Promising mcl-1 inhibiting phytocomponents for imatinib resistant CML therapy- an *in-silico* analysis

Anusha[1], Rashi Srivastava[2], Rama Chandra Prasad[1*]

[1] Department of Life Sciences, Garden City University, Bangalore, India, 560049

[2]Department of Chemical and Biochemical Engineering, Indian Institute of Technology, Patna, India, 801106

***Corresponding author**

Dr. L.A. Rama Chandra Prasad,
Assistant Professor,
Department of Life Sciences, Garden City University,
India, Bangalore-560049
ramachandra.prasad@gardencity.university

Abstract

Cancer holds immense significance in today's medical landscape. Chronic Myeloid Leukemia (CML), a type of blood cancer, impacts the bone marrow and blood cells explicitly. Developing tyrosine kinase inhibitors (TKIs) has dramatically improved patient outcomes. TKIs, such as Imatinib, precisely target and inhibit the activity of the BCR-ABL tyrosine kinase. By inhibiting the BCR-ABL tyrosine kinase, TKIs effectively suppress the abnormal proliferation of leukemic cells, induce their apoptosis, and restore normal hematopoiesis. However, despite the remarkable efficacy of TKIs, the development of Imatinib resistance remains a challenge in CML treatment. To combat this resistance issue, second and third-generation TKIs also developed; however, most people develop side effects, including nausea, vomiting, diarrhea, cardiac complications, and lung infection. Thus, the necessity of herbal drug compounds increased to combat this issue. Recent research has shown that Imatinib-resistant CML patients show over-expression of Myeloid cell leukemia-1 (MCL-1). MCL-1 has been validated as an effective target for cancer therapy. Therefore, the objective of this study is to specifically target MCL-1 in order to aid in the treatment

of individuals with Imatinib-resistant CML. By unlocking the hidden potential of known compounds, *in-silico* drug repurposing opens doors to more efficient and targeted therapies, ultimately benefiting patients worldwide. In this study, we have opted *in-silico* methods for identifying herbal drug compounds against MCL-1. 500 compounds were opted, and compounds that follow Lipinski rules of 5 were subjected to molecular docking using PDB ID: 4wmr for MCL-1. We found three potent phytochemicals showing less than -8.8 kcal/mol binding energy. The best one, Corynan-17-oic acid, was further subjected to molecular dynamics simulation analysis. Simulation results confirm that Corynan-17-oic acid can inhibit the activity of MCL-1. Hence, this study is a substantial research effort to find a promising herbal drug against MCL-1 targeting CML that is warranted in the future.

Keywords: *CML, MCL-1, Molecular docking & Simulation, Phytocomponents, Corynan-17-oic acid*

Introduction

Chronic Myeloid Leukemia is a type of blood cancer that affects the bone marrow and blood cells, driven by the BCR-ABL fusion gene resulting from the Philadelphia chromosome translocation. This fusion gene produces an abnormal tyrosine kinase that encourages the proliferation and survival of leukemic cells (Bartram et al., 1983; Grant et al., 2022). A key factor in cancer progression and treatment resistance is the evasion of apoptosis, a programmed cell death process that removes damaged cells (Qian et al., 2022). BCL-2 family proteins are crucial in regulating apoptosis by maintaining mitochondrial integrity, which is essential for initiating the mitochondrial-dependent apoptotic pathway (Qian et al., 2022). The equilibrium between pro-apoptotic and anti-apoptotic BCL-2 family members is vital in determining cell fate. Pro-apoptotic proteins like BIM can overpower anti-apoptotic proteins such as BCL-2, MCL-1, and BCL-XL, activating BAX and BAK proteins that permeabilize the mitochondrial outer membrane, leading to cell death (Singh & Lim, 2022). Recent advances in cancer research have focused on developing inhibitors for anti-apoptotic BCL-2 proteins, particularly BH3-mimetics. These small molecules imitate BH3-only proteins by binding to anti-apoptotic BCL-2 proteins and releasing pro-apoptotic proteins, thereby inducing apoptosis (Wang et al., 2021). Among the anti-apoptotic proteins, MCL-1

stands out due to its short half-life and unique role in regulating mitochondrial metabolism. MCL-1 overexpression is commonly seen in various hematologic malignancies, such as plasma cell myeloma, acute myeloid leukemia (AML), and lymphoma, where it enhances cell survival (Kim et al., 2009; Thomas et al., 2010; Wei et al., 2020). BCR/ABLin CML enhances mcl-1 promoter activity, mcl-1 mRNA expression, and the MCL-1 protein in Ba/F3 cells (Alchberger et al., 2005; Fukuchi et al., 2001).

