International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 15(2): March-April 2025

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences

Research Article……!!!

Received: 12-11-2024; Revised: 23-02-2025; Accepted: 27-02-2025

STRUCTURE-BASED DRUG DISCOVERY FOR CHRONIC LYMPHOCYTIC LEUKEMIA: VIRTUAL SCREENING AND MOLECULAR DYNAMICS SIMULATION TARGETING MUTANT BCL-2 RECEPTOR

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Keywords:

Chronic lymphocytic leukemia,Bcl-2protein,PDB-REDO,virtual screening, molecular dynamic simulation ,FDA-approved drugs, protein-ligand interaction

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ABSTRACT

Chronic Lymphocytic Leukemia (CLL) is a prevalent hematological malignancy characterized by the overexpression of the anti-apoptotic Bcl-2 protein, making it a critical therapeutic target. This study aims to identify potential FDA-approved inhibitors of Bcl-2 using a structure-based drug discovery approach. The methodology involved protein refinement of the Bcl-2 receptor (PDB ID: 6GL8) using PDB-REDO, followed by virtual screening of FDA-approved drugs through the DrugRep platform. Molecular docking analyses identified Nebivolol (-7.5), Pexidartinib (-7.1), and Cilostazol (-7.1) as the top-scoring compounds based on binding affinity. To further assess their stability and interaction dynamics, molecular dynamics (MD) simulations were conducted, incorporating iMODS analysis to evaluate molecular deformability, atomic fluctuations, structural stability, and residue motion correlations. The findings highlight the potential repurposing of these drugs for CLL treatment, providing a foundation for further experimental validation. This study underscores the significance of structure-based virtual screening and MD simulations in accelerating drug discovery for CLL, offering promising insights into novel therapeutic interventions.

Introduction

Chronic lymphocytic leukemia (CLL) is the most prevalent form of adult blood cancer, characterized by the progressive accumulation of dysfunctional B lymphocytes[1]. This malignant proliferation results from a combination of genetic mutations, impaired apoptosis, and an altered tumor microenvironment, ultimately leading to immune dysfunction and disease progression[2]. Despite advancements in treatment, CLL remains largely incurable, necessitating the exploration of novel therapeutic strategies[3].

One of the key contributors to CLL pathogenesis is the B-cell lymphoma 2 (Bcl-2) protein, an essential regulator of apoptosis[4]. Overexpression of Bcl-2 promotes prolonged cell survival by inhibiting programmed cell death, thereby facilitating the persistence of malignant cells[5]. Moreover, mutations in the Bcl-2 gene can further drive cancer progression and contribute to therapeutic resistance, posing significant challenges in disease management[6]. Notably, the presence of Bcl-2 mutations has been linked to resistance against venetoclax, a selective Bcl-2 inhibitor currently used in CLL therapy, underscoring the need for alternative treatment strategies[7].

Existing therapies for CLL, including chemotherapy, monoclonal antibodies, and targeted agents, have limitations such as acquired resistance, adverse side effects, and incomplete remission[8]. Therefore, repurposing FDA-approved drugs offers a promising approach to overcoming these challenges by identifying new therapeutic candidates with established safety profiles and potential efficacy against mutant Bcl-2[9].

This study aims to employ structure-based drug discovery (SBDD) methodologies, including virtual screening and molecular dynamics simulations, to identify FDA-approved drugs that can effectively target the mutant Bcl-2 receptor in CLL[10]. By leveraging computational techniques, this research seeks to accelerate the identification of viable therapeutic candidates, paving the way for improved treatment strategies against drug-resistant CLL[11]

2. Materials and Methods

2.1. Protein Structure Preparation

The structural foundation of this study is the Bcl-2 receptor (PDB ID: 6GL8) complexed with the potent inhibitor S55746, selected due to its critical role in the pathophysiology of Chronic Lymphocytic Leukemia (CLL), where its anti-apoptotic function promotes cancer cell survival. Targeting Bcl-2 with small-molecule inhibitors like S55746 has demonstrated therapeutic potential, making it a promising candidate for virtual screening and molecular dynamics simulations[12]. To enhance the structural accuracy and reliability of the selected Bcl-2 receptor, the protein structure was refined using PDB-REDO, which optimizes geometry by improving electron density fitting, correcting atomic positions, and refining B-factors, thereby ensuring biologically relevant conformations. Structural validation was performed using SAVES v6.0, incorporating Ramachandran Plot Analysis to assess backbone dihedral angles

(φ, ψ), ERRAT to evaluate non-bonded atomic interactions, and Verify3D to confirm atomic model compatibility with the amino acid sequence[13]. These refinement and validation steps confirmed the stability and structural integrity of the Bcl-2 receptor, ensuring its suitability for subsequent computational analyses in structure-based drug discovery for CLL[14].

2.2. Receptor-Based Virtual Screening

To identify potential inhibitors of the Bcl-2 receptor, the refined PDB structure was uploaded to the DrugRep platform for receptor-based virtual screening of FDA-approved compounds. Molecular docking simulations were conducted to assess the binding affinity of various compounds, with docking scores serving as the primary selection criterion, where lower scores indicate stronger interactions[15]. The analysis identified Nebivolol (-7.5), Pexidartinib (-7.1), and Cilostazol (-7.1) as the top-scoring compounds, demonstrating significant binding affinity to the Bcl-2 receptor. These compounds exhibited strong receptor interactions, suggesting their potential as Bcl-2 inhibitors[16]. Further investigations, including molecular dynamics simulations and binding free energy calculations, are required to confirm their stability and potential efficacy in targeting Bcl-2 for Chronic Lymphocytic Leukemia (CLL) treatment[17].

2.3. Molecular Dynamics (MD) Simulations

Molecular dynamics (MD) simulations were performed to gain deeper insights into the stability and dynamic behavior of Bcl-2-ligand complexes[18]. The preparation phase included solvation, ionization, and energy minimization to ensure a biologically relevant environment for accurate computational analysis[19]. Post-simulation analyses were conducted using iMODS, a computational tool designed to assess protein flexibility, stability, and conformational dynamics[20]. Various parameters were examined, including molecular deformability, which identified flexible and rigid regions, and B-factors, which measured atomic displacement to determine high-mobility or restricted-movement regions[21]. Eigenvalue calculations provided an indication of structural stability, where higher eigenvalues signified greater rigidity and lower flexibility[22].

