

Discovery of Small Molecules Targeting VEGF Using Virtual Screening as Potential Therapies for Vascular Malformations

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ABSTRACT: Vascular malformations comprise a diverse group of congenital vascular disorders that often decrease the quality of life through symptoms of pain, hemorrhage, and organ dysfunction. The protein VEGF modulates vascular remodeling and angiogenesis, as well as vascular permeability and tissue repair, by binding to receptors; excessive VEGF signaling in the presence of vascular malformations often intensifies structural abnormalities. Targeting VEGF can therefore help mitigate the effects of vascular malformations and slow progression, yet VEGF inhibitors remain underexplored. Initial druggability tests were conducted with geometric, energetic, and machine learning based site prediction tools to find and evaluate several potential ligand-binding pockets. To further match compound candidates with the potential sites, Pharmit's pharmacore-based virtual screening tool was used to discover 15 possible drugs with satisfactory overlays on the 7 selected interactions of the VEGF protein. Each candidate virtually simulated molecular docking using SwissDock, and all 15 had satisfactory or better binding affinity and orientation. Prioritizing medical relevance, Lipinski's Rule of Five was applied using SwissADME to eliminate 7 candidates. Finally, ProTox-II's silico toxicity test showed that 5 of the 8 remaining drugs had tolerable levels of toxicity according to their predicted LD50 and toxicity class data. The findings shed light on 5 new VEGF inhibitors that demonstrate strong efficacy, availability, and safety. These drugs represent potential and progress in inhibiting vascular malformation growth and development as a new form of non-surgical therapy for such.

KEYWORDS: Chemistry, Medicinal Chemistry, Drug Discovery, VEGF, Vascular Malformation.

■ Introduction

Vascular malformations are congenital abnormalities in blood vessels that stem from a variety of rare diseases and disorders.¹⁻³ These abnormalities often form during fetal development but can sometimes form as a result of an injury during adolescence or pregnancy.^{4,5} Genetic mutations, such as hereditary hemorrhagic telangiectasia (HHT), can also contribute to the likelihood of a vascular malformation.⁶⁻⁸ The severity of such malformations can range from asymptomatic lesions to severe lumps causing pain, bleeding, or organ dysfunction.⁹⁻¹¹ Despite progress in classification and treatment, essential information, such as precise causes or ideal treatment, for vascular malformations continues to remain unknown.^{10,12,13}

Current standard strategies used to address vascular malformations are limited and cannot serve as long-term solutions.¹⁴⁻¹⁶ Traditional approaches like surgical excisions, sclerotherapy, and embolization are effective in localized control but fail to exhibit preventative care.^{7,15} Drug therapies such as the mTOR inhibitor sirolimus are promising and are considered the most optimal treatment, but their potential side effects and prolonged treatment present issues in use.^{1,14,17} The absence of any FDA-approved pharmaceuticals for the treatment of vascular malformations also poses an issue for patients.^{13,18} Thus, there is an urgent need for the development and discovery of safer, long-term treatments with greater efficacy.^{10,19}

To address such concerns, recent efforts to find drugs have centered around the identification and inhibition of relevant molecular pathways.^{9,20} Inhibitors for the PI3K/AKT/mTOR and RAS/MAPK/ERK pathways are seen as those with the

most potential, due to their importance in endothelial cell proliferation, migration, and differentiation.^{3,21} PIK3CA inhibitor alpelisib and other MEK inhibitors have promising preclinical trial results that could reduce lesion size or alleviate symptoms.^{19,22} However, the challenges of shifting scientific findings to accessible treatments with long-term safety remain and prevent such drugs from becoming mainstream therapies.

The signaling protein vascular endothelial growth factor (VEGF) regulates angiogenesis and is related to processes such as embryonic development. VEGF can trigger the proliferation and migration of new blood vessels through its binding to receptors such as VEGFR-1 and VEGFR-2.^{15,22} While VEGFR-1 plays the role of regulating VEGFR-2, VEGFR-2 is the main receptor that drives endothelial cell angiogenesis. The main process related to VEGF remains angiogenesis, but its signaling also relates to vascular permeability and tissue repair.^{4,17} With its diverse functions, VEGF remains relevant for physiological and pathological vascular processes.

