# *In silico* analysis and molecular docking studies of mannich base Lawsone derivatives functionalized ZnO nanocomposites (LSDs) against BRAF and CDK4 proteins

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## *Abstract*

*Cyclin-dependent kinase 4 (CDK4) is a potent anti-cancer therapeutic target because of its role in cell proliferation. BRAF is the most important member of the RAF kinases family. 66% of melanomas and 7% of all cancers have been found to have BRAF mutations. Therefore, selective inhibition of CDK4 and BRAF may enhance therapeutic efficacy. The Lawsone derived Mannich base functionalized ZnO nanocomposites (LM@ZnO) have been successfully synthesized. To apprehend the LM@ZnO as an antitumor agent, we have performed in silico studies against BRAF and CDK4 proteins.*

*Molecular docking studies of all LM@ZnO revealed greater binding energy when compared to the standard drug doxorubicin which displayed -110.6 and -107.212 kcal/mol against CDK4 and BRAF proteins respectively. This study validates that anticancer efficacy is not always dependent on antioxidant activity; there could be another mechanism that leads to cell growth suppression during the cell cycle.*

**Keywords:** *In silico*, Molecular docking, Molecular dynamic Simulation, Anticancer, CDK4, BRAF, ZnO nanocomposites, Mannich base derivatives

## Introduction

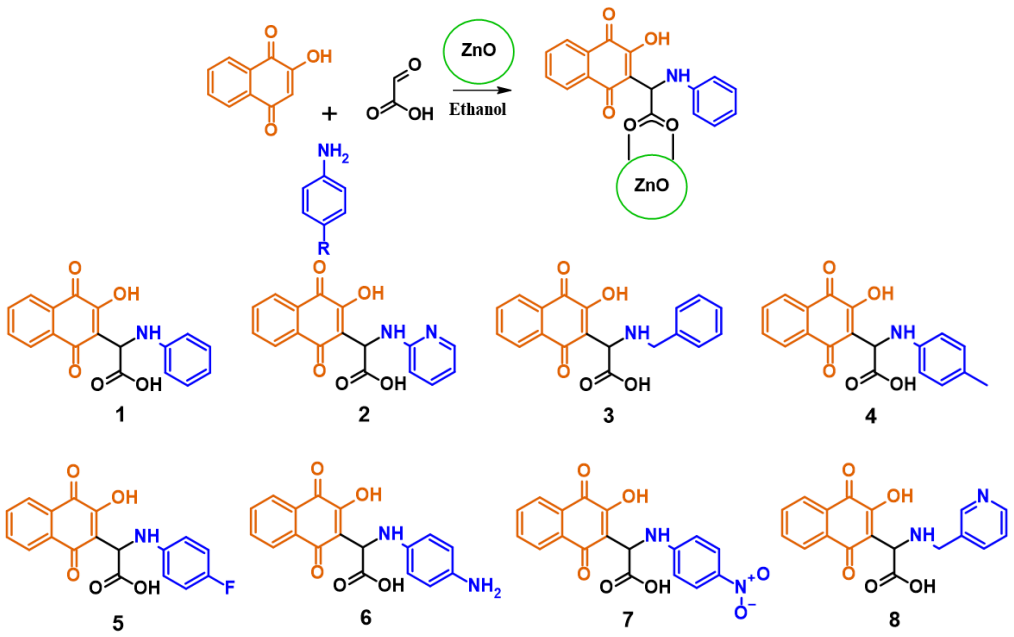
The second most common cause of death in the world is cancer, a serious health issue that can affect any part of the body[14]. Nearly 10 million deaths, or one in six deaths, were caused by cancer in 2020, making it the leading cause of death globally. It is regarded as a serious public health issue that will be accountable for 1 in 8 deaths for men and 1 in 11 deaths for women respectively[10]. The most fatal form of skin cancer is melanoma[21]. Melanoma, the deadliest form of skin cancer, grows in the cells (melanocytes) that produce melanin, the pigment that gives your skin its color. Melanoma can also develop in the eyes and in rare cases, inside the body, such as the nose or throat[12]. More than 300,000 people were affected by cutaneous melanoma in 2020 and there were about one million cases over the previous five years, according to data on the disease's incidence[25]. The naphthoquinones class of natural compounds, which are members of the quinone family, function as a component in a number of biochemical systems, including the human immune system[19]. Numerous naphthoquinones have demonstrated the capacity to lessen the stemness and metastatic potential of cancer. In comparison to other classes of naphthoquinones, furano-naphthoquinones, a subclass of naphthoquinones distinguished by the presence of an additional furan ring, have shown improved anti-cancer potency. Pharmacological properties as antibacterial, antifungal, antitumoral, or antiprotozoal agents are present in a number of naphthoquinones[17].

