In silico investigation of new drug design and development for the treatment of lung cancer with natural Quercetin and its derivatives

B. Pharm (Honors) Project Report

[In the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy]

Submitted To

Department of Pharmacy

Faculty of Allied Health Science

Daffodil International University

Submitted By

Student ID: 191-29-1503

Batch: 21th, Section: A

Department of Pharmacy

Faculty of Allied Health Science

Daffodil International University

April 2023

A P P R O VA L

This document confirms that the project titled **"In silico investigation of new drug design and development for the treatment of lung cancer with natural Quercetin and its derivatives"** has been submitted to the Department of Pharmacy, Faculty of Allied Health Science, Daffodil International University. It fulfills the partial requirements for the degree of Bachelor of Pharmacy and has been approved for its style and content.

BOARD OF EXAMINERS

Chairman

_ _

Professor Dr. Muniruddin Ahmed

Professor and Head

Department of Pharmacy

Faculty of Allied Health Science

Daffodil International University

Examiners

_ **Internal Examiner-1**

_ **Internal Examiner-2**

_ **External**

ACKNOWLEDGEMENT

First and foremost, I express my gratitude to **ALLAH** for providing me with wisdom, strength, peace of mind, and good health, and for helping me overcome all difficulties in completing this project.

I owe my deepest gratitude to my supervisor, **Md. A.K. Azad**, Assistant Professor at the Department of Pharmacy, Daffodil International University, for his unwavering support, guidance, and motivation throughout the completion of my project.

I am also grateful to **Prof. Dr. Muniruddin Ahmed**, Head of the Department of Pharmacy, for his valuable support and assistance in developing the department.

I am forever thankful to my **parents** for their spiritual support throughout my life. I am also thankful for the constant encouragement, support, and guidance of all my **teachers** from the Department of Pharmacy at Daffodil International University.

I am grateful to my senior brother, **Shopnil Akash**, who taught me computational studies for drug design and helped me gather necessary information for my project.

Finally, I express my thanks and appreciation to my **friends and well-wishers** who have always trusted me, supported me, and provided me with unconditional inspiration.

DECLARATION

I, **Saila Kabir Maeesa**, affirm that the project work titled " **In silico investigation of new drug design and development for the treatment of lung cancer with natural Quercetin and its derivatives**" was conducted under my own effort, and the observations and conclusions presented in this work are based on my observations. This project work was conducted under the supervision of **Mr. Md. A.K. Azad**, Assistant Professor & Coordinator M. Pharm, Department of Pharmacy, Daffodil International University, to partially fulfill the requirements for the Bachelor of Pharmacy degree. I confirm that this work has not been previously submitted, either in full or in part, to any other university or institution, for the purpose of obtaining any other degree or professional qualification, except as explicitly stated.

Supervised By

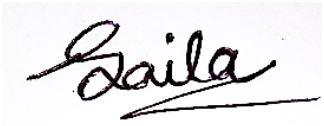
Md. A.K. Azad

Assistant Professor & Coordinator M. Pharm

Department of Pharmacy

Daffodil International University

Submitted By



Saila Kabir Maeesa

Student ID: 191-29-1503

Batch: 21th, Section: A

Department of Pharmacy

Daffodil International University

DEDICATION

ABSTRACT

Background: Developing new drugs is a time-consuming and risky process that requires significant financial investment, resources, and labor. To discover potent new drugs, the process must be performed step-by-step. Computational approaches are increasingly popular in drug discovery due to their ability to combine physical and biochemical processes, potentially increasing success rates and reducing costs.

Aim of this study: Lung cancer is a significant global public health issue, and the need for new, effective, and safe treatments is critical**.** Quercetin, a natural compound, and its derivatives have demonstrated potent anticancer activity, making them an attractive option for lung cancer treatment.

Method: This research utilized the latest computer-aided drug-design approaches, such as online data collection (Pass prediction, Lipinski rule, Pharmacokinetics, and Drug likeness, ADMET), structure optimization, and molecular docking, to identify new bioactive compounds for treating lung cancer.

Findings: The study began by calculating the biological pass prediction spectrum to select the target protein, and cancerous proteins were identified through a high probability of active scores. Docking scores for lung cancer were -7.1 to -10.5 kcal/mol, and drug-likeness, ADME, and toxicity prediction were also evaluated.

Conclusion: The results indicate that the natural quercetin and its derivatives have the potential as effective inhibitors for treating lung cancer due to their strong anticancer activity, good pharmacokinetic properties, and lack of toxicity.

Keywords: Computational, Design, Quercetin, Lung cancer, Docking.

TABLE OF CONTENTS

CHAPTER ONE: INTRODUCTION

CHAPTER TWO: AIM OF THE STUDY

CHAPTER THREE: LITERATURE REVIEW

CHAPTER FOUR: COMPEUTATIONAL METHODS & WORKING PROCEDURE

CHAPTER FIVE: RESULTS AND DISCUSSION

CHAPTER SIX: CONCLUSION

CHAPTER SEVEN: REFERENCES

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1
INTRODUCTION

1.1. Introduction

Cancer is the second leading cause of mortality in the United States, after only heart disease. In 2022, the United States is anticipated to have 1.9 million new cancer cases and 609,360 cancer deaths, for a total of 1,670 fatalities each day. Lung cancer is the second most prevalent cancer in both men and women in the United States. According to the American Cancer Society, there will be around 238,340 new cases of lung cancer in the United States in 2023. (117,550 in men and 120,790 in women). Lung cancer claimed the lives of around 127,070 people (67,160 in men and 59,910 in women) [1]. Patients with lung cancer are more likely to develop severe COVID-19 pneumonia and face greater problems [2], [3]. COVID-19 pneumonia is sometimes diagnosed on CT scans in cancer patients who have no distinctive COVID-19 symptoms, and the differential diagnosis between COVID-19 pneumonia and lung cancer can be difficult [4], [5].

With ongoing developments in molecular biology and biochemistry, we have achieved significant progress in the treatment of lung cancer. Standard treatment (such as surgery, radiation, chemotherapy, targeted therapy, and immunotherapy) has undeniably improved the prognosis of lung cancer populations. Immunotherapy, in particular, has given more and more good news to innumerable lung cancer patients [6]. But still, they have only proven limited efficacy and cannot heal completely.

Multidrug resistance in cancer is a concern that occurs because of genetic alterations, specific growth factors, increased drug effluence, increase in the metabolic rate of xenobiotics. This can decrease the effects of therapies and also disrupt the development of a targeted and specific remedy [7]. Furthermore, cancer radiation treatment demonstrates its efficacy by preferentially destroying tumor cells while sparingly affecting neighboring healthy cells, resulting in a variety of unpleasant symptoms such as skin peeling, blister development, itching, and even alopecia [8]. As a result, choosing an effective cancer therapy medication is necessary, based on a full understanding of the illness and the limits of current therapeutic alternatives.

Natural products are well-known as cancer remedies. Cancer vaccines, immune-checkpoint inhibitors, and adoptive immune-cell therapy with natural products have all been documented to have an impact on cancer immunotherapy. And the process is mostly attributable to the alteration of the tumor-immunosuppressive microenvironment, which is a critical role in assisting tumors to evade detection and assault by the immune system and cancer immunotherapy [9].

