment in which they would potentially bind and be active. These rational filtering and prioritizing helped us to reduce the number of hits from 13,650 to 111. The LibDock score, binding energy, -PLP1, and -PLP2 values of SM-1 and Sorafenib were compared with the novel ligands (NL) **(Table 1)**. Based upon the LibDock score, binding energy and novelty index, 5 new ligands, **(Figure 3)** were proposed herein for synthesis and subsequent biological evaluations in the future investigations, viz. (E)-2-(3-amino-3-oxopropyl)-5-((Z)-(3,4-dimethoxyphenyl)diazenyl)pent-4-enoic acid (NL-1), (S)-2-(3,4-dimethoxy phenylsulfon amido)-N1-hydroxypentanediamide (NL-19), (2S,4R)-2-(3-amino-3-oxopropyl)-4-(3,4-di methoxyphenyl)hex-5-enoic acid (NL-30), (R)-5-amino-2-(2-((3,4-dimethoxybenzoyl) oxy)hydrazinyl)-5-oxopentanoic acid (NL-33) & (R)-5-amino-2-(3,4-dimethoxybenzamido)-5-oxopentanoic acid (NL-111).

Table 1: Docking parameters of Sorafenib, SM-1 and Hit molecules are explained with novelty index of the hit/ novel compounds. The list is arranged according to the highest LibDock score. GS= Grow Scaffold; SH= Scaffold Hopping

Code	LibDock Score	Binding Energy	-PLP1	-PLP2	Total PLP score	Ligand Novelty Index	Fragment similarity	Lead optimization method	Point of Modification
4ASD	103.02	-157.31	121.64	108.7	230.34	-	-	-	-
SM-1	98.03	-29.35	79.56	76.27	155.83	-	-	-	-
NL-1	129.29	-121.96	114.64	103.99	218.63	13	0.81	SH	Sulfonamide Fragment
NL-19	119.24	-46.96	86.85	77.81	164.66	1	0.06	GS	Grow point-3
NL-30	116.42	-91.07	114.17	113.08	227.25	47	0.12	SH	Sulfonamide Fragment
NL-33	115.98	-84.85	96.63	86.4	183.03	1	0.59	SH	Sulfonamide Fragment
NL-111	103.41	-180.88	81.03	74.21	155.24	21	0.11	SH	Sulfonamide Fragment

