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Molecular Docking and *In-Silico* Toxicity Analysis of Classical Alkylating Agents Against VEGF: Uncovering the Anti-Angiogenic Potential

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Abstract

This study explored the novel anti-angiogenic effect of the classical alkylating agents using their ability to directly interact with Vascular Endothelial Growth Factor (VEGF), the key controller of tumor angiogenesis and resistance to combinations of chemotherapy. The alkylating agents commonly used in clinical practice, namely, Mitomycin C, Melphalan, Chlorambucil, and Cyclophosphamide, were evaluated with the assistance of the molecular docking program, AutoDock Vina, integrated within YASARA, for the structural visualization and interaction profiling. Mitomycin C showed the highest binding affinity to VEGF (−7.4 kcal/mol) as it exhibited large-scale interaction with key residues of the cystine-knot motif mediating VEGF binding to VEGFR, which implies the possibility of inhibiting the interaction with the receptor. Melphalan, Chlorambucil and Cyclophosphamide showed moderate binding via hydrophobic and aromatic interactions inside the VEGF functional cleft, and Cyclophosphamide weak peripheral interactions. These results reveal that some alkylating agents might have direct anti-angiogenic actions beyond their known DNA-damaging processes that explain clinical findings of vascular inhibition on treatment. Furthermore, In-silico toxicity study also confirmed the mutagenic, carcinogenic, and organ-targeted toxicities of selected alkylating agents. Moreover, ADME profiling also showed specific absorption, permeability, and metabolic behaviors, which are associated with pharmacological activity. Together, these studies highlight Mitomycin C as a promising dual-mechanism candidate, which can both destroy tumor DNA and regulate angiogenesis by binding the VEGF. These findings support the use of alkylating chemotherapeutics as economical anti-angiogenic agents and provides a basis to the design and experimental validation of hybrid drugs which combine cytotoxic and anti-vascular action.

Introduction

VEGF is a vital part of tumor angiogenesis. Tumors bigger than a few millimeters in diameter rely on the endothelial cell growth, migration, and tube formation through VEGF-mediated endothelial cell proliferation [1]. The VEGF induced vasculature is characterized by structural abnormalities, increased permeability, and a high susceptibility to tumor invasion and dissemination, resulting in the establishment of a microenvironment that supports malignancy [2]. Considering this paramount biological position, VEGF has become a key target of cancer treatment. Not only does inhibition of VEGF curtails the process of neovascularization, it also impairs the delivery of nutrients, the growth of tumors, and the metastatic process. Moreover, VEGF is directly involved in resistance to treatment (tumors with high VEGF concentrations have poor responses to chemotherapy and radiotherapy because of reduced drug penetration, high hypoxia, and activation of cell-survival pathways [3]. Such hypoxia further triggers the expression of VEGF which creates a vicious cycle where angiogenesis and therapeutic resistance mutually reinforce each other. Suppressing the VEGF can be useful in counteracting this cycling in normalizing the aberrant vasculature, normalizing oxygenation and augmenting delivery of chemotherapeutic agent, thus, maximizing the therapeutic response [4].

VEGF also is quite important in regulating the immune microenvironment. High concentrations of VEGF inhibit the maturation of dendritic cells, stimulate the growth of regulatory T-cells, and prevent the infiltration of the cytotoxic T-cells thus facilitate in tumor immune evasion [5].

Countermeasures of VEGF blockade include the restoration of immune surveillance, the increased availability of tumors to T-cells, and antigen presentation. This immunomodulatory benefit has made VEGF inhibitors useful as adjunct to immune checkpoint inhibitors resulting in better therapeutic efficacies in renal cell carcinoma, hepatocellular carcinoma, and non-small cell lung cancer. Bevacizumab, ramucirumab and VEGFR-targeting tyrosine kinase inhibitors have clinically been used as VEGF-targeted agents and have become standard treatments for various solid tumors [6]. Their proven effectiveness in angiogenesis inhibition, slowing tumor progression, and dampening metastasis attests to the therapeutic value of VEGF inhibition and adds weight to VEGF as an approved molecular target toward drug development [7]. Following the massive costs, toxicity, and resistance linked with dedicated anti-VEGF biologics and kinase inhibitors, the possibility that traditional alkylating agents can also suppress VEGF and brings a whole new approach to cancer therapy. Mitomycin C, Melphalan, Chlorambucil, and Cyclophosphamide are well known alkylating agents that have the ability to cause DNA damage and apoptosis [8]. Recent observations, such as the docking analysis indicate that they can also have indirect anti-angiogenic effects. In the event that these agents prove to be in a position to directly interact with VEGF or interfere with VEGF-VEGFR interactions. The possibility arises that they form a dual- functionality therapeutic approach that simultaneously prevents tumor cell proliferation and interrupts angiogenesis. Such a dual activity is especially relevant in tumors in which VEGF signaling is one of the factors that make tumors aggressive and resistant to treatment. Recent observations (Mitomycin C interact to VEGF with strong affinity) provides molecular support for prior clinical findings indicating that alkylating agents may reduce tumor vascularity [9].

