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ORIGINAL RESEARCH

In Silico Effects of Thalidomide and Its Analogs on BCL-2 and BRCA1: Molecular Docking, Molecular Dynamics and Imaging Studies

Erkut TAMTÜRK, Serap YALÇIN AZARKAN

[1] Department of Biology, Faculty of Art and Sciences, Erciyes University, 38100, Kayseri, Türkiye.
[2] Department of Pharmacology, Faculty of Medicine, Kırşehir Ahi Evran University, 40100, Kırşehir, Türkiye.

ABSTRACT

In recent years, *in silico* studies in molecular biology have emerged as a leading approach that accelerates experimental processes and provides economic advantages, particularly in biomedical research. As a computer-aided strategy, *in silico* methods contribute significantly to key areas such as the identification of novel therapeutic targets in cancer research, the visualization and analysis of molecular interactions, and the evaluation of the drug-likeness potential of chemical compounds. This study aimed to analyze the interactions of thalidomide and its analogs (lenalidomide, pomalidomide) with cancer-related proteins BCL-2 and BRCA1 using *in silico* approaches, in order to identify potential inhibitor candidates. Molecular docking analyses were performed using AutoDock Vina, and molecular dynamics (MD) simulations were conducted for 50 ns on the WebGro molecular dynamics computation site. System stability was assessed by RMSD, RMSF, and Rg analyses. Docking results showed that the binding energies of thalidomide, lenalidomide and pomalidomide with the BCL-2 protein were –6.8, –6.6, and –6.7 kcal/mol, respectively. For the BRCA1 protein, the binding energies were –5.3, –5.5, and –6.7 kcal/mol, respectively. MD simulations demonstrated that both free proteins and protein–ligand complexes maintained compact and stable structures throughout the simulation. The RMSD values stabilized between 0.3 and 0.5 nm, supporting the dynamic stability of the systems. Notably, thalidomide and lenalidomide enhanced the structural stability of the BCL-2 complexes. In conclusion, thalidomide derivatives exhibit moderate binding affinity and complex stability to BCL-2 and BRCA1 proteins, suggesting that these compounds can be considered as candidates for anti-cancer therapeutic agents. Future *in vitro* and *in vivo* studies are expected to further validate the biological relevance of these findings.

Keywords: *In silico* analysis. Molecular docking. Molecular dynamic. Thalidomide. Bcl-2.

Talidomid ve Analoglarının BCL-2 ve BRCA1 Üzerindeki Silico Etkileri: Moleküler Yerleştirme, Moleküler Dinamikler ve Görüntüleme Çalışmaları

ÖZET

Moleküler biyolojide *in silico* çalışmalar, son yıllarda özellikle biyomedikal araştırmalarda deneysel süreçleri hızlandıran ve ekonomik avantaj sağlayan yöntemlerden biri olarak öne çıkmaktadır. Bilgisayar destekli bir yaklaşım olan *in silico* yöntemler; kanser araştırmalarında yeni hedeflerin belirlenmesi, moleküler etkileşimlerin görselleştirilerek analiz edilmesi ve kimyasal bileşiklerin ilaç adayı potansiyellerinin değerlendirilmesi gibi temel alanlarda önemli katkılar sunmaktadır. Bu çalışmanın amacı, talidomid ve analoglarının (lenalidomid, pomalidomid) kanserle ilişkili BCL-2 ve BRCA1 proteinleriyle etkileşimlerini *in silico* yöntemlerle analiz ederek potansiyel inhibitör adaylarını belirlemektir. Moleküler docking analizleri AutoDock Vina ile, moleküler dinamik (MD) simülasyonları ise WebGro moleküler dinamik hesaplama sitesi kullanılarak 50 ns süreyle yürütülmüştür. Sistem kararlılığı RMSD, RMSF ve Rg analizleriyle değerlendirilmiştir. Docking sonuçlarına göre, BCL-2 proteiniyle talidomid, lenalidomid ve pomalidomidin bağlanma enerjileri sırasıyla –6,8, –6,6 ve –6,7 kcal/mol'dür. BRCA1 proteiniyle talidomid -5,3, lenalidomid -6,7, pomalidomid -5,5 olarak hesaplanmıştır. MD simülasyonları hem serbest proteinlerin hem de protein-ligand komplekslerinin kompakt ve kararlı yapılarını koruduğunu göstermiştir. RMSD değerlerinin 0,3–0,5 nm aralığında sabitlenmesi, sistemlerin dinamik stabilitesini desteklemiştir. Özellikle talidomid ve lenalidomidin BCL-2 komplekslerinde yapısal stabiliteyi artırdığı belirlenmiştir. Sonuç olarak, talidomid türevlerinin BCL-2 ve BRCA1 proteinlerine orta düzeyde bağlanma afinitesi ve kompleks stabilitesi göstermesi, bu bileşiklerin anti-kanser terapötik ajan adayları olarak değerlendirilebileceğini ortaya koymaktadır. Gelecekteki *in vitro* ve *in vivo* çalışmalar bu bulguların biyolojik geçerliliğini güçlendirecektir.

