Functional fragmentation of approved drugs to design novel VEGFR-2 Inhibitors

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Abstract: Many approved drugs acting as VEGFR-2 inhibitors were used to derive functional fragments for present fragment-based design of novel inhibitors. Fragments can be regarded as functional when derived from approved or experimental drugs and not just based on structure similarity. Various fragments received from approved drugs have been cross linked using a systematic combination method to produce many novel molecules which are closely related to the structures of approved drugs. These novel candidates have been evaluated and compared using virtual screening against all approved drugs used as source compounds. Novel compounds have outperformed the approved drugs in all aspects of results obtained after virtual screening including molecular docking scores, drug-likeness, ADME and toxicity parameters. The best novel molecule named as PO19_SU29_PO21 have recorded a docking re-rank score of -175.833 against its counter approved drug Ponatinib with re-rank score of -145.239. Cys-919, Glu-885 and Asp-1046 are the amino acids involved in H-bond formation with novel molecules. The same set of amino acids has been found to form H-bond with other approved drugs. Novel molecules have shown improved drug-likeness when compared with approved drugs. ADME studies suggest that all top ranked novel molecules have provided better Blood-Brain-Barrier (BBB) values. Toxicity parameters have been almost identical for novel and approved drugs

Keywords: Fragment-based drug design (FBDD), vascular endothelial growth factor receptor-2 (VEGFR-2) Inhibitors, virtual screening and molecular docking.

Introduction

Cancer is considered to be a group of over 100 distinct diseases which affects various tissues and cell types. However, all forms of cancer are symbolized by anomalous cell growth which results from a comparatively small number of rooted or environmentally-induced genetic anomalies [1]. Understanding the mechanism of carcinogenesis and angiogenesis can provide us with essential routes for cancer prevention. Various types of the cancers are mainly caused due to various environmental factors and the rest are caused due to inherited genes [2]. Environmental factors include lifestyle, economic and behavioral factors. Various other factors such as usage of tobacco (causing 25-30% cancers), obesity (30-35% cancers), infections (15-20% cancers), radiations (causing up to 10 % cancers), stress, lack of physical activity and environmental pollution are some of the other prime factors responsible for cancer [3]. Vascular endothelial growth factor (VEGF) is a signal protein produced by cells that stimulates vasculogenesis and angiogenesis. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate. Serum concentration of VEGF is high in bronchial asthma and diabetes mellitus [4]. VEGF's normal function is to create new blood vessels during embryonic development, new blood vessels after injury, muscle following exercise, and new vessels (collateral circulation) to bypass blocked vessels.

Cancer cells can penetrate blood and lymphatic vessels, circulate intravenously and can proliferate at some another site making cancer a threatening disease [5]. In 1970s, it was proposed by Dr. Judah Folkman of the Harvard Medical School that cancer can be treated with anti-angiogenic agents [6]. Vascular endothelial growth receptors (VEGFRs) have established as key factors in angiogenesis, found necessary for tumor outgrowth and can be targeted for tumor growth regression and inhibition. It has verified in a series of experiments that VEGFRs are the principal stimulators of angiogenesis [7]. During the meager supply of oxygen in tissues, the cells produce vascular endothelial growth factor (VEGF) which binds to VEGFRs and their complex acts as a signaling protein to restore and supply oxygen to outgrowing tumor cells.

Present work includes a systematic approach of fragmentation and their possible cross-linking to generate all possible chemical structures which could act as VEGFR-2 inhibitors. Present work has included eleven most promising and FDA approved drugs acting as VEGFR-2 inhibitors which are currently being prescribed. These eleven approved VEGFR-2 inhibitors include Afatinib [8-14], Axitinib [15], Cobozantinib [16-18], Imanitinb [19,20], Lenvatinib [21,22], Pazopanib [23-30], Ponatinib [31-33], Regorafenib [34,35], Sorafenib [36-41], Sunitinib [42], and Vandetanib [43-48]. The purpose of selecting these eleven approved drugs in present fragment-based drug design is to include only selective and functional fragments. General practice of fragment-based drug design indicates use of fragments which are structurally similar to the lead fragment. These structurally similar fragments can be derived using random search which is purely based on chemoinformatics search algorithms. These randomly searched and selected fragments may or may not be functional. Therefore, present studies have derived fragments from only

approved drugs acting on the same biological target. Approved inhibitors when allowed to produce fragments will surely generate functional fragments. These functional fragments derived from approved VEGFR-2 inhibitors are further cross-linked to generate novel molecule structures.

