Biointerface Research in Applied Chemistry
Open-Access Journal (ISSN: 2069-5837)

Article
Volume 14, Issue 6, 2024, 149
https://doi.org/10.33263/BRIAC146.149

Computational Insights into *VEGFR1* Inhibitors: Redefining Cancer Treatment through Dual-Targeted Therapy

Boutaina Addoum [1,*], Meryem El Azzouzi [1], Hajar El Ahanidi [2], Meryem Jafari [3], Abdelilah laraqui [4], Mohammed Attaleb [1], Laila Benbacer [1], Abdelhakim Elmakssoudi [5], Mohammed El Mzibri [1], Imane Chaoui [1]

[1] Biology and Medical Research Unit, National Center of Energy, Sciences and Nuclear Techniques, Rabat 10001, Morocco; addoumboutaina72@gmail.com;
[2] Department of Pathology and Immunology, University of Geneva, Geneva, Switzerland; hajar.elahanidi@unige.ch;
[3] Laboratory of Biology of Human Pathologies, Genomic Center for Human Pathologies, Department of Biology, Mohammed V University, Faculty of Sciences of Rabat, Morocco; meryem.jafari@um5r.ac.ma;
[4] Royal School of Military Health Service, Sequencing Unit, Laboratory of Virology, Center of Virology, Infectious, and Tropical Diseases, Mohammed V Military Teaching Hospital, Rabat, Morocco; Loranjad@yahoo.fr;
[5] Department of Chemistry, Laboratory of Organic Synthesis, Extraction, and Valorization, Faculty of Sciences Aïn Chock, University Hassan II of Casablanca, Morocco; h.elmakssoudi@gmail.com;
* Correspondence: addoumboutaina72@gmail.com;

Scopus Author ID 57209023468
Received: 5.08.2024; Accepted: 6.10.2024; Published: 13.12.2024

Abstract: Worldwide, cancer remains a significant health concern, and great efforts are made continuously to identify new drugs to increase therapeutic efficacy, limit treatment side effects, and overcome drug resistance. In this field, dual/multi-targeted therapy has emerged as a forefront strategy in combating cancers, focusing on disrupting specific molecular pathways essential for tumor growth and progression. In this computational study, we have focused our interest on identifying, among 44 approved anti-cancer drugs, potential VEGF-R1 inhibitors, thereby exhibiting inhibitory effects on angiogenesis. The binding affinity of the tested compounds to the extracellular domain of *VEGF-R1* has been performed by using PyRx software and showed that Cladribine, Etoposide, Allopurinol, and Altretamine were the most effective compounds, characterized by promising binding energies ranging between -8.9 to -9.2 kcal/mol. Our computational study clearly showed that Etoposide, Cladribine, Altretamine, and Allopurinol exhibit a great potential affinity to *VEGF-R1*, suggesting their potential inhibition of *VEGF/VEGF-R1* complex that could be of great interest to suppress and block the angiogenic pathway in tumors. Further in silico analyses and experimental investigations are needed to explore the anti-angiogenic effect of these small anti-cancer compounds deeply.

Keywords: dual-target; therapy; VEGF-R1; angiosinesis; inhibitors; computational.

1. Introduction

Scientific evidence has shown that tumor cells actively recruit stromal cells, vascular cells, and fibroblasts to create a microenvironment that fosters tumor growth. Moreover, to promote tumor growth, cancer cells will induce angiogenesis during early tumor development by recruiting bone marrow-derived endothelial progenitor cells and activating different growth factors involved in the angiogenic signaling [1]. Thus, inhibiting angiogenesis and vascular growth would deprive the tumor of oxygen and nutrients, which is a promising approach to blocking tumor development [1,2].

Vascular endothelial growth factor (VEGF) plays a crucial role in tumor vascularization and metastasis [1]. This 45 kDa homodimeric glycoprotein is a member of the tyrosine kinase receptors family [1], which includes *VEGF-A, VEGF-B, VEGF-C, VEGF-D* [1], and placental growth factor

(PlGF) [1,2]. Among these, *VEGF-A* primarily drives tumor angiogenesis [3]. Its angiogenic effects arise from direct interactions with endothelial cells, facilitated by its binding to two similar tyrosine kinase receptors, *VEGF* receptor-1 (*VEGF-R1*) and VEGF receptor-2 (*VEGF-R2*) [1–3].

Likewise, the activation of *VEGF-R1* engages in diverse biological mechanisms, including chemotaxis, the secretion of inflammatory cytokines, the recruitment of medullary progenitor cells to injury sites [1], the secretion of growth factors [2], interaction with *PlGF* [3], and the activation of proteolytic enzymes [4–6]. Also, *VEGFR-2* serves as the main receptor transmitting angiogenic signals, and inhibition of *VEGFR-2* signaling can attenuate angiogenesis by disturbing signaling pathways [7]. *VEGFR-2* exhibits greater potency than *VEGFR-1* and displays kinase activity approximately tenfold stronger than *VEGFR-1* [8]. However, the downside of its activity is that this receptor is not specific for tumor angiogenesis. It regulates physiological functions such as blood pressure or pulmonary hypertension [8–10]. Unlike *VEGFR2*, the pro-angiogenic activity of *VEGFR1* seems more restricted to pathological phenomena. In this context, it appears to be an interesting therapeutic target, and consequently, targeting this receptor seems a strategy of choice to block tumor growth without inducing too many side effects [10].