Anahtar Kelimeler: I*n silico* analizler. Moleküler kenetlenme. Moleküler dinamik. Talidomid. Bcl-2.

Dr. Erkut TAMTÜRK
Erciyes University
E-mail:erkuttamturk@gmail.com

AUTHORS' ORCID INFORMATION
Erkut TAMTÜRK: 0000-0002-6771-0715
Serap YALÇIN AZARKAN: 0000-0002-9584-266X

Cancer is one of the most common diseases worldwide and remains at the center of research. Deaths from metastatic cancers account for 90% of cancer deaths. Focusing on the underlying causes of these deaths and fighting cancer more effectively are among the main objectives of research. Additionally, improving the quality of life and extending the

lifespan of cancer patients is extremely important[1]. Breast cancer, particularly in women, causes deaths due to the formation of malignant tumors. The unique epidemiological characteristics of breast cancer and its increasing molecular heterogeneity have made it a priority area for oncological research[2]. In recent years, *in silico* studies, which are frequently used in fields such as molecular biology, chemistry, and pharmacy, are also gaining importance. A significant portion of cancer research is also comprised of *in silico* studies. Molecular docking studies are one of the *in silico* methods that have been frequently used recently, especially in drug development studies. As Mahmudov et al. note, molecular docking "is the basis of drug design; a widely used, reliable, and short-time-consuming method for determining the binding position and protein–ligand interactions"[3]. Molecular binding studies, which provide a wealth of theoretical information, including the interactions of particles at the atomic level and binding sites on proteins, are becoming increasingly widespread[4]. These computational analyses aim to determine the tendency of a molecule to bind to another molecule and form a stable complex, as well as the binding energy released in this process. Moreover, the docking study supported the experimental enzyme inhibition data, demonstrating consistency between binding energy scores and *in vitro* Ki/IC_{50} values[3]. The primary goal of this method is to predict the binding affinity and potential interaction sites between a small molecule (ligand) and a target macromolecule (usually a protein or nucleic acid). Molecular binding is critically important in drug design, as it determines the binding interactions of molecules and provides essential information for the discovery of new drugs[5]. There are fundamentally two different types of molecular bonding. These are rigid and flexible connections. In the case of rigid binding, both the ligand and the receptor are treated as inflexible substances. In flexible binding energy, it considers the conformational changes of the ligand and, in some special cases, the receptor. This approach allows for more accurate identification of molecular interactions[6,7]. The types of molecular bonding are shown in Table I.

Table I. Types of Molecular Docking

Characteristics	Rigid Docking	Flexible Docking
Definition	The ligand and receptor are modeled in fixed conformations.	The ligand and/or receptor exhibit conformational flexibility.
Computational Cost	Low	High
Realism	Low – does not fully represent the dynamic nature.	High – provides a more accurate representation of molecular interactions.
Use Cases	Systems with minimal conformational changes.	Dynamic and flexible systems.
Example Software	DOCK 5	AutoDock, FlexX 6

Chemotherapy is one of the most commonly used treatment approaches in cancer therapy. It can be administered as neoadjuvant therapy before surgery or as adjuvant therapy after surgery. Compared to adjuvant chemotherapy, neoadjuvant chemotherapy can effectively cause tumor regression[8,9]. Although chemotherapy is widely used, it is a treatment approach with serious side effects. A study involving over a thousand patients found that adjuvant or neoadjuvant chemotherapy administered to patients with metastatic cancer caused serious side effects in 44.5% of cases[10]. Therefore, any approach that reduces the side effects of chemotherapy or serves as an alternative to it is important. Thalidomide is a drug that is clinically beneficial in many types of cancer[11]. Thalidomide was first used in the 1950s as a sedative or anti-nausea medication for pregnant women. However, the subsequent birth of children with serious birth defects provoked considerable controversy. Four decades later, it was discovered that thalidomide was a promising drug for cancer[12]. One effective way to analyze the interactions of drugs used in cancer treatment with target proteins in more detail is through molecular dynamics (MD) simulations. The primary goal of these simulations is to gain comprehensive information about the system's stability, molecular folding processes, and overall structural behavior by tracking the atomic-level movements of chemical compounds over specific time intervals.