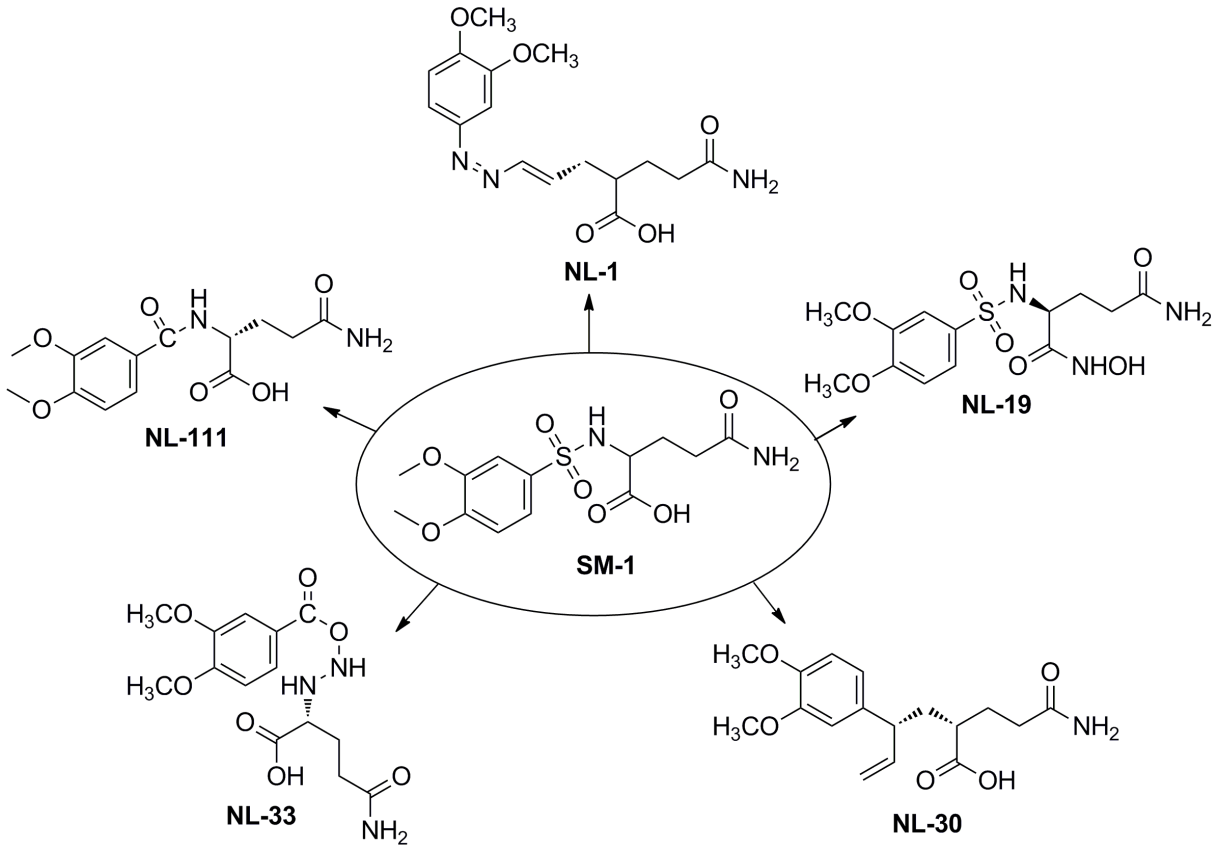

Figure 3: New ligands designed from SM-1 based on Docking with VEGFR-2

The docking interaction study of *Sorafenib*, *SM-1***(Figure 4)**, *NL-1***(Figure 5)**, *NL-19* **(Figure 6)**, *NL-30* **(Figure 7)**, *NL-33* **(Figure 8)**, and *NL-111* **(Figure 9)** are reported in the **Table 2**.

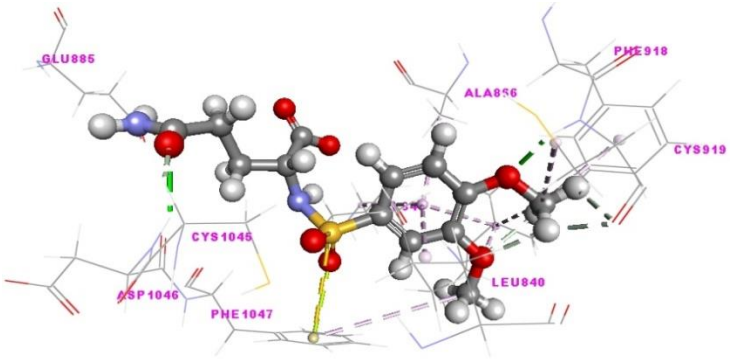

Figure 4: Docking interaction of SM-1 with VEGFR-2.
Green dotted lines represent Hydrogen bonds.

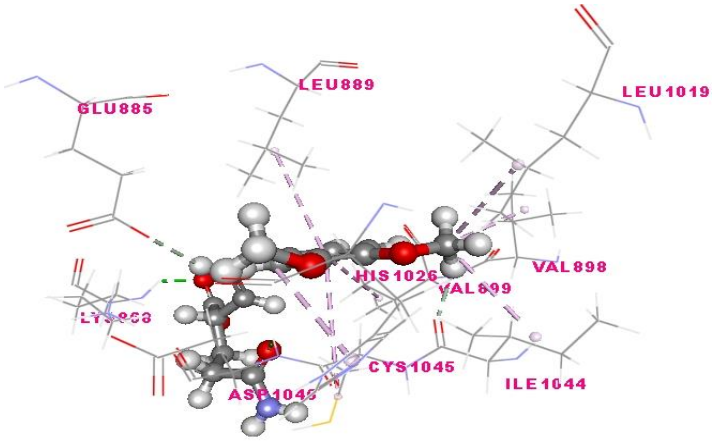

Figure 5: Docking interaction of NL-1 with VEGFR-2.
Green dotted lines represent Hydrogen bonds.

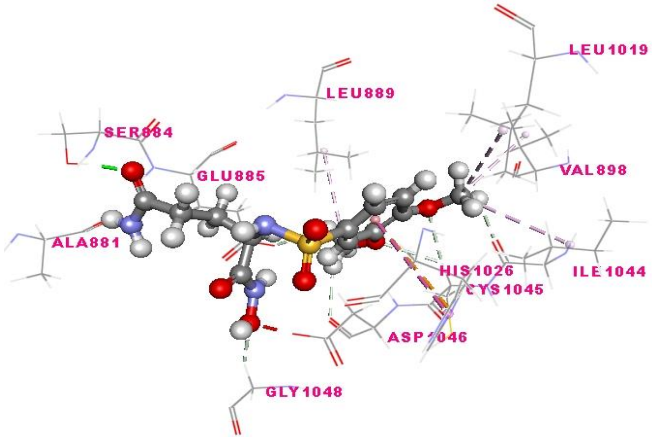

Figure 6: Docking interaction of NL-19 with VEGFR-2.
Green dotted lines represent Hydrogen bonds.