The clinical implication of such dual action is enormous. The cost-effective alternative to anti-angiogenic drugs specific to VEGF is the reuse of alkylating agents as anti-VEGF, which would allow lowering the cost of treatment, and decrease

dependence on biologics. Their established clinical potential and the safety profiles that are well-characterized make them convenient candidates to be included into current treatment regimens. Further, alkylating agent mediated direct VEGF inhibition can be helpful in overcoming resistance to conventional anti-VEGF therapies, where the cytotoxic properties of alkylating agents are able to kill tumor and endothelial cells, but may also prevent the induction of other angiogenic pathways. Such complex inhibition can reduce chances of angiogenic escape as well as increase long-term therapeutic efficacy. It is in light of these opportunities that the current study aims to explore the largely untapped potential of classical alkylating agents, namely Mitochondrine C, Melphalan, Chlorambucil and Cyclophosphamide to directly engage and regulate the activity of one of the most significant tumor angiogenic factors Vascular Endothelial Growth Factor (VEGF). This research aimed at preparation and optimization of ligands and VEGF structures, conducting high-precision molecular docking to determine binding affinities, interaction residues, binding modes and validating interaction profiles by comprehensive 3D and 2D visualizations. In addition to this, the experiment aimed to compare the in-silico toxicity of these alkylating agents, especially organ toxicity, stress response pathways, and nuclear receptor interaction. Furthermore, it also aimed to analyze their ADME properties to gain more insightful knowledge about absorption and permeability and general drug-likeness. It was hoped that through these integrative computational studies, one can gain an understanding of whether this chemotherapeutics have direct anti-angiogenic effects that would guide drug repurposing efforts or would be used to design future VEGF-targeting agents.

Materials and Methods

Ligand and target preparation

PubChem was queried to download 4 alkylating agents including mitomycin C (CID 5746), melphalan (CID 460612), chlorambucil (CID 2708), and cyclophosphamide (CID 2907). Avogadro2 was used to minimize the energy to get most stable conformation [10] and the files were stored in SDF format to further analyze them.

The target for the computational study was Vascular Endothelial Growth Factor (VEGF-A). The three-dimensional structure of the VEGF homodimer was obtained from the Protein Data Bank. Initially, protein preparation was done with BIOVIA Discovery Studio, which included the removal of all the crystallographic water molecules, ions, and any non-native ligand or co-factor. This is done to achieve a clean starting structure by avoiding possible steric clashes and spurious interactions that may affect the docking pose or scoring of novel compounds. The hydrogen atoms were introduced and partial charges attributed to the corresponding force field which produced a complete protein structure to be used in virtual screening.

2.2 Molecular docking

In YASARA, the receptor was imported and prepared by deleting crystallographic waters, salts and any non-biological heteroatoms, fixing missing side chains and backbone atoms, adding hydrogen and running YASARA with its built-in force field to relax steric clashes as per known study [11]. To identify the best pocket to bind in VEGF-A, the binding-site finder of YASARA was applied. AutoDock Vina was used in a docking module of YASARA [12]; a grid box, the size of which is completely surrounded by the binding area that is predicted or known (an additional 6-8 Aº covering ligand flexibility) was established. There was a series of independent runs (20 runs) with docking parameters and exhaustiveness being augmented in order to sample the space comprehensively. The best poses of all ligands were stored. The poses of the top results after docking were re-scored and refined by minimizing each

protein-ligand complex within YASARA to a local minimum to remove any remaining clashes.

To visualize the best complexes, they were opened in PyMOL to generate publication-quality cartoons, surface maps and distance measurement of hydrogen bonds and ionic contacts [13]. Regular coloring and labeling of the residues communicating with the ligands were developed and the high-resolution images were exported. Lastly, the LigPlot generated 2D interaction diagrams (now using the PDB of the minimized complex as the input) to list all the hydrogen bond, hydrophobic contacts, and interacting residues in a simple schematic [14]. These plots, paired with PyMOL 3D views and YASARA energy analyses, provide a complete and reproducible docking workflow.

2.3 Toxicity study

The toxicity study of both alkylating agents was analyzed using PROTOX online tools [15]. In a toxicity study, we study organ toxicity, toxicity end points, nuclear receptor signaling pathways, stress response pathways, molecular initiating events, and metabolism. We also draw a radar diagram that completely elaborates the mode of action of both drugs.

2.4 ADME properties of alkylating agents

The online server [16] was used to analyze the physicochemical, lipophilicity, water solubility, pharmacokinetics, and drug likeness of both alkylating agents.

3. Results and Discussions

The present molecular docking study was undertaken to explore whether classical alkylating agents, which are primarily known for their DNA-damaging cytotoxicity, may also possess a previously underexplored ability to directly bind and modulate VEGF (a master regulator of angiogenesis). The need for this project arises from the fact that tumor vasculature plays a central role in cancer progression, and although several anti-VEGF biologics exist, they are expensive, prone to resistance, and not universally effective [17]. Repurposing existing chemotherapeutic agents to achieve additional anti-angiogenic effects, particularly by directly interacting with VEGF, provides a cost-effective and mechanistically novel avenue for combined cytotoxic and anti-vascular therapy. In this study, the alkylating agents Mitomycin C, Melphalan, Chlorambucil, and Cyclophosphamide were docked to VEGF using an extensive set of fifty runs per ligand (given in supplementary file in Table S1), providing a comprehensive assessment of binding affinities, interaction residues, dissociation constants, and contact surface characteristics.