Further analysis included variance analysis, which quantified atomic position variations across simulation frames, highlighting key dynamic features[23].Covariance maps illustrated correlations in residue motion, identifying coordinated movements essential for functional conformational changes[24]. Additionally, elastic network models represented overall protein flexibility and identified possible motion pathways critical for inhibitor binding[25]. The MD simulations and iMODS analysis provided valuable insights into the stability, flexibility, and binding efficiency of selected inhibitors within the Bcl-2 receptor binding site[26]. These findings will guide further refinement through binding free energy calculations and experimental validation, aiding in the identification of promising candidates for Chronic Lymphocytic Leukemia (CLL) treatment[27].

3. Results and Discussion

3.1. Protein Validation Results

3.1.1. PDB-REDO Results

Table 1: Comparison of Structural Validation Metrics between Original and PDB-REDO Refined Models

Validation Metrics from PDB-REDO	Original	PDB-REDO
Crystallographic Refinement		
R	0.1787	0.1470
R-free	0.1953	0.1817
Bond length RMS Z-score	1.302	0.444
Bond angle RMS Z-score	1.108	0.641
Model Quality		
Ramachandran plot normality	70	88
Rotamer normality	84	95
Coarse packing	N/A	N/A
Fine packing	N/A	N/A
Bump severity	95	95
Hydrogen bond satisfaction	N/A	N/A

The refinement statistics from PDB-REDO demonstrate a significant improvement in model quality compared to the original structure, as evidenced by a reduction in the R-value (0.1787 to 0.1470) and R-free (0.1953 to 0.1817), indicating better agreement between the crystallographic data and the refined model while reducing overfitting (Table 1). Bond geometry improved substantially, with the bond length RMS Z-score decreasing from 1.302 to 0.444 and the bond angle RMS Z-score reducing from 1.108 to 0.641, correcting structural distortions and leading to a more chemically sound model. Model quality percentile scores further support these enhancements, with Ramachandran plot normality increasing from 70% to 88%, reflecting improved backbone geometry, and rotamer normality rising from 84% to 95%, indicating fewer steric clashes and more favorable side-chain conformations. While bump severity remained stable at 95%, suggesting pre-existing control over steric hindrance, data on packing efficiency and hydrogen bonding optimization were unavailable. Overall, these refinements significantly enhance the structural accuracy and reliability of the protein model, which is crucial for subsequent computational analyses, including virtual screening and molecular dynamics simulations, ensuring precise binding predictions and mechanistic insights in structure-based drug discovery for Chronic Lymphocytic Leukemia.

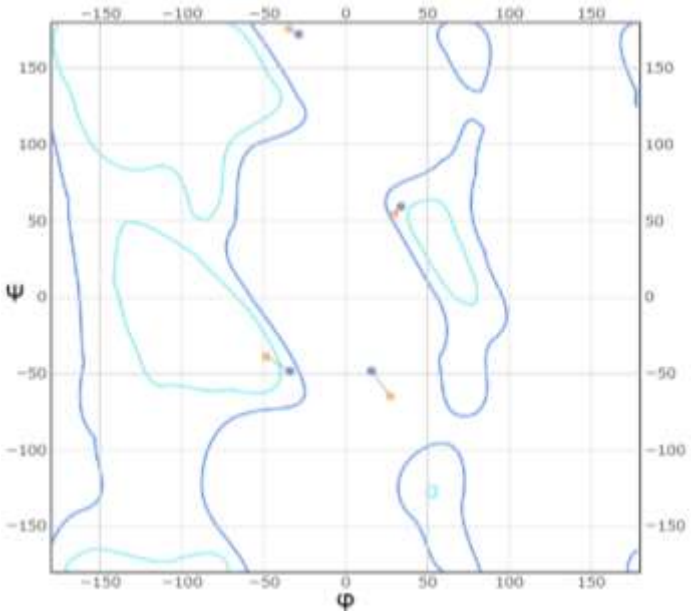


Figure 1:Kleywegt-Like Ramachandran Plot for chronic Lymphocytic leukemia

The Kleywegt-like Ramachandran plot provides an assessment of the backbone dihedral angles (ϕ, ψ) of the protein structure, highlighting potential conformational outliers and serving as a valuable tool for evaluating structural refinement by identifying residues that deviate from energetically favorable regions (Figure 1). In the context of Chronic Lymphocytic Leukemia (CLL), where the structural accuracy of target proteins like the Bcl-2 receptor is crucial for drug discovery, this plot aids in quality validation by depicting contour regions that indicate allowed and favored conformations, with blue and cyan lines representing the most probable regions. Outliers, marked as individual data points, suggest residues with non-ideal backbone conformations, potentially arising from crystallographic errors or inherent protein flexibility. The provided plot reveals a few residues outside the favored regions, which could correspond to functionally significant sites, such as binding pockets or flexible loop regions, while also indicating a need for further refinement or validation through molecular dynamics simulations to enhance structural accuracy. Overall, this plot confirms the model's quality while pinpointing areas requiring optimization, which is essential for structure-based drug discovery in CLL, where even minor structural deviations can impact ligand binding and therapeutic efficacy

