People's Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research
University of Martyr Hamma Lakhdar El Oued
Faculty of Natural and Life Sciences
Department of Biology

Graduation Thesis
For an Academic Master's Degree
Field: Natural and Life Sciences
Department: Biological Sciences
Specialization:

Theme

Investigating the Therapeutic Potential of Coumarin-Based Compounds: In Silico Toxicological Assessment, ADMET Profiling, and Molecular Docking Simulations for the Inhibition of SARS-CoV-2 Main Protease

Prepared by students:

- Arar Sabrine
- Bech Nadjia
- Dardouri Chaima
- Mansouri Hadjer

Under the supervision:

- LANEZ Elhafnaoui
- Housseyn CHAOUA

Academic year : 2024-2025

الحمد لله الذي منَّ علينا بالعون والتوفيق حتى أتممنا هذا العمل، فكان فضله سبحانه وتعالى خير مُعينٍ لنا في كل خطوة.

وخالص الشكر والعرفان نُهديه إلى الأستاذ الدكتور العانز الحفناوي، على توجيهاته القيّمة وصبره في إشرافه على رسالتنا، فكان خير مُرشدٍ ومُعين.

كما نتقدم بجزيل الامتنان إلى أساتذة كلية علوم الطبيعة والحياة بجامعة الشهيد حمة لخضر – الوادي، على ما بذلوه من جهودٍ في تعليمنا وتأهيلنا، فكانوا بحقٍّ روّادَ علمٍ ونبراسَ معرفة.

ولا يفوتنا أن نتوجه بخالص الشكر والتقدير إلى رئيسة لجنة المناقشة الدكتورة علوش جنات، على حضورها الكريم وتقييمها الموضوعي، وإلى الدكتور تواتي السعيد، على ما تفضل به من ملاحظات بنّاءة واقتراحات قيمة، والتي سيكون لها بالغ الأثر في إثراء هذا العمل وتجويده.

واللهَ نسأل أن يجزيهم خير الجزاء، وأن يُمكّننا من تطبيق ما تعلَّمناه في خدمة العلم والمجتمع.

الإهداء

بسم الله الرحمن الرحيم

﴿ وَآخِرُ دَعْوَاهُمْ أَنِ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ ﴾

والصلاة والسلام على أشرف الأنبياء والمرسلين

الحمد لله الذي بفضله تتم الصالحات، وبإذنه تُختتم المسيرات

ها نحن اليوم نقف شامخين أمام حلم التخرج، نهديه بكل حبٍّ وفخرٍ إلى:

من ساندونا في الرخاء والشدّة، فكانوا لنا سندًا لا يُنسى.

آبائنا وأمهاتنا، الأحياء منهم والأموات، الذين ربّونا على القيم وسهروا لينيروا طريقنا.

أساتذتنا الكرام الذين أضاءوا بعلمهم دروبنا، فكانوا لنا قدوةً ومعلماً.

ها قد وصلنا لليوم المنتظر، يوم الكفاح والدموع والأمل.

طريقٌ طويلٌ قطعناه معًا، ضحكنا وبكينا، تعبنا وقمنا، حتى منّ الله علينا بالنجاح.

أشكر كلّ لحظة عشناها معًا، وأعتذر عن أيّ زللٍ غير مقصود.

أسأل الله أن يحقّق أحلامنا، وأن يكتب لنا التوفيق في مسيرتنا القادمة.

والسلام عليكم ورحمة الله وبركاته

Abstract:

This study investigates the potential of coumarin derivatives as inhibitors of SARS-CoV-2, the virus responsible for COVID-19, using advanced in silico methods. A virtual library of 256 coumarin-based molecules was generated via SmiLib v2.0 by introducing diverse functional groups (OH, CN, NO_2, NH_2) to the coumarin scaffold.

Pharmacokinetic and toxicity profiles were assessed using SwissADME and ProTox-II, prioritizing compounds with favorable ADMET properties. Molecular structures were optimized through Density Functional Theory (DFT) calculations (B3LYP/6-311G++(d,p)) using Gaussian 16W software.

Molecular docking simulations targeting SARS-CoV-2 Main Protease (Mpro) were performed using Schrödinger Maestro (Glide module). The most active compounds, such as Com19 (IC_{50} = 40.31 μM), demonstrated strong binding affinity and inhibitory potential. Stability and interaction dynamics of the ligand-protein complexes were validated through Molecular Dynamics (MD) simulations under simulated physiological conditions.

The integrated computational approach — combining virtual screening, ADMET evaluation, quantum chemical calculations, molecular docking, and MD simulations — highlights coumarin derivatives as promising antiviral candidates against SARS-CoV-2 and offers a rational framework for future drug development efforts

Keywords:

Coumarin derivatives, SARS-CoV-2, COVID-19, In silico methods, Virtual library, Functional groups (OH, CN, NO_2, NH_2), *Pseudomonas aeruginosa* ,Pharmacokinetics, Toxicity, SwissADME, ProTox-II, Density Functional Theory (DFT), Molecular Docking, Schrödinger Maestro, SARS-CoV-2 Main Protease (Mpro), Half-maximal Inhibitory Concentration (IC_{50}), Molecular Dynamics (MD) Simulations, Antiviral candidates, Drug development.

Résumé:

Cette étude explore le potentiel des dérivés de la coumarine en tant qu'inhibiteurs du SARS-CoV-2, le virus responsable de la COVID-19, à l'aide de méthodes in silico avancées. Une bibliothèque virtuelle de 256 molécules à base de coumarine a été générée via SmiLib v2.0 en introduisant divers groupes fonctionnels (OH, CN, NO_2, NH_2) sur le squelette de la coumarine .

Les profils pharmacocinétiques et toxicologiques ont été évalués à l'aide de SwissADME et ProTox-II, en privilégiant les composés présentant des propriétés ADMET favorables. Les structures moléculaires ont été optimisées par des calculs de la théorie de la fonctionnelle de la densité (DFT) (B3LYP/6-311G++(d,p)) à l'aide du logiciel Gaussian 16W .

Des simulations de docking moléculaire ciblant la protéase principale du SARS-CoV-2 (Mpro) ont été effectuées avec Schrödinger Maestro (module Glide). Les composés les plus actifs, tels que Com19 (IC_{50} = 40,31 μM), ont montré une forte affinité de liaison et un potentiel inhibiteur significatif. La stabilité et la dynamique d'interaction des complexes ligand-protéine ont été validées par des simulations de dynamique moléculaire (MD) dans des conditions physiologiques simulées .

L'approche computationnelle intégrée combinant criblage virtuel, évaluation ADMET, calculs chimiques quantiques, docking moléculaire et simulations MD met en évidence les dérivés de la coumarine comme des candidats antiviraux prometteurs contre le SARS-CoV-2 et offre un cadre rationnel pour le développement futur de médicaments.

Mots-clés :

Dérivés de la coumarine, SARS-CoV-2, COVID-19, Méthodes in silico, Bibliothèque virtuelle, Groupes fonctionnels (OH, CN, NO_2, NH_2), *Pseudomonas aeruginosa*, Pharmacocinétique, Toxicité, SwissADME, ProTox-II, Théorie de la fonctionnelle de la densité (DFT), Docking moléculaire, Schrödinger Maestro, Protéase principale du SARS-CoV-2 (Mpro), Concentration inhibitrice médiane (IC_{50}), Simulations de dynamique moléculaire (MD), Candidats antiviraux, Développement de médicaments.

ملخص:

تبحث هذه الدراسة في إمكانية استخدام مشتقات الكومارين كمثبطات لفيروس كورونا المستجد (SARS-CoV-2)، الفيروس المسؤول عن مرض كوفيد-19، باستخدام أساليب حاسوبية متقدمة. تم إنشاء مكتبة افتراضية تضم 256 جزيئًا قائمًا على الكومارين عبر SmiLib v2.0، وذلك بإدخال مجموعات وظيفية متنوعة (OH، CN، NO_2، NH_2) إلى هيكل الكومارين.

تم تقييم خصائص الحركية الدوائية والسمية باستخدام SwissADME وProTox-II، مع إعطاء الأولوية للمركبات ذات خصائص ADMET المواتية. تم تحسين الهياكل الجزيئية من خلال حسابات نظرية الكثافة الوظيفية (DFT) (B3LYP/6-311G++(d,p)) باستخدام برنامج Gaussian 16W.

أُجريت عمليات محاكاة الالتحام الجزيئي التي تستهدف البروتياز الرئيسي لفيروس كورونا المستجد (SARS-CoV-2) باستخدام Schrödinger Maestro (وحدة Glide). أظهرت المركبات الأكثر نشاطًا، مثل Com19 (IC_{50} = 40.31 ميكرومولار)، ألفة ارتباط قوية وقدرة تثبيطية. وتم التحقق من ثبات وتفاعل مركبات البروتين-الربيطة من خلال محاكاة الديناميكيات الجزيئية (MD) في ظل ظروف فسيولوجية مُحاكاة. يُسلّط النهج الحسابي المتكامل الذي يجمع بين الفحص الافتراضي، وتقييم ADMET، والحسابات الكيميائية الكمومية، والالتحام الجزيئي، ومحاكاة الديناميكيات الجزيئية الضوء على مشتقات الكومارين كمرشحات واعدة لمكافحة فيروس SARS-CoV-2، ويُقدّم إطارًا منطقيًا لجهود تطوير الأدوية المستقبلية.

الكلمات المفتاحية:

مشتقات الكومارين، SARS-CoV-2، كوفيد-19، طرق حاسوبية، مكتبة افتراضية، المجموعات الوظيفية (OH، CN، NO_2، NH_2)، الزائفة الزنجارية، حركية الدواء، السمية، SwissADME، ProTox-II، نظرية الكثافة الوظيفية (DFT)، الالتحام الجزيئي، شرودنجر مايسترو، البروتياز الرئيسي لـ (Mpro) SARS-CoV-2، نصف التركيز المثبط الأقصى (IC_{50})، محاكاة الديناميكيات الجزيئية (MD)، مرشحات مضادات الفيروسات، تطوير الأدوية.

Table of Contents

List of Tables

List of Figures

Introduction General

The emergence of the novel coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has posed a significant global health challenge since its outbreak in late 2019. Characterized by a high transmission rate and the capacity to induce severe respiratory symptoms and systemic complications, SARS-CoV-2 prompted an unprecedented surge in scientific research aimed at understanding the virus's molecular mechanisms and developing effective therapeutic strategies. While numerous drugs and vaccines have been developed and deployed with varying degrees of success, the continuous emergence of viral mutations and the limitations of current treatments underscore the ongoing need for the identification of novel antiviral agents.

In this context, natural products and their synthetic derivatives have garnered substantial attention for their broad-spectrum biological activities, including antiviral, antibacterial, anticancer, and anti-inflammatory properties. Among these, coumarins, a class of benzopyrone compounds widely distributed in nature, have demonstrated a wide range of pharmacological effects. Structurally characterized by a fused benzene and α-pyrone ring, coumarins exhibit excellent drug-likeness and have been investigated for their potential as inhibitors of viral proteases, helicases, and other essential viral proteins.

Advancements in computer-aided drug design (CADD) have significantly accelerated the process of drug discovery, enabling researchers to predict and optimize the interaction between bioactive compounds and their molecular targets. In this study, we employ a suite of in silico approaches to evaluate the potential of coumarin-based derivatives as inhibitors of key SARS-CoV-2 targets. The ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis was utilized to assess the pharmacokinetic and toxicological properties of the selected molecules, ensuring their drug-likeness and safety profiles.

Subsequently, density functional theory (DFT) calculations were performed to investigate the electronic properties and reactivity descriptors of the compounds, such as the energy gap (HOMO-LUMO), molecular electrostatic potential (MEP), and dipole moments.

These descriptors provide crucial insights into the molecules' chemical behavior and their propensity to interact with biological targets.

Molecular docking studies were carried out to predict the binding modes and interaction patterns of the coumarin derivatives with selected SARS-CoV-2 proteins, particularly the main protease (Mpro) and RNA-dependent RNA polymerase (RdRp). The docking scores and binding affinities obtained help to identify the most promising compounds based on their predicted inhibitory potential.

To further validate the stability and reliability of the ligand–protein complexes, molecular dynamics (MD) simulations were conducted. This approach enables the observation of the time-dependent behavior of the complexes in a simulated biological environment, providing dynamic insights into the conformational stability, binding stability, and interaction persistence over time.

Collectively, this research aims to provide a comprehensive in silico evaluation of coumarin-based derivatives as potential antiviral candidates against SARS-CoV-2. By integrating ADMET screening, quantum chemical calculations, molecular docking, and molecular dynamics simulations, the study offers valuable perspectives into the rational design and optimization of coumarin-derived antiviral agents. The outcomes are anticipated to contribute meaningfully to ongoing efforts in antiviral drug discovery, particularly in the context of emerging infectious diseases such as COVID-19.

PART I: LITERATURE REVIEW

Chapter I: SARS COV-2

1. Introduction to SARS-CoV-2

1.1. Definition (SARS-CoV-2)

The human body is exposed to a variety of infectious microorganisms, such as viruses, bacteria, fungi, protozoa, and helminths, which cause tissue damage through different mechanisms. Viruses are unique among these five types of infectious organisms in that they can manipulate the host-cell machinery in a unique way and continuously evolve to survive and prosper in all species.[1]

COVID-19 is the disease caused by a new coronavirus called SARS-CoV-2. WHO first learned of this new virus on 31 December 2019, following a report of a cluster of cases of 'viral pneumonia' in Wuhan, People's Republic of China [2].

Since December 2019, a novel coronavirus disease had rapidly spread throughout China, leading to a global outbreak, and causing considerable public health concern.World Health Organisation (WHO) announced the outbreak of COVID-19 as a global public health emergency on 30 January 2020. In India, the first case of COVID-19 was reported on January 27, 2020, in Kerala district. Since then, there is a wide variation in the reporting of cases across the country. The case reporting is based on the SARS-CoV-2 antigen testing by Real-Time Reverse Transcription Polymerase Chain Reaction (RT-qPCR) or by Rapid Antigen Test (RAT)[3].

Coronavirus (CoV) is clustered under the viral family group that causes disease in mammals and birds. A pandemic novel coronavirus was named as ''Corona Virus Disease 2019'' (2019-nCoV) by World Health Organization (WHO) in Geneva, Switzerland. As its RNA pattern is closer to SARS, the 2019 Coronavirus is renamed as SARSCoV- 2 pandemic. It belongs to the subfamily Orthocoronavirinae inside the family Coronaviridae, order Nidovirales, and the realm Riboviria [4]. A two-dimensional view of Corona beneath a transmission electron microscopy reveals a characteristic look of ''paying homage to a crown'' around the virions. This lead to naming the virus ''Corona'', meaning ''crown'' or ''halo'' in Latin

This is the deadly third-generation virus in Corona family preceded by severe acute respiratory syndrome (SARS) in 2003, killed almost 10% of total affected patients (8429) across 29 international locations and Middle East Respiratory Syndrome (MERS) in 2012, even more lethal with a mortality rate of 30% of the infected patients [4] .

1.2. Historical Background

Coronaviruses are enveloped positive sense RNA viruses ranging from 60 nm to 140 nm in diameter with spike like projections on its surface giving it a crown like appearance under the electron microscope; hence the name coronavirus [3]. Four corona viruses namely HKU1, NL63, 229E and OC43 have been in circulation in humans, and generally cause mild respiratory disease.There have been two events in the past two decades wherein crossover of animal betacorona viruses to humans has resulted in severe disease. The first such instance was in 2002–2003 when a new coronavirus of the β genera and with origin in bats crossed over to humans via the intermediary host of palm civet cats in the Guangdong province of China. This virus, designated as severe acute respiratory syndrome coronavirus affected 8422 people mostly in China and Hong Kong and caused 916 deaths (mortality rate 11%) before being contained. Almost a decade later in 2012, the Middle East respiratory syndrome coronavirus (MERS-CoV), also of bat origin, emerged in Saudi Arabia with dromedary camels as the intermediate host and affected 2494 people and caused 858 deaths (fatality rate 34%) [5].