Recent findings have shown a link between excessive VEGF signaling and the growth of vascular malformations.^{21,22} Abnormally high VEGF signaling often disrupts normal vessel development and stability, which creates structurally anomalous vessels. Especially in AVMs, elevated VEGF levels can also exacerbate vascular malformations and foster further deviation.^{5,12} The obstruction of VEGF signaling, on the other hand, using agents like bevacizumab has proven to decelerate lesion growth or even reverse early-stage vascular malformations.^{3,21} VEGF signaling also often triggers other pathways related to vascular malformations, such as the PI3K/AKT/

mTOR and RAS/MAPK/ERK pathways, enhancing the effectiveness of VEGF drug targets as candidates for vascular malformation therapy.^{9,19} The discoveries related to VEGF signaling and vascular malformation open up a new field of research for therapeutic strategies in addressing vascular malformations.^{10,16,18}

The 2025 review “Genetic Insights Into Hemorrhagic Stroke and Vascular Malformations: Pathogenesis and Emerging Therapeutic Strategies” reveals VEGF’s therapeutic potential in vascular malformation treatment.²³ The discovery of an intersection between RAS/MAPK/ERK pathways and VEGF signaling to trigger and manage function. There was also a strong link between VEGF drug candidacy and effective treatment for specifically AVMs and CCMs. The use of VEGF-inhibiting drugs such as bevacizumab was shown to normalize vascular permeability and to lessen lesion growth. Such efficiency was also extended to effectively address HHT through VEGF regulation.

Another 2025 study, “Extracranial arteriovenous malformations: towards etiology-based therapeutic management,” focused on the treatment of AVMs by reducing VEGF over-expression.²⁴ Increased VEGF levels were shown to cause a greater risk of hemorrhage and vascular malformation relapse even after embolization. Preclinical settings demonstrated how bevacizumab reduced dysplasia, and early human clinical trials also yielded efficacy. Such promising findings prompted the Bevacizumab phase II-III trials to begin to evaluate optimal dosing.

The recent 2025 paper “Vascular endothelial growth factor signaling in health and disease: from molecular mechanisms to therapeutic perspectives” focused on the phosphorylation of VEGFR2 to study how VEGF signals endothelial cell growth.²⁵ Specific phosphorylation sites on VEGFR2, such as Y951 or Y1214, were shown to be critical in processes like angiogenesis seen in vascular malformations. Mice with targeted mutations at specific phosphorylation sites were able to reduce vascular leakage and improve vessel stability, highlighting the possibility of precise interventions for VEGFR1/VEGFR2 signaling to treat vascular malformations.

■ Methods

Geometric-Based Binding Site Identification:

The ProteinPlus tool was accessed through the internet: ProteinPlus is a tool that identifies possible binding sites on different proteins based on their size and shape.²⁶ It mainly uses volume and surface area to determine how ideal the site is. To identify the VEGF protein, its PDB, a protein data bank code that is uniquely assigned to each discovered protein, of “4AF8” was typed into the blank text box. After clicking “go!”, the “DoGSiteScorer Binding Site Detection” and then the “DoGSiteScorer” were clicked to access the actual geometric-based binding site identification tool. As there are no special complications, standard settings were set and then “Calculate” was clicked. To see each respective site’s size, shape, location, and configuration in a different color, “show all pockets” was checked off.

Energetic-Based Binding Site Identification:

The FTSite tool was accessed through the internet: FTSite is a tool that identifies possible binding sites on different proteins based on their interactions with van der Waals probes.²⁷ To distinguish the identification of VEGF from other previous identifications, the job name was titled “VEGF.” To identify the VEGF protein, its PDB ID of “4AF8” was typed into the blank text box. After clicking “Find My Binding Site”, each binding site candidate was shown in a different color, but no additional information was given.

Machine Learning Site Identification:

The PrankWeb tool was accessed through the internet: PrankWeb is a tool that displays a molecular model of the protein with its possible binding sites.²⁸ It uses a variety of factors to score each site, but the amino acids making up each site can also be accessed. “Experimental Structure” was clicked to access the machine learning site identification tool. To identify the VEGF protein, its PDB of “4AF8” was typed into the “PDG” text box. After clicking “Submit”, a model of the VEGF protein appeared, which could be moved and zoomed in to show specific details in the protein and its sites, as well as the amino acids that make up the protein and its sides.

Virtual Screening:

The Pharmit tool was accessed through the internet: Pharmit is a virtual screening tool that uses interactions in different proteins to match them with existing small-molecule drugs.²⁹ To identify the VEGF protein, its PDB of “4AF8” was typed into the “PDB” text box. After clicking “submit” and turning “Receptor Surface Opacity” to the lowest, a model of the interactions within the VEGF protein appeared. “Receptor Surface Opacity” is the opacity of the VEGF protein, and setting it to low ensured that the interactions are more visible. Clicking “ON” for all “Hydrogen Donor”, “Hydrogen Acceptor”, and “Hydrophobic” interactions balanced the the seven interactions are balanced between too many and too few so that there were a large amount of results, but also so that all the results had low enough RMSDs.