One of the naturally occurring naphthoquinone derivatives known for its biological activity is Lawsone (2-hydroxy-1,4-naphthoquinone). A number of its derivatives have antitumor, antibacterial and antifungal activity[6,26]. This substance, which is found in *Lawsonia inermis*, is a precursor in the synthesis of compounds with potential biological activity. Although many naturally occurring substances with biological activity have been studied, many of them lack the necessary safety, carcinogenicity and mutagenicity to be used therapeutically. Today, it is possible to alter the chemical structures of active substances to create compounds with increased therapeutic activity and decreased toxicity[5]. Naphthoquinones and their derivatives' distinctive structural, biological and functional characteristics have drawn a lot of attention, especially from a medicinal perspective[15].

The recent advances in computational approaches have significantly developed the rationale for identifying and designing pharmacologically active natural molecules that can target proteins of interest. The natural compounds obtained from plants can be repurposed by computational approaches to prove their potential. Here, in the present study, we have used, mannich base Lawsone derivatives functionalized ZnO nanocomposites (LSDs). To apprehend the LSDs as an antitumor agent, we have performed *in silico* studies against BRAF and CDK4 proteins. In the present study.

## Material and Methods

**General procedure for the one-pot synthesis of Lawsone derived Mannich base functionalized ZnO nanocomposites (LM@ZnO):** Synthesis of Lawsone derived Mannich base functionalized ZnO nanocomposites is presented in figure 1.




Lawsone derived Mannich base functionalized ZnO nanocomposites

**Figure 1: Schematic representation of Synthesis of Lawsone derived Mannich base functionalized ZnO nanocomposites**

***In silico* anticancer evaluation of the Lawsone derivatives functionalized ZnO composites:** For rational drug design and discovery as well as in mechanistic studies, molecular docking is an intriguing framework for identifying drug biomolecular interactions. This helps to identify a molecule's preferred orientation when it is getting ready to interact with a macromolecule's (a protein's) binding pocket[3]. The docking procedure's data can be used to calculate the stability, free energy and binding energy of two molecules[16]. *In silico* molecular docking has developed into a successful drug discovery strategy for the disclosure of structure-based medicines[11]. The most well-known and widely used programme for molecular docking is Auto Dock. Auto Dock is an open-source programme for computational docking and virtual screening of small compounds to receptors[22].

In order to gather preliminary data on the binding energy, free energy and stability of ligands with particular proteins (CDK4 and BRAF), docking experiments were conducted. A typical cell cycle kinase that forms complexes with D-type cyclins is called cyclin-dependent kinase 4 (CDK4). CDK4 is a potent anti-cancer therapeutic target because of its role in cell proliferation. BRAF is the most important member of the RAF kinases family. 66% of melanomas and 7% of all cancers have been found to have BRAF mutations[7]. Therefore, selective inhibition of CDK4 and BRAF may enhance therapeutic efficacy.

**Ligand Preparation:** The Marvin sketch was used to create all of the Lawsone derivatives and each ligand molecule's functional group ($OH^-$) was given with ZnO. Doxorubicin (DOX), a common medication, was obtained from Pubchem. Doxorubicin was one of the molecules that was saved in the DOT MOL file and subjected to molecular docking analysis. Later, PyRx was used to perform energy minimization on all of the molecules in order to complete the normalization process and background adjustments. Additionally, the Marvin view, a potent chemical viewer for 2D or 3D chemical structures and related data, was used to examine each molecule. The chosen ligands were then converted into 3D structures using Biovia Discovery software for later use (Figure 2) displaying the structures of doxorubicin and Lawsone derivatives.

After causing DNA double-strand breaks and before DNA is re-ligated, doxorubicin inhibits topoisomerase II∝, causing DNA damage. For many years, it was believed that doxorubicin and its structural analogues' remarkable anticancer activity were primarily caused by this mode of action. N, N dimethyl doxorubicin, like aclarubicin, is a potent anticancer drug without the severe side effects seen in mouse models of doxorubicin. The type and placement of the substituent at the phenyl moiety determine the platelet anti-aggregate activity of Lawsone derivatives LS1 to LS8.

**Protein Preparation:** The three-dimensional structure of CDK4 and BRAF is presented (Figure 3) which was obtained from the PDB databank (PDB IDs: 2W96 and 1UWH) and was solved using the X-Ray diffraction method with a resolution of $5A^0$ against our chosen Lawsone derivatives. The Discovery Studio Visualizer was used to add polar hydrogens to the recovered protein structure prior to docking.

It is possible to evaluate the accuracy of the predicted protein structure using the Ramachandran plot. The Ramachandran plot of the CDK4 and BRAF proteins, shown in figures 4a and 4b revealed that 85.2 and 82.8 percent of the residues respectively were located in the highly preferred regions.