2. SYMPTOMS

A wide range of symptoms is found in COVID-19 patients, ranging from mild/moderate to severe, rapidly progressive, and fulminant disease. Symptoms of COVID-19 are non-specific and disease presentation can range from asymptomatic to severe pneumonia. Incidence of asymptomatic cases ranges from 1.6% to 51.7% and these people do not present typical clinical symptoms or signs and do not present apparent abnormalities in lung computed tomography. The most common symptoms of COVID-19 are fever, cough, myalgia, or fatigue and atypical symptoms include sputum, headache, haemoptysis, vomiting, and diarrhoea. Some patients may present with sore throat, rhinorrhoea, headache, and confusion a few days before the onset of fever, indicating that fever is a critical symptom, but not the initial manifestation of infection. Furthermore, some patients experience loss of smell (hyposmia) or taste (hypogeusia), which are now being considered early warning signs and indications for self-isolation [6].

- The most common symptoms of COVID-19 are

• Fever.

• Dry cough.

• Fatigue.

- Other symptoms that are less common and may affect some patients include

• Loss of taste or smell.
• Nasal congestion.
• Conjunctivitis (also known as red eyes).
• Sore throat.
• Headache.
• Muscle or joint pain.
• Different types of skin rash.
• Nausea or vomiting.
• Diarrhea.
• Chills or dizziness.

- Symptoms of severe COVID-19 disease include:

• Shortness of breath.
• Loss of appetite.
• Confusion.
• Persistent pain or pressure in the chest.
• High temperature (above 38 °C).

- Other less common symptoms are

• Irritability.
• Confusion.
• Reduced consciousness (sometimes associated with seizures).
• Anxiety.
• Depression.
• Sleep disorders.
• More severe and rare neurological complications such as strokes, brain inflammation, delirium and nerve damage.

People of all ages who experience fever and/or cough associated with difficulty breathing or shortness of breath, chest pain or pressure, or loss of speech or movement should seek medical care immediately. If possible, call your health care provider, hotline or health facility first, so you can be directed to the right clinic [1].

3. EPIDEMIOLOGY

All ages are at risk of getting the illness. This is because the ailment is transmitted through large droplets that result from coughing and sneezing by symptomatic individuals. In some instances, the infection can happen from asymptomatic individuals and before the beginning of symptoms. As of March 2020, the WHO announced that there are about 87,317 cases of COVID-19 globally as well as confirmed cases of deaths is 2,977. This implies that the disease symptoms are mild, as only 3.42 per cent of patients with it have died because of the virus. At the same time, the high number of incidences and deaths have been identified in China. It is that 92 per cent of the total number of occurrences have been reported in Asia, mainly China. Importantly, the confirmed incidences are clinically identified and laboratory-confirmed. Further, outside Asia, the number of cases and deaths differs due to the on-going nature of the disease, population density, degree of testing and reporting, and timing of reducing strategies. The features of COVID-19 are categorized into the host of the virus, transmission mode and incubation period. In the first place, the Chinese horseshoe bat is the natural hosts and the terminal hosts are humans. In addition, the transmission is from individual to individual through aerosol droplets. Lastly, the incubation period varies from two to fourteen days. Therefore, COVID-19 cumulative incidence differs depending on the country and incidences have been confirmed in almost all continents [7].

4. TRANSMISSION

Zoonotic transmission initially appeared to be a plausible cause as majority of early cases had a history of exposure to wet markets [8]. However, by the end of January 2020, the number of people who developed the disease without exposure to the market or another person with respiratory symptoms increased. The spread of the disease among persons who did not visit Wuhan and among healthcare workers suggested a person-to-person spread of the virus [9][10] . The exact mode of transmission of this virus is unknown. But, as with other respiratory viruses, droplet borne infection, either directly or indirectly, through fomites is probably the predominant mode of transmission. At present, there is no evidence for airborne transmission of the virus.12 13 Although virus particles have been detected in stool samples of both symptomatic and convalescing patients, the risk of feco-oral transmission is unclear [11].

- **Period of Infectivity**

The duration for which a patient with COVID-19 remains infective is unclear. Viral load in the oropharyngeal secretions is highest during the early symptomatic stage of the disease [11][12] . The patient can continue to shed the virus even after symptom resolution [11] . In a study from China, the median duration of virus shedding was 20 days (interquartile range [IQR] 17.0–24.0) amongst the survivors [13]. A study of viral dynamics in mild and severe cases revealed that mild cases tend to clear the viruses early, while severe cases can have prolonged viral shedding [14]. Data from studies using twin respiratory and fecal sampling have shown viral shedding can persist in stools for more than 4 weeks even when respiratory samples are negative [15]. Xu et al identified male sex, delayed hospitalization after illness, and invasive mechanical ventilation as risk factors for prolonged viral shedding [11] . Transmission during the asymptomatic phase has also been reported. In a study from Singapore, 6.4% of the 157 locally acquired cases of COVID-19 wereattributed to transmission during the asymptomatic phase of the disease [16].

5. DIAGNOSIS

Diagnostic testing for COVID-19 is conducted to find out whether a person is infected with the SARS-CoV-2 virus, responsible forCOVID-19 infection. Your healthcare practitioner may recommend you the same if:

• You are experiencing symptoms of COVID 19 such as high fever, cough, shortness of breath, excessive fatigue, etc.

• You have long-term health conditions such as asthma, heart diseases, etc. and experience a sudden worsening of symptoms.

• You have come in contact with someone tested positive for COVID 19 recently.

• You are a healthcare worker working in a hospital environment.

• You require hospitalization for treatment or surgery of existing medical conditions.

In general, there are two types of tests for diagnosing COVID-19 namely, Antigen or rapid testing and Molecular or PCR testing. The antigen test is often used as a pointof- care test, less expensive and yields quicker results within minutes. However, there is a higher chance of false-

negative results as compared to molecular testing. Molecular testing yields more accurate results but are time-consuming [17].

6. TREATMENT

Initially, early in the pandemic, the understanding of COVID-19 and its therapeutic management was limited, creating an urgency to mitigate this new viral illness with experimental therapies and drug repurposing. Since then, due to the intense efforts of clinical researchers globally, significant progress has been made which has led to a better understanding of not only COVID-19 and its management but also has resulted in the development of novel therapeutics and vaccine development at an unprecedented speed [18].

Chapter II: COUMARIN

1. Introduction to Coumarins

1.1 Definition and Historical Background

Coumarins are a class of benzopyrone compounds characterized by a fused benzene and α-pyrone ring system. The name "coumarin" is derived from the French word *coumarou*, the vernacular name for the tonka bean (*Dipteryx odorata*), which is one of the primary natural sources of the compound [19]. First isolated in the 19th century, coumarin itself was identified as the substance responsible for the sweet smell of tonka beans and sweet clover [20] . Since then, extensive research has been carried out to explore the chemical properties and pharmacological potential of coumarins and their derivatives [21] .

1.2 Natural Occurrence and Sources

Coumarins are widely distributed in nature, predominantly found in plants belonging to families such as Fabaceae, Rutaceae, and Apiaceae [20] . They occur in various parts of the plant, including roots, leaves, seeds, and fruits. Natural coumarins are typically found as glycosides and can be released through enzymatic hydrolysis. In addition to plants, some microbial species are known to biosynthesize coumarin-related structures [22] . Their broad presence in natural systems has contributed significantly to their recognition as bioactive molecules with diverse therapeutic potentials.

1.3 General Chemical Structure and Classification

The basic skeleton of coumarin comprises a benzene ring fused to a α-pyrone (lactone) ring. This structural motif serves as a versatile scaffold for chemical modification, leading to a wide array of derivatives with different substituents on the aromatic or lactone ring. Based on structural variations, coumarins are generally classified into several categories[23] , including:

- **Simple coumarins** (e.g, coumarin, umbelliferone)
- **Furanocoumarins** (e.g, psoralen, bergapten)
- **Pyranocoumarins**
- **Benzocoumarins**
- **Bis-coumarins** (compounds containing two coumarin units)

These structural subclasses contribute to the wide range of biological activities attributed to coumarins [23].

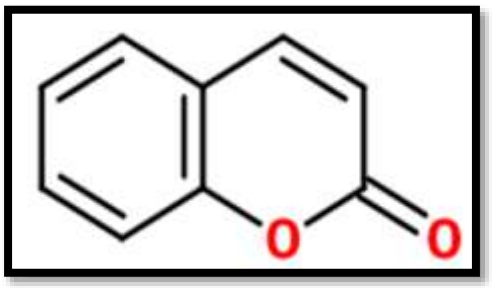

Figure 1. Structure of coumarin

1.4 Biosynthesis Pathways in Plants

The biosynthesis of coumarins [24] in plants typically begins with the phenylpropanoid pathway, where phenylalanine undergoes deamination to cinnamic acid via phenylalanine ammonia-lyase (PAL). This is followed by a series of hydroxylation and glycosylation reactions that lead to the formation of umbelliferone and other coumarin derivatives [21]. These biosynthetic transformations often occur in response to environmental stimuli such as pathogen attack, UV exposure, or oxidative stress, underlining the ecological role of coumarins as plant defense agents [25].

2. Pharmacological Properties of Coumarins

2.1 Overview of Biological Activities

Coumarins and their derivatives exhibit a broad spectrum of biological activities, making them valuable lead compounds in pharmaceutical research. Their pharmacological versatility is largely attributed to their ability to interact with multiple biological targets, modulate enzyme functions, and penetrate biological membranes [23]. The core structure of coumarin allows for extensive chemical modifications, which can significantly enhance or modulate their bioactivity. Some of the most notable biological effects include anti-inflammatory, anticoagulant, antioxidant, antimicrobial, and anticancer activities [26].

2.2 Antiviral Properties

In recent years, significant attention has been directed toward the antiviral potential of coumarins. Several natural and synthetic coumarins have been reported to inhibit the replication of a variety of viruses [27], including human immunodeficiency virus (HIV), hepatitis viruses

(HBV, HCV), dengue virus, and more recently, coronaviruses [28]. The mechanisms of antiviral action vary depending on the derivative and the virus but may include inhibition of viral proteases, interference with viral entry or uncoating, and modulation of host immune responses. The molecular versatility of coumarins enables them to be tailored as selective inhibitors of key viral enzymes, such as proteases and polymerases, essential for viral replication [29].

2.3 Antibacterial, Antifungal, and Anticancer Effects

Beyond their antiviral capabilities, coumarins demonstrate notable antibacterial and antifungal effects. They have been shown to disrupt microbial membrane integrity, inhibit nucleic acid synthesis, and interfere with quorum sensing pathways in bacteria [30]. Coumarins such as novobiocin are already used clinically as antibiotics. In oncology, coumarin derivatives exhibit cytotoxic properties by inducing apoptosis [31], inhibiting angiogenesis, and modulating cell cycle regulators. Some coumarin-based compounds have been developed into anticancer agents or are under investigation in clinical trials.

2.4 Structure–Activity Relationships (SAR)

A deep understanding of the structure–activity relationships (SAR) of coumarins is fundamental to rational drug design. Modifications at specific positions on the coumarin scaffold (e.g., hydroxyl, methoxy, prenyl, or halogen substitutions) significantly influence biological activity [23]. For example, hydroxylation at positions 6 and 7 often enhances antioxidant and anti-inflammatory properties, while furan or pyran ring fusion increases lipophilicity and bioavailability. These SAR insights guide the synthesis of new analogues with improved efficacy, selectivity, and pharmacokinetic propertiesb [32].

3. Coumarins as Antiviral Agents

3.1 Mechanisms of Antiviral Action

Coumarin derivatives exert antiviral effects through multiple mechanisms, depending on the virus type and the molecular modifications of the coumarin core [33]. These mechanisms include:

- **Inhibition of viral enzymes**, such as proteases (e.g., 3CLpro), reverse transcriptase, and RNA-dependent RNA polymerase (RdRp), which are critical for viral replication.

- **Blocking viral entry and fusion** with host cells by interfering with envelope proteins or host receptors.
- **Interruption of nucleic acid synthesis**, either by direct binding or by chelating essential cofactors (e.g., metal ions).
- **Modulation of host immune responses**, such as enhancing interferon signaling or inhibiting pro-inflammatory cytokine cascades.

These multitarget actions make coumarins attractive candidates for the development of broad-spectrum antiviral agents [34].

3.2 Previously Reported Antiviral Coumarin Derivatives

Numerous studies have identified coumarin derivatives with significant antiviral activity. For example:

- **Mesuol**, a naturally occurring coumarin, has been reported to inhibit HIV-1 replication by targeting the NF-κB pathway [28].
- **Daphnetin**, a dihydroxycoumarin, exhibits inhibitory activity against HBV and influenza virus by suppressing viral antigen expression.
- **Esculetin** and **scopoletin** show activity against herpes simplex virus (HSV) by interfering with early gene expression and viral replication.

In addition, synthetic coumarin derivatives have been engineered to improve potency and specificity, particularly through modifications that enhance binding to viral targets or improve pharmacokinetic profiles [13-14].

3.3 Coumarins Against RNA Viruses

The recent global focus on RNA viruses, particularly SARS-CoV-2, has renewed interest in coumarin-based antivirals [36]. RNA viruses rely heavily on highly conserved enzymes such as RdRp and viral proteases (e.g., 3CLpro), which are promising drug targets [37]. Several coumarins have shown potential inhibitory activity against these enzymes in *in silico* and *in vitro* assays. For instance:

- Molecular docking studies have demonstrated the ability of coumarins to form stable interactions with the catalytic residues of SARS-CoV-2 main protease.
- QSAR and molecular dynamics studies support their favorable binding energies and conformational stability.

These findings position coumarins as candidates for repurposing or further derivatization in the context of COVID-19 drug discovery.

3.4 Challenges in Antiviral Drug Development with Coumarins

Despite their promising bioactivity [38], several challenges exist in the development of coumarin-based antivirals:

- **Low aqueous solubility** and **variable bioavailability** can limit their effectiveness in vivo.
- Some coumarins are metabolized into **toxic or reactive intermediates**, which may raise safety concerns.
- The **selectivity** of coumarins for viral vs. host targets must be carefully optimized to minimize cytotoxicity.

To address these issues, computational modeling, nanoformulation, and prodrug strategies are actively being explored to improve the drug-likeness and safety profile of coumarin derivatives [39].

4. In Silico Approaches in Drug Discovery

4.1 Overview and Importance

In silico methods [40] have emerged as indispensable tools in modern drug discovery and development. These computational approaches facilitate the identification, optimization, and evaluation of drug candidates by simulating molecular interactions, predicting pharmacokinetic behaviors, and estimating potential toxicity—all prior to laboratory testing [41]. The advantages of in silico techniques include reduced cost, shorter development timelines, improved precision, and the ability to virtually screen large compound libraries.

In the context of antiviral drug discovery, in silico approaches are particularly valuable for rapidly identifying molecules that can inhibit viral targets such as proteases, polymerases, or host entry factors.

4.2 ADMET Prediction

ADMET [42] (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling is a critical component of early drug development. Poor ADMET properties are responsible for the

majority of clinical trial failures [43]. In silico ADMET tools (e.g., SwissADME, pkCSM, ADMETlab) allow researchers to predict:

- **Oral bioavailability and gastrointestinal absorption**
- **Blood-brain barrier permeability**
- **Metabolic stability and interaction with cytochrome P450 enzymes**
- **Toxicological endpoints**, including hepatotoxicity, mutagenicity, and carcinogenicity

These predictions help eliminate compounds with undesirable profiles and prioritize those with optimal drug-likeness for further development.