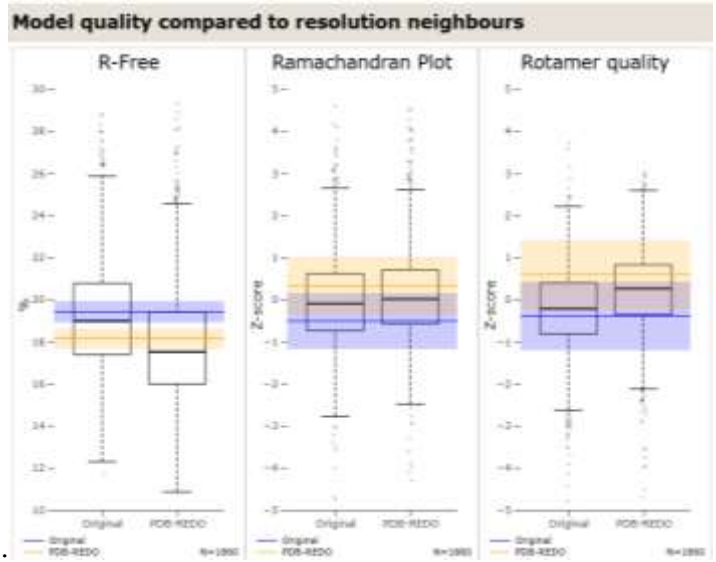


Figure 2: Model Quality Compared to Resolution Neighbours

The boxplot visualization compares key structural quality metrics—R-Free, Ramachandran plot Z-score, and Rotamer quality Z-score—between the original and PDB-REDO refined models, contextualized against resolution-matched neighboring structures (N = 1860), providing insights into the effectiveness of PDB-REDO refinement in improving model accuracy and stereochemical quality (Figure 2). The R-Free values, which measure model accuracy against unseen crystallographic data, show a notable reduction post-refinement, with a lower median and narrower distribution, indicating an improved fit to experimental data and reduced structural uncertainty, which is crucial for structure-based drug discovery in Chronic Lymphocytic Leukemia (CLL) by enhancing confidence in binding site conformations. The Ramachandran plot Z-score shifts toward more favorable values, reducing backbone dihedral angle deviations and outliers, demonstrating improved backbone geometry necessary for reliable molecular docking and dynamics simulations. Similarly, the rotamer quality Z-score indicates optimized side-chain conformations, with reduced steric clashes and improved chemical accuracy, essential for precise ligand interactions in targeting mutant Bcl-2 receptors in CLL. Comparing the original and refined models against resolution-matched neighbors, the refined model consistently exhibits improved or maintained quality across key parameters, with narrower distributions and fewer extreme outliers, suggesting that PDB-REDO not only enhances structural accuracy but also stabilizes the model within high-quality structural standards. These refinements directly contribute to the reliability of computational drug screening and molecular simulations, ensuring a robust and accurate structural model for subsequent investigations in drug discovery.

3.2. Protein Validation Results

3.2.1. SAVESV6.1 Results

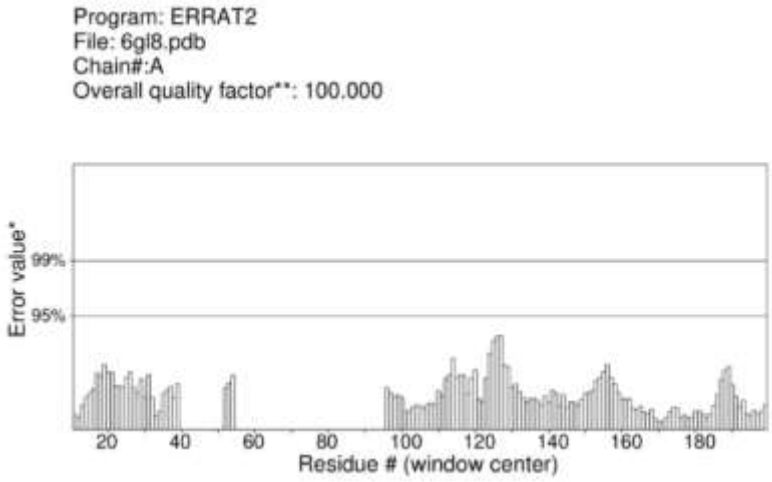


Figure 3: ERRAT2 Quality Analysis of Chains A of Bcl-2 receptor

The structural validation of the modeledBcl-2 receptor(file"6GL8.pdb")was assessed using ERRAT2, which evaluates model reliability based on non-bonded atomic interactions. The analysis yielded a quality factor of 100, indicating exceptionally high accuracy, surpassing the 95% threshold typical of high-resolution structures (Figure 3). The error value distribution across residues demonstrated minimal deviations, confirming well-optimized backbone and side-chain interactions with negligible steric clashes. The absence of major outliers suggests strong alignment with high-resolution experimental structures, reinforcing the model's reliability for downstream computational studies such as molecular docking and molecular dynamics (MD) simulations. This high confidence ensures that ligand-binding interactions and conformational analyses will be based on a structurally robust template. Given these results, the validated model provides a strong foundation for structure-based drug discovery targeting the mutant Bcl-2 receptor in chronic lymphocytic leukemia (CLL). Future validation steps, including Ramachandran plot analysis, MolProbity assessment, and MD-based stability studies, will further refine and confirm the structural fidelity of the model.

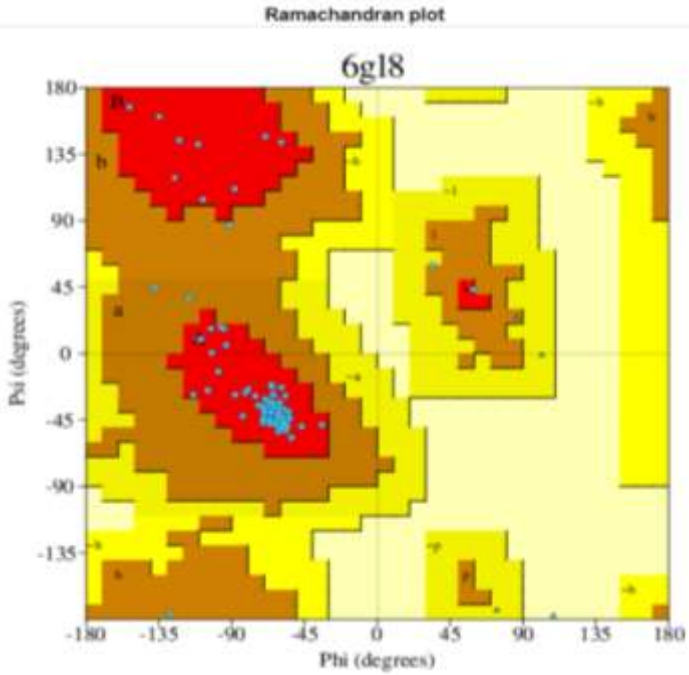


Figure 4: Ramachandran Plot for Protein structure Analysis of Bcl-2 receptor

The Ramachandran plot for the Bcl-2 mutant receptor (PDBID:6GL8) provides a comprehensive evaluation of the backbone dihedral angles (Φ and Ψ) of the protein structure, crucial for assessing its conformational stability (Figure 4). The majority of the residues are positioned within the most favored (red) and additionally allowed (brown) regions, indicating that the modeled structure maintains a highly stable and energetically favorable conformation. A minimal presence of residues in the disallowed (yellow) regions suggests limited steric hindrance or conformational strain, reinforcing the structural integrity of the protein. These results confirm the reliability of the receptor model for subsequent molecular docking and molecular dynamics simulations in the context of structure-based drug discovery for chronic lymphocytic leukemia.