Quercetin, a natural flavonoid molecule found in fruits and vegetables, is a powerful cancer-fighting agent. It has been shown to decrease proliferation and trigger apoptosis in both A549 and H1299 human lung cancer cells [10]. This bioactive molecule is also composed of broad-spectrum biological actions, including potent anticancer [11], and its derivatives have antibacterial [12], anti-viral [13], and

anti-fungal [14] properties. Because quercetin and its derivatives had previously reported potential bioactivities, this molecule has been taken up and changed by adding or substituting other functional groups. In this study, quercetin has been presented as a potentially useful anticancer treatment.

The in-silico techniques for early evaluation are interesting because they offer the potential to boost success rates while simultaneously cutting R&D costs. Drug research & development costs millions of dollars and takes more than 10-15 years, and only a few drugs meet the FDA's efficacy and safety standards after extensive experimental testing [15]. As a result, in this study, I propose quercetin and its nine derivatives as an improved computational technique for identifying novel prospective drugs for the treatment of lung cancer.

CHAPTER 2
AIM OF THE STUDY

2.1. Main Objective

- To establish a new natural drug molecule for the treatment of Lung Cancer.

2.2. Specific Objective

- To run computational studies to detect some potential ligands within a short period.
- To complete pre-clinical trial steps of drug design.
- To find a drug with the least side effects against lung cancer.
- To study the dynamic behavior of drug-receptor complexes.
- To assess the overall satisfaction and cost-effectiveness of a new drug design.

CHAPTER 3
LITERATURE REVIEW

3.1. Lung cancer

Lung cancer is a major cause of cancer-related deaths globally, with a five-year survival rate of only 15%. Lung cancer accounts for more deaths each year than breast, pancreas, colon, and prostate cancers combined. It is the second most common cancer in both men and women, and around 14% of all new cancers in the USA are lung cancers. The incidence of lung cancer is higher among older people, with an average age at diagnosis of 70 years. The incidence of lung cancer peaked in 1990 in men and 2000 in women, but since then, mortality in men has been decreasing, while mortality in women has only recently started to decrease [16]. Socioeconomic factors are strongly correlated with the incidence and mortality of lung cancer across various countries, with higher rates of incidence and mortality seen in countries with higher levels of socioeconomic development and productivity. Smoking is the primary risk factor for lung cancer, responsible for over 80% of all cases [17]. Other factors that contribute to lung cancer development include air pollution, indoor emission of fuel burning, environmental exposure to radon, asbestos, some metals such as chromium, cadmium, and arsenic, and some organic chemicals [18]. The most common type of lung cancer is non-small cell lung cancer (NSCLC), which comprises three histological subtypes: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. Around 90% of patients with NSCLC have a history of tobacco smoking [19].

3.2. Mutations in Lung cancer cells

KRAS is the most commonly occurring oncogene in non-small cell lung cancer (NSCLC), which is a molecular subset that has seen unsuccessful attempts at targeted treatment approaches such as farnesyl transferase inhibition, downstream MEK inhibition, and synthetic lethality screens [20]. Biomarkers such as epidermal growth factor receptor (EGFR) mutations and anaplastic large-cell lymphoma kinase (ALK) rearrangements are now regularly used in managing NSCLC [21]. However, KRAS mutations are more prevalent in lung adenocarcinomas, unlike EGFR and EML4-ALK mutations, and have not been effectively targeted by current treatment methods [22]. The enzyme cytochrome P450 2A13 (CYP2A13) found in human lungs can convert the nicotine-related procarcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) into compounds that modify DNA and ultimately lead to the development of lung cancer [23].

3.3. Therapeutic activity of Quercetin and its derivatives

Quercetin (L- 01) is a flavonoid commonly found in food and herbal supplements, with a variety of potential uses in treating or preventing medical conditions such as cardiovascular disease, hypercholesterolemia, and cancer. While clinical trials have not shown consistent effectiveness, quercetin supplements are generally well-tolerated and have not been linked to liver injury [24], [25]. Additionally, quercetin has the ability to stabilize the cells responsible for histamine release in the body, resulting in an anti-inflammatory and antihistamine impact [26]. Quercetin can cause cell cycle arrest in numerous types of cancer cells by directly binding to various protein regulators that control the cell cycle. This binding can impact any of the different stages of cell cycle progression, leading to growth arrest at the G1, G1/S, or G2/M phase through interactions with different regulators [27].

There are several derivatives of quercetin with potential therapeutic activities. Quercetin-3-rhamnoside (L- 02) has anti-inflammatory, antioxidative, and neuroprotective effects, while quercitrin can induce apoptosis of colon cancer cells [28]. Quercetin 3-galactoside (L- 03) has anti-inflammatory properties and can inhibit the apoptosis pathway [29], also has a role as a hepatoprotective agent and a plant metabolite [30]. Quercetin 7-O-beta-L-rhamnopyranoside (L- 04) and Quercetin 7-O-alpha-D-rhamnopyranoside (L- 05) have antioxidant and anticancer properties [31], [32]. Quercetin 3-O-beta-L-fucopyranoside (L- 06) is related to beta-L-fucose and has antiviral effects [33], while Quercetin-3-O-alpha-L-rhamnopyranoside (L- 07) has immunomodulatory properties against influenza A virus and anticancer properties [34], [35]. Quercetin 5-rhamnoside (L- 08) has antioxidant and anti-inflammatory effects, and Quercetin 7-O-glucoside (L- 09) has antioxidant and anticancer properties [36]. Finally, Quercetin-3-D-xyloside (L- 10), also called Reinutrin, has antiproliferative effects on cancer cell lines and can arrest human leukemic T cells in the late G1 phase of the cell cycle via type II estrogen receptors [37].

CHAPTER 4
COMPUTATIONAL METHODS & WORKING PROCEDURE

Using cutting-edge technology, computational approaches are used in the early phases of the drug discovery process to augment experimental investigation by offering insightful information into the understanding of chemical systems virtually [38]. Here, I used the protocols that offer the docking–MD combination as a logical approach to the aim of discovering a new drug.

4.1. Pass prediction spectrum

PASS (Prediction of Activity Spectra for Substances) is a computational tool used in drug design and discovery to predict the biological activity of a molecule based on its structural formula. It is useful in the early stages of drug design to identify promising lead compounds with the desired biological activity. The PASS online tool, has been developed for the purpose of predicting the physiological and biochemical properties of organic molecules based on their structural formulas. It is capable of predicting over 4,000 categories of physiological and biochemical activity with a high accuracy rate of more than 95% for drug-like molecules. The tool provides Pa and Pi values, which are typically limited to a maximum value of 1 and are not equal to each other [39].

