

In silico study of Sobuzoxane for VEGF-B mediated lymphangiogenesis targeting triple negative breast cancer

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Abstract. Radiation resistance is a major limitation in breast cancer therapy, although the chemotherapeutic drugs are well regarded for its efficiency in targeting rapidly proliferating cancer cells. Molecule such as 9-amino camptothecin was explored as radiosensitizer to address this concern of resistance. Pharmacophore modelling was used in this study to modify the structure of this molecule to get a derivative named Sobuzoxane which showed promising radiosensitizing activity and ability of mimic OH-lactone ester. In triple negative breast cancer, VEGF-B is known for vascular remodeling and modulating endothelial survival. Hence, it indirectly supports the tumour proliferation. Therefore, ligands were optimized to increase the VEGF-B targeting ability by refining hydrogen bond donors, acceptors, hydrophobic interactions, and aromatic features. The selected hit was further used for molecular docking, MD simulation and ADMET analysis. It was found that Sobuzoxane has higher binding affinity towards VEGF-B compared to the 9-amino camptothecin, hence could minimize the protein destabilization. Number of hydrogen bonds between VEGF-B-Sobuzoxane complex could further be increased by the incorporation of a neutralizing antibody Fab fragment. This indicates greater stability and specificity within the complex. Structural stability and persistence of protein-ligand complex were analysed by molecular dynamic simulation study for 100 ns. The stable binding was evident by the persistent interaction profile visualized throughout the MD simulation. Moreover, strong ligand-protein interaction potential along with good solubility was established by ADME analysis. In-silico analysis conducted in this study recognised Sobuzoxane as a potential radiosensitizer targeting VEGF-B for angiogenesis-mediated triple negative breast cancer. Finally, this study provides new understanding about the increased stability of Sobuzoxane-VEGF-B complex in presence of a neutralizing antibody Fab fragment as a result of enhanced hydrogen bonding.

1 Introduction

Breast cancer is widespread and a major cause of high amount of morbidity and mortality related to cancer in India. Angiogenesis supports tumour growth, survival and adaptive responses to therapeutic stress. Hence, it plays a crucial role in the cancer progression in breast cancer. Main modulator of angiogenesis and lymphangiogenesis are vascular endothelial growth factors (VEGF). Among these factors VEGF-B has been reported to modulate endothelial cell survival and vascular remodeling. Consequently, it can indirectly support tumour progression and vascular maintenance (Arjunan et al., 2018).

VEGF mediated lymphangiogenesis is closely related to triple negative breast cancer which is a key indicator of aggressive disease and poor clinical outcome (Adams et al., 2000). Mutations such as Single nucleotide polymorphisms (SNPs) in VEGF gene may lead to the alteration of VEGF-B protein activity which again may influence the lymphangiogenesis and treatment resistance (Hovinga KE et al., 2005). Increased concentration of VEGF proteins creates difficulty in diagnosis and treatment of breast cancer using hormonal therapy

(Jain et al., 2009). However, it is noteworthy that VEGF-B is not the primary factor for the induction of lymphangiogenesis. Rather it is a modulator whose role in lymphangiogenesis depends on the microenvironmental conditions and tumour variant.

Ionizing radiation (IR), since its discovery in 18th century has emerged as foundation mode of treatment for various types of cancers including breast cancer (Winter et al., 2024). Radiation therapy is used for the elimination of residual cancer cells and prevention of reoccurrence at post-surgery stage. IR disrupt cellular homeostasis thus enhance cell death by forming single and double stranded breaks in the DNA through generating reactive oxygen species such as hydroxyl radicals. Moreover, IR stimulates stress responses and apoptosis by affecting plasma membrane, subcellular organelles, and intracellular signalling pathways. In addition, non-irradiated cell in the vicinity may also get influenced by the irradiated cells thorough direct intercellular signalling or necrotic signaling pathways. As a result the non-targeted cells surrounding the cancer cells also face chromosomal instability, mutations, and apoptotic cell death (Tang et al., 2018). However, tumour cells often were found to develop radio resistance

leading to the failure or reduced efficacy of irradiation.

One of the key contributors to radioresistance is VEGF-mediated angiogenic signaling. VEGF expression has been reported to increase following radiation exposure in several experimental tumor models, including Lewis lung carcinoma, esophageal adenocarcinoma, squamous cell carcinoma, and glioblastoma, both in vitro and in vivo. In vitro studies have shown that the accumulation of VEGF protein reduces radiation-induced cell death in human malignancies, while anti-VEGF treatments potentiate radiation-mediated cytotoxicity in endothelial cells. These observations suggest that radiation-induced VEGF expression protects tumor vasculature from radiation damage, thereby contributing to radioresistance (Filippelli et al., 2025). VEGF-B (2vwe) forms a homodimer, with each monomer contributing to a binding site at the “poles” of the dimer. These epitopes are the same areas used by VEGF to bind its receptor (VEGFR-1) in homologous structures.