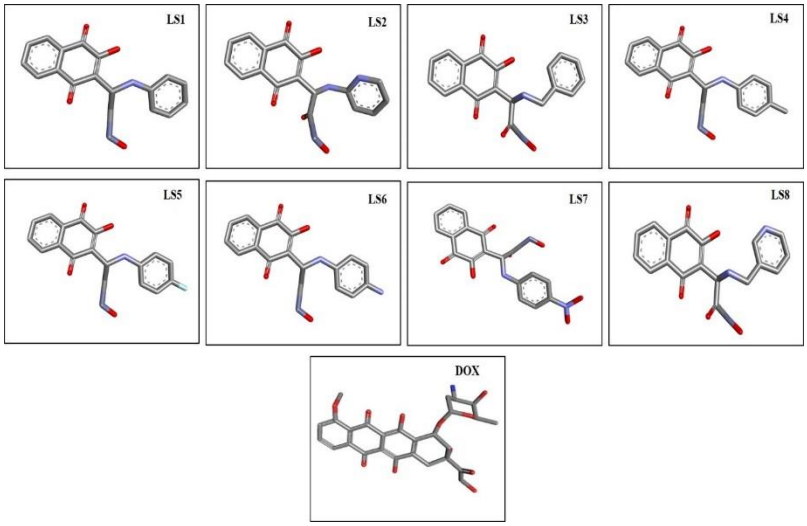



**Figure 2: 3D Structures of Lawsone derivatives LS1 to LS8 and DOX**

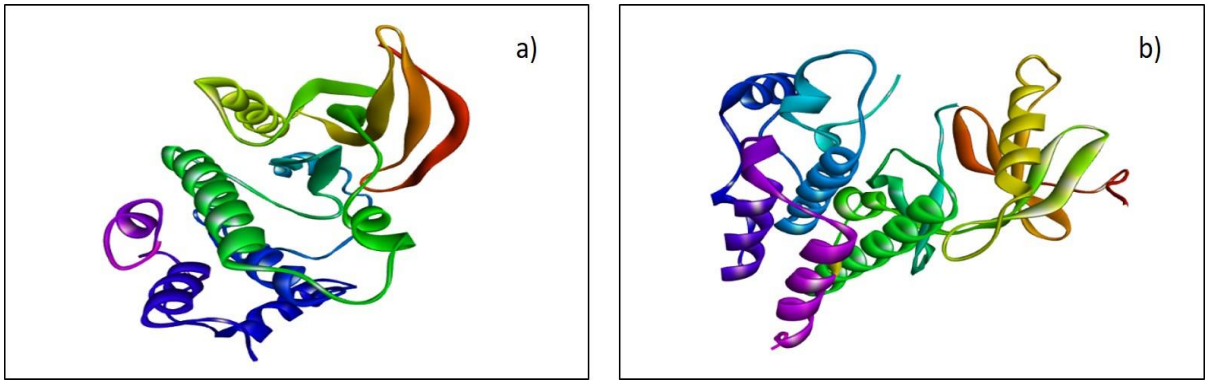
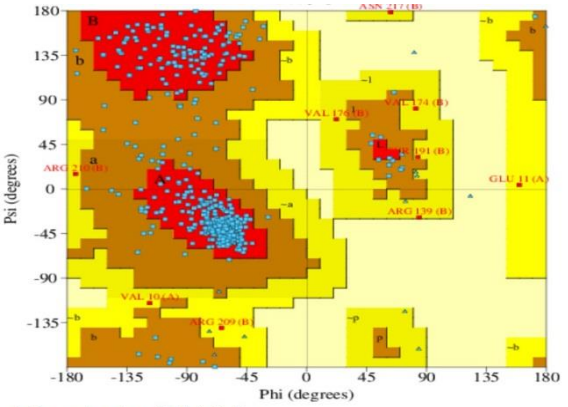


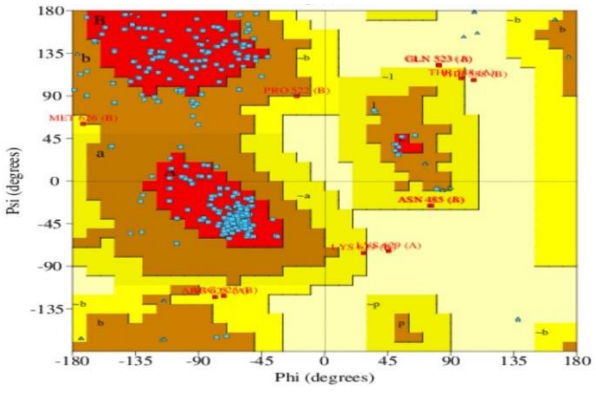

**Figure 3: 3D structure of a) CDK4 and b) BRAF proteins**




1. Ramachandran Plot statistics

| | | No. of residues | %-tage |
|---|---|---|---|
| Most favoured regions | [A,B,L] | 391 | 85.2%* |
| Additional allowed regions | [a,b,l,p] | 59 | 12.9% |
| Generously allowed regions | [~a,~b,~l,~p] | 9 | 2.0% |
| Disallowed regions | [XX] | 0 | 0.0% |
| Non-glycine and non-proline residues | | 459 | 100.0% |
| End-residues (excl. Gly and Pro) | | 8 | |
| Glycine residues | | 19 | |
| Proline residues | | 30 | |
| Total number of residues | | 516 | |

