

Use of natural molecules as anti-angiogenic inhibitors for vascular endothelial growth factor receptor

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Abstract:

Angiogenesis refers to the formation of new blood vessels, controlled by certain chemicals, which on stimulation repairs damaged cells or form new ones. Other chemicals, called angiogenesis inhibitors, signal the process to stop, having only mild side effects and are non toxic to most healthy cells. In our study, attempt was made to find potent anti-angiogenic inhibitor (pazopanib was considered as a reference drug) for vascular endothelial growth factor receptor (VEGFR-1/FLT-1), which served as a molecular target, using natural agents targeting biological processes important in cancer. Hundreds of natural molecules were initially screened based on lipinski's rule of five and the satisfying ones were taken for receptor-ligand interaction study using docking tools like HEX and quantum. Around fifteen molecules were taken as lead molecule and their binding pocket on VEGF was analyzed using SwissPDBviewer and Q-site finder. The investigational drug pazopanib was found to be interacting with leucine 32 and glutamine 30 in terms of hydrogen bond with the distance of 1.86 and 2.49 Å respectively. Ames test for the molecules was predicted for probability of mutagenicity on molecular systems such as blood, cardiovascular system, gastrointestinal system; kidney, liver and lung were considered for further screening of the molecules. The natural molecules curcumin, epigallocatechin gallate (EGCG), barrigtozenol and finasteride were showing reliable interaction with VEGFR and their pharmacokinetics parameters were comparatively good than the pazopanib. The dietary product curcumin and EGCG can be cancer chemopreventive agents and the natural molecules barrigtozenol and finasteride can be effective inhibitors for VEGFR.

Keywords: Angiogenesis, VEGF, chemopreventive agent, pharmacokinetics study, Lipinski's rule.

Background:

Cancer is an abnormal growth of cells which tend to proliferate in an uncontrolled way and, in some cases, leads to metastasize [1]. Improvements in cancer-directed therapies and management strategies have led to significant gains in survival over the past several decades. While additional progress is unquestionably necessary, a substantial proportion of adult cancer patients (an estimated 64%) currently reach 5-year survival, and the number of cancer survivors has grown steadily, increasing from 3 million in 1971 to more than 10 million in 2002 [2]. Angiogenesis, the process of new blood vessel formation, is involved in many physiological and pathological settings such as ischemia, diabetes, atherosclerosis,

and cancer [2]. Recent advances in the development of angiogenesis-based therapies for treatment of angiogenesis-associated diseases have provided new hope in a wide variety of human diseases ranging from eye diseases to cancer. One group of growth factor receptors critically implicated in angiogenesis is vascular endothelial growth factor receptors VEGFR-1/FLT-1 (fms-like tyrosine kinase) and VEGFR-2/KDR/FLK-1 (fetal liver kinase) are the prototypes of a gene family encoding structurally related receptors, FLT-3/FLK-2 and FLT-4/VEGFR-3 and, a subfamily of receptor tyrosine kinases (RTKs) [3]. VEGF primarily utilizes its receptor VEGFR2 (also known as Flk-1 or KDR) to induce angiogenic responses by activating a variety of signaling cascades, including

activation of PI3K-Akt, PLC- γ -PKC, and MAPK [4]. Proangiogenic factors can positively regulate VEGF-VEGFR2 signaling. Sphingosine-1 phosphate via its receptor S1P1, placental growth factor via its receptor VEGFR1, and laminar flow via Src can transactivate VEGFR2 [5-8]. VEGFR2 activity is also regulated by direct interactions with other proteins, including co-receptor neuropilins [9], adhesion molecule vascular endothelial-cadherin (VE-cadherin) [10], and integrins. VE-cadherin is in complex with VEGFR2 and is critical for VEGF-induced survival (PI3K-Akt) signaling [11]. VE-cadherin-VEGFR2 also regulates EC permeability [12, 13]. Interestingly, a complex of VEGFR2, VE-cadherin, and PECAM-1 (CD31) has been shown to be a mechanosensor that functions upstream of integrin signaling and transduces shear stress-mediated angiogenesis and vascular remodeling [14]. It is also seen proinflammatory cytokine TNF via its receptor TNFR2 transactivates VEGFR2 [15, 16]. However, negative regulations of VEGFR2 by protein-protein interactions are less understood, and an endogenous inhibitor that directly binds to and modulates VEGFR2 activity has not been identified [17]. Nature is an infinite sphere of which the center is everywhere and the circumference nowhere and henceforth we have chosen around two hundred natural molecules derived from natural source that can be used to treat cancer.

