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Computational Pharmacokinetic and Docking Analysis of Curcumin and Piperine as Inhibitors of E3 Ubiquitin Ligases for Lung Cancer

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ABSTRACT

Lung cancer is the world's most prevalent disease leading to several fatalities. An enormous amount of effort has been made to increase the disease-free survival rate, in this screening study for early diagnosis therapy. In this study, the selected hypothetical proteins of E3 ubiquitin ligase involved in lung cancer were studied. The sequence and structural annotations were determined by ExpasyProtParam and SOPMA software. The structural three-dimensional (3D) of these proteins were predicted through homology modeling using swiss-modeller. The natural compound inhibitors like piperine and curcumin were studied for binding mechanisms and pharmacokinetic analysis. The natural inhibitor piperine has the highest binding affinity towards the A0A0A8K9F1 hypothetical protein.

Keywords
Ubiquitin-Protein Ligases, Lung Neoplasms, piperine, Curcumin, hypothetical protein, drug development

Introduction

Lung cancer is a malignant tumor with uncontrolled development of cells in the tissue. It is the most prevalent cancer diagnosed in males, whereas in females it is the third most prevalent cancer. [1]. In 2012, around 14 million fresh instances and 8.2 million cancer-related fatalities were discovered, as reported by the WHO (World Health Organization). It is anticipated that the number of cases will increase over the next twenty decades, around 70 % [2].

The detection of lung cancer symptoms is often at the late stage when the prognosis is poor [3, 4].The leading cause is smoking with the highest 85% of all lung cancer cases. Hazardous chemicals in cigarette smoke have been proven to induce cancer. Other variables that may be triggered include radiation, asbestos, and living in an environment of neighbouring pollution as well as the virus of human immunodeficiency. Unlike non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC) is about 10–15% of pulmonary infections. The average 5-year survival range of NSCLC is 9% to 15% [5,6,7].

The human murine double minute 2 (MDM2) genes with a genomic size of 34 kb on chromosome 12q13-14 is a negative p53 regulator [8]. MDM2 is an E3 ubiquitin-protein ligase that mediates the ubiquitination of p53/TP53 leading to proteasome degradation [9]. Overexpression of this gene can lead to the excessive inactivation of p53, in another word it allowing the damage of the cells to evade the control of the cell checkpoint and becomes the carcinogenic cells [8]. Previous research revealed that an early incident of lung carcinogenesis was the accumulation of MDM2 in preneoplastic lung lesions. The MDM2 gene codifies an E3 ubiquitin ligase that acts as a strong inhibitor of the tumor suppressor P53, the master regulator of the development of the cell cycle, in a feedback loop that attracts excellent interest and attention

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as a prospective goal for tumor therapy. Natural products are a wealthy source for the discovery and growth of new therapeutic and preventive agents against human cancers, particularly those from medicinal and food crops. Curcumin is a polyphenol plant that occurs naturally has been proven to exhibit antioxidant, anti-inflammatory, anti-cancer, and chemopreventive properties with a very minimal side effect [10, 11, 12]. Recent studies show that Piperine has been used for such diseases as diarrhea, cardiovascular disease, insomnia it aids against respiratory disorders such as fever, asthma, and cold. Despite that, it has been approved that Piperine can exhibit antioxidant, anti-cancer, anti-inflammatory, and chemo preventive [13, 14]. Thus, in this study, we used Piperine and curcumin as an inhibitor natural compound against E3 ubiquitin ligase proteins targeting lung cancer.

Due to advancement in sequencing technology like next-generation sequencing technology, there is a tremendous increase in genome sequences of biological species are available in the database. As a result, there is increasing in protein sequence data deposited in sequence databases compared to experimentally isolated protein structure in Protein Data Bank (PDB) [15, 16]. It is usually expected that hypothetical proteins will be expressed from an open reading frame (ORF). These proteins have no experimental solid evidence of their functions [17]. Currently, 50% of a genome's proteins are presumed to be hypothetical proteins [18]. This promotes the use of the experimental information in the research of a hypothetical protein [18].

In this study, the lung cancer hypothetical protein of J3KN53, D0EKL1,A0A0A8K9F1was selected as the primary amino acid sequences. This protein was subjected to extensive studies to identify structural and molecular characteristics. Furthermore, the prediction of a good quality model of J3KN53, D0EKL1,A0A0A8K9F1 has been made by using the protein homology modeling techniques and successive of the computer-aided active site prediction for targeted against lung cancer.

Methodology

Retrieval of Sequence

The selected hypothetical protein (J3KN53, D0EKL1, A0A0A8K9F1) of E3 ubiquitin-protein ligase Mdm2 of lung cancer were retrieved from the UniProt database. Information about these proteins was accessible in Uniprot. For further proteomic analysis, the sequence of amino acids was then retrieved in the FASTA format.

