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Theme

Computational Drug Discovery and Toxicological Profiling of Novel Quinoline Derivatives: Insights from ADMET Analysis, Molecular Docking, and Dynamics Simulations for Antiviral Applications

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HABA Islam **LABID Narimane** **THAMER Amina**

Abstract

This study explores the antiviral potential of a novel series of quinoline derivatives using an integrated computational approach, with a particular focus on their inhibitory activity against the 2′-O-methyltransferase (MTase) enzyme of the yellow fever virus (YFV). The MTase enzyme, which plays a critical role in viral RNA capping and immune evasion, was selected as a strategic target (PDB ID: 3EVA). A logical approach was used to generate a structurally diverse library of quinoline analogs, followed by virtual screening of their pharmacokinetic properties and drug-likeness using the SwissADME and pkCSM platforms.

Molecular docking analyses revealed that several compounds exhibited strong binding affinities to the MTase active site, interacting with conserved catalytic residues. The most promising candidates—Q11, Q12, and Q15—were further subjected to molecular dynamics (MD) simulations to validate the stability of the ligand–enzyme complexes. These compounds demonstrated stable binding behavior throughout the 100-nanosecond simulation period, as evidenced by root-mean-square deviation (RMSD) values remaining below 2 Å.

Overall, the results suggest that the proposed quinoline derivatives possess favorable drug-like properties and exhibit strong potential for targeting the MTase enzyme of the yellow fever virus, making them promising scaffolds for the development of future antiviral agents.

Keywords: Quinoline derivatives, Yellow fever virus, 2′-O-methyltransferase (MTase), Molecular docking, Molecular dynamics, Antiviral agents, ADMET profile.

RÉSUMÉ :

Cette étude explore le potentiel antiviral d'une nouvelle série de dérivés de la quinoléine par une approche computationnelle intégrée, en se concentrant plus particulièrement sur leur activité inhibitrice contre l'enzyme 2′-O-méthyltransférase (MTase) du virus de la fièvre jaune (VFJ). L'enzyme MTase, qui joue un rôle essentiel dans le coiffage de l'ARN viral et l'évasion immunitaire, a été sélectionnée comme cible stratégique (ID PDB : 3EVA). Une approche logique a été utilisée pour générer une banque d'analogues de la quinoléine structurellement diversifiée, puis un criblage virtuel de leurs propriétés pharmacocinétiques et de leur similarité médicamenteuse a été réalisé à l'aide des plateformes SwissADME et pkCSM.

Des analyses d'amarrage moléculaire ont révélé que plusieurs composés présentaient de fortes affinités de liaison au site actif de la MTase, interagissant avec des résidus catalytiques conservés. Les candidats les plus prometteurs – Q11, Q12 et Q15 – ont ensuite été soumis à des simulations de dynamique moléculaire (MD) afin de valider la stabilité des complexes ligand-enzyme. Ces composés ont démontré un comportement de liaison stable tout au long de la période de simulation de 100 nanosecondes, comme en témoignent les valeurs de l'écart quadratique moyen (RMSD) restant inférieures à 2 Å.

Dans l'ensemble, les résultats suggèrent que les dérivés de quinoléine proposés possèdent des propriétés pharmacologiques favorables et présentent un fort potentiel pour cibler l'enzyme MTase du virus de la fièvre jaune, ce qui en fait des supports prometteurs pour le développement de futurs agents antiviraux.

Mots-clés : Dérivés de quinoléine, Virus de la fièvre jaune, 2′-O-méthyltransférase (MTase), Amarrage moléculaire, Dynamique moléculaire, Agents antiviraux, Profil ADMET.

الملخص

تستكشف هذه الدراسة الإمكانات المضادة للفيروسات لسلسلة جديدة من مشتقات الكينولين باستخدام نهج حاسوبي متكامل، مع التركيز بشكل خاص على نشاطها المثبط ضد إنزيم 2'-O-ميثيل ترانسفيراز (MTase) لفيروس الحمى الصفراء (YFV). وقد اختير إنزيم MTase، الذي يلعب دورًا حاسمًا في تغطية الحمض النووي الريبي الفيروسي والتهرب المناعي، كهدف استراتيجي (معرف قاعدة بيانات البروتينات: EVA3). واستُخدم نهج منطقي لإنشاء مكتبة متنوعة هيكليًا من نظائر الكينولين، تلاها فحص افتراضي لخصائصها الحركية الدوائية وتشابهها الدوائي باستخدام منصتي SwissADME وpkCSM.

أظهرت تحليلات الالتحام الجزيئي أن العديد من المركبات أظهرت ألفة ارتباط قوية بالموقع النشط لإنزيم MTase، متفاعلةً مع بقايا المحفزات المحفوظة. خضعت المركبات المرشحة الواعدة - Q11 وQ12 وQ15 - لمزيد من عمليات محاكاة الديناميكيات الجزيئية (MD) للتحقق من استقرار معقدات الربيطة والإنزيم. أظهرت هذه المركبات سلوك ارتباط مستقرًا طوال فترة المحاكاة التي استمرت 100 نانوثانية، كما يتضح من بقاء قيم انحراف الجذر التربيعي المتوسط (RMSD) أقل من 2 أنجستروم.

بشكل عام، تشير النتائج إلى أن مشتقات الكينولين المقترحة تمتلك خصائص دوائية مواتية، وتُظهر إمكانات قوية لاستهداف إنزيم MTase لفيروس الحمى الصفراء، مما يجعلها ركائز واعدة لتطوير عوامل مضادة للفيروسات في المستقبل.

الكلمات المفتاحية: مشتقات الكينولين، فيروس الحمى الصفراء، 2'-O-ميثيل ترانسفيراز (MTase)، الالتحام الجزيئي، الديناميكيات الجزيئية، العوامل المضادة للفيروسات، ملف ADMET.

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Summary

Background and Significance of the Study

Despite the availability of a highly effective vaccine, Yellow Fever Virus (YFV) remains a significant public health concern in many tropical and subtropical regions. YFV is a positive-sense, single-stranded RNA virus of the Flaviviridae family, and continues to cause recurrent outbreaks, particularly in Africa and South America. The lack of approved antiviral therapies for YFV has prompted a renewed interest in the identification of viral targets for therapeutic intervention[1]. One such target is the 2'-O-methyltransferase (MTase) enzyme, a conserved non-structural protein responsible for methylating the 5'-cap structure of viral RNA, which is essential for viral replication and evasion of host immune responses. Inhibition of this enzyme may impair viral replication and restore innate immune recognition of uncapped viral RNAs[2][3]

Quinoline-based compounds are known for their broad-spectrum pharmacological activities, including notable antiviral properties. The rigid aromatic framework of quinoline allows for hydrophobic interactions with nucleic acids and viral proteins, making it a privileged scaffold in antiviral drug design .

Several quinoline-containing drugs, such as saquinavir and indinavir, have demonstrated clinical success against other viral pathogens, yet their application against flaviviruses, particularly YFV, remains underexplored[4] .In this context, the current study aims to design and evaluate novel quinoline derivatives targeting the MTase enzyme of YFV using an in-silico structure-based drug discovery approach, including virtual screening, molecular docking, and molecular dynamics simulations. The findings offer a promising foundation for further optimization of quinoline scaffolds as potent antiviral candidates against yellow fever.

Objectives and Scope of the Research

The primary objective of this research is to explore the antiviral potential of a newly developed series of quinoline analogues targeting Yellow Fever Virus (YFV) through an integrative computational approach. The study begins with the construction of a virtual combinatorial library, where structural modifications are systematically introduced to the quinoline scaffold with the goal of enhancing antiviral efficacy and optimizing pharmacokinetic properties, as supported by previous work on quinoline derivatives exhibiting antiviral activities . Following library generation, in silico pharmacokinetic and toxicological profiling will be conducted using established tools such as SwissADME and ProTox-II to predict absorption, distribution, metabolism, excretion (ADME), and toxicity parameters, ensuring the selection of drug-like candidates with acceptable safety profiles . Virtual screening techniques will then be employed to prioritize compounds for molecular docking studies against the YFV methyltransferase enzyme, a critical target involved in viral RNA capping and immune evasion. Molecular docking will provide insights into the binding affinity, key interacting residues, and plausible mechanisms of action, in line with previous studies on flavivirus methyltransferase inhibition[5].

To further validate the stability and dynamic behavior of the top-ranked ligand–protein complexes, This comprehensive computational strategy aims to facilitate the identification of potent YFV inhibitors and contribute to the rational design of novel antiviral therapeutics.

Part I.

LITERATURE REVIEW

Chapter I

Introduction

Introduction

Yellow fever is a viral hemorrhagic disease caused by the yellow fever virus (YFV), a member of the Flaviviridae family, and remains a significant public health threat in tropical regions of Africa, South America, and parts of Asia. The virus is transmitted primarily by mosquitoes and can lead to severe illness, including liver damage, hemorrhagic fever, and multi-organ failure. Although an effective vaccine exists, there is no specific antiviral treatment for yellow fever, and outbreaks continue to pose a risk to vulnerable populations.

Given the lack of targeted antiviral therapies, there is a pressing need for the identification and development of novel compounds capable of inhibiting the virus replication and improving clinical outcomes.

Quinoline derivatives have emerged as a promising class of compounds in the search for antiviral agents due to their broad-spectrum activity against various viral infections. The quinoline scaffold, characterized by its bicyclic aromatic structure, has been shown to exhibit antimicrobial, antimalarial, and antiviral activities. These compounds possess unique chemical features that make them suitable for modification and optimization, allowing for the design of derivatives with enhanced potency and selectivity.

In recent years, the interaction of quinoline derivatives with flavivirus proteins, particularly those involved in the viral replication cycle, has gained attention. Yellow fever virus, like other flaviviruses, relies on key proteins such as the NS3 protease/helicase, NS5 RNA-dependent RNA polymerase, and the E protein for its replication and infectivity. These viral proteins represent attractive targets for drug development, and the ability of quinoline derivatives to interact with these proteins could offer a novel therapeutic approach for treating yellow fever.

This thesis aims to explore the interaction of quinoline-based compounds with yellow fever virus proteins, using advanced in silico techniques to model and predict the binding affinity of various quinoline derivatives. By applying molecular docking, molecular dynamics simulations, and quantitative structure-activity relationship (QSAR) modeling, this study seeks to identify promising candidates that may inhibit the key steps of the yellow fever virus life cycle. Through a detailed analysis of the binding interactions between quinoline derivatives and viral targets, this research aims to contribute to the rational design of new antiviral agents and further our understanding of the molecular mechanisms underlying viral inhibition.

The scope of this work includes an investigation of quinoline derivatives potential to interfere with viral protein function, assess their binding stability, and evaluate their pharmacokinetic properties. By combining computational approaches with experimental validation, this study provides a comprehensive framework for the development of quinoline-based antivirals for yellow fever. The outcomes of this research could pave the way for novel therapeutic strategies in the fight against yellow fever and other flavivirus-related diseases.