**Figure 4a: Ramachandran plot for the modelled CDK4 protein**




1. Ramachandran Plot statistics

| | | No. of residues | %-tage |
|---|---|---|---|
| Most favoured regions | [A,B,L] | 381 | 82.8%* |
| Additional allowed regions | [a,b,l,p] | 68 | 14.8% |
| Generously allowed regions | [~a,~b,~l,~p] | 7 | 1.5% |
| Disallowed regions | [XX] | 4 | 0.9%* |
| Non-glycine and non-proline residues | | 460 | 100.0% |
| End-residues (excl. Gly and Pro) | | 8 | |
| Glycine residues | | 36 | |
| Proline residues | | 24 | |
| Total number of residues | | 528 | |

**Figure 4b: Ramachandran plot for the modelled BRAF protein**

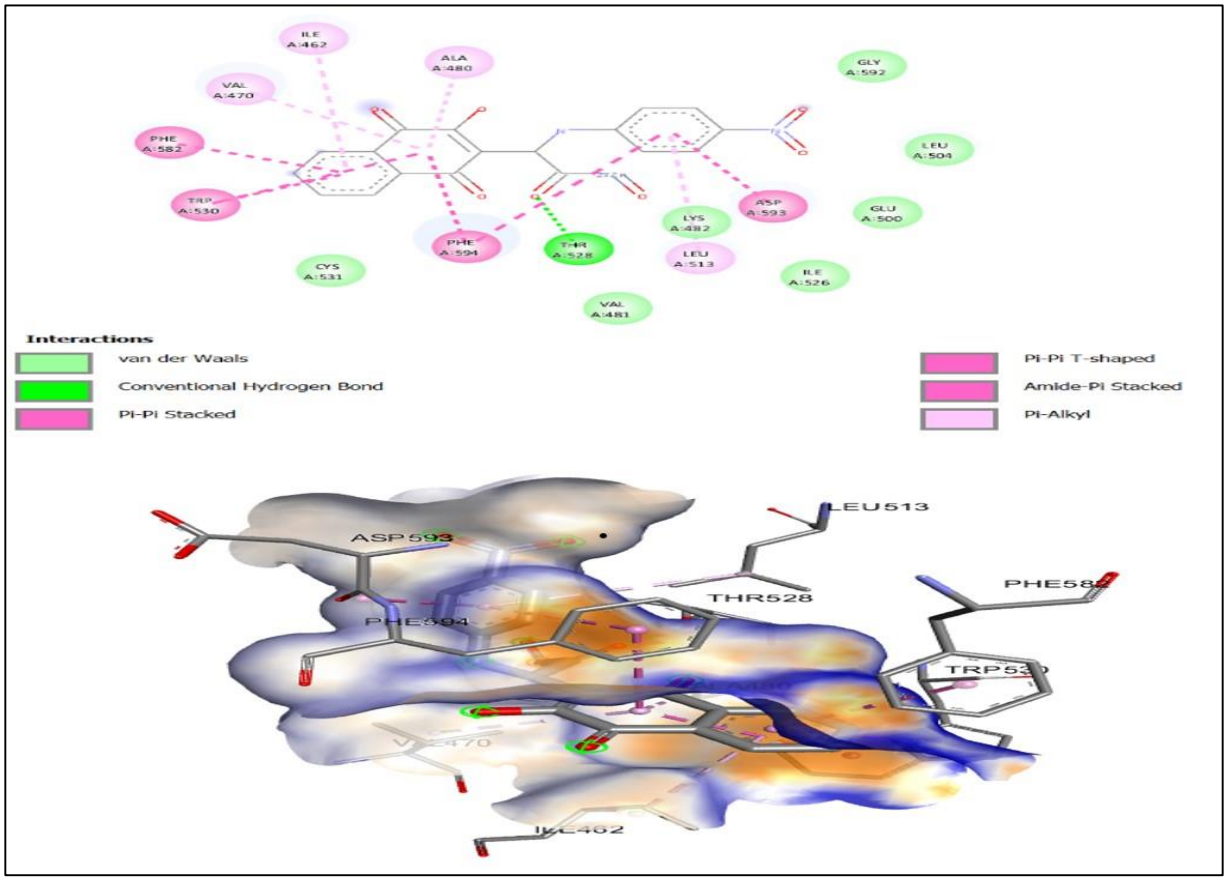



**Figure 5a: 2D and 3D interaction of highest docked LS7with BRAF protein.**

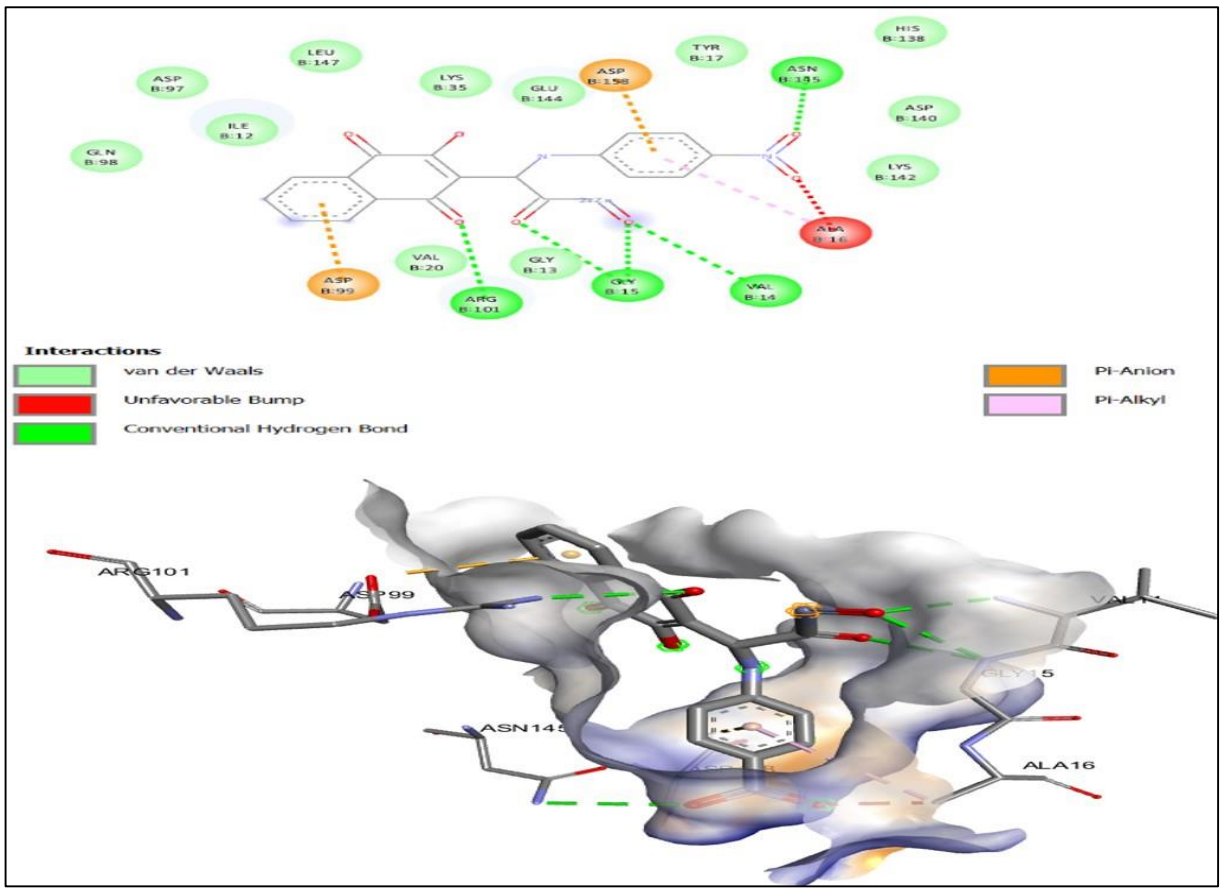