Molecular Docking:

The ChemDiv website was accessed through the internet to identify each small molecule. The each name small molecule “X###-####” was typed into the blank text box. After clicking “enter” and clicking “compound profile”, finding and copying “smiles” noted the SMILES, or the code that describes the structure of a compound or molecule in a way that is readable by online chemistry tools, for further processes. The SwissDock tool was accessed through the internet: SwissDock is a tool that virtually runs molecular docking to see if selected compounds are able to dock and bind to a protein target.³⁰ To identify the small molecule, its SMILES from was typed in the “Provide a SMILES” box, and “Prepare ligand” was clicked to prepare the small molecule. To identify the VEGF protein, its PDB of “4AF8” was typed in the “Provide a PDB ID” box, and “Prepare target” was clicked to prepare the protein. The “Define search space” was used to remodel the cross-section,

and trial and error was led to the initial values 22, 23, 32 for the "Search box center" box and the values 44, 46, 51 for the "Search box size" box. Clicking "Check parameters" provided an estimated time, and, depending on the small molecule, if the estimated time was over 1:00:00, going back to "Define search space" and shrinking the cross-section size until the estimated time was under 1:00:00 made the process more efficient. To be notified of the results after the listed time, email and docking name were entered before clicking "START DOCKING."

Drug Properties:

The SMILES for each small molecule was found and copied before accessing the SwissADME tool through the internet: SwissADME is a tool that finds drug properties to see if they follow Lipinski's rules and can serve as a satisfactory drug.³¹ The previously copied SMILES was pasted in the "Enter a list of SMILES here:" box, and "Run!" was clicked.

Toxicity:

The SMILES for each small molecule was found and copied before accessing the ProTox-II tool through the internet: ProTox-II tool is a tool that identifies the level of toxicity in different drugs.³² Clicking "TOX PREDICTION" led to a "Canonical Smiles" box where the previously copied SMILES was pasted, and clicking "smiles", "All", and "Start-Tox Prediction" began the process.

Results and Discussion

Geometric-Based Binding Site Identification:

Generally, a 300-700 Å² surface area is considered "druggable" for binding sites based on geometric analysis.³³ Smaller or larger sizes are often difficult to bind small molecules to efficiently. With surface areas smaller than 300 Å², a lack of contact points can obstruct proper binding.³⁴ On the other hand, surface areas greater than 700 Å² can lead to weak or nonspecific binding, as well as insufficient concavity to contain the drug.³⁵ Thus, when analyzing the 10 possible sites found based on geometric-based identification, G_P6, G_P7, G_P8, and G_P9 are all undruggable due to their less than 300 Å² surface area (Table 1). While G_P0 is also larger than typical druggable sites, it remains the ideal candidate due to its location (Figure 1). As the site is a pocket with most of its sizable surface area being enclosed within the protein, concerns about concavity and nonspecific binding are better addressed.

Table 1: This table contains data representing the 10 binding sites found through geometric analysis. Each row lists the volume (Å³), surface area (Å²), color code, and druggability based on the previous factors. G_P0 is the most ideal binding site candidate.

	G_P0	G_P1	G_P2	G_P3	G_P4	G_P5	G_P6	G_P7	G_P8	G_P9
Volume (Å ³)	873.09	543.23	440.38	279.19	174.4	173.18	131.84	126.89	121.41	101.18
Surface Area (Å ²)	910.38	647.67	535.62	480.86	380.47	356.91	271.37	277.96	224.56	273.48
Drug Score	0.83	0.86	0.8	0.57	0.33	0.31	0.19	0.27	0.34	0.13
Color	Yellow	Purple	Neon Green	Red	Blue	Muddy Green	Pink	Teal	Orange	Indigo

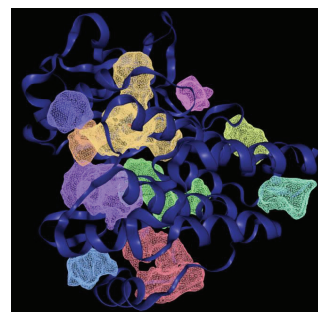


Figure 1: The protein plus site identified 10 different binding sites on the VEGF protein based on the size and location of each pocket. Each pocket is color-coded and has "druggability" evaluated based on accessibility and suitability for small molecules. From left to right and top to bottom, the colors of the pockets are: indigo, yellow, pink, orange, muddy green, purple, neon green, teal, blue, red. The yellow pocket shown in the figure is the most ideal binding site candidate.

Energetic-Based Binding Site Identification:

For a binding site to be "druggable" based on energy, sites are identified based on the levels of energy and interactions within pockets. Size and location are less important than the presence of sufficient interactions that allow for small molecules to reach the pockets and bind to the protein to initiate inhibition. In analyzing the resulting figure, three colored pockets near the center of the protein prove the protein is druggable (Figure 2).