Material and Method

Molecular docking analyses

The binding energies of the chemicals thalidomide, lenalidomide and pomalidomide to the Bcl-2 (1GJH) and BRCA-1 (1IYJ) proteins were calculated using the Autodock Vina program[7]. The aim was to gain a better understanding of the molecular effects of drugs on proteins by calculating the binding energies of thalidomide and its analogs to cancer-related proteins. The two- and three-dimensional structures of thalidomide and its analogs were obtained from the online data storage site PubChem (https://pubchem.ncbi.nlm.nih.gov/)[13,14]. The three-dimensional structures of the proteins were obtained from the online protein data storage site, the Protein Data Bank (https://www.rcsb.org/search/browse) [15]. Two- and three-dimensional images of thalidomide's binding sites to proteins were created using the Discovery Studio Visualizer 2025 free program. Binding energy of -5 kcal/mol and above was considered as weak, -6 kcal/mol and -7 kcal/mol as moderate binding, and -8 kcal/mol and above as high binding.

Molecular Dynamic Analyses

The WebGro online molecular dynamics simulation preparation site was used for molecular dynamics analyses. The single and ligand-bound molecular stability and folding states of the proteins were measured and recorded within a 50 ns (nanosecond) time interval[16-22].

Results

Molecular Docking Analyses

The binding energies of thalidomide and its analogs, lenalidomide and pomalidomide, to cancer-related proteins BCL-2 (1GJH) and BRCA-1 (1IYJ) have been determined and their binding positions shown. The binding energies of thalidomide and its derivatives to BCL-2 and BRCA1 proteins are given in Table II. The binding site of thalidomide to the BCL-2 protein is shown in Figure 1, and the number of hydrogen bonds is shown in Figure 2. The binding site of pomalidomide to the BCL-2 protein is shown in Figure 3, and the number of hydrogen bonds is shown in Figure 4. The binding site of Lenalidomide to the BCL-2 protein is shown in Figure 5, and the number of hydrogen bonds is shown in Figure 6.

Table II. Binding Energies of Thalidomide and Its Derivatives to BCL-2 (1GJH) and BRCA1 (1IYJ) Proteins

Protein	Ligand	Binding Energy (AutoDock Vina) (kcal/mol)
BCL-2 (1GJH)	Thalidomide	-6.8
BCL-2 (1GJH)	Lenalidomide	-6.6
BCL-2 (1GJH)	Pomalidomide	-6.7
BRCA-1 (1IYJ)	Thalidomide	-5.3
BRCA-1 (1IYJ)	Lenalidomide	-6.7
BRCA-1 (1IYJ)	Pomalidomide	-5.5

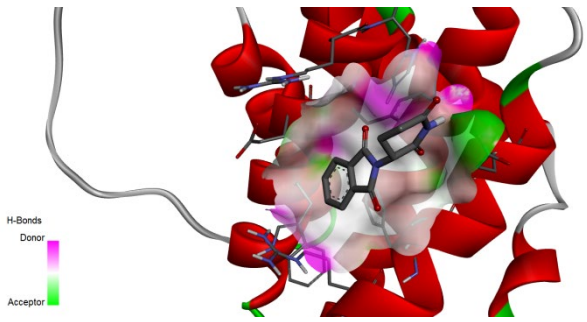
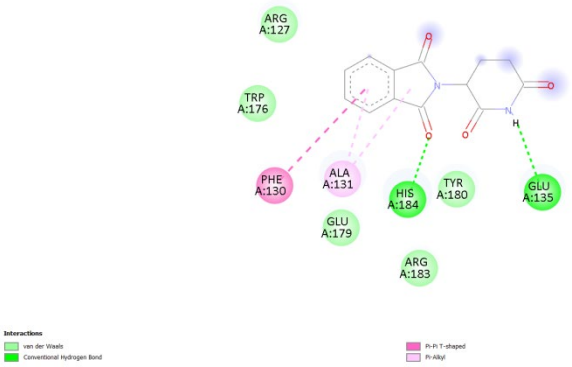