Camptothecin analogs are chemotherapeutic agents that exert their anticancer effects by stabilizing the camptothecin–topoisomerase I–DNA complex. This stabilization occurs when the 5'-phosphoryl end of an enzyme-catalyzed DNA break covalently binds to a tyrosine residue of topoisomerase I. Topoisomerase I binds with damaged DNA with comparatively higher affinity than undamaged DNA. This highly stable complex causes cell death by inducing double stranded break in DNA through interference in replication fork during S-phase of the cell cycle. OH-lactone ring found in camptothecin derivatives are crucial factor for their biological activity as radiosensitizer (Dhiman et al., 2025).

This understanding of binding mechanism of camptothecin derivatives highlights the requirement of developing drug derivatives with chemotherapeutic and radiosensitizing property. Inhibition of VEGF signaling enhances the efficacy of radiation therapy by increasing radiosensitivity of the tumour cells. Although VEGF-B is not the principal modulator of angiogenesis and lymphangiogenesis, but it influences the vascular survival and metabolic adaptation within the tumour microenvironment. Radiation exposure has been shown to induce VEGF expression, while blockade of VEGF activity using neutralizing antibodies prior to irradiation synergistically improves tumor control. This radiosensitization effect is attributed to enhanced sensitivity of tumor endothelial cells and favorable modifications of the tumor microenvironment, including improved oxygenation and suppression of post-radiation angiogenesis.

In the current pharmacophore study, 9-amino camptothecin was used as a template to design derivatives which can mimic the E-ring moiety. Sobuzoxane is one such derivative which is supposed to form a covalent ternary complex with DNA and topoisomerase I. As a result, irreversible single stranded breaks in DNA are formed during replication. This phenomenon resembles the mechanism of radiosensitizers previously demonstrated through pharmacophore modelling (Sunseri et al., 2016). The identified pharmacophore hits were subsequently evaluated using molecular docking, molecular dynamics (MD) simulations, and ADMET profiling. MD simulations were employed to assess the dynamic stability and interaction behavior of the VEGF-B–Sobuzoxane complex, while ADMET analysis provided insights into drug-likeness and pharmacokinetic feasibility. Collectively, this in silico approach aims to provide mechanistic insight into VEGF-B–targeted radiosensitization and to identify Sobuzoxane as a promising lead compound for further experimental validation in triple-negative breast cancer models.

2 Methodology

2.1 Pharmacophore study

For the pharmacophore modeling the VEGF –B protein structure was obtained from X-ray crystal complex with the small-protein molecule antagonist (PDB ID: 2VWE) and ligand were collected from the pubchem in order to produce 9-aminocamptothecin derivatives with Radiosensitization activity. In pharmit, the 9-amino camptothecin were converted into different camptothecin derivatives by adding hydrogen donor, aromatic and also hydrophobic groups. Ten hits were selected based on the h bond and RMSD value and these ligands are downloaded for doing docking by Pyrx software. Pharmacophore models were generated and screened using Pharmit, and candidate molecules were ranked based on feature matching, spatial overlap, and RMSD relative to the reference pharmacophore. The initial screening yielded multiple candidates, from which ten hits were selected based on (i) optimal pharmacophore feature alignment, (ii) low RMSD values indicating stable pocket accommodation, (iii) absence of steric clashes or unfavorable electrostatic interactions, and (iv) retention of functional motifs relevant to camptothecin-based radiosensitization. These ten compounds were subsequently advanced for molecular docking, molecular dynamics simulations, and ADME analysis.

2.2 Pyrx -Molecular docking and Visualization

Molecular docking were accomplished using AutoDock Vina implemented within the PyRx (Virtual Screening software for Computational Drug Discovery) workflow. 3D -coordinates of human VEGF-B protein was obtained from the RCSB Protein Data Bank and prepared by removal of water (HETATM) molecules, non-essential heteroatoms and co-crystallized proteins; polar hydrogens were added prior to conversion to PDBQT. Firstly, import receptor as PDB and from that generate PDBQT files. Then import the 10 hits as ligand molecules in sdf format which should also convert or minimize to produce PDBQT files. In Pyrx, the Vina tab can be used to add prepared ligands as well as protein. After that set the grid in such a way that the grid box will cover whole protein-ligand complex. This pyrx can the vina and data can be recorded. Redocking were done using Autodock 4 and Autodock vina and results were similar at each iteration (2-3 H-bonds). Camptothecin analogs such as 9-amino camptothecin, 9-hydroxy camptothecin and 10-Methoxy camptothecin contain the OH lactone ring were used as control ligands for these studies.

The visualization step is conducted by applying Discovery Studio to gather H-bonds, 2D diagrams, hydrophobic contacts, and electrostatic interactions. The option view hierarchy were used in order to incorporate the final ligand and protein molecules. The important point related to visualization are it also shows whether hits make contacts with the key interface residues 2VWE. It also provides the details such as H-bond donors/acceptors interactions, hydrophobic contacts and salt bridges. The 2VWE structure also contains neutralising antibody fab fragment, with and without these antibodies the docking visualization were performed.