4.3 Density Functional Theory (DFT) Analysis

DFT is a quantum mechanical modeling method used to investigate the electronic structure of molecules [44]. In drug discovery, DFT is employed to:

- Optimize molecular geometries
- Evaluate electronic properties such as HOMO-LUMO gap, dipole moment, and molecular electrostatic potential (MEP)
- Predict reactive sites and interaction potentials with biological targets

For coumarins, DFT analysis provides valuable insights into their electronic configuration, reactivity, and interaction potential with viral enzymes, thereby guiding rational drug design.

4.4 Molecular Docking

Molecular docking is a computational method used to predict the binding orientation and affinity of small molecules (ligands) to target proteins [45]. It provides a structural basis for understanding drug-target interactions and ranking candidate compounds based on their binding scores. Tools such as AutoDock, AutoDock Vina [46], and Glide are widely used to simulate these interactions.

In the case of SARS-CoV-2 [47], docking studies typically target the main protease (3CLpro), spike protein, or RdRp. Docking coumarin derivatives against these proteins helps identify potential inhibitors by assessing binding energy, hydrogen bonding, and hydrophobic interactions.

4.5 Molecular Dynamics (MD) Simulation

MD simulations provide detailed information on the time-dependent behavior of biomolecular systems [48]. After docking, MD simulations are employed to evaluate the **stability** and **flexibility** of ligand-protein complexes in a simulated physiological environment [49].

Key parameters analyzed include:

- **Root Mean Square Deviation (RMSD)** to assess structural stability
- **Root Mean Square Fluctuation (RMSF)** to examine residue flexibility
- **Radius of gyration (Rg)** for compactness
- **Hydrogen bond interactions** over time
- **Binding free energy estimations** using MM-PBSA or MM-GBSA methods

These analyses validate docking predictions and help refine compound selection.

5. Application of In Silico Approaches to Coumarins Targeting SARS-CoV2

5.1 Rationale for Targeting SARS-CoV-2

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, has prompted an urgent search for effective antiviral agents [50]. The virus relies on several essential proteins for its replication and pathogenesis, most notably:

- The **main protease (Mpro or 3CLpro)**: crucial for proteolytic processing of viral polyproteins.
- The **RNA-dependent RNA polymerase (RdRp)**: essential for viral RNA replication.
- The **spike (S) protein**: facilitates viral entry via interaction with the ACE2 receptor on host cells.

These viral proteins present ideal molecular targets for drug development. Natural compounds, especially coumarins, have shown promising binding affinity to these targets in silico, making them candidates for further optimization and testing [51].

5.2 Virtual Screening and Molecular Docking Studies

Numerous docking studies have been conducted to assess the binding affinity of coumarin derivatives to SARS-CoV-2 targets. These studies involve:

- Preparing the 3D structures of ligands and target proteins.
- Identifying active sites using crystallographic data or predictive tools.

- Running docking simulations using tools like AutoDock Vina or Glide.
- Analyzing binding energies and interactions (hydrogen bonds, π–π stacking, hydrophobic contacts).

Results from various reports indicate that coumarins can bind strongly to the active sites of SARS-CoV-2 Mpro and RdRp [52]. Structural features such as hydroxyl substitutions, fused ring systems, and electron-rich moieties contribute significantly to enhanced binding interactions.

5.3 ADMET and Drug-Likeness Evaluation

Following docking, in silico ADMET profiling is used to evaluate the pharmacokinetic and safety characteristics of the top-performing coumarin derivatives. Most coumarins show favorable drug-likeness based on Lipinski's Rule of Five, good gastrointestinal absorption, and low predicted toxicity [53]. However, structural modifications are sometimes necessary to improve metabolic stability and reduce potential for hepatotoxicity or mutagenicity.

5.4 DFT Insights into Electronic Properties

DFT calculations provide a deeper understanding of the electronic behavior of selected coumarin candidates. The HOMO-LUMO gap, dipole moment, and molecular electrostatic potential maps offer insight into reactivity and likely interaction sites with viral proteins [54].

Compounds with lower HOMO-LUMO energy gaps generally exhibit greater chemical reactivity and stronger binding capabilities, which correlate with improved docking results.

5.5 Molecular Dynamics Simulation for Complex Stability

To assess the dynamic behavior and stability of the coumarin–protein complexes, MD simulations are conducted under physiological conditions [55]. Over simulation times (e.g., 100 ns), key metrics are analyzed:

- **RMSD** confirms the conformational stability of the protein-ligand complex.
- **RMSF** identifies flexible regions and potential conformational shifts.
- **Hydrogen bonding profiles** indicate persistent interactions supporting stable binding.
- **MM-PBSA or MM-GBSA** methods provide estimates of binding free energy to quantify interaction strength.

Such analyses validate docking predictions and reinforce the suitability of coumarins as promising inhibitors of SARS-CoV-2.

5.6 Summary and Prospects

The integration of molecular docking, ADMET screening, DFT analysis, and MD simulations provides a comprehensive framework for evaluating coumarin derivatives against SARS-CoV-2 [56]. These computational strategies enable the rapid identification of lead compounds with optimal binding affinity, drug-likeness, and stability. Future directions involve experimental validation and the synthesis of novel coumarin-based analogues with improved antiviral efficacy [57].

6. Molecular Docking Study of Coumarin Derivatives Against SARS-CoV-2 Targets

Molecular docking is a computational technique that predicts the preferred orientation of a ligand when bound to a target macromolecule, usually a protein, and estimates the strength of the interaction. In the context of drug discovery, docking serves as a pivotal tool for identifying and optimizing bioactive compounds based on their binding affinity and interaction patterns within the active site of therapeutic targets [58].

6.1 Theoretical Basis of Molecular Docking

The underlying principle of molecular docking is rooted in molecular recognition—the ability of two molecules, such as a drug and an enzyme, to interact through specific non-covalent forces [59]. These include hydrogen bonds, hydrophobic contacts, van der Waals forces, π–π stacking, and electrostatic interactions. Docking algorithms attempt to reproduce these interactions by searching conformational and orientational space to identify the most energetically favorable ligand–protein complex [60].

The docking process consists of two essential components:

- **Search algorithm**: explores different ligand conformations and orientations within the binding site.
- **Scoring function**: evaluates the binding affinity of each pose by calculating an energy-based score, which approximates the Gibbs free energy of binding.

6.2 Relevance to SARS-CoV-2 Therapeutics

In the battle against SARS-CoV-2, molecular docking has been extensively applied to screen and prioritize small molecules that can inhibit essential viral proteins [61]. Key viral targets include:

- **Main protease (3CLpro)**: indispensable for processing polyproteins into functional units necessary for replication.
- **RNA-dependent RNA polymerase (RdRp)**: crucial for the replication and transcription of viral RNA.
- **Spike (S) protein**: mediates viral entry by binding to the ACE2 receptor on host cells.

The rationale for docking coumarin-based molecules against these proteins stems from the structural versatility and biological potential of the coumarin scaffold, which has demonstrated inhibitory activity in other viral contexts. Docking studies enable the identification of favorable binding interactions, predict the binding mode, and help rationalize the biological activity of these derivatives [62].

6.3 Theoretical Evaluation of Binding Affinity

Docking simulations yield a quantitative estimate of the ligand's binding affinity, often reported as the binding energy in kcal/mol [63]. A lower binding energy indicates a more stable and potentially more effective inhibitor. In addition to binding energy, qualitative assessment of interaction profiles provides insights into the molecular determinants of binding. For instance, ligands forming stable hydrogen bonds with catalytic residues or interacting with key amino acid side chains within the binding pocket are likely to exhibit potent inhibitory effects [64].

6.4 Structure–Activity Relationships and Coumarin Scaffold

The coumarin core, characterized by a benzopyran-2-one ring system, offers numerous sites for functionalization, thereby influencing the molecule's interaction with biological targets. Substituents at specific positions can modulate electronic distribution, steric accessibility, and hydrogen-bonding capacity, all of which affect docking performance. Theoretical studies suggest that hydroxyl, methoxy, and halogen groups can enhance binding by improving complementarity with the protein's active site [65].

The ability to explore structure–activity relationships (SAR) through docking enables rational design, guiding the synthesis of optimized coumarin derivatives with improved interaction profiles and binding energies.

6.5 Limitations and Considerations

Despite its utility, molecular docking is inherently limited by the static nature of the protein and ligand conformations used in the simulations [66]. Proteins are dynamic entities, and their flexibility can significantly influence ligand binding. Furthermore, scoring functions may not always accurately capture the entropic contributions or solvation effects involved in real biological systems.

Therefore, docking is most effective when integrated with other computational methods—such as molecular dynamics (MD) simulations—to validate the predicted binding modes and assess the temporal stability of the interactions under near-physiological conditions [67].

7. Molecular Dynamics Simulations: Theoretical Aspects and Application to SARS-CoV-2 Systems

Molecular dynamics (MD) simulations provide a powerful tool for studying the behavior of biomolecules at the atomic level by simulating the physical movements of atoms and molecules over time. Unlike molecular docking, which offers static snapshots of ligand–protein interactions, MD simulations allow the study of the time-dependent behavior of a system, including flexibility, conformational changes, and the stability of the ligand–protein complex under near-physiological conditions [68].

7.1 Theoretical Foundations of Molecular Dynamics Simulations

MD simulations are based on Newton’s laws of motion, where the position and velocity of each atom are calculated at each time step based on the forces acting on it. These forces arise from interatomic interactions such as bond stretching [68], angle bending, van der Waals forces, electrostatic interactions, and hydrogen bonding. The main objectives of MD simulations in drug design and biochemistry are to:

- Explore the conformational space of proteins and ligands,
- Assess the stability of ligand–protein complexes over time,
- Provide insight into the thermodynamics of the binding process,

- Identify potential allosteric sites and conformational changes in proteins that could influence drug efficacy.

In MD simulations, the system is treated as a collection of interacting particles (atoms) that evolve with time. The movement of these particles is tracked using an integration algorithm, most commonly the Verlet algorithm, which computes the trajectory of the particles by solving the equations of motion.

7.2 Relevance to SARS-CoV-2 Target Interaction Studies

For SARS-CoV-2 drug discovery, MD simulations are essential for understanding the dynamics of the viral proteins and the ligands interacting with them. This is especially important because viral proteins, such as the main protease (3CLpro), RNA-dependent RNA polymerase (RdRp), and the spike protein, are highly flexible, undergoing conformational changes during their normal function [69]. These dynamic processes influence the binding of potential inhibitors and their ability to disrupt viral activity.

MD simulations provide a deeper understanding of:

- **Protein flexibility**: SARS-CoV-2 proteins exhibit dynamic structural flexibility, which is crucial for their biological function. MD simulations capture this flexibility, revealing how the proteins adapt upon binding to ligands.
- **Binding site dynamics**: The flexibility of the binding pocket or active site of the target protein can significantly impact the stability and affinity of the ligand. MD simulations allow the assessment of ligand–protein interactions over longer timescales (nanoseconds to microseconds), which is not possible in static docking simulations.
- **Solvent effects**: Water molecules play a key role in biological interactions, often mediating protein–ligand interactions. MD simulations account for the dynamic nature of the solvent, providing more realistic binding predictions.

7.3 Molecular Dynamics in the Coumarin Derivatives–SARS-CoV-2 Complex

When simulating the interaction of coumarin-based derivatives with SARS-CoV-2 proteins, MD simulations provide insights into the structural stability of the complex and its interactions under physiological conditions. Several key features are examined [70]:

- **Ligand stability**: The interaction between coumarin derivatives and the SARS-CoV-2 target proteins is assessed by monitoring the root-mean-square deviation (RMSD) and root-mean-

square fluctuation (RMSF) of the ligand and protein, respectively. This allows for a quantitative measure of how the ligand's position and conformation change over time.

- **Binding energy over time**: During the simulation, the binding energy of the ligand to the protein is recalculated at each time step. A stable binding energy profile suggests a strong and consistent interaction, which is a key feature for identifying promising therapeutic candidates.
- **Protein-ligand interactions**: MD simulations also allow for the analysis of key interactions, such as hydrogen bonds, hydrophobic interactions, and salt bridges, over time. These interactions are important for the sustained binding of the ligand and the inhibition of viral function.

7.4 Role of MD in Refining Docking Results

MD simulations serve as a complementary tool to molecular docking by validating and refining the binding modes predicted through docking. While docking provides an initial prediction of binding poses, MD simulations allow for the evaluation of the stability of these poses in a dynamic environment [71]. MD helps to confirm whether the predicted interactions are maintained over time and if any conformational changes occur that might impact the binding affinity.

By integrating MD simulations with docking, it is possible to obtain a more comprehensive understanding of the ligand–protein interactions, improving the accuracy of virtual screening results and guiding the design of more potent inhibitors [72].

7.5 Limitations and Challenges of MD Simulations

Despite their ability to simulate real-time molecular interactions, MD simulations have certain limitations [73]:

- **Time scale**: MD simulations are limited by computational power and typically simulate time scales of nanoseconds to microseconds. Biological processes, such as protein folding or large conformational changes, may require longer simulation times.
- **Force field accuracy**: The results of MD simulations depend heavily on the force fields used to describe the atomic interactions. Inaccurate force fields may lead to erroneous predictions, especially in complex systems like protein–ligand interactions.

- **Sampling issues**: The ability to explore the full conformational space of a protein or ligand is constrained by the chosen initial structure and the inherent limitations of the simulation's time frame.

Despite these challenges, MD simulations remain a crucial tool in drug design, offering invaluable insights into the dynamic interactions between ligands and their protein targets.

Part II: EXPERIMENTAL STUDY

Chapter I : MATERIALS AND METHODS

1. Generation of Combinatorial Library

To expand the structural diversity of potential coumarin analogues, a virtual screening workflow was implemented using SmiLib v2.0 software [74]. Utilizing the coumarin scaffold as the core framework, a range of functional groups including hydroxyl (OH), cyanide (CN), nitro (NO_2), and amino (NH_2) groups, in addition to vacant linkers were systematically incorporated to construct a wide array of molecular derivatives. As a result, a total of 256 unique coumarin-based molecules were enumerated.

To prioritize compounds with desirable pharmacokinetic and toxicity characteristics, the generated coumarin analogues were evaluated using predictive screening tools such as SwissADME [75] and ProTox II [76]. These platforms enabled the assessment of key pharmacokinetic parameters relevant to human use, including aqueous solubility, blood-brain barrier (BBB) permeability, cytochrome P450 (CYP) enzyme interactions, and intestinal absorption.

The combinatorial library of coumarin analogues, used for the aforementioned virtual screening and subsequent molecular docking studies, was generated according to the following protocol:

1.1. Selection of Scaffold

A scaffold structure of coumarin was chosen and served as a starting point for diversification.

1.2. Identification of Building Blocks

A selection of building blocks and functional groups suitable for attachment to the core scaffold was identified to facilitate the generation of structurally diverse molecular entities. These building blocks encompass a range of chemical moieties, including methyl, hydroxyl, amino, and halogen groups, enabling broad chemical variation in the resulting analogues.

1.3. Enumeration of Combinations

The enumeration of all possible combinations between the scaffold and the selected building blocks was performed using the SmiLib v2.0 software [77]. This process systematically integrates each building block with the scaffold, resulting in the generation of a large and diverse set of unique molecular structures.