**Figure 5b: 2D and 3D interaction of Highest docked LS7 with CDK4 protein**

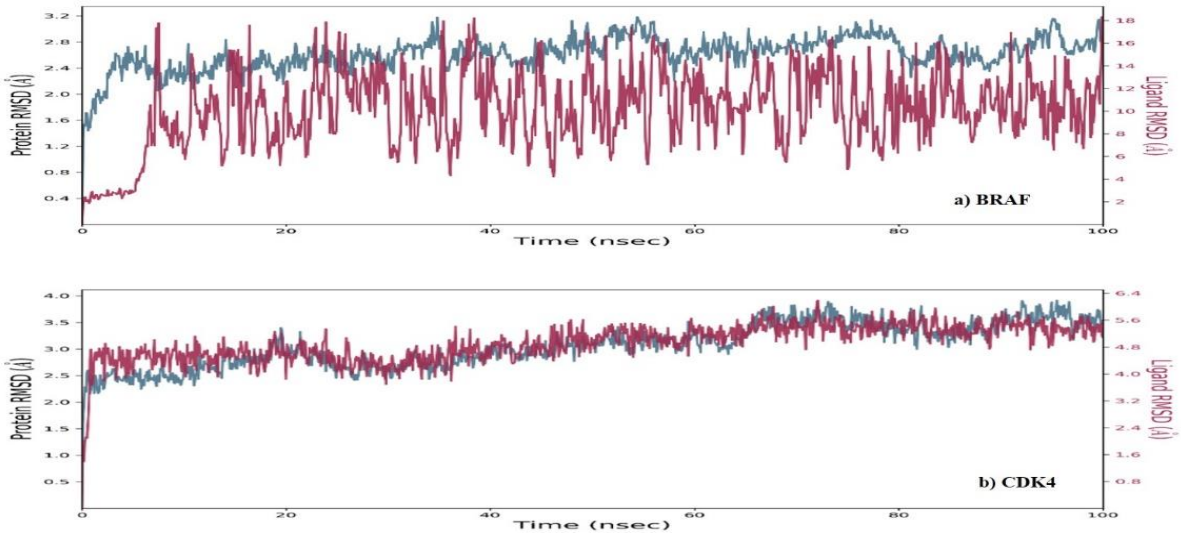



**Figure 6: Represents the RMSD of LS7 against a) BRAF b) CDK4 proteins**

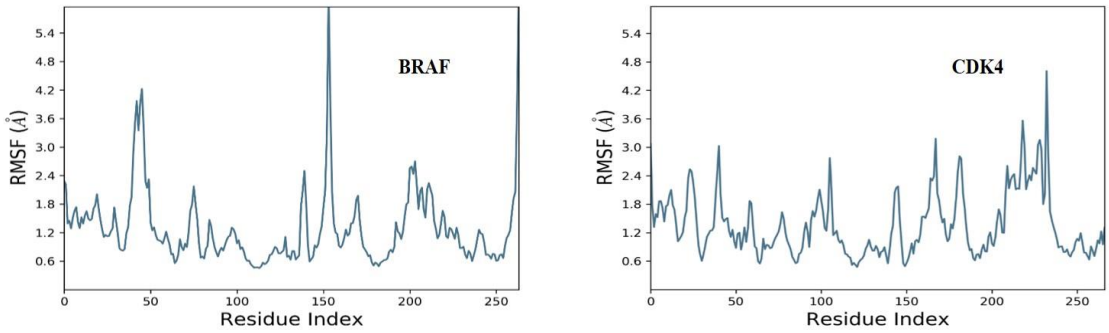



**Figure 7: RMSF plot of the LS7-BRAF and CDK4 complexes calculated from MD simulation from 1-100ns.**

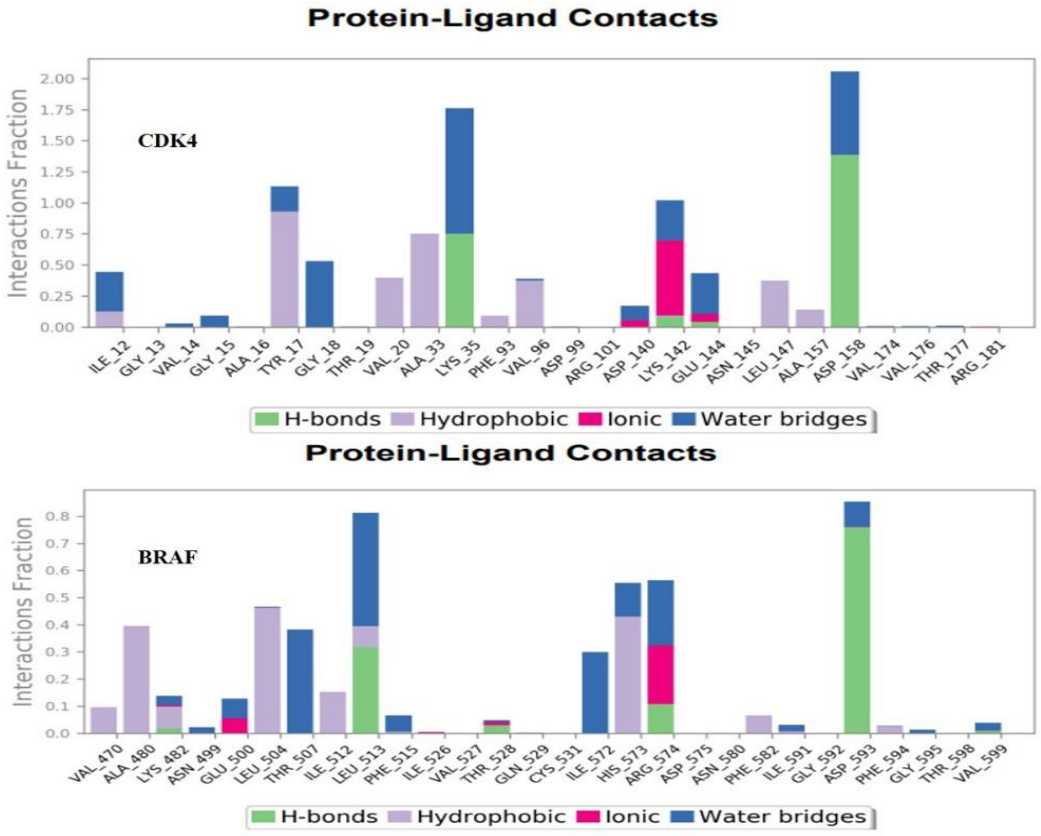



**Figure 8: Represents the Ligand-protein interactions during MD simulation.**

## Results and Discussion

**Molecular Docking Analysis:** A crucial step in molecular docking is to estimate and to find the right target for effective docking. A suitable docking tool can be used to look for the best binding site between the ligand and the target protein using the known three-dimensional structure of the target protein[23]. Several docking tools for nanoparticle-based molecular docking are available (NBMD). The user-friendly and open-source iGEMDOCK programme is being used in this investigation. The proteins will be examined after docking is complete[9]. So, following docking, the results were recorded and the ranking was finished using the binding energy function.

The various interaction scores obtained using the iGEMDOCK software for LSDs (LS1-LS8) against BRAF and CDK4 proteins are shown in tables 1 and 2. Fitness is defined as the total energy of a predicted pose in the binding site. The van der Waals, hydrogen bonding and electrostatic energies are added together to create the iGEMDOCK scoring function. The strongest negative values imply a more potent active ingredient.