1.Yellow fever disease

Yellow fever virus is an acute viral disease caused by a virus belonging to the Flaviviridae family. It is transmitted to humans through the bites of mosquitoes, particularly the Aedes aegypti mosquito . The disease is known by several names, such as "yellow hemorrhagic fever," "yellow plague," and "black disease," reflecting its hemorrhagic nature and its devastating impact throughout history . The disease begins after an incubation period of 3 to 6 days, and symptoms appear suddenly and include high fever, severe headache, severe muscle pain, especially in the back, nausea, vomiting, and redness of the eyes and face . Some patients may recover at this stage, but others enter the toxic phase, which begins after a temporary remission and includes the return of fever, yellowing of the skin and eyes (jaundice), internal and external bleeding, and kidney and liver failure, which can lead to death . Yellow fever goes through three main stages: the acute stage, which typically lasts between 3 and 4 days, followed by a remission phase where symptoms disappear in some patients. The third, toxic, stage occurs in approximately 15% of infected individuals and is fatal in up to 60% of cases without medical care. There is no specific treatment for the virus, and if left untreated, it can lead to death due to severe bleeding, vital organ failure, or coma, Poor prevention is one of the most prominent causes of the disease's spread. This includes not receiving the highly effective vaccine, along with neglecting to control the vector mosquitoes, the prevalence of stagnant water, and a lack of health awareness, especially in endemic areas. Traveling without vaccination to areas where the infection is prevalent Early detection plays a pivotal role in reducing mortality and helping infected individuals overcome the toxic stage. It also contributes to containing the spread of the virus by isolating infected individuals, initiating mosquito control campaigns, and warning the population,thus breaking the chain of infection[6].

2.Symptoms of yellow fever disease

YF infection can cause the onset of different clinical features, ranging from a self-limited or mild febrile illness with flu-like symptoms in most of the cases to severe hemorrhage and liver disease. The analysisof data on asymptomatic infections, mild disease, severe disease (fever with jaundice or hemorrhagic symptoms), and fatalities collected in 11 studies involving Africa and South America during the period 1969–2011 was used by the group of Johansson[7],to estimate the probability of each infection outcome. In more detail, in cases of YF virus infections, the probability of being asymptomatic was 55%, whereas the probabilities of developing mild and severe diseases were 33% and 12%, respectively. The probability of death for people experiencing severe disease was 47%. Symptoms include fever, headache, jaundice, muscle pain, nausea, vomiting, and fatigue. Such variety in the clinical spectrum makes YF diagnosis difficult. In those patients presenting a severe infection, hemorrhagic fever can develop leading to the death of the infected subjects. The Case fatality rate has been estimated as 20 – 50% in patients with severe symptoms[1].

3. Causes of yellow fever disease

Yellow fever is caused by the yellow fever virus (YFV), a member of the Flaviviridae family This virus is transmitted to humans through the bites of infected female mosquitoes, particularly the Aedes aegypti mosquito, which breeds in stagnant water near human habitations,Infection occurs when the mosquito bites an infected monkey or human, who then acquires the virus and transmits it to other people [8]Traveling or living in tropical regions of South America and Africa is a major risk factor, given the endemicity of the virus in these areas The absence of a vaccine or a weakened immune system also increases susceptibility to infection[9] Furthermore, the diversity of virus transmission patterns contributes to the continued spread of the disease, as there are three main cycles: the forest pattern (between monkeys and mosquitoes), the urban pattern (between humans and mosquitoes), and the intermediate or savanna pattern (between monkeys and humans), which promotes the spread of the virus in different environments [10].

4. Treatment of Yellow Fever disease

Yellow fever is an acute viral disease transmitted through the bites of infected mosquitoes. There is currently no specific treatment for the virus, and treatment focuses on providing supportive care to patients. This includes monitoring and managing symptoms,

including controlling fever and pain, and preventing serious complications such as bleeding or kidney and liver failure :

4.1 Supportive Care

Supportive care focuses on providing comfort and adequate hydration through fluids. Dehydration should be avoided by using oral or intravenous fluids as needed. The patient's health condition is monitored regularly to ensure that complications such as bleeding or organ failure do not develop[1].

4.2 Antipyretics

To treat fever associated with the disease, paracetamol (acetaminophen) is used as a fever reducer and pain reliever. It is recommended to avoid the use of aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs) (such as ibuprofen and naproxen), as they can increase the risk of bleeding in patients with yellow fever[6].

4.3 Medical Monitoring

In severe cases of yellow fever, the patient may require hospitalization to monitor vital functions such as blood pressure, heart rate, and liver and kidney function. Advanced support is provided if bleeding or vital organ failure occurs[8].

4.4Medications

There are no approved antiviral medications for the treatment of yellow fever. However, some medications, such as ribavirin and NITD008, have been studied in animal models, but have not shown sufficient efficacy or are toxic for human use in treatment against the virus[11][12].

4.5 Prevention

The yellow fever vaccine is considered the most effective means of preventing the disease. The vaccine is administered once and provides long-lasting protection against the virus. Vaccination is recommended for travelers to endemic areas or areas experiencing outbreaks [9]

5.Yellow Fever Virus in Human Infection

5.1 Introduction and Classification:

Flaviviruses are among the most prominent viruses affecting humans. They are single-stranded, positive-sense RNA viruses belonging to the family Flaviviridae and are primarily transmitted via arthropod vectors such as mosquitoes. Yellow Fever Virus (YFV) is a major cause of hemorrhagic disease in tropical regions, causing severe infections that in some cases lead to fatal complications

5.2 Structural Characteristics:

Yellow Fever Virus is a single-stranded, positive-sense RNA virus enveloped by a lipid bilayer and belongs to the genus Flavivirus. It is transmitted to humans primarily via the Aedes aegypti mosquito and is the causative agent of Yellow Fever, predominantly affecting regions in Africa and South America

The viral genome is approximately 11 kilobases in length and encodes a polyprotein that is subsequently cleaved into structural proteins (C, prM, E) and non-structural proteins (NS1–NS5). The envelope protein E plays a crucial role in mediating the virus's attachment to and fusion with host cell membranes [13]

5.3 Pathogenic Factors:

The virus primarily targets liver cells, causing necrosis within hepatic lobes, which can lead to jaundice, internal hemorrhage, and multi-organ failure. Viral proteins such as NS3 and NS5 significantly contribute to its pathogenicity; NS5, in particular, interferes with immune signaling pathways, enabling the virus to replicate efficiently within host cells

5.4 Interaction with the Immune System:

Upon entering the human body, Yellow Fever Virus induces an innate immune response characterized by the production of interferons. However, its non-structural proteins (notably NS5) inhibit this response by interfering with the JAK-STAT signaling pathway, thereby reducing the effectiveness of type I interferons. This immune evasion mechanism allows the virus to multiply within host cells without early inhibition by the immune system[14]

6. Yellow fever virus methyltransferase:

Methyltransferase (MTase) is a key functional component located at the amino terminus of the yellow fever virus (YFV) non-structural protein NS5. It plays a pivotal role in the capping process of viral RNA, an essential step for ensuring viral genome stability and increasing the efficiency of its translation within the host cell[2] This enzyme catalyzes two distinct methylation reactions: the first is methylation of the N7 nitrogen atom of the guanine base, and the second is methylation of the 2′-O group of the ribose sugar, using S-adenosyl-L-methionine (SAM) as the methyl group source[15] These modifications are essential for the formation of the 5′-terminal cap (5′ RNA cap) on the viral genome, which provides additional stability to the RNA, improves its translation efficiency, and helps the virus evade the host's innate immune mechanisms[2] Structurally, this enzyme adopts a classic Rossmann fold and contains a conserved active site that interacts with both SAM and the RNA substrate Given its importance in the viral life cycle, this enzyme is a promising target for the development of antivirals, as its disruption can disrupt RNA maturation processes and thus halt viral replication. Numerous structural and functional studies on MTase enzymes in viruses belonging to the Flaviviridae family, including yellow fever virus, have revealed fine details regarding binding sites and catalytic residues that can be exploited in the rational design of antiviral drugs[5]

7. Antiviral potential of quinoline

It is known to possess several biological activitiess such as antimalarial ,antibacterial, anticancer . A broad spectrum biological activity of quinoline is illustrated in Several antiviral drugs such as saquinavir, indiravir containing quinoline scaffold are marketed these days but still this area is widely unexplored. Drugs acting specifically on virus targets are available but only for some viral infections. Various molecules have been developed but they are usually associated with drug resistance, cytotoxicity thats by there is an urge to develop more efficient antiviral drugs. Owing to its immense pharmacological activity, extensive research has been done for the synthesis of quinoline molecule and its derivatives over the years.

Although several methods have been developed, they are associated with certain limitations thus there is a need for developing[16].

8. Synthesis route of quinolone

The synthesis of quinoline derivatives has long been a focus of organic chemistry, due to their broad pharmacological relevance. Among the classical and widely used synthetic methods

is the Skraup synthesis, which is one of the oldest and most reliable routes for producing quinoline structures .

Step 1: Formation of quinoline ring (Skraup reaction)

The Skraup reaction involves the cyclization of aniline with glycerol in the presence of sulfuric acid and an oxidizing agent such as nitrobenzene. Under heating conditions, this reaction forms quinoline as the main product:

Aniline + Glycerol + H_2SO_4 + Nitrobenzene → Quinoline .

Step 2: Electrophilic nitration of quinoline

Quinoline can then undergo nitration using a mixture of concentrated nitric and sulfuric acids to produce 5-nitroquinoline, targeting the electron-rich position on the aromatic system:

Quinoline + HNO_3/H_2SO_4 → 5-Nitroquinoline.

Step 3: Amination and derivatization

5-Nitroquinoline can be further transformed by reduction (using Sn/HCl or Fe/AcOH) to yield 5-aminoquinoline ,which serves as an important intermediate for several antimalarial drugs (e.g., chloroquine, amodiaquine).

5-Nitroquinoline + Sn/HCl → 5-Aminoquinoline[17].

Molecular description:

The core structure of quinoline consists of a benzene ring fused with a pyridine ring, forming a rigid, planar, and aromatic system. Substituents at positions 2, 4, 5, and 8 are particularly relevant for modulating biological activity. The nitrogen in the pyridine ring can act as a hydrogen bond acceptor, crucial for interactions with enzymes and DNA [18]

Structure-activity relationship (SAR):

Substitution at the 5-position with electron-donating or electron-withdrawing groups significantly affects antimicrobial or anticancer properties. For instance, nitro and amino groups at position 5 have been associated with enhanced DNA intercalation and inhibition of topoisomerase enzymes [17].

Quinoline derivatives and biological activity Several novel quinoline derivatives have been synthesized based on this scaffold. Notably, 5-aminoquinoline–triazole hybrids have

shown promising antiplasmodial and anticancer properties. These compounds inhibit Plasmodium falciparum growth and have low cytotoxicity toward human cells. Recent studies report successful inhibition of drug-resistant strains, positioning these conjugates as promising leads for future drug development (Figure 1) [19]

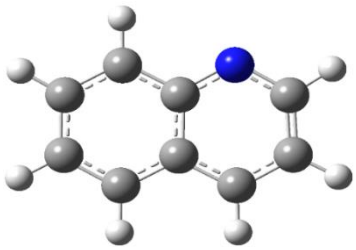


Figure 1. 3D structure of Quinoline

9. In silico approaches

refer to a set of computational methods used to simulate and analyze biological, chemical, and physical processes in a virtual environment. The term “in silico” is derived from the Latin word for silicon, indicating the role of computer-based technologies as an alternative to traditional in vivo (within the living organism) and in vitro (outside the organism) experiments[20] .

These approaches have become indispensable in modern science due to their predictive capabilities, cost efficiency, and ethical advantages. Predictive modeling, for instance, allows researchers to simulate complex biological systems using mathematical algorithms, providing a platform for hypothesis testing and drug response forecasting before entering the lab [21]

In silico studies are also significantly more cost-effective compared to experimental methods, enabling rapid screening of vast datasets and chemical libraries with minimal financial and material resources[22].