The findings indicated that Mitomycin C showed the strongest affinity to VEGF with a maximum binding energy of about -7.4 kcal/mol, which was much higher than the rest of the agents under experimentation (the optimum docking run of each ligand to VEGF is presented in Table 1). The dissociation constants of the same in the low-micromolar to sub-micromolar range show strong and stable interactions. Mitomycin was able to form substantial contacts with residues Gly59, Cys60, Cys61, Asn62, Asp63, Glu64, Leu66 and Cys68. These residues lie in VEGF cystine-knot motif, which is an essential functional region and it helps in maintaining VEGF dimer in place and it is directly involved in receptor recognition. Further contacts with Asp34, Phe36, Ile46 and Ser50 indicate that Mitomycin binds to a shallow groove between the central strands of beta and the so-called adjoining loops. These interface sites are reported to belong to the VEGF receptor binding surface, and it suggests that the binding of Mitomycin may reasonably disrupt the formation of VEGF–VEGFR complexes. The 3D orientation of Mitomycin is such that the ligand is entrenched in the middle of disulfide-rich loops, with various hydrogen bonds with the carbonyl and methoxy groups of the ligand, with hydrophobic packing taking place via the residues, such as Phe36 and Ile46.

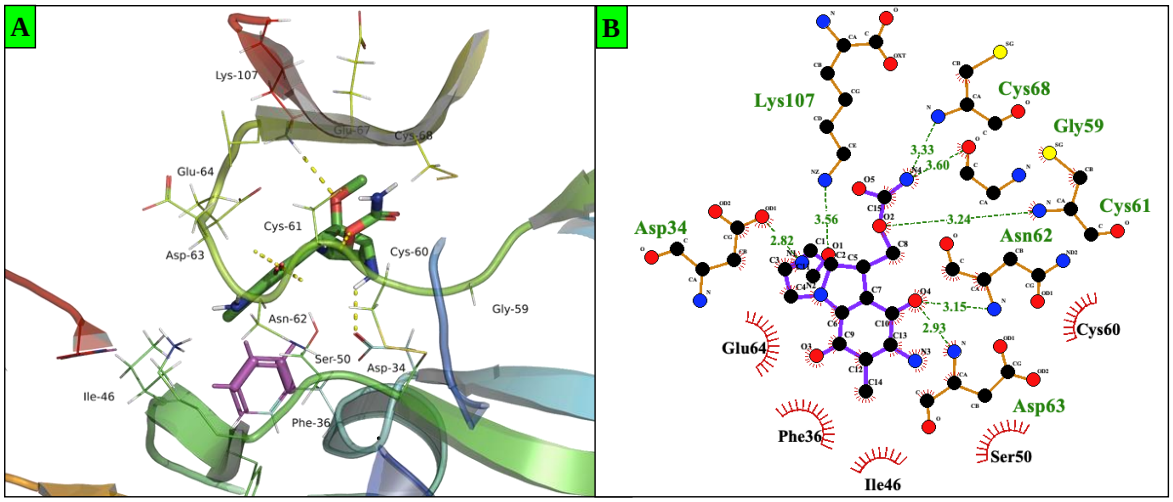

Figure 1 Docking of Mitomycin agents against VEGF protein chain (A: 3D visualization & B: 2D visualization).

Mitomycin presents typical hydrogen bonding with Asp63 and Glu64 in a 2D interaction view, which are complemented by van der Waals and hydrophobic interactions, which stabilize the ligand in the VEGF cleft

Although Melphalan exhibited lower binding energy than Mitomycin, it nevertheless showed moderate affinity with optimum docking energies of about -5.7 kcal/mol. Its interactions were predominantly with residues Asp34, Ile35, Phe36, Glu42, Tyr45, Ile46, and Phe47, which is a mix of hydrophobic aromatic residues and acidic residues that complete its amino-bearing side chain. The phenyl ring of Melphalan is exactly in the same plane as the hydrophobic patch formed by Phe36 and Tyr45 allowing π -pi stacking that stabilizes the ligand in the VEGF pocket. Hydrogen bonding with Asp34 and Glu64 also provides positions to it.

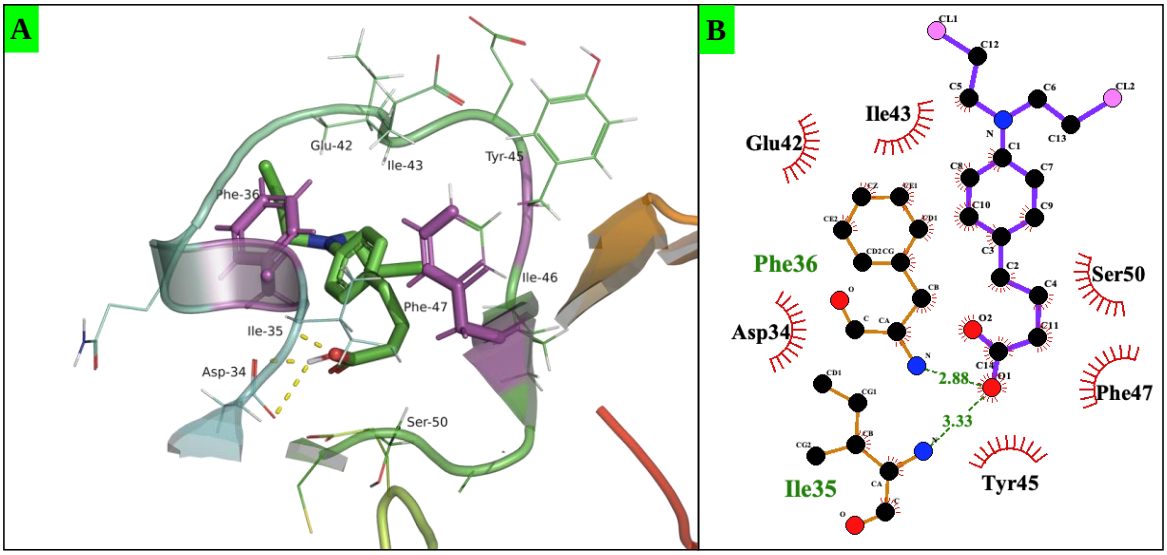

Figure 2 Docking of Melphalan agents against VEGF protein chain (A: 3D visualization & B: 2D visualization).