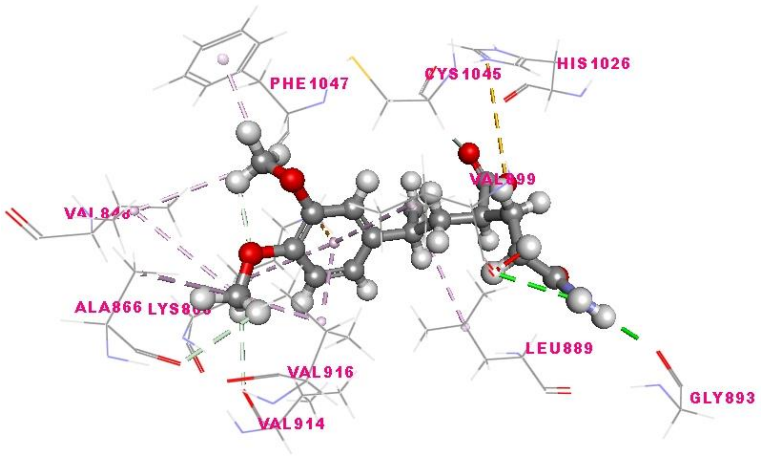

Figure 7: Docking interaction of NL-30 with VEGFR-2.
Green dotted lines represent Hydrogen bonds.

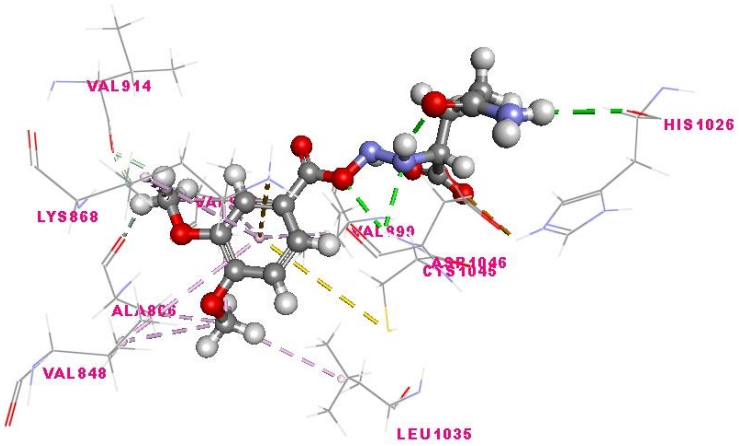

Figure 8: Docking interaction of NL-33 with VEGFR-2.
Green dotted lines represent Hydrogen bonds.

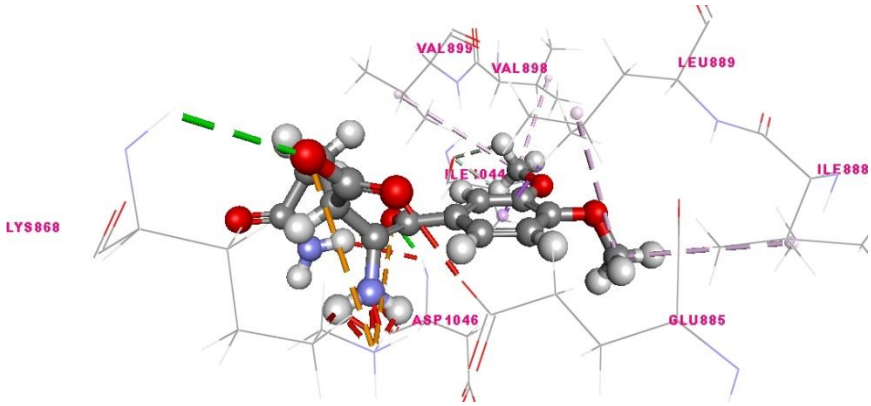

Figure 9: Docking interaction of NL-111 with VEGFR-2.
Green dotted lines represent Hydrogen bonds.

Table 2: Non-bond Interactions of the Sorafenib (reference), SM-1 and Hit compounds with the amino acid residues of VEGFR-2 protein