The Fab fragment structure was used as a structural template representing the VEGF-B binding interface. The docking study aimed to explore potential competitive binding interactions within the VEGF-B epitope region rather than to simulate physiological antibody–ligand binding in vivo.

2.3 molecular dynamics simulation

The molecular dynamics simulations of the protein peptide complex were visualized to predict the structural and interaction potential of the complex. The 3d structure of the protein was retrieved from the Protein Data Bank (PDB) (Burley, S. K., et al.2019). Missing residues were added using homology modeling for the protein structure to validate the structural integrity before moving on to simulations (Eswar, N., et al. 2006).

Molecular docking was performed using AutoDock Vina, with the grid box centered on the VEGF binding interface and dimensions of $24 \times 24 \times 24 \text{ \AA}$ to ensure full coverage of the ligand-accessible region. Exhaustiveness value of 8 were selected for the docking analysis. Considering its established reliability for protein-ligand interaction analysis, GROMACS with the CHARMM36 force field were used for molecular dynamics simulations. CGenFF server were used for generating parameters for subuzoxane. A solution of the complex was made in TIP3P water box with a 10 \AA buffer. The solution was neutralized at physiological ionic strength by adding counter ions. Steepest descent algorithm was used for the energy minimization until the maximum force became less than $1000 \text{ kJ}\cdot\text{mol}^{-1}\cdot\text{nm}^{-1}$. Equilibrium were carried out for 100 ps each for NVT and NPT ensembles. Following that, production run was carried out for 100 ns at 300 K of temperature and 1 bar of pressure. RMSD, radius of gyration, and potential energy profiles were used for the evaluation of system stability and convergence.

A simulation time of 100 ns selected for this analysis to ensure the stabilization of protein-ligand complex and to obtain adequate samples for confirmation. Prior studies related to MD simulation of protein-ligand systems of VEGF family used 50-100ns simulation time to successfully assess structural stability, binding interaction and dynamic behaviour. In the current study, trajectory analysis including RMSD, RMSF, hydrogen bonding, and radius of gyration was used to confirm the effectiveness of 100 ns simulation time for evaluation of the complex stability of protein-ligand system.

2.4 ADME study

Swiss ADME, a well-recognised tool for pharmacokinetics analysis of anticancer molecules, were used for the prediction key parameters such as lipophilicity, solubility, bioavailability, and permeability of subuzoxane. Camptothecin derivatives were analysed for their physicochemical properties, potential efficacy and safety profiles. Predictive model such as BOILED-Egg were used for the evaluation of drug-likeness, aiding in the design and development of novel anticancer agents.

3 Results and Discussion

3.1 Pharmacophore study

The hydrogen bond interactions between 9-amino-camptothecin and VEGF-B reveal an important role in stabilizing the ligand within the binding site. There are hydrogen bonds formed with Ser50 and Leu35, where the hydroxyl (OH) group of Ser50 accepts a hydrogen bond from the carbonyl oxygen of the ligand, and the backbone amide group of Leu35 forms a hydrogen bond with another carbonyl group of the ligand.

All together the interactions act as an anchor to the ligand, determinedly helps in the hydrophilic pocket and maintain its correct orientation. As for stabilization, the weak polar connection is also deals with Gln46, contributing minor one. These hydrogen bonds lead to the enhancement of the binding specificity and strengthen the 9-amino camptothecin-VEGF -B complex (**Fig. 1**).

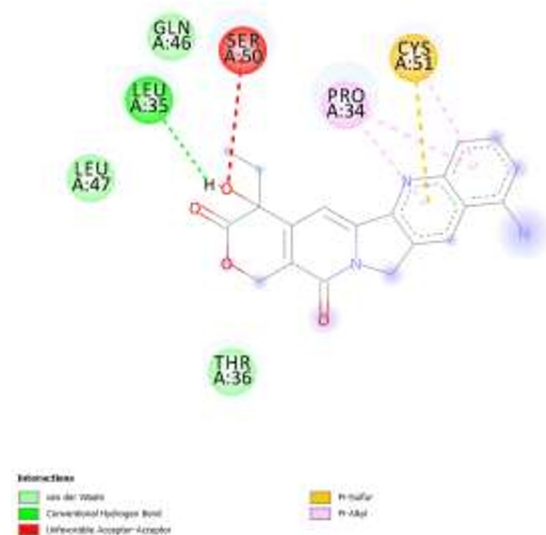


Fig. 1. Docked result of 9-aminocamptothecin and VEGF-B protein

However, one unfavorable interaction an acceptor-acceptor bond was observed near Ser50, where two electronegative oxygen atoms from the ligand and the residue are positioned too closely, resulting in a slight electrostatic repulsion. This interaction marginally reduces the local hydrogen bonding affinity but does not significantly affect the overall stability of the complex. Despite this minor negative induction, stabilizing hydrogen bonds, along with supportive π -sulfur and hydrophobic interactions, predominantly contribute to the binding, indicating that 9-amino-camptothecin maintains a stable and energetically favorable association with the VEGF-B active site. The negative influence exerted by Ser50 on the ligand

interaction highlights the need for an additional or modified drug molecule, as identified through pharmacophore modeling of 9-amino-camptothecin, to achieve enhanced complex stability and improved radiosensitizing potential.