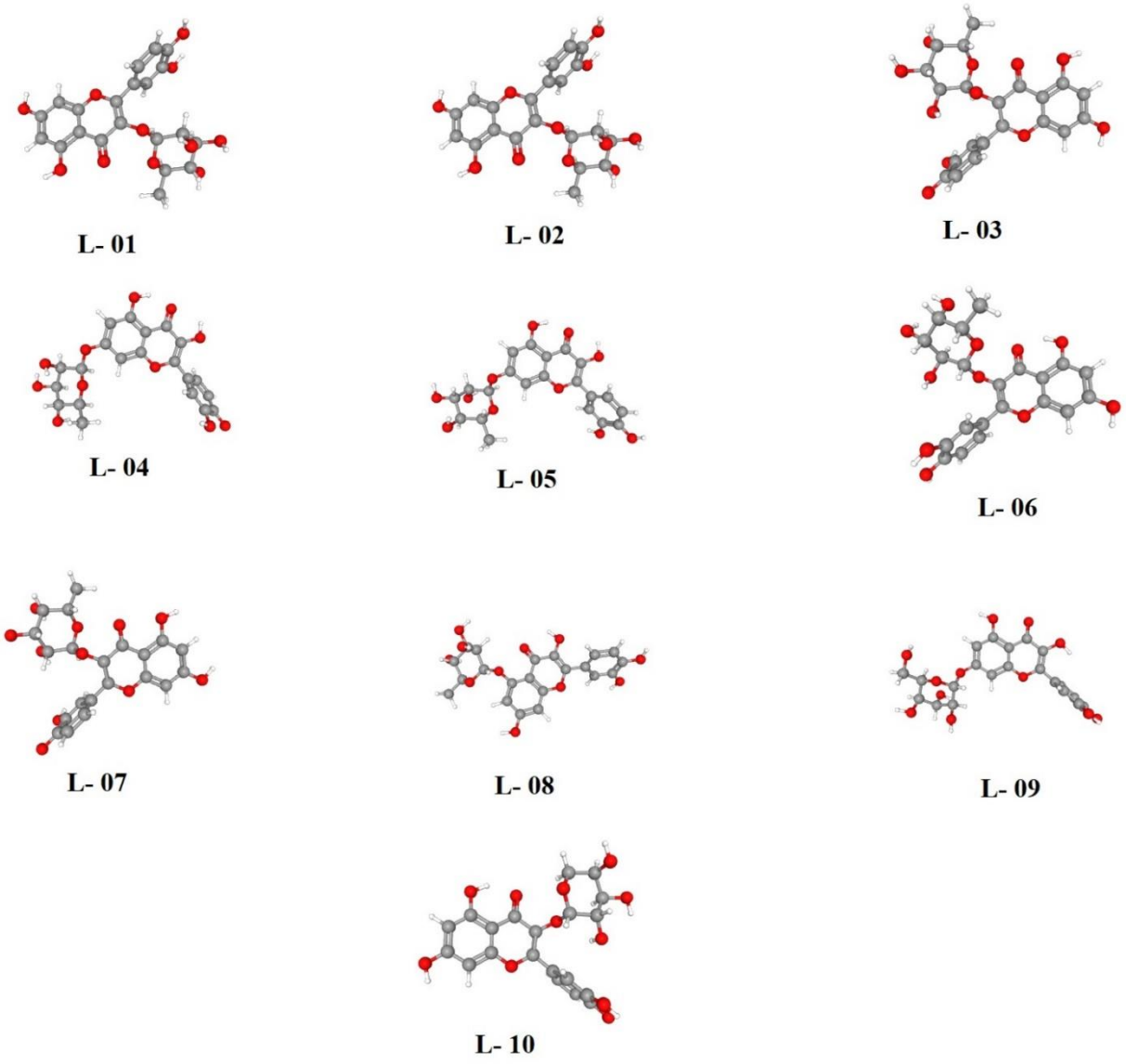
4.2. Lipinski rule, Pharmacokinetics, and Drug likeness

Swiss ADME was employed to predict the pharmacokinetic and drug-likeness properties of the bioactive compounds, owing to its high impact and adaptable features [40]. One of the major pharmacokinetic parameters evaluated for any bioactive compound is the Lipinski Rule, which uses the "rule of five" to determine its characteristics. According to this rule, a chemical is considered drug-like if it meets all five of the following criteria: its molecular mass is less than 500 g/mol, it has no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, and its octanol-water partition coefficient log P is not greater than 5 [41].

4.3. Optimization of Ligands Structures

Initially, the chemical structures were checked using ChemDraw Professional. Next, the optimization of these compounds was carried out using vibrational frequencies obtained from the B3LYP functional and DND basis semi-core pseudo-potentials [42]. The optimized versions of the 10 compounds were then saved as a PDB file for additional computational analyses, such as molecular docking, molecular dynamics, and ADMET properties.

Figure 1: Optimized 3D chemical structures of Quercetin and derivatives.

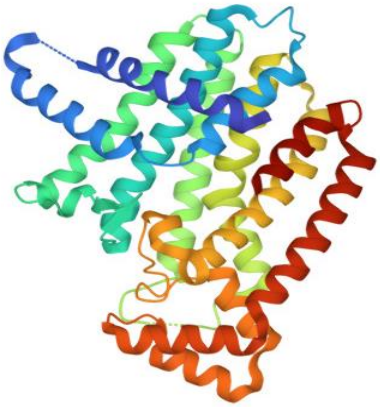
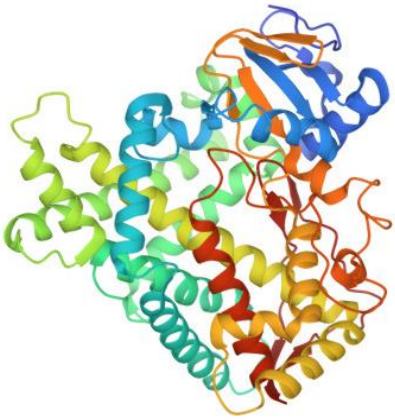


4.4. Preparation of protein, and molecular docking studies

The PDB format was used to obtain the three-dimensional structure of Human Farnesyl Diphosphate Synthase PDB ID 4N1Z (https://www.rcsb.org/structure/4N1Z) and Human Lung Cytochrome P450 2A13 with indole bound PDB ID 2P85 (https://www.rcsb.org/structure/2P85) from the protein data bank. The proteins were then uploaded to the PyMOL 2020 application, where unwanted substances such as drugs or heteroatoms were minimized before saving the structures in

PDB format [43]. The structures were then uploaded to the PyRx software for molecular docking with ligands. After the docking process was complete, both the macromolecule and ligand frameworks were saved in the pdbqt format specified by BIOVIA Discovery Studio 2020 for analysis of the docking outcome and identification of non-bonding interactions between ligands and amino acid residues of the receptor protein [44]. Figure 2 provides additional information on the proteins used in the study.

Figure 2: Three-dimensional structure of selected Lung cancer target proteins used in this study.

Lung Cancer (PDB ID: 4N1Z)	**Lung cancer (PDB ID: 2P85)**
Organism: Homo sapiens	Organism: Homo sapiens
Resolution: 2.35 Å	Resolution: 2.35 Å
3D structure 	3D structure 
Ref. [45]	Ref. [46]

4.5. Determination of ADMET Data

ADMET (absorption, distribution, metabolism, excretion, and toxicity) are pharmacokinetic parameters that describe the processes by which a drug is absorbed, distributed, metabolized, and eliminated by the body, as well as its potential for toxicity. Researchers can use ADMET data to determine various factors, such as the amount of drug that is absorbed by the human intestine, how much of the drug crosses the blood-brain barrier, and how much enters and exits the central nervous system [47]. The online web tool pkCSM is a tool that has been used to develop ADMET data. This data is typically calculated and measured based on the chemical properties and structures of the molecules being studied [48].