Developing tyrosine kinase inhibitors has dramatically improved patient outcomes (Manley et al., 2002; Rossari et al., 2018). TKIs, such as Imatinib, precisely target and inhibit the activity of the BCR-ABL tyrosine kinase. By inhibiting the BCR-ABL tyrosine kinase, TKIs effectively suppress the abnormal proliferation of leukemic cells, induce their apoptosis, and restore normal hematopoiesis (Manley et al., 2002; Rossari et al., 2018). However, despite the remarkable efficacy of TKIs, the development of Imatinib resistance remains a challenge in CML treatment (Aguilera & Tsimberidou, 2009; Amarante-Mendes et al., 2022). To combat this resistance issue, second and third-generation TKIs also developed; however, most people develop side effects, including nausea, vomiting, diarrhea, cardiac complications, and lung infection (Aguilera & Tsimberidou, 2009; Amarante-Mendes et al., 2022). Thus, the necessity of herbal drug compounds increased to combat this issue. Recent research has shown that Imatinib-resistant CML patients show over-expression of MCL-1 (Tantawy et al., 2023). MCL-1 has been validated as an effective target for cancer therapy. Clinical trials have been initiated for several MCL-1 inhibitors, including AZD5991 (NCT03218683), S64315 (NCT02979366), AMG 176 (NCT03797261), and AMG 397 (NCT3465540) (Tantawy et al., 2023). However, the possibility of identifying a safe treatment window for this novel class of Mcl-1 inhibitors is another crucial concern. Therefore, this study aims to specifically target MCL-1 to aid in treating individuals with Imatinib-resistant CML. *In-silico* drug repurposing opens doors to more efficient and targeted therapies by unlocking the hidden potential of known compounds, ultimately benefiting patients worldwide.

Materials and Methodology

Protein Selection and Ligand Dataset Preparation

MCL-1 wild-type protein has been chosen from the RCSB-PDB database (https://www.rcsb.org/). The X-ray 3D crystal structure of PDB ID 4wmr has 150 amino acid lengths with a resolution of 1.70 Å. Further, PubChem server database (https://pubchem.ncbi.nlm.nih.gov/) provided the 3D structures of 500 phytochemicals, most of which are found in herbal plants. These plants include *Alstonia scholaris, Berberis vulgaris*, *Nigella sativa, Camellia sinensis, Catharanthus roseus, Annona reticulata, Eleusine coracana,* and *Ziziphus nummularia*. Each ligand that was chosen was examined using ADME analysis.

ADME/T Analysis

Drug research and development require a thorough understanding of drug absorption, distribution, metabolism, excretion, and toxicity (ADMET). A high-quality drug candidate should exhibit suitable ADMET characteristics at a therapeutic dosage and have enough activity against the therapeutic target. The DruLiTo open-source software was used to calculate the drug-likeness of compounds in order to determine their molecular properties for humans (Swati et al., 2024) Only those ligands that satisfy Lipinski's five requirements were investigated for molecular docking. Additionally, a publicly accessible, verified, and tested software tool called ProTox II (Swati et al., 2024) was utilized to estimate the toxicity of the final chosen molecule.