2. Filtering and Validation

A series of filters and selection criteria were applied to the enumerated molecules to prioritize candidates exhibiting favorable attributes such as drug-likeness, synthetic accessibility, and structural diversity. Compounds that violated established chemical constraints or displayed undesirable physicochemical properties were excluded from further consideration.

3. Pharmacokinetic and Toxicity Prediction

Computational tools such as SwissADME and ProTox II were employed to predict key pharmacokinetic parameters, including aqueous solubility, blood-brain barrier (BBB) permeability, and cytochrome P450 (CYP) binding affinity. In parallel, the potential toxicity of the generated molecules was evaluated using in silico toxicity prediction models.

3.1. Final Selection:

A subset of compounds was selected from the combinatorial library based on their predicted pharmacokinetic behavior, toxicity profiles, and structural diversity. These selected molecules were advanced to virtual screening and molecular docking studies to identify potential lead candidates with promising biological activity.

3.2. Database Generation:

All prioritized compounds were compiled into a structured database, encompassing their 2D molecular representations, chemical identifiers, and associated physicochemical and pharmacokinetic properties. This database serves as a foundational input for virtual screening tools in the subsequent phases of the computational drug discovery pipeline.

By adhering to this workflow, researchers can systematically generate a chemically diverse and pharmacologically relevant library of compounds optimized for virtual screening and lead identification in drug discovery

3.3. Screening for pharmacokinetics-toxicity

We employed the SwissADME tool, provided by the Swiss Institute of Bioinformatics, to predict the pharmacokinetic properties of the generated coumarin analogs [78]. This analysis aimed to assess whether these compounds could act as inhibitors of various cytochrome P450

(CYP) isoforms. Furthermore, we evaluated pharmacokinetic parameters related to intestinal absorption, P-glycoprotein activity, and blood-brain barrier permeability [79]. To estimate the toxicity profile, we utilized the Protox II web server to predict the median lethal dose (LD50) for coumarin and its designed analogs. Candidate molecules were selected from the combinatorial library based on key pharmacological and toxicological criteria, with particular emphasis on LD50 values to assess their potential impact on human health.

4. Structural Optimization

In the initial stage of our investigation, we focused on refining the geometries of the selected coumarin analogues using molecular mechanics. This process involved the application of computational algorithms to optimize the spatial configuration of atoms within the molecules, enabling us to identify their most stable conformations and corresponding energy minima [80].

Following this preliminary geometry optimization, we performed a comprehensive re-optimization using Density Functional Theory (DFT), a widely utilized quantum mechanical approach in computational chemistry. Specifically, we employed the B3LYP functional, recognized for its accuracy in predicting molecular properties, in conjunction with the 6-311G++(d,p) basis set, which offers a detailed description of electron density distributions.

All DFT calculations were conducted using the Gaussian 16W software package, a robust platform for performing quantum chemical computations and in-depth analysis of molecular properties. To provide a benchmark for validation and comparison, we retrieved the three-dimensional structure of parent coumarin, a naturally occurring aromatic compound with well-established biological activities, from the PubChem database (https://pubchem.ncbi.nlm.nih.gov).

This reference molecule allowed us to evaluate and compare the geometric and electronic properties of the designed coumarin analogues. By utilizing these computational techniques and leveraging publicly available structural data, we aimed to gain deeper insights into the structural stability, electronic characteristics, and potential reactivity of the coumarin derivatives. This approach facilitated the assessment of their suitability as potential antiviral agents, particularly in the context of targeting SARS-CoV-2, and highlighted their promise for further in silico investigations.

5. Protein selection

The protein selected for investigation in this study is the SARS-CoV-2 major protease (Mpro (Cov-19)), an enzyme essential for viral replication and transcription. Mpro is responsible for cleaving viral proteins at specific sites and synthesizing non-essential proteins necessary for replication and transcription. Given its role in shedding light on the viral life cycle and the lack of relevant documentation in humans, Mpro is widely recognized as a highly specific and cultural target for developing viral cultures against COVID-19.

The three-dimensional structure of Mpro was retrieved from the UniProt database under accession number P0DTD1, which is similar to the SARS-CoV-2 major protease. This protein was selected for its structural accuracy and suitability for structure-to-structure drug design. The retrieved data were used as the basis for a formal submarine docking ticket to identify coumarin analogs selected for the protease site (Figure 1).

By targeting a specific site directly involved in the viral replication mechanism, a study is underway to explore the potential puncturing properties of coumarin compounds. The detailed structural insights from the welcome docking provide predictive information about binding and affinity, which could clearly support the use of coumarin derivatives as antiviral agents against the novel coronavirus (SARS-CoV-2). This contributes to the broader investigation of natural product-based structures suitable for maximizing the benefits of drug development.

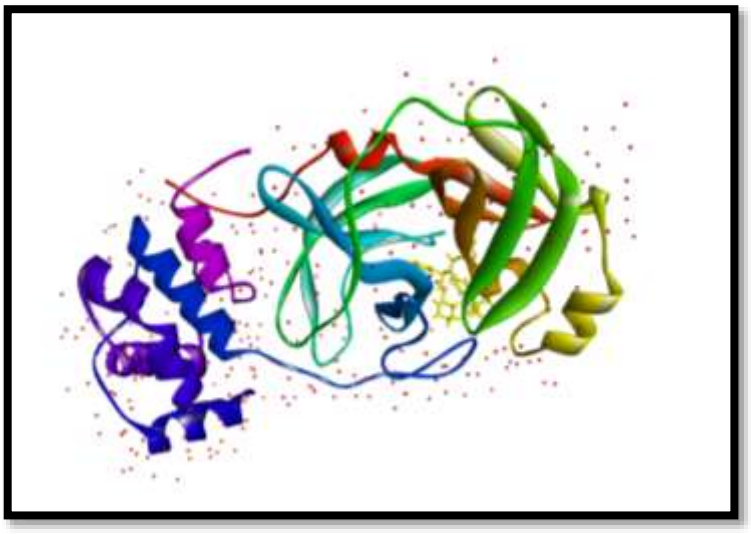

Figure 2. Three-dimensional structure of SARS-CoV-2 (COV-19)

6. Steps of Molecular docking

Molecular docking simulations were carried out utilizing the Schrödinger Maestro software suite [81], following a series of well-defined steps aimed at predicting the binding orientations and affinities of ligands toward their respective protein targets. To define the ligand–receptor interaction region, a Receptor Grid was subsequently generated using the Receptor Grid Generation tool within Maestro. This grid was centered around the active site, specified by coordinates (x, y, z). For both receptor systems under investigation, the grid dimensions were configured to 60×60×60 points along the x, y, and z axes, respectively. Docking studies were then performed using the Glide module within Maestro [82], enabling detailed analysis of ligand binding modes and interaction energies.

Step 1: Preparation of Protein Structures

The crystallographic three-dimensional (3D) structures of the target proteins were initially imported into the Maestro workspace and visualized using the Maestro interface. Protein structures were then prepared using the Protein Preparation Wizard at a physiological pH of 7.0. During this step, all water molecules and non-relevant ligands were carefully removed. The protonation states of amino acid residues were assessed and adjusted accordingly. Missing hydrogen atoms were added, and appropriate bond orders were assigned to ensure structural accuracy and readiness for docking simulations.

Step 2: Protein Grid Generation

To define the region for ligand docking, the active site or binding pocket within the protein structure was identified. A receptor grid was then generated around this site using the Receptor Grid Generation tool in Maestro. This grid serves as the defined spatial domain within which the ligand is allowed to explore potential binding conformations, effectively restricting the docking search to the biologically relevant region of the protein.

Step 3: Ligand Structure Preparation

Ligand structures were prepared using the LigPrep module in Schrödinger Maestro to ensure a comprehensive exploration of binding poses during the docking process.

3D Conformer Generation: Multiple 3D conformers of each ligand were generated to capture various spatial orientations and potential binding modes.

Structure Cleaning: All solvent molecules and irrelevant entities were removed, retaining only the ligand structure for accurate protein-ligand interaction analysis.

Geometry Optimization: The geometrical parameters, including bond lengths, bond angles, and dihedral angles, were optimized to produce energetically favorable conformations compatible with the protein active site.

Protonation and Ionization States: Ligands were protonated or deprotonated to reflect physiological pH conditions, and various ionization states were generated to account for possible pH-dependent interactions.

Energy Minimization: Final ligand geometries were subjected to energy minimization using appropriate force fields to reduce steric clashes and optimize the overall conformation.

Step 4: Molecular Docking Simulation

Ligands were docked into the defined binding pocket of the target protein using the Glide docking algorithm within Maestro. Docking poses were generated and ranked based on GlideScore, which estimates the binding affinity of each ligand toward the protein receptor.

Step 5: Post-Docking Refinement and Scoring

Docking poses were further refined using more accurate scoring methods and energy calculations. Post-docking minimization was applied to improve the ligand conformations within the binding site. Final scoring was performed using advanced scoring functions such as GlideScore and Prime MM-GBSA, providing more precise estimations of binding free energies.

Step 6: Analysis and Visualization

The docking results were analyzed to identify the most favorable protein–ligand interactions and binding modes. Docked poses were visualized using Maestro's molecular visualization tools to assess key interactions (e.g., hydrogen bonds, hydrophobic contacts). Ligands were selected for further analysis or experimental validation based on docking scores, interaction profiles, and structural complementarity with the protein target.

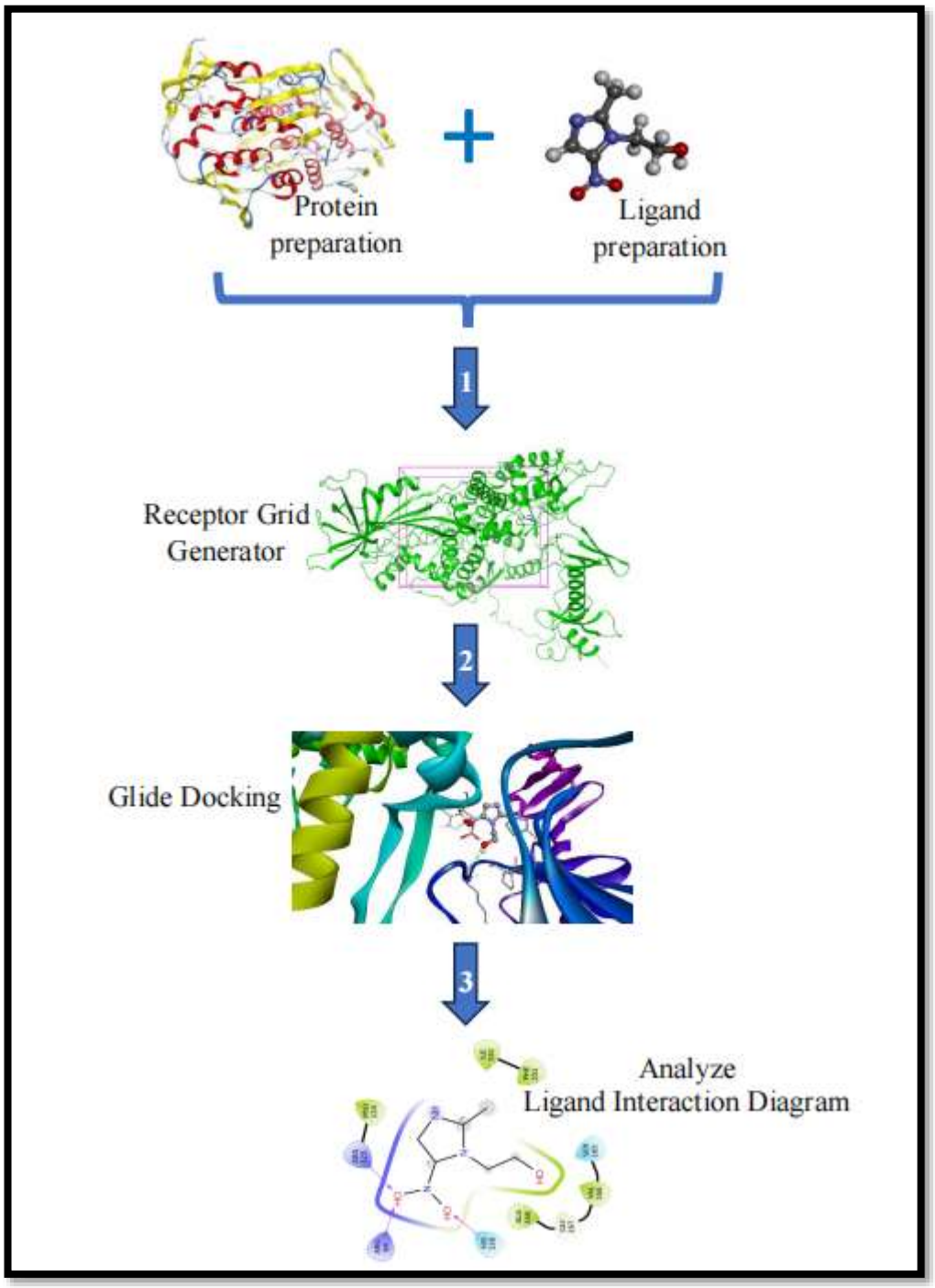

Figure 3. Molecular docking steps with Maestro

Chapter II: RESULTS & DISCUSSION

1. Virtual screening

The generation of a series of coumarin-based analogues was initiated using the core scaffold structure depicted in Figure 2 as the molecular framework. A variety of functional groups, including hydroxyl OH, nitro NO_2, NH_2, and CN, along with vacant linker sites, were employed to diversify the chemical space. The corresponding simplified molecular input line entry system (SMILES) representations for both the scaffold and the functional groups were compiled and integrated into the SmiLib software platform. This systematic combinatorial approach resulted in the construction of a virtual library comprising 256 coumarin derivatives in SMILES format. The generated library allowed for the exploration of a wide array of structural modifications, yielding analogues with varied physicochemical properties.

Subsequent in silico evaluation of these compounds was conducted to investigate their biological potential, particularly focusing on their antimicrobial efficacy against *Pseudomonas aeruginosa*, a clinically relevant opportunistic pathogen. This phase of analysis was critical for identifying compounds with promising therapeutic potential, laying the groundwork for further experimental validation and potential application in the development of antimicrobial agents targeting resistant bacterial strains.

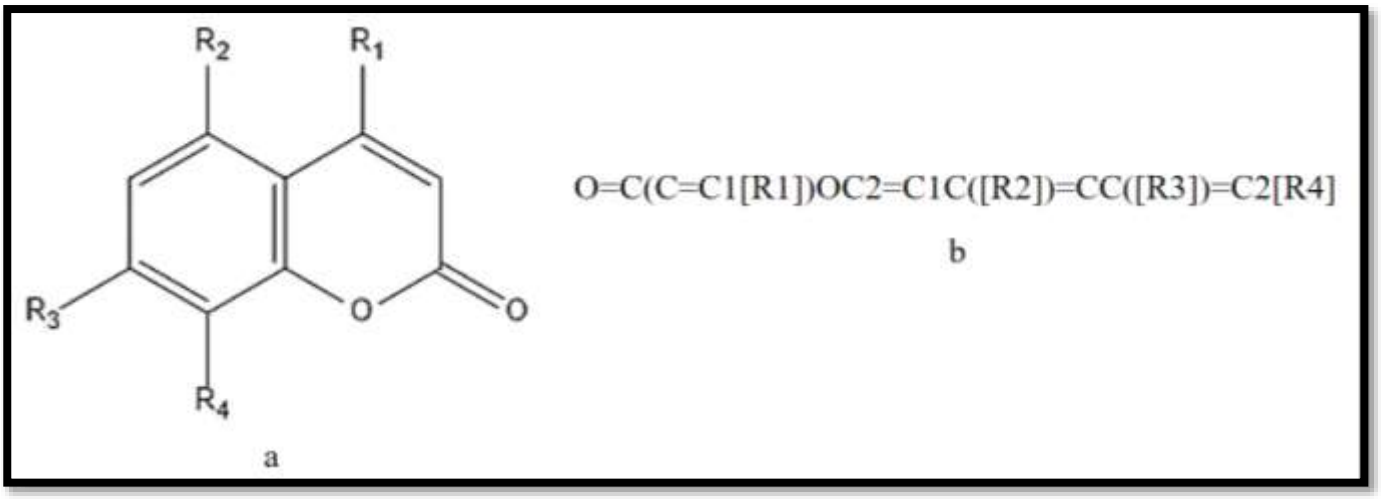

Figure 4. Scaffold structure used for the enumeration of coumarin analogues, (a) molecular structure presentation (b) SMILES presentation

All generated compounds were subjected to a rigorous virtual screening protocol to evaluate their potential as antimicrobial agents targeting *Pseudomonas aeruginosa*, a pathogenic bacterium a clinically relevant opportunistic pathogen. The initial phase of this screening involved a comprehensive toxicity assessment performed via the ProTox II web-based platform [83]. This tool enabled the prediction of key toxicity indicators, including toxicity class (TC) and median lethal dose (LD_{50}) the dose at which 50% of a test population is expected to succumb to the compound when administered acutely.