2. Methodology

3D structure of VEGFR-2 receptor as target protein has been retrieved from Protein Data Bank (PDB) available as 3WZE (www.rcsb.org/3WZE) [49]. The complex structure of VEGFR-2 and Sorafenib has been determined using X-ray diffraction method at high resolution of 1.9 Angstrom. Synchroton generated X-rays of single wavelength were used with PIXEL detector to determine 3D structure of the complex in vapor diffusion method.

All the approved and selected eleven VEGFR-2 inhibitors represent chemical structures carrying functional pharmacophoric groups in form of fragments. Fragmentation schemes of all selected and approved eleven VEGFR-2 inhibitors have been produced in figure 1 below.

Functional fragments were derived from approved VEGFR-2 inhibitors. These fragments were collected based on their pharmacophoric entities which represent a significant substitution in the original approved inhibitors. Fragments were found to offer a crucial role in determining target-inhibitor interactions. Molecular docking was conducted using Molegro Virtual Docker (MVD) [50] to confirm the significant role of these fragments against VEGFR-2 inhibition via H-bond, Vander Waal interactions, electrostatic interactions, hydrophobic and steric interactions. Chemical structure and scheme of fragmentation has been presented in figure 1 below. Figure 1 shows the three fragments (F1, F2 and F3) of each of all 11 FDA approved drugs which are Afatinib [A], Axitinib [B], Cabozantinib [C], Imatinib [D], Lenvatinib [E], Ponatinib [F], Pazopanib [G], Regorafenib [H], Sorafenib [I], Sunitinib [J] and Vandetanib [K] respectively.