Figure 1:
Molecular visualization of thalidomide bound to the Bcl-2 protein. The protein is shown with red α-helices and grey loops. The ligand appears as a color-coded ball-and-stick model within the semi-transparent binding pocket. Hydrogen bonds between the ligand and surrounding residues are indicated, highlighting key interaction sites.

a.



b.

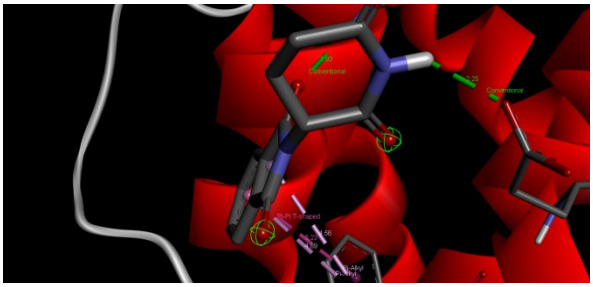


Figure 2:
Two-dimensional representation of thalidomide binding to the Bcl-2 protein. The diagram shows key interactions between the ligand and nearby residues (ARG127, TRP176, PHE130, ALA131, GLU179, HIS184, TYR180, ARG183). Interaction types are color-coded: pink for Pi–Pi, green for hydrogen bonds, and light green for van der Waals contacts.

*b. Conventional hydrogen bonds are indicated by green dashed lines, with measured bond lengths of **1.93 Å** for the interaction with His and **2.25 Å** for the interaction with Glu. These distances fall within the typical range for stable hydrogen bonding, supporting the reliability of the observed binding mode.*

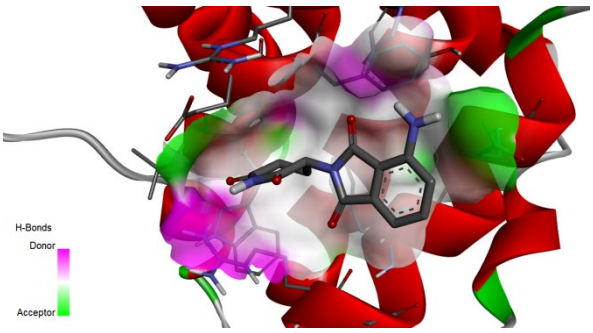


Figure 3:
Molecular visualization of pomalidomide bound to the Bcl-2 protein. The protein is shown with red α-helices and grey loops, and the ligand as a color-coded stick model within the semi-transparent binding pocket. Hydrogen bonds (magenta and green) highlight key interactions and structural complementarity.

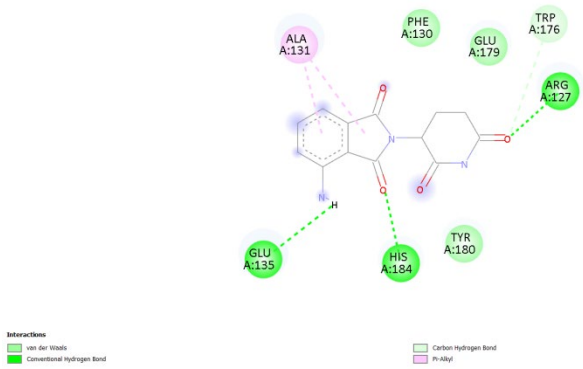


Figure 4:
Two-dimensional representation of pomalidomide binding to the Bcl-2 protein. The diagram shows interactions between the ligand and nearby residues (ALA131, PHE130, TRP176, GLU137, ARG127, GLU135, HIS184, TYR180). Interaction types are color- and line-coded: green for hydrogen bonds, pink for Pi–Alkyl, solid green for C–H bonds, and purple for van der Waals contacts.