Despite significant advancements in medical knowledge, the effectiveness of cancer treatment remains unsatisfactory. Previously, the primary focus was developing highly specific inhibitors targeting individual receptor tyrosine kinases (RTKs). However, there is a consensus that molecules capable of interfering with multiple targets simultaneously may prove more effective than single-target agents, representing a transition from "one compound - one target" to "cocktail therapy" and, more recently, to the multi-target approach. In this field, the US Food and Drug Administration (FDA) had approved Sorafenib and Sunitinib, targeting *VEGFR*, *PDGFR*, *FLT-3*, and *c-Kit*, ushering a new generation of anti-cancer drugs acting on multiple molecular targets and capable of inhibiting various pathways [11–15].

Imatinib was the first US FDA-approved tyrosine kinase inhibitor (TKI) and has been employed in the treatment of chronic myeloid leukemia (CML) and advanced anaplastic thyroid cancer. Imatinib functions by blocking the activity of tyrosine kinases *Bcr-Abl*, *c-KIT*, and platelet-derived growth factor (PDGF) through binding to their ATP-binding site [16,17]. Thereafter, great interest was given to identifying new TKIs targeting tumorous and vascular endothelial cell kinase receptors, blocking cell proliferation, and encountering angiogenesis pathways [18]. In this field, we have planned to investigate the potential inhibitory effects of 45 FDA-approved small molecules on *VEGFR1*, a privileged target in cancer treatment, which can modulate or block signaling pathways involved in angiogenesis, and compare them with Bevacizumab, used as reference anti-*VEGFR1* drugs like. This computational study aims to identify anticancer drugs that simultaneously target different key oncogenic pathways for better control of cancer cells and ensure efficient cancer treatment.

2. Materials and Methods

2.1. Virtual screening software.

The computational analysis was carried out using several software/webservers and online libraries, as reported in Table 1.

Table 1. Software and web servers were used in our study.

In-silico tools	Type	Prediction	Availability	Open-source license	Reference
PyRx	Software	Virtual Screening(docking and scoring)	Freely available	http://pyrx.sourceforge.net/	[19]

In-silico tools	Type	Prediction	Availability	Open-source license	Reference
Open Babel version 2.3	Software	Chemical Tool Box to search, convert, analyze, or store data from molecular modeling, chemistry, and solid-state materials.	Freely available	http://openbabel.org.	[20]
Biova	Software	Target preparation Visualization of Ligand-protein interaction.	Freely available		[21]
RCSB PDB	Database	Efficient Tool to explore, visualize, and analyze the experimentally determined 3D structure of macromolecules (mainly proteins and nucleic acid) by X-ray crystallography or NMR spectroscopy.	Freely available	http://pdb.org/	[22]
PuChem NCBI	Open chemistry database	Collect data about molecules such as chemical structure, toxicity, and physicochemical properties via CID(compound ID)	Freely available	https://pubchem.ncbi.nlm.nih.gov/	[23]
ChEMBL	Open chemical-genomic database	The human chemogenomic database combines chemical, bioactivity, and genomic datasets to facilitate the conversion of genomic insights into potent pharmaceuticals	Freely available	https://www.ebi.ac.uk/chembl/	[24,25]
Therapeutic Target Database (TDD)	Online database	Database that offers details on identified and investigated therapeutic targets, as well as information on the associated diseases, pathways, and drugs designed for each target.	Freely available	https://idrblab.org/ttd/	[26]

2.2. Molecular docking.

2.2.1. Target prediction.

Swiss Target Prediction is an online tool established in 2014 to anticipate the targets of small bioactive molecules within humans and other vertebrates [27]. The prediction relies on the concept of similarity, employing reverse screening methodology. This prediction is founded on a combination of 2D and 3D similarity with a library of 370000 known actives on more than 3000 proteins [27,28]. The updated SwissTargetPrediction interface can be obtained for free through www.swisstargetprediction.ch [28].

2.2.2. Preparation of target.

The X-ray crystal data of target receptor tyrosin kinase *VEGFR1* (PDB id: 4CL7) was retrieved from the Protein Data Bank (RCSB) (http://www.rcsb.org/pdb). The selected conformation of VEGFR-1 is complex to Cobalt [29]. The 4CL7 structure was edited to remove water and heteroatoms using Accelyrs Discovery Studio Visualizer.

2.2.3. Extraction and preparation of tested molecules.

The SDF molecular structures were uploaded using ChEMBL or PubChem, and energy minimization was performed for all compounds using open Babel [30]. The 45 molecules utilized in this study are listed in Table 2, referenced by their respective codes in the NCBI/PubChem database.

Table 2. The PubChem CID of the studied compounds and the control compound Bevacizumab.

N°	Molecules	PubChem ID	N°	Molecules	PubChem ID
1	Abemaciclib	46220502	23	Clofarabine	119182
2	Abiraterone	132971	24	Cobimetinib	16222096
3	Acalabrutinib	71226662	25	Copanlisib tris-HCl	135565596
4	Allopurinol	135401907	26	Cyclophosphamide	2907
5	Altretamine	2123	27	Cytarabine-HCl	6252
6	Amifostine	2141	28	Dacarbazine	135398738
7	Aminolevulinic-HCl	123608	29	Daunorubicin	30323
8	Anastrozole	2187	30	Darolutamide	67171867
9	Apalutamide	24872560	31	Decitabine	451668
10	Avaprinitib	118023034	32	Dasatinib	3062316