Molecular Docking and Interaction Study

PyMOL software (Chetanath Neupane et al., 2020) was used to remove the water molecules and other heteroatoms from the protein molecule. Moreover, the protein was minimized by utilizing the AMBER ff14SB forcefield in Chimera software (Chetanath Neupane et al., 2020). Using AutoDock tools (ADT), the final steps in protein and ligand preparation were adding hydrogen polarities, Gasteiger and Kollman charges, and missing residues to the structures of the proteins and ligands (Swati et al., 2024). The receptor's active site pocket was located by utilizing the Autoligand module of ADT and BIOVIA Discovery Studio Visualizer 2020 (Srivastava et al., 2021) to acquire the coordinates for the ligand's potential binding location. The active site was taken into account when creating a grid box in ADT. Autodock Vina docking tool was used for molecular docking that took the atomic coordinates of the target protein and selected ligand, thus predicting the most suitable docking conformation of the two (Swati et al., 2024). The interaction was visualized on

BIOVIA Discovery Studio Visualizer 2020 (Srivastava et al., 2021; Swati et al., 2024). This program helps to project 3D structure to a 2D image, thus facilitating close inspection of 2D interactions of the protein-ligand complex.

Molecular Dynamics Simulation Study

The molecular dynamics (MD) simulation process was meticulously executed in several stages to ensure precise and stable outcomes. Initially, the CHARMM36 force field (Lee et al., 2016) was employed within the GROMACS 2021.4 software (Frisch et al., 2016), with parameter and topology files generated using CGenFF (Vanommeslaeghe & MacKerell, 2012). The system was then enclosed in a cubic water box of 10 Å dimensions, established via the gmx editconf module, and subsequently solvated using the TIP3P water model with the gmx solvate module. Energy minimization followed, utilizing the steepest descent algorithm over 50,000 steps. To neutralize the system, sodium and chloride ions were introduced.

Equilibration of the system was conducted in two distinct steps. The first step involved the NVT ensemble, where the V-rescale thermostat (Bussi et al., 2007) maintained the temperature at 298 K for 100 ps. The second step used the NPT ensemble, employing the V-rescale thermostat and the Berendsen barostat (Berendsen et al., 1984) to maintain conditions at 358 K and 1 atm for another 100 ps. The production run was then carried out in the NPT ensemble using the V-rescale thermostat and the Parrinello-Rahman barostat (Parrinello & Rahman, 1981), sustaining a temperature of 300 K for 50 ns. Post-simulation, the resulting trajectories were analyzed with various GROMACS utilities, ensuring thorough examination and interpretation of the simulation data.

Results and Discussions

ADME analysis and Active site prediction

Considering less studied phytochemicals from the selected plants, we studied 500 potential lead molecules to find their efficacy against MCL-1. Lipinski's rule of five determines the oral route of administering a drug in humans. After screening molecules using DruLito software, it was identified that 215 molecules were suitable for lead molecules, owing to Lipinski's rule of 5.

The active site of protein 4wmr was identified using Biovia Discovery Studio and AutoDock tools (Figure 1). The grid box's X, Y, and Z centers were -3.68477, -10.8415,

and 5.63033, respectively. The size of the box was 40 in all three directions.

Molecular Docking Analysis

Prior to docking analysis, we minimized the selected proteins. After minimization, the final potential energy came down for 4wmr to -18153.7 kJ/mol with an RMSD value of 0.144319. Further, we have selected a compound, namely Obatoclax, as our positive control, which is widely reported to be active against MCL-1. The binding energy of three compounds, namely Corynan-17-oic acid, Liriodenine, and Cyclointergin, was lower than Obtaoclax (Table 1). Corynan-17-oic acid was found to possess the lowest binding energy and thus was selected for simulation study along with Obatoclax.

Protein-ligand interaction and Toxicity analysis

The Corynan-17-oic acid and Obatoclax were first subjected to interaction analysis. The overall interacting residues with the nature of interaction obtained from Biovia Discovery Studio are depicted in Figures 2 and 3. Corynan-17-oic acid shows two possible hydrogen bonds with Leu267 and Val253; however, no hydrogen bonds are shown in the interaction with Obtaoclax. Corynan-17-oic acid and Obatoclax interacted with 6 and 10 amino acids on the active site through the van der Waals force of attraction. Further, both ligands do not show any unfavorable interaction with protein.