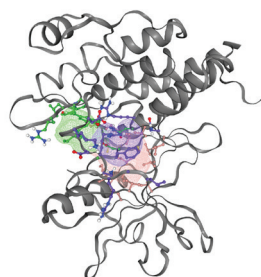


Figure 2: The FTSite identified binding sites marked by colors, based on the energies of sites on the protein. All three colored pockets can serve as sufficient binding sites.

Machine Learning Site Identification:

In terms of the machine learning tools used by Prankweb to identify binding sites, a virtual model of the protein is analyzed to factor in location and amino acids making up pockets and assign "scores." Scores above 1.0 are considered satisfactory, and scores above 5.0 are considered ideal. As ML_P1, ML_P2, and ML_P3 are each above 1.0 (Table 2), VEGF displays satisfactory sites, and ML_P0's score of 38.53 provides the most ideal binding site for small molecules (Figure 3).

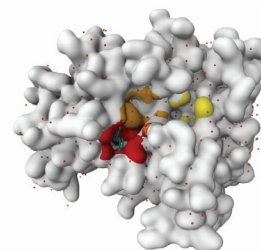


Figure 3: The Prankweb site identified four, color-coded binding sites based on machine learning, with key factors for each pocket being spatial distribution and amino acid composition. Each pocket was scored and ranked based on such factors. The red pocket is the most ideal binding site candidate.

Table 2: This table lists each pocket's color, number of amino acids, and a few of the amino acids making up the pocket. The greatest comprehensive score ranks the four pockets based on the listed properties.

	ML_P0	ML_P1	ML_P2	ML_P3
Ranking	1	2	3	4
Score	38.53	1.55	1.44	1.06
Color	Red	Yellow	Orange	Blue
Number of Residues	37	7	8	6
Amino Acids	GLY117, LEU180, ASP191	SER249, ASP203, GLU242	ASP201, PHE192, ARG196	ARG172, SER231, MET217

Virtual Screening:

Using the tool Pharmit on a pharmacore map with 7 interaction spheres (Figure 4), an RMSD score is given to each compound that is virtually screened based on how well it layers with the protein. RMSD scores, or root mean square deviation scores, are, in simplest terms, calculations of the average distance between two sets of values. In the context of Pharmit and pharmacore mapping, RMSD scores are used to calculate how well compounds can fit a protein.³⁶ Such is done by aligning the two in the most ideal overlay and comparing distances between corresponding atoms (Figures 5-19). Beyond simple molecular structures, however, the pharmacore map uses existing interactions within the VEGF molecule to ensure that the small molecules are able to interact and potentially bind with the protein.

Scores between 0.5 and 0.7 would be considered acceptable. Of the 15 drug candidates that were virtually screened, only ChemDiv-4393-0009 has an RMSD score below 0.7 (Figure 5). However, since the other 14 candidates each have RMSD scores that have a deviation of less than 0.1, greater than the acceptable range of 0.5-0.7 (Table 3), the virtual screening can be considered successful for these candidates as well.

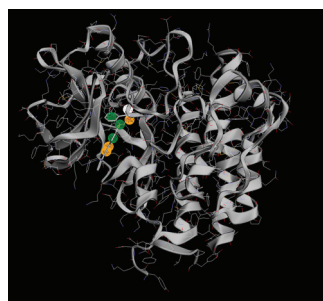


Figure 4: The protein VEGF is shown on a pharmacore map with 7 main interactions in different colors: white represents a hydrogen donor, yellow represents hydrogen acceptors, and green represents hydrophobic interactions.

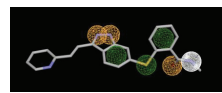


Figure 5: Compound 4393-000. Interacting with the target.

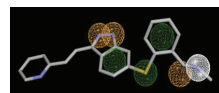


Figure 6: Compound V029-1633. Interacting with the target.

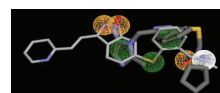


Figure 7: Compound 8017-4590. Interacting with the target.

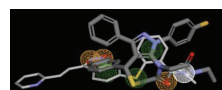


Figure 8: Compound V015-7427. Interacting with the target.

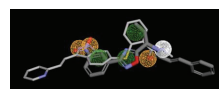


Figure 9: Compound V027-6087. Interacting with the target.

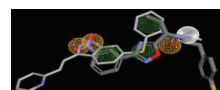


Figure 10: Compound V027-2157. Interacting with the target.

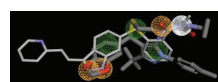


Figure 11: Compound V015-2773. Interacting with the target.

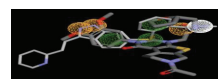


Figure 12: Compound K305-0525. Interacting with the target.