Pazopanib served as a reference drug in our study. Pazopanib (GW786034B; GlaxoSmithKline) is a novel orally available, small-molecule tyrosine kinase inhibitor of VEGF receptor -1, -2, and -3 with IC₅₀ values of 10, 30, and 47 nM, respectively [18]. An initial nonrandomized, dose-escalation phase I study with pazopanib (GSK-VEG10003) showed stable disease or partial responses in relapsed/refractory patients with renal cell (RCC), Hurthle cell, neuroendocrine, GIST, adeno lung carcinoma, chondrosarcoma, leiomyosarcoma, and melanoma. Remarkably, of 12 patients with RCC, 7 patients had stable disease or tumor reduction and 1 patient had a partial response. Adverse side effects included manageable hypertension, tiredness and hair de-pigmentation. Pazopanib inhibits VEGF-triggered signaling pathways in both tumor and endothelial cells, thereby blocking in vitro MM cell growth, survival, and migration, and inhibits VEGF-induced up-regulation of adhesion molecules on both endothelial and tumor cells, thereby abrogating endothelial cell-MM cell binding and associated cell proliferation [19].

Here we have explored natural compounds inhibiting the process of angiogenesis by targeting VEGFR. Also, quantitative structure-activity relationships (*i.e.* partition coefficient log *p* value) was studied to identify the low toxic natural compounds that has good drug likeness properties compared to the reference drug pazopanib, which are detailed in further sections.

Methodology:

Pubchem

Pubchem chemical database was used to retrieve natural molecules which are targeting important biological process in cancer. Around two hundred molecules were studied in this category. The chemical structure of the molecules was retrieved in SD (Structure Data) format. Physical and chemical properties of all two hundred molecules were screened from the structure page of pubchem.

Lipinski's rule of five

Lipinski's rule-of-five analysis helped to raise awareness about properties and structural features that make molecules more or less drug-like. The guidelines were quickly adopted by the pharmaceutical industry as it helped apply ADME (Absorption, Distribution, Metabolism, and Excretion) considerations early in preclinical development and could help avoid costly late-stage preclinical and clinical failures. The guidelines predict that poor absorption or permeation of an orally administered compound is more likely if the compound meets the following criteria:

- Molecular mass greater than 500 Da
- High lipophilicity (expressed as cLogP greater than 5)
- More than 5 hydrogen bond donors
- More than 10 hydrogen bond acceptors

The molecules which are not meeting the above criteria were taken for docking studies (Figure 1a).

Protein Data Bank

Vascular endothelial growth factor (VEGF) is a homodimeric hormone that induces proliferation of endothelial cells through binding to the vascular endothelial growth factor receptor. Thus VEGFR acts as a target for molecular medicine. Crystal structure of vascular endothelial growth factor receptor (PDB id: 1FLT) (Figure 1b) was retrieved from protein data bank.

HEX

Molecules which are screened based on lipinski's rule features were subjected to receptor-ligand interaction study using HEX (Version 5.1) docking tool. Marvin sketch, chemical structure drawing tool, was used for file conversion and PDB (Protein Data Bank) format of small molecules were prepared for docking in HEX. The protein-ligand complex of the molecules was saved for active site analysis. Figure 1c is showing the docking score of top nine molecules out of two hundred agents targeting biological processes important in cancer.

SwissPDBViewer

The receptor-ligand complex of the molecules was subjected to active site analysis using SwissPDBviewer (Version 4.0.1) to find the amino acids contributing for binding pocket (Figure 1d). The pazopanib, an investigational drug for VEGFR is interacting with Leucine-32 and Glutamine -30 in terms of hydrogen bond. The hydrogen bond of all complexes was found by using this tool. Table 1 (see supplementary material) represents the binding site results along with the Hydrogen bond distance.