Furthermore, these approaches contribute to the reduction of animal testing, offering ethical alternatives that comply with the 3Rs principles (Replacement, Reduction, and Refinement) [23]

Among the key techniques under the in silico umbrella are molecular modeling and simulation, which involve the construction and dynamic analysis of 3D molecular structures such as proteins and ligands [24]

Bioinformatics plays a crucial role by extracting meaningful insights from biological data including gene sequences, protein structures, and expression profiles [25]

while cheminformatics supports the prediction of chemical behavior, biological activity, and toxicity of compounds based on their structural properties [26]

Systems biology further integrates computational and experimental data to model interactions within complex biological networks, enabling the study of emergent properties at various levels of biological organization [27]

Overall, in silico approaches represent a transformative shift in scientific research, enhancing the pace and precision of discovery across biology, chemistry, and pharmaceutical science.

10. Molecular Docking

Molecular docking is a computer-aided modeling technique used to predict the preferred orientation and binding affinity of a small molecule (ligand) when bound to a target protein (receptor). This approach plays a fundamental role in structure-based drug design, as it helps identify and optimize compounds with high binding potential[28][29]The main steps involved in molecular docking are:

Preparation of ligands and receptors: Ligands and target proteins are preprocessed by removing water molecules, adding hydrogen atoms, assigning correct protonation states, and minimizing energy to ensure an optimal geometry for docking.

Grid generation: A 3D grid is generated to define the active site of the receptor, allowing the docking algorithm to focus on relevant regions of the protein structure where ligand binding is most likely.

Scoring function: Scoring functions are mathematical models that estimate the binding affinity of ligand poses based on factors such as van der Waals forces, hydrogen bonding, hydrophobic effects, electrostatic interactions, and desolvation energy.

Search algorithm: Docking software explores different spatial conformations and orientations of ligands using search algorithms such as genetic algorithms, Monte Carlo methods, or systematic sampling.

Pose generation and refinement: Multiple poses of each ligand are generated within the binding site. The top-ranked poses—those with the lowest energy—are selected for further analysis and optimization.

Analysis and visualization: The resulting ligand-receptor complexes are analyzed to identify critical binding interactions such as hydrogen bonds, π–π stacking, and hydrophobic contacts. Visualization tools like PyMOL or Discovery Studio are used to interpret the molecular interactions.

Molecular docking is widely applied in virtual screening of compound libraries, lead optimization, and elucidation of molecular mechanisms of drug action. Its ability to predict binding affinity and orientation makes it a valuable tool in the rational design of novel therapeutic agents [28][30]

10.1. Types of Molecular Docking

here are several types of molecular docking methods, each with its own approach and application. Some of the main types of molecular docking include:

Rigid Docking: In rigid docking, both the ligand and receptor are treated as rigid entities, meaning their conformations do not change during the docking process. This method is computationally efficient but may overlook important conformational changes in the binding site .

10.1.1. Flexible Docking

Flexible docking allows for limited flexibility in either the ligand, receptor, or both during the docking process. This approach considers conformational changes in the binding site and can provide more accurate predictions of binding modes and affinities.

10.1.2. Induced Fit Docking

Induced fit docking accounts for conformational changes in both the ligand and receptor upon binding. It involves iterative cycles of docking and energy minimization to optimize the binding pose and predict induced conformational changes in the receptor.

10.1.3. Ligand-Based Docking

In ligand-based docking, the ligand conformation is flexible while the receptor is kept rigid. This approach is useful when the receptor structure is unknown or unavailable, allowing researchers to predict ligand binding modes based on known ligand-receptor interactions.

10.1.4. Protein-Ligand Docking

Protein-ligand docking involves the docking of small molecules (ligands) into the binding sites of proteins (receptors) to predict their binding affinity and mode of interaction. This method is widely used in drug discovery to screen libraries of small molecules for potential drug candidates.

10.1.5. Protein-Protein Docking

Protein-protein docking predicts the binding modes and interactions between two or more protein molecules. It is used to study protein-protein interactions, protein complexes, and signaling pathways, providing insights into biological processes and disease mechanisms

10.1.6. Flexible Receptor Docking

Flexible receptor docking allows for conformational changes in the receptor structure during the docking process. It considers flexibility in specific regions of the receptor that may undergo conformational changes upon ligand binding, such as loop regions or side-chain movements.

Each type of molecular docking method has its advantages and limitations, and the choice of method depends on the specific research question, the availability of structural information, and computational resources. Integrating multiple docking approaches can enhance the accuracy and reliability of predictions in drug discovery and structural biology studies[31]**.**

11. Maestro Molecular Docking Software

Maestro is a molecular modeling platform developed by Schrödinger, Inc., offering a comprehensive suite of computational chemistry tools including molecular docking, virtual screening, and molecular dynamics simulations. The docking module within Maestro enables the prediction of ligand binding modes and affinities with protein targets, facilitating the exploration of molecular interactions critical to drug discovery [32]

11.1. Graphical user interface (GUI):

Maestro features a highly intuitive graphical interface that allows researchers to set up, execute, and analyze docking simulations. It supports interactive 3D visualization and manipulation of protein-ligand complexes [33]

11.2 .Scoring functions:

The software integrates multiple scoring functions—empirical, physics-based, and knowledge-based—to evaluate binding affinities and predict the most favorable ligand binding poses within the protein active site [34]

11.3. Flexible docking protocols:

Maestro supports a range of docking methodologies including rigid docking, flexible docking, induced fit docking, and standard protein-ligand docking. Users can customize parameters such as ligand flexibility, receptor flexibility, and conformational sampling techniques.

11.3.1. Ligand preparation:

The platform includes tools for ligand preprocessing, such as conformational sampling, energy minimization, and protonation state prediction, ensuring chemical accuracy prior to docking.

11.3.2. Receptor preparation:

Maestro facilitates protein preparation by adding missing atoms, optimizing side-chain conformations, assigning bond orders, and removing non-essential entities like crystallographic water molecules from the active site [35]

11.3.3. Analysis and visualization:

Post-docking analysis includes interaction diagrams, binding score reports, and detailed 3D representations of binding modes. This enables users to identify key molecular interactions and assess ligand efficacy visually and quantitatively [36]

Overall, Maestro delivers a powerful and integrated platform for structure-based drug design, enabling efficient identification of promising lead compounds for experimental validation.

12. ADME and Toxicity

ADME-Tox is an integrated concept encompassing two fundamental dimensions of pharmacological evaluation: Absorption, Distribution, Metabolism, and Excretion (ADME),

alongside Toxicity (Tox). These parameters collectively determine the pharmacokinetic behavior and safety profile of candidate drugs, forming an essential cornerstone in the early stages of drug discovery and development [37]

Absorption refers to the process by which a drug permeates into the systemic circulation from its administration site. The efficiency of absorption is influenced by the physicochemical characteristics of the compound such as molecular weight, lipophilicity, aqueous solubility, and permeability. Moreover, biological factors such as gastrointestinal pH, enzymatic degradation, and the activity of efflux transporters like P-glycoprotein can significantly modulate absorption rates [38]

Distribution encompasses the dissemination of a drug throughout the body's compartments following absorption. This process is governed by factors including cardiac output, regional blood flow, tissue affinity, and plasma protein binding. Highly lipophilic compounds tend to accumulate in fatty tissues, while hydrophilic drugs may remain confined to the vascular compartment [39]

Distribution is critical for ensuring sufficient drug concentrations at the therapeutic target site.

Metabolism primarily occurs in the liver through phase I (functionalization) and phase II (conjugation) reactions, mediated by cytochrome P450 enzymes and other metabolic systems. These biotransformations can activate or inactivate the parent drug and sometimes produce toxic intermediates. Extrahepatic metabolism also takes place in tissues such as the intestine, lungs, and kidneys[40]Excretion refers to the clearance of drugs and their metabolites from the body, predominantly through renal (urinary) or biliary (fecal) routes. Minor routes include pulmonary excretion, sweat, and saliva. The excretion pattern influences the drug's half-life and risk of accumulation upon repeated dosing, which is essential for dosage regimen design[41]

Toxicity involves the adverse or undesired biological effects elicited by a drug, which may be dose-dependent or idiosyncratic. Toxic manifestations can affect multiple organ systems, with common categories including hepatotoxicity, nephrotoxicity, cardiotoxicity, neurotoxicity, and genotoxicity. Toxicity assessment integrates in vitro cell-based assays, in vivo animal models, and increasingly, in silico predictive models to evaluate compound safety profiles[42]

A thorough assessment of ADME-Tox characteristics facilitates the identification of liabilities in drug candidates, allowing for rational optimization and selection before clinical trials. Computational tools, including quantitative structure–activity relationship (QSAR) models and machine learning algorithms, have become indispensable in predicting these properties and reducing attrition rates during pharmaceutical development.

Chapter II

Quinoline: A Historical Overview and Development of Scientific Uses

1. Introduction to Quinoline Derivatives

1.1 Chemical Structure of Quinoline

Quinoline, a heterocyclic compound, consists of a fused bicyclic structure comprising a benzene ring (C6H5) and a pyridine ring (C5H4N)[43]. The fundamental structural motif of quinoline includes a nitrogen atom in the pyridine ring at the 1-position, making it a nitrogen-containing aromatic compound. Quinoline and its derivatives are significant due to their versatile chemical properties and biological activities, particularly as potential drug candidates.

The chemical structure of quinoline is highly adaptable, with a variety of substitution positions available for further modification. The pyridine nitrogen atom is crucial for the chemical reactivity of the molecule, contributing to its nucleophilic properties and enhancing its ability to interact with various biological targets. The benzene ring, on the other hand, is a hydrophobic moiety that aids in π–π stacking interactions[44], particularly in drug design.

Quinoline derivatives can be synthesized by introducing functional groups such as hydroxyl, amino, alkoxy, halogens, or heteroatoms to the quinoline scaffold[45]. These modifications can significantly alter the molecule's electronic properties, solubility, and bioactivity, thereby expanding the chemical space for potential drug discovery. The quinoline scaffold has been utilized in the design of various bioactive compounds, demonstrating a broad spectrum of pharmacological effects[46].

1.2 Biological Significance of Quinoline Derivatives

Quinoline-based derivatives have been extensively studied for their biological activities, especially their role as antimalarial, antiviral, anti-inflammatory, and anticancer agents. Their wide range of biological effects can be attributed to their ability to interact with various cellular and viral targets, which makes them valuable in drug discovery.

Antimalarial Activity: Quinoline derivatives are well-known for their antimalarial properties, with chloroquine and hydroxychloroquine being some of the most prominent examples[47]. These drugs interfere with the parasite's ability to detoxify heme within the red blood cells, leading to its accumulation and eventual death of the malaria parasite. This mechanism is primarily attributed to the ability of quinoline derivatives to intercalate into the heme polymer in the malaria parasite's digestive vacuole[48].

Antiviral Properties: Quinoline derivatives have shown promise in the inhibition of several viral targets, including HIV, hepatitis C, and flaviviruses like the Yellow Fever Virus

(YFV). The antiviral action is often mediated by the ability of quinolines to bind to viral enzymes, such as proteases or polymerases, thus disrupting the viral life cycle. For example, quinoline-based compounds have been identified as inhibitors of viral proteases, enzymes critical for viral replication and maturation[16].

Anti-inflammatory and Anticancer Activity: Quinoline derivatives also demonstrate potent anti-inflammatory and anticancer properties. The anti-inflammatory action is primarily through the inhibition of cyclooxygenase enzymes (COX), while the anticancer effects are attributed to the ability to interfere with cell division and apoptosis in cancer cells. Studies have shown that quinoline derivatives can induce oxidative stress and DNA damage, leading to cancer cell death, making them candidates for cancer therapy[49].

Other Pharmacological Properties: Quinoline derivatives have also been studied for their potential to treat other diseases, including diabetes, cardiovascular diseases, and neurological disorders. Their diverse pharmacological profile makes them valuable scaffolds for drug discovery, particularly in the development of broad-spectrum antiviral agents.