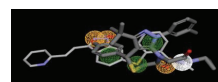


Figure 13: Compound V013-5298. Interacting with the target.

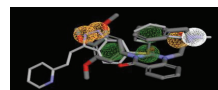


Figure 14: Compound C260-0238. Interacting with the target.

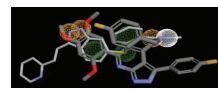


Figure 15: Compound G923-0287. Interacting with the target.

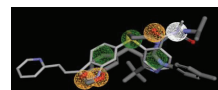


Figure 16: Compound V010-4661. Interacting with the target.

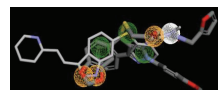


Figure 17: Compound V004-4933. Interacting with the target.

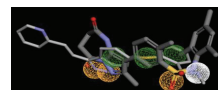


Figure 18: Compound L299-0508. Interacting with the target.

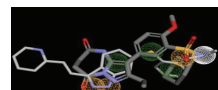


Figure 19: Compound L299-3577. Interacting with the target.

Table 3: The table lists each compound, its SMILES, corresponding figure, and data from the virtual screening. With small molecule mass and interaction, an RMSD score is given to evaluate how well each candidate interacts with the protein. ChemDiv-4393-0009 is the most ideal candidate, but all 14 are acceptable.

Compound	RMSD	Mass	RBnds	Figure	SMILES
ChemDiv-4393-0009	0.673	429	9	5	<chem>C1(C(=O)C2=CC=C(C(=C2)F)C(C2=CC=CC=C2F)N(C(=O)C=O)CCCCC(O)=O</chem>
ChemDiv-V029-1633	0.704	415	8	6	<chem>CCC(C)(C1ncc2ccccc2)[N+](O-)=O)no1)NC(c1ccccc1[C@H])=O</chem>
ChemDiv-8017-4590	0.723	442	9	7	<chem>Cc1ncc([N+](O-)=O)n1Cc1nnc(n1c1ccccc1)SCC(NC1CCCC1)=O</chem>
ChemDiv-V015-7427	0.745	557	7	8	<chem>C1CC1NC(CN1C(CSC(c2ccc3c(c2)OCO3)c2c(c3cccc3)nn(c3ccc(cc3)F)c12)=O)=O</chem>
ChemDiv-V027-6087	0.749	392	8	9	<chem>CC(C)(c1ncc2ccccc2)[N+](O-)=O)ccc2n1)NC(C=O)c3ccccc3=O)C</chem>
ChemDiv-V027-2157	0.754	398	8	10	<chem>CC(C)(C1ncc2ccccc2)[N+](O-)=O)no1)NC(Cc1cccc1)F=O</chem>
ChemDiv-V015-2773	0.759	533	7	11	<chem>Cc1ccc(cc1)n1c2c(Cc3ccc4c(c3)OCO4)SCC(N2C(CN2CC2)=O)=O)c(C(C)(C)C)n1</chem>
ChemDiv-K305-0525	0.759	543	9	12	<chem>CC(N1CCc2c3C(N(C(N(CCC4CCCCO4)=O)c3sc2C1)=O)c1ccc(cc1)OC)OC)=O</chem>
ChemDiv-V013-5298	0.762	533	7	13	<chem>Cc1cccc(c1)n1c2c(Cc3ccc4c(c3)OCO4)SCC(N2C(CN2CC2)=O)=O)c(C(C)(C)C)n1</chem>
ChemDiv-C260-0238	0.762	475	9	14	<chem>COc1cc(cc1OC)OC)N1C(c2cccc2N(C(NC2c2cccc2)=O)C1=O)=O</chem>
ChemDiv-G923-0287	0.763	531	9	15	<chem>COc1cc(cc1c1cc(C(NC2ccc(cc2)F)=O)c2c(n1)n[nH]c2c1ccc(cc1)F)OC)OC</chem>
ChemDiv-V010-4661	0.764	535	7	16	<chem>CC(C)NC(CN1C(CSC(c2ccc3c(c2)OCO3)c2c(C(C)(C)C)nn(c3ccc(C)cc3)c12)=O)=O</chem>
ChemDiv-V004-4933	0.771	609	9	17	<chem>COc1ccc(cc1)n1c2c(Cc3ccc4c(c3)OCO4)SCC(N2CC(NC2cccc2)=O)=O)c(c2cccc2)n1</chem>
ChemDiv-L299-0508	0.775	467	4	18	<chem>Cc1cc(C)c(c(C)c1)NS(c1ccc(cc1)c1C(C)nn2C(C(C(Nc12)=O)=O)(=O)=O</chem>
ChemDiv-L299-3577	0.776	461	6	19	<chem>CCc1c(c2ccc(c2)S)(NC2CCCCC2)(=O)OC)c2NC(CCC(n2n1)=O)=O</chem>