We have performed toxicity analysis to identify potential toxicity caused by either Corynan-17-oic acid or Obatoclax. Obatoclax was found to be an active agent to cause mutagenicity and ecotoxicity. Corynan-17-oic acid was inactive for most toxicity, including hepatotoxicity, carcinogenicity, cardiotoxicity, immunotoxicity, ecotoxicity, and mutagenicity. The radar Chart for both ligands for their ADME properties is shown in Figure 4. Corynan-17-oic acid molecular properties are within the acceptable range; however, Obatoclax defies one parameter of the molecular properties.

Molecular Dynamics Simulations Analysis

Molecular Dynamics simulations are a powerful tool for investigating the stability and dynamics of protein-ligand complexes under physiological conditions. By running 50 ns of MD simulations on protein-ligand complexes, researchers can obtain detailed insights into the interactions and stability of the complexes over time. In this study, the protein 4wmr was analyzed in complex with

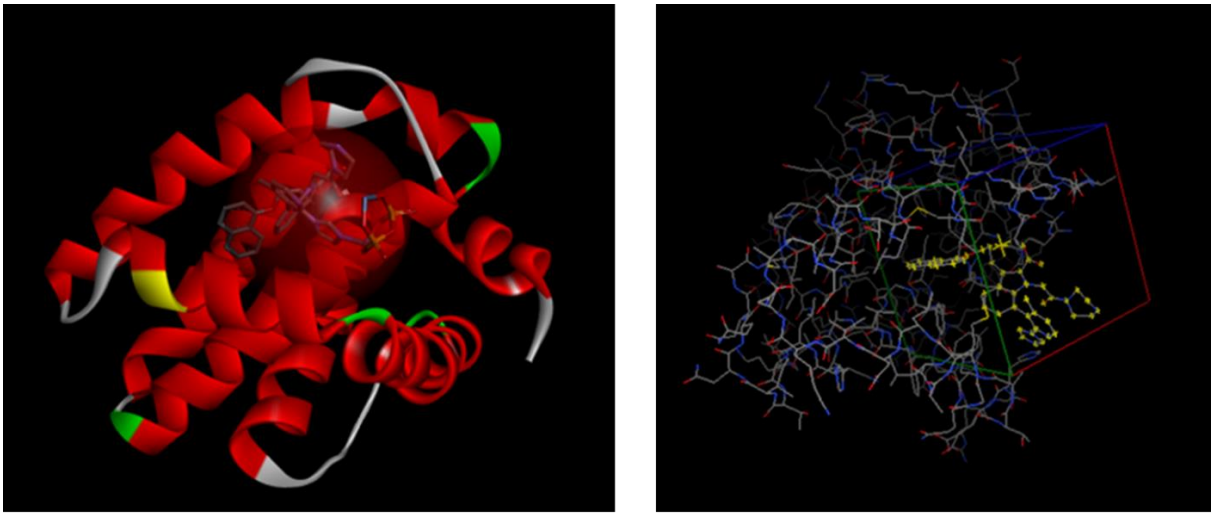
two ligands: Corynan-17-oic acid and Obatoclax. The efficacy of these ligands within the binding cavity of the protein was assessed using several critical parameters derived from the simulation trajectories. Root Mean Square Deviation (RMSD) measures the average deviation of the protein and ligand atom positions from a reference structure over time, providing insights into the overall structural stability of the protein-ligand complex. Root Mean Square Fluctuation (RMSF) offers information on the flexibility of specific residues within the protein, helping identify regions with significant fluctuations that could impact ligand binding stability. The number and stability of hydrogen bonds between the protein and the ligand are crucial for maintaining the integrity of the complex, with hydrogen bond interactions being vital to understanding the stabilization mechanisms. Lastly, the Radius of Gyration (RGyr) measures the compactness of the protein structure, where stable RGyr values suggest the protein maintains its overall shape throughout the simulation. By analyzing these parameters, researchers can evaluate the dynamic stability of the protein-ligand complexes and gain valuable insights into the movements and interactions that influence their stability.