Primary Structural Annotation

An ExPASy server tool, ProtParam (http:/web.expasy.org/protparam/), has been fully utilized to analyze the physicochemical properties of the protein sequence retrieved.For further analysis, this tool can predict the physicochemical characteristics including molecular weight, theoretical pI, aliphatic index, amino acid composition, GRAVY, instability index, and extinction coefficients.

Secondary Structural Annotation

The secondary structure was accessed using GOR secondary structure prediction tool [19]. Self-optimized prediction method with alignment (SOPMA) has been used to predict secondary structure. It is solely a tool for predicting a protein's secondary structure based on the protein primary sequence query. The GOR method predicts the secondary structure of alpha helix, beta sheets, turns, or random coils at each site by analyzing the sequences.

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Homology Structure Validation

The protein sequence was used for homology modeling by using the SWISS-MODEL server. It was used to predict the three-dimensional structure of the selected hypothetical proteins [20]. With the model obtained, the modelled protein was accessed for stereo-chemical quality with the Ramachandran plot method.

Ligand Analysis and ADMET Test

The chemical structures of Piperine and Curcumin were obtained from ChEMBL Database (https://www.ebi.ac.uk/chembl/). This molecule's information was downloaded in SMILE format for further analysis. The pharmacokinetics analysis of the piperine and curcumin was predicted using SWISS-ADMET and PRE-ADMET server for analyzing the toxicity properties of the targeted drugs

Molecular Docking

To validate the drug-target association, the molecular docking simulation was carried out using DockingServer. It provides an easy to use web-based interface that holds all elements of ligand and protein set-up molecular docking. Using Auto Dock tools, essential hydrogen atoms, Kollman united atom type charges and solvent parameters were added.The Auto Grid program generated affinity (grid) maps of 60X60X60Å grid points and 0.375 Å spacing.Simulations for docking were carried out using the Lamarckian Genetic Algorithm (LGA) and the local search method Solis and Wets.For each GA run, fifty generations have been set and each run has a population size of 150. A maximum mutation rate is 0.02 and 0.08 rates were crossed to produce docking paths for the subsequent generation.

Results and Discussion

Sequence Retrieval

The protein sequence of J3KN53, D0EKL1, A0A0A8K9F1 were retrieved from the UniProt KB database 2121and the sequence was saved in the FASTA file format. The details of the protein include gene name, organism name, UniProt KB ID, and sequence length were shown in table 1. Based on table 1, these proteins were taken from the same gene MDM2 which was E3 ubiquitin-protein ligase Mdm2. The protein shows it's from the same organism but each protein has a different sequence of length.

Table 1: Selected hypothetical protein of human lung cancer protein.

Gene Name	Organism Name	Uniprot KB ID	Sequence Length
MDM2	Homo Sapiens	J3KN53	466
MDM2	Homo Sapiens	D0EKL1	466
MDM2	Homo Sapiens	A0A0A8K9F1	455

Primary Structural Annotation

The ProtParam server of the ExPASy was used to evaluate the theoretical physiochemical properties of the hypothetical protein's amino acid sequence. The result of these three

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physicochemical properties proteins was enlisted in table 2. As table 2 shows, the J3KN53, D0EKL1, A0A0A8K9F1 protein has more or less equal to 50,000 pI/Mw of molecular weight. This low molecular weight makes it ideal for a drug target. The total number of positively charged residues (Arg+Lys) was higher than the total number of negatively charged residues (Asp+Glu) for each protein. On the other hand, these proteins show more than 60 for the instability index, which leads to unstable proteins [21]. The aliphatic index of the proteins is likely the same for each protein. A protein's aliphatic index is designated as the relative volume of aliphatic side chains (alanine, valine, isoleucine, and leucine) [22]. These proteins had a negative score for GRAVY indicative of a hydrophilic and soluble protein.

Table 2: Physiochemical characterization of the selected hypothetical proteins (J3KN53, D0EKL1, and A0A0A8K9F1).

Parameters	**Value of J3KN53**	**Value of D0EKL1**	**Value of A0A0A8K9F1**
Numbers of Amino Acid	466	466	455
Molecular Weight	52393.42	52469.58	50862.68
Theoretical PI	4.50	4.50	4.50
Total number of negatively charged residues (Asp + Glu)	46	46	46
Total number of positively charged residues (Arg + Lys)	90	90	90
Instability Index (II)	61.35	60.65	65.47
Aliphatic Index	63.73	63.73	60.81
Grand average of hydropathicity (GRAVY)	-0.800	-0.786	-0.860

Homology Structure & Validation

The 3D structure of selected proteins was predicted by using the SWISS Model server since their crystal structure unavailable in PDB. 3D protein structure provides important insights into the molecular functional basis and thus allows for an effective design of experiments [23]. Therefore, a high resolution of a protein's 3D structure is the key to understanding and manipulating the protein's biochemical and cellular functions [24]. Ramachandran plot was obtained from the SAVES server.