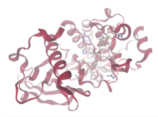
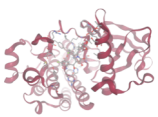
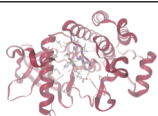
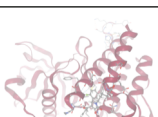
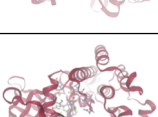
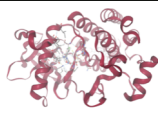
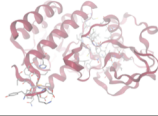
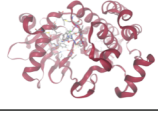
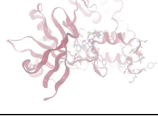
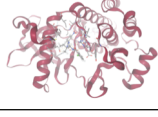
Molecular Docking:

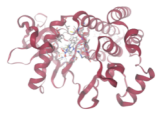
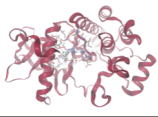
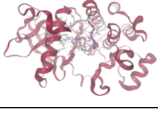
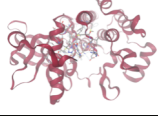
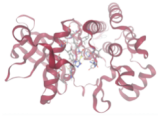
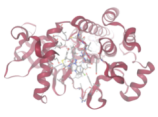
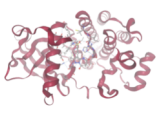
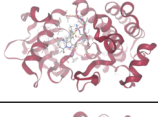
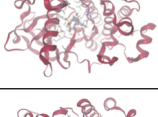
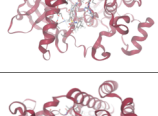
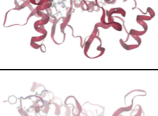
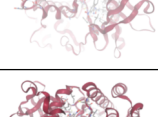
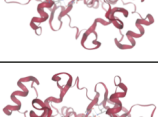
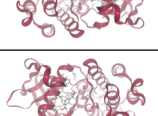
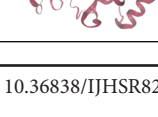
Molecular docking is one of the most important experiments in a drug discovery project. Using a virtual model of the protein and compound, realistic simulations are run between the drug and every possible docking point and approach on the VEGF protein's surface. Testing docking evaluates how well a compound can approach and bind to the protein in order to begin any sort of inhibition.³⁷ The results from this experiment portray the true feasibility of small molecule drugs in acting as a possible therapeutic for vascular malformations.

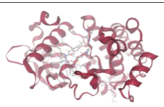
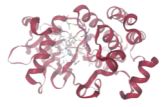
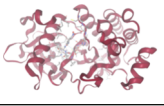
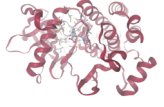
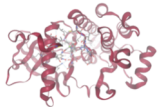
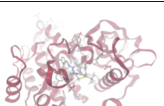
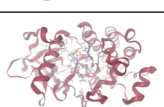
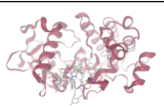
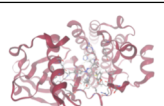
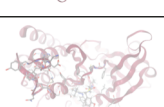
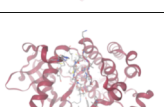
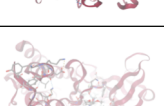
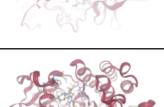
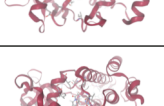
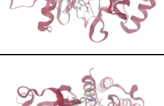
To determine the spontaneity of docking each drug candidate, the SwissDock tool was used to simulate molecular docking across the entire VEGF protein's surface. The parameters for each molecular docking simulation were set after

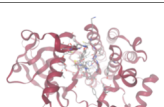
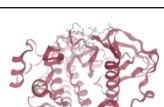
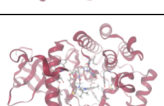
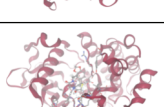
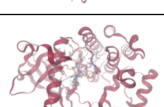
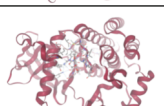
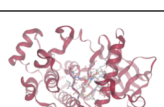
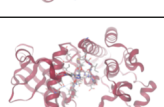
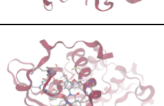
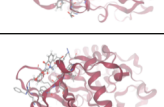
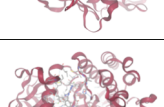
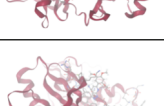
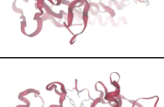
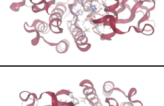
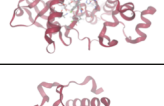
various trials and adjustments to determine the minimum parameters that would account for the maximum surface area. Each candidate's attempt at docking was given a SwissParam score, with an absolute value of 7-8 being satisfactory, 9 being great, and 10 being ideal (Tables 4-18).³⁸ As the compound ChemDiv-V027-6087 had a SwissParam score of -10.0058 (Table 8), it is considered an ideal candidate. Additionally, there were also 4 candidates that had SwissParam scores of -9, which provided great results as well (Table 6, Table 9, Table 13, Table 14). The 10 other candidates each had scores ranging from -7 to -8, with none having an absolute value less than 7, so all 15 compounds can be considered for the next phase of research.