RMSD is a widely used metric in MD simulations to measure atoms' average movement or deviation between two reference frames. It provides valuable information about the stability of protein-ligand complexes during MD simulations, which are typically conducted over various nanosecond timescales. Generally, less fluctuation and stable backbone atom RMSD are considered favorable evidence for the stability of the docked complex. Graphical analysis of the protein backbone RMSD values interprets the stability of the docking complex over time (Figure 5). The average RMSD values for the native protein (4wmr), 4wmr-Obatoclax, and 4wmr-Corynan-17-oic acid are 4.66, 4.83, and 4.79 nm, respectively. The fluctuation of RMSD obtained from all three simulations ranges from 1 to 3 Å, which is considered consistent and indicative of the system reaching equilibrium. This stable RMSD suggests that the protein-ligand complexes maintain their structural integrity over the simulation period, thereby supporting their potential stability under physiological conditions.

Ligand Name	PubChem ID	Binding Energy (kcal/mol)

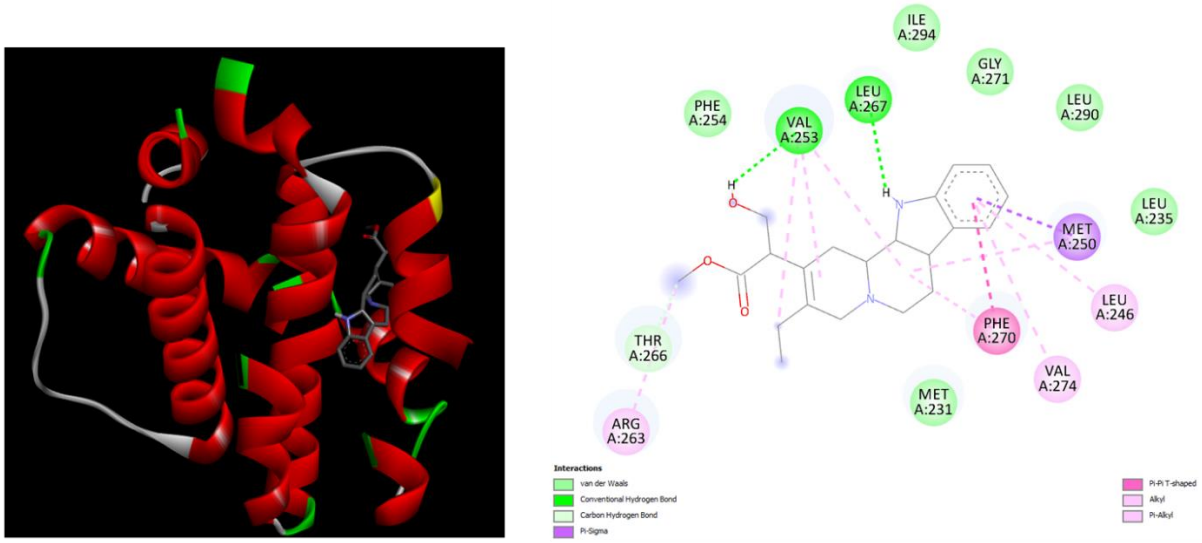
Obatoclax (Control compound)	11404337	-8.7
Corynan-17-oic acid	5377267	-9.1
Liriodenine	10144	-8.9
Cyclointegrin	44258672	-8.9

Table 1: Binding energy of top compounds obtained through molecular docking study.