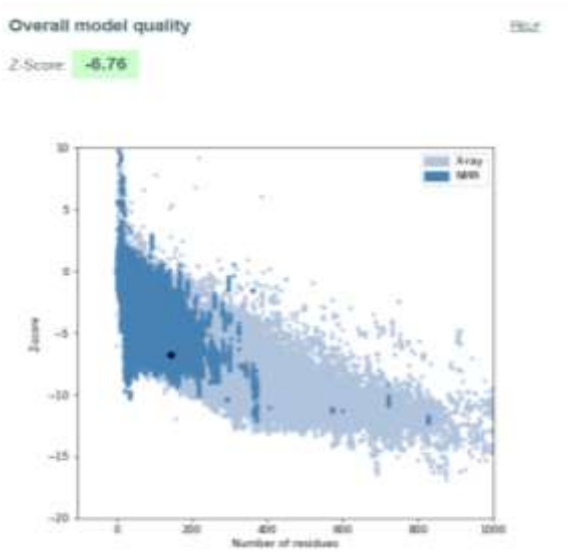


Figure 5: ProSA Z-Score Validation for Bcl-2 receptor(PDB ID:6GL8)

The structural validation of the modeledBcl-2 receptor, a key target in chronic lymphocytic leukemia (CLL), was assessed using ProSA (Protein Structure Analysis), which evaluates model quality based on energy-based scoring. The obtained Z-score of -6.76 falls within the range of experimentally determined NMR and X-ray crystal structures, indicating a reliable and well-optimized model (Figure 5). The scatter plot comparison, displaying distributions of X-ray (light blue) and NMR (dark blue) structures, confirms that the modeled structure aligns with known protein structures of similar size, reinforcing its stability and accuracy for further computational analyses. As a critical metric for global model quality, the Z-score suggests that the model is structurally sound, ensuring its suitability for structure-based drug discovery targeting mutant Bcl-2 in CLL. This validation supports the credibility of subsequent molecular docking and molecular dynamics (MD) simulations, enhancing ligand-binding predictions and drug candidate evaluations. Given that Bcl-2 overexpression is a key driver of CLL pathogenesis, validating its structural integrity is crucial for identifying potent inhibitors. Further validation, including Ramachandran plot analysis, MolProbity, and MD-based stability studies, will refine and confirm its suitability for computational drug design. These findings provide a strong foundation for developing targeted therapies aimed at disrupting Bcl-2-mediated apoptosis evasion in CLL cells, potentially advancing therapeutic interventions.

Figure 6: Local Model Quality Assessment for Bcl-2 receptor (PDB ID:6GL8)

The local model quality assessment of the modeled Bcl-2 receptor, a key target in chronic lymphocytic leukemia (CLL), was evaluated using knowledge-based energy calculations, providing insights into the structural stability and reliability of different regions within the protein model (Figure 6). The energy distribution plot, analyzed using two window sizes—10 (light green) and 40 (dark green)—demonstrates that most regions exhibit negative energy values, indicating a stable and well-folded structure with minimal steric clashes or unfavorable interactions. The absence of significant positive energy spikes further supports the structural integrity of the model, as unfavorable regions are not observed. Small fluctuations in the light green curve (window size 10) represent local variations in stability, which are smoothed in the dark green curve (window size 40), reinforcing an overall well-optimized conformation. These findings confirm the suitability of the model for further computational studies, including molecular docking and molecular dynamics (MD) simulations, which require structurally reliable templates for accurate ligand-binding predictions. Since Bcl-2 plays a pivotal role in apoptosis regulation and CLL progression, ensuring its structural accuracy is critical for designing targeted inhibitors. Additional validation methods, such as Ramachandran plot analysis, MolProbity assessment, and MD-based refinement, will further strengthen confidence in the model's reliability for structure-based drug discovery.

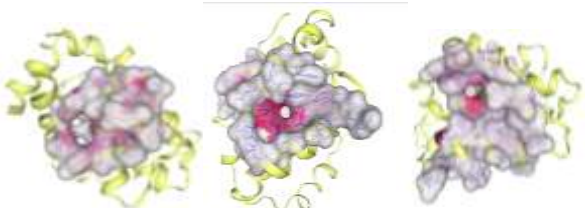
3.3. Results of Virtual Screening Outcomes

Table 2: Top-Ranked FDA-Approved Drugs Identified Through Virtual Screening Against chronic lymphocytic leukemia Bcl-2 receptor (PDB ID:6GL8)