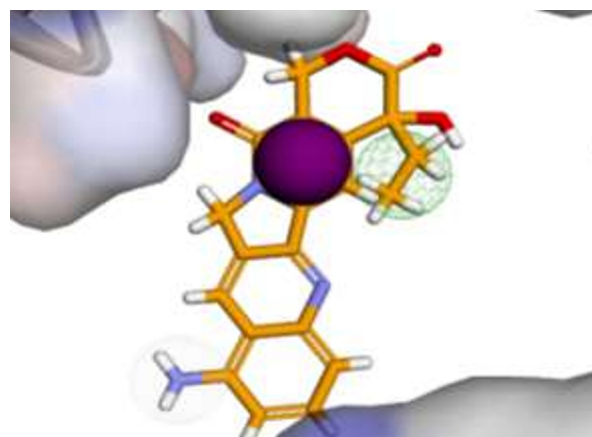


Fig. 2. Pharmacophore analysis of ligand and protein complex (9-amino camptothecin- VEGF-B)

Ligand recognition and binding affinity are both critical factor for the VEGF-B-9-amino-camptothecin complex. Aromatic rings, hydrogen bond donors, hydrogen bond acceptors and hydrophobic regions were identified as the prevailing interaction features by pharmacophore map. Hence, alterations in these features were carried out for the current study. The π - π stacking and π -alkyl interactions resulted from the precise alignment of the aromatic moiety of 9-amino-camptothecin and the hydrophobic cavity of VEGF-B. These interactions further stabilize the ligand within the active site. Hydroxyl and carbonyl groups of the camptothecin act as hydrogen donor and acceptor, respectively. As a consequence, these groups contribute to specific polar interactions with key amino acid residues such as Ser50 and Leu35. The green mesh spheres visualized in pharmacophore map represent the hydrophobic regions of the ligand. These regions are complementary to the proteins receptor sites's geometry. Therefore, it indicates an optimal fit for the ligand within receptor site. Largely, the pharmacophore model exhibits that 9-amino-camptothecin possesses an ideal balance of aromatic and hydrogen-bonding characteristics, supporting stable and selective binding to the VEGF-B active site.

The initial pharmacophore screening yielded ten hits, including PubChem compounds 138593275, 165313195, 123920291, 161374626, 18304982, 10595439, 89283400, 142416801, and 139513953. Among these, Sobuzoxane (PubChem CID: 68022665) was selected for further investigation. This selection was based on its unique chemical framework and its limited prior exploration compared to other 9-aminocamptothecin

derivatives. Sobuzoxane, a bis(dioxopiperazine) analog, exhibited a notably low RMSD value of 0.00314, indicating high structural stability within the binding pocket. Additionally, its reported dual function as a topoisomerase II catalytic inhibitor and anti-angiogenic agent highlights its potential relevance in radiosensitization and the modulation of VEGF-B-mediated angiogenic pathways. Owing to these attributes, Sobuzoxane was identified as a promising lead molecule for subsequent docking, pharmacophore modelling, molecular dynamics simulation, and ADME analyses aimed at evaluating its therapeutic potential in N1–N8 breast cancer models.

3.2 Pyrx -Molecular docking and Visualization

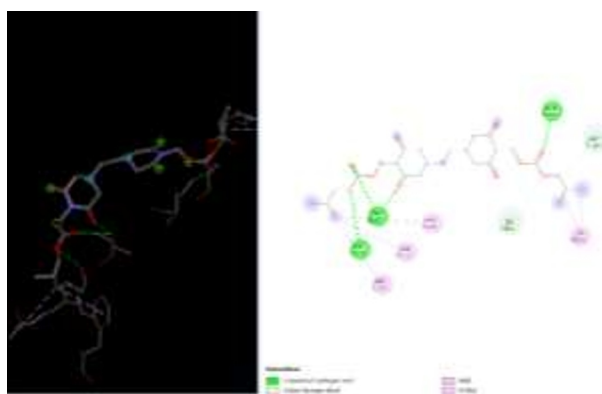


Fig. 3. 2D diagram shows the different types of interactions between Sobuzoxane and VEGF-B protein

The docking analysis between Sobuzoxane and VEGF-B (Fig.:3) reveals a strong and specific hydrogen-bonding (Table: 1) network that enhances the stability of the ligand–protein complex compared to the previous one. The 2D interaction diagram shows that Sobuzoxane forms multiple conventional hydrogen bonds with key residues such as Ser115, and Asn187, Thr 137 while additional interactions with Asp169 and Gly162 further reinforce the binding orientation which is showed in the Fig. 4. These polar interactions help anchor Sobuzoxane firmly within the active groove of VEGF-B, aligning its carbonyl and hydroxyl groups with complementary donor and acceptor sites in the protein’s active region.