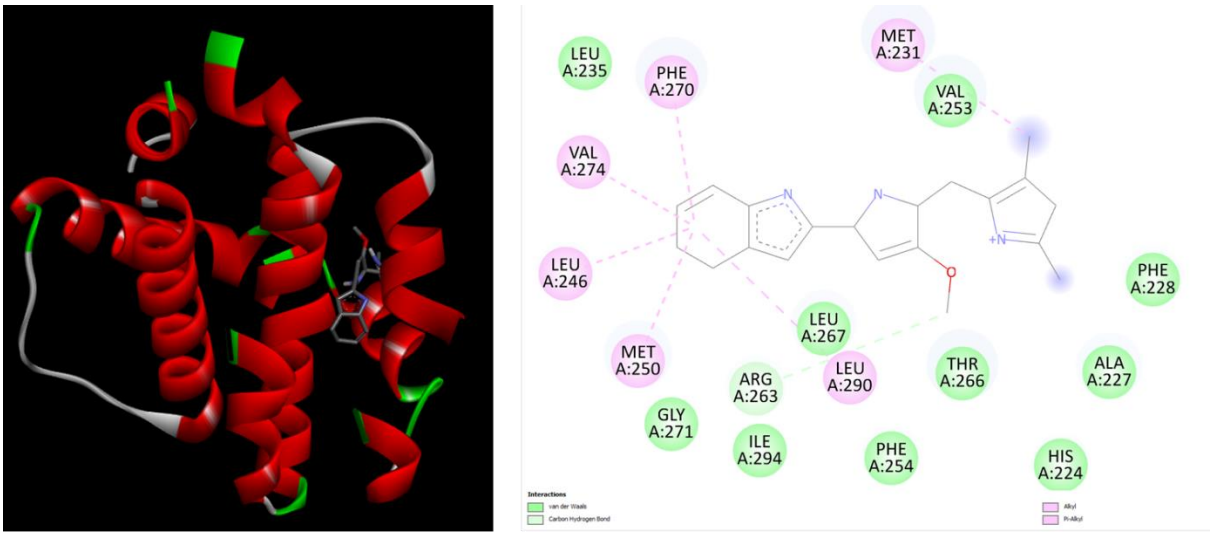
(A) **(B)**

Figure 1: Active site obtained for 4wmr. (A): Discovered through Biovia Discovery studio; (B): Discovered through AutoDock tools autoligand module.



(A) (B)

Figure 2: Protein-Corynan-17-oic acid interaction. (A): Three dimensional interaction; (B): Two dimensional interaction.



(A) (B)

Figure 3: Protein-Obatoclax interaction. (A): Three dimensional interaction; (B): Two dimensional interaction.

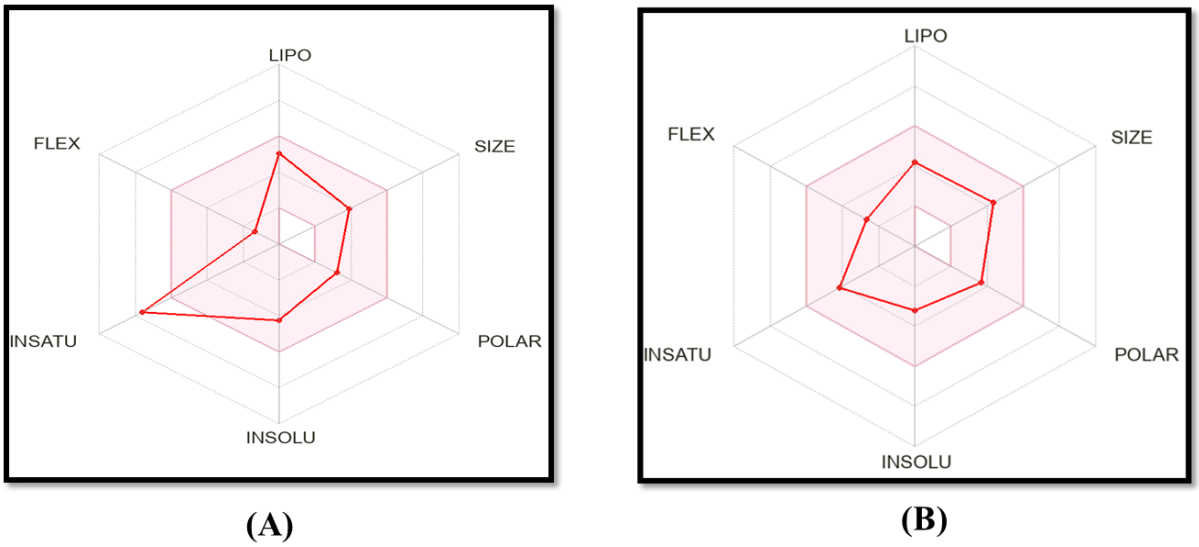


Figure 4: Radar Chart specifying molecular properties of ligands. (A): Obatoclax; (B): Corynan-17-oic acid.