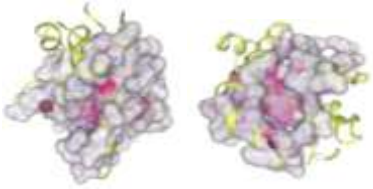
SR. NO	ID	Name	Score	SR. NO	ID	Name	Score
1	DB04861	Nebivolol	-7.5	2	DB12978	Pexidartinib	-7.1
3	DB01166	Cilostazol	-7.1	4	DB04820	Nialamide	-6.9
5	DB01238	Aripiprazole	-6.6	6	DB05812	Abiraterone	-6.6
7	DB04209	Dequalinium	-6.6	8	DB00342	Terfenadine	-6.6
9	DB09128	Brexpiprazole	-6.6	10	DB00857	Terbinafine	-6.6
11	DB01184	Domperidone	-6.5	12	DB00163	Vitamin E	-6.4
13	DB12867	Benperidol	-6.4	14	DB00450	Droperidol	-6.4
15	DB01079	Tegaserod	-6.4	16	DB08901	Ponatinib	-6.4
17	DB13931	Netarsudil	-6.4	18	DB08877	Ruxolitinib	-6.3
19	DB00738	Pentamidine	-6.3	20	DB13953	Estradiol benzoate	-6.2
21	DB12313	Dopexamine	-6.2	22	DB01046	Lubiprostone	-6.1
23	DB00426	Famciclovir	-6.1	24	DB00735	Naftifine	-6.1
25	DB08981	Fenbufen	-6.1	26	DB12554	Mebeverine	-6.1
27	DB14075	Imidurea	-6.1	28	DB03756	Doconexent	-6.0
29	DB00398	Sorafenib	-6.0	30	DB01132	Pioglitazone	-6.0
31	DB11125	Benzethonium	-6.0	32	DB15465	Benzhydrocodone	-6.0
33	DB08976	Floctafenine	-5.9	34	DB00757	Dolasetron	-5.9
35	DB11672	Curcumin	-5.9	36	DB08896	Regorafenib	-5.9
37	DB01136	Carvedilol	-5.8	38	DB00656	Trazodone	-5.8
39	DB09039	Eliglustat	-5.8	40	DB00706	Tamsulosin	-5.8
41	DB11963	Dacomitinib	-5.8	42	DB00577	Valaciclovir	-5.8
43	DB11642	Pitolisant	-5.7	44	DB05154	Pretomanid	-5.7
45	DB00755	Tretinoin	-5.7	46	DB09195	Lorpiprazole	-5.7
47	DB01026	Ketoconazole	-5.7	48	DB09082	Vilanterol	-5.7
49	DB00775	Tirofiban	-5.7	50	DB00523	Alitretinoin	-5.7
51	DB06684	Vilazodone	-5.7	52	DB00448	Lansoprazole	-5.7
53	DB04953	Ezogabine	-5.7	54	DB13781	Xamoterol	-5.7
55	DB01053	Benzylpenicillin	-5.7	56	DB04574	Estronesulfate	-5.7
57	DB06016	Cariprazine	-5.7	58	DB06774	Capsaicin	-5.7
59	DB01380	Cortisone acetate	-5.7	60	DB00805	Minaprine	-5.7
61	DB01393	Bezafibrate	-5.6	62	DB02546	Vorinostat	-5.6
63	DB04794	Bifonazole	-5.6	64	DB01122	Ambenonium	-5.6
65	DB01247	Isocarboxazid	-5.6	66	DB00433	Prochlorperazine	-5.6
67	DB09209	Pholcodine	-5.6	68	DB00918	Almotriptan	-5.6

69	DB00298	Dapiprazole	-5.6	70	DB00412	Rosiglitazone	-5.6
71	DB07776	Flavone	-5.5	72	DB04841	Flunarizine	-5.5
73	DB11186	Pentoxyverine	-5.5	74	DB06711	Naphazoline	-5.5
75	DB01187	Iophendylate	-5.5	76	DB04224	Oleic Acid	-5.5
77	DB11660	Latanoprostenebunod	-5.4	78	DB14646	Prednisone acetate	-5.4
79	DB00914	Phenformin	-5.4	80	DB00211	Midodrine	-5.4
81	DB14196	N-Cyclohexyl-N'-phenyl-1,4-phenylenediamine	-5.4	82	DB11583	Cetalkonium	-5.4
83	DB00461	Nabumetone	-5.4	84	DB06777	Chenodeoxycholic acid	-5.4
85	DB13216	Oxolamine	-5.3	86	DB00571	Propranolol	-5.3
87	DB00619	Imatinib	-5.3	88	DB00929	Misoprostol	-5.3
89	DB00178	Ramipril	-5.3	90	DB05271	Rotigotine	-5.2
91	DB00278	Argatroban	-5.1	92	DB00268	Ropinirole	-5.0
93	DB01091	Butenafine	-5.0	94	DB00746	Deferoxamine	-5.0
95	DB13943	Testosterone cypionate	-5.0	96	DB13532	Cyclopenthiazide	-4.9
97	DB00984	Nandrolone phenpropionate	-4.7	98	DB15091	Upadacitinib	-4.3
99	DB00688	Mycophenolate mofetil	-4.2	100	DB00256	Lymecycline	-4.1

The molecular docking results presented in the table 2 highlight the binding affinities of various drug compounds against the mutant Bcl-2 receptor, a key target in chronic lymphocytic leukemia (CLL). Among the screened compounds, Nebivolol (DB04861) exhibited the strongest binding affinity with a docking score of -7.5 kcal/mol, suggesting its potential as a lead compound. Other notable candidates, such as Pexidartinib (DB12978) and Cilostazol (DB01166), showed promising interactions with docking scores of -7.1 kcal/mol. Additionally, several FDA-approved drugs, including Aripiprazole, Abiraterone, and Terfenadine, demonstrated moderate binding affinities ranging from -6.6 to -6.4 kcal/mol, indicating possible repurposing opportunities. Interestingly, structurally diverse compounds such as Ponatinib, Ruxolitinib, and Sorafenib, known kinase inhibitors, also exhibited favorable docking scores, reinforcing their potential role in Bcl-2 inhibition. While these results provide an initial insight into potential drug candidates, further validation through molecular dynamics simulations and experimental assays is essential to confirm their binding stability and therapeutic relevance in CLL treatment.



Curpocket ID:C1Curpocket ID:C2Curpocket ID:C3
Cavity volume(A3) :649 Cavity volume(A3):332 Cavity Volume(A3):323



Curpocket ID:C4Curpocket ID:C5
Cavity volume (A3) :159 Cavity volume (A3) :156

Figure 7: Structural Analysis of Binding Pockets in Bcl-2 receptor: Insights for Targeted Drug Design

The cavity analysis of the mutant Bcl-2 receptor, performed using Curpocket, identified five distinct binding pockets (C1–C5) with varying cavity volumes (Figure 7). Among these, Curpocket ID: C1 exhibited the largest cavity volume **(**649 Å^3**),** suggesting it as the primary binding site with the highest potential for ligand accommodation. This makes C1 an ideal target for structure-based drug discovery, as larger cavities often provide greater flexibility for ligand binding and optimization. The second-largest cavity, Curpocket ID: C2 (332 Å^3), along with Curpocket ID: C3 (323 Å^3), represents additional potential binding sites that could accommodate small-molecule inhibitors. The smaller cavities, Curpocket ID: C4 (159 Å^3) and C5 (156 Å^3), may serve as allosteric sites or secondary binding regions that could contribute to the modulation of Bcl-2 activity. The identification of these cavities is crucial for virtual screening and molecular docking studies, as it allows for a targeted approach in selecting compounds that fit optimally within these pockets. Further molecular dynamics simulations will be necessary to assess the stability, flexibility, and druggability of these cavities, providing deeper insights into their therapeutic potential for chronic lymphocytic leukemia (CLL) treatment.