2. Quinoline Derivatives in Drug Discovery

2.1 Medicinal Chemistry of Quinoline Derivatives

The medicinal chemistry of quinoline derivatives focuses on modifying the quinoline scaffold to optimize biological activity while minimizing undesirable properties such as toxicity or poor solubility. Quinoline derivatives have long been explored for their antimicrobial, antimalarial, antiviral, and anticancer activities. The medicinal chemistry approach is rooted in understanding the pharmacophore of quinoline, which interacts with specific biological targets, and optimizing it for increased efficacy and selectivity.

A key aspect of medicinal chemistry in quinoline derivatives is the introduction of substituents to modify the molecule's physicochemical properties. Functional groups such as hydroxyl (-OH), methoxy (-OCH3), amino (-NH2), halogens (Cl, F, Br), and carbonyl groups have been incorporated into the quinoline ring system, each affecting the compound's bioactivity. For instance, halogenated quinolines can enhance lipophilicity and, consequently, cellular membrane penetration, a critical factor in antiviral drug efficacy. On the other hand, polar groups, such as hydroxyl or amino functionalities, improve water solubility and bioavailability.

The modification of the quinoline scaffold to improve its activity against specific targets requires careful structural consideration. Substituents at various positions on the ring (such as

positions 2, 4, 6, or 7) can dramatically alter the pharmacological profile of the compound. For example, in antimalarial drug design, quinoline derivatives are optimized to increase their potency by enhancing their ability to intercalate into the heme group of the malaria parasite[50]. Similarly, antiviral activity is improved by designing quinoline derivatives that can effectively bind to viral proteases or RNA-dependent RNA polymerase (RdRp), crucial enzymes involved in viral replication.

A significant part of medicinal chemistry is the study of **structure–activity relationships (SAR)**, which allows researchers to understand the correlation between the chemical structure of quinoline derivatives and their biological activity. SAR studies provide crucial insights into how different substituents affect the compound's ability to bind to its target, its solubility, toxicity, and overall pharmacokinetics. This information is vital for the design of the next generation of quinoline-based drugs[51].

2.2 Pharmacological Applications of Quinoline Derivatives

Quinoline derivatives have a broad range of pharmacological applications, with their most notable use being in the treatment of infectious diseases, particularly malaria. However, their antiviral, anti-inflammatory, and anticancer properties make them attractive scaffolds for therapeutic development in other fields as well.

Antimalarial Activity: Quinoline derivatives, particularly chloroquine and hydroxychloroquine, have historically been the cornerstone of malaria treatment. These compounds exert their antimalarial effects by interfering with the detoxification of heme inside the malaria parasite, causing heme accumulation that is toxic to the parasite. Quinoline-based antimalarial drugs are effective against the Plasmodium species responsible for malaria, and ongoing research aims to overcome resistance mechanisms that have emerged[52].

Antiviral Activity: Quinoline derivatives have gained attention for their ability to inhibit various viral enzymes, including proteases and polymerases, essential for the viral replication cycle. For instance, some quinoline derivatives have shown activity against the hepatitis C virus (HCV) by inhibiting its NS3/4A protease. Additionally, in the context of flaviviruses such as the Yellow Fever Virus (YFV), quinoline derivatives have been studied for their ability to block viral replication by targeting the viral RNA polymerase or protease[53].

Anti-inflammatory and Immunomodulatory Effects: Quinoline derivatives possess anti-inflammatory activity by inhibiting key enzymes such as cyclooxygenases (COX-1 and COX-2), which are responsible for the production of prostaglandins, mediators of

inflammation. By reducing prostaglandin synthesis, quinoline derivatives can alleviate symptoms of inflammatory diseases such as rheumatoid arthritis. Furthermore, some quinolines have demonstrated the ability to modulate the immune response, making them valuable in autoimmune disorders and inflammatory conditions[54].

Anticancer Activity: The anticancer potential of quinoline derivatives stems from their ability to inhibit tumor cell growth through various mechanisms. These include induction of apoptosis (programmed cell death), inhibition of mitotic division, and disruption of cancer cell metabolism. Quinoline derivatives have been shown to exert cytotoxic effects on various cancer cell lines, including breast, liver, and lung cancers. Additionally, some quinoline-based compounds also target the angiogenesis process, preventing the formation of new blood vessels necessary for tumor growth[55].

Neuroprotective and Cardiovascular Benefits: Emerging evidence suggests that quinoline derivatives may also possess neuroprotective properties, making them promising candidates for treating neurodegenerative diseases like Alzheimer's and Parkinson's disease. Their ability to modulate oxidative stress and inhibit neuroinflammation has been linked to their potential for neuroprotection. Furthermore, quinoline derivatives have been investigated for their cardiovascular effects, including the potential to regulate blood pressure, prevent atherosclerosis, and reduce cardiac arrhythmias[56].

2.3 Role of Quinoline Derivatives in Antiviral Research

The antiviral potential of quinoline derivatives has been a topic of increasing interest, especially with the rise of novel viral infections. Quinoline derivatives have been shown to inhibit the replication of several viruses by targeting key viral enzymes, making them attractive candidates in the development of broad-spectrum antiviral agents.

Mechanism of Action Against Viruses: Quinoline derivatives inhibit viral replication through multiple mechanisms. They may directly interact with viral enzymes such as proteases, polymerases, or helicases, or they may interfere with the virus's ability to enter host cells. By targeting these essential viral functions, quinoline derivatives disrupt the virus's ability to replicate and propagate.

Specificity for Flaviviruses: Flaviviruses, including Yellow Fever Virus (YFV), Zika Virus, and Dengue Virus, have been identified as key targets for quinoline-based antiviral agents. The similarity in the structure and function of the enzymes within the flavivirus family makes them ideal candidates for drug discovery. Quinoline derivatives have shown promise in

inhibiting viral proteases and RNA polymerases, essential for the replication and transcription of viral RNA.

For instance, in the case of YFV, quinoline derivatives may bind to the viral NS3 protease, preventing the maturation of viral proteins and thus inhibiting the virus's ability to reproduce and infect host cells. Moreover, quinolines may interfere with viral entry into host cells by disrupting interactions between the viral envelope and host cell receptors.

Broad-Spectrum Antiviral Activity: Quinoline derivatives are not only effective against flaviviruses but have also demonstrated activity against other viral families, including coronaviruses, retroviruses, and herpesviruses. Their ability to interact with a wide variety of viral enzymes positions quinoline derivatives as potential broad-spectrum antiviral agents, capable of combating a wide range of viral pathogens[4].

3. Structure–Activity Relationship (SAR) of Quinoline Derivatives

3.1 Importance of SAR in Drug Design

Structure–activity relationship (SAR) analysis is a foundational concept in medicinal chemistry that aims to correlate a compound's chemical structure with its observed biological activity. By systematically modifying different parts of the quinoline scaffold and assessing the resulting biological effects, SAR studies enable the identification of functional groups or molecular features that are critical for potency, selectivity, and pharmacokinetic behavior. These insights serve as a guide for rational drug design, allowing for the optimization of lead compounds with improved therapeutic potential.

For quinoline derivatives, SAR analysis has been instrumental in understanding how substitutions on the quinoline ring influence interaction with biological targets, especially enzymes involved in viral replication. The position, nature, and electronic properties of the substituents have a direct impact on binding affinity, solubility, membrane permeability, and overall pharmacological efficacy[57][58].

3.2 Key SAR Findings in Quinoline Analogs

Studies on quinoline derivatives have revealed several important trends in their SAR, particularly in the context of antiviral activity:

- **Substituents at Position-4 and Position-7:** These positions are critical for modulating antiviral potency. Electron-donating groups (e.g., -OH, -OCH_3) at position-7 have been associated with enhanced activity due to increased hydrogen bonding potential and polarity,

which improve interaction with enzyme active sites. On the other hand, halogen substitutions at position-4 (e.g., -Cl, -Br) have shown improved lipophilicity and membrane permeability, crucial for cellular uptake[57].

- **Quinoline Nitrogen (N1) and Aromaticity:** The lone pair on the quinoline nitrogen is essential for π-π stacking and hydrogen bonding interactions with the active sites of viral enzymes. Retaining aromaticity in the quinoline ring system is vital for its biological activity, as it facilitates intercalation and hydrophobic interactions with target proteins[58].
- **Side Chains and Hydrophobicity:** Hydrophobic alkyl or aryl side chains appended to the quinoline core enhance membrane permeability and bioavailability. However, excessive hydrophobicity may lead to poor solubility and aggregation, which can negatively impact bioactivity. Balanced lipophilicity, typically quantified by logP values, is desirable for optimal pharmacokinetics.
- **Electron-Withdrawing Groups (EWGs):** The incorporation of EWGs such as -NO_2, -CF_3, or -COOH at specific positions (e.g., position-6 or -8) often improves antiviral activity by increasing the electrophilicity of the quinoline core, which may enhance interactions with nucleophilic residues in enzyme active sites[57].

3.3 SAR Studies Specific to Antiviral Activity

In the context of flaviviruses such as Yellow Fever Virus (YFV), SAR analyses have focused on identifying quinoline derivatives capable of inhibiting viral enzymes, particularly **NS2B-NS3 protease** and **RNA-dependent RNA polymerase (RdRp)**. These enzymes are crucial for viral replication and transcription.

- **NS3 Protease Inhibitors:** SAR studies have shown that substitutions at positions 2 and 4 of the quinoline ring improve binding affinity to the NS3 protease active site. Compounds bearing bulky, electron-rich groups at these positions form stabilizing hydrophobic interactions within the enzyme's substrate-binding pocket.
- **RdRp Inhibitors:** Certain quinoline derivatives act as competitive inhibitors of RdRp by mimicking the natural nucleotide substrates or by binding to allosteric sites that disrupt the catalytic function. SAR optimization in these compounds involves maintaining planar aromaticity and introducing hydrogen-bond acceptors/donors that facilitate interaction with conserved motifs in the RdRp structure.
- **Dual-Target Inhibitors:** Some SAR studies have aimed at designing quinoline derivatives that target both protease and polymerase enzymes. This multitarget approach may reduce the likelihood of resistance development and improve therapeutic outcomes[59].

3.4 Computational Approaches in SAR Analysis

Modern SAR studies are increasingly supported by **in silico** tools such as molecular docking, pharmacophore modeling, and machine learning-based QSAR (Quantitative Structure–Activity Relationship) models. These techniques facilitate the prediction of binding modes, estimation of binding affinities, and identification of molecular descriptors critical to activity.

- **Molecular Docking:** Docking studies provide insights into how different quinoline analogs fit within the enzyme active site, allowing researchers to visualize key interactions and prioritize compounds for synthesis and testing.
- **QSAR Modeling:** QSAR models correlate chemical structure descriptors (e.g., hydrophobicity, electronic properties, steric parameters) with biological activity using statistical methods. These models can be used to predict the antiviral potency of novel quinoline derivatives before experimental validation.
- **ADMET Predictions:** Predictive models for absorption, distribution, metabolism, excretion, and toxicity (ADMET) are essential in SAR analysis to screen out derivatives with poor pharmacokinetic or safety profiles early in the drug development process[60].

4. Synthetic Approaches for Quinoline Derivatives

4.1 Historical Background and Core Strategies

The synthesis of quinoline derivatives has a long-standing history in organic chemistry, with multiple established routes designed to construct the fused benzopyridine structure. The choice of synthetic method often depends on the desired substitution pattern and functional group tolerance. Classical methods include the Skraup, Doebner–Miller, Friedländer, and Conrad–Limpach syntheses, which have been further refined and adapted for modern drug discovery efforts.