N°	Molecules	PubChem ID	N°	Molecules	PubChem ID
11	Azacitidine	9444	33	Dexrazoxane	71384
12	Bendamustine-HCl	77082	34	Doxorubicin	31703
13	Brigantine	68165256	35	Duvelisib	50905713
14	Busulfan	2478	36	Enasidenib	89683805
15	Capecitabine	60953	37	Encorafenib	50922675
16	Capmantinib	25145656	38	Entrectinib	25141092
17	Carboplatin	426756	39	Epirubicin-HCl	65348
18	Carmustine	2578	40	Erlotinib-HCl	176871
19	Celecoxib	2662	41	Estramustine phosphate	259329
20	Cerentinib	57379345	42	Etoposide	36462
21	Chlorambucil	2708	43	Exemestane	60198
22	Cladribine	20279	44	Mitomycin C	5746
45. Ref	Bevacizumab	24801581			

2.2.4. Docking and scoring via PyRx software.

In the present study, we used the *VEGF* binding zone, which is located in the extracellular d2 domain of *VEGFR1*[31].This is a buried area of the receptor composed of hydrophobic, polar, and aromatic residues that can interact with ligands. The detailed analysis of this region allowed the identification of three sub-pockets: A, B, and C [31-33]. Docking was conducted to generate a range of possible conformations and orientations for the studied ligands within the binding site of *VEGFR-1*. The protein structure was prepared in PyRx software, resulting in a PDBQT file containing hydrogen atoms for all polar residues. Rotatable bonds were assigned to the ligands and stimulated the interaction L-P, and then scoring calculations were performed using the Lamarckian Genetic Algorithm (LGA) method. The docking site on the protein target was defined by establishing a grid box with dimensions of (X, Y, Z): 81 Å × 61 Å × 64 Å and a grid spacing of 0.375 Å was positioned at the binding pocket of *VEGFR-1* [31-33]. The best conformation, determined by the lowest docked energy, was selected after completing the docking search. Ten runs with AutoDock Vina were executed for each ligand structure, with the best pose saved for each run. The final affinity value was determined by averaging the affinities of the best poses. This computational investigation continued by analyzing the 2D conformation of complex "*VEGFR1*-Ligand" and the different bounds/residues involved in this interaction. Also, bond lengths were detected using Discovery Studio Visualizer [32-33], which must list the authority that provided approval and the corresponding ethical approval code.

3. Results and Discussion

3.1. Target prediction.

SwissTarget prediction serves as an online tool designed to anticipate the macromolecular targets of bioactive small molecules, encompassing proteins sourced from humans, mice, and rats. Its utility extends to deciphering complex molecular pathways underlying specific bioactive traits, rationalizing potential side effects, forecasting target effects, and assessing the viability of repurposing therapeutically relevant molecules. In the context of the current investigation, the selection process prioritized the top 13 targets based on their pronounced affinity for kinases (Figure S1 of supplementary material). Notably, Ceritinib emerged as a standout, with a 94% binding probability to interact with kinase receptors, followed by Capecitabine and Cabmantinib, with 34% and 30% interaction possibilities, respectively. Conversely, Altretamine demonstrated a notable affinity towards family A protein G-coupled receptors, with an interaction probability of 63.9%. Of particular interest is the reference drug Bevacizumab, which exhibits an affinity interaction of 26.7% towards the kinase receptor. Our finding showed that the examined molecules may serve as selective inhibitors targeting

the protein kinase family, including TKRs, widely reported to play a critical role in molecular pathways governing cell survival and differentiation [34].

3.2. Molecular docking investigation.

The binding affinity results of the ligands against the selected angiogenesis targets are shown in Table 3. The docking scores of the compounds range from −4.6 to −9.2 kcal/mol. Accordingly, Cladribine and Etoposide achieved the highest binding affinity score of −9.2 kcal/mol, closely followed by Altretamine and Allopurinol with docking scores of −9.0 and −8.9 kcal/mol for *VEGFR*, respectively. Other interesting drugs, including Estramustine phosphate, Enasidenib, Capecitabine, Celecoxib, and Brigatinib, also showed high binding affinities.

Table 3. List of the scoring and residues implicated in the interaction between the studied compound and the VEGFR target.