Doxorubicin, a reference medication, was used to compare the binding score with the LSDs (LS1-LS8) and it was found that the total energy for the BRAF and CDK4 proteins was -107.212 and -110.6 kcal/mol respectively. According to the results, LS7 binds to BRAF and CDK4 with the highest binding energies of -120.165 and -122.571 kcal/mol respectively. LSDs were discovered to have scores that were comparable to the norm and showed best-fit score values. The figures 5a and 5b represent various interactions of LS7 ligand, doxorubicin and amino acids in the active sites of BRAF and CDK4. Further the highest docked composite is subjected to ADMET analysis.

**ADMET Analysis:** A potent molecule must be delivered to its target in sufficient concentration and remain there in a bioactive form for an extended period of time for the predicted biological activities to take place for it to be useful as a medicine. When a large number of compounds are being investigated but there is limited access to physical samples, drug development increasingly involves assessing absorption, distribution, metabolism and excretion (ADME)[4]. Swiss ADME is a web-based server that calculates various physicochemical descriptors, drug-like nature predictions, ADME parameters, medicinal chemistry friendliness and pharmacokinetic features to support drug discovery.

The detailed results for each category for the hit compound mentioned earlier are shown in table 3. The compounds physicochemical characteristics fall within the drug-likeness range and do not violate any of the Lipinski rule criteria. A common metric used to increase the ability of medications to cross the blood barrier is topological polar surface area (TPSA) which is defined as the surface sum of all polar atoms[18].

**Table 1**
**iGEMDOCK score for BRAF**

| Ligand | | Total Energy | Vander Waals | H-bond |
|---|---|---|---|---|
| BAX-LS1 | | -98.1581 | -92.2209 | -5.93717 |
| BAX-LS2 | | -114.429 | -108.559 | -5.8701 |
| BAX-LS3 | | -99.0836 | -90.5895 | -8.4941 |
| BAX-LS4 | | -107.704 | -106.833 | -0.87096 |
| BAX-LS5 | | -104.302 | -98.7062 | -5.59606 |
| BAX-LS6 | | -105.439 | -95.9388 | -9.5 |
| BAX-LS7 | | -120.165 | -110.47 | -9.29751 |
| BAX-LS8 | | -109.435 | -99.1788 | -10.2558 |
| Doxorubicin | | -107.212 | -82.91 | -24.302 |

**Table 2**
**iGEMDOCK score for CDK4**

| Ligand | Total Energy | Vander Waals | H-bond |
|---|---|---|---|
| BAX-LS1 | -99.2537 | -69.5638 | -29.6899 |
| BAX-LS2 | -103.729 | -86.557 | -17.1715 |
| BAX-LS3 | -106.455 | -85.4327 | -21.0223 |
| BAX-LS4 | -114.138 | -99.8194 | -14.3189 |
| BAX-LS5 | -99.4094 | -92.1097 | -7.29967 |
| BAX-LS6 | -109.529 | -84.7629 | -24.7664 |
| BAX-LS7 | -122.571 | -84.9496 | -36.668 |
| BAX-LS8 | -118.188 | -99.9886 | -18.1995 |
| Doxorubicin (Control) | -110.6 | -87.16 | -23.70 |

**Table 3**
**ADMET analysis of highest docked LS7**

| **Physicochemical Properties** | | **Lipophilicity** | |
|---|---|---|---|
| Formula | C18H12N2O6Zn | Ilogp | 0 |
| MW | 417.68 | XLOGP3 | 1.72 |
| Heavy atoms | 27 | WLOGP | 1.8 |
| Aromatic heavy atoms | 12 | MLOGP | 0.84 |
| Fraction Csp3 | 0.17 | SILICOS-IT | 0.68 |
| Rotatable bonds | 6 | Consensus Log Po/w | 0.4 |
| H-bond acceptors | 8 | **Pharmacokinetics** | |
| H-bond donors | 1 | GI absorption | High |
| Molar Refractivity | 88.89 | BBB permeant | No |
| TPSA | 94.5 $Å^2$ | Pgp substrate | No |
| **Water Solubility** | | CYP1A2 inhibitor | No |
| ESOL (Log S) | -3.45 | CYP2C19 inhibitor | No |
| ESOL Solubility (mg/ml) | 1.50E-01 | CYP2C9 inhibitor | No |
| ESOL Class | Soluble | CYP2D6 inhibitor | No |
| Ali Log S | -3.32 | CYP3A4 inhibitor | No |
| Ali Solubility (mg/ml) | 2.00E-01 | log Kp (skin permeation) | -7.63 cm/s |
| Ali Solubility (mol/l) | Soluble | **Druglikeness** | |
| Ali Class | Soluble | Lipinski | Yes; 0 violation |
| Silicos-IT LogSw | -3.84 | Ghose | Yes |
| Silicos-IT Solubility (mg/ml) | 5.98E-02 | Veber | Yes |
| Silicos-IT Solubility (mol/l) | 1.43E-04 | Egan | Yes |
| Silicos-IT class | Soluble | Muegge | Yes |
| | | Bioavailability Score | 0.56 |
| **Medicinal Chemistry** | | | |
| PAINS | | 1 | |
| Lead likeness | | 1 | |
| Synthetic Accessibility | | 3.84 | |