Q site finder

Crystal structure of VEGFR is not co-crystallized, so Q-site finder was used to find out the possible binding pockets in the VEGFR (PDB id: 1FLT). Around ten sites were generated by this tool and the site 7 (Figure 1e) may be an active site for this receptor as the binding site results from SwissPDBviewer and Q-site finder are correlating well in amino acid contribution for making binding pockets.

Quantum

The small molecules which were making hydrogen bond with the VEGFR were taken for docking study using commercial docking tool Quantum. Further screening of the molecules was based on the docking score comparison between HEX and

Quantum. **Figure 1f** is showing the docking score of barrigotegenol, epigallocatechin, finasteride and curcumin along

with investigational drug pazopanib.

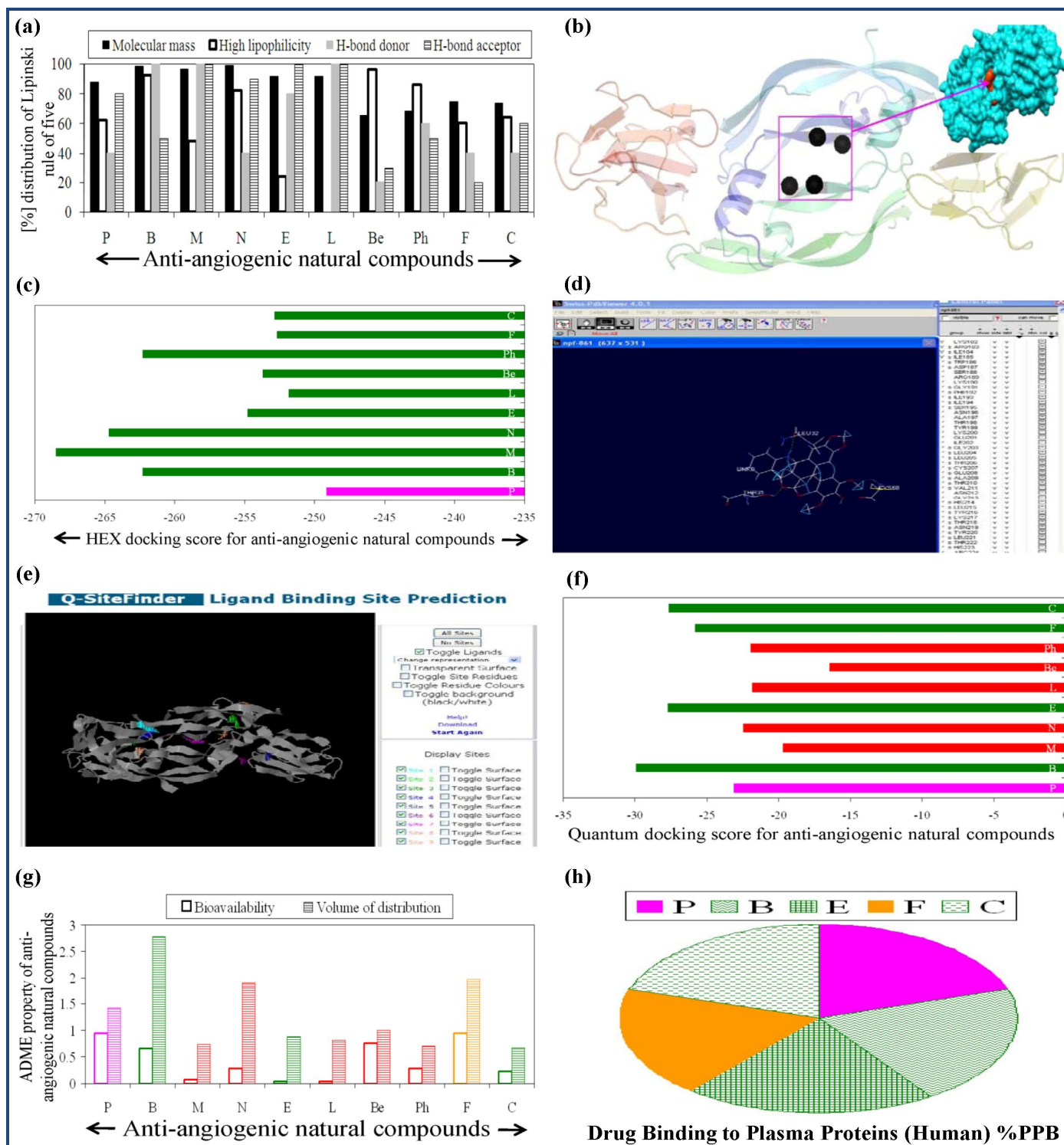


Figure 1: (a) Bar graph representing Lipinski rule of five for top 9 anti-angiogenic natural compounds screened through HEX; (b) Cartoon representation of VEGF receptor (PDB Id: 1FLT), binding site highlighted in black balls and inset representation of binding site in red; (c) Bar graph representing HEX docking score for top 9 anti-angiogenic natural compounds; (d) Graphical representation of amino acid and hydrogen bond analysis for the anti-angiogenic natural compound (Npfr_86i) using SwissPDBViewer; (e) Binding site analysis for the VEGF receptor using Q-site server prediction; (f) Bar graph representing QUANTUM docking score for top 9 anti-angiogenic natural compounds screened through HEX, (g) ADME property of top 9 anti-angiogenic natural compounds screened through HEX; (h) Pie-chart representation for percentage distribution of drug binding to plasma protein – humans, (The Compound Id represented in the bar graphs along with the color code used is same as represented in Table 1).

ADME analysis

ADME Boxes (Version 4.0) predictors are desktop software modules based on exacting data analyses and carefully built expert models for calculating vital properties. Most of the ADME Boxes modules combine several such independent models, based on sometimes even independent data sets along with experimental data for the most similar compounds that seek to provide maximum information for the decision, a consensus of all the results being a perfect suggestion that would be the right one. Twelve major pharmacokinetics and pharmacodynamics features were predicted for molecules which are showing good interaction with VEGFR, then the important features like bioavailability (**Figure 1g**), solubility, drug plasma binding protein and volume of distribution (**Figure 1g**) was considered for comparison studies.