Melphalan has a lower level of embedded orientation in 3D space than Mitomycin, although both are close to the same VEGF functional region. Aromatic stacking is generally demonstrated by the 2D interaction plot, along with hydrogen bonds and slightly strong van der Waals contacts. This intermediate affinity helps to substantiate available biological evidence that Melphalan has some anti-angiogenic properties in clinical conditions, including intra-arterial chemotherapy of tumors of the eye, where the vascular upkeep of the tumor is a contributing factor to its regression.

Chlorambucil that has binding energies of around -5.5 kcal/mol also exhibited moderate interaction with VEGF but had less extensive interaction with VEGF than Melphalan. Its long flexible shape enabled the insertion half-way into the VEGF hydrophobic cleft into interaction with residues Asp34, Phe36, Glu42, Tyr45, Ile46, and Phe47. Its aromatic ring formed hydrophobic interactions with Melphalan, but did not have the extra stabilizing hydrogen bonds to increase the affinity. Chlorambucil's 3D orientation tends to situate the ligand in a semi-surface-exposed position, providing fewer penetration contacts with VEGF's cystine knot motif. In 2D interaction diagrams, Chlorambucil usually forms only one or two hydrogen bonds and relies mostly on hydrophobic contacts. This explains its intermediate affinity and suggests that although Chlorambucil might influence endothelial behavior indirectly, its direct VEGF binding is weaker compared to Mitomycin and Melphalan.

Table 1 Best Docking Run for Each Ligand Against VEGF

Ligand	**BBE[a]**	**BE[b]**	**D.C[c]**	**CS[d]**	**Key Interacting Residues**
Mitomycin C	-7.409	0.3087	3.71×10^{6}	279.39	GLY59, CYS60, CYS61, ASN62, ASP63, GLU64, GLU67, CYS68, LYS107, ASP34, PHE36, ILE46, SER50
Melphalan	-5.716	0.3008	6.46×10^{7}	330.62	ASP34, ILE35, PHE36, TYR45, ILE46, PHE47, SER50, ASN62, ASP63, GLU64, GLY65, HIS223, ARG224, THR226
Chlorambucil	-5.58	0.2937	8.12×10^{7}	242.24	ASP34, ILE35, PHE36, GLU42, ILE43, TYR45, ILE46, PHE47, SER50
Cyclophosphamide	-4.537	0.3241	4.72×10^{8}	230.36	ASP34, ILE35, PHE36, GLU42, ILE43, TYR45, ILE46, PHE47, SER50

Best Binding Energy (kcal/mol); b: Binding Efficiency (kcal/mol atom); c: Dissociation constant (pM) and d: contact surface area ($Å^2$)

Cyclophosphamide demonstrated the weakest binding affinity among all tested compounds, with energies around -4.3 to -4.5 kcal/mol and minimal contact surfaces. This result aligns well with its known pharmacology, as Cyclophosphamide is a prodrug that becomes active only after hepatic biotransformation, and thus the parent compound is not expected to bind strongly to protein targets such as VEGF. The interactions observed involve mainly Glu64, Asp63, Cys60, and Asn62, but are limited to weak van der Waals contacts or single hydrogen bonds.

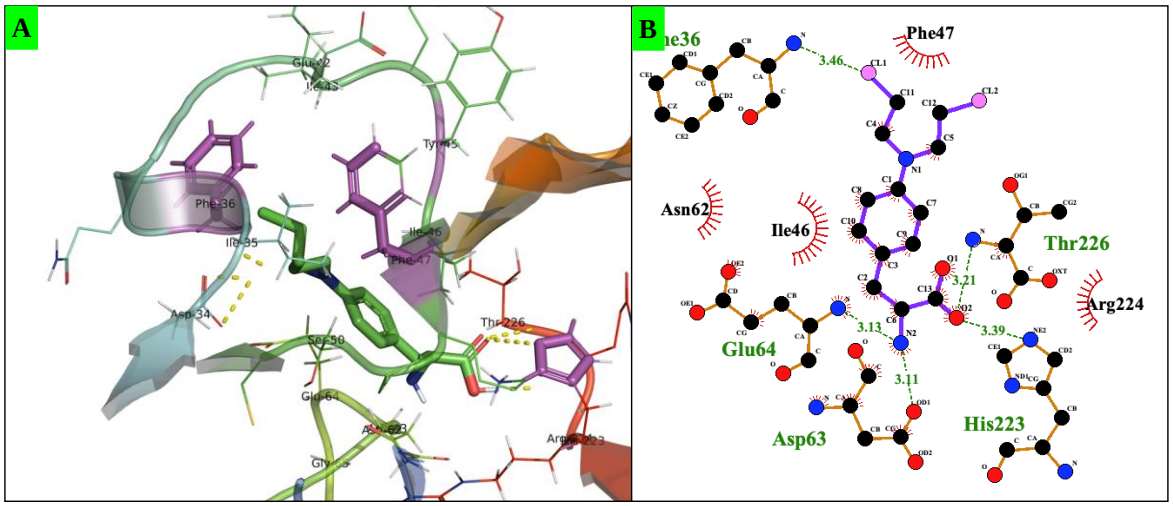

Figure 3. Docking of Chlorambucil agents against VEGF protein chain (A: 3D visualization & B: 2D visualization).