Additionally, these polar contacts, weak π -alkyl and alkyl interactions with, Phe117, Pro126, Val127, and Lys166 contribute hydrophobic stabilization, ensuring optimal fitting of the ligand in the partially binding sites. Overall, these

interactions profile point out that Sobuzoxane exhibits a strong binding affinity toward VEGF-B through a balanced combination of hydrogen bonding and hydrophobic interactions, suggesting its potential as a radio sensitizing inhibitor capable of modifying VEGF-B-mediated angiogenic signalling even in antibody without conditions. It is important to acknowledge that docking against the Fab fragment does not fully recapitulate physiological VEGF receptor interactions. The Fab structure was employed as a structural surrogate to identify potential epitope-level interactions. Further validation using VEGFR-bound VEGF-B complexes or cellular assays would be required to confirm biological relevance.

Table 1. Features of 9-Amino camptothecin and Sobuzoxane

| Important Features | 9-Aminocamptothecin | Sobuzoxane |
|---------------------------------|---------------------|-----------------------|
| 5-membered lactone ring | Yes | No but E-ring mimicry |
| H-bond | One | Four |
| Stabilizes of complex | Less | High |
| Unfavorable interactions | Yes | No |
| Binding to catalytic pocket | Yes | Yes |
| Mimics polarity and spatial fit | Less | High |

3.3 Molecular dynamics simulation

VEGF-sobuzoxane interaction remained intact for the duration of the 100 ns simulation; RMSD fluctuated initially (Fig.: 4) (i.e., within the first 15 ns) and then stabilized and occasionally experienced minor mid-trajectory fluctuations. Transient RMSD spikes at approximately 30-55 ns were indicative of transient loop reorganization proximal to the area where the ligand binds to VEGF. Analysis of the root mean square fluctuation (RMSF) (Fig.: S1) data indicated that the highest degree of flexibility occurred among residues located on surface loops, while the rigid structural elements (β -sheets) exhibited minimal structural deviation. Residues in contact with the ligand demonstrated moderate levels of fluctuation suggesting some level of stability imparted by the

presence of sobuzoxane. H-bond analysis revealed the formation of intermittent 0-3 H-bonds with short-lived yet recurring interactions between polar atoms of sobuzoxane and VEGF. Radius of Gyration (R_g) (Shown in Fig. :5) increased slightly during the time frame when structural expansion occurred and then returned towards the baseline value, indicating a return to a more compact state. Surface Accessible Solvent Area (SASA) values decreased gradually from early to late simulation frames, suggesting a small amount of surface burial of VEGF residues due to the accommodation of sobuzoxane.

Principal Component Analysis (PCA) identified two major clusters of conformationally distinct structures (Fig.: S2) that represented an early expanded state and a later compact, stable state of the protein-ligand system. Motion of the protein along principal component 1 (PC1) reflected the open and close motion of the loop regions surrounding the ligand binding pocket. Ligand dissociation did not occur over the course of the entire 100 ns simulation. Sobuzoxane maintained stable hydrophobic interactions with the protein, contributing to the persistent nature of binding. Periodic reorientations of the ligand were associated with temporary increases in polar contacts with the protein. The conformations which exhibited post-fluctuation stability (i.e., after initial movement) show sobuzoxane as being positioned within a shallow binding pocket; the trends in the interaction energy indicate that the main stabilizing factor for keeping sobuzoxane bound to the protein is through Van Der Waal's forces. The secondary structure of the protein remains unaltered throughout the simulation without the occurrence of any global unfolding. Localized rearrangements are found to occur in the loop areas of the protein and there is little to no evidence of changes in the overall structure of the protein. Overall, the results from the simulation suggest that there exists a dynamically stable interaction between VEGF and Sobuzoxane (illustrated in Fig. :6).

Although a single 100 ns production run was performed, the structural parameters showed stable convergence without significant drift after equilibration, supporting the reliability of the observed interaction profile. Nevertheless, independent replicate simulations could further strengthen statistical robustness and will be considered in future studies.

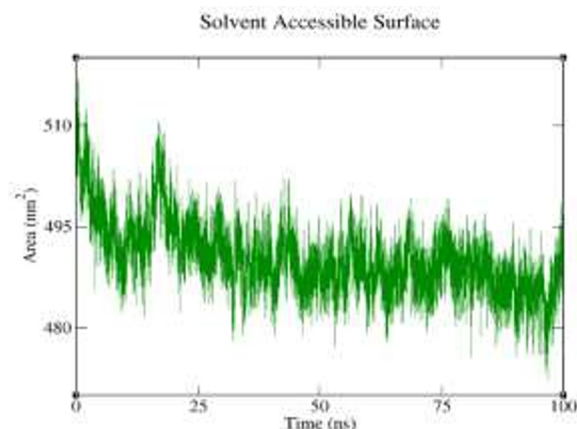


Fig. 4. Time evolution of solvent-accessible surface area (SASA) of the VEGF-B–Sobuzoxane complex over the 100 ns molecular dynamics simulation. The gradual decrease and subsequent stabilization of SASA values indicate progressive burial of solvent-exposed residues and attainment of a compact, dynamically stable protein–ligand conformation.