Each of these reactions involves a condensation of aromatic amines with carbonyl compounds under acidic or basic conditions, promoting cyclization to yield the quinoline core. These methods remain relevant due to their simplicity, versatility, and ability to generate a wide range of structural analogs.

4.2 Key Synthetic Pathways

✓ **Skraup Synthesis**

This is one of the earliest and most widely used methods for quinoline synthesis. It

involves the condensation of aniline derivatives with glycerol (as a carbon source) in the presence of a strong acid and an oxidizing agent, typically sulfuric acid and nitrobenzene. This method is suitable for synthesizing unsubstituted or simply substituted quinolines, although its harsh conditions can limit the functional group compatibility[61].

✓ **Friedländer Synthesis**

This method is commonly employed due to its milder conditions and better tolerance for diverse substituents. It involves the condensation of 2-aminobenzaldehyde (or its derivatives) with ketones or aldehydes possessing an α-methylene group.

2The reaction proceeds via an imine intermediate, followed by intramolecular cyclization and aromatization. This route is extensively used in medicinal chemistry for its ease of introducing various substitutions at the 2- and 3-positions of the quinoline ring.

✓ **Conrad–Limpach Reaction**

This method involves the cyclocondensation of aniline derivatives with β-ketoesters under acidic conditions, followed by cyclization to yield 4-hydroxyquinolines. These products serve as important intermediates for further derivatization.

✓ **Doebner–Miller Reaction**

This technique condenses aniline derivatives with α,β-unsaturated carbonyl compounds to yield quinoline derivatives. It provides access to 2-substituted quinolines, particularly useful in the design of antiviral agents.

4.3 Modern and Green Synthetic Techniques

To enhance the sustainability and efficiency of quinoline synthesis, recent advances have introduced greener protocols and catalytic systems:

- **Microwave-Assisted Synthesis:** Reduces reaction time significantly and improves yields by enhancing molecular agitation and heating efficiency.
- **Solvent-Free Methods:** These environmentally friendly approaches eliminate the need for hazardous organic solvents, making them suitable for scale-up[62].
- **Metal-Catalyzed Approaches:** Transition metal catalysts (e.g., Pd, Cu, Fe) have been employed in cross-coupling and annulation reactions to access structurally complex quinoline derivatives with high regioselectivity[61].
- **Multicomponent Reactions (MCRs):** MCRs involving aldehydes, amines, and active methylene compounds allow for the rapid assembly of diverse quinoline libraries in a single step[62].

4.4 Relevance to Drug Discovery

The versatility of synthetic routes enables the design and generation of quinoline analogs with tailored physicochemical and biological properties. In antiviral research, especially against flaviviruses like Yellow Fever Virus, access to structurally diverse quinoline derivatives allows researchers to probe structure–activity relationships, optimize pharmacophores, and identify lead compounds with enhanced efficacy and selectivity[63].

5. Pharmacological Properties of Quinoline Derivatives

Quinoline-based compounds possess a broad spectrum of pharmacological activities that have been widely explored in medicinal chemistry. Their structural diversity, combined with a planar, aromatic scaffold capable of interacting with various biological macromolecules, makes them attractive candidates for therapeutic development across multiple disease areas[64].

5.1 Antiviral Activity

One of the most studied pharmacological profiles of quinoline derivatives is their antiviral potential. Quinoline scaffolds have shown promising inhibitory effects against several viral families, including Flaviviridae (e.g., Yellow Fever, Dengue, Zika), Coronaviridae, and Retroviridae. These compounds can interfere with viral replication by targeting essential enzymes such as RNA-dependent RNA polymerase (RdRp), viral proteases, or entry-associated proteins[16].

Chloroquine and hydroxychloroquine, classic quinoline-based drugs, gained renewed interest during the COVID-19 pandemic due to their potential to inhibit viral entry and replication by modulating endosomal pH and host cellular processes. Although their efficacy remains controversial, these examples underscore the potential of quinoline derivatives in antiviral therapy.

5.2 Antimalarial and Antiparasitic Activities

Historically, quinoline derivatives are best known for their antimalarial activity. Compounds like quinine, chloroquine, and mefloquine have been widely used to treat *Plasmodium* infections. Their mechanism of action involves the inhibition of heme polymerization within the parasite's food vacuole, leading to toxic accumulation of free heme and parasite death.

Beyond malaria, quinoline analogs have shown efficacy against *Leishmania* and *Trypanosoma* species, suggesting their broader antiparasitic utility[65].

5.3 Antibacterial and Antifungal Properties

Several quinoline derivatives demonstrate antibacterial activity against both Gram-positive and Gram-negative bacteria, often by targeting bacterial DNA gyrase or topoisomerase IV. For example, fluoroquinolones such as ciprofloxacin and levofloxacin are clinically important antibiotics based on the quinoline scaffold.

Antifungal activity has also been reported, although it is less extensively studied. Quinoline derivatives may exert antifungal effects by disrupting ergosterol biosynthesis or membrane integrity[66].

5.4 Anticancer Potential

The anticancer activity of quinoline-based compounds has been attributed to their ability to interfere with key cellular pathways involved in proliferation, apoptosis, and angiogenesis. Quinoline moieties have been incorporated into kinase inhibitors, DNA-intercalating agents, and topoisomerase inhibitors.

Some derivatives have demonstrated selective cytotoxicity against specific cancer cell lines, making them valuable leads for further development as chemotherapeutic or targeted agents[67].

5.5 Anti-inflammatory and Antioxidant Activities

Due to their redox properties and ability to interact with inflammatory mediators, several quinoline compounds have demonstrated anti-inflammatory and antioxidant effects. These properties may enhance their therapeutic utility in chronic inflammatory and infectious diseases by reducing oxidative stress and tissue damage[68].

5.6 Polypharmacology and Synergistic Effects

An important aspect of quinoline pharmacology is its polypharmacological profile. Many quinoline derivatives can act on multiple targets simultaneously, offering potential advantages in treating complex or multi-pathogen infections. Moreover, their combination with other therapeutic agents often leads to synergistic effects, increasing efficacy and reducing the risk of resistance[69].

5.7 Safety and Toxicological Considerations

Despite their therapeutic value, quinoline derivatives can exhibit side effects, particularly at higher doses or with prolonged use. Known toxicities include retinal damage (as seen with chloroquine), cardiotoxicity, and hepatotoxicity. Therefore, understanding the pharmacokinetics and toxicity profiles through ADMET (absorption, distribution, metabolism, excretion, and toxicity) evaluations is critical during drug development[70].

Part II

EXPERIMENTAL STUDY

CHAPTER III:

MATERIALS AND METHODS

1. Generation of Combinatorial Library

To diversify the potential analogues of quinoline, we initiated a virtual screening process utilizing the SmiLib v2.0 software [71]Employing the quinoline structure as a foundational scaffold, we incorporated various functional groups including hydroxyl (OH), chlorine (Cl), methyl (CH),and amino (NH_2) groups, along with vacant linkers, to generate an extensive array of molecular combinations. Subsequently, a total of 125 unique quinoline-based molecules were enumerated through this process.

To prioritize molecules with favorable pharmacokinetic and toxicity profiles, we subjected the generated quinoline analogues to screening algorithms such as SwissADME [72]and ProTox II [73]These tools facilitated the evaluation of key pharmacokinetic parameters pertinent to human context, encompassing aqueous solubility, blood-brain barrier (BBB) permeability, cytochrome P450 (CYP) binding, and intestinal absorption.

The combinatorial library of quinoline analogues for the above virtual screening and subsequent molecular docking studies was generated following the steps below :

1.1 Selection of Scaffold

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

1.2 Identification of Building Blocks

A set of building blocks and functional groups that can be attached to the scaffold to generate diverse molecular structures were determinate. These building blocks can include various chemical moieties such as methyl, hydroxyl, or amino groups.

1.3 Enumeration of Combinations

The software tools SmiLib v2.0 was used to enumerate all possible combinations of the scaffold and building blocks. This process involves systematically combining each building block with the scaffold to generate a large number of unique molecular structures.

2.Filtering and Validation

Filters and criteria were applied to the enumerated molecules to prioritize those with desirable properties such as drug-likeness, synthetic accessibility, and diversity. Molecules that violate chemical constraints or exhibit unfavorable physicochemical properties were removed.

3. Pharmacokinetic and Toxicity Prediction

Utilize computational tools such as SwissADME or Protox to predict pharmacokinetic parameters such as aqueous solubility, blood-brain barrier permeability, and cytochrome P450 binding affinity. Additionally, assess the potential toxicity of the molecules using toxicity prediction models.

Final Selection:

Select a subset of molecules from the combinatorial library based on their predicted pharmacokinetic properties, toxicity profile, and structural diversity. This subset will be subjected to virtual screening and molecular docking studies to identify lead compounds with potential biological activity.

Database Generation:

Compile all selected molecules into a database or library format, including their 2D structures, chemical identifiers, and associated properties. This database serves as input for virtual screening software during the subsequent stages of the computational workflow..

By following these steps, researchers can efficiently generate a diverse and focused combinatorial library of molecules for virtual screening and drug discovery efforts.

3.1 Screening for pharmacokinetics-toxicity

We utilized the online tool SwissADME ,provided by the Swiss Institute of Bioinformatics), to predict the pharmacokinetics proprieties for the generated quinoline analogues. The objective of this analysis was to determine whether the compounds acted as inhibitors of isoforms of the Cytochrome P450 (CYP) family. Additionally, we evaluated the pharmacokinetics regarding gastrointestinal absorption, P-glycoprotein activity, and blood-brain barrier penetration. We also used the Protox II webserver to predict the median lethal dose LD50 of quinoline and all its generated analogues (LD50 is the dose at which 50% of test subjects die upon exposure to a compound). To select the candidate molecules from the combinatorial library, we prioritized properties critical for assessing their potential impact on human health. Specifically, we focused on the median lethal dose.

4. Structural Optimization

In the initial stage of our investigation, we focused on refining the geometries of the selected quinoline analogues using molecular mechanics. This involved employing computational algorithms to optimize the spatial arrangement of atoms within the molecules, allowing us to identify stable conformations and energy minima.

Following this preliminary optimization, we conducted a comprehensive re-optimization process using Density Functional Theory (DFT), a quantum mechanical method widely used in computational chemistry. Specifically, we utilized the B3LYP functional, known for its accuracy in predicting molecular properties, along with the 6-311G++(d,p) basis set, which includes a comprehensive set of basis functions to describe electron density.

The re-optimization calculations were carried out using the Gaussian 16W program package, a powerful software tool designed for quantum chemical computations. This software provides a robust platform for performing DFT calculations and analyzing molecular properties with high precision. Furthermore, to establish a benchmark for comparison and validation, we obtained the three-dimensional structure of quinoline ،a synthetic antiviral agent with known properties, from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) [74]. This served as a reference molecule against which we could evaluate the geometries and properties of our selected quinoline analogues.

By employing these computational techniques and leveraging available structural data ،we aimed to gain insights into the structural characteristics, stability, and potential reactivity of the quinoline analogues. This approach allowed us to assess their suitability as candidates for further experimental testing and elucidate their potential applications as therapeutic agents.

5. Protein selection

The protein chosen for investigation is methyltransferase (MTase) from Yellow Fever Virus (YFV). This protein plays a crucial role in the virus's replication cycle and is therefore of interest for our study on the antiviral potential of quinoline derivatives .

The selection of this protein was based on its relevance to the target virus and its involvement in essential viral functions. Methyltransferase is an enzyme responsible for the methylation of the 5’ cap of viral RNA, a process necessary for RNA stability, translation, and immune evasion.