CHAPTER 5
RESULTS & DISCUSSION

5.1. Pass prediction spectrum Data

We used the PASS (prediction of activity spectra for molecules) online tool to gather information on the efficacy of our molecules in terms of antiviral, antibacterial, antifungal, and antineoplastic activity. Our tested molecules have demonstrated potent antineoplastic activity with a Pa value greater than 0.800+, except ligand 1. Among the compounds, ligand number 8 displayed the highest Pa value (0.860). The Pa value represents the likelihood of being active, while pa indicates the likelihood of being inactive [49]. For the lead compounds mentioned, the Pa value ranges for antiviral activity were 0.398 to 0.505, for antibacterial activity were 0.387 to 0.642, for antifungal activity were 0.490 to 0.740, and for antineoplastic activity were 0.797 to 0.860. Based on the higher probability of being active or a higher Pa score, lung cancer was selected as the target disease for further investigation (Table 1).

Table 1. Data of PASS prediction

SI No.	PubChem ID	Antiviral (Hepatitis B)		Antibacterial		Antifungal		Antineoplastic	
		Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi
1.	5280343 (Quercetin)	0.498	0.005	0.387	0.033	0.490	0.032	0.797	0.012
2.	5353915	0.421	0.012	0.642	0.007	0.740	0.008	0.854	0.007
3.	5359430	0.421	0.012	0.642	0.007	0.740	0.008	0.854	0.007
4.	72193658	0.456	0.008	0.633	0.007	0.728	0.008	0.856	0.006
5.	72193676	0.456	0.008	0.633	0.007	0.728	0.008	0.856	0.006
6.	5320852	0.421	0.012	0.642	0.007	0.740	0.008	0.854	0.007
7.	6325794	0.421	0.012	0.642	0.007	0.740	0.008	0.854	0.007
8.	102052885	0.449	0.009	0.625	0.008	0.714	0.009	0.860	0.006
9.	5381351	0.505	0.004	0.601	0.009	0.700	0.010	0.835	0.008
10.	5878729	0.398	0.015	0.563	0.011	0.698	0.010	0.834	0.008

5.2. Data of Lipinski rule, Pharmacokinetics, and Drug likeness

Lipinski's five-rule is commonly used to investigate drug likeness, assuming that lower bioavailability is a major reason for potential drugs to fail and not reach the target site [50]. It has been estimated that a significant number of targeted medicines fail due to their negative effect, resulting in significant medication costs, time, and human resources being wasted [51]. And, these concerns can be minimized through the use of computational methodologies. To be considered a good and effective oral bioactive molecule, any bioactive molecule should adhere to the Lipinski rule. Ligand 1 was found to have superior bioavailability and comply

with the Lipinski rule among all the reported compounds. On the other hand, ligands 2-9 had low bioavailability and violated the Lipinski rule due to mismatches in the hydrogen bond acceptor and donor. Nevertheless, the molecular weight and Log P value were within the range for all compounds (Table 2).

Table 2: Data on Lipinski rule, pharmacokinetics, and drug-likeness.

SI. No.	CID	Molecular Weight (g/mol)	Hydrogen bond acceptor	Hydrogen bond donor	Consensus Log $P_{o/w}$	Lipinski rule		Bioavailability
						Result	violation	
1.	5280343	302.24	7	5	1.23	Yes	0	0.55
2.	5353915	448.38	11	7	0.16	Yes	2	0.17
3.	5359430	448.38	11	7	0.19	Yes	2	0.17
4.	72193658	448.38	11	7	0.55	Yes	2	0.17
5.	72193676	448.38	11	7	0.51	Yes	2	0.17
6.	5320852	448.38	11	7	0.32	Yes	2	0.17
7.	6325794	448.38	11	7	0.37	Yes	2	0.17
8.	102052885	448.38	11	7	0.09	Yes	2	0.17
9.	5381351	464.38	12	8	-0.37	Yes	2	0.17
10.	5878729	434.35	11	7	-0.00	Yes	2	0.17

5.3. Molecular docking and interaction analysis

The process of molecular docking is an established method to determine how two molecules interact and determine the appropriate ligand configuration for forming a minimum energetic complex. A bioactive molecule is typically considered to have a general and standard value of -6.0 kcal/mol [52]. In this study, ten targeted ligands and one standard (Capmatinib) were docked against two Lung cancer proteins. Through this in silico experiment, it was found that all ligands in Table 3 showed good binding affinity to the target, but ligands 2, 5, 7, and 9 displayed the most biological activity. Ligands 5 and 9 had the highest docking scores of -10.1 and -10.5, respectively, against lung cancer (PDB 4N1Z), while ligands 2 and 7 had scores of -9.8 and -8.8, respectively, against lung cancer (PDB 2P85). This suggests that these bioactive molecules may be effective anticancer agents for lung cancer.

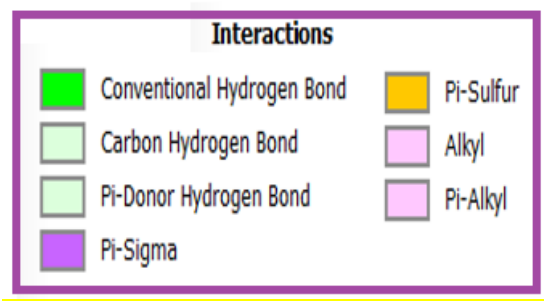
Table 3: Binding affinity against lung cancer proteins.

SI. No.	CID	**Lung cancer (PDB ID: 4N1Z)**	**Lung cancer (PDB ID: 2P85)**
		Binding affinity (Kcal/mol)	Binding affinity (Kcal/mol)
1.	5280343	-9.3	-8.4
2.	5353915	-7.3	-9.8
3.	5359430	-7.4	-7.3
4.	72193658	-8.5	-7.9
5.	72193676	-10.1	-7.9
6.	5320852	-7.1	-7.2
7.	6325794	-7.2	-8.8
8.	102052885	-8.2	-8.2
9.	5381351	-10.5	-8.4
10.	5878729	-7.5	-7.8
11.	Standard Capmatinib	-9.3	-8.7

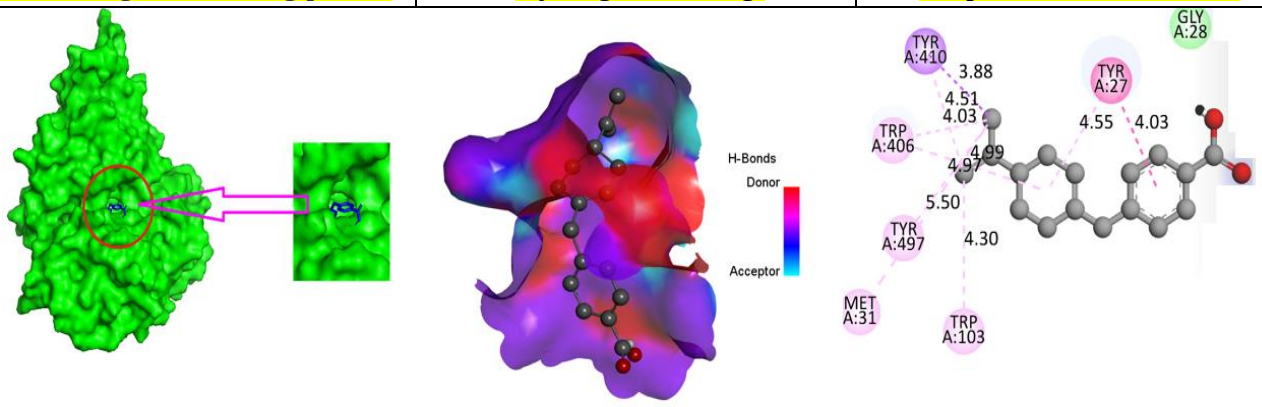
5.4. Protein-ligand interaction and Molecular docking process