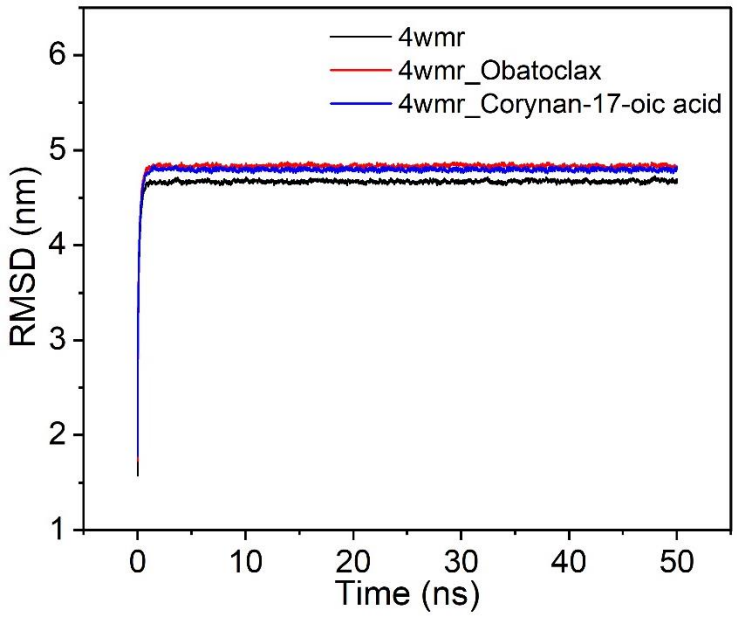


Figure 5: RMSD plot obtained for native protein (4wmr), 4wmr- Obatoclax, and 4wmr- Corynan-17-oic acid.

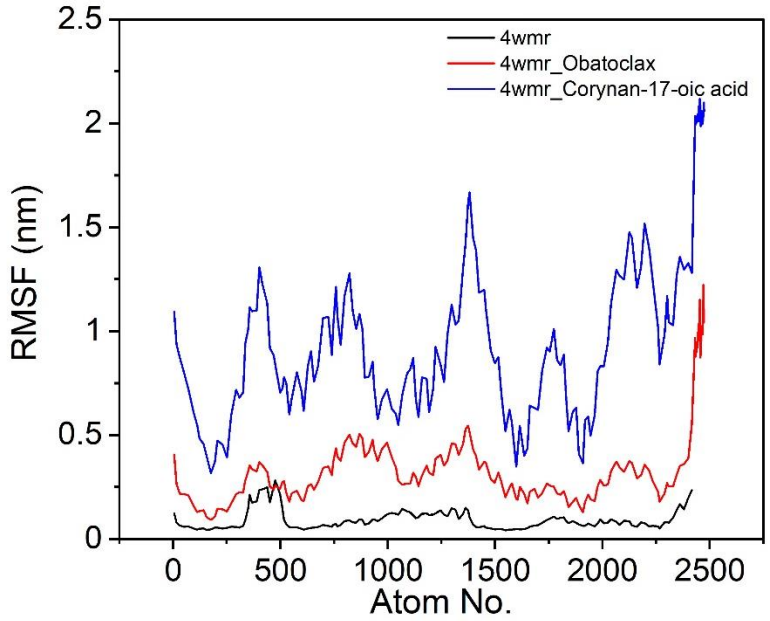


Figure 6: RMSF plot obtained for native protein (4wmr), 4wmr- Obatoclax, and 4wmr- Corynan-17-oic acid.