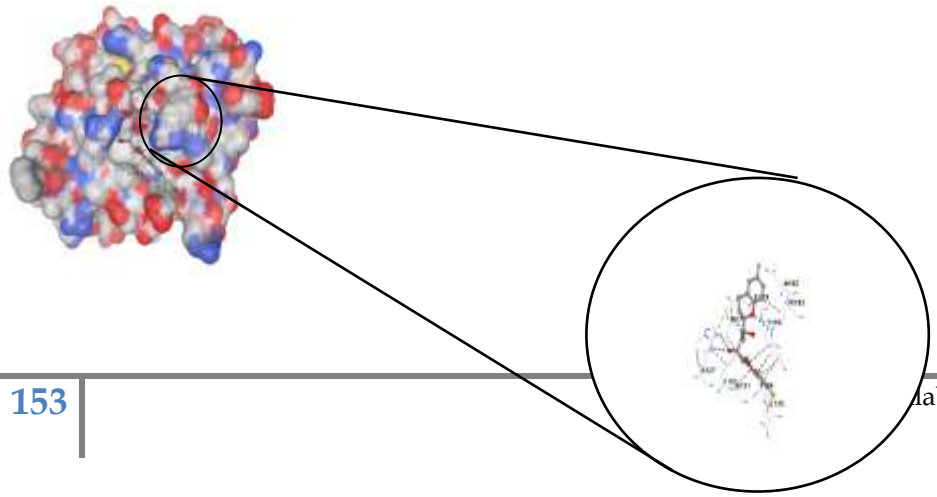


Figure 8: Non-Covalent Interactions Between Bcl-2 receptor and Nebivolol: Insights into Binding Mechanisms

The molecular docking visualization in the image depicts the interaction between a ligand and the mutant Bcl-2 receptor, highlighting key binding interactions (Figure 8). The ligand is stabilized within the binding pocket through hydrogen bonds (blue dashed lines) with residues E79, E135, and Y184, indicating strong polar interactions. Additionally, hydrophobic interactions (black dashed lines) involving W117, F130, and A131 suggest a favorable binding environment, enhancing ligand affinity. The presence of positively charged residues like R127 and R183 may contribute to electrostatic interactions, further stabilizing the complex. These findings support the identified binding cavity's drugability, aligning with the Curpocket cavity analysis. The combination of hydrogen bonding, hydrophobic forces, and electrostatic interactions suggests that the ligand effectively engages with the Bcl-2 receptor, making it a promising candidate for further validation through molecular dynamics simulations and experimental assays to assess its potential in chronic lymphocytic leukemia (CLL) therapy.

3.4. Molecular Dynamics Simulation Analysis

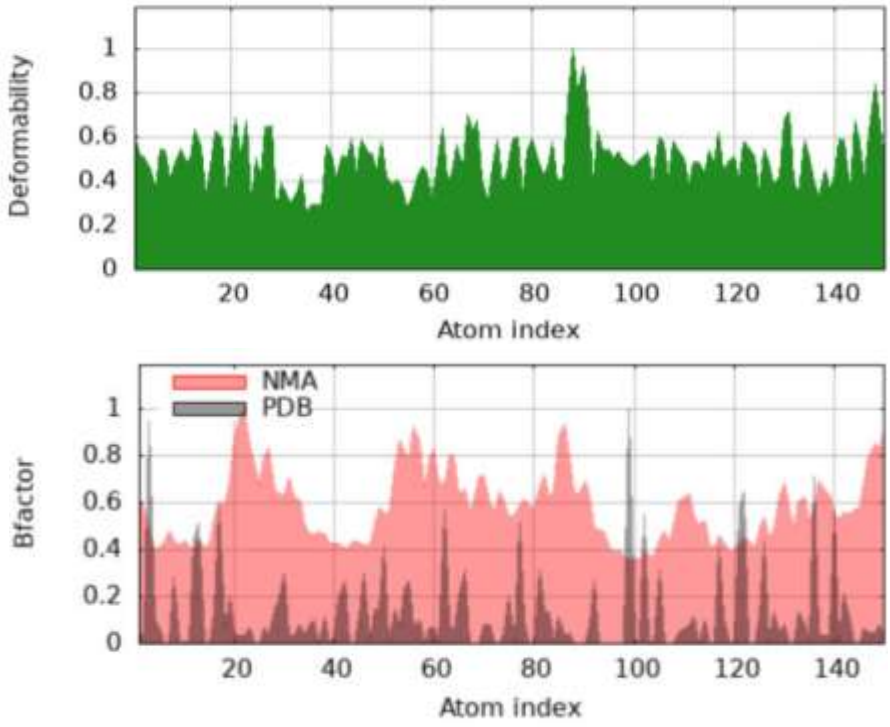


Figure 9: Comparative Analysis of Molecular Deformability and B-factors

The analysis of B-factor and mobility provides crucial insights into the flexibility and structural dynamics of the mutant Bcl-2 receptor, which are essential for structure-based drug discovery (Figure 9). The deformability plot (green) indicates regions

of high flexibility along the protein backbone, with peaks suggesting potential hinge points that may influence ligand binding and conformational changes. The comparison of experimental B-factors from the PDB and calculated normal mode analysis (NMA) values (red) further validates these mobility trends, showing agreement in fluctuation patterns, particularly in flexible loop regions. Such structural mobility assessments are instrumental in identifying druggable sites, optimizing ligand interactions, and refining molecular dynamics simulations to improve drug design for chronic lymphocytic leukemia.

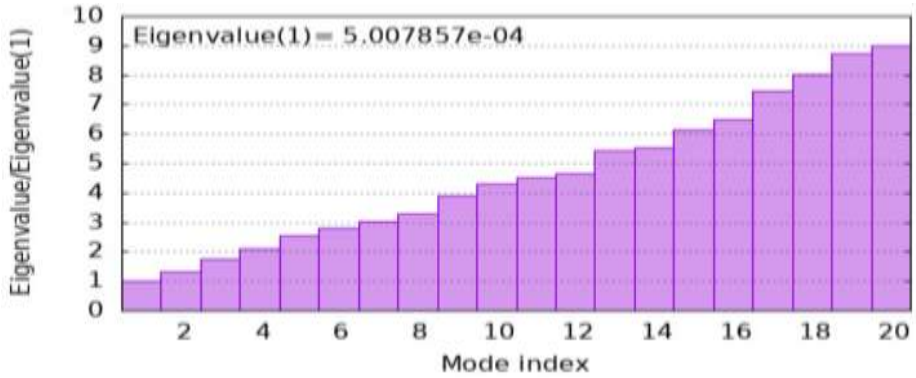


Figure 10: Normalized Eigenvalues for Different Normal Modes of the Structure

The eigenvalue distribution depicted in the histogram provides critical insights into the global motion of the mutant Bcl-2 receptor, as obtained from normal mode analysis (NMA). The increasing trend of eigenvalues with mode index suggests that lower-indexed modes correspond to large-scale collective motions, while higher-indexed modes represent localized, higher-frequency fluctuations (Figure 10). The smallest eigenvalue (5.007857e-04) corresponds to the most dominant and functionally relevant motion, which may be associated with conformational changes critical for ligand binding. Understanding these vibrational modes aids in identifying key flexible regions that could influence molecular interactions, making this analysis essential for refining molecular dynamics simulations and enhancing structure-based drug discovery strategies for chronic lymphocytic leukemia.