In 3D visualizations, Cyclophosphamide occupies a peripheral region of the VEGF surface rather than the core binding pocket. Its 2D interaction network is sparse, lacking the hydrophobic or aromatic interactions required for strong affinity. This supports the idea that Cyclophosphamide's anti-angiogenic effects seen in metronomic therapy arise from systemic immunomodulation and endothelial progenitor suppression rather than from direct VEGF binding.

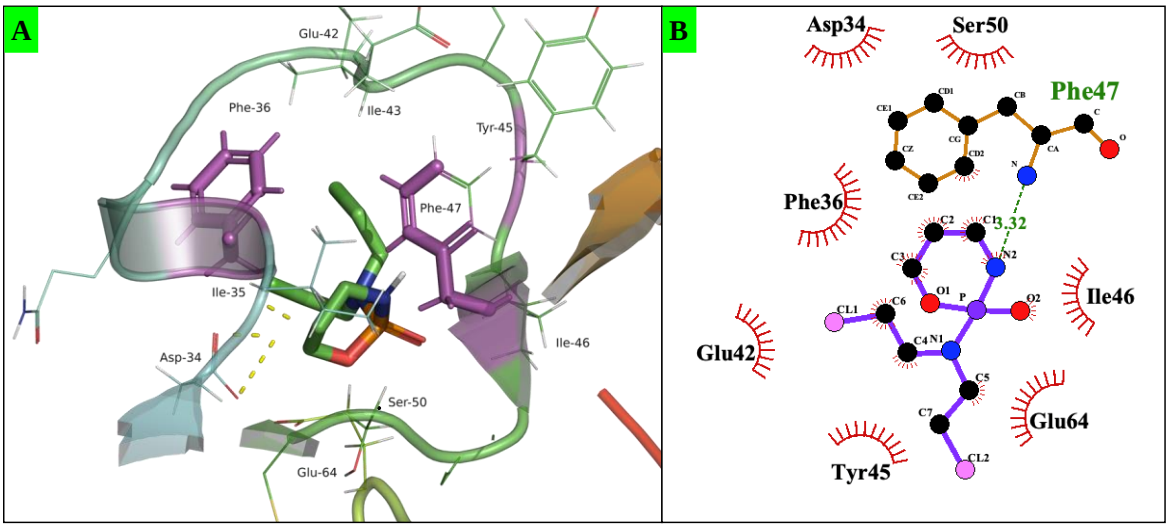

Figure 4. Docking of Cyclophosphamide agents against VEGF protein chain (A: 3D visualization & B: 2D visualization).

Overall the docking studies indicate that Mitomycin C possesses the most promising VEGF interaction profile, energetically and structurally. It is always bound in the residues that are vital in VEGF–VEGFR-mediated interactions, suggesting that it may be competitively restrictive to receptors. This is important since the anticancer effects of Mitomycin are well-known and are mostly accredited to DNA crosslinking and because of the strong affinity to VEGF, it may be its dual effect; direct suppression of tumor vasculature and cytotoxic DNA damage. The intermediate binding of Melphalan and Chlorambucil also implies that the drugs may partially regulate the VEGF activity and mostly by acting on DNA alkylation. This is consistent with growing evidence that certain classical chemotherapies have secondary anti-angiogenic effects whose mechanism has not been well understood. The low-affinity binding of cyclophosphamide evidences the existing body of knowledge according to which the anti-vascular effects of cyclophosphamide are not directed to the receptor.

This work is significant because it helps in drug rediscovery and mechanistic oncology. The fact that some alkylating agents can bind VEGF directly outlines a novel aspect of their pharmacology that could be the reason behind the clinical observations of increased tumor regression in vascular-rich cancers undergoing treatment with Mitomycin or Melphalan. Therapeutically, these compounds can be considered as adjunct therapy to the known anti-VEGF drugs to achieve additive or synergistic angiogenesis inhibition. The docking study also shows particular amino acids to be binding sites. Asp34, Phe36, Cys60, Cys61, Cys68, Asp63 and Glu64 are the same amino acids to be learnt as being constituted as being predictable as ligand contacts, to be employed in the creation of novel derivatives or VEGF inhibitors. In addition, the results are stimulated to corroborate these results in-vitro with endothelial proliferation, VEGFVEGFR binding, and tube-formation assays and in-vivo angiogenesis testing.

In-Silico Toxicity Investigation

The in-silico toxicity profile that was produced on mitomycin C, melphalan, cyclophosphamide, and chlorambucil indicates well-reported toxicological behaviors of alkylating agents as shown in supplementary Table S2 and the resulting Radar Chart in Figure 5. In all four compounds, the models are able to provide high probabilities of mutagenicity, carcinogenicity and cytotoxicity as is expected of DNA-crosslinking chemotherapeutics. As an example, mitomycin C has a high predicted mutagenicity (0.81) and carcinogenicity (0.78) as per the already established literature which characterizes it as an DNA-alkylating quinone and has the ability to cause inter-strand DNA crosslinks, chromosomal aberration and micronuclei [18]. These assumptions are supported by research that suggested that mitomycin C is one of the strongest clinically used clastogenic agents. Likewise predictions of melphalan, cyclophosphamide and chlorambucil were similar to previously known risks of therapy-related myelodysplasia and secondary leukemias, a known class effect of bifunctional alkylators [19].

The predictions of the model on organ-specific toxicity are also in line with the known clinical trends. The active prediction of respiratory toxicity (0.86–0.96) is consistent with the findings of literature on pulmonary fibrosis, interstitial pneumonitis, and endothelial damage with all four agents, especially mitomycin C and cyclophosphamide. It is also positive in the predictions of nephrotoxicity with compounds (0.53 0.83) in agreement with the fact that alkylators induce renal tubular injury and glomerular dysfunction, particularly with high cumulative doses [20]. Cardiotoxicity is not predicted in case of mitomycin C, but active in case of melphalan (0.63), which is in line with the fact that high dose of melphalan can cause acute cardiac effects, but mitomycin C has relatively low cardiotoxic potential.