The binding site between the receptor and ligand was identified using Discovery Studio version 2021 and displayed in Figure 3. Initially, auto-docking was performed on the protein and ligand to determine the binding sites, amino acid residues, and obstruction of the active site. The complex formation between the protein and ligand in the binding pocket is clearly visible, including the hydrogen bonding donor or acceptor regions [53]. Additionally, 2D images of active amino acid residues were generated for Lung cancer proteins (PDB ID 4N1Z and 2P85) with each ligand. For example, for PDB ID 4N1Z, ligand 5 binds with A: TRP-103, A: MET-31, A: TYR-497, A: TRP-406, A: TYR-410, A: TYR-27, and A: GLY-28, while ligand 9 binds with A: ARG-225, A: ALA-390, A: TYR-27, A: TRP-406, A: TYR-410, and A: TRP-103. Similarly, for PDB ID 2P85, ligand 2 binds with A: ILE-291, A: PHE-275, A: ILE-135, A: PHE-279, A: GLN-288, and A: TRP-287, while ligand 7 binds with A: VAL-133, A: PHE-15, and A: LEU-12. All amino acid residues and their bond distances for each compound are available in the supplementary file.

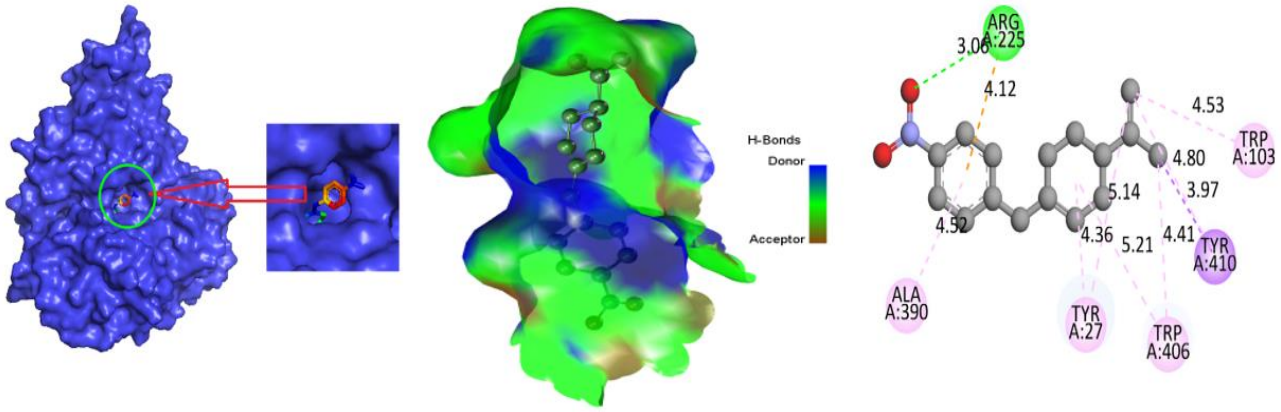
Figure 3: Docking interactions between the proposed compounds.



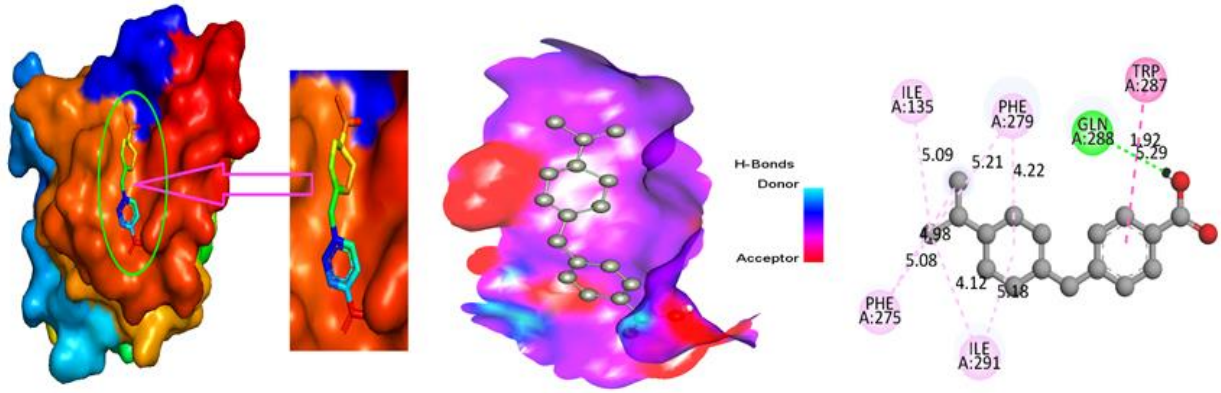
Protein ligands binding pocket	Hydrogen bonding	2D picture of active sites



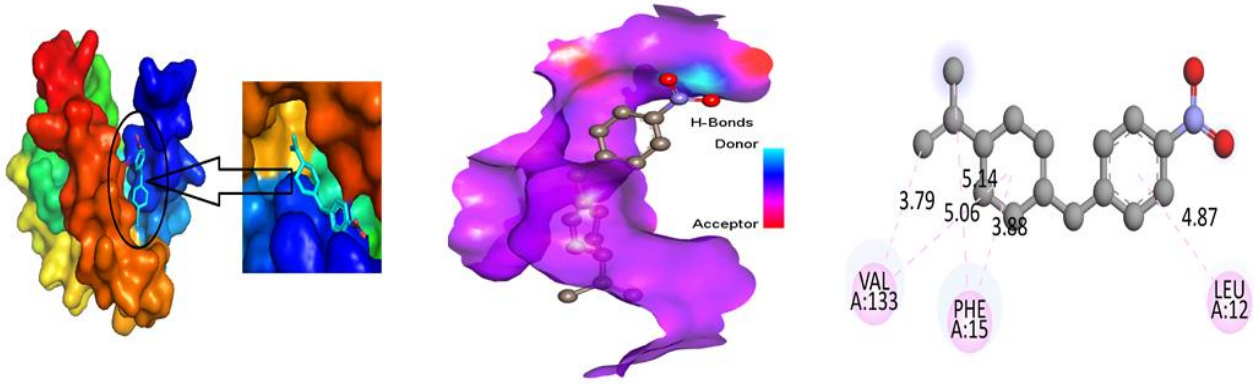
Lung cancer (PDB ID: 4N1Z) with ligand 05 (CID 72193676)



Lung cancer (PDB ID: 4N1Z) with ligand 09 (CID 5381351)