The toxicity assessment offered essential insights into the safety profiles of the synthesized coumarin derivatives. Compounds that demonstrated favorable toxicity parameters namely, a lower toxicity classification and a higher median lethal dose (LD_{50}) relative to the parent compound, coumarin were selected for subsequent pharmacokinetic evaluation. Conversely, analogues that did not satisfy these toxicity benchmarks were excluded from further analysis.

The selected compounds were subsequently subjected to pharmacokinetic prediction using the SwissADME online platform. Key parameters such as gastrointestinal absorption, blood–brain barrier permeability, and drug-likeness were evaluated to assess their drug development potential. By integrating the outcomes of both toxicity and pharmacokinetic screenings, the most promising coumarin analogues exhibiting potential antimicrobial activity against Pseudomonas aeruginosa were identified for further investigation (Table 1).

Table 1. Selected coumarin analogues

Entry	Code	R1	R2	R3	R4
1	Com1	NH2	NO2	NH2	OH
2	Com2	NH2	OH	NH2	OH
3	Com3	NH2	CN	CN	OH
4	Com4	CN	CN	CN	OH
5	Com5	OH	CN	CN	OH
6	Com6	NH2	OH	CN	OH
7	Com7	CN	OH	CN	OH
8	Com8	NH2	NH2	OH	OH
9	Com9	OH	NH2	OH	OH
10	Com10	CN	CN	OH	OH

11	Com11	CN	OH	OH	CN
12	Com12	NH2	OH	OH	CN
13	Com13	CN	CN	OH	CN
14	Com14	NH2	CN	OH	CN
15	Com15	OH	NH2	OH	CN
16	Com16	NH2	OH	CN	CN
17	Com17	NH2	OH	OH	NH2
18	Com18	OH	NH2	OH	NH2
19	Com19	CN	NH2	CN	NH2
20	Com20	OH	NH2	NH2	OH

An additional selection criterion involved the use of the Protox online server, which provides predictions for various toxicological endpoints, including cytotoxicity, mutagenicity, carcinogenicity, hepatotoxicity, and immunotoxicity. These predictions are consistent with the classification standards established by the Globally Harmonized System (GHS) for chemical hazard classification.

The in silico toxicity evaluation of the selected coumarin analogues revealed significant variability in their predicted toxicological profiles. Most of the compounds (Com1–Com20) were classified under toxicity class 5, indicating relatively low acute toxicity. Notably, Com9, Com18, and Com20 were assigned to class 6 with LD50 values exceeding 5000 mg/kg, suggesting a safer profile compared to the rest. In contrast, the parent compound Com ‹showed a markedly higher toxicity, falling under class 3 with a significantly lower LD50 of 196 mg/kg, warranting caution in its potential therapeutic use.

In terms of specific toxicological endpoints, the majority of the analogues demonstrated a high probability of immunotoxicity (scores > 0.95), which may limit their suitability for systemic administration without further optimization. Cytotoxicity scores varied across the series, with compounds such as Com5, Com7, Com10, and Com11 exhibiting relatively higher values (> 0.75), indicating a need for further in vitro validation to assess their safety margins.

Interestingly, mutagenicity and carcinogenicity predictions remained moderate to low for most compounds, with values generally close to 0.5. However, Com10 and Com11 showed

slightly elevated mutagenic potential (0.79), which could be attributed to structural features warranting further structural refinement or SAR analysis.

Hepatotoxicity scores were relatively consistent across the dataset, with only a few compounds (e.g., Com7, Com11, Com13) showing moderate to high probability values (> 0.65). These findings highlight the importance of monitoring liver toxicity in subsequent preclinical studies.

Overall, compounds such as Com9, Com18, and Com20 emerged as promising candidates due to their high LD50 values, low toxicity class, and balanced toxicity profiles across multiple endpoints. Conversely, Com‹ may require chemical modification or structural optimization due to its higher toxicity potential.

These in silico predictions provide a foundational screening for the prioritization of coumarin derivatives in drug development pipelines and highlight the need for complementary experimental validation to confirm the computational findings.

Table 2. Toxicity prediction probability, median lethal dose, and toxicity class of selected coumarin analogues

Entry	Code	Hepato	Carcino	Imminu	Muta	Cyto	LD50	TC
1	Com1	0.51	0.54	0.66	0.92	0.56	5000	5
2	Com2	0.50	0.56	0.97	0.50	0.65	3200	5
3	Com3	0.50	0.56	0.99	0.58	0.65	3200	5
4	Com4	0.66	0.56	0.99	0.76	0.67	3200	5
5	Com5	0.71	0.51	0.97	0.71	0.79	3200	5
6	Com6	0.52	0.59	0.99	0.52	0.63	3200	5
7	Com7	0.72	0.51	0.99	0.77	0.77	3200	5
8	Com8	0.50	0.56	0.79	0.51	0.64	3200	5
9	Com9	0.52	0.54	0.82	0.50	0.56	5370	6
10	Com10	0.71	0.56	0.99	0.79	0.72	3200	5

11	Com11	0.71	0.56	0.99	0.79	0.78	3200	5
12	Com12	0.53	0.57	0.99	0.52	0.64	3200	5
13	Com13	0.66	0.56	0.99	0.76	0.67	3200	5
14	Com14	0.50	0.56	0.99	0.58	0.65	3200	5
15	Com15	0.50	0.54	0.95	0.52	0.59	3200	5
16	Com16	0.51	0.58	0.99	0.51	0.64	3200	5
17	Com17	0.50	0.56	0.95	0.50	0.65	5000	5
18	Com18	0.50	0.55	0.89	0.52	0.60	5370	6
19	Com19	0.53	0.73	0.99	0.51	0.62	3800	5
20	Com20	0.52	0.56	0.92	0.56	0.62	5370	6
21	Com	0.69	0.83	0.99	0.53	0.55	196	3

LD50 (mg/kg), - (Inactive toxic class (probability score)), + (Active toxic class (probability score)), TC: Toxicity Class

The evaluation of pharmacokinetic and toxicity profiles of selected compounds plays a pivotal role in the drug development process for several key reasons. Firstly, a comprehensive understanding of a compound's pharmacokinetics encompassing its absorption, distribution, metabolism, and excretion (ADME) provides critical insights into its in vivo behavior. This includes important parameters such as bioavailability and half-life, which are instrumental in establishing appropriate dosing regimens and predicting therapeutic efficacy.

Secondly, the assessment of toxicity is essential to ensure the safety of candidate compounds for human use. Toxicological evaluation involves identifying potential adverse effects on major physiological systems, including hepatic, renal, cardiovascular, and nervous systems. Early detection of toxic effects allows researchers to address potential safety concerns proactively and prioritize compounds with favorable toxicity profiles for continued development.

Moreover, integrated pharmacokinetic and toxicity profiling facilitates the selection of lead compounds with optimal drug-like characteristics suitable for progression into preclinical and clinical stages. Compounds demonstrating adequate bioavailability, favorable half-life, and

minimal toxicity are more likely to succeed in clinical applications, thereby improving the overall efficiency and success rate of the drug development pipeline.

The tabulated data presented in Table 3 provides a comprehensive overview of the pharmacokinetic, toxicity profiles obtained for the selected analogues of coumarin, and its 20 synthetic analogues (Com1–Com21) were evaluated using in silico tools. The assessment focused on key ADME (Absorption, Distribution, Metabolism, and Excretion) parameters: gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, P-glycoprotein (gp.P) substrate status, and inhibition potential for major cytochrome P450 (CYP) isoforms (1A2, 2C19, 2C9, 2D6, and 3A4). Each compound was categorized based on predicted outcomes using computational pharmacokinetics models. The goal was to identify candidates with favorable oral bioavailability and minimal potential for adverse drug-drug interactions.

The data indicate that most of the coumarin analogues possess high GI absorption, suggesting potential for effective oral bioavailability. Notably, Com1 is the only compound with low GI absorption, which may limit its therapeutic use via oral administration.

BBB permeability is absent in all compounds except Com21, suggesting low central nervous system (CNS) penetration for most analogues. This property can be advantageous for non-CNS-targeted therapies, reducing the risk of neurotoxicity.

Only Com4, Com13, and Com20 were identified as CYP1A2 inhibitors, and none of the compounds inhibited other tested CYP isoforms (2C19, 2C9, 2D6, or 3A4). This implies a relatively low risk of CYP-mediated drug-drug interactions for most analogues, with the exception of the aforementioned three compounds, which may impact drugs metabolized by CYP1A2.

Com21 is distinct for showing both BBB permeability and gp.P substrate status, which may indicate CNS activity and potential involvement in efflux transport mechanisms, potentially influencing bioavailability and efficacy.

The compounds such as Com2, Com3, Com5–Com12, and Com14–Com19 show promising pharmacokinetic profiles due to high GI absorption, no BBB penetration, non-substrate status for gp.P, and no CYP inhibition.

Table 3. Evaluation of Pharmacokinetics properties of coumarin and its generated analogues

Entry	Molucule	G1 absorption	BBB permeant	P-gp substrate	CYP inhibitor				
					1A2	2C19	2C9	2D6	3A4
1	Com1	Low	No	No	No	No	No	No	No
2	Com2	High	No	No	No	No	No	No	No
3	Com3	High	No	No	No	No	No	No	No
4	Com4	High	No	No	Yes	No	No	No	No
5	Com5	High	No	No	No	No	No	No	No
6	Com6	High	No	No	No	No	No	No	No
7	Com7	High	No	No	No	No	No	No	No
8	Com8	High	No	No	No	No	No	No	No
9	Com9	High	No	No	No	No	No	No	No
10	Com10	High	No	No	No	No	No	No	No
11	Com11	High	No	No	No	No	No	No	No
12	Com12	High	No	No	No	No	No	No	No
13	Com13	High	No	No	Yes	No	No	No	No
14	Com14	High	No	No	No	No	No	No	No
15	Com15	High	No	No	No	No	No	No	No
16	Com16	High	No	No	No	No	No	No	No
17	Com17	High	No	No	No	No	No	No	No
18	Com18	High	No	No	No	No	No	No	No
19	Com19	High	No	No	Yes	No	No	No	No
20	Com20	High	No	No	No	No	No	No	No
21	Com	High	Yes	No	Yes	No	No	No	No

The in-silico assessments of toxicological and pharmacokinetic parameters presented in Tables 2 and 3 identify compounds Com9, Com18, and Com20 as the most promising coumarin analogues for further investigation. These compounds exhibit high lethal dose (LD50) values and are categorized in lower toxicity classes, suggesting favorable safety profiles compared to the reference compound (Com, 21), which showed significantly higher predicted toxicity, including a low LD50 and assignment to a more hazardous toxicity class. Additionally, Com9, Com18, and Com20 demonstrate low predicted probabilities for key toxicological endpoints such as hepatotoxicity, mutagenicity, and cytotoxicity, indicating reduced potential for adverse effects.

From a pharmacokinetic perspective, these compounds also show high gastrointestinal (GI) absorption and minimal predicted interaction with cytochrome P450 (CYP) enzymes, thereby reducing the likelihood of drug-drug interactions. However, Com20 warrants careful monitoring due to its predicted CYP1A2 inhibition potential, while Com1 should be excluded from further development due to poor oral absorption. Importantly, none of the prioritized compounds are predicted to permeate the blood-brain barrier (BBB), minimizing the risk of central nervous system side effects, except for Com21, which may be considered for CNS-targeted applications.

Overall, these findings support the prioritization of Com9, Com18, and Com20 for further in vitro and in vivo studies. Structural modifications and SAR analysis may be necessary for compounds with elevated immunotoxicity or hepatotoxicity predictions, and the reference compound Com(21) may require chemical refinement to mitigate its toxicological liabilities. The integration of computational toxicology and ADME profiling underscores the utility of in-silico screening in early-stage drug discovery and reinforces the potential of coumarin-based scaffolds in therapeutic development.

3. Physicochemical properties

The physicochemical characteristics of coumarin analogues (Com1- Com20), as detailed in Table 4, play a crucial role in predicting drug-likeness and oral bioavailability. Key parameters evaluated include molecular weight, lipophilicity, water solubility, and hydrogen bonding capacity. All compounds exhibit molecular weights within the acceptable threshold for oral drug candidates (<500 Da), ranging from 146.14 g/mol (Com) to 280.17 g/mol (Com20), indicating favorable pharmacokinetic potential [84].

The analogues display minimal molecular flexibility, with 0 to 1 rotatable bonds, which is considered advantageous for membrane permeability and efficient oral absorption [85]. The number of hydrogen bond donors (HBDs) and acceptors (HBAs) for most compounds remains within Lipinski's Rule of Five criteria (≤5 HBDs and ≤10 HBAs), supporting their potential for good absorption and systemic distribution [86].

Molar refractivity values for the compounds fall within an appropriate range (42.48 - 62.14), suggesting suitable polarizability and molecular dimensions conducive to drug-like behavior. Lipophilicity, expressed as log P, spans from 0.05 to 0.65 for the majority of analogues, reflecting a favorable hydrophilic-lipophilic balance. The parent compound, Com, displays a relatively higher log P value of 1.82, which, although within the acceptable range, may influence membrane permeability and requires further investigation [87].

In terms of aqueous solubility, the compounds demonstrate moderate water solubility (log S = 1.63 - 2.29), with Com exhibiting the highest solubility value, supporting its formulation potential despite its increased lipophilicity. Compounds such as Com3, Com4, Com5,Com10 and Com14 exhibit particularly favorable physicochemical profiles, combining balanced lipophilicity and solubility, thereby positioning them as strong candidates for further drug development.

The unique profile of Com characterized by its low molecular weight and elevated lipophilicity warrants additional evaluation to fully assess its permeability and in vivo bioavailability. Collectively, these findings underscore the importance of maintaining low molecular complexity and balanced physicochemical attributes in the rational design of coumarin-based drug candidates for oral therapeutic applications.