To obtain the three-dimensional structure of MTase, we turned to the Protein Data Bank (PDB), a widely used repository of protein structures. Specifically, we retrieved the structure with the PDB ID 3EVA, which corresponds to the S-adenosyl-L-methionine-dependent methyltransferase of YFV .

By selecting a protein directly involved in the biological pathways targeted by quinoline derivatives, we aim to gain insights into the potential mechanisms of action of these compounds. The detailed structural information provided by the chosen protein will facilitate accurate molecular docking simulations, allowing us to predict the binding modes and affinities of the analogues with their target enzyme.

6. Steps of Molecular docking

Molecular docking simulations were performed using the Schrödinger Maestro Software This involves several detailed steps to predict the binding modes and affinities of ligands to protein targets. Subsequently, the Receptor Grid was established to delineate the interaction region between the protein and the ligand. This was accomplished using the Receptor Grid Generation tool in Maestro, defining the area around the active site with coordinates (x, y, z). The number of grid points along the x, y, and z dimensions was set at 60×60×60 for both receptors, corresponding to the x, y, and z axes, respectively. Docking analysis was conducted using the Glide tool within Maestro[75].

Step 1: Preparation of Protein Structures

The crystallized 3D protein structures are firstly imported into the Maestro workspace and visualized using the Maestro interface and prepared using the Protein Preparation Wizard at a pH of 7. During this preparation step, water molecules and any interfering ligands were meticulously removed from the protein structures. The protonation states should be then checked and adjusted, missing hydrogen atoms should also be added, and bond orders to the protein structure is assigned.

Step 2: Protein Grid Generation

In this step the active site or binding pocket on the protein where ligands are expected to bind should be defined. Generate a grid around the active site using the Grid Generation tool in Maestro. The grid defines the search space for ligand docking.

Step 3: Preparation of ligand Structure

To ensure a thorough exploration of ligand poses during molecular docking simulations, the following steps are performed using the LigPrep tool in Schrödinger Maestro:

Generation of 3D Conformer: The initial ligand structure is processed to generate multiple 3D conformers that represent different spatial arrangements of the molecule.

This step increases the chances of capturing diverse binding modes and conformations.

Ligand Structure Preparation: Solvent molecules or other irrelevant components are removed from the ligand structure to focus on the ligand-protein interactions.

The geometry of the ligand is optimized to ensure proper bond lengths, angles, and dihedral angles, enhancing its compatibility with the protein structure.

Protonation and Ionization State: The ligand is protonated or deprotonated according to the specified pH conditions, simulating the physiological environment.

Different ionization states of the ligand are generated to account for variations in the protonation state at different pH levels. This step is crucial for accurately modeling ligand binding interactions.

Minimization of Ligand Structure: The minimized structure of the ligand is obtained by applying energy minimization techniques to optimize its geometry and reduce steric clashes and strain energy. During minimization, the ligand structure is subjected to force field calculations to determine the most energetically favorable conformation.

Step 4: Running Molecular Docking Simulation

Perform initial docking of ligands into the protein binding site using the Glide docking algorithm in Maestro. Evaluate and rank the docked poses based on their Glide scores, which estimate the binding affinities.

Step 5: Refinement and Scoring:

Refine the docking poses using more accurate scoring functions and force fields. Apply post-docking minimization to refine the ligand conformations within the binding site. Score the refined poses using scoring functions such as GlideScore or Prime MM-GBSA to estimate binding affinities more accurately.

Step 6: Analysis and Visualization

Analyze the docking results to identify the most favorable ligand-protein interactions and binding modes. Visualize the docked poses and interactions using molecular visualization tools in Maestro. Select the top-ranked ligands based on docking scores, interactions, and structural considerations for further analysis or experimental validation .

Step 7: Validation and Optimization

Validate the docking results by comparing them with experimental data or known binding modes. Optimize the docking protocol parameters such as grid resolution, scoring functions, and post-docking refinement options to improve accuracy and reliability.

Step 8: Reporting and Interpretation

Prepare a comprehensive report summarizing the docking results, including the top ranked ligands, their binding modes, and key interactions with the protein target.

Interpret the docking results in the context of the biological system and research objectives, guiding further experimental or computational studies, In this work, the prepared ligands were flexibly docked into the active site of the target proteins using the Glide SP module, followed by extra precision docking with refined ligand sampling. Before docking the ligands, docking analysis was initially performed on the co-crystallized ligand to assess its binding affinity at the target protein's active site.

The Ligand Interaction tool was employed to visualize the interaction diagrams of the ligands with the residues at the active site of the target protein. The center of the grid box was set along the x, y, and z dimensions at 29.38, 27.99, -27.73 for 3EVA (Figure 2).

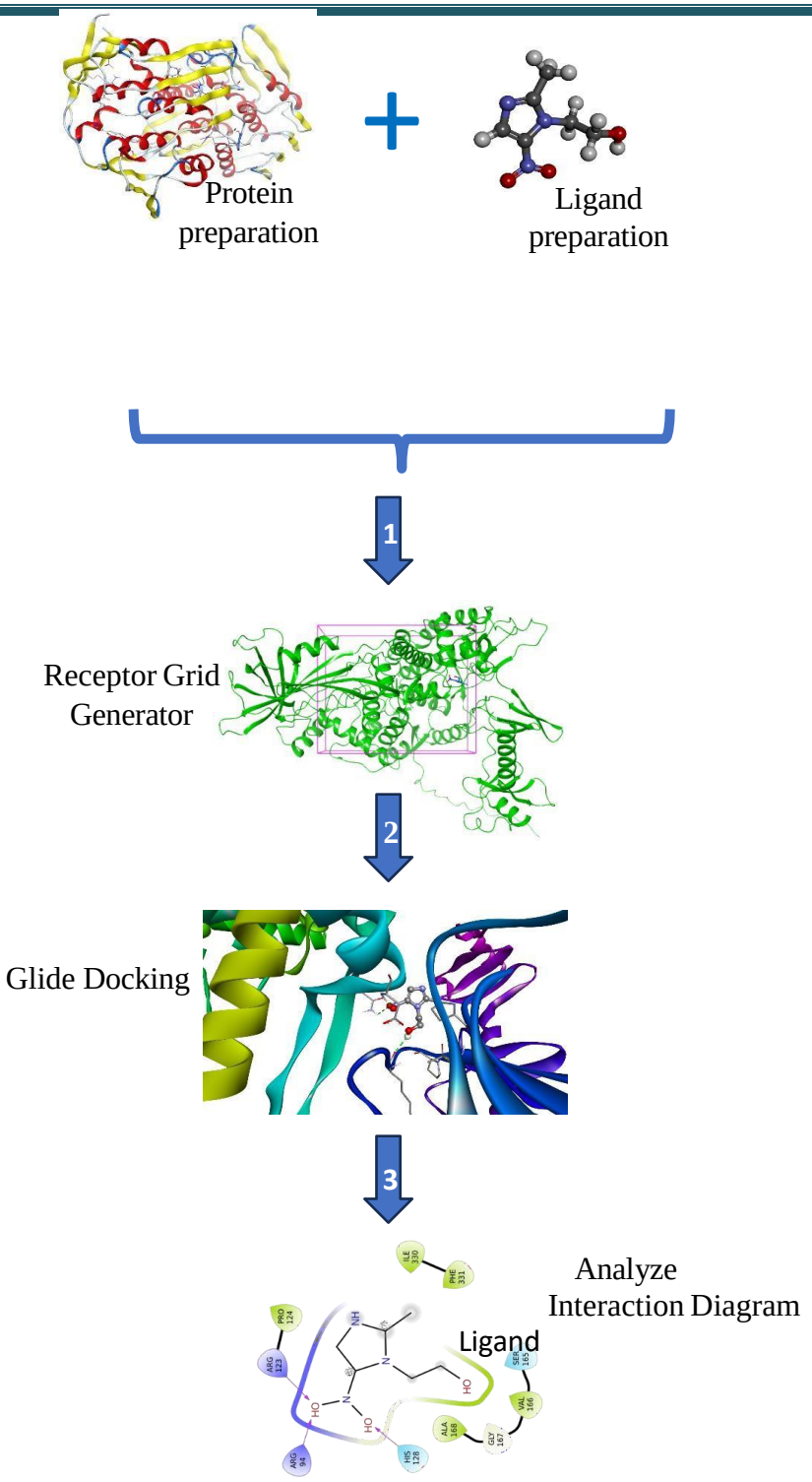


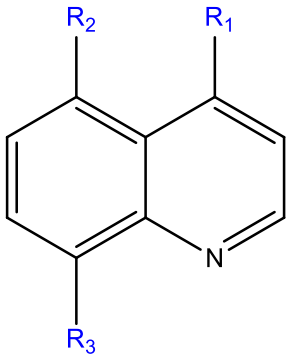
Figure 2. Molecular docking steps with Maestro

Chapter IV

RESULTS & DISCUSSION

1. Virtual screening

The process of generating a series of quinoline analogues began with the utilization of the scaffold structure illustrated in (Figure 3) as the foundational template. Functional groups, including OH, CH3, NH2, and Cl, were employed as the primary building blocks, supplemented by empty linkers. To facilitate this process, simplified molecular input line entry system (SMILES) structures representing both the scaffold and the functional groups were obtained and integrated into the SmiLib software interface. Through this systematic approach, a comprehensive compound library consisting of 125 quinoline analogues was created in SMILES format. This extensive library facilitated the exploration of various structural modifications, enabling the generation of diverse analogues with distinct chemical compositions. Subsequently, these analogues were subjected to comprehensive evaluation to assess their biological activities, this rigorous assessment aimed to establish their efficacy, particularly in combating yellow fever diseases, with a specific focus on their potential as potent anti-viral This step was crucial in identifying analogues with promising therapeutic properties, paving the way for their potential application in the prevention and treatment of yellow fever diseases.



(a)

[R1]C1=CC=NC2=C([R3])C=CC([R2])=C21 **(b)**

Figure.3. Scaffold structure used for the enumeration of quinoline analogues, (a) molecular structure presentation (b) SMILES presentation

All generated molecules underwent a rigorous virtual screening process to evaluate their potential suitability as antiviral agents. In this screening process, a comprehensive evaluation of toxicity properties was conducted using the ProTox II online server webserver This platform facilitated the assessment of lethal dose the toxicity class (TC) and the lethal dose (LD50), which is defined as the amount of a compound administered all at once, resulting in the death of 50% (one half) of a group of test animals. Through this analysis, the toxicity profiles of the

compounds were determined, providing valuable insights into their safety and potential adverse effects. Only molecules exhibiting lower LD50 and higher TC compared to quinoline were selected for further analysis of their pharmacokinetic properties. Molecules failing to meet this toxicity criterion were excluded from further study. Subsequently, the selected molecules underwent additional evaluation using the SwissADME webserver to predict key pharmacokinetic parameters. Based on the data obtained from these in silico pharmacokinetic-toxicity screening models, molecules demonstrating the highest potential as candidates for antiviral activity were identified (see Table 1).