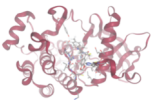
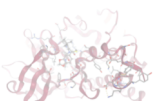
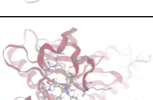
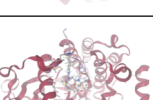
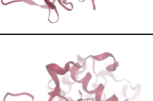
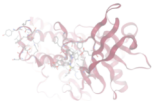
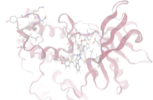
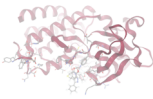
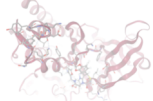
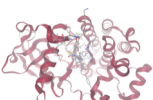
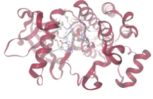
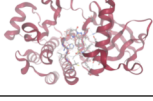
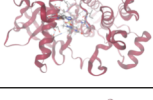
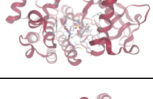
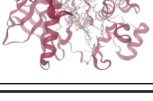
Table 4: Molecular docking of compounds on the VEGF protein.

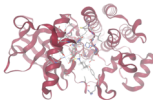
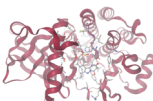
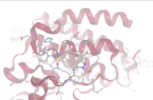
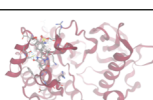
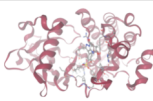
Compound	Result	Cluster Number	SwissParam Score	Binding Pose of Compound to VEGF
ChemDiv-4393-0009	Satisfactory	0	-7.4869	
		3	-7.3202	
		5	-7.4940	
		6	-7.8000	
		7	-7.4028	
ChemDiv-V029-1633	Satisfactory	5	-8.8704	
		3	-8.4975	
		4	-8.3086	
		6	-7.8442	
		1	-7.7287	

ChemDiv-8017-4590	Great	1	-9.1069	
		4	-8.8363	
		0	-8.7276	
		3	-8.6509	
		6	-8.0580	
ChemDiv-V015-7427	Satisfactory	0	-8.2343	
		1	-8.2016	
		2	-8.1032	
		4	-7.9576	
		0	-7.9241	
ChemDiv-V027-6087	Ideal	1	-10.0058	
		2	-9.4998	
		0	-9.0186	
		3	-8.8561	
		4	-8.7069	

ChemDiv-V027-2157	Great	0	-9.7904	
		2	-9.1912	
		1	-8.7009	
		6	-8.6618	
		3	-8.5877	
ChemDiv-V015-2773	Satisfactory	0	-8.2597	
		2	-8.2429	
		2	-7.9334	
		3	-7.5495	
		5	-7.4495	
ChemDiv-K305-0525	Satisfactory	1	-8.5544	
		5	-8.3300	
		3	-8.2011	
		8	-7.8659	
		0	-7.8548	

ChemDiv-V013-5298	Satisfactory	0	-8.1580	
		4	-7.4057	
		0	-7.3134	
		8	-7.3049	
		1	-7.2493	
ChemDiv-C260-0238	Great	0	-9.1128	
		3	-8.9344	
		4	-7.8125	
		1	-7.7466	
		5	-7.4370	
ChemDiv-G923-0287	Great	2	-9.4199	
		1	-8.7616	
		0	-8.7069	
		4	-8.4481	
		3	-8.3458	

ChemDiv-V010-4661	Satisfactory	8	-7.9318	
		0	-7.4391	
		7	-7.3112	
		6	-7.1171	
		5	-7.0050	
ChemDiv-V004-4933	Satisfactory	0	-8.3058	
		1	-8.0859	
		4	-7.9022	
		2	-7.8650	
ChemDiv-L299-0508	Satisfactory	8	-7.8318	
		1	-8.2658	
		0	-8.2153	
		6	-7.7609	
		2	-7.7220	
		7	-7.6990	

ChemDiv-L299-3577	Satisfactory	0	-8.0102	
		2	-7.6432	
		6	-7.2487	
		8	-7.2319	
		9	-7.1352	

Drug Properties:

While structure and compatibility are important for binding with proteins, small molecules must first permeate through membranes and cycle out of the body system after their work is done. Factors such as hydrogen bonds, mass, and solubility each affect how permeable compounds are, according to observations made by Christopher A. Lipinski in his Rule of 5.³⁹ As his conditions are maximums, any compounds with qualities less than his standards are more likely to be permeable and absorbable. Thus, the 8 qualified candidates are proven fully adequate in all aspects, and the only trial that remains is to evaluate their toxicity.