N°	Molecules	Binding affinity (kcal/mol)	Residues implicated in interaction L-P
1	Abemaciclib	-6,9	Glu 64 Glu 67 Asn 62 Asp 63 Cys 61 Cys 68 Gly 59
2	Abiraterone	-4,7	Cys 61 Cys 68 Asn 62 Asp 63
3	Acalabrutinib	-7,7	Asp 34 Glu 31 Leu 32 Gly 38 Ile 29 Cys 60 Cys 61 Cys 68
4	Allopurinol	-8,9	ILE 29 Gly 59 Glu 30 Glu 31 Arg 36 Gly 59 Cys 57
5	Altretamine	-9	Asp 63 Asn 62 Cys 60 Cys 61 Cys 68 Gly 58 Ile 29 Leu 32
6	Amifostine	-5,1	Cys 61 Cys 68 Asn 62 Phe 36 Ile 46
7	Amin acid hydro	-5,1	Gly 58 Gly 59 Leu 32 Cys 68 Ile 29
8	Anastrozole	-7,1	Glu 30 Leu 32 Thr 31 Gly 39 Arg 58
9	Apalutamide	-7,3	Asp 63 Leu 66 Asn 62 Cys 61 Glu 64 Phe 36 Ile 43
10	Avaprinitib	-4,8	Cys 61 Asn 62 Gly 59 Glu 64 Phe 36 Asp 34
11	Azacitidine	-4,3	Cys 61 Leu 66 Asn 62 Asp 63 Glu 64 Asp 34 Phe 36 Lys 107
12	Bendamustine-HCl	-7,5	Leu 32 Glu 64 Ser 50 Cys 51 Cys 60 Cys 61 Cys 68
13	Brigantine	-8	Glu 64 Gly 59 Cys 61 Cys 68 Glu 64 Ile 46 Phe 36
14	Busulfan	-7,5	Lys 107 Phe 36 Cys 68 Asp 34 Asp 63 Asn 62 Leu 46 Glu 64 Ile 46
15	Capicitabine	-8,6	Phe 36 Glu 46 Asp 34 Asp 63 Asn 62 Leu 66 Lys 107 Cys 61 Cys 68
16	Capmantinib	-7,4	Lys 107 Asp 63 Asn 62 Glu 64 Asp 34 Cys 68
17	Carboplatin	-5,7	Cys 60 Glu 64 Ile 46 Phe 36
18	Carmustine	-5,4	Arg 56 Gly 59 Leu 66 Leu 32 Thr 31
19	Celecoxib	-8	Asp 63 Lys 107 Glu 64 Asn 62 Cys 61 Cys 68 Asn 62 Ile 46 Phe 36
20	Cerentinib	-4,6	Asp 63 Lys 107 Glu 64 Asn 62 Cys 61 Cys 68 Ile 46 Phe 36
21	Chlorpbucil	-7,1	Gly 58 Gly 59 Asp 63 Cys 60 Cys 61 Cys 68 Asn 62 Leu 32 Ile 29
22	Cladribine	-9,2	Asn 62 Cys 60 Cys 61
23	Clofarabine	-5	Leu 32 Gly 59 Glu 30 Leu 32 Thr 31
24	Cobimetinib	-7,2	Cys 51 Cys 60 Ser 50 Glu 64 Asp 34
25	Copanlisib tris-Hcl	-7	Asn 62 Glu 64 Cys 60 Cys 51 Cys 61
26	Cyclophosphamide	-5,1	Asp 34 Phe 36 Glu 64 Leu 66
27	Cytarabine-HCl	-5,7	Glu 64 Lys 107 Cys 61 Gly 59 Asp 34 Cys 51 Cys 60
28	Dacarbazine	-7,2	Asn 62 Gly 58 Gly 59 Leu 66 Leu 32 Gly
29	Daounorubicin	-7,9	Cys 61 Cys 68 Leu 66 Asn 62 Asp 63
30	Darolutamide	-4,2	Asp 34 Glu 64 Ser 50 Cys 60 Cys 68
31	Decitabine	-7,3	Ile 46 Glu 64 Ser 50 Cys 60 Cys 68
32	Dasatinib	-7	Leu 66 Asp 34 Leu 32 Cys 60 Cys 61 Cys 68 Gly 59
33	Dexrazoxane	-6,5	Asp 34 Asp 63 Glu 64
34	Doxorubicin	-4,9	Leu 66 Gly 59 Asp 63 Ser 56 Glu 64 Cys 61 Asn 62 Asp 34
35	Duvelisib	-4,6	Thr 31 Gly 59 Glu 30 Glu 64 Asp 61 Leu 32 Leu 66 Cys 51 Cys 60 Ile 29
36	Enasidenib	-8,6	Thr 31 Glu 37 Val 33 Gly 58
37	Encorafenib	-6,4	Glu 64 Glu 67 Asn 62 Asp 63 Cys 61 Cys 68 Gly 59
38	Entrectinib	-6,8	Asn 62 Asp 63Cys 61 Cys 68
39	Epirubicin-HCl	-4,5	Asp 34 Cys 60 Cys 61
40	Erolontib-HCl	-7,8	Ile 29 Asp 34 Ser 50
41	Estramustine Phosphate	-8,8	Asn 62 Asp 34 Asp 63 Glu 64 Ile 29 Leu 32 Gly 58 Cys 60 Cys 61 Cys 68
42	Etoposide	-9,2	Lys 107 Leu 66 Asp 63 Asn 62 Cys 60
43	Exemestane	-5,1	Cys 60 Cys 61 Asn 62 Ser 50 Ile 46 Phe 36
44	Mitomycin C	-7,9	Gly 58 Gly 59 Ile 29 Leu 32 Thr 31Glu 30

N°	Molecules	Binding affinity (kcal/mol)	Residues implicated in interaction L-P
45	Bevacizumab	-8,5	Glu 30 Glu 64 Thr 31 Gly 59 Leu 32 Leu 66 Asp 63 Cys 51 Cys 60 Ile 29

Figure 1 presents the 2D diagram of the interaction between the top 9 compounds and the target 4CL7, ranked by their binding energies. A tight junction is observed with Cladribine, Etoposide, Altretamine, and Allopurinol, which creates a stronger connection than Bevacizumab, which is used as a reference drug. Analysis of binding liaisons showed that Etoposide engaged with the target active site through two conventional hydrogen bonds with residues Glu 3 and Thr 31, as well as three Pi-alkyl interactions with residues Ile 29 and Arg 56, followed by halogen bonds with residues Gly 59 and Cys 57. Also, Cladribine formed three hydrogen bonds (Asn 62-Cys 61-Asp 63), a carbon bond with Leu 32, and Pi-alkyl interactions with residues Ile 29, Cys 68, and Cys 60, with the active site of 4CL7. Altretamine established three hydrogen bonds (Cys 68-Cys 59-Cys 61) and Pi-alkyl interactions with Ile 46. Indeed, Allopurinol formed five hydrogen bonds (Cys 68-Cys 61-Asn 62-Aqp 63-Leu 66) and Pi-alkyl interactions with Phe 36. Estramustine Phosphate interacted through five hydrogen bonds (Cys 68-Cys 61-Asn 62-Aqp 63-Lys 107) and two Pi-alkyl interactions with Phe 36 and Ile 46. Enasidenib formed two hydrogen bonds (Cys 61-Asn 62) and Pi-alkyl interactions with Cys 60. Capecitabine engaged through two hydrogen bonds (Gln 37 and Thr 31) and Pi-alkyl interactions with Val 33, as well as a carbon-hydrogen bond with Gly 58. Also, celecoxib formed four hydrogen bonds (Leu 66-Lys 107-Asp 63-Asn 62) and Pi-alkyl interactions with Cys 60. Brigantib established five hydrogen bonds with Cys 61-Cys 68-Leu 66-Asn 62-Asp 63. Bevacizumab, used as a reference drug, engaged in four hydrogen bonds (Leu 32-Thr 31-Gly 59-Asp 63), Pi-alkyl interactions with Ile 29 and Cys 60, and carbon bonds with Leu 66, as well as halogen bond with Glu 30.