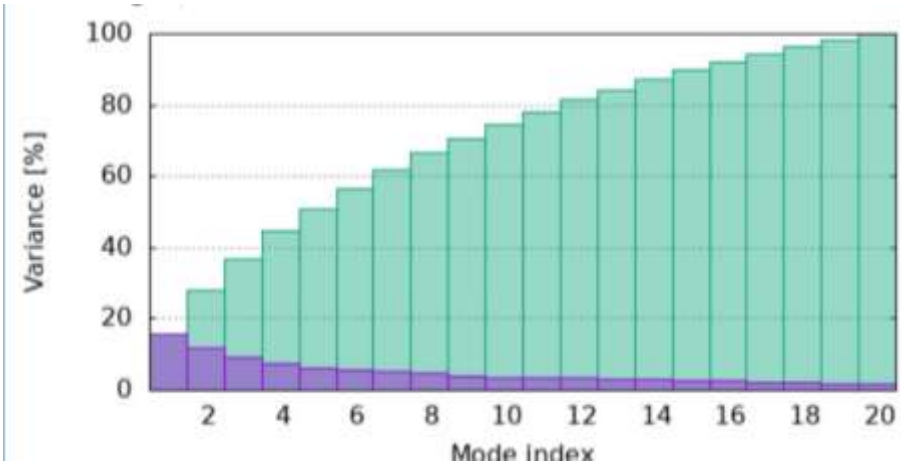


Figure 11: Variance Associated with Normal Modes of the Structure

The variance distribution plot illustrates the contribution of each normal mode to the overall structural flexibility of the mutant Bcl-2 receptor. The lower-indexed modes exhibit significantly higher variance, indicating that they correspond to large-scale, collective motions essential for the receptor's functional dynamics, including ligand binding and conformational transitions (Figure 11). In contrast, the higher-indexed modes contribute minimally to variance, representing localized, high-frequency fluctuations with limited biological relevance. This analysis reinforces the importance of dominant low-frequency modes in protein dynamics, aiding in the identification of flexible and druggable regions, which is crucial for molecular docking, virtual screening, and structure-based drug discovery in chronic lymphocytic leukemia.

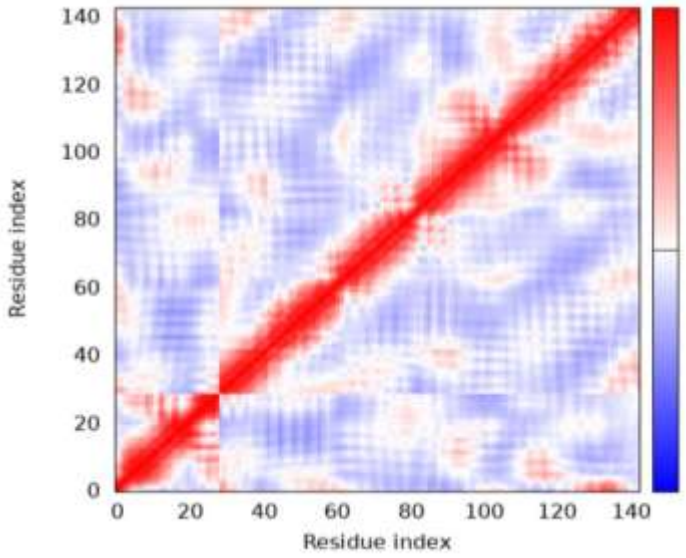


Figure 12: Covariance Map of Residue Coupling in chronic lymphocytic leukemia

The cross-correlation matrix depicted in the heatmap provides valuable insights into the dynamic behavior and inter-residue interactions of the mutant Bcl-2 receptor.

The red regions along the diagonal indicate strong correlated motions between neighboring residues, reflecting structural rigidity and cooperative movements essential for maintaining protein stability (Figure 12). Conversely, the presence of blue regions signifies anti-correlated motions, suggesting flexible regions that may undergo significant conformational changes. Such dynamic behavior is crucial in identifying potential binding sites and allosteric regulation mechanisms. This analysis enhances our understanding of the receptor's functional dynamics, aiding in the refinement of molecular docking strategies and optimizing structure-based drug discovery for chronic lymphocytic leukemia**.**

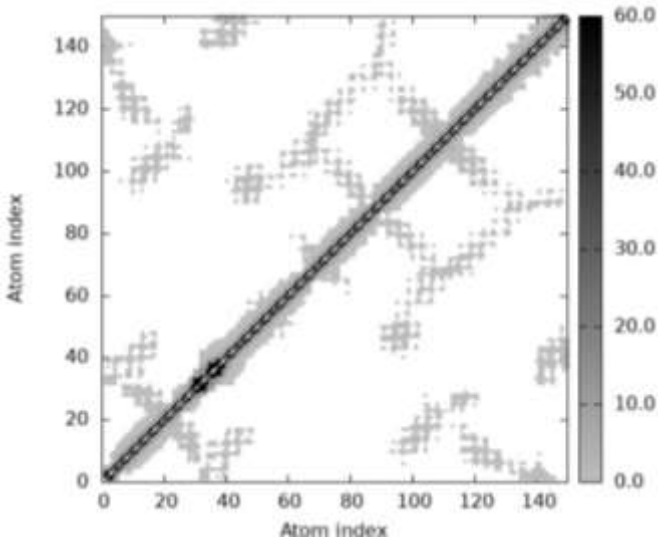


Figure 13: Elastic Network Model of Atomic Connections in Ebola Glycoprotein

The contact map shown in the image provides a detailed representation of atomic interactions within the mutant Bcl-2 receptor, offering insights into its structural stability and flexibility (Figure 13). The diagonal dominance indicates strong interactions between sequentially adjacent residues, which are essential for maintaining the protein's backbone conformation. The off-diagonal contacts suggest long-range interactions, which play a crucial role in stabilizing the protein's tertiary structure. Regions with high contact density may represent key structural motifs, while sparse regions could indicate flexible or disordered segments. This analysis is instrumental in understanding the receptor's structural integrity, aiding in molecular docking studies and optimizing drug design strategies for chronic lymphocytic leukemia