Table 4. Physicochemical Properties of coumarin and its generated analogues

Molecule	Water solubility (Log *S*)	Lipophilicity (Consensus Log *Po*/w)	Molar refractivity	H-bond acceptor	H-bond donor	Rotatable bonds	Molecular weight
Com1	-2.21	-0.13	62.14	5	3	1	237.17
Com2	-1.69	0.05	55.34	4	4	0	208.17
Com3	-2.05	0.50	58.34	5	2	0	227.18
Com4	-1.99	0.63	58.65	6	1	0	237.17

Com5	-2.26	0.64	55.96	6	2	0	228.16
Com6	-1.63	0.22	55.65	5	3	0	218.17
Com7	-2.26	0.63	55.96	6	2	0	228.16
Com8	-1.69	0.13	55.34	4	4	0	208.17
Com9	-1.90	0.27	52.96	5	4	0	209.16
Com10	-2.26	0.64	55.96	6	2	0	228.16
Com11	-2.26	0.65	55.96	6	2	0	228.16
Com12	-1.63	0.22	55.65	5	3	0	218.17
Com13	-1.99	0.63	58.65	6	1	0	237.17
Com14	-2.05	0.51	58.34	5	2	0	227.18
Com15	-1.63	0.30	55.65	5	3	0	218.17
Com16	-1.70	0.36	58.34	5	2	0	227.18
Com17	-1.69	0.06	55.34	4	4	0	208.17
Com18	-1.69	0.10	55.34	4	4	0	208.17
Com19	-1.84	0.35	60.72	4	2	0	226.19
Com20	-1.69	0.10	55.34	4	4	0	280.17
Com	-2.29	1.82	42.48	2	0	0	146.14

4. Druglikeness

The druglikeness of coumarin and its synthetic analogues was systematically assessed using a suite of established computational filters, including the Lipinski, Veber, Egan, Ghose, and Muegge rules (Table 5). These filters evaluate a compound's oral drug candidacy based on its physicochemical and pharmacokinetic attributes. Each compound was also assigned a bioavailability score, and any violations of the individual rules were recorded to facilitate a comprehensive evaluation of their drug-like potential [88].

All analogues (Com1–Com20) demonstrated a consistent bioavailability score of 0.55, which is considered moderate and generally acceptable for orally administered drugs [89]. This uniformity suggests a broadly favorable potential for oral bioavailability across the compound series [90].

Regarding rule violations:

- Com1 was the only analogue to breach both the Egan and Veber rules, indicating potential issues related to molecular flexibility and permeability.

- The parent compound, Com (21), failed to meet the criteria set by the Muegge and Ghose filters, suggesting concerns related to structural complexity or physicochemical balance.

- All remaining analogues (Com2–Com20) successfully complied with all five drug-likeness filters, indicating robust drug-like properties.

Of particular note is the absence of any violations of Lipinski's Rule of Five among all tested compounds—a strong indicator of potential suitability for oral administration and an essential benchmark in early drug discovery [42].

As highlighted in Table 5, the findings support the prioritization of Com2–Com20 for further drug development due to their strong adherence to drug-likeness criteria and favorable bioavailability scores. Com1 warrants additional investigation to determine whether structural modifications could resolve its Egan and Veber rule violations. Meanwhile, the parent compound Com may require more substantial structural optimization to overcome its deficiencies and become a viable candidate for drug development.

Table 5. Druglikeness properties of coumarin and its generated analogues

Molecule	Lipinski ≠violation	Ghose ≠violation	Veber ≠violation	Egan ≠violation	Muegge ≠violation	Bioavailability Score
Com1	0	0	1	1	0	0.55
Com2	0	0	0	0	0	0.55
Com3	0	0	0	0	0	0.55
Com4	0	0	0	0	0	0.55
Com5	0	0	0	0	0	0.55
Com6	0	0	0	0	0	0.55
Com7	0	0	0	0	0	0.55
Com8	0	0	0	0	0	0.55
Com9	0	0	0	0	0	0.55
Com10	0	0	0	0	0	0.55

Com11	0	0	0	0	0	0.55
Com12	0	0	0	0	0	0.55
Com13	0	0	0	0	0	0.55
Com14	0	0	0	0	0	0.55
Com15	0	0	0	0	0	0.55
Com16	0	0	0	0	0	0.55
Com17	0	0	0	0	0	0.55
Com18	0	0	0	0	0	0.55
Com19	0	0	0	0	0	0.55
Com20	0	0	0	0	0	0.55
Com	0	2	0	0	1	0.55

Following the prediction of pharmacokinetic and toxicity profiles, the half maximal inhibitory concentration (IC_{50}) values of all selected compounds against SARS-CoV-2 target enzymes were calculated using AutoDock 4.2 and AutoDock Tools 1.5.6 software [91][92]. These molecular docking simulations provided insights into the binding affinity and inhibitory potential of each compound.

The obtained data are presented in Table 6.

Table 6. Half-maximal inhibitory concentration of coumarin and selected generated analogues

Molecule	**COV-19**
	IC50 (µM)
Com1	248.33
Com2	150.53
Com3	135.24
Com4	75.68
Com5	97.54
Com6	159.49
Com7	115.08
Com8	251.27
Com9	121.94

Com10	62.5
Com11	118.69
Com12	152.24
Com13	81.99
Com14	91.63
Com15	82.83
Com16	83.19
Com17	123.87
Com18	112.77
Com19	40.31
Com20	114.55
Com	186.52

In Table 6 presents the half-maximal inhibitory concentration (IC_{50}) values for coumarin (Com) and its synthetic analogues (Com1–Com20) against *Pseudomonas aeruginosa*, a clinically significant opportunistic pathogen. These analogues were initially designed and evaluated for their inhibitory activity against SARS-CoV-2, specifically targeting the virus's main protease (Mpro (cov-19)), using molecular modeling techniques to generate structural variants of the parent coumarin compound.

A lower IC_{50} value corresponds to higher potency, indicating a stronger interaction with the viral target.

The IC_{50} values among the tested compounds varied widely, reflecting differences in antiviral activity. Com19 exhibited the strongest inhibitory effect, with an IC_{50} of 40.31 µM, suggesting a high binding affinity to the SARS-CoV-2 (cov-19). Other analogues, such as Com10 (62.5 µM), Com13 (81.99 µM), Com15 (82.83 µM), and Com16 (83.19 µM), also demonstrated notable antiviral activity within the low micromolar range. Conversely, compounds like Com1 (248.33 µM) and Com8 (251.27 µM) showed significantly weaker inhibition, potentially due to unfavorable steric hindrance or suboptimal electronic properties that reduce binding efficiency.

The observed variability in IC_{50} values underscores the influence of structural modifications on the biological activity of coumarin derivatives. Specific substitutions likely enhance hydrogen

bonding or hydrophobic interactions with the protease's active site, thereby improving inhibitory potency. These results highlight the importance of structure activity relationship (SAR) analysis to identify key functional groups and molecular features that contribute to enhanced efficacy.

This study identifies coumarin-based analogues, particularly Com19, as promising candidates for further development as SARS-CoV-2 (cov-19).

5. Computational perspective

In the forthcoming section, we will conduct a structure optimization of the most potent compounds, namelyCom4, Com10, and Com19, to prepare them for molecular docking analysis. This process involvesrefining the geometry of the ligands to ensure accurate predictions of their interactions with target receptors. By optimizing the molecular structures, we aim to enhance the reliability and validity of subsequent docking simulations, ultimately facilitating a more comprehensive understanding of the ligand-receptor interactions and their potential therapeutic implications.

5.1. Geometry optimization

Obtaining the optimized structure of small molecules is very crucial for elucidating their exact binding behavior. Therefore, to comprehensively understand this behavior, the structures of the highly potent compounds Com4, Com10, and Com19, along with the reference drug metronidazole, were fully optimized using the DFT/B3LYP method as outlined in the literature overview section. The resulting optimized ground state geometries of these compounds are depicted in Figure 4

.

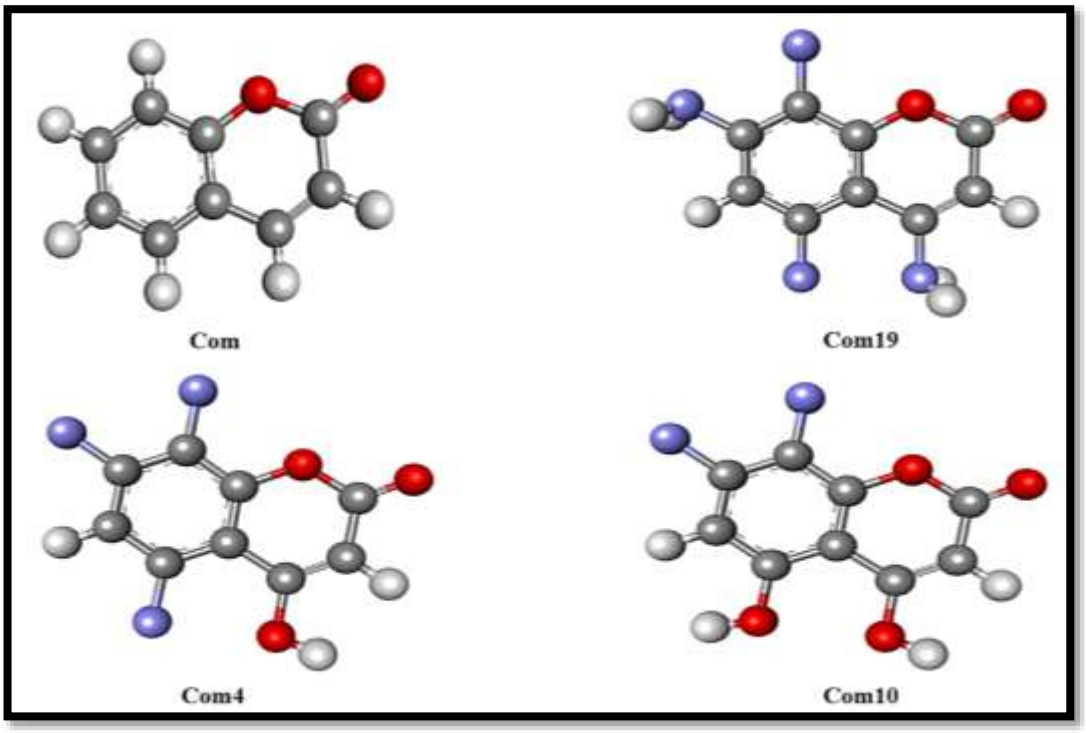

Figure 5. The optimized 3D-structure of Com4, Com10, Com19, and the reference drug Com at DFT/B3LYP method

6. Molecular docking study

To elucidate the mode of interaction between the most potent Coumarin analogues, namely Com4, Com10, and Com19, in addition to the standard drug Com, with the receptors Cov-19, molecular docking simulations were performed. These simulations aim to predict the most stable conformation of the most potent coumarin analogues when bound to the selected target receptors SARS-CoV-2 (COV-19). The protein sequence of COV-19 from *Pseudomonas aeruginosa* was retrieved from UniProt, their accession number is P0DTD1. Figure 5**.**

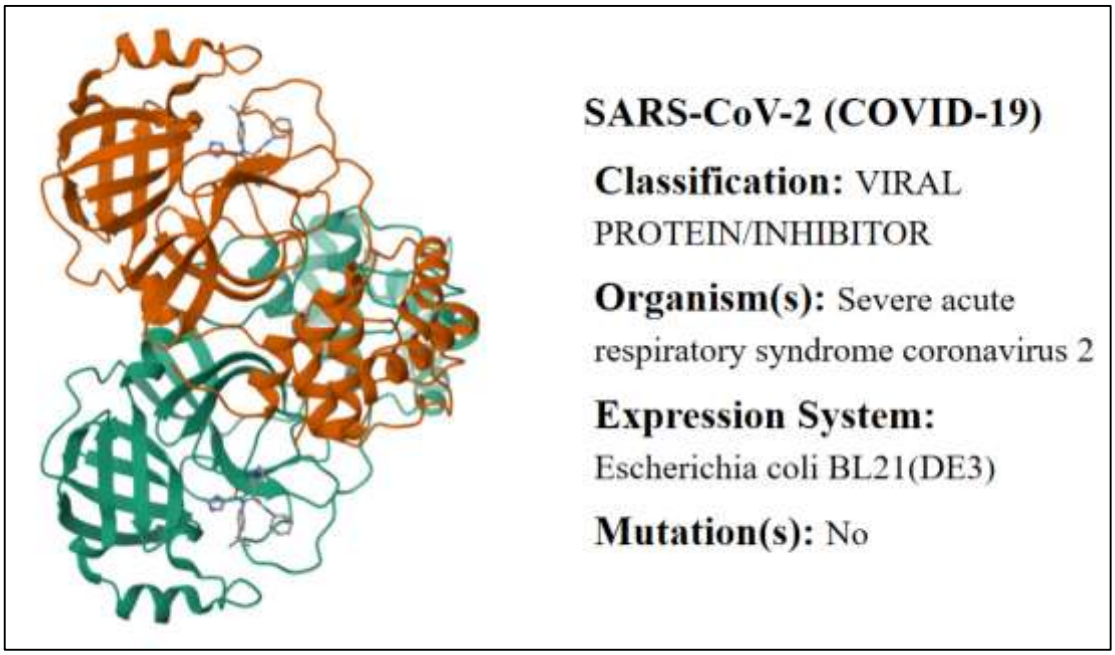

Figure 6. Three-dimensional structure of coumarin derivatives in complex with SARS-CoV-2 main protease (Mpro (COV-19))

The binding site for SARS-CoV-2 main protease (Mpro) was predicted using the Ligand Binding Site Prediction tool available at https://prankweb.cz. Molecular docking studies were performed utilizing Schrödinger Maestro software, specifically employing the Glide Standard Precision (SP) module [93]. Ligands were pre-processed using the LigPrep module within Schrödinger, applying the OPLS3 force field, as reported by Harder et al. This step included generating up to 32 stereoisomers per ligand and determining appropriate ionization states at physiological pH (7.0 ± 2.0). The receptor protein, expressed in Escherichia coli BL21 (DE3) and derived from the SARS-CoV-2 virus, was prepared using the Protein Preparation Wizard. This preparation involved the addition of polar hydrogens, removal of water molecules and ions, assignment of bond orders, and protonation state prediction using PROPKA at pH 7.0 [94]. A positional restraint of 0.3 Å RMSD was applied to heavy atoms during optimization using the OPLS3 force field. The receptor's active site was defined by generating a grid box centered on the predicted binding site. Subsequently, ligands were docked into the prepared protein structure using the Glide SP algorithm, allowing for the ranking and analysis of ligand-protein interactions. The resulting docking model, illustrated in Figure 5, provides critical insight into the binding conformations of coumarin derivatives within the Mpro active site.