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Ligand Analysis and ADMET Test

The ligands of Piperine and curcumin were retrieved from ChEMBL. The ADMET properties of each molecule were predicted with SWISS ADME and PREADME. The small molecules are Piperine and Curcumin. Figure 1 shows the structure for both of the small molecules. Meanwhile, table 3 shows the details of properties that were predicted with SWISS ADME and PREADMET. The result contains the name of the molecule with important ADMET parameters such as molecular weight, solubility, Blood-Brain Barrier (BBB), bioavailability, synthetic accessibility, etc., were obtained for each molecule. These include the physicochemical properties, lipophilicity, water-solubility, pharmacokinetics, drug-likeness, and medicinal chemistry.

Table 3: SWISS-ADME and PREADMET properties of Piperine and Curcumin.

Compound/Properties		**Piperine**	**Curcumin**
Physiochemical	Molecular Weight	285.34	368.38
	TPSA	38.77	96.22
Druglikeness	Bioavailability Score	0.55	0.56
	Lipinski	Yes	-
Absorption	CYP 2C19	Yes	
	CYP 2C9		
	CYP 3A4		
	Buffer Solubility	5803.83	
	Caco2	22.2815	
	BBB	1.49973	
	MDCK	204	
	Plasma Protein Binding	-1	
Lipophilicity	M Log P	2.39	1.47
Medical Chemistry Synthetic		2.92	3.42
Water Solubility		Very	Moderately

Based on table 3, both servers of SWISS ADME and PRE-ADMET was used to determine the properties of the ligands. From table 3, it shows that the absorption of these two molecules has the same result [25]. BBB indicates the prediction of the penetration of the blood-brain barrier to cross by the blood-brain barrier. This is crucial in the pharmaceutical field, as CNS-active compounds have to pass through it and CNS-inactive compounds should not pass through it to prevent side effects of CNS. These ligands are positive for BBB which did not give the side effect. [26, 27]. Meanwhile, the plasma protein binding shows a negative result which it has difficult to reach the plasma membrane.

Molecular Docking using docking server

Ligand-receptor docking is a molecular docking strategy to reproduce chemical potentials that determine bound conformation preference and free energy of binding between a ligand and its

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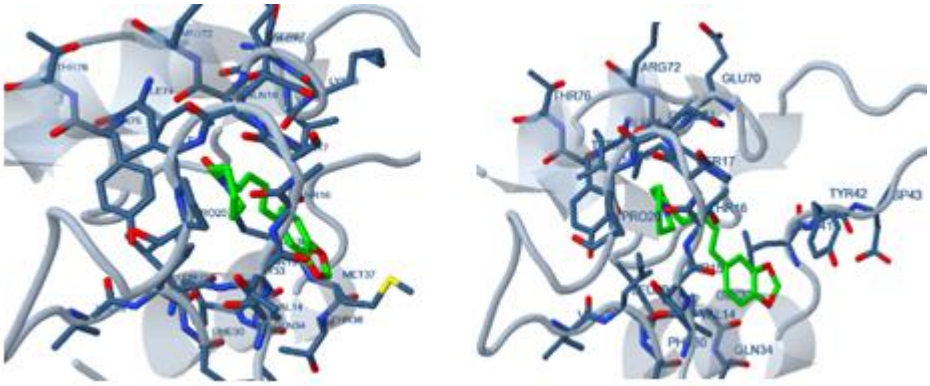
receptor. Table 4 and 5 includes the details of docking results. It was observed that Piperine with 0A0A8K9F1 has the highest binding affinity (-7.94 kcal/mol) than other docked compounds.

Table 4: Docking result of Piperine with Hypothetical Protein**.**

Properties	**J3KN53**	**D0EKL1**	**A0A0A8K9F1**
Est Free Energy of Binding (kcal/mol)	-6.89	-6.61	-7.94
Est. Inhibition Constant,Ki (uM)	8.95	14.26	1.51
vdW + Hbond + desolv Energy (kcal/mol)	-7.72	-7.41	-8.92
Electrostatic Energy (kcal/mol)	-0.00	-0.01	-0.0
Total Intermolec. Energy (kcal/mol)	-7.72	-7.43	-8.92
Frequency	30%	30%	80%
Interact Surface	736.778	740.817	703.567