4. Conclusion

The structural refinements performed using PDB-REDO significantly enhanced the quality and accuracy of the Bcl-2 receptor model, crucial for structure-based drug discovery in Chronic Lymphocytic Leukemia (CLL). The reduced R-value (0.1787 to 0.1470) and R-free (0.1953 to 0.1817) indicate a better fit to crystallographic data while minimizing overfitting, and improvements in bond geometry, with bond length RMS Z-score decreasing from 1.302 to 0.444 and bond

angle RMS Z-score from 1.108 to 0.641, further enhance structural reliability. Model quality metrics validate these refinements, as seen in the increased Ramachandran plot normality (70% to 88%) and rotamer normality (84% to 95%), signifying optimized backbone and side-chain conformations. The Kleywegt-like Ramachandran plot analysis reveals minimal conformational outliers, reinforcing structural accuracy, while comparisons with resolution-matched neighboring structures confirm improvements in R-free, backbone dihedral angles, and rotamer quality, demonstrating PDB-REDO's effectiveness in refining the model to high structural standards. These enhancements strengthen the reliability of molecular docking and dynamics simulations, ensuring precise binding predictions and mechanistic insights. Ultimately, the optimized Bcl-2 receptor model provides a strong foundation for structure-based drug discovery in CLL, aiding in the identification and validation of therapeutic candidates with improved accuracy and reliability, with future in vitro and in vivo studies essential to confirm their clinical potential.

The structural validation of the modeled Bcl-2 receptor confirms its high accuracy and reliability for structure-based drug discovery in Chronic Lymphocytic Leukemia (CLL). ERRAT2 analysis yielded a quality factor of 100, indicating exceptional model reliability with minimal deviations and negligible steric clashes, ensuring a well-optimized backbone and side-chain conformation. The Ramachandran plot analysis further supports this, with the majority of residues occupying favored and allowed regions, reinforcing structural stability. ProSA validation produced a Z-score of -6.76, aligning the model with experimentally determined structures, while local model quality assessments using energy calculations confirmed a stable and well-folded conformation with no significant steric hindrances. These results validate the model's suitability for downstream computational analyses, including molecular docking and molecular dynamics (MD) simulations, crucial for predicting ligand-binding interactions with high confidence. Given the critical role of Bcl-2 in apoptosis regulation and CLL progression, ensuring its structural accuracy is essential for designing potent inhibitors. Future refinements through MolProbity assessment, extended MD simulations, and additional validation techniques will further enhance the model's reliability, providing a robust foundation for targeted drug discovery against mutant Bcl-2 in CLL.

The structure-based drug discovery approach employed in this study has identified several FDA-approved drugs with potential inhibitory activity against the mutant Bcl-2 receptor, a critical target in chronic lymphocytic leukemia (CLL). Among the top-ranked compounds, Nebivolol (DB04861) exhibited the highest binding affinity (-7.5 kcal/mol), followed by Pexidartinib (DB12978) and Cilostazol (DB01166), demonstrating strong interactions within the identified binding cavities. Notably, kinase inhibitors such as Ponatinib, Ruxolitinib, and Sorafenib also displayed favorable docking scores,

reinforcing their potential for Bcl-2 inhibition and possible therapeutic repurposing.

Cavity analysis using Curpocket identified five distinct binding pockets, with **C1 (649 Å³)** emerging as the most promising site for ligand binding due to its larger volume and structural accessibility. The presence of additional binding sites (C2–C5) suggests alternative targeting strategies, including allosteric modulation. Molecular docking visualization further confirmed key non-covalent interactions, including hydrogen bonding, hydrophobic forces, and electrostatic interactions, which contribute to ligand stability within the receptor's active site.

Molecular dynamics simulation provided deeper insights into the receptor's structural flexibility and binding stability, highlighting crucial fluctuations in flexible loop regions that may impact ligand accommodation. Normal mode analysis (NMA) indicated that **low-**frequency collective motions play a significant role in receptor conformational dynamics, further supporting the potential druggability of the identified binding sites. The covariance and elastic network model analyses revealed correlated and anti-correlated residue motions, emphasizing key regions involved in ligand binding and receptor stability.

Overall, these findings provide a strong foundation for further experimental validation of the identified drug candidates. The integration of molecular docking, cavity analysis, and molecular dynamics simulations underscores the feasibility of repurposing existing FDA-approved drugs for CLL treatment. Future studies should focus on in vitro and in vivo evaluations to confirm the efficacy and therapeutic relevance of these potential inhibitors, ultimately advancing precision medicine approaches in CLL therapy.

5. Acknowledgment

We express our heartfelt gratitude to the Ashokrao Mane College Of Pharmacy Peth Vadgaon for their support and encouragement throughout this study.

6. Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

7. Conflict of Interest

The authors declare no conflicts of interest related to this study.

8. Author Contributions

Vaishnavi D. Halake: Conceptualization, literature review, and manuscript drafting.

Shonak S. Adivarekar: Study supervision, manuscript editing, and correspondence.

Tejaswini M. Biraje: Data collection and analysis, manuscript formatting.

Babaso V. Udugade: Review of literature and figure preparation.

9. Ethics Approval

Not applicable. This study does not involve human or animal subjects.

10. Data Availability

The data supporting the findings of this study are available within the manuscript

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HOW TO CITE THIS ARTICLE

Vaishnavi Halake*, Ishwari Nimbalkar, Shonak Adivarekar, Tejaswini Biraje, Babaso Udugade: Structure-based drug discovery for chronic lymphocytic leukemia: virtual screening and molecular dynamics simulation targeting mutant bcl-2 receptor. International Journal of Institutional Pharmacy and Life Sciences, Vol 15[2] March-April 2025 : 141-162.