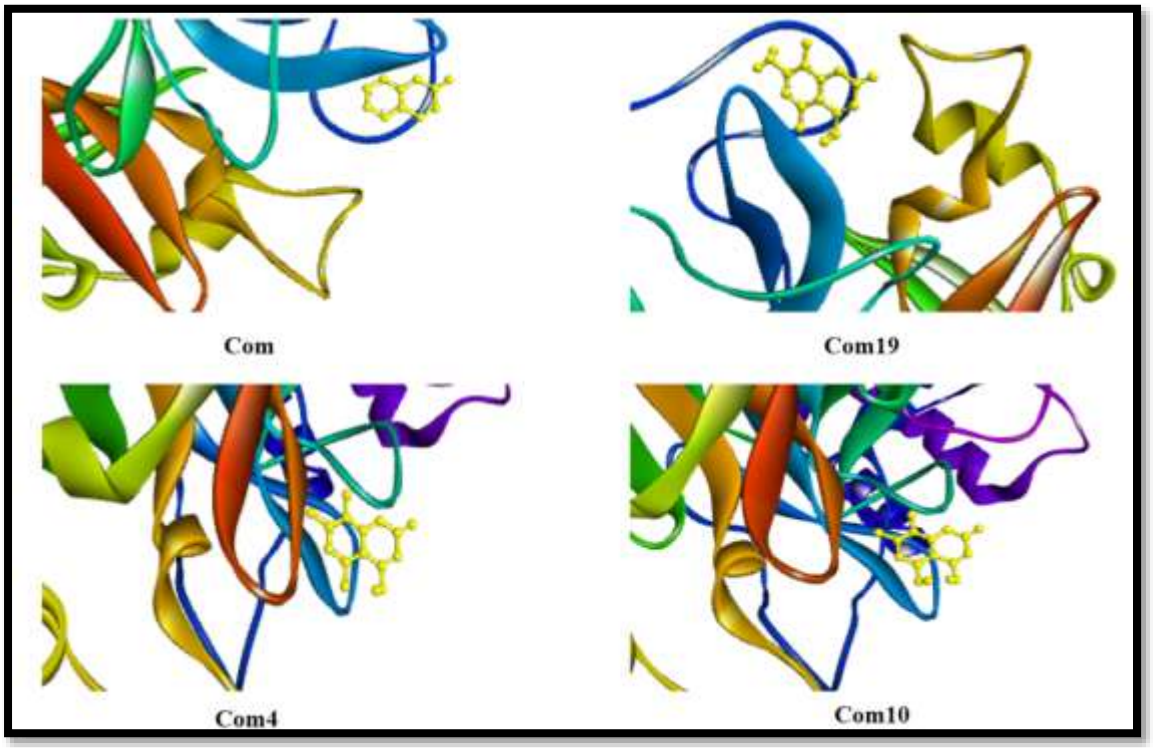

Figure 7. 3D interaction of coumarin and the selected most potent analogues with SARS-CoV-2 main protease (Mpro (COV-19)) where the red lines represent the H-bonds

The distinct binding interactions between coumarin and its most potent analogues (Com, Com4, Com10, and Com19) with the SARS-CoV-2 main protease (Mpro) were elucidated through 3D molecular docking analysis, as depicted in Figure 6. The formation of multiple hydrogen bonds, highlighted by red lines, underscores their pivotal role in stabilizing the ligand–receptor complexes. These coumarin derivatives adopted diverse binding conformations within the active site of Mpro, facilitating favorable hydrogen bonding with key amino acid residues. The observed differences in binding orientation and interaction profiles can be attributed to structural variations among the analogues, which significantly influenced their binding affinities. These findings provide valuable mechanistic insights into the potential of coumarin-based compounds as Mpro inhibitors and are consistent with interaction patterns reported in earlier studies on related enzymatic targets.

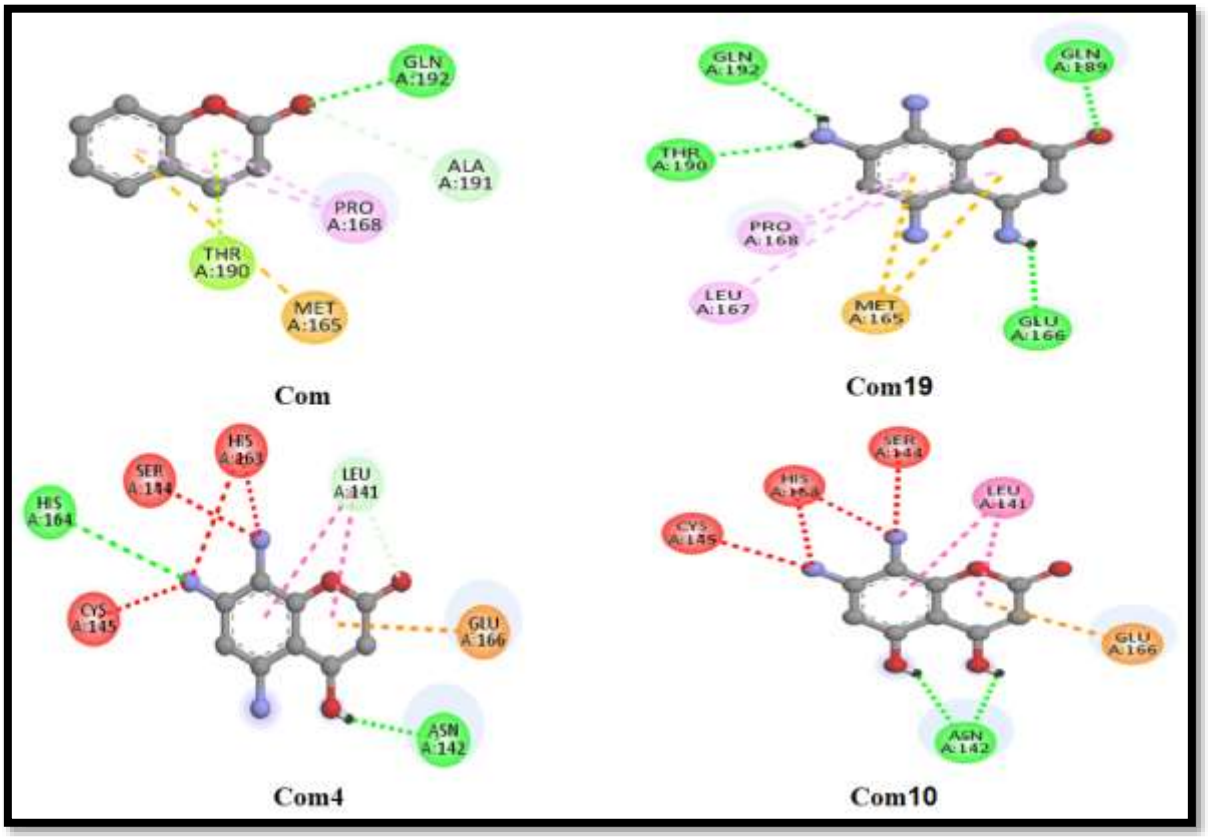

Figure 8. 2D interaction of coumarin and the selected most potent analogues with the target protein of SARS-CoV-2 (Mpro (COV-19)) where the red lines represent the H-bonds

The detailed analysis presented in Table 7 encompasses a comprehensive examination of the binding free energies, Molecular docking was performed to assess the binding affinity and interaction profiles of coumarin and selected analogues (Com4, Com10, Com19, and the parent compound Com) with the SARS-CoV-2 main protease (Mpro, PDB ID: 6LU7). Ligand structures were optimized using the MMFF94 force field, and docking was centered on the catalytic dyad (His41 and Cys145). Binding energies (ΔG) were calculated, and interactions were visualized using Discovery Studio and LigPlot+(Figure 7).

Compound selection was based on IC_{50} data, with Com19 and Com10 showing the strongest inhibitory activity. Com10 formed hydrogen bonds with key residues including Cys145 and His163, while Com19 interacted with Glu166 and Thr190, indicating stable binding within the active site. Com4 also demonstrated effective binding, whereas the parent compound Com showed limited interaction, correlating with lower potency.

Com19 showed the most favorable binding energy (ΔG = –6.0 kcal/mol), followed by Com10 (–5.74 kcal/mol), Com4 (–5.62 kcal/mol), and the parent compound Com (–5.09 kcal/mol). These results highlight the potential of Com10 and Com19 as lead SARS-CoV-2 inhibitors, with strong binding affinities and favorable interaction patterns that support further development.

Table 7. Molecular docking and bond interactions analysis of Com4, Com10, Com19, and the control Com, candidates with the receptors Cov-19

Cov-19		
Ligand code	**Center grid box x, y, z**	**ΔG(kcal/mol)**
Com4	**-21.42, 17.40, -27.73**	**-5.62**
Com10		**-5.74**
Com19		**-6.0**
Com		**-5.09**

The molecular docking results identified Com19 and Com10 than Com4, as the most promising SARS-CoV-2 inhibitors, exhibiting the lowest binding energies and strongest interactions within the conserved active site.indicating stable and specific binding through hydrogen bonds, hydrophobic interactions, and π-π stacking. The combined docking and biological data support the prioritization of Com19 and Com10 for further structural optimization aimed at improving pharmacokinetic properties and target specificity.

Conclusion

Coumarine and its dervatives exhibit promising antiviral properties in in vitro and in silico studies targeting SARS-COV-2 .Thèse compounds have shown potential inhibiting key viral proteins such as main protease (Mpro),RNA -dependent RNA polymerase (RdRp),and spike protein interaction .However while the computational and laboratory data are encuraging there is currently no conclusive clinical evidence supporting coumarin's efficacy or safty in treating covid-19in humans .Therfore further preclinical and clinical studies are required to validate its therapeutic potential and determine optimal dosage and safty profiles .

References:

[1] M. A. Almutairi, “The coronavirus disease 2019 (COVID-19) outbreak: Challenges for pediatric dentistry,” *J Res Med Dent Sci*, vol. 9, pp. 116–121, 2021.

[2] A. Santamaria, J. Chang, and C. Savarain, “SARS-CoV-2 among the potential viral triggers for Vogt-Konayagi-Harada disease: first case report and literature review,” *Ocul. Immunol. Inflamm.*, vol. 30, no. 7–8, pp. 1869–1875, 2022.

[3] B. Nagargoje, A. Palod, J. Dixit, K. Yelikar, S. Andurkar, and S. Badgujar, “Seroprevalence of COVID-19 in a city in India: A community-based cross-sectional study,” *J Res Med Dent Sci*, vol. 9, no. 9, pp. 48–53, 2021.

[4] D. K. Singh, A. Garg, S. Bagri, P. Choudhary, D. Kuber, and A. K. Kem, “COVID 19 presentation and effect of associated co-morbidities on severity of illness at a dedicated COVID hospital in North India,” *J Res Med Dent Sci*, vol. 9, pp. 49–54, 2021.

[5] T. Singhal, “A review of coronavirus disease-2019 (COVID-19),” *indian J. Pediatr.*, vol. 87, no. 4, pp. 281–286, 2020.

[6] S. Dane and M. Akyuz, “Symptom spectrum and the evaluation of severity and duration of symptoms in patients with COVID-19,” *J Res Med Dent Sci*, vol. 9, pp. 262–266, 2021.

[7] A. A. Milibari, “Current situation of coronavirus disease:(COVID-19) review article,” *Heal. Sci. J.*, pp. 1–4, 2020.

[8] A. S. R. Souza *et al.*, “General aspects of the COVID-19 pandemic,” *Rev. Bras. Saúde Matern. Infant.*, vol. 21, no. Suppl 1, pp. 29–45, 2021.

[9] Q. Li *et al.*, “Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia,” *N. Engl. J. Med.*, vol. 382, no. 13, pp. 1199–1207, 2020.

[10] J. F.-W. Chan *et al.*, “A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster,” *Lancet*, vol. 395, no. 10223, pp. 514–523, 2020.

[11] Y. Yueniwati *et al.*, "Low molecular weight heparin doses and ventilator airway necessity in the COVID-19 patients: Systematic review and meta-analysis," *Open Access Maced. J. Med. Sci.*, vol. 10, no. F, pp. 241–245, 2022.

[12] K. K.-W. To *et al.*, "Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study," *Lancet Infect. Dis.*, vol. 20, no. 5, pp. 565–574, 2020.

[13] F. Zhou *et al.*, "Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study," *Lancet*, vol. 395, no. 10229, pp. 1054–1062, 2020.

[14] Y. Liu *et al.*, "Viral dynamics in mild and severe cases of COVID-19," *Lancet Infect. Dis.*, vol. 20, no. 6, pp. 656–657, 2020.

[15] Y. Wu *et al.*, "Prolonged presence of SARS-CoV-2 viral RNA in faecal samples," *lancet Gastroenterol. Hepatol.*, vol. 5, no. 5, pp. 434–435, 2020.

[16] Z.-D. Tong *et al.*, "Potential presymptomatic transmission of SARS-CoV-2, Zhejiang province, China, 2020," *Emerg. Infect. Dis.*, vol. 26, no. 5, p. 1052, 2020.

[17] O. Filchakova, D. Dossym, A. Ilyas, T. Kuanysheva, A. Abdizhamil, and R. Bukasov, "Review of COVID-19 testing and diagnostic methods," *Talanta*, vol. 244, p. 123409, 2022.

[18] M. Cascella, M. Rajnik, A. Aleem, S. C. Dulebohn, and R. Di Napoli, "Features, evaluation, and treatment of coronavirus (COVID-19). Statpearls. 2022," *NCBI Bookshelf ID NBK554776 PMID 32150360*, 2022.

[19] T. M. Costa, L. B. B. Tavares, and D. de Oliveira, "Fungi as a source of natural coumarins production," *Appl. Microbiol. Biotechnol.*, vol. 100, pp. 6571–6584, 2016.

[20] M. J. Matos, L. Santana, E. Uriarte, O. A. Abreu, E. Molina, and E. G. Yordi, "Coumarins—an important class of phytochemicals," *Phytochem. characterisation role Hum. Heal.*, vol. 25, pp. 533–538, 2015.

[21] L. Wu, X. Wang, W. Xu, F. Farzaneh, and R. Xu, "The Structure and Pharmacological Functions of Coumarins and Their Derivatives," *Curr. Med. Chem.*, vol. 16, no. 32, pp. 4236–4260, 2009, doi: 10.2174/092986709789578187.

[22] O. M. Tsivileva, O. V Koftin, and N. V Evseeva, “Coumarins as fungal metabolites with potential medicinal properties,” *Antibiotics*, vol. 11, no. 9, p. 1156, 2022.

[23] F. Annunziata, C. Pinna, S. Dallavalle, L. Tamborini, and A. Pinto, “An overview of coumarin as a versatile and readily accessible scaffold with broad-ranging biological activities,” *Int. J. Mol. Sci.*, vol. 21, no. 13, pp. 1–83, 2020, doi: 10.3390/ijms21134618.

[24] I. A. Stringlis, R. De Jonge, and C. M. J. Pieterse, “The age of coumarins in plant–microbe interactions,” *Plant Cell Physiol.*, vol. 60, no. 7, pp. 1405–1419, 2019.

[25] M. Zaynab, J. Khan, R. Al-Yahyai, M. Sadder, and S. Li, “Toxicity of coumarins in plant defense against pathogens,” *Toxicon*, p. 108118, 2024.

[26] V. Flores-Morales, A. P. Villasana-Ruíz, I. Garza-Veloz, S. González-Delgado, and M. L. Martinez-Fierro, “Therapeutic effects of coumarins with different substitution patterns,” *Molecules*, vol. 28, no. 5, p. 2413, 2023.

[27] Z. Li, D. Kong, Y. Liu, and M. Li, “Pharmacological perspectives and molecular mechanisms of coumarin derivatives against virus disease,” *Genes Dis.*, vol. 9, no. 1, pp. 80–94, 2022.

[28] F. Aktar, A. Karim, R. N. Chowdhury, S. A. Sultana, and M. S. Amran, “Recent Advances in Dengue, Viral Hepatitis, and HIV Infections and Related Management Strategies,” *Avicenna J. Med. Biochem.*, vol. 12, no. 2, pp. 172–187, 2024.

[29] T. Bajaj, *Discovery of Small Molecule Drugs against Crucial Proteins, Papain-like Protease (PLpro) and Non-structural protein 15 (Nsp15), from SARS-CoV-2 virus*. University of California, Berkeley, 2024.

[30] C. Montagner, S. M. de Souza, C. Groposo, F. Delle Monache, E. F. A. Smânia, and A. Smânia Jr, “Antifungal activity of coumarins,” *Zeitschrift für Naturforsch. C*, vol. 63, no. 1–2, pp. 21–28, 2008.

[31] E. Küpeli Akkol, Y. Genç, B. Karpuz, E. Sobarzo-Sánchez, and R. Capasso, “Coumarins and coumarin-related compounds in pharmacotherapy of cancer,” *Cancers (Basel).*, vol. 12, no. 7, p. 1959, 2020.