Sl. No.	Compound	Category	Types of interaction	Residues	Bond Distance (Å)
1	Sorafenib (Reference)	Hydrogen Bond	Conventional	ASP1046	2.22495
				CYS919	1.87747
		Hydrophobic	π-Alkyl	VAL848	4.8000
				ALA866	4.2864
				LEU1035	4.3093
				VAL848	5.4569
				LYS868	4.3936
				VAL899	5.2995
				VAL916	3.8730
				LEU840	4.6062
			Alkyl-Alkyl	LEU840	4.73152
			π-Sigma	LYS868	2.66117
		Electrostatic	π-Cation	LYS868	4.43416
		Halogen	Fluorine	LEU840	3.53656
2	**SM-1**	Hydrogen Bond	Conventional	HIS1026	2.72193
				ARG1027	2.04097
				ASP1046	2.72887
		Electrostatic	Attractive Charge	LYS868	4.65977
3	**NL-1**	Hydrogen Bond	Conventional	LYS868	2.35161
				ASP1046	2.58787
		Hydrophobic	Pi-Alkyl	LEU889	5.07156
				VAL899	4.97029
				CYS1045	5.26222
4	**NL-19**	Hydrogen Bond	Conventional	SER884	1.76121
				ALA881	1.94397
		Hydrophobic	π –π stacked	HIS1026	5.0893
		Electrostatic	π- cation	HIS1026	4.71361
5	**NL-30**	Hydrogen Bond	Conventional	VAL899	2.78625
				GLY893	1.98807
		Hydrophobic	Pi-Alkyl	LYS868	4.93612
				VAL899	4.8123
				VAL916	3.97804
			Alkyl	LEU889	4.84501
		Electrostatic	Attractive Charge	HIS1026	4.99443
			π- cation	LYS868	4.99443

6	**NL-33**	Hydrogen Bond	Conventional	ASP1046 ASP1046 HIS1026	2.4822 2.39738 2.42006
		Hydrophobic	Pi-Alkyl	VAL848 LYS868 VAL899 VAL916	5.4942 5.02164 4.84825 4.18131
		Electrostatic	Salt Bridge	HIS1026	3.2238
			π-Cation	LYS868	4.60445
		Other	π- Sulphur	CYS1045	5.57059
7	**NL-111**	Hydrogen Bond	Conventional	LYS868 ASP1046	3.03343 1.72143
		Electrostatic	Attractive Charge	LYS868 LYS868	4.97013 5.03992
		Hydrophobic	π-σ	LEU889	2.04576

The LibDock score of NL-1 is highest with 129.299, NL-19 and NL-33 have a ligand novelty index of 1, NL-30 has the highest ligand binding affinity of -227.25, and NL-111 has the best binding energy with -180.889. In this present study, the index of novelty ranks ranges from 1 to 392, index 1 being the most novel. We have chosen these five novel ligands for the chance of becoming promising hit compounds as they bear at least any one of the best features described above. However, novel ligands above the threshold values for SM-1 are of prime interest.

Exploring molecular interactions of Sorafenib reveals that it binds to different functional states of VEGFR2, viz. the DFG-out state of VEGFR-2. It forms two conventional hydrogen bonds; one with ASP1046 (H-bond acceptor); this region extends as the hydrophobic back pocket of the receptor, and CYS919 (H-bond acceptor) and one halogen (Fluorine) hydrogen bond with hydrophobic LEU840 **(Table 2)**. The pyridine ring and the substituted phenyl ring, which are attached with the ethereal linkage is placed on the more hydrophobic region of the receptor forming π-alkyl interaction with the residues like VAL848, ALA866, LEU1035, VAL899, VAL916, and LEU840. Three non-conventional hydrogen bonds are also seen, *i.e.,* with Cys1045, Phe1047 and Glu917. Similarly, SM-1 forms three hydrogen bonds with HIS1026, ARG1027, and ASP1046 residues. This region is a deeply buried hydrophobic pocket created by the movement of PHE1047 residue of the "DFG'' motif induces the "DFG-out'' conformation of the receptor. The novel ligands share the same space as SM-1 and orients with the favored DFG out conformation in Sorafenib. Exceptionally, NL-33 shares a π- Sulphur interaction with ASP1046. Comparing the binding free energy of the compounds shows that the values are most approximate to the standard. Physicochemical properties of the hits showed that the novel ligands are more polar than the drug Sorafenib; hence their ADME must be favored. The hydrogen bond acceptor and donor features are most similar to Sorafenib. **(Table 3)**

Table 3: Comparison of physicochemical properties of Sorafenib (reference), SM-1 and Hit compounds

Sl. No.	Code	M.W.	clogP	HBA	HBD
1	Sorafenib	464.82	4.11	7	3
2	SM-1	346.36	-0.35	8	2
3	NL-1	321.33	-0.35	7	2
4	NL-19	361.371	-0.56	8	4
5	NL-30	321.37	2.21	5	2
6	NL-33	341.32	-0.78	9	4
7	NL-111	324.33	0.34	6	4

Conclusion

It can be concluded from the present investigation that lead optimization of a prospective anticancer drug candidate, SM-1 has been proved to be encouraging. The Insilco method has given a wide variety of novel ligands with favored interaction with VEGF-R2, and the compounds were found to be synthetically accessible. Hence, the selected compounds may be synthesized and tested for antiangiogenic activity and anticancer activity in Multiple Myeloma and other relevant cell lines.

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Conflict of interest

None

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Ethics statement

None

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