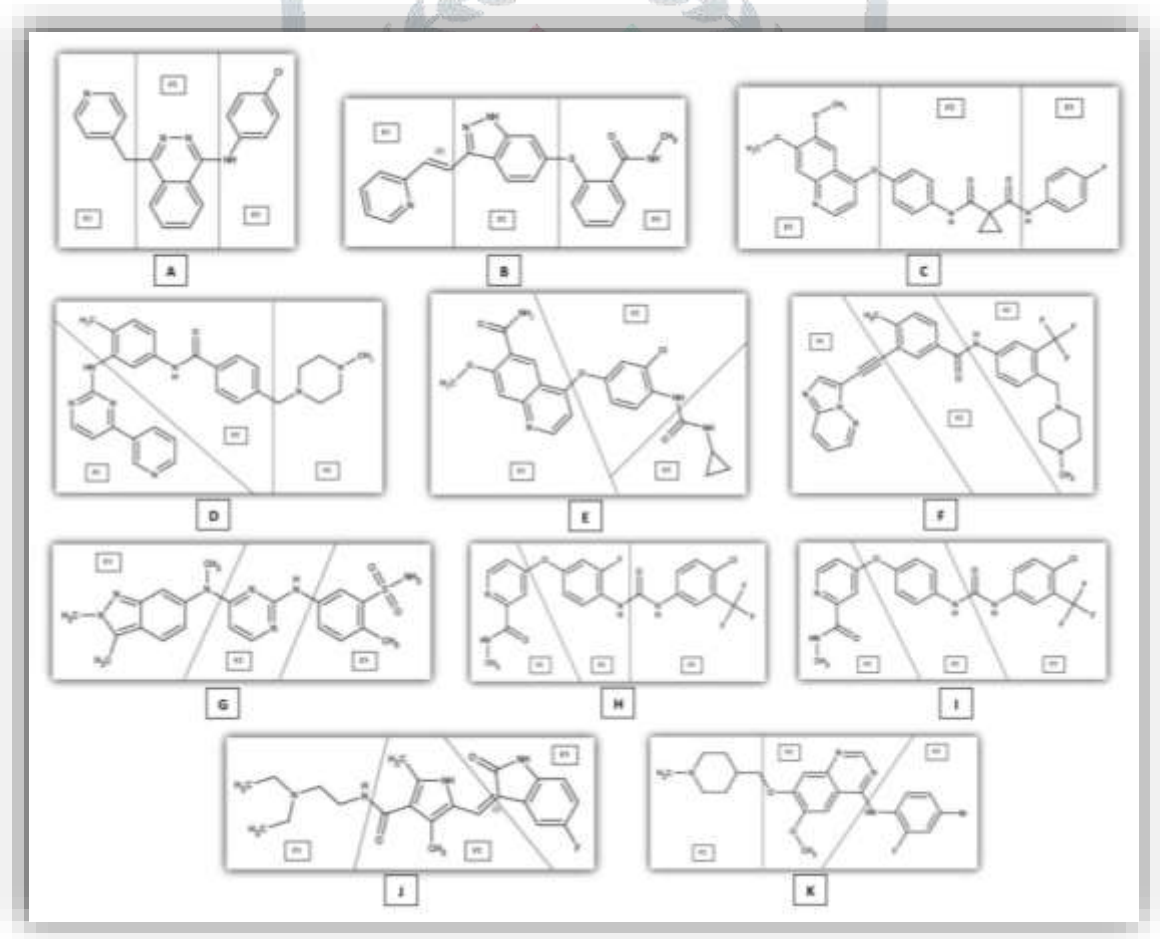


Figure 1: Chemical structure and scheme of fragmentation of FDA approved VEGFR-2 inhibitors

Fragments received from above approved inhibitors were collected and combined in a definite order to generate novel chemical structures. Combination method utilizes a variation in single fragment of structure of an approved VEGFR-2 inhibitor keeping the other two fragments constant at a time. Cross-linking of fragments was achieved using a method given below in table 1. Table 1

shows combination scheme where only one fragment has been replaced by similar fragments keeping the other two fragments constant for a given approved VEGFR-2 Inhibitor.

AF1 = F1 fragment of Afatinib,

AF2= F2 fragment of Afatinib,

AF3= F3 fragment of Afatinib

Similarly, AX (4,5,6),CA (7,8,9), IM (10,11,12), LE (13,14,15), PA (16,17,18), PO (19,20,21), RE (22,23,24), SO (25,26,27), SU (28,29,30) and VA (31, 32,33) are three fragments of other remaining 10 approved VEGFR-2 inhibitors included in cross-linking with fragments of Afatinib to design novel VEGFR-2 Inhibitors.

Table 1: Fragment cross-linking scheme using Ponatinib fragments.

Combination Scheme 1	**Combination Scheme 2**	**Combination Scheme 3**
PO19_PO20_AF3	PO19_AF2_PO21	AF1_PO20_PO21
PO19_PO20_AX6	PO19_AX5_PO21	AX4_PO20_PO21
PO19_PO20_CA9	PO19_CA8_PO21	CA7_PO20_PO21
PO19_PO20_IM12	PO19_IM11_PO21	IM10_PO20_PO21
PO19_PO20_LE15	PO19_LE14_PO21	LE13_PO20_PO21
PO19_PO20_PA18	PO19_PA17_PO21	PA16_PO20_PO21
PO19_PO20_RE24	PO19_RE23_PO21	RE22_PO20_PO21
PO19_PO20_SO27	PO19_SO26_PO21	SO25_PO20_PO21
PO19_PO20_SU30	PO19_SU29_PO21	SU28_PO20_PO21
PO19_PO20_VA33	PO19_VA32_PO21	VA31_PO20_PO21

Molecules obtained after cross-linking of functional fragments are further submitted for structure novelty check in Pubchem database. Few structures were found as 100% identical in Pubchem database while for most of them no identical structure was found. It proves that most of the chemical structures which were designed using functional-fragmentation and cross-linking in present fragment-based drug design attempt are novel. These novel molecules can be more promising if found to inhibit VEGFR-2 effectively when compared to the inhibition strength of their counter FDA approved molecules. All of these novel molecules have been completely designed using functional and existing fragments which were derived from approved drug candidates.

3. Results and Discussions

All the novel molecules along with approved VEGFR-2 inhibitors were included in ligand database which was submitted to virtual screening. Virtual screening typically involves the use of computational programs of receptor-ligand binding and drug likeness properties. Molecular docking scores usually measures binding affinity of a ligand towards a biological target like protein, enzyme or receptor. Docking scores are direct measures of sum of attractive and repulsive energies of interactions taking place between atoms or pharmacophoric groups of ligand and counter atoms or groups of amino acids of biological target structure. These attractive and repulsive forces include a variety of physiochemical forces like H-bond, electrostatic interactions, Vander Waal's force, hydrophobic, hydrophilic and steric interactions.