SwissADME tests compounds for absorption to ensure that drugs can reach their protein targets. Lipinski's Rule factors multiple qualities of a compound, such as molecular mass and hydrogen bond donors, to determine if it is permeable. Following the maximum of 5 hydrogen bond donors, the maximum of hydrogen bond acceptors, the maximum LogP value of 5, and the maximum molecular mass of 500 daltons, 8 of the 15 compounds are realistically able to be absorbed by the body and circulated to reach proteins, while 7 of the 15 compounds fail to be realistic candidates (Table 5).

Table 5: Qualities of ChemDiv compounds and passing/failing of Lipinski's Rule. 8 candidates "pass" and 7 candidates "fail".

ChemDiv	Hydrogen Bond Donors	Hydrogen Bond Acceptors	Molecular Mass	Consensus LogP	ESOL Water Solubility	Lipinski's Rule
4393-0009	2	7	429.41	3.41	-4.56	Yes
V029-1633	1	6	414.84	3.50	-5.34	Yes
8017-4590	1	6	441.51	2.03	-4.15	Yes
V015-7427	1	6	556.61	4.20	-6.41	No
V027-6087	1	6	392.41	3.08	-4.84	Yes
V027-2157	1	7	398.39	3.02	-4.63	Yes
V015-2773	1	5	532.65	4.30	-6.36	No
K305-0525	1	7	542.60	2.11	-3.81	No
V013-5298	1	5	532.65	4.30	-6.36	No
C260-0238	1	6	475.49	2.81	-4.62	Yes
G923-0287	2	8	530.52	4.92	-6.22	No
V010-4661	1	5	534.67	4.33	-6.53	No
V004-4933	1	7	608.66	4.15	-6.59	No
L299-0508	2	5	466.55	3.31	-4.91	Yes
L299-3577	2	7	460.55	2.41	-4.11	Yes

Toxicity:

As a drug cannot be fully “safe”, the best method to measure hazard is the level of toxicity, and whether the human body can handle the compound without experiencing serious side effects. Thus, the LD50 dosage observes how much dosage of the compound would be lethal for half of a test sample, meaning that a higher LD50 indicates a safer drug. The predicted toxicity class describes a range of values, with the class of 4 being mildly hazardous but not acutely dangerous. That said, the generally accepted LD50 dosage is 500 mg/kg, but anything above 1000 is considered more ideal.⁴⁰ While all 8 compounds are still viable, the compounds ChemDiv-V027-2157, ChemDiv-L299-0508, and ChemDiv-L299-3577 have LD50 scores close to the more hazardous 500 mg/kg dosage and so cannot be considered successful candidates (Table 6). Of the 8 viable candidates, ChemDiv-V027-6087 is the most ideal, as the LD50 dosage is safely at 1000 mg/kg, and the molecular dock-

Table 6: Level and kinds of toxicity of each compound, followed by a figure comparing each drug to FDA-approved toxicity levels. The most ideal candidate is ChemDiv-V027-6087.

ChemDiv	Predicted LD50 (mg/kg)	Predicted Toxicity Class	Figure
4393-0009	1230	4	
V029-1633	1000	4	
8017-4590	1000	4	
V027-6087	1000	4	

V027-2157	649	4	
C260-0238	2000	4	
L299-0508	560	4	
L299-3577	630	4	

Table 7: Structures of the 5 small molecule drug candidates.

ChemDiv	V027-6087	4393-0009	V029-1633	8017-4590	C260-0238
Structure					

Conclusion

The results of this project were successful in identifying 5 small-molecule drug candidates to target VEGF as potential treatments for vascular malformations. The overall ideal compound is ChemDiv-V027-6087, as its results in each of the experiments were highest when taken from a holistic point of view. With the best molecular docking SwissParam score of -10.0058 and a safe 1000 LD50 dosage, the candidate is able to bind, permeate, and inhibit the VEGF protein to reduce vascular malformation symptoms and stagnate further development. These results provide a new foundation for further research in long-term therapeutics for those with vascular malformations. However, it must be noted that as the majority of these experiments were conducted through computer simulations and in silico models, further validation through *in vitro* and *in vivo* studies must be conducted to account for complex biological variables, such as off-target effects, systemic toxicity, and metabolic stability.

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