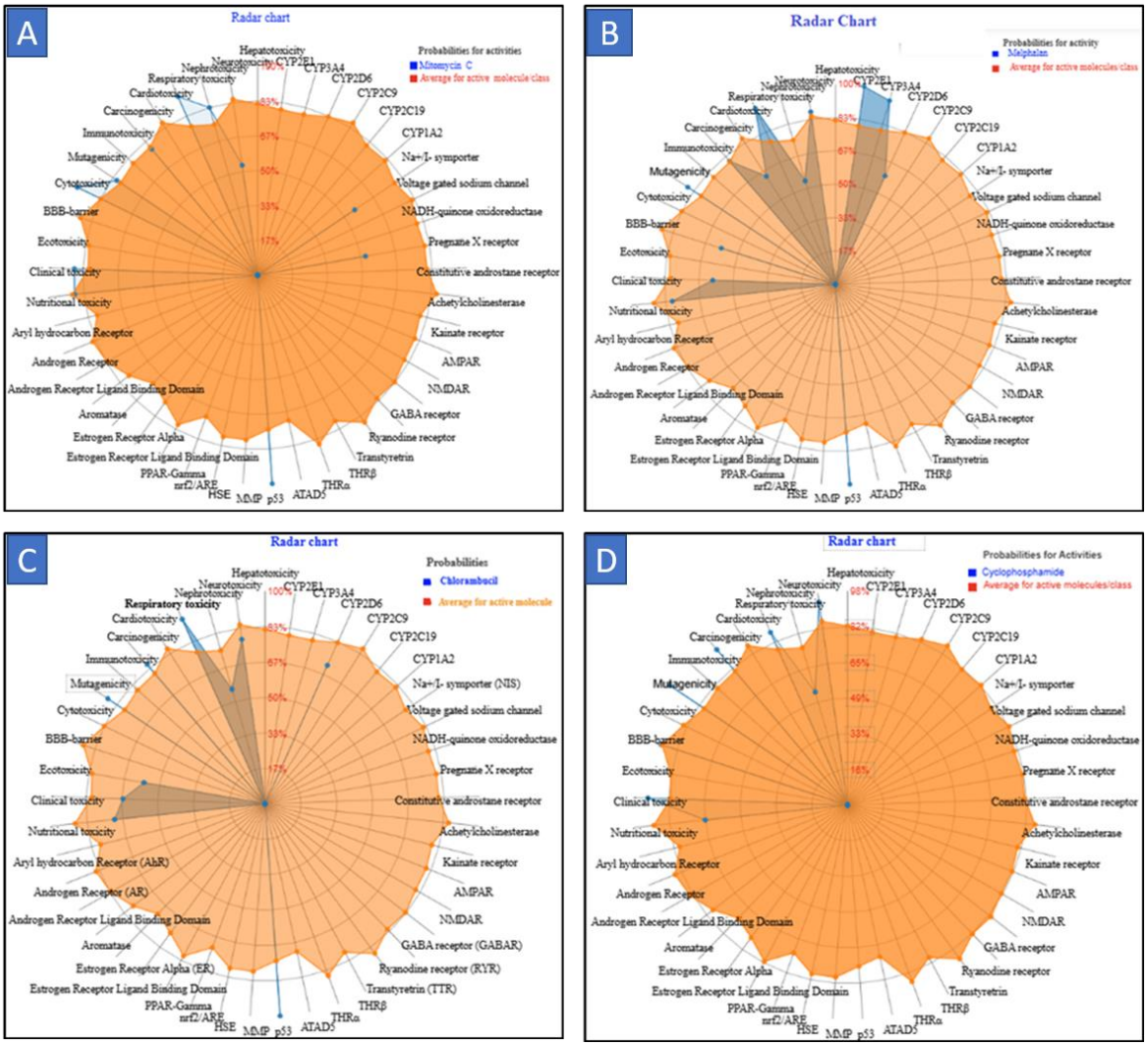

Figure 5. Radar chart for n-silco toxicity

The inactivity of most of the nuclear receptor interactions indicates that endocrine disruption is not of great feature of the toxicity of these compounds. All the androgen, estrogen, PPAR- g and AhR pathway predictions are inactive with very high probabilities (0.9-0.99). This coincides with pharmacological effects of the alkylating agents that are not generally involved in ligand mediated receptor signaling. Equally, the molecular initiating events involving neuroreceptors, including GABA, NMDA, or AMPA receptors, are estimated to be inactive (0.680.99), which confirms the clinical result of the drugs not being directly neuroreceptor-modulating in producing clinical neurotoxicity; neurotoxicity in clinical practice is normally secondary to damage to the DNA and to oxidative stress instead of directly acting on the neuroreceptors.

It is also interesting to note that the activation of p53 pathway has been consistently predicted with a score of active with the highest probability (1.0) with mitomycin C, melphalan, and chlorambucil. This observation is biologically feasible, because DNA crosslinkers are potent triggers of p53-mediated responses to DNA damage, which causes either apoptosis or cell-cycle arrest. It has been established that mitomycin C is strong in activating p53 and p21 and Bax downstream pathways [21]. This mechanistic understanding supports the usefulness of the in-silico predictions.