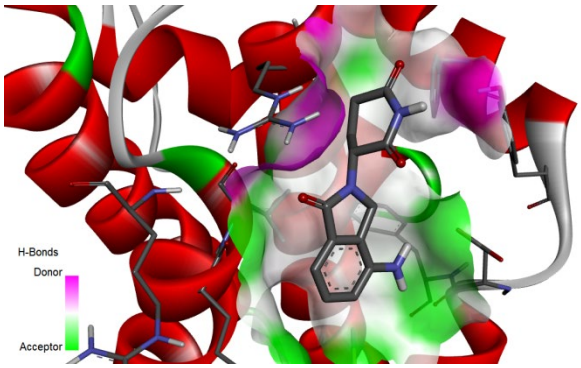


Figure 5:
Molecular visualization of lenalidomide bound to the Bcl-2 protein. The protein is shown with red α-helices and grey loops, and the ligand as a color-coded ball-and-stick model within a semi-transparent binding pocket. Green and magenta regions indicate hydrophobic and hydrogen-bond donor areas, illustrating the ligand's binding mode and interaction environment.

Molecular Dynamic Analyses

Molecular dynamics simulations were performed to evaluate the structural properties of BRCA1 (PDB ID: 1IYJ) and BCL-2 (PDB ID: 1GJH) proteins. In this context, the hydrogen bonds, stability, and folding behavior of proteins have been analyzed in detail. Additionally, the dynamic properties of the ligand-protein complexes formed by these proteins with thalidomide, lenalidomide, and pomalidomide were examined to evaluate the structural effects of these compounds after binding. The graphs of Rg (Radius of gyration), hydrogen bonds, and RMSD (Root mean square) for the Bcl-2(1GJH) protein are shown in Figure 7. The Rg (Radius of gyration), hydrogen bond, and RMSD (Root mean square) graphs of the Bcl-2(1GJH)-Thalidomide protein-ligand complex is shown in Figure 8. The Rg (Radius of gyration), hydrogen bond, and RMSD (Root mean square) graphs of the Bcl-2(1GJH)-lenalidomide protein-ligand complex is shown in Figure 9.

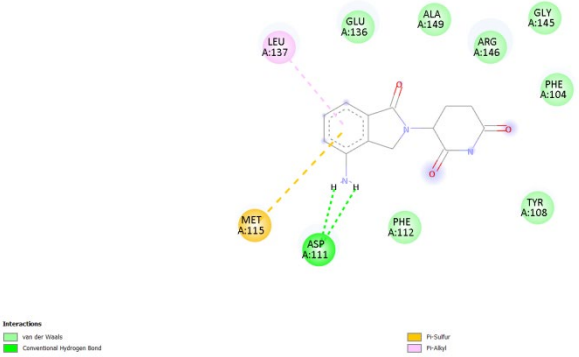


Figure 6:
Lenalidomid'in Bcl-2 proteine bağlanmasının iki boyutlu temsili. Diyagram, ligand ile çevresindeki kalıntılar (MET115, ASP111, PHE104, LEU137, PHE112) arasındaki etkileşimleri göstermektedir. Etkileşim türleri renk ve çizgi kodlarıyla belirtilmiştir: yeşil van der Waals, sarı hidrojen bağı, turuncu Pi–kükürt ve mor Pi–alkil etkileşimlerini ifade eder.

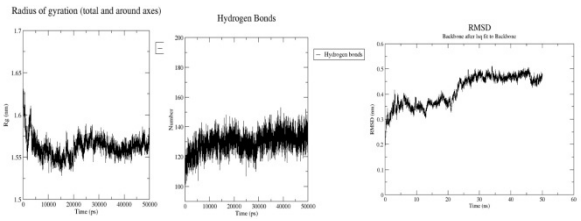


Figure 7:
Rg (Radius of gyration), hydrogen bonds and RMSD (Root mean square) graphs of Bcl-2(1GJH) protein. The figure shows molecular dynamics simulation results. Left graph: The radius of gyration (Rg) fluctuates between 1.55–1.60 nm, indicating preserved compactness. Middle graph: The number of hydrogen bonds varies but remains consistently present, supporting structural stability. Right graph: The backbone RMSD stays within 0.35–0.5 nm without a clear increase, confirming overall stability.