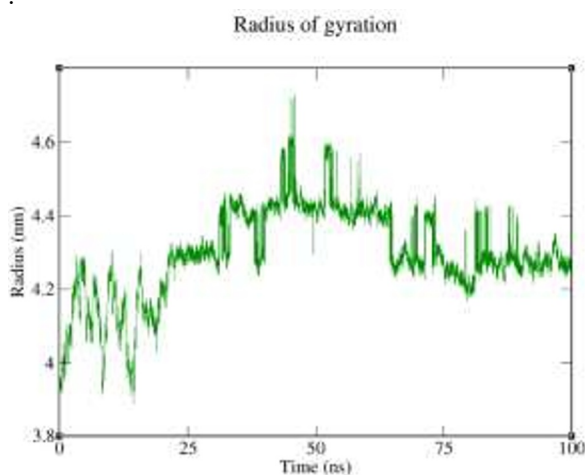


Fig. 5. Radius of gyration (R_g) profile of the VEGF-B–Sobuzoxane complex during the 100 ns molecular dynamics simulation. The overall stabilization of R_g values indicates maintenance of structural compactness and absence of global unfolding throughout the simulation.

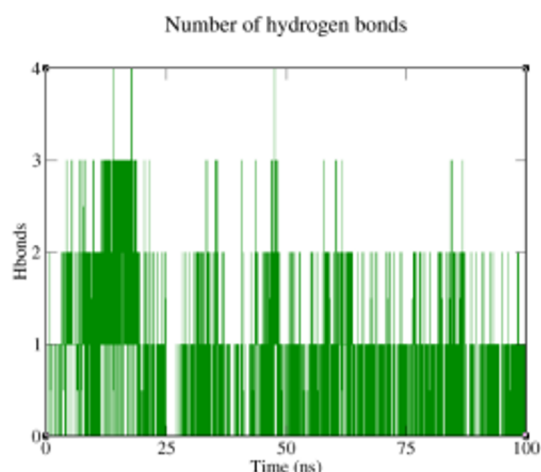


Fig. 6. Time evolution of the number of hydrogen bonds between VEGF-B and Sobuzoxane during the 100 ns molecular dynamics simulation. The presence of recurring hydrogen bonds indicates sustained intermolecular interactions contributing to complex stability.

Convergence of the molecular dynamics trajectory was assessed using multiple complementary qualitative indicators rather than explicit numerical convergence metrics. Principal component analysis revealed that, following an initial exploratory phase, the VEGF–Sobuzoxane complex transitioned into a dominant compact conformational basin that persisted throughout the later stages of the 100 ns simulation. The PC1–PC2 projection showed saturation of the essential conformational space, with no progressive drift or emergence of additional metastable states after equilibration, indicating stabilization of the sampled dynamics. Consistent with this behavior, the relative free-energy landscape exhibited a single, highly populated low-energy basin that remained thermodynamically favored, with a stable free-energy separation of approximately 1.5–3.0 kcal·mol⁻¹ from the early expanded state. The persistence of this basin, together with restricted collective motions localized mainly to loop regions surrounding the ligand-binding pocket, supports convergence of the essential dynamics and reliable characterization of the VEGF–Sobuzoxane interaction within the simulated timescale.

The present computational analysis provides mechanistic insight into how Sobuzoxane may contribute to radiosensitization by modulating VEGF-B–mediated survival signaling rather than merely inhibiting angiogenesis in a generic sense. Pharmacophore mapping and docking results demonstrate that Sobuzoxane establishes an expanded hydrogen-bonding network.

Additionally, results show that sobuzoxane eliminated unfavourable electrostatic interactions observed in case of 9-amino camptothecin. These findings are further reinforced by the findings of molecular dynamics simulation results. MD simulation shows sustained ligand retention, RMSD convergence, stable radius of gyration, persistent hydrogen-bond occupancy, and limited binding-site flexibility over 100 ns. These findings indicate that sobuzoxane is resistant towards radiation like stress-induced conformational agitations. Such stable engagement indicates that ligand has potential to interfere with VEGF-B VEGFR-1 dependent endothelial survival and metabolic adaptation pathways. Therefore, sobuzoxane may indirectly damage post-radiation vascular recovery and enhance radiation efficacy by destabilizing these pro-survival signals of tumour. The current study is not only reiterating the established angiogenesis paradigms, but also it is linking the stability of the molecular level interaction to the radiosensitizing ability. Thus, it is providing a rational foundation for the future experimental validation.