The predictions of the metabolism give more mechanistic background. It is suggested that mitomycin C should be inactive among all major CYP450 isoforms, including CYP3A4 and CYP2D6 (0.63–0.98), which is in line with known information that bioactivation of mitomycin C largely relies on reducing enzymes in the form of NADPH-cytochrome P450 reductase and not oxidative CYP metabolism. Contrarily,

melphalan demonstrates an expected CYP3A4 activity (0.95), which is in line with the literature that indicated that melphalan is subjected to slight cytochrome-mediated degradation in the liver [22]. The well-known drug cyclophosphamide that depends on CYP2B6, CYP2C9, and CYP3A4 to be activated is expected to act on CYP2D6 and CYP3A4 with moderate to high probabilities (0.59 and 0.95), due to its known metabolic activation route [23].

ADME analysis of ligands

The absorption, distribution, metabolism and excretion (ADME) profile of mitomycin C, cyclophosphamide, melphalan and chlorambucil depict clear and understandable differences in physicochemical properties, absorption potential and overall drug-likeness which clarifies the pharmacological and toxicity profiles. The highest molecular weight and the greatest number of hydrogen-bond acceptors and donors make mitomycin C exhibit a markedly negative logP value (–2.41), which shows high levels of polarity and low levels of membrane permeability. This property explains why the compound is very soluble and has been predicted to have low gastrointestinal (GI) absorption since polar and hydrophilic compounds are not expected to cross lipid membranes easily. The CYP and Melphalan have a moderate lipophilicity (log P of 1.06 and 1.92, respectively) and this relates to the high predicted GI absorption. By contrast, the most lipophilic drug (logP 3.38)—chlorambucil—is considered to be soluble and has the most desirable prediction of permeability profile. These differences in lipophilicity and water solubility are consistent with their clinical uses, since all of cyclophosphamide, melphalan and chlorambucil are well-absorbed by the oral route, whereas mitomycin C is administered parenterally due to its low oral bioavailability.

This is not only due to the fact that cyclophosphamide, melphalan, and chlorambucil, but not mitomycin C, are predicted to permeate the blood-brain barrier (BBB), as a result of the physicochemical characteristics of the drug: lower polarity and increased lipophilicity leads to penetration into the central nervous system (CNS). Melphalan that is reportedly penetrating the BBB to a small extent in a clinical environment is both moderately lipophilic, and of the right size, confirming these predictions. The failure of mitomycin C in penetrating the BBB is expected because of the high polarity and its hydrophilicity. The estimates of skin permeation (log Kp) align with predicted patterns also, with chlorambucil having the greatest permeation potential due to its lipophilicity and mitomycin C having the lowest permeability due to its extreme hydrophilicity.

Each of the four compounds satisfies Lipinski rule, with a zero violation [24], indicating good theoretical oral drug-likeness although an unfavorable practical effect of mitomycin C is as a result of its inability to diffuse as opposed to being limited by a rule. The bioavailability scores have a subtler picture: the predictive score of mitomycin C, cyclophosphamide and melphalan is moderate (0.55), indicating its acceptable but not ideal oral exposure, whereas the higher score of chlorambucil (0.85) can be attributed to its favorable lipophilicity, membrane permeability, and effective absorption. All these ADME properties explain many of the observed differences in pharmacokinetics of these compounds in the clinic and complement the toxicity predictions: more lipophilic compounds and those with greater systemic exposure (melphalan and chlorambucil) often have broader organ distribution with attendant toxicity and mitomycin C is hydrophilic and still causes significant toxicity through reductive activation in selected organs, such as the kidneys and lungs.

Table 2 ADME properties of Mitomycin, Cyclophosphamide, Melphalan and Chlorambucil

Properties	**Physicochemical**			
	Mitomycin	**Cyclophosphamide**	**Melphalan**	**Chlorambucil**
Formula	$C_{15}H_{18}N_4O_5$	C7H16Cl2N2O2P	$C_{13}H_{18}C_{l2}N_2O_2$	C14H19Cl2NO2
Molecular weight	334.33	262.09 g/mol	305.20 g/mol	304.21 g/mol
No. of heavy atoms	24	14	19	19
H. bond donors	03	02	02	02
H. bond acceptors	06	04	03	01
Molar refractivity	86.95	64.13	78.91	81.01
	Lipophilicity			
Log p o/w (WLOGP)	-2.41	1.06	1.92	3.38
	Water Solubility			
Class	Very soluble	Very soluble	Very soluble	Soluble
Log S (SILICOS-IT)	-1.19	-2.23	-4.04	-5.16
	Pharmacokinetics			
GI absorption	Low	High	High	High
BBB permeant	No	Yes	Yes	Yes
Log Kp (skin permeation)	-8.62 cm/s	-7.39 cm/s	-8.02 cm/s	-6.95 cm/s
	Drug likeness			
Lipinski	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
Bioavailability score	0.55	0.55	0.55	0.85

Conclusion

The present in-silico study offers strong indications that classical alkylating agents, especially Mitomycin C and recognized its capacity to engage with VEGF directly and potentially disrupt VEGF-Receptor signaling. The strong binding affinity of Mitomycin C as well as the interaction with the key residues of cystine-knot motif indicates that Mitomycin C could competitively inhibit the binding of VEGF receptors providing a mechanistic explanation of its observed vascular-suppressive effects. Also, Melphalan and chlorambucil have shown considerable interactions with VEGF and that effects may not be restricted to cytotoxicity. Conversely, low binding affinity of Cyclophosphamide confirms the hypothesis that the vascular activity of the compound is mostly indirectly related, i.e. immunomodulatory and endothelial progenitor cell depleting. A combination of these docking findings with toxicity and ADME studies provides a consistent pharmacological picture where binding strength, activation of metabolism, and physicochemical characteristics are in line with the clinical behavior of any given agent. Moreover, the presence of the conserved VEGF residues used in ligand interactions is an important structural information that can be utilized in rational drug design. Taken together, this research supports the idea that some alkylating agents can be used as dual-action therapeutics, which can destroy tumor DNA integrity and regulate angiogenesis at the same time. Such results open the path of the repurposing of the known chemotherapeutics as low-cost anti-VEGF substitutes and encourage additional in vitro and in vivo validation to determine their suitability as multifunctional anti-cancer agents.