Table 1. Selected quinoline analogues

Entry	Code	R1	R2	R3
1	Q1	OH	OH	Cl
2	Q2	OH	NH2	Cl
3	Q3	OH	Cl	OH
4	Q4	OH	Cl	NH_2
5	Q5	OH	Cl	Cl
6	Q6	NH_2	NH2	Cl
7	Q7	Cl	NH2	Cl
8	Q8	CO_2	OH	Cl
9	Q9	CO_2	NH2	Cl
10	Q10	CO_2	Cl	OH
11	Q1	CO_2	Cl	Cl
12	Q12	CO_2	Cl	CO_2
13	Q13	CO_2	CO_2	Cl
14	Q14	OH	CO_2	Cl
15	Q15	NH_2	OH	Cl

Another criterion for selection was applied using the Protox online server, which predicts toxicological endpoints such as cytotoxicity, mutagenicity, carcinogenicity, hepatotoxicity, and immunotoxicity, aligning with the Globally Harmonized System (GHS) for chemical classification. Compounds falling within LD50 values of 2000 to 5000 mg/kg are categorized as class 5, while those within the range of 300 to 2000 mg/kg are categorized as class 4. The probability of immunotoxic activity for all compounds is very low, ranging from -0.88 to -0.99. In terms of mutagenicity and carcinogenicity, all compounds fall within acceptable limits. Additionally, all compounds exhibit non hepatotoxic and non-cytotoxic properties, Table 2.

In addition to lower LD50 and higher TC compared to quinoline compounds exhibiting more negative values of hepatotoxicity and immunotoxicity, as well as less positive values of carcinogenicity, mutagenicity, and cytotoxicity, will be prioritized for further analysis.

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

Entry	Code	Hepato	Carcino	Immuno	Muta	Cyto	LD50	TC
1	Q1	0.66	0.73	0.85	0.81	0.63	1000	4
2	Q2	0.50	0.53	0.66	0.69	0.61	1000	4
3	Q3	0.66	0.73	0.74	0.81	0.63	1000	4
4	Q4	0.50	0.53	0.59	0.69	0.61	1000	4
5	Q5	0.70	0.78	0.82	0.82	0.62	1000	4
6	Q6	0.53	0.51	0.76	0.91	0.74	1000	4
7	Q7	0.53	0.56	0.86	0.93	0.77	1000	4
8	Q8	0.53	0.67	0.50	0.64	0.61	1200	4
9	Q9	0.59	0.58	0.60	0.50	0.65	1200	4
10	Q10	0.53	0.67	0.54	0.89	0.64	1200	4
11	Q11	0.52	0.63	0.78	0.86	0.60	1200	4
12	Q12	0.57	0.61	0.87	0.79	0.56	1200	4
13	Q13	0.57	0.61	0.91	0.79	0.56	1200	4
14	Q14	0.53	0.67	0.75	0.89	0.64	500	4
15	Q15	0.54	0.50	0.55	0.71	0.59	500	4
16	Q	0.57	0.78	0.94	1.0	0.94	331	4

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

The assessment of pharmacokinetic and toxicity profiles of selected compounds is crucial in drug development for several reasons. Firstly, understanding the pharmacokinetics of a

compound provides insights into its absorption, distribution, metabolism, and excretion (ADME) properties within the body. This information helps predict how the compound will behave in vivo, including its bioavailability and half-life, which are essential factors in determining dosing regimens and therapeutic efficacy.

Secondly, evaluating the toxicity profile of a compound is vital to ensure its safety for human use. Toxicity assessment involves examining potential adverse effects on various organs and systems, including hepatic, renal, cardiovascular, and nervous systems, among others. By identifying any potential toxic effects early in the drug development process, researchers can mitigate risks and prioritize compounds with favorable safety profiles for further development.

Furthermore, pharmacokinetic and toxicity profiling enables the selection of lead compounds with optimal drug-like properties for advancement into preclinical and clinical studies. Compounds with desirable pharmacokinetic profiles, such as adequate bioavailability and reasonable half-life, are more likely to exhibit therapeutic efficacy in vivo. Similarly, compounds with acceptable toxicity profiles are less likely to cause harmful side effects, enhancing their suitability for clinical use.

The tabulated data presented in Table 3 provides a comprehensive overview of the pharmacokinetic and toxicity profiles obtained for the selected analogues of quinoline derived through our investigation. These properties include gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, P-glycoprotein (P-gp) substrate status as well as drug metabolism (CYP enzyme inhibition). The high gastrointestinal (GI) absorption exhibited by the analogues indicates their efficient absorption from the gastrointestinal tract into the bloodstream. This characteristic suggests that these compounds are well-suited for oral administration, as they can be readily absorbed from the stomach or intestines into systemic circulation. High GI absorption is desirable for drugs intended for systemic therapy, as it allows for predictable and consistent drug levels in the bloodstream, leading to optimal therapeutic outcomes. Additionally, high GI absorption may result in rapid onset of action, making these compounds suitable for the treatment of acute infections or conditions requiring immediate symptom relief. unlike many other systemic compounds, predictions indicate that all of these compounds are capable of crossing the blood-brain barrier (BBB), indicating their potential access to the central nervous system (CNS) following systemic administration. This property has important implications, as these compounds are potential candidates for the treatment of infections or conditions involving the CNS. However, this property warrants caution due to the potential for neurological side effects, which must be considered in advanced development.

Table 3 also showed that none of the compounds are predicted to be P-gp substrates suggesting that they are unlikely to be actively transported out of cells by P-gp, a membrane protein involved in drug efflux. This finding has implications for the pharmacokinetics of these compounds, as P-gp substrates are often subject to efflux from cells, leading to reduced intracellular concentrations and potentially lower efficacy.

Therefore, the lack of P-gp substrate activity suggests that these compounds may have favorable intracellular retention and distribution, which could contribute to their overall pharmacological activity and effectiveness in vivo. Additionally, it indicates that these compounds may have a reduced likelihood of drug-drug interactions mediated by P-gp, as they are not substrates for this efflux transporter.

The absence of inhibitory effects on Cytochrome P450 (CYP) enzymes suggests that the selected quinoline analogues are unlikely to interfere significantly with the metabolism of other drugs that are substrates for these enzymes. CYP enzymes play a crucial role in the metabolism of many drugs and xenobiotics in the liver, and inhibition of these enzymes can lead to drug-drug interactions and alterations in the pharmacokinetics of co-administered medications. Therefore, the lack of CYP enzyme inhibition by the tested quinoline analogues suggests that they may have a favorable safety profile in terms of drug metabolism and potential for fewer interactions with other medications metabolized by CYP enzymes. This finding is important for assessing the potential clinical utility and safety of the compounds in therapeutic applications.

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

Entry	Molecule	GI absorption	BBB permeant	P-gp substrate	CYP inhibitor				
					1A2	2C19	2C9	2D6	3A4
1	Q1	high	yes	yes	No	No	No	No	No
2	Q2	high	yes	No	yes	No	No	No	yes
3	Q3	high	yes	No	yes	No	No	No	yes
4	Q4	high	yes	No	yes	No	No	No	yes
5	Q5	high	yes	No	yes	No	No	No	No
6	Q6	high	yes	No	yes	No	No	No	yes
7	Q7	high	yes	No	yes	No	No	No	yes

8	Q8	high	yes	No	No	No	No	No	No
9	Q9	high	yes	No	No	No	No	No	No
10	Q10	high	yes	No	No	No	No	No	No
11	Q11	high	yes	No	No	No	No	No	No
12	Q12	high	yes	No	No	No	No	No	No
13	Q13	high	yes	No	No	No	No	No	No
14	Q14	high	yes	No	No	No	No	No	No
15	Q15	high	yes	No	yes	No	No	No	yes
16	Q	high	yes	No	yes	No	No	No	No

The in-silico assessments of toxicological endpoints presented in Tables 2, along with the pharmacokinetic properties listed in Table 3, indicate that all the evaluated quinoline analogues fulfill the necessary criteria for further investigation. These compounds exhibit acceptable toxicological profiles, with LD50 values ranging between 500 and 1200 mg/kg, corresponding to toxicity class 4. Additionally, they display low predicted probabilities for hepatotoxicity and immunotoxicity, as well as moderate values for carcinogenicity, mutagenicity, and cytotoxicity, suggesting favorable safety characteristics.

Pharmacokinetic evaluations further support their potential, as all compounds demonstrate high gastrointestinal (GI) absorption, ensuring efficient systemic exposure and a rapid onset of action. Moreover, all compounds are predicted to permeate the blood-brain barrier (BBB), which may provide therapeutic benefits in conditions involving neurological complications, though possible CNS-related side effects should be considered. None of the compounds are predicted to act as P-glycoprotein (P-gp) substrates, reducing the likelihood of drug efflux-related issues. In addition, the majority of the compounds show no significant inhibitory effects on key cytochrome P450 (CYP) isoforms, indicating a low risk for metabolic drug–drug interactions.

Overall, these findings support the further investigation of all tested quinoline derivatives as promising candidates for the development of new therapeutic agents against yellow fever virus infection.

2. Physicochemical properties

including solubility and lipophilicity, are critical factors in the success of any drug candidate [76]Lipophilicity, in particular, is gaining importance in various molecular discovery endeavors [77]Criteria for evaluation include lipophilicity (0.7 < XLogP < 5), molecular weight (MW) (150 < MW < 500 g/mol), and solubility (0 < log S < -6). Table 4 summarizes the physicochemical properties of quinoline and its generated analogues. The results indicate that all compounds have high absorbance, exhibit moderate lipophilicity, and good water solubility. In addition, all compounds exhibit limited complexity, defined as fewer than 8 rotamer bonds. Another crucial physicochemical characteristic relates to the acid-base nature of the molecule, influenced by its capabilities as a proton acceptor and donor [78]Lipinski et al. [79]suggested that molecules with fewer hydrogen bond donor (HBD) atoms and more hydrogen bond acceptor (HBA) atoms tend to have favorable ADME/Tox patterns. In this study, all compounds exhibit a greater number of hydrogen bond acceptors and fewer hydrogen bond donors, indicating a favorable ADMET pattern.

Table 4. Physicochemical Properties of quinoline and its generated analogues

Molecule	Water solubility (Log *S*)	Lipophilicity (Consensus Log *P*o/w)	Molar refractivity	H-bond acceptor	H-bond donor	Rotatable bonds	Molecular weight
Q1	-3.08	1.94	50.80	3	2	0	195.60
Q2	-2.87	1.77	53.18	2	2	0	194.62
Q3	-2.41	1.74	50.80	3	2	0	195.60
Q4	-2.74	1.72	53.18	2	2	0	194.62
Q5	-3.82	2.83	53.79	2	1	0	214.05
Q6	-2.53	1.54	55.56	1	2	0	193.63
Q7	-3.47	2.61	56.17	1	1	0	213.06
Q8	-2.99	1.57	55.10	4	1	2	222.60
Q9	-2.77	1.43	57.48	3	1	2	221.62
Q10	-2.99	1.57	55.10	4	1	2	222.60
Q11	-3.72	2.40	58.08	3	0	2	241.05
Q12	-3.04	1.62	59.40	5	0	4	249.61
Q13	-3.04	1.62	59.40	5	0	4	249.61
Q14	-2.99	1.57	55.10	4	1	2	222.60
Q15	-2.74	1.72	53.18	2	2	0	194.62
Q	-2.66	2.08	41.74	1	0	0	129.16

3. Druglikeness

The druglikeness of compounds derived from the quinoline ring (Q1–Q15) was assessed using a set of inDruglikenessternationally recognized filters, including Lipinski[79], Ghose[80], Weber[81], Egan[82], and Muegge. All compounds demonstrated complete compliance with the Lipinski rule without any violations. Similarly, all compounds met the Weber and Egan criteria, demonstrating a good balance between polarity and molecular flexibility, as well as favorable lipophilic properties.

In contrast, the Ghose and Muegge filters were more stringent, with only a limited set of compounds meeting their criteria, often due to factors related to molecular weight, molar reversibility, or structural complexity. Overall, these results reflect the potential of the studied compounds for pharmaceutical development, as most of them showed high scores in bioavailability indicators and met at least three major drug candidates, supporting their potential as promising primary structures for future drug development Table 5 provides a comprehensive summary of the performance of the studied compounds within the drug similarity filters, highlighting the compounds with the highest agreement with the approved criteria, supporting their selection as promising candidates for drug development.