The molecule also has a high lipophilicity which speeds up transport and allows it to reach the target site[2]. Importantly, the molecule does not interfere with all of the cytochrome P450 isoforms involved in drug removal via metabolic biotransformation and has good gastrointestinal absorption. Additionally, it was demonstrated that the molecule complied with all of the druggability criteria put forth by Lipinski[15], Veber et al[23] and Muegge et al[17]. The bioavailability rating for the compound is 0.55. This suggests that at least 10% of the molecule's bioavailability is likely to be there. From the perspective of synthetic chemistry, compound synthesis is straight forward. The substance is also anticipated to interact with just one biological target as opposed to a number of targets and should be free of pan-assay interference compounds (PAINS)[8].

**Molecular Dynamic Simulation (MDS):** The MDS of selected proteins with the highest docked ligand was carried out by employing the Desmond 2019 program, an open source solvent MD package with in-built optimized potential for liquid simulation (OPLS)[1]. Using a predefined water model (simple point charges, SPC) as the solvent in a cubic box with periodic boundary conditions that specified the box's shape and size as 10 Å *x*10 Å *x*10 Å distance, the simulation setup was carried out. To balance the systems, 1.5 mM of NaCl ions were added to the water-filled container. Next, the model system was relaxed before the simulation using the Desmond program's molecular dynamics tool, with a simulation time set up for 100 ns under typical NTP (constant number of Particles-N, pressure-P and temperature-T) conditions.

The Nose-Hoover thermostat algorithm was used to maintain a constant temperature of 300 K throughout the simulation[24]. The simulation interactive diagram tool went on to conduct a further graphical analysis of the simulation data, which revealed information about the characteristics of the protein-ligand complex during the simulation period. To further validate our findings in docking, root mean square deviations (RMSD), root mean square fluctuation (RMSF) and H-bond interaction data were examined.

Desmond Schrodinger's 2019.2 was used to run 100-ns MD simulations in order to pinpoint crucial hotspot residues at the protein-ligand interface and to assess the stability of the highest docked ligand's binding to BRAF and CDK4 protein complexes (Figure 6) displaying the root mean square deviations (RMSDs) for the simulations. According to the plots, the complexes are stable because their RMSDs stabilize before 10 ns and continue to do so for the duration of the simulation, which lasts 100 ns. The local changes

along the protein chain can be characterized using the root mean square fluctuations (RMSFs). The RMSFs of complexes are shown in figure 7 which suggest that the loop and tail regions of the protein fluctuate more than any other region. Less than 6 Å of overall minimal fluctuations was found throughout the simulation.

Hydrogen bonds are crucial for ligand binding. Because they have such a significant impact on drug specificity, metabolization and adsorption, hydrogen-bonding properties must be taken into account when developing new drugs (Figure 8) showing the formation of an H-bond between the highest docked ligand and the active site residues of CDK4 and BRAF. The highest docked ligand LS7 displayed 6 H-bonds with BRAF protein and 4 H-bonds with CDK4 (LYS-35, LYS-142, GLY-144 and ASP-158) (LYS-482, LEU-513, THR-528, ARG-574, ASP-593 and VAL-599).

Newly synthesized LSDs (LS1-LS8) demonstrated good docking scores against BRAF and CDK4 target proteins. When docked with the proteins BRAF and CDK4, LS7 displayed the highest docking score out of the 8 newly synthesized LSDs. The ADMET analysis of LS7 confirms that it interacts with a single biological target as opposed to several targets with a bioavailability score of 0.55.

Further, a 100ns molecular dynamic simulation was performed to ascertain the stability of the ligand with proteins. The highest docked ligand in this study, LS7, demonstrated its stability with proteins before 10 ns. When LS7's stability was compared to that of the BRAF and CDK4 proteins, LS7 demonstrated comparably strong stability with CDK4. This finding supports further testing of these recently created molecules for antioxidant and antiproliferative activity.

## Conclusion

Lawsone has the potential to be developed into an effective anticancer pharmaceutical agent with low toxicity. A molecular modeling study was carried out to understand the binding energy and binding stability of LSDs against BRAF and CDK4 proteins in order to investigate the anti-tumor activity of recently synthesized LSDs. Based on the binding mode analysis performed during molecular docking, all of the newly synthesized LSDs displayed interactions with BRAF and CDK4 that were comparable to those of doxorubicin. In the meantime, ADMET analysis was performed on the highest docked LSD (LS7) and the results displayed that the substance has both drug-like and physicochemical properties.

Results from molecular dynamic simulations displayed that ligand (LS7) is more stable with CDK4 than BRAF protein. All of the LSDs predicted to behave as lead compounds for *in vitro* analysis, according to the *in silico* study. Additionally, performance evaluation is necessary to validate the outcomes of *in vitro* analysis in the medical industry and create better models for figuring out the long-lasting effects of LSDs in people. Therefore, it is necessary to support the framework of *in vivo* research with thorough results.

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