Novel molecules designed using functional fragment-based approach as described above were used in virtual screening in present studies. The first impression of molecular docking results confirms that the novel molecules designed using functional fragment-based approach have scored reasonable higher binding affinities when compared with approved drugs presently being used in cancer treatment as VEGFR-2 inhibitors. Table 2 includes molecular names of novel designed ligands; re-rank scores, number of H-bonds, amino acids participating in H-bond bonds, steric interactions and amino acids participating in steric interactions. PO19_SU29_PO21 is a novel candidate designed using fragments of Ponatinib and Sunitinib which has scored top rank in virtual screening with Re-rank score -175.833. The second ranked novel candidate (PO19_RE23_PO21) has scored Re-rank score -167.435. PO19_RE23_PO21 has been designed using similar fragments of Ponatinib where middle fragment was derived from Regorafenib. It is a noteworthy observation in docking results that all the top ranked novel ligands are only those which have been designed using Fragment 1(Ponatinib) and Fragment 3 (Ponatinib). All the top ranked novel candidates have only variation in middle fragment 2 which are predominantly derived from Sunitinib, Regorafenib and Lenvatinib. Another significant observation visible from Re-rank scores of out of 11 FDA approved drugs confirms that only Ponatinib, Sunitinib, Regorafenib and Lenvatinib could show higher interactions during docking. All the fragments used in designing of top five novel ligands based on re-rank scores are derived from these top ranked FDA approved drugs i.e. Ponatinib, Sunitinib, Regorafenib and Lenvatinib. Table 2 is showing the Re-rank scores, number of H-bonds, amino acids involved in H-bond and amino acids involved steric interactions of top ranked five poses of novel ligands designed using functional fragment-based approach and top ranked FDA approved drugs acting as VEGFR-2 inhibitors.

Table 2: Molecular docking results having Re-rank scores, number of H-bonds & steric interactions with different amino acid residues of best 5 novel candidates and four FDA approved drugs

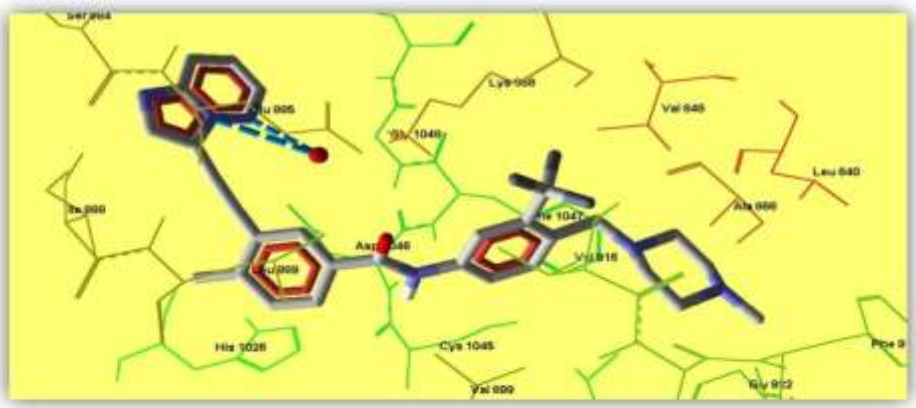
S.N.	Molecule Name	Re-rank Score	Number of Hydrogen Bonds & Amino Acid Residues	Steric Interactions & Amino Acid Residues
1	PO19_SU29_PO21	-175.833	**3**{Cys-919, Glu-885 & Asp-1046}	**9**{Glu-917, Val-916, Leu-889, Glu-885, Asp-1046,Val-898 & lle-1044}
2	PO19_RE23_PO21	-167.435	**3**{Cys-919, Glu-885 & Asp-1046}	**6**{Cys-919, Val-848, Asp-1046,His-1026 & Arg-1027}
3	PO19_SU29'_PO21	-166.196	**1**{Cys-919}	**6**{Cys-919,Asp-1046, Val-848, Leu-889, Val-898 & lle-1044}
4	PO19_LE14_PO21	-164.537	**3**{Cys-919, Glu-885 & Asp-1046}	**7**{Cys-919, Glu-885, Asp-1046, lle-1044, Cys-1024 & lle-1025}
5	PO19_LE14'_PO21	-164.309	**2**{Cys-919 & Glu-885}	**4**{Cys-919, Glu-885 & His-1026}
6	Ponatinib	-145.239	----	**9**{Cys-919, Glu-885, Asp-1046, lle-888, Leu-889, Cys-1045, Val-916, Val-848 & Lys-868}
7	Sunitinib	-130.095	**2**{Asp-1046 & Glu-917}	**3**{Glu-917, Val-916 & Lys-868}
8	Regorafenib	-138.983	**4**{Cys-919, Glu-885 & Asp-1046}	**4**{Cys-919, Phe-1047, Val-899 & lle-1044}