References

Duffy, A.M., D.J. Bouchier-Hayes, and J.H. Harmey, Vascular endothelial growth factor (VEGF) and its role in non-endothelial cells: autocrine signalling by VEGF, in Madame Curie Bioscience Database [Internet]. 2013, Landes Bioscience.

Maiuolo, J., et al., The contribution of gut microbiota and endothelial dysfunction in the development of arterial hypertension in animal models and in humans. International Journal of Molecular Sciences, 2022. **23**(7): p. 3698.

Soleimanjahi, H. and A. Habibian, Novel Anti-angiogenic Strategies in Cancer Drug Development, in Anti-Angiogenesis Drug Discovery and Development: Volume 5. 2020, Bentham Science Publishers. p. 125-150.

Zhao, Y. and A.A. Adjei, Targeting angiogenesis in cancer therapy: moving beyond vascular endothelial growth factor. The oncologist, 2015. **20**(6): p. 660-673.

Yang, J., J. Yan, and B. Liu, Targeting VEGF/VEGFR to modulate antitumor immunity. Frontiers in immunology, 2018. **9**: p. 978.

Patel, S.A., et al., Molecular mechanisms and future implications of VEGF/VEGFR in cancer therapy. Clinical Cancer Research, 2023. **29**(1): p. 30-39.

Lee, C., et al., Vascular endothelial growth factor signaling in health and disease: from molecular mechanisms to therapeutic perspectives. Signal Transduction and Targeted Therapy, 2025. **10**(1): p. 170.

Fu, D., J.A. Calvo, and L.D. Samson, Balancing repair and tolerance of DNA damage caused by alkylating agents. Nature Reviews Cancer, 2012. **12**(2): p. 104-120.

Andrés-Guerrero, V., et al., Current perspectives on the use of anti-VEGF drugs as adjuvant therapy in glaucoma. Advances in Therapy, 2017. **34**(2): p. 378-395.

Snyder, H.D. and T.G. Kucukkal, Computational chemistry activities with Avogadro and ORCA. Journal of Chemical Education, 2021. **98**(4): p. 1335-1341.

Rehman, M.F.u., et al., Effectiveness of natural antioxidants against SARS-CoV-2? Insights from the in-silico world. Antibiotics, 2021. **10**(8): p. 1011.

Rubab, S.L., et al., Synthesis, crystal structure, DFT calculations, Hirshfeld surface analysis and in silico drug-target profiling of (R)-2-(2-(1, 3-dioxoisoindolin-2-yl) propanamido) benzoic acid methyl ester. Molecules, 2023. **28**(11): p. 4375.

Yuan, S., et al., PyMOL and inkscape bridge the data and the data visualization. Structure, 2016. **24**(12): p. 2041-2042.

Patil, R., et al., Optimized hydrophobic interactions and hydrogen bonding at the target-ligand interface leads the pathways of drug-designing. PloS one, 2010. **5**(8): p. e12029.

Banerjee, P., et al., ProTox 3.0: a webserver for the prediction of toxicity of chemicals. Nucleic Acids Research, 2024. **52**(W1): p. W513-W520.

Walles, M., A. Connor, and D. Hainzl, ADME and safety aspects of non-cleavable linkers in drug discovery and development. Current Topics in Medicinal Chemistry, 2017. **17**(32): p. 3463-3475.

Ribatti, D., A.G. Solimando, and F. Pezzella, The anti-VEGF (R) drug discovery legacy: improving attrition rates by breaking the vicious cycle of angiogenesis in cancer. Cancers, 2021. **13**(14): p. 3433.

Bağatir, G., et al., A CLASTOGENICITY STUDY WITH MITOMYCIN-C IN APLASTIC ANEMIA: DISTINGUISHING FANCONI ANEMIA. Nobel Medicus Journal, 2024. **20**(2).

GREENE, M.H., et al., Melphalan may be a more potent leukemogen than cyclophosphamide. Annals of internal medicine, 1986. **105**(3): p. 360-367.

Li, L., et al., Anticancer therapy and lung injury: molecular mechanisms. Expert review of anticancer therapy, 2018. **18**(10): p. 1041-1057.

Cheng, S.-Y., et al., Mitomycin C and decarbamoyl mitomycin C induce p53-independent p21WAF1/CIP1 activation. International Journal of Oncology, 2016. **49**(5): p. 1815-1824.

Diabetes, N.I.o., Digestive, and K. Diseases, LiverTox: clinical and research information on drug-induced liver injury. 2012: National Institute of Diabetes and Digestive and Kidney Diseases.

Tran, A., et al., Serious haematological toxicity of cyclophosphamide in relation to CYP2B6, GSTA1 and GSTP1 polymorphisms. British journal of clinical pharmacology, 2008. **65**(2): p. 279-280.

Chen, X., et al., Analysis of the physicochemical properties of acaricides based on Lipinski's rule of five. Journal of computational biology, 2020. **27**(9): p. 1397-1406.