Lung cancer (PDB ID: 2P85) with ligand 02 (CID 5353915)



Lung cancer (PDB ID: 2P85) with ligand 07 (CID 6325794)

5.5. ADMET Data Investigation

ADMET plays a crucial role in the development of effective bioactive molecules because a drug with poor ADMET properties can be toxic [54]. Up to 60% of drugs in clinical trials fail due to poor ADMET performance, making it a critical step in drug design and discovery [55]. The ADMET properties of reported novel quercetin derivatives were predicted using pkCSM and Table 4 presents the information on their ADMET properties, including water solubility, absorption in the gastrointestinal tract, the volume of distribution, blood-brain barrier permeability, CYP450 enzyme inhibition or substrate activity, total clearance, renal substrate activity, and toxicity. The quercetin derivatives have high water solubility, with Log S values ranging from -2.881 to -2.905. However, none of the derivatives can pass through the blood-brain barriers. The absorption rate ranges from 33.633% to 77.207%, where only Ligand 1 have high absorption in the gastrointestinal tract. The VDss values of the molecules suggest moderate distribution, and only ligand 1 can inhibit the CYP450 1A2 inhibitor, whereas no ligand is CYP450 2D6 substrate. The total clearance rates range from 0.364 to 0.407 ml/min/kg, indicating a drug with a maximum clearance rate of 0.407 ml/min/kg can be cleared from the body, and none of the drugs can be excreted through the renal OCT2 substrate. None of the ligands exceed the AMES toxicity, skin sensitization and hepatotoxicity level.

Table 4: Summary of ADMET prediction.

SI. No.	CID	Distribution		Metabolism				Excretion		Toxicity		
		Water solubility Log S	Human Intestinal Absorption (%)	VDss (human)	BBB Permeability	CYP450 1A2 Inhibitor	CYP450 2D6 substrate	Total Clearance (ml/min/kg)	Renal OCT2 substrate	AMES toxicity	Skin Sensitization	Hepatotoxicity
1.	5280343	-2.925	77.207	1.559	-1.098	Yes	No	0.407	No	No	No	No
2.	5353915	-2.903	52.709	1.517	-1.495	No	No	0.364	No	No	No	No
3.	5359430	-2.903	52.709	1.517	-1.495	No	No	0.364	No	No	No	No
4.	72193658	-2.905	47.394	1.536	-1.636	No	No	0.376	No	No	No	No
5.	72193676	-2.905	47.394	1.536	-1.636	No	No	0.376	No	No	No	No
6.	5320852	-2.903	52.709	1.517	-1.495	No	No	0.364	No	No	No	No
7.	6325794	-2.903	52.709	1.517	-1.495	No	No	0.364	No	No	No	No
8.	102052885	-2.9	56.352	1.573	-1.641	No	No	0.387	No	No	No	No
9.	5381351	-2.881	33.633	1.526	-1.623	No	No	0.388	No	No	No	No
10.	5878729	-2.903	51.884	1.508	-1.473	No	No	0.364	No	No	No	No

CHAPTER 6
CONCLUSION

6.1. Conclusion

The study results indicate that certain derivatives are potent inhibitors of the cancer proteins, suggesting their potential for treating Lung cancer. The binding affinities of ligands 5 and 9 in PDB 4N1Z have increased to -10.1 and -10.5 kcal/mol, respectively, while ligands 2 and 7 in PDB 2P85 have gained -9.8 and -8.8 binding energies, respectively, indicating that the proposed quercetin derivatives have excellent binding energy and stability in the active site of experimental proteins. The crystal structure of lung cancer and the bioactive quercetin derivatives demonstrate that the compounds have different active amino acids, including tyrosine-410, arginine-225, phenylalanine-279, glycine-288, leucine-12, and valine-133, which contribute to their strong binding energy than the standard and exhibit excellent pharmacokinetic properties, proper absorption, favorable metabolic transformation, and reduced adverse effects. Given these positive attributes, it is suggested that further research should be conducted to develop novel molecules based on these derivatives.

CHAPTER 7
REFERENCES

7.1. References

[1] "Lung Cancer Statistics | How Common is Lung Cancer?" https://www.cancer.org/cancer/lung-cancer/about/key-statistics.html (accessed Mar. 06, 2023).

[2] L. V. Mitrev, A. Banerjee, N. Van Helmond, L. V. Mitrev, A. Banerjee, and N. Van Helmond, "Correlation of Country Characteristics and Government Response Measures With COVID-19 Mortality During the First Phase of the Global COVID-19 Pandemic: A Worldwide Ecological Study," *Cureus*, vol. 13, no. 10, Oct. 2021, doi: 10.7759/CUREUS.18689.

[3] R. Fusco *et al.*, "Artificial Intelligence and COVID-19 Using Chest CT Scan and Chest X-ray Images: Machine Learning and Deep Learning Approaches for Diagnosis and Treatment," *J. Pers. Med. 2021, Vol. 11, Page 993*, vol. 11, no. 10, p. 993, Sep. 2021, doi: 10.3390/JPM11100993.

[4] K. Yoo *et al.*, "Incidental COVID-19 in the radiology department: Radiographic findings of COVID-19 in asymptomatic patient undergoing CT staging for breast cancer," *Radiol. Case Reports*, vol. 15, no. 9, pp. 1614–1617, Sep. 2020, doi: 10.1016/J.RADCR.2020.05.080.

[5] C. Catania, V. Stati, and G. Spitaleri, "Interstitial pneumonitis in the COVID-19 era: a difficult differential diagnosis in patients with lung cancer," *https://doi.org/10.1177/0300891620951863*, vol. 107, no. 3, pp. 267–269, Aug. 2020, doi: 10.1177/0300891620951863.

[6] Z. Li, Z. Feiyue, and L. Gaofeng, "Traditional Chinese medicine and lung cancer——From theory to practice," *Biomed. Pharmacother.*, vol. 137, p. 111381, May 2021, doi: 10.1016/J.BIOPHA.2021.111381.

[7] D. S. Aldeghaither *et al.*, "A mechanism of resistance to antibody-targeted immune attack," *Cancer Immunol. Res.*, vol. 7, no. 2, pp. 230–243, Feb. 2019, doi: 10.1158/2326-6066.CIR-18-0266/470947/AM/A-MECHANISM-OF-RESISTANCE-TO-ANTIBODY-TARGETED.

[8] C. Allen, S. Her, and D. A. Jaffray, "Radiotherapy for Cancer: Present and Future," *Adv. Drug Deliv. Rev.*, vol. 109, pp. 1–2, Jan. 2017, doi: 10.1016/J.ADDR.2017.01.004.

[9] S. Dong, X. Guo, F. Han, Z. He, and Y. Wang, "Emerging role of natural products in cancer immunotherapy," *Acta Pharm. Sin. B*, vol. 12, no. 3, pp. 1163–1185, Mar. 2022, doi: 10.1016/J.APSB.2021.08.020.

[10] H. Guo *et al.*, "Quercetin induces pro-apoptotic autophagy via SIRT1/AMPK signaling pathway in human lung cancer cell lines A549 and H1299 in vitro," *Thorac. Cancer*, vol. 12, no. 9, pp. 1415–1422, May 2021, doi: 10.1111/1759-7714.13925.