[32] A. M. Said, Y. E. Mansour, R. R. Soliman, R. Islam, and S. S. Fatahala, “Design, synthesis, molecular modeling, in vitro and in vivo biological evaluation of potent anthranilamide derivatives as dual P-glycoprotein and CYP3A4 inhibitors,” *Eur. J. Med. Chem.*, vol. 273, p. 116492, 2024.

[33] M. Z. Hassan, H. Osman, M. A. Ali, and M. J. Ahsan, “Therapeutic potential of coumarins as antiviral agents,” *Eur. J. Med. Chem.*, vol. 123, pp. 236–255, 2016.

[34] W.-C. Chen *et al.*, “A new coumarin derivative plays a role in rhabdoviral clearance by interfering glycoprotein function during the early stage of viral infection,” *Cell. Signal.*, vol. 51, pp. 199–210, 2018.

[35] S. Sandhu, Y. Bansal, O. Silakari, and G. Bansal, “Coumarin hybrids as novel therapeutic agents,” *Bioorg. Med. Chem.*, vol. 22, no. 15, pp. 3806–3814, 2014.

[36] I. da Silva Santos *et al.*, “Natural and Synthetic Coumarins as Potential Drug Candidates Against SARS-CoV-2/CoViD-19,” *Curr. Med. Chem.*, vol. 32, no. 3, pp. 539–562, 2025.

[37] E. Singh *et al.*, “A comprehensive review on promising anti-viral therapeutic candidates identified against main protease from SARS-CoV-2 through various computational methods,” *J. Genet. Eng. Biotechnol.*, vol. 18, no. 1, p. 69, 2020.

[38] R. S. Keri, B. S. Sasidhar, B. M. Nagaraja, and M. A. Santos, “Recent progress in the drug development of coumarin derivatives as potent antituberculosis agents,” *Eur. J. Med. Chem.*, vol. 100, pp. 257–269, 2015.

[39] M. R. Hossain *et al.*, “Esculetin unveiled: Decoding its anti-tumor potential through molecular mechanisms—A comprehensive review,” *Cancer Rep.*, vol. 7, no. 1, p. e1948, 2023.

[40] B. Shaker, S. Ahmad, J. Lee, C. Jung, and D. Na, “In silico methods and tools for drug discovery,” *Comput. Biol. Med.*, vol. 137, p. 104851, 2021.

[41] A. M. B. Amorim, L. F. Piochi, A. T. Gaspar, A. J. Preto, N. Rosário-Ferreira, and I. S. Moreira, “Advancing drug safety in drug development: bridging computational predictions for enhanced toxicity prediction,” *Chem. Res. Toxicol.*, vol. 37, no. 6, pp. 827–849, 2024.

[42] L. L. G. Ferreira and A. D. Andricopulo, “ADMET modeling approaches in drug discovery,” *Drug Discov. Today*, vol. 24, no. 5, pp. 1157–1165, 2019.

[43] G. Xiong *et al.*, "ADMETlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties," *Nucleic Acids Res.*, vol. 49, no. W1, pp. W5–W14, 2021.

[44] M. Mirnezhad, R. Ansari, and S. R. Falahatgar, "Quantum effects on the mechanical properties of fine-scale CNTs: an approach based on DFT and molecular mechanics model," *Eur. Phys. J. Plus*, vol. 135, no. 11, p. 908, 2020.

[45] J. Fan, A. Fu, and L. Zhang, "Progress in molecular docking," *Quant. Biol.*, vol. 7, pp. 83–89, 2019.

[46] T. Lanez, M. Lanez, R. Lanez, E. Lanez, and B. Talbi-Lanez, "Computational Screening of Novel Nitroimidazole Candidates: Targeting Key Enzymes of Oral Anaerobes for Anti-Parasitic Potential," *Curr. Drug Discov. Technol.*, 2024.

[47] M. M. Lamers and B. L. Haagmans, "SARS-CoV-2 pathogenesis," *Nat. Rev. Microbiol.*, vol. 20, no. 5, pp. 270–284, 2022.

[48] A. R. Tejedor, R. Collepardo-Guevara, J. Ramírez, and J. R. Espinosa, "Time-dependent material properties of aging biomolecular condensates from different viscoelasticity measurements in molecular dynamics simulations," *J. Phys. Chem. B*, vol. 127, no. 20, pp. 4441–4459, 2023.

[49] M. Pavan, S. Menin, D. Bassani, M. Sturlese, and S. Moro, "Qualitative estimation of protein–ligand complex stability through thermal titration molecular dynamics simulations," *J. Chem. Inf. Model.*, vol. 62, no. 22, pp. 5715–5728, 2022.

[50] W. Feng, W. Zong, F. Wang, and S. Ju, "Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): a review," *Mol. Cancer*, vol. 19, pp. 1–14, 2020.

[51] R. Orioli, F. Belluti, S. Gobbi, A. Rampa, and A. Bisi, "Naturally inspired coumarin derivatives in Alzheimer's Disease Drug Discovery: latest advances and current challenges," *Molecules*, vol. 29, no. 15, p. 3514, 2024.

[52] R. Abdizadeh, F. Hadizadeh, and T. Abdizadeh, "In silico analysis and identification of antiviral coumarin derivatives against 3-chymotrypsin-like main protease of the novel coronavirus SARS-CoV-2," *Mol. Divers.*, vol. 26, no. 2, pp. 1053–1076, 2022.

[53] R. Eswaramoorthy, H. Hailekiros, F. Kedir, and M. Endale, “In silico molecular docking, DFT analysis and ADMET studies of carbazole alkaloid and coumarins from roots of clausena anisata: a potent inhibitor for quorum sensing,” *Adv. Appl. Bioinforma. Chem.*, pp. 13–24, 2021.

[54] S. K. Mohamed *et al.*, “XRD/DFT, Hirshfeld surface analysis and molecular modelling simulations for unfolding reactivity of newly synthesized vanillin derivatives: excellent optical, NLO and protein binding efficiency,” *J. Biomol. Struct. Dyn.*, pp. 1–19, 2024.

[55] S. Lyndem *et al.*, “A comprehensive in vitro exploration into the interaction mechanism of coumarin derivatives with bovine hemoglobin: Spectroscopic and computational methods,” *J. Photochem. Photobiol. A Chem.*, vol. 436, p. 114425, 2023.

[56] D. Dastan and A. Ebadi, “Sesquiterpene Coumarins as Promising Antiviral Agents: An In-silico Study,” *Anti-Infective Agents*, 2025.

[57] P. P. Bhagat and T. N. Bansode, “Coumarin Derivatives: Pioneering New Frontiers in Biological Applications,” *Curr. Org. Chem.*, vol. 29, no. 10, pp. 794–813, 2025.

[58] T. Katsila, G. A. Spyroulias, G. P. Patrinos, and M.-T. Matsoukas, “Computational approaches in target identification and drug discovery,” *Comput. Struct. Biotechnol. J.*, vol. 14, pp. 177–184, 2016.

[59] M. T. Muhammed and E. Aki-Yalcin, “Molecular docking: Principles, advances, and its applications in drug discovery,” *Lett. Drug Des. Discov.*, vol. 21, no. 3, pp. 480–495, 2024.

[60] R. G. Coleman, M. Carchia, T. Sterling, J. J. Irwin, and B. K. Shoichet, “Ligand pose and orientational sampling in molecular docking,” *PLoS One*, vol. 8, no. 10, p. e75992, 2013.

[61] A. Citarella, A. Scala, A. Piperno, and N. Micale, “SARS-CoV-2 Mpro: A potential target for peptidomimetics and small-molecule inhibitors,” *Biomolecules*, vol. 11, no. 4, p. 607, 2021.

[62] M. K. Sahu, A. K. Nayak, B. Hailemeskel, and O. E. Eyupoglu, “Exploring recent updates on molecular docking: Types, method, application, limitation & future prospects,” *Int. J. Pharm. Res. Allied Sci.*, vol. 13, no. 2–2024, pp. 24–40, 2024.

[63] O. V Stroganov, F. N. Novikov, V. S. Stroylov, V. Kulkov, and G. G. Chilov, “Lead finder: An approach to improve accuracy of protein− ligand docking, binding energy estimation, and virtual screening,” *J. Chem. Inf. Model.*, vol. 48, no. 12, pp. 2371–2385, 2008.

[64] I. Luque and E. Freire, “Structural stability of binding sites: consequences for binding affinity and allosteric effects,” *Proteins Struct. Funct. Bioinforma.*, vol. 41, no. S4, pp. 63–71, 2000.

[65] L. T. Todorov and I. P. Kostova, “Coumarin-transition metal complexes with biological activity: current trends and perspectives,” *Front. Chem.*, vol. 12, p. 1342772, 2024.

[66] V. Salmaso and S. Moro, “Bridging molecular docking to molecular dynamics in exploring ligand-protein recognition process: An overview,” *Front. Pharmacol.*, vol. 9, p. 923, 2018.

[67] G. Grassmann *et al.*, “Computational approaches to predict protein–protein interactions in crowded cellular environments,” *Chem. Rev.*, vol. 124, no. 7, pp. 3932–3977, 2024.

[68] M. S. Badar, S. Shamsi, J. Ahmed, and M. A. Alam, “Molecular dynamics simulations: concept, methods, and applications,” in *Transdisciplinarity*, Springer, 2022, pp. 131–151.

[69] J. Huang, W. Song, H. Huang, and Q. Sun, “Pharmacological therapeutics targeting RNA-dependent RNA polymerase, proteinase and spike protein: from mechanistic studies to clinical trials for COVID-19,” *J. Clin. Med.*, vol. 9, no. 4, p. 1131, 2020.

[70] S. A. Mir, R. K. Meher, and B. Nayak, “Molecular modeling and simulations of some antiviral drugs, benzylisoquinoline alkaloid, and coumarin molecules to investigate the effects on Mpro main viral protease inhibition,” *Biochem. Biophys. Reports*, vol. 34, p. 101459, 2023.

[71] K. Liu and H. Kokubo, “Exploring the stability of ligand binding modes to proteins by molecular dynamics simulations: A cross-docking study,” *J. Chem. Inf. Model.*, vol. 57, no. 10, pp. 2514–2522, 2017.

[72] A. A. T. Naqvi, T. Mohammad, G. M. Hasan, and M. I. Hassan, “Advancements in docking and molecular dynamics simulations towards ligand-receptor interactions and structure-function relationships,” *Curr. Top. Med. Chem.*, vol. 18, no. 20, pp. 1755–1768, 2018.

[73] P. W. Hildebrand, A. S. Rose, and J. K. S. Tiemann, “Bringing molecular dynamics simulation data into view,” *Trends Biochem. Sci.*, vol. 44, no. 11, pp. 902–913, 2019.

[74] E. Lionta, G. Spyrou, D. K Vassilatis, and Z. Cournia, “Structure-based virtual screening for drug discovery: principles, applications and recent advances,” *Curr. Top. Med. Chem.*, vol. 14, no. 16, pp. 1923–1938, 2014.

[75] W.-X. Gao, H.-J. Feng, B.-B. Guo, Y. Lu, and G.-X. Jin, "Coordination-directed construction of molecular links," *Chem. Rev.*, vol. 120, no. 13, pp. 6288–6325, 2020.

[76] S. O. Oselusi, A. Christoffels, and S. A. Egieyeh, "Cheminformatic characterization of natural antimicrobial products for the development of new lead compounds," *Molecules*, vol. 26, no. 13, p. 3970, 2021.

[77] M. Karthikeyan, D. Pandit, and R. Vyas, "ChemScreener: A distributed computing tool for scaffold based virtual screening," *Comb. Chem. High Throughput Screen.*, vol. 18, no. 6, pp. 544–561, 2015.

[78] A. M. Udrea *et al.*, "Advanced bioinformatics tools in the pharmacokinetic profiles of natural and synthetic compounds with anti-diabetic activity," *Biomolecules*, vol. 11, no. 11, p. 1692, 2021.

[79] Y. Zhang and L. Z. Benet, "The gut as a barrier to drug absorption: combined role of cytochrome P450 3A and P-glycoprotein," *Clin. Pharmacokinet.*, vol. 40, pp. 159–168, 2001.

[80] M. Christen and W. F. Van Gunsteren, "On searching in, sampling of, and dynamically moving through conformational space of biomolecular systems: A review," *J. Comput. Chem.*, vol. 29, no. 2, pp. 157–166, 2008.

[81] S. A. A. Anand, C. Loganathan, K. Saravanan, and S. Kabilan, "Comparison of molecular docking and molecular dynamics simulations of 1, 3-thiazin-4-one with MDM2 protein," *Int. Lett. Chem. Phys. Astron.*, vol. 60, pp. 161–167, 2015.

[82] M. P. Repasky, M. Shelley, and R. A. Friesner, "Flexible ligand docking with Glide," *Curr. Protoc. Bioinforma.*, vol. 18, no. 1, pp. 8–12, 2007.

[83] V. Bhat and J. Chatterjee, "The use of in silico tools for the toxicity prediction of potential inhibitors of SARS-CoV-2," *Altern. to Lab. Anim.*, vol. 49, no. 1–2, pp. 22–32, 2021.

[84] J. A. Arnott and S. L. Planey, "The influence of lipophilicity in drug discovery and design," *Expert Opin. Drug Discov.*, vol. 7, no. 10, pp. 863–875, 2012.

[85] D. S. Nielsen, N. E. Shepherd, W. Xu, A. J. Lucke, M. J. Stoermer, and D. P. Fairlie, "Orally absorbed cyclic peptides," *Chem. Rev.*, vol. 117, no. 12, pp. 8094–8128, 2017.

[86] C. Laurence, K. A. Brameld, J. Graton, J.-Y. Le Questel, and E. Renault, "The p K BHX database: toward a better understanding of hydrogen-bond basicity for medicinal chemists," *J. Med. Chem.*, vol. 52, no. 14, pp. 4073–4086, 2009.

[87] M. K. Bayliss *et al.*, "Quality guidelines for oral drug candidates: dose, solubility and lipophilicity," *Drug Discov. Today*, vol. 21, no. 10, pp. 1719–1727, 2016.

[88] G. W. Caldwell, "Compound optimization in early-and late-phase drug discovery: acceptable pharmacokinetic properties utilizing combined physicochemical, in vitro and in vivo screens," *Curr. Opin. Drug Discov. Dev.*, vol. 3, no. 1, pp. 30–41, 2000.

[89] C.-Y. Jia, J.-Y. Li, G.-F. Hao, and G.-F. Yang, "A drug-likeness toolbox facilitates ADMET study in drug discovery," *Drug Discov. Today*, vol. 25, no. 1, pp. 248–258, 2020.

[90] S. Gupta, R. Kesarla, and A. Omri, "Formulation strategies to improve the bioavailability of poorly absorbed drugs with special emphasis on self-emulsifying systems," *Int. Sch. Res. Not.*, vol. 2013, no. 1, p. 848043, 2013.

[91] S. Ghahremanian, M. M. Rashidi, K. Raeisi, and D. Toghraie, "Molecular dynamics simulation approach for discovering potential inhibitors against SARS-CoV-2: A structural review," *J. Mol. Liq.*, vol. 354, p. 118901, 2022.

[92] V. G. Maltarollo, J. C. Gertrudes, P. R. Oliveira, and K. M. Honorio, "Applying machine learning techniques for ADME-Tox prediction: a review," *Expert Opin. Drug Metab. Toxicol.*, vol. 11, no. 2, pp. 259–271, 2015.

[93] J. Bhachoo and T. Beuming, "Investigating protein–peptide interactions using the Schrödinger computational suite," *Model. Pept. Interact. methods Protoc.*, pp. 235–254, 2017.

[94] E. Alexov *et al.*, "Progress in the prediction of pKa values in proteins," *Proteins Struct. Funct. Bioinforma.*, vol. 79, no. 12, pp. 3260–3275, 2011.