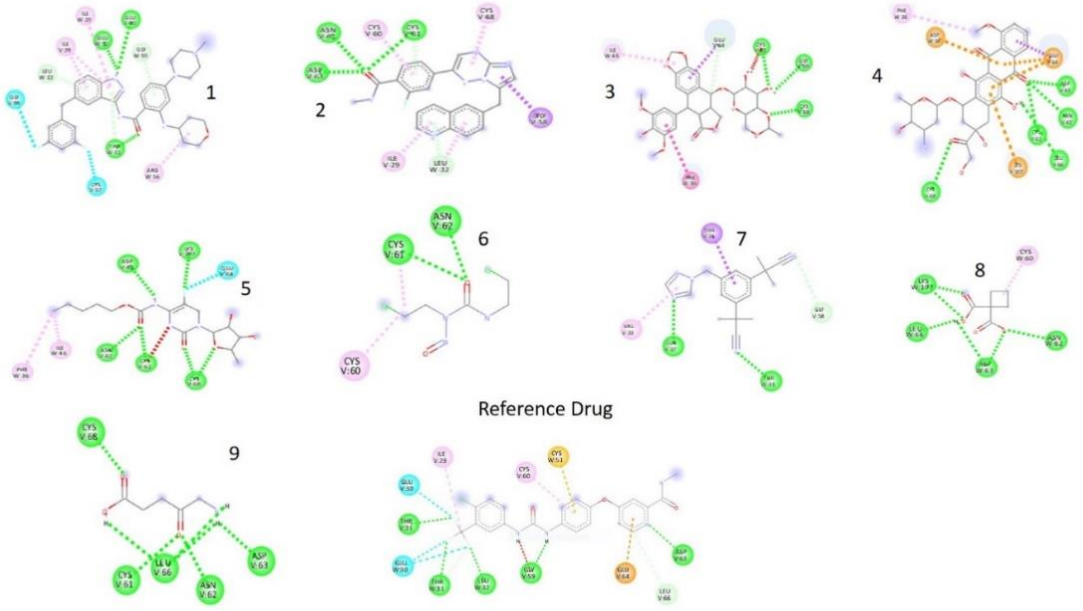

Figure 1. 2D diagram of the 9 best-docked compounds and the reference drug (Bevacizumab) into the VEGF-R1 pocket.1: Etoposide; 2: Cladribine; 3: Altretamine; 4: Allopurinol; 5: Estramustine Phosphate; 6: Enasidenib; 7: Capecitabine; 8: Celecoxib; 9: Brigantib; Reference Drug: Bevacizumab. The 2D diagram of all 45 studied compounds is provided in the supplementary material section.

3.3. Discussions.

Precision medicine, also known as targeted therapy, impedes the proliferation of cancer cells by selectively interfering with molecules and pathways vital for cancer advancement, as opposed to the broad impact on all rapidly dividing cells seen in traditional chemotherapy. Conventional

chemotherapy rests on single-target therapy, widely reported as ineffective with huge limitations [35]. Currently, great efforts are made to identify multi-targeting agents that are more effective in treating complex diseases and drug-resistant cancers [36-38]. Accordingly, with the support of AI tools [38-39], polypharmacology has the potential to unveil new off-targets for existing drugs, providing insights into drug side effects and toxicities and facilitating drug repurposing by identifying new indications or therapeutic targets for established drugs [40].

In many human cancers, *VEGF-R1* plays a pivotal role in tumor angiogenesis [41] and leads it to be a promising target for cancer control. In this field, our approach aimed to computationally stimulate the inhibition of the *VEGF/VEGFR1* interaction by targeting VEGF at the receptor recognition site. Regradignly, molecular docking was used to assess the affinity of 44 FDA-approved anti-cancer compounds, with diverse mechanisms of action and proven efficacy against various types of cancer, on the *VEGF-R1*. Of note, Bevacizumab (Avastin®), which was previously recognized for its anticancer behavior and owns a good affinity for the selected target, was used as a reference drug [41]. Bevacizumab is a humanized monoclonal antibody approved by the FDA and capable of selectively binding to circulating *VEG*, thereby inhibiting *VEGF*'s binding to its cell surface receptors [41,42]. In this field, it's widely accepted that protein-protein interactions play a key role in various biological processes and thus offer numerous opportunities in medicinal chemistry. The development and/or identification of molecules capable of modulating a protein-protein interaction remains a significant scientific challenge today. Thus, inhibiting the interaction between *VEGF* and *VEGFR* remains a potential target, leading to a decrease in the microvascular development of tumor blood vessels and restricting the blood supply to the tumor tissues [42].