[11] P. Biswas *et al.*, "A Comprehensive Analysis and Anti-Cancer Activities of Quercetin in ROS-Mediated Cancer and Cancer Stem Cells," *Int. J. Mol. Sci. 2022, Vol. 23, Page 11746*, vol. 23, no. 19, p. 11746, Oct. 2022, doi: 10.3390/IJMS231911746.

[12] S. A. N. Aljadaan, R. S. Elias, and R. A. Al-Anssari, "Investigation of the antioxidant and antibacterial activity of novel quercetin derivatives," *Biointerface Res. Appl. Chem.*, vol. 10, no. 6, pp. 7329–7336, Dec. 2020, doi: 10.33263/BRIAC106.73297336.

[13] A. Di Petrillo, G. Orrù, A. Fais, and M. C. Fantini, "Quercetin and its derivates as antiviral potentials: A comprehensive review," *Phyther. Res.*, vol. 36, no. 1, pp. 266–278, Jan. 2022, doi: 10.1002/PTR.7309.

[14] N. Lenard, T. M. Henagan, T. Lan, A. Nguyen, and D. Bhattacharya, "Antimicrobial Activity of Quercetin: An Approach to Its Mechanistic Principle," *Mol. 2022, Vol. 27, Page 2494*, vol. 27, no. 8, p. 2494, Apr. 2022, doi: 10.3390/MOLECULES27082494.

[15] M. M. Rahman, M. R. Karim, M. Q. Ahsan, A. B. R. Khalipha, M. R. Chowdhury, and M. Saifuzzaman, "USE OF COMPUTER IN DRUG DESIGN AND DRUG DISCOVERY: A REVIEW," *Int. J. Pharm. Life Sci.*, vol. 1, no. 2, Sep. 2012, doi: 10.3329/IJPLS.V1I2.12955.

[16] R. L. Siegel, K. D. Miller, and A. Jemal, "Cancer statistics, 2018," *CA. Cancer J. Clin.*, vol. 68, no. 1, pp. 7–30, Jan. 2018, doi: 10.3322/CAAC.21442.

[17] D. Kale, H. Gilbert, S. S.-A. behaviors reports, and undefined 2019, "An exploration of the barriers to attendance at the English Stop Smoking Services," *Elsevier*, Accessed: Apr. 03, 2023. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S2352853218300968.

[18] Y. Guo *et al.*, "Lung cancer incidence and ambient air pollution in China: a spatial age–period cohort study 1990–2009," *Lancet*, vol. 386, p. S5, Oct. 2015, doi: 10.1016/S0140-6736(15)00583-8.

[19] W. D. Travis, E. Brambilla, and G. J. Riely, "New pathologic classification of lung cancer: Relevance for clinical practice and clinical trials," *J. Clin. Oncol.*, vol. 31, no. 8, pp. 992–1001, Mar. 2013, doi: 10.1200/JCO.2012.46.9270.

[20] H. Adderley, F. Blackhall, C. L.- EBioMedicine, and undefined 2019, "KRAS-mutant non-small cell lung cancer: Converging small molecules and immune checkpoint inhibition," *Elsevier*, Accessed: Apr. 03, 2023. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S2352396419301288.

[21] R. Sweis, S. Thomas, B. Bank, P. Fishkin, C. M.- Cureus, and undefined 2016, "Concurrent EGFR mutation and ALK translocation in non-small cell lung cancer," *cureus.com*, Accessed: Apr. 03, 2023. [Online]. Available: https://www.cureus.com/articles/4140-concurrent-egfr-mutation-and-alk-translocation-in-non-small-cell-lung-cancer.

[22] Y. Xia *et al.*, "A combination therapy for kras-Driven lung adenocarcinomas using lipophilic bisphosphonates and rapamycin," *Sci. Transl. Med.*, vol. 6, no. 263, Nov. 2014, doi: 10.1126/SCITRANSLMED.3010382.

[23] B. Smith, J. Sanders, … P. P.-J. of B., and undefined 2007, "Structure of the human lung cytochrome P450 2A13," *ASBMB*, Accessed: Apr. 03, 2023. [Online]. Available: https://www.jbc.org/article/S0021-9258(19)57677-3/abstract.

[24] A. Olry, L. Meunier, B. Délire, D. Larrey, Y. Horsmans, and H. Le Louët, "Drug-Induced Liver Injury and COVID-19 Infection: The Rules Remain the Same," *Drug Saf.*, vol. 43, no. 7, pp. 615–617, Jul. 2020, doi: 10.1007/S40264-020-00954-Z/METRICS.

[25] S. Li, H. Cao, D. Shen, Q. Jia, C. Chen, and S. L. Xing, "Quercetin protects against ox-LDL-induced injury via regulation of ABCAl, LXR-? and PCSK9 in RAW264.7 macrophages," *Mol. Med. Rep.*, vol. 18, no. 1, pp. 799–806, Jul. 2018, doi: 10.3892/MMR.2018.9048.

[26] "Quercetin Information | Mount Sinai - New York." https://www.mountsinai.org/health-library/supplement/quercetin (accessed Apr. 03, 2023).

[27] R. Rather, M. B.-C. medicine, and undefined 2020, "Quercetin as an innovative therapeutic tool for cancer chemoprevention: Molecular mechanisms and implications in human health," *Wiley Online Libr.*, vol. 9, no. 24, pp. 9181–9192, Dec. 2018, doi: 10.1002/cam4.1411.

[28] J. Mottaghipisheh, M. Iriti, and F. Cacciola, "Sephadex® LH-20, Isolation, and Purification of Flavonoids from Plant Species: A Comprehensive Review," *mdpi.com*, 2020, doi: 10.3390/molecules25184146.

[29] M. Sabitha, K. Krishnaveni, … M. M.-J. of D., and undefined 2021, "In-silico ADMET predicated Pharmacoinformatics of Quercetin-3-Galactoside, polyphenolic compound from Azadirachta indica, a sacred tree from Hill Temple in," *jddtonline.info*, 2021, doi: 10.22270/jddt.v11i5-S.5026.

[30] R. Bencheraiet, A. Kabouche, Z. Kabouche, R. Touzani, and M. Jay, "Flavonol 3-O-Glycosides from Three Algerian Bupleurum Species.," *acgpubs.org*, vol. 6, no. 2, pp. 171–174, 2012, Accessed: Apr. 03, 2023. [Online]. Available: https://acgpubs.org/doc/2018080810282525-RNP-1011-485.pdf.

[31] E. Gansukh, A. Nile, D. Kim, J. Oh, S. N.-F. chemistry, and undefined 2021, "New insights into antiviral and cytotoxic potential of quercetin and its derivatives–a biochemical perspective," *Elsevier*, Accessed: Apr. 03, 2023. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0308814620313704.

[32] N. Żurek, K. Pycia, A. Pawłowska, I. K.- Antioxidants, and undefined 2022, "Phytochemical Screening and Bioactive Properties of Juglans regia L. Pollen," *mdpi.com*, 2022, doi: 10.3390/antiox11102046.

[33] "quercetin 3-O-beta-L-fucopyranoside (CHEBI:75886)." https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:75886 (accessed Apr. 03, 2023).