A **B**

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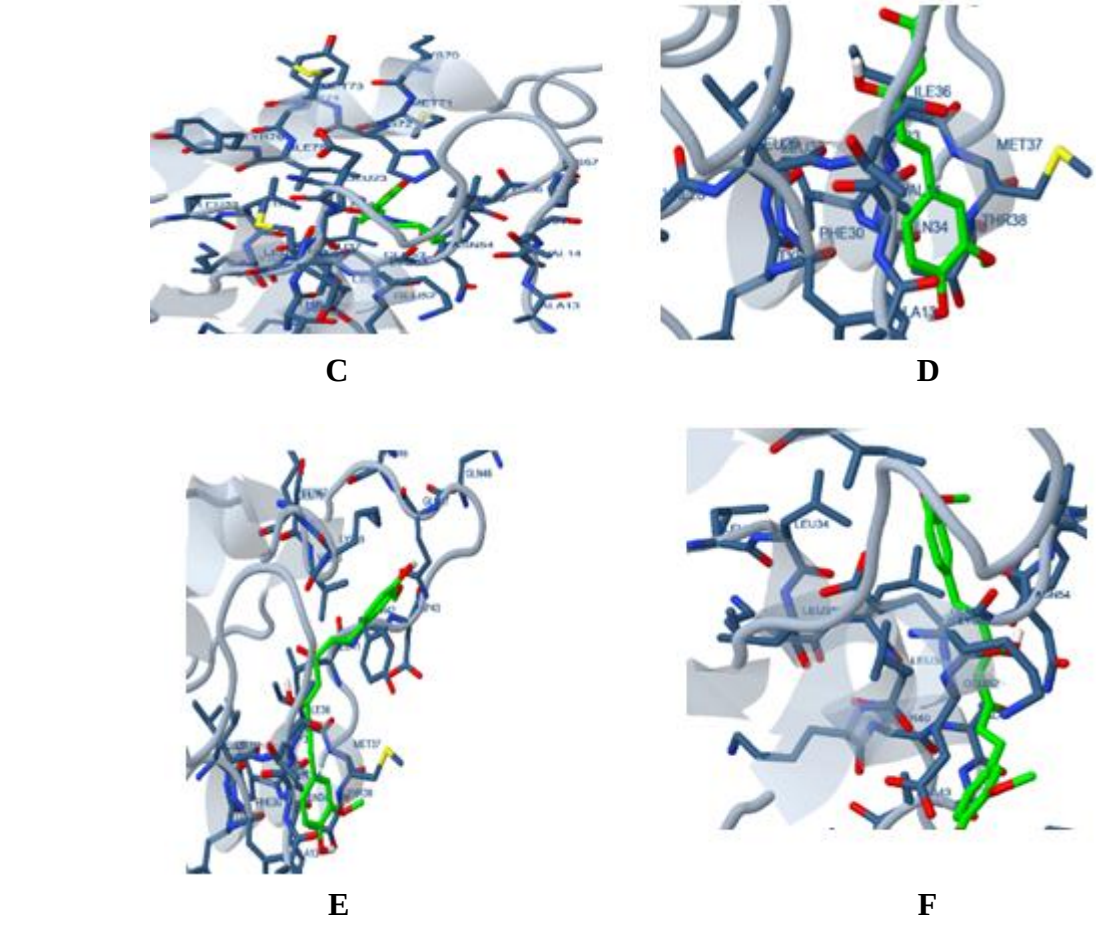

Figure 1: 2D picture of Hypothetical Protein binds with the ligand. (A) J3KN53 bind with Piperine, (B) D0EKL1 bind with Piperine, (C) A0A0A8K9F1 bind with Piperine, (D) J3KN53 bind with Curcumin, (E) D0EKL1 bind with Curcumin, and (F) A0A0A8K9F1 bind with Curcumin.

Table 5: Docking result of Curcumin with Hypothetical Protein.

Properties	**J3KN53**	**D0EKL1**	**A0A0A8K9F1**
Est Free Energy of Binding (kcal/mol)	-4.51	-4.88	-5.13
Est. Inhibition Constant,Ki (uM)	493.89	262.81	174.15
vdW + Hbond + desolv Energy (kcal/mol)	-6.64	-6.37	-7.43

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Electrostatic Energy (kcal/mol)	-0.0	-0.03	-0.22
Total Intermolec. Energy (kcal/mol)	-6.65	-6.40	-7.65
Frequency	20%	30%	30%
Interact Surface	870.457	792.584	988.283

CONCLUSIONS

In this work, selected hypothetical proteins of E3 ligases involved in lung cancer were analysed using *in silico*approach. The small molecules, curcumin, and piperine were selected as natural inhibitors. Computational methods have been used to obtain ligand-receptor binding and to predict ADMET for selected hypothetical proteins predictive against lung cancer. Based on this analysis curcumin and piperine were found compatible with these three proteins. The most compatible interaction is piperine with A0A0A8K9F1 because it showed the highest binding affinity which was -7.94 kcal/mol. This information could be supportive of a new drug design for lung cancer, in which these potential inhibitors should interact.

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