9	Lenvatinib	-130.068	**6**{Cys-919, Glu-885, Asp-1046 & Leu-840}	**5**{Cys-919, Glu-917, Phe-1047 & Leu-840}

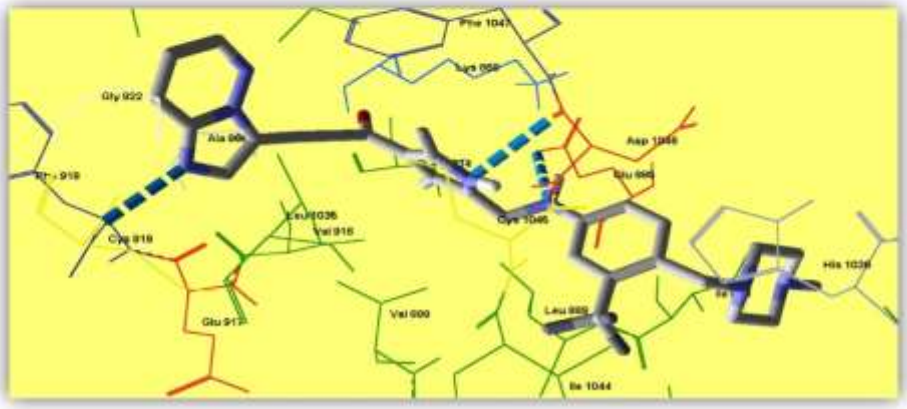
Based on the interaction profile of ligands and participating amino acids of VEGFR-2 protein structure it can be concluded that Cys-919 can be regarded as one of the key amino acid which has contributed in H-bond with almost every ligand in the table 2. Glu-885 is another amino acid residue of VEGFR-2 which has participated selectively in almost all top ranked novel molecules and FDA approved drugs. Asp-1046 is third amino acid in the list of H-bond forming amino acids (figure 2).

Similarly, there are steric interactions which usually records hindrance factor or clashes among atoms or functional groups of ligands with amino acids of VEGFR-2. Steric interactions do not favor attractive scores of binding affinity. Although the negative binding affinity scores (negative Re-rank scores) signify the fact that attractive forces are dominant over repulsive forces. Cys-919, Val-916, Leu-889, Glu-885, Ile-1044 and Asp-1046 are most repetitive amino acids involved in steric interactions (figure 3).

Presence and participation of identified amino acids of VEGFR-2 can be easily confirmed from diagrams below (Figure 2, 3 and 4). Conformations of top ranked ligands in terms of their selective poses show change in orientation when Fragments F1 and F3 derived from Ponatinib are kept constant and F2 kept changing. Results also confirm the role of middle fragment F2 derived from Sunitinib, Regorafenib and Lenvatinib which provide required flexibility and orientation in structure of novel candidates.

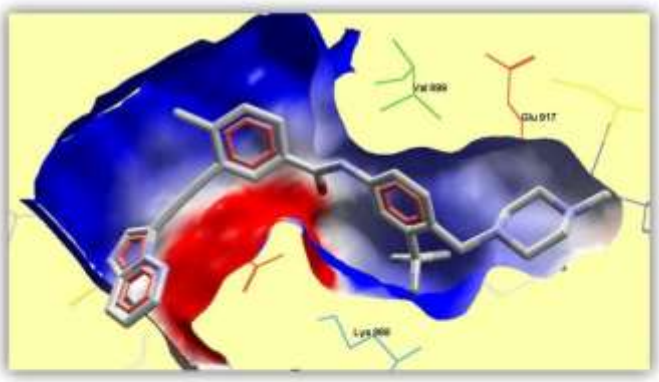


(H-bond Interactions of Ponatinib)