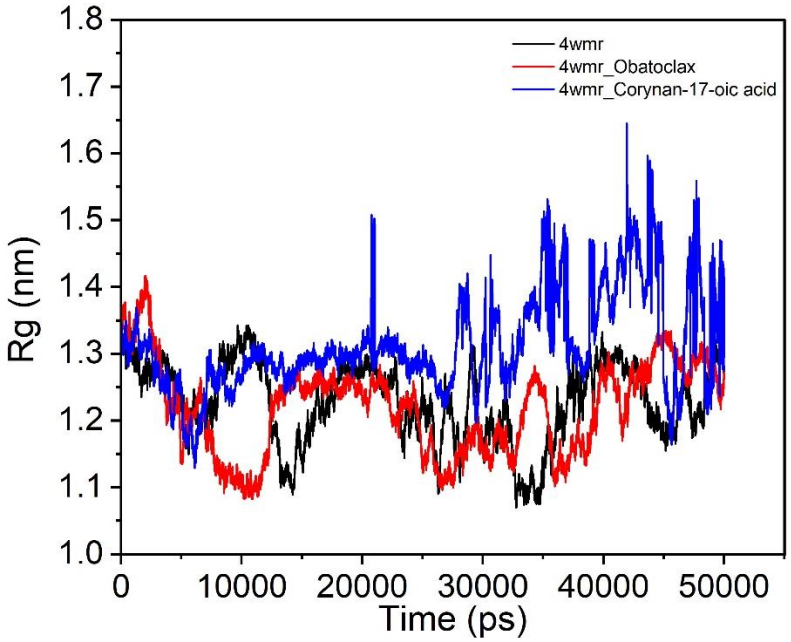


Figure 7: Radius of gyration plot obtained for native protein (4wmr), 4wmr- Obatoclax, and 4wmr- Corynan-17-oic acid.

RMSF determines the residue-by-residue alterations of a protein during the course of a simulation. It is a critical metric for protein characterization as it provides detailed information about the local changes in the protein chain. RMSF focuses on residue fluctuations, revealing the significance of these fluctuations in the flexibility of functionally important residues. Generally, low RMSF values are associated with stability, while high RMSF values indicate greater flexibility. Significant alterations were observed throughout the simulation at the N-terminal regions known for being flexible. The native protein and the protein complexes with Obatoclax and Corynan-17-oic acid showed RMSF fluctuations within an acceptable and consistent range. After binding with Obatoclax and Corynan-17-oic acid, the protein's conformation was affected, as indicated by changes in the pattern of the c-alpha RMSF trajectories compared to the native protein (Figure 6). The RMSF graph revealed that the native protein and each complex exhibited comparable fluctuations, particularly near the amino acids present in the protein's active site, suggesting that binding did not significantly destabilize these critical regions.

The RGyr parameter, determined from MD simulation trajectories, is an important measure for assessing the stability of protein-ligand complexes. It represents the protein's tertiary structure and overall size, crucial for understanding its compactness and folding. During MD simulation, a constant and consistent fluctuation of RGyr suggests a well-folded protein. The RGyr graph, generated for all complexes, showed no aberrant or exceptional deviations in the protein complexes with ligands or the free apo-protein. As shown in Figure 7, the RGyr values obtained from the simulations for all three complexes were reported to be between 12-16 Å. Although a slight deviation was initially observed in the protein-ligand complexes, the values eventually achieved stability, indicating the system's compactness and suggesting that the protein maintains its structural integrity throughout the simulation.

Conclusions

This study aims to find an effective treatment for individuals with Imatinib-resistant chronic myeloid leukemia by targeting the anti-apoptotic protein MCL-1. Utilizing in-silico drug repurposing methods, we seek to identify known compounds with potential therapeutic effects, ultimately contributing to more efficient and targeted therapies for patients worldwide. Initially, we screened 500 herbal compounds and applied Lipinski's

Rule of 5 to ensure drug-likeness, which resulted in 215 suitable compounds. These compounds were subjected to molecular docking studies using PDB ID: 4wmr for MCL-1, identifying three potent phytochemicals with binding energies less than -8.8 kcal/mol. Corynan-17-oic acid showed the most promising results, with a binding energy of –9.1 kcal/mol. This compound and the positive control Obtaoclax were further analyzed through molecular dynamics simulation, confirming its potential to inhibit MCL-1 activity. Given that Obtaoclax has multiple side effects, it would be beneficial to explore the efficacy of Corynan-17-oic acid against MCL-1 using wet lab techniques. This study represents a significant step towards finding a promising herbal drug targeting MCL-1 in CML treatment.

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