3.4 ADME analysis

The Swiss ADME analysis of Sobuzoxane reveals that it contains favorable solubility plus strong molecular interaction potential but shown limitations in pharmacokinetic domain as compared to 9-amino camptothecin (Shown in the table :2). The physicochemical properties indicate a molecular weight of 514.53 g/mol with 12 hydrogen bond acceptors and 15 rotatable bonds, indicating high flexibility and strong polar character. The compound possesses topological polar surface area of 152.30 Å² and its moderate consensus Log P value of 0.79 denote balanced amphiphilicity, enabling it to interact efficiently within both hydrophilic and hydrophobic regions of the VEGF-B active pocket. The solubility profile, as indicated by ESOL and SILICOS-IT models, classifies So Sobuzoxane as moderately to highly soluble, which supports its applicability in aqueous formulations. ADME analysis revealed that sobuzoxane violates multiple classical drug-likeness criteria, including elevated molecular weight, a high number of rotatable bonds, and increased hydrogen bond acceptors, which are generally associated with reduced oral bioavailability. These properties suggest that sobuzoxane may not be suitable as a conventional orally administered small-molecule drug. However, such limitations do not preclude its biological efficacy, particularly in non-oral or parenteral administration contexts, which are common for anticancer agents especially as prodrug-based and formulation-assisted therapies.

Table 2. Comparative study of 9-amino camptothecin and Sobuzoxane

| Physicochemical properties | 9-amino camptothecin | Sobuzoxane |
|----------------------------|----------------------|------------|
| Molecular weight | 363.37 | 514.53 |
| Rotatable bonds | 4 | 15 |
| Hydrogen bond acceptors | 5 | 12 |
| Hydrogen bond donors | 2 | 0 |

In terms of pharmacokinetics, Sobuzoxane displays low gastrointestinal absorption (Table: 3) and is not permeable to the blood–brain barrier, aligning as well with its design for peripheral relatively than central activity. It is not a P-gp substrate, reducing the risk of efflux-mediated clearance, via it shows inhibition of CYP2D6 and CYP3A4, suggesting potential for limited metabolic contacts. This compound violates some drug-likeness rules, highly due to its high molecular weight and polarity, resulting in a low bioavailability score such as 0.17. Nonetheless, there exist a absence of PAINS alerts and moderate synthetic accessibility (3.80) shows chemical stability and feasibility for structural optimization. Overall, Sobuzoxane shows promising solubility and strong ligand–protein interaction capacity, supporting its potential as a lead radiosensitizer compound targeting VEGF-B, with future optimization required to improve its absorption and bioavailability.

Table 3. pharmaco kinetics study of both the drug molecules

| Pharmaco Kinetics Parameters | 9-amino camptothecin | Sobuzoxane |
|------------------------------|----------------------|------------|
| GI Absorption | High | Low |
| BBB Permeant | No | No |
| Cy P450 1A2 Inhibitor | YES | No |
| P-gp substrate | Yes | No |
| CYP2C9 Inhibitor | No | No |
| CYP3A4 Inhibitor | No | Yes |
| CYP2C19 Inhibitor | No | No |
| CYP2D6 Inhibitor | No | Yes |
| Log Kp | -7.76 cm/s | -8.04 cm/s |

Sobuzoxane demonstrates favorable aqueous solubility across multiple predictive models (ESOL: LogS -3.28, Ali: -4.79, SILICOS-IT: -1.74), classifying it as soluble to moderately soluble, which is consistent with its low consensus

LogP (0.79) and high polar character. However, despite this solubility advantage, the compound exhibits low gastrointestinal absorption and poor bioavailability (0.17) due to its high molecular weight (514.53 g/mol), elevated topological polar surface area (TPSA = 152.30 Å²), and excessive molecular flexibility (15 rotatable bonds). These parameters exceed classical permeability thresholds and collectively limit passive membrane diffusion, explaining the apparent contradiction between solubility and absorption. Furthermore, multiple violation of drug-likeness filters (Lipinski, Ghose, Veber, Muegge) consistently point toward restricted oral drug-likeness rather than flawed ADME prediction. Importantly, Sobuzoxane is not a P-gp substrate, reducing efflux-related clearance risk, and lacks PAINS alerts, supporting chemical validity. CYP2D6 and CYP3A4 inhibition suggests possible metabolic interactions, reinforcing the need for controlled dosing or formulation strategies. ADME profile positions sobuzoxane as a highly soluble, target-stable, but permeability-limited molecule, aligning with its proposed role as a parenterally administered or formulation-optimized radiosensitization lead, rather than an orally optimized small-molecule drug.

4 Conclusion

The current study provides a computational framework for understanding the interaction between sobuzoxane and VEGF-B at the molecular level. Molecular docking and molecular dynamics simulations indicate that sobuzoxane forms a dynamically stable complex with VEGF-B, characterized by stubborn van der Waals interactions, limited loop flexibility, and a shift toward a compact low-energy conformational state. The current findings highlight the ability of sobuzoxane as a conformational modulator of VEGF-B. The kind of modulation that can potentially influence angiogenic signaling under radiation like cellular stress. However, the conclusion is drawn based on the current in silico analysis only. To determine the radiosensitizing efficacy of sobuzoxane in appropriate cancer models, experimental validation through biochemical, cellular, and radiobiological tests are essential. Thus, the current investigation provides the groundwork for future experimental studies.