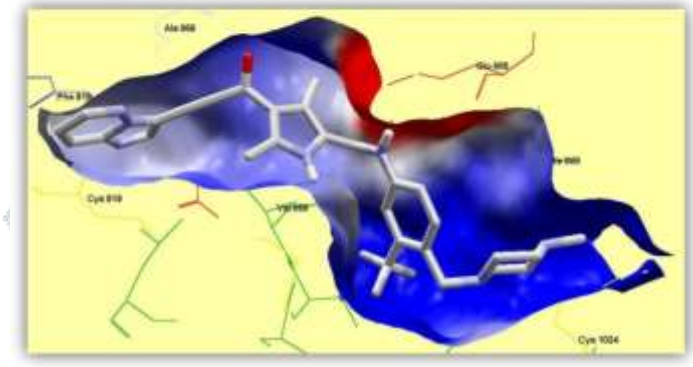


(H-bond Interactions of PO19_SU29_PO21)

Figure 2: Hydrogen bond interaction of Ponatinib and top ranked novel molecule designed using FBDD.

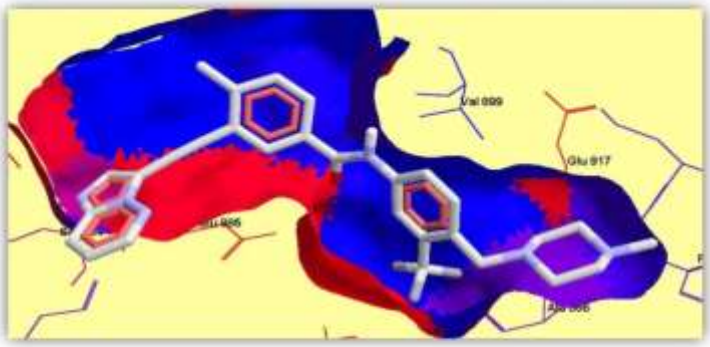


(Electrostatic Interactions of Ponatinib)

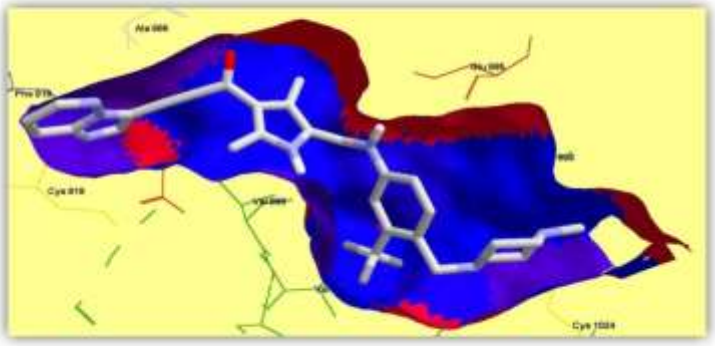


(Electrostatic Interactions of PO19_SU29_PO21)

Figure 3: Electrostatic interaction of Approved VEGFR-2 inhibitor and top ranked novel molecule designed using FBDD.



(Hydrophobic Interactions of Ponatinib)



(Hydrophobic Interactions of PO19_SU29_PO21)

Figure 4: Hydrophobic interaction of Ponatinib and top ranked novel molecule designed using FBDD.

A comparative estimation of drug-like parameters for top ranked novel molecules designed by functional fragmentation and FDA

approved drugs has been produced below in the table 3. Drug-likeness parameters have produced interesting results. Ponatinib being FDA approved drug failed to score qualifying criteria in drug-likeness. Ponatinib was identified as Mid-structure by MDDR Like Rule instead Drug-like mark. Ponatinib has violated MDDR rule in terms of No Rotatable Bonds. Surprisingly, all top ranked best poses of newly designed molecules based on the same Ponatinib molecule have qualified the MDDR Like rule without violating any features. All the newly designed molecules have been confirmed as drug-like under MDDR Like Rule. Rule of five has also found top ranked newly designed molecules suitable as drug-like molecules. Ponatinib has been again found under 'Violated' tag with issues related to molecular weight and AlopP98_value. Table 3 below shows promising character of newly designed VEGFR-2 inhibitor candidates using functional fragmentation based approach. Therefore, as a strong recommendation based on present results, these newly identified molecules should be synthesized and tested in vitro for their possible VEGFR-2 inhibition profiles.

Table 3: Comparison of drug-likeness parameters of newly designed molecules and selective FDA approved drugs.