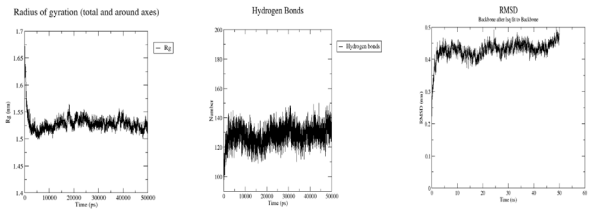


Figure 8:
Rg (Radius of gyration), hydrogen bonds and RMSD (Root mean square) plots of the Bcl-2(1GJH)-Thalidomide protein-ligand complex. Left graph: The radius of gyration (Rg) remains stable with minor fluctuations, indicating preserved compactness. Middle graph: The number of hydrogen bonds fluctuates but persists throughout, supporting structural stability. Right graph: The backbone RMSD ranges between 0.35–0.5 nm without a clear upward trend, confirming overall stability during the simulation.

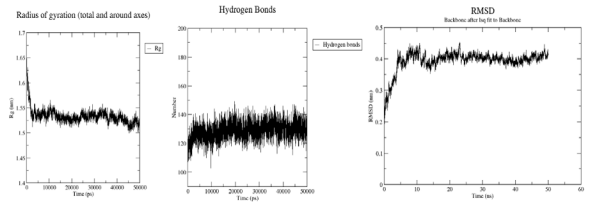


Figure 9:
Rg (Radius of gyration), hydrogen bonds and RMSD (Root mean square) plots of the Bcl-2(1GJH)-Lenalidomide protein-ligand complex. Rg graph: The radius of gyration (Rg) fluctuates between 1.50–1.55 nm, remaining overall stable and indicating preserved compactness. Hyrdogen bonds graph: The number of hydrogen bonds varies within 100–140, suggesting persistent internal interactions that support structural stability. RMSD graph: The backbone RMSD increases initially and then stabilizes mostly around 0.4 nm, indicating that the protein reaches a stable conformation after early adjustments.

Discussion and Conclusion

Determining the binding energies of thalidomide and its analogs to the BCL-2 (1GJH) and BRCA1 (1IYJ) proteins is extremely important for analyzing the effects of these chemicals. In docking studies, it was found that thalidomide, lenalidomide, and pomalidomide have high binding energies to the BCL-2 (1GJH) protein. It was observed that thalidomide and pomalidomide showed moderate binding to the BRCA1 (1İYJ) protein, while lenalidomide bound with higher energy bonds compared to thalidomide and pomalidomide. As a result of the analysis of the imaging, the hydrogen bonds and hydrophobic interactions of thalidomide and its analogs with BRCA1 and BCL-2 proteins are shown in two and three dimensions. Based on these analyzes, it can be concluded that thalidomide and its analogs exhibit a high degree of interaction with the BRCA1 and BCL-2 proteins. Following molecular docking studies and molecular imaging analyses, molecular dynamics studies constitute a significant dimension of *in silico* studies. Although a protein has a large number of three-dimensional structures, it can reach its required conformational structure quickly. This situation allows the process to be completed in a much shorter time. The speed at which these proteins reach a stable structure, their folding rates, and how they differ from other proteins in these contexts are of great importance for designing new proteins or better understanding their effects. The radius of gyration (Rg) is a frequently used measure in molecular dynamics studies, indicating how far or spread out the atoms of a protein are from the protein's center. In other words, Rg analysis provides information about the compactness of the protein[23]. When the Rg graph is examined, it is observed that the Bcl-2 protein generally exhibits a stable image within the 50 ns range, typically having an Rg value of 1.55-1.60 nm. From this, it can be inferred that the Bcl-2 protein exhibits a stable and compact structure. Thalidomide or lenalidomide binding to proteins also did not change the Rg value. The hydrogen bond graphs of the Bcl-2 protein fluctuate between an average of 120 and 130 at 50 ns. Initially, the number of bonds, which was relatively lower, increased even further over time and formed a stable structure. The number of hydrogen bonds also varies between 100 and 140 in the Bcl-2 protein, the Bcl-2-thalidomide complex, and the Bcl-2-lenalidomide complex. This situation also indicates that the system exhibits a stable structure. One of the most frequently used approaches in molecular biology for comparing the structures of molecules, and a measure of the structural stability of molecules over time, is RMSD analysis[24]. It is observed that the RMSD value of the Bcl-2 protein starts at 0.3 and approaches 0.5 over time. An increase in this value indicates an increase in stability. In the Bcl-2-thalidomide and Bcl-2-lenalidomide complexes, the graph starting at 0.3 approached a value of 0.5 in a shorter time. This situation could lead to the conclusion that thalidomide and lenalidomide increase protein stability.