Toxicity analysis

Tox Boxes (Version 2.0) is new high quality toxicity prediction software. The software provides predictions for three basic toxicity endpoints: acute toxicity, genotoxicity and organ specific health effects. Toxic effects of molecules are predicted solely from the chemical structure. Tox Boxes employ large and validated databases, robust Structure- Activity Relationship (QSAR) models in combination with expert knowledge in organic chemistry and toxicology. Ames test parameter was used for finding mutagenicity of the molecules. Health effects in blood, cardiovascular, gastrointestinal system, kidney, liver, and lungs were predicted and LD50mouse analysis used for finding acute toxicity of the molecules. **Table 1** is showing the ADME and toxicity properties of the top nine molecules out of two hundred agents targeting biological processes important in cancer.

Results:

Agents targeting biological processes important in cancer were taken for our study. Initial screening of the molecules was based on Lipinski's rule of five. Around 101 molecules were screened by applying this rule. All these molecules were subjected to receptor-ligand interaction study using HEX docking tool. The investigational drug pazopanib docking score was used as a reference in this study. Molecules which are showing better interaction with the vascular endothelial growth factor receptor (1FLT) than the pazopanib were subjected to binding site analysis using swissPDBviewer and Q-site finder. The investigational drug pazopanib is interacting with leucine 32 and glutamine 30 in terms of hydrogen bond with the distance of 1.86 Å and 2.49 Å respectively. Around ten possible binding pockets of VEGFR were predicted using Q-site finder. Analyzing the binding site results from SwissPDBviewer and Q-site finder it was clear that the molecules were showing interaction only in binding site 7. The following amino acids were observed towards contributing for the binding pocket in crystal structure of vascular endothelial growth factor receptor Cys-57, Gly-59, Leu-32, Glu-30, Thr-31, Arg-56, Ile-29. The molecules which are interacting with any one of these residues from the binding site analysis were selected for docking in Quantum, a commercial tool for finding receptor-ligand interaction. Absorption, distribution, metabolism, excretion and toxicity features of the top ten molecules which were showing interaction with VEGFR in both HEX and Quantum analysis were predicted using ADME Box and TOX Box tool.