By comparing the results generated by the "PyRx" software with a visual assessment of the "Ligand-4CL7" complex acquired *via* the "Biova Discovery Studio Visualizer", 4 drugs including Etoposide, Cladribine, Altretamine, and Allopurinol were identified as the most potent inhibitors, owing to their significant binding affinity ranging between -8.9 to -9.2 kcal/mol. These compounds exhibited interactions with various target residues, forming hydrogen, hydrophobic, and electrostatic bonds. Visual analysis results corroborate our theoretical calculations, affirming their capability to inhibit the activity of *VEGF* by binding to its receptor, *VEGFR1* (4CL7), which can potentially disrupt the angiogenesis pathway.

Our *in silico* results are in agreement with those previously reported. Indeed, Magdalena Kluska et al. have shown that the Etoposide, a semi-synthetic derivative of podophyllotoxin isolated from the dried roots and rhizomes of *Podophyllum emodi*, is an anti-cancer agent with a wide spectrum and is currently used in chemotherapy of stomach cancer, ovarian cancer, acute leukemia, Hodgkin lymphoma, some sarcoma forms and neuroblastoma [43]. Etoposide is a specific inhibitor of topoisomerase II [44], inhibits mitosis and cell cycle in the S or G2 phase [45], and participates in the apoptosis pathway via the activation of cytochrome C/Caspase 9 [46].

Cladribine, the 2-chloro-2'-deoxyadenosine, has been reported as a potential inhibitor of DNA synthesis by inhibiting adenosine deaminase [47]. This purine nucleoside analog can arrest the cell cycle at the G1 phase, induce the expression of p21 and p27 [47], and inactivate the factor *STAT 3* [48]. Of particular interest, Xu et al. have shown that Cladribine can induce apoptosis by stimulating the endoplasmic reticulum (ER) stress signaling pathway in diffuse large B-cell lymphoma cells (DLBCL) [49].

In the current *in-silico* investigation, Altretamine was also identified as an anti-VEGF-R1 inhibitor[50]. The FDA approved this synthetic s-triazine derivative as antineoplastic, while its mechanism of cytotoxicity remains elusive. The N-demethylation of Altretamine might generate

reactive intermediates that form covalent bonds with DNA, leading to DNA damage and blocking the metabolic biomarkers Glutathione peroxidase 4 (GPX4) [51].

In a separate study, Yasuda et al. outlined the cytotoxic effects of Allopurinol on human hormone-refractory prostate cancer cells [52]. Additionally, their derivatives targeted various pathways, including cyclin kinase 1 (CK1) [53], hepatocyte growth factor receptor (c-MET) [54], vascular endothelial growth factor receptor 2 (VEGF-R 2) [55], Fms-like tyrosine kinase 3 (FLT3) and Wnt (Wingless-related integration site)/β-catenin signaling [55-56], epidermal growth factor receptor (EGFR) [57], MARK (mitogen-activated protein kinases) signaling [58].

To complete our virtual screening, we performed the structure-activity relationship (QAR) of the selected molecules, and the results were very informative. Indeed, the anticancer activity of Etoposide can be related to the presence of a phenyl ring and the presence of hydroxyl groups at ortho and para positions [59]. The antitumoral effect of Cladribine is attributed to its chemical structure [60], particularly the replacement of hydrogen with chlorine at the 2-position of the purine ring [60]. Moreover, the computational study reveals that Altretamine exhibits anti-*VEGF* activity, likely owing to its structural resemblance to the alkylating agent triethylenemelamine[61], known for its antineoplastic properties [61]. Despite the lack of precise understanding regarding how this synthetic s-triazine induces cytotoxicity, it's distinguished by an extended half-life, which is also attributed to its molecular structure [61].

Also, our docking screening identified Allopurinol (4-hydroxy pyrazole (3,4-d)pyrimidine) as a potential VEGF-R1 inhibitor [61]. The anti-angiogenic activity of this nucleobase analog can be related to its complex structure, combining a pyrazole moiety with a hydroxyl-substituted pyrimidine ring [61].

The present study is very informative and highlights the potential ability of 4 small molecules, currently used in cancer chemotherapy, to target VERGF-R1 and, therefore, inhibit *VEGF/VEGFR1* interaction. These molecules, including Alteramine, Allopurinol, Cladribine, and Etoposide, have thus emerged as promising « scaffolds » for the design of novel VEGF-R1 antagonists. In this field, adding active substituents like methoxy [61], hydroxyl, or halogens [62] to these potentially active compounds will impact the stability of these analogs and may increase their binding strength with the d2 domain of *VEGF-R1* and potentially enhance their pharmacological activities [63-66].

The identification of new molecules targeting *VEGF-R1* will enlarge the cancer therapeutic arsenal with new anti-angiogenic drugs and overcome the emergence of drug-resistant clones. Indeed, genetic mutations affecting TKRs can initiate tumorigenesis by activating the mitogenic signaling pathways and deregulating the apoptosis process. Accordingly, the potential of these compounds to block or modulate the abnormalities in these pathways is widely recognized as a promising strategy in drug design [67]. The main limitation of this computational study is the lack of Quantitative Structure-Activity Relationship (QSAR) studies, allowing us to understand their potential mechanism of action and to provide access to other more potent molecules [68-69].

Further dynamic stimulation (MD) studies are needed to complete this current work. MD simulations serve as a computational approach that utilizes Newton's laws to analyze the motions of water, ions, small molecules, macromolecules, and more complex systems. These simulations are essential for examining structural motions that depend on temperature and solute/solvent interactions, which are critical for studying the recognition patterns of ligand-protein or protein-protein complexes. MD simulations are particularly beneficial in drug design, offering insights into the structural cavities needed to develop new compounds with higher affinity for their targets. Moreover, MD simulations assist in refining the three-dimensional (3D) structures of targets, enhancing the sampling of binding

poses and providing more accurate affinity values by incorporating biological conditions that include structural motions, as opposed to traditional docking methods [70-71].