[34] P. Mehrbod *et al.*, "Immunomodulatory properties of quercetin-3-O-α-L-rhamnopyranoside from Rapanea melanophloeos against influenza a virus," *BMC Complement. Altern. Med.*, vol. 18, no. 1, Jun. 2018, doi: 10.1186/S12906-018-2246-1.

[35] K. Greeshma, R. T.-J. of M. Liquids, and undefined 2022, "Experimental and theoretical approach on green synthesized zinc oxide nanoparticles from combined leaf extracts of Catharanthus roseus and Morinda Citrifolia for," *Elsevier*, Accessed: Apr. 03, 2023. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0167732222001738.

[36] M. Azeem, M. Hanif, K. Mahmood, N. Ameer, F. R. S. Chughtai, and U. Abid, "An insight into anticancer, antioxidant, antimicrobial, antidiabetic and anti-inflammatory effects of quercetin: a review," *Polym. Bull.*, vol. 80, no. 1, pp. 241–262, Jan. 2023, doi: 10.1007/S00289-022-04091-8.

[37] "Quercetin 3-O-β-xyloside-d3 | 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-3-(β-D-xylopyranosyloxy)-4H-1-benzopyran-4-one-d3; Reynoutrin-d3; 3,3',4',5,7-Pentahydroxyflavone 3β-D-xylopyranoside-d3; Quercetin 3-O-β-xyloside-d3; Quercetin 3-β-D-xylopyranoside-d3; Quercetin-3-O-β-D-xylopyranoside; Reinutrin-d3 | C20H15D3O11 | TRC." https://www.trc-canada.com/product-detail/?Q509702#:~:text=Product Description&text=Quercetin has antiproliferative effects on,phase of the cell cycle. (accessed Apr. 03, 2023).

[38] L. Santos, R. Ferreira, E. C.-D. screens for drug, and undefined 2019, "Integrating molecular docking and molecular dynamics simulations," *Springer*, Accessed: Apr. 03, 2023. [Online]. Available: https://link.springer.com/protocol/10.1007/978-1-4939-9752-7_2.

[39] S. Ivanov, A. Lagunin, … A. R.-J. of C., and undefined 2018, "ADVERPred–Web service for prediction of adverse effects of drugs," *ACS Publ.*, Accessed: Apr. 03, 2023. [Online]. Available: https://pubs.acs.org/doi/abs/10.1021/acs.jcim.7b00568.

[40] A. Daina, O. Michielin, V. Z.-N. acids research, and undefined 2019, "SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules," *academic.oup.com*, Accessed: Apr. 03, 2023. [Online]. Available: https://academic.oup.com/nar/article-abstract/47/W1/W357/5491750.

[41] G. B. Santos, A. Ganesan, and F. S. Emery, "Oral Administration of Peptide-Based Drugs: Beyond Lipinski's Rule," *ChemMedChem*, vol. 11, no. 20, pp. 2245–2251, Oct. 2016, doi: 10.1002/CMDC.201600288.

[42] B. D.-J. of P. C. Matter and undefined 2010, "Time dependent density functional theory with DMol3," *iopscience.iop.org*, Accessed: Apr. 03, 2023. [Online]. Available: https://iopscience.iop.org/article/10.1088/0953-8984/22/38/384208/meta.

[43] N. Guex, M. P.- electrophoresis, and undefined 1997, "SWISS-MODEL and the Swiss-Pdb Viewer: an environment for comparative protein modeling," *Wiley Online Libr.*, vol. 21, no. 15,

pp. 14–2723, Dec. 1997, doi: 10.1002/elps.1150181505.

[44] "Accelrys Discovery Studio Modeling Environment, Release... - Google Scholar." https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Accelrys+Discovery+Studio+Modeling+Environment%2C+Release+4.0%2C+2013.+&btnG= (accessed Apr. 04, 2023).

[45] Y. Xia *et al.*, "A combination therapy for KRAS-driven lung adenocarcinomas using lipophilic bisphosphonates and rapamycin," *science.org*, Accessed: Apr. 04, 2023. [Online]. Available: https://www.science.org/doi/abs/10.1126/scitranslmed.3010382.

[46] B. Smith, J. Sanders, … P. P.-J. of B., and undefined 2007, "Structure of the human lung cytochrome P450 2A13," *ASBMB*, Accessed: Apr. 04, 2023. [Online]. Available: https://www.jbc.org/article/S0021-9258(19)57677-3/abstract.

[47] L. L. G. Ferreira and A. D. Andricopulo, "ADMET modeling approaches in drug discovery," *Drug Discov. Today*, vol. 24, no. 5, pp. 1157–1165, May 2019, doi: 10.1016/J.DRUDIS.2019.03.015.

[48] D. E. V. Pires, T. L. Blundell, and D. B. Ascher, "pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures," *J. Med. Chem.*, vol. 58, no. 9, pp. 4066–4072, May 2015, doi: 10.1021/ACS.JMEDCHEM.5B00104.

[49] D. A. Filimonov *et al.*, "Prediction of the biological activity spectra of organic compounds using the pass online web resource," *Chem. Heterocycl. Compd.*, vol. 50, no. 3, pp. 444–457, 2014, doi: 10.1007/S10593-014-1496-1.

[50] D. J. Hosfield *et al.*, "Stereospecific Lasofoxifene Derivatives Reveal the Interplay between Estrogen Receptor Alpha Stability and Antagonistic Activity in ESR1 Mutant Breast Cancer Cells," *Elife*, vol. 11, May 2022, doi: 10.7554/ELIFE.72512.

[51] M. Athar, A. N. Sona, B. D. Bekono, and F. Ntie-Kang, "Fundamental physical and chemical concepts behind 'drug-likeness' and 'natural product-likeness,'" *Phys. Sci. Rev.*, vol. 4, no. 12, Dec. 2019, doi: 10.1515/PSR-2018-0101/HTML.

[52] S. M. A. Kawsar, A. Kumer, N. S. Munia, M. A. Hosen, U. Chakma, and S. Akash, "Chemical descriptors, PASS, molecular docking, molecular dynamics and ADMET predictions of glucopyranoside derivatives as inhibitors to bacteria and fungi growth," *search.proquest.com*, vol. 15, no. 2, pp. 184–203, 2022, doi: 10.25135/acg.oc.122.2203.2397.

[53] H. M. Patel *et al.*, "Quantitative structure–activity relationship (QSAR) studies as strategic approach in drug discovery," *Springer*, vol. 23, no. 12, pp. 4991–5007, 2014, doi: 10.1007/s00044-014-1072-3.

[54] M. Mazumdar, D. Fournier, D. Zhu, … C. C.-B., and undefined 2009, "Binary and ternary crystal structure analyses of a novel inhibitor with 17β-HSD type 1: a lead compound for breast cancer therapy," *portlandpress.com*, Accessed: Apr. 04, 2023. [Online]. Available:

https://portlandpress.com/biochemj/article-abstract/424/3/357/44987.

[55] T. Hou, J. Wang, W. Zhang, … W. W.-C. medicinal, and undefined 2006, "Recent advances in computational prediction of drug absorption and permeability in drug discovery," *ingentaconnect.com*, vol. 13, no. 22, pp. 2653–2667, Aug. 2014, doi: 10.2174/092986706778201558.