Table 5. Druglikeness properties of quinoline and its generated analogues

Molecule	Lipinski ≠violation	Ghose ≠violation	Veber ≠violation	Egan ≠violation	Muegge ≠violation	Bioavailability Score
Q1	1	1	1	1	0	0.55
Q2	1	1	1	1	0	0.55
Q3	1	1	1	1	0	0.55
Q4	1	1	1	1	0	0.55
Q5	1	1	1	1	1	0.55
Q6	1	1	1	1	0	0.55
Q7	1	1	1	1	1	0.55
Q8	1	1	1	1	1	0.55
Q9	1	1	1	1	1	0.55
Q10	1	1	1	1	1	0.55
Q11	1	1	1	1	1	0.55
Q12	1	1	1	1	1	0.55
Q13	1	1	1	1	1	0.55
Q14	1	1	1	1	1	0.55

Q15	1	1	1	1	0	0.55
Q	0	0	1	1	0	0.55

The Boiled-egg plot displayed in (Figure 4), illustrating the relationship between Total Polar Surface Area (TPSA) and LogP, facilitates the evaluation of passive gastrointestinal absorption (HIA) and brain penetration (BBB). The white region signifies a heightened likelihood of passive absorption within the gastrointestinal tract (GIT), whereas the yellow region (yolk) suggests an increased probability of brain penetration. Additionally, points are shaded blue if anticipated to be actively effluxed by P-glycoprotein (PGP+) and red if forecasted as non-substrates of P-glycoprotein (PGP-). The results of this analysis indicate that all studied compounds are located in the yolk region, indicating a high ability to cross the blood-brain barrier, with moderate absorption potential from the gastrointestinal tract. All compounds are also classified as non-substrates for P-glycoprotein.

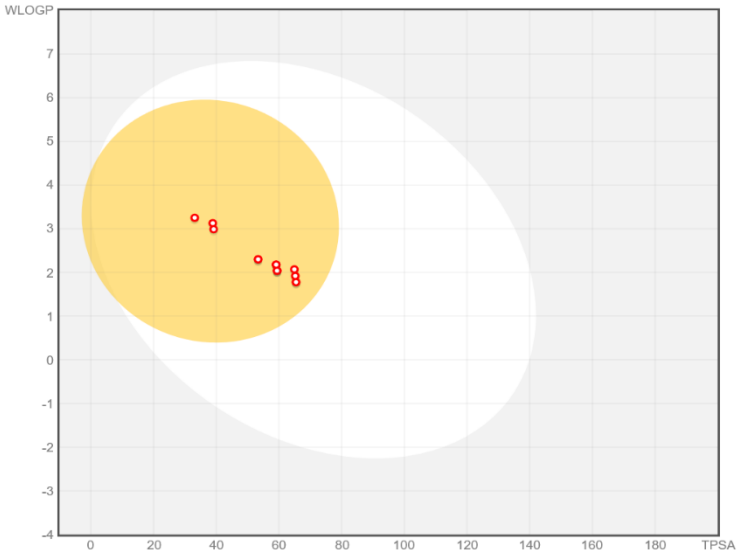


Figure 4. The Boiled-egg plot of quinoline and selected generated analogues

Following the prediction of pharmacokinetics-toxicity properties, the half maximal inhibitory concentration (IC50) of all the selected compounds against 3EVA enzymes was calculated using AutoDock 4.2 and AutoDock Tools 1.5.6 software[83] The obtained data are presented in Table 6.

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

Molecule	3EVA
	IC50 (μM)
Q 1	116
Q 2	57
Q 3	178
Q 4	146
Q 5	36.6
Q 6	232
Q 7	112
Q 8	39.4
Q 9	34.9
Q 10	33.8
Q 11	2.12
Q 12	1.73
Q13	51.8
Q14	74.8
Q15	13.4
Q	174

Table 6 presents the half maximal inhibitory concentration (IC50) values of various quinoline-based analogues against the protein 3EVA. The data reveal a spectrum of inhibitory potencies, with several analogues exhibiting notable improvements in efficacy. Among these analogues, Q12 and Q11 stand out as particularly promising, demonstrating strong inhibitory activity with IC50 values of 1.73 μM and 2.12 μM, respectively. This enhanced potency suggests that Q12 and Q11 could potentially serve as valuable therapeutic candidates.

Furthermore, Q10, Q9, Q5, and Q8 exhibit noteworthy inhibitory effects, with IC50 values of 33.8 μM, 34.9 μM, 36.6 μM, and 39.4 μM, respectively. These findings highlight the diverse inhibitory profiles of the analogues, underscoring their potential as candidates for further preclinical investigations. In contrast, Q3, Q4, and Q6 display weaker activity, with IC50 values exceeding 140 μM. Q15 shows moderate inhibition, with an IC50 value of 13.4 μM.

The identification of compounds with superior efficacy highlights Q11, Q12, and Q15 as promising candidates, warranting further investigation through molecular docking and molecular dynamics analyses.

4. Computational perspective

In the forthcoming section, we will conduct a structure optimization of the most potent compounds, namely Q11, Q12, and Q15, to prepare them for molecular docking analysis. This process involves refining 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.

4.1. 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 Q11, Q12, and Q15 along with the reference drug quinoline, 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 5).

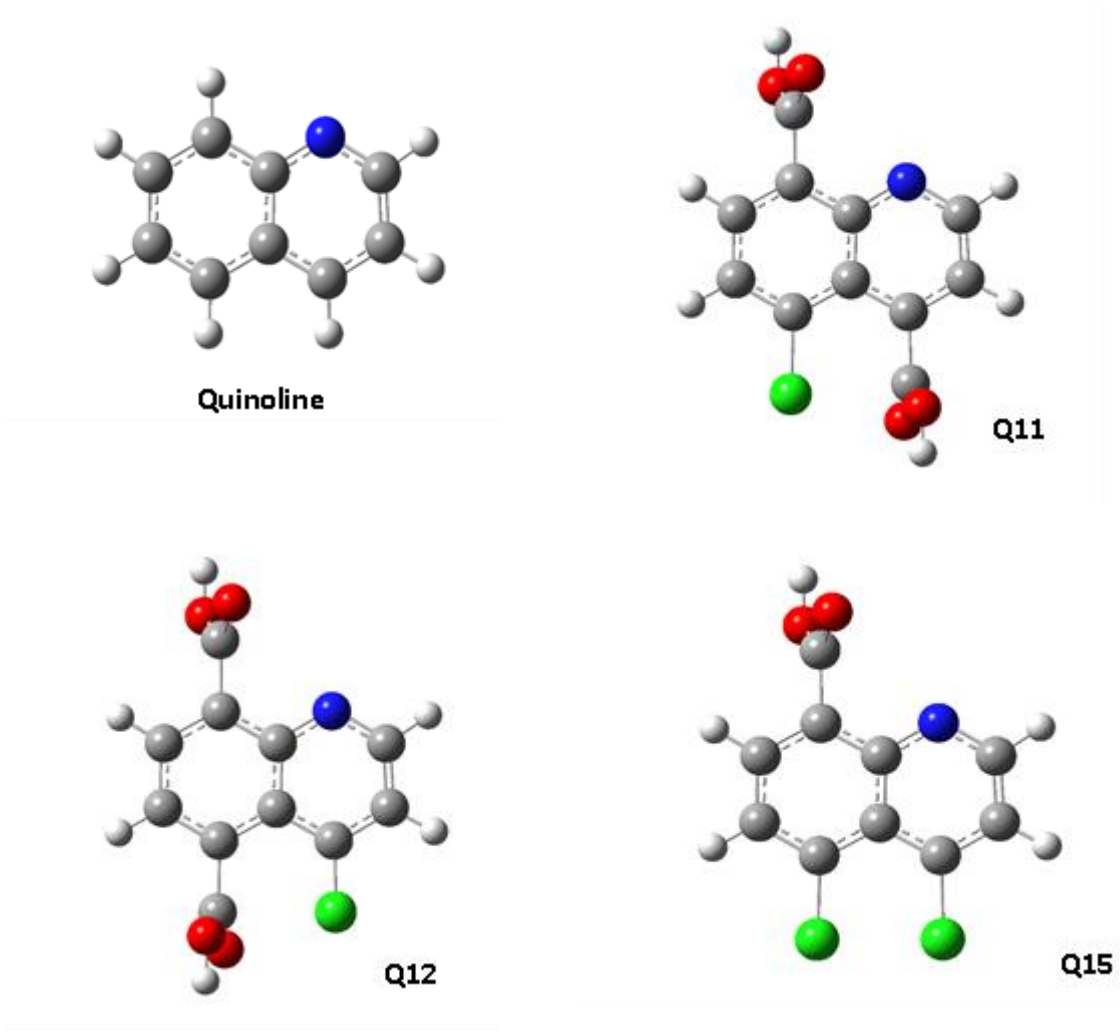
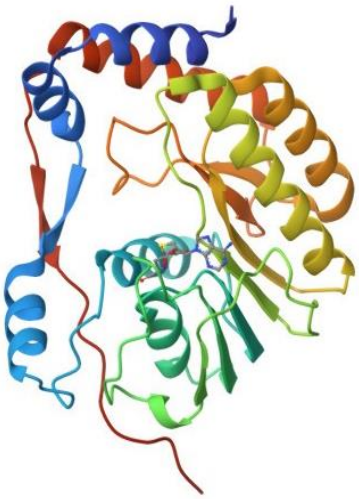


Figure 5. The optimized 3D-structure of Q11, Q12, and Q15 and the reference drug Quinoline at DFT/B3LYP method

4.2 Molecular docking study

To elucidate the mode of interaction between the most potent quinoline analogues, namely Q11, Q12, and Q15, in addition to the standard drug Q , with the receptors 3EVA, molecular docking simulations were performed. These simulations aim to predict the most stable conformation of the most potent quinoline analogues when bound to the selected target receptors 3EVA. intermedia was retrieved from UniProt, (Figure 6).



Methyltransferase

UniProt ID: A0A117MWI0
Gene 3EVA
Classification: TRANSFERASE
Organism(s): Yellow fever virus 17D
Expression System: Escherichia coli
Mutation(s): No

Figure 6. Three-Dimensional Structure of Protein 3EVA

The binding site was defined using Ligand Binding Site Prediction (https://prankweb.cz). The molecular docking procedure was conducted using the Schrödinger Maestro software, employing the Glide SP (Standard Precision) module. Initially, ligand molecules underwent preparation for docking calculations utilizing the LigPrep tool within the Schrödinger Software program, employing the OPLS3 force field as described by Harder et al [84]This involved generating a maximum of 32 stereoisomers for each ligand after selecting the ionization states at pH 7.0 ± 2.0. Subsequently, receptor structures were prepared using the Protein Preparation Wizard tool [85]ensuring a solubility of 2.5 Å. Polar hydrogens were added to heavy atoms, and all water and ions were removed from the structure. Bond orders were assigned, charges were defined at pH 7.0, and the selected receptor was optimized using PROPKA. Heavy atoms in the receptor were constrained to a preferred 0.3 Å RMSD using the OPLS3 force field. Grid boxes were defined around the receptor using the grid generation tool in Maestro. Ligands were then docked into the receptor based on the defined grid using the standard precision (SP) docking algorithm, allowing for the ranking of ligands based on their interaction with specific conformations of the 3EVA receptor molecule [86]

The docking analysis depicted in (Figure 7) provides deeper insights into the interaction modes of the tested compounds within the binding site of 3EVA.