	Druglikeness parameters				
S.N.	**Mol. Structure/ Mol. Code**	**MDDR Like Rule**	**MDDR Like Rule Violation Fields**	**Rule of Five**	**Rule of Five Violation Field**
1	PO19_SU29_PO21	Drug-like	----	Suitable	Molecular_weight
2	PO19_RE23_PO21	Drug-like	----	Suitable	Molecular_weight
3	PO19_SU29'_PO21	Drug-like	----	Suitable	Molecular_weight
4	PO19_LE14_PO21	Drug-like	----	Violated	Molecular_weight, AlopP98_value
5	PO19_LE14'_PO21	Drug-like	----	Violated	Molecular_weight, AlopP98_value
6	Ponatinib	Mid-structure	No_Rotatable_bonds	Violated	Molecular_weight, AlopP98_value
7	Sunitinib	Drug-like	----	Suitable	----
8	Regorafenib	Mid-structure	No_Rotatable_bonds	Suitable	----
9	Lenvatinib	Mid-structure	No_Rotatable_bonds	Suitable	----

There is a significant level of difference observed in Blood-Brain-Barrier (BBB) values of newly designed candidates and selective approved drugs acting as VEGFR-2 inhibitors. The average value of BBB for a CNS acting drug is estimated to be 2.1. All newly designed molecules were calculated to carry BBB value above 2.1 except the first PO19_SU29_PO21. Their BBB values confirm their improved CNS penetration ability by many folds when compared to BBB values of selective approved drugs like Ponatinib, Sunitinib, Regorafenib and Lenvatinib (Table 4). It can be clearly understood from this observation that a single fragment change can bring major changes in physio-chemical and biological properties of a drug like molecule.

Similarly, Predicted Caco2 activity shows slightly higher values for newly designed molecules when compared to Caco2 activity of selective approved drugs. Human Intestinal Absorption (HIA) remains close to each other which show newly molecules are equally absorbable from intestine as approved drugs. Similar equivalent values have been calculated for MDCK, Plasma Protein

Binding, Skin Permeability, SKlogD Value, SKlogP Value and buffer values SKlogS (Table 4) for newly designed candidates and approved drugs.

Table 4 clearly indicates the comparison of Absorption, Distribution, Metabolism and Excretion (ADME) parameters of newly designed molecules and selective FDA approved drugs.

Table 4: Comparison of ADME parameters of newly designed molecules and selective FDA approved drugs.

	ADME parameters								
Molecule Code/Name	**Blood-Brain-Barrier (BBB)**	**Caco2**	**HIA**	**MDCK**	**Plasma Protein Binding**	**Skin Perme-ability**	**SKlogD Value**	**SK logP Value**	**SK logS buffer**
PO19_SU29_PO21	2.00	39.86	94.03	0.05	83.55	-2.42	3.61	5.17	-4.48
PO19_RE23_PO21	2.39	40.99	96.09	0.06	87.73	-2.19	4.70	6.27	-10.22
PO19_SU29'_PO21	2.35	36.98	93.92	0.05	84.84	-2.49	3.29	4.49	-3.22
PO19_LE14_PO21	3.48	45.77	96.39	0.05	90.24	-2.12	5.24	6.80	-10.74
PO19_LE14'_PO21	3.39	46.75	96.42	0.05	84.03	-2.14	5.59	7.15	-15.24
Ponatinib	0.20	33.72	97.20	0.05	86.76	-2.15	4.46	6.02	-2.97
Sunitinib	0.91	34.77	90.09	7.81	64.22	-4.44	1.40	2.96	-3.71
Regorafenib	1.32	22.44	93.52	0.08	91.05	-2.50	4.71	4.71	-5.25
Lenvatinib	0.05	18.99	93.21	0.35	86.65	-4.22	3.16	3.16	-5.92

Conclusion

Present fragment-based design method has utilized functional fragments derived from established (FDA approved) drugs which acts as VEGFR-2 inhibitors. A systematic fragmentation of eleven approved drugs was performed to generate functional fragments. These functional fragments were further cross linked in a definite order to yield many structural combinations of novel molecules which have not been reported in medicinal chemistry when search in Pubchem repository. These novel molecules were further submitted to virtual screening using molecular docking, drug-likeness, ADME studies and toxicity studies. Comparative virtual screening results have confirmed better binding affinities of newly designed molecules over approved VEGFR-2 inhibitors presently used to treat cancer. Improved docking scores in terms of secondary valency interactions confirm that present functional fragmentation based approach have produced novel molecules possessing better binding affinities than established drugs available in the market. insilico analysis of their drug-likeness, ADME and toxicity parameters have found these novel candidates to be safe and appropriate for further synthesis, in vitro and in vivo studies.

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