4. Conclusions

Our computational investigation focused on the *VEGF/VEGFR* system reveals that Etoposide, Cladribine, Altretamine, and Allopurinol, widely used cancer chemotherapy, exhibit great inhibition of *VEGF-R1*, suggesting their potential for effectively suppressing and blocking the angiogenic pathway.

These results are, therefore, very promising and offer captivating perspectives to improve affinity, conformational stability, and biological activities of the selected compounds. These observations could be further completed by experimental investigations employing human cancer cell lines and anticancer bioassays.

Funding

This research received no external funding.

Acknowledgments

None.

Conflicts of Interest

None.

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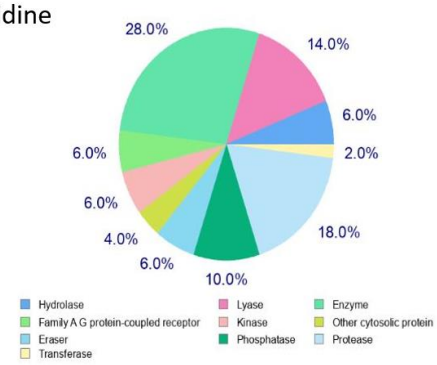
Supplementary materials

1.Aazacitidine



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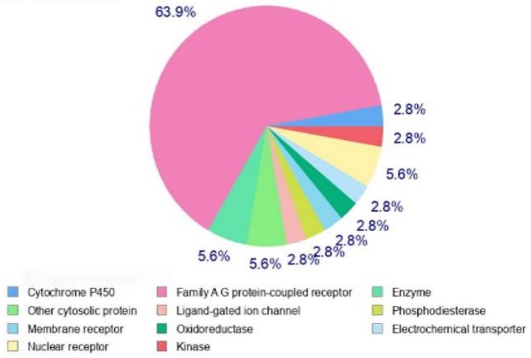

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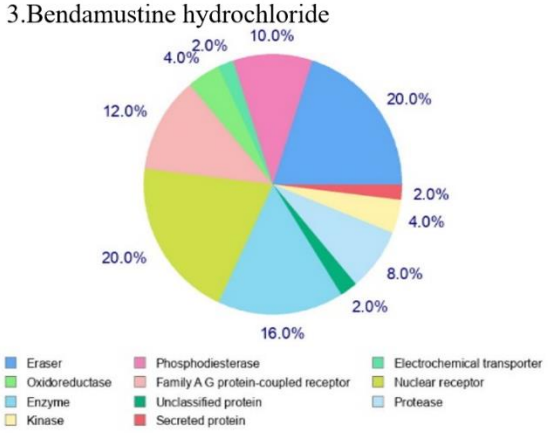

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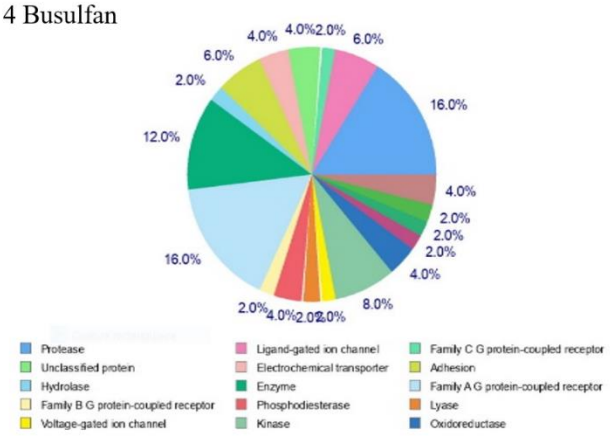

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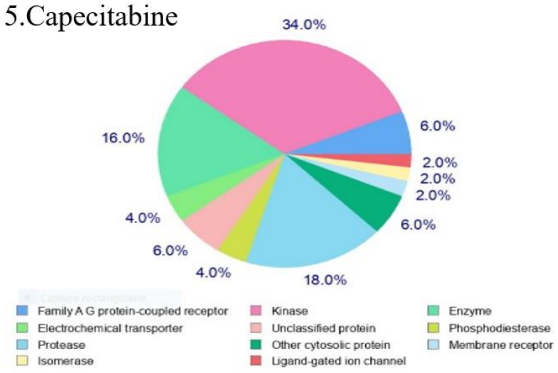

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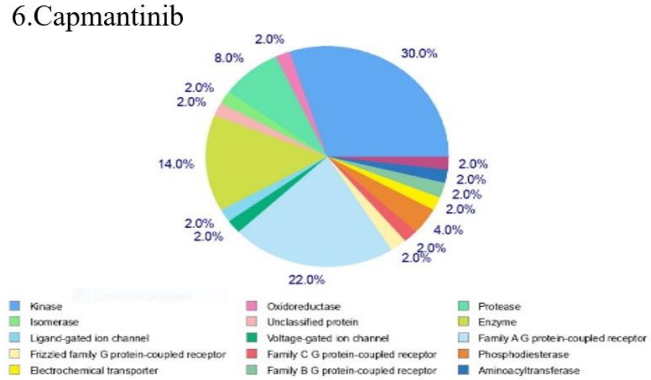
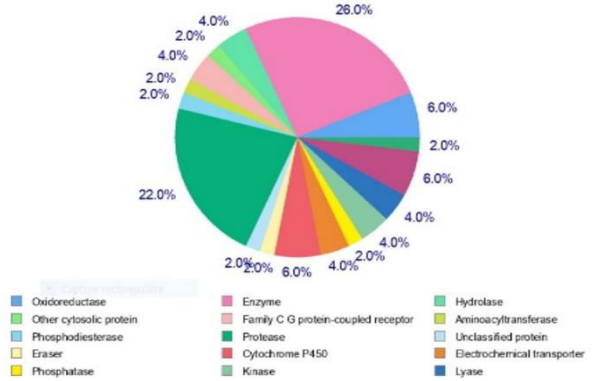
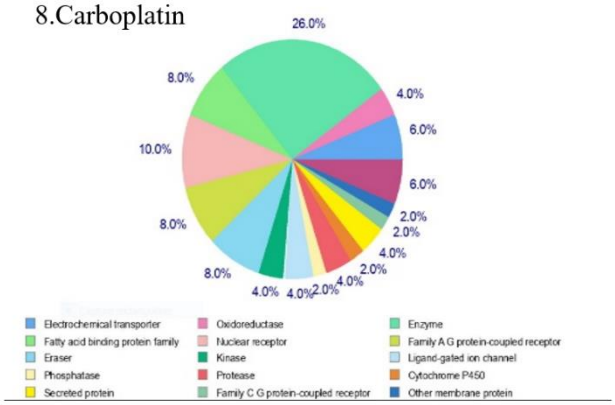