The results of the *in silico* analyses performed revealed interactions between thalidomide and its analogs with the cancer-related proteins BRCA1 and BCL-2. Thalidomide was introduced in Europe in 1954 as a sedative agent and was used to alleviate nausea during pregnancy[25]. However, in the following years, its side effects caused a great uproar. A significant increase in limb malformations (amelia and phocomelia) was observed in newborns following the use of thalidomide during pregnancy[26,27]. The

investigation of thalidomide's effects on cancer occurred after these processes. Promising results have emerged in malignancies such as Kaposi's sarcoma and myelofibrosis, although further study is needed. It was also concluded that it has varying degrees of effects on other malignancies[28]. In studies on breast cancer, it was concluded that thalidomide had no or very little effect[26]. Mutations in tumor suppressor genes such as BRCA1 and BRCA2 are closely associated with breast cancer[29]. BCL-2 protein is considered a prognostic marker in breast cancer[30]. Based on this information, it is important to investigate the effect of thalidomide on BRCA1 and BCL-2 proteins *in silico*. This study demonstrates the high binding affinity of thalidomide and its analogs to BCL-2 and BRCA1, their ability to enhance molecular folding and stability, and the need for further research into the effects of thalidomide and its analogs on breast cancer. If *in silico* studies are supported by *in vitro, in vivo*, and clinical studies, more information can be obtained about the therapeutic effects of thalidomide and its analogs. Additionally, the effects of thalidomide and its derivatives on other proteins, miRNAs, or molecules associated with cancer will also contribute to the literature.

In conclusion, the comprehensive *in silico* analyses of thalidomide and its clinically relevant derivatives, lenalidomide and pomalidomide, provided detailed insights into their molecular interaction behaviors with key target proteins. Molecular docking results demonstrated that all compounds exhibited moderate to strong binding affinities, particularly toward the anti-apoptotic protein BCL-2 and the tumor suppressor BRCA-1. Among these, thalidomide showed the most favorable interaction with BCL-2 (−6.8 kcal/mol), followed closely by pomalidomide (−6.7 kcal/mol) and lenalidomide (−6.6 kcal/mol), indicating comparable inhibitory potential within this protein family. In contrast, lenalidomide displayed the highest affinity toward BRCA-1 (−6.7 kcal/mol), surpassing pomalidomide (−5.5 kcal/mol) and thalidomide (−5.3 kcal/mol). These findings suggest that small structural modifications among thalidomide analogs significantly influence their binding affinity and protein selectivity.

Molecular visualization confirmed that all ligands occupied the active binding pockets of their respective proteins, forming stable hydrogen bonds and hydrophobic interactions with key amino acid residues responsible for biological activity. Molecular dynamics (MD) simulations further validated the docking results by demonstrating consistent structural stability of both the free protein and the protein–ligand complexes. For the native BCL-2 (1GJH) protein, the radius of gyration (Rg) fluctuated between 1.55–1.60 nm, hydrogen bond counts remained steady throughout the 50 ns simulation, and RMSD values stayed within 0.35–0.5 nm without upward drift, confirming overall stability. Similarly, the BCL-2–thalidomide complex maintained stable Rg and RMSD profiles (Rg ≈ 1.50–1.55 nm; RMSD ≈ 0.35–0.5 nm) and persistent hydrogen bonding, indicating that ligand binding did not disrupt the protein's structural compactness. The BCL-2–lenalidomide complex also showed stable conformations, with Rg values fluctuating within 1.50–1.55 nm, hydrogen bond counts between 100–140, and RMSD stabilizing around 0.4 nm after an initial equilibration phase, confirming a stable bound state throughout the simulation trajectory.

Researcher Contribution Statement:
Idea/Concept: S.Y.A., Design:E.T.; Data Collection/Processing: E.T., S.Y.A.; Analysis/Interpretation: E.T, S.Y.A.
Literature Review: E.T.; Drafting/Writing: E.T., S.Y.A.; Critical Review: E.T., S.Y.A. All authors have read and agreed to the published version of the manuscript.
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Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.
Ethics Committee Approval Information:
The authors state that this study did not require ethics committee approval because it does not involve human participants, animal experiments, or any procedure necessitating ethical review.
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