5 Declaration

5.1 Ethical Approval

It does not apply to this manuscript.

5.2 Funding

Not applicable

5.3 Competing Interests

The authors declare that they have no competing interests

Reference

1. A. Dhiman, D. Rana, D. Benival, and K. Garkhal, "Comprehensive insights into glioblastoma multiforme: Drug delivery challenges and multimodal treatment strategies," *Therapeutic Delivery*, vol. 16, no. 1, pp. 87–115, Jan. 2025.
2. A. Filippelli, "Targeting the endothelium: Novel therapeutic approaches using natural extracts, VEGF-mimetic peptides, and tumor angiogenic dynamics," [Journal name not specified].
3. A. V. Kirichenko and T. A. Rich, "Radiation enhancement by 9-aminocamptothecin: The effect of fractionation and timing of administration," *International Journal of Radiation Oncology Biology Physics*, vol. 44, no. 3, pp. 659–664, Jun. 1999.
4. J. Adams et al., "Vascular endothelial growth factor (VEGF) in breast cancer: Comparison of plasma, serum, and tissue VEGF and microvessel density and effects of tamoxifen," *Cancer Research*, vol. 60, no. 11, pp. 2898–2905, 2000.
5. J. D. Winter, V. Reddy, W. Li, T. Craig, and S. Raman, "Impact of technological advances in treatment planning, image guidance, and treatment delivery on target margin design for prostate cancer radiotherapy: An updated review," *British Journal of Radiology*, vol. 97, no. 1153, pp. 31–40, Jan. 2024, doi: 10.1093/bjr/tqad041.
6. J. Sunseri and D. R. Koes, "Pharmit: Interactive exploration of chemical space," *Nucleic Acids Research*, vol. 44, no. W1, pp. W442–W448, 2016, doi: 10.1093/nar/gkw287.
7. K. E. Hovinga et al., "Radiation-enhanced vascular endothelial growth factor (VEGF) secretion in glioblastoma multiforme cell lines—A clue to radioresistance?" *Journal of Neuro-Oncology*, vol. 74, no. 2, pp. 99–103, Sep. 2005.
8. L. Jain et al., "The role of vascular endothelial growth factor SNPs as predictive and prognostic markers for major solid tumors," *Molecular Cancer Therapeutics*, vol. 8, no. 9, pp. 2496–2508, Sep. 2009, doi: 10.1158/1535-7163.MCT-09-0302.
9. L. Tang et al., "Role of metabolism in cancer cell radioresistance and radiosensitization methods," *Journal of Experimental & Clinical Cancer Research*, vol. 37, p. 87, 2018, doi: 10.1186/s13046-018-0758-7.
10. M. J. Abraham et al., "GROMACS: High-performance molecular simulations through multi-level parallelism from laptops to supercomputers," *SoftwareX*, vols. 1–2, pp. 19–25, 2015, doi: 10.1016/j.softx.2015.06.001.
11. N. Eswar et al., "Comparative protein structure modeling using Modeller," *Current Protocols in Bioinformatics*, vol. 15, no. 1, pp. 5.6.1–5.6.30, 2006, doi: 10.1002/0471250953.bi0506s15.
12. P. Arjunan, X. Lin, Z. Tang, Y. Du, A. Kumar, L. Liu, X. Yin, L. Huang, W. Chen, Q. Chen, Z. Ye, S. Wang, H. Kuang, L. Zhou, K. Xu, X. Chen, H. Zeng, W. Lu, Y. Cao, Y. Liu, C. Zhao, & X. Li, "VEGF-B is a potent antioxidant," *Proc. Natl. Acad. Sci. U.S.A.* 115 (41) 10351-10356, <https://doi.org/10.1073/pnas.1801379115> (2018).
13. S. Dallakyan and A. J. Olson, "Small-molecule library screening by docking with PyRx," in *Chemical Biology: Methods and Protocols*, J. E. Hempel, C. H. Williams, and C. C. Hong, Eds. New York, NY, USA: Springer, 2015, pp. 243–250, doi: 10.1007/978-1-4939-2269-7_19.
14. S. Karaman S, M. Detmar, "Mechanisms of lymphatic metastasis." *J Clin Invest.* 2014 Mar;124(3):922-8. doi: 10.1172/JCI71606. Epub 2014 Mar 3. PMID: 24590277; PMCID: PMC3938272.
15. S. K. Burley et al., "Protein Data Bank: The single global archive for 3D macromolecular structure data," *Nucleic Acids Research*, vol. 47, no. D1, pp. D520–D528, 2019, doi: 10.1093/nar/gky949.
16. W. L. DeLano, *The PyMOL Molecular Graphics System*. Schrödinger LLC, 2002.