7.Carmustine



8.Carboplatin



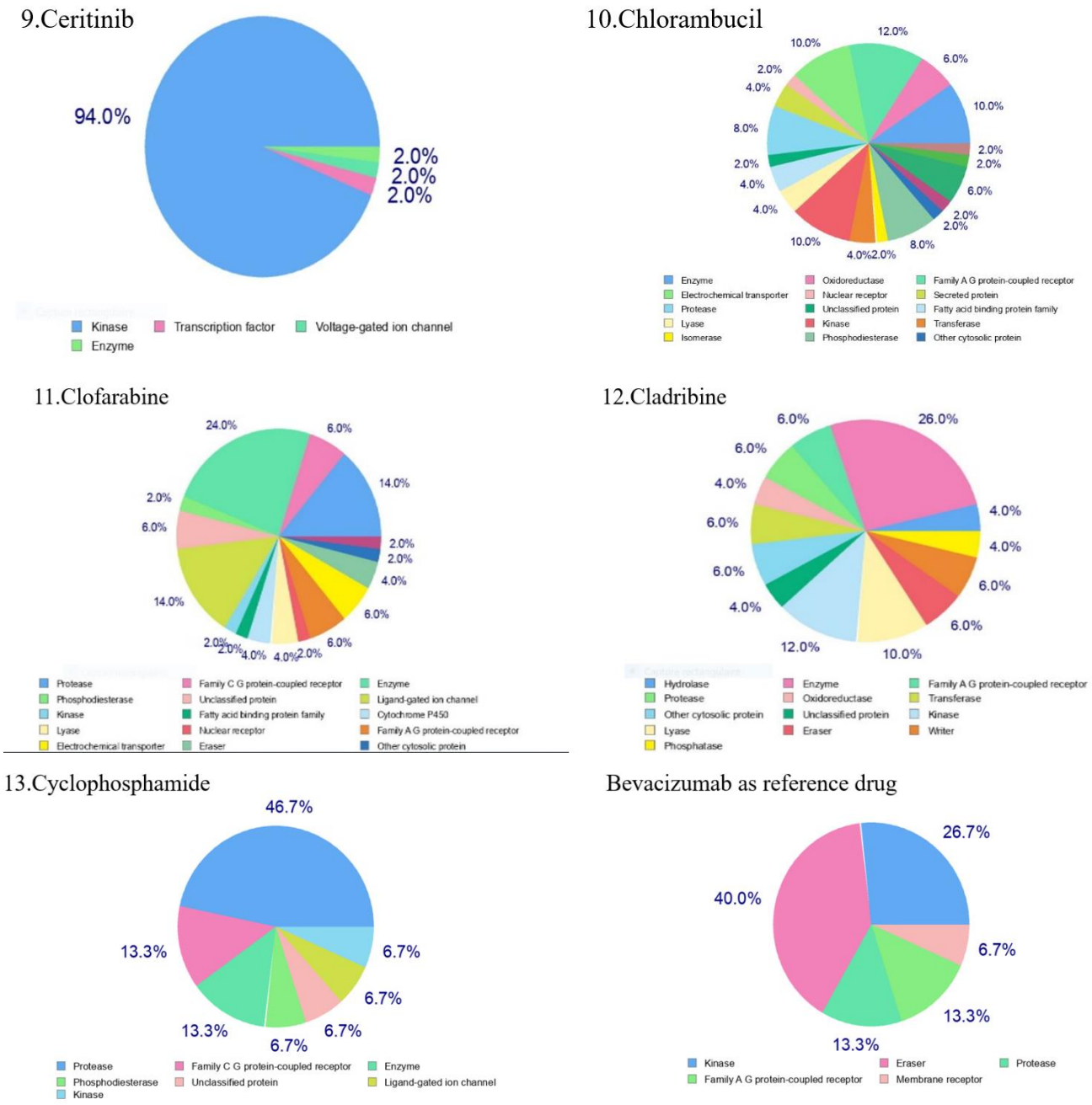


Figure S1. Swiss target Pi-chart compares the top 13 studied compounds against Bevacizumab. 1 Aazacitidine, 2 Altretamine, 3 Bendamustine hydrochloride, 4 Busulfan, 5 Capecitabine, 6 Capmantinib, 7 Carmustine, 8 Carboplatin, 9 Ceritinib, 10 Chlorambucil, 11 Clofarabine, 12 Cladribine, 13 Cyclophosphamide, and Bevacizumab used as reference drug.