For toxicity prediction, Ames test was considered for initial screening of the molecules based on their ability to induce mutation. Ames test is for determining if a chemical is a mutagen. The use of the Ames test is based on the assumption that any substance that is mutagenic may also turn out to be a carcinogen; that is, to cause cancer. The molecules which were showing the ability to induce mutation were rejected in the toxicity based screening. Ames test prediction value for pazopanib used as a reference and the molecules showing value less than the pazopanib were selected as lead molecules. Further health effects of these lead molecules in blood, cardiovascular system, gastrointestinal system, kidney, liver and lung were predicted. The LD50 values also were predicted for selecting reliable molecule for ADME analysis. The reference investigational drug is good in solubility, stability and absorption but no significant first pass metabolism in liver and intestine and no active transport. Plasma binding protein and volume of distribution prediction of pazopanib was unreliable in distribution analysis. The natural molecules curcumin, EGCG, finasteride and barrigtogenol were considered as final reliable molecules based on their ADME and Toxicity features. These molecules considered as better ligands for VEGFR based on their interaction, pharmacokinetics and pharmacodynamics features.

Conclusion:

Tumors cannot grow or spread without the formation of new blood vessels, scientists are trying to find ways to stop angiogenesis. Studies are going on to find out natural and synthetic angiogenesis inhibitors, also called antiangiogenic agents, in the hope that these chemicals will prevent or slow down the growth of cancer by blocking the formation of new blood vessels. In our study we predicted the angiogenic inhibition activity of natural agents targeting biological processes in cancer on vascular endothelial growth factor receptor. The natural molecules curcumin, epigallocatechin gallate (EGCG), barrigtogenol and finasteride were showing reliable pharmacokinetics and pharmacodynamics features than the investigational drug pazopanib were taken as outcome of our work. The dietary product curcumin and epigallocatechin gallate (EGCG) can be cancer chemopreventive agents and the natural molecules barrigtogenol and finasteride can be effective inhibitors for vascular endothelial growth factor receptor.

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Conflict of interest:

Authors don't have any conflict of interest.

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Supplementary material:

Table 1: Table representing amino acids contributing for binding pocket and chemo-preventive analysis for the top 9 anti-angiogenic natural compounds screened through HEX.

Serial no	Molecule (Compound Id)	Amino acid base (H bond distance in Å ⁰)	Ames test	Health effect						rank
				Blood	Cardiovascular system	Gastrointestinal system	Kidney	Liver	Lungs	
1	Pazopanib (P)	LEU 32 (1.63), GLY 59 (1.73)	0.048	0.57	0.75	0.93	0.27	0.23	0.50	Ref
2	Lactoferrin (L)	GLU 30 (2.21), PRO 28 (2.57), HIS 27 (2.03)	0.642	0.93	0.99	1.00	0.93	0.98	0.99	-
3	Benzophenone 3 (Be)	LEU 32 (2.77), GLY 52 (2.03)	0.099	0.11	0.29	0.28	0.33	0.04	0.55	-
4	Phytoestrogens (Ph)	GLY 59 (2.71)	0.105	0.31	0.41	0.30	0.87	0.49	0.40	-
5	Finasteride (F)	LEU 32 (1.56), GLY 59 (2.17)	0.001	0.82	0.91	0.65	0.92	0.94	0.98	4
6	Curcumin (C)	PHE 47 (2.01)	0.033	0.62	0.95	0.24	0.42	0.69	0.51	1
7	Epigallocatechin Gallate [EGCG] (E)	GLU 13 (1.21)	0.037	0.78	0.92	0.98	0.83	0.67	0.96	2
8	Milk Thistle (M)	GLY 59 (2.22)	0.262	0.99	0.93	1.00	0.87	0.90	0.95	-
9	Npfl-86i (N)	LEU 32 (2.44), CYS 32 (2.46), THR 31 (2.34)	0.237	0.77	0.97	1.00	0.73	0.98	0.99	-
10	Barringtonol (B)	GLU 30 (2.50), 1.59)	0.000	0.80	1.00	0.99	1.00	0.77	1.00	3

	GOOD		MODERATE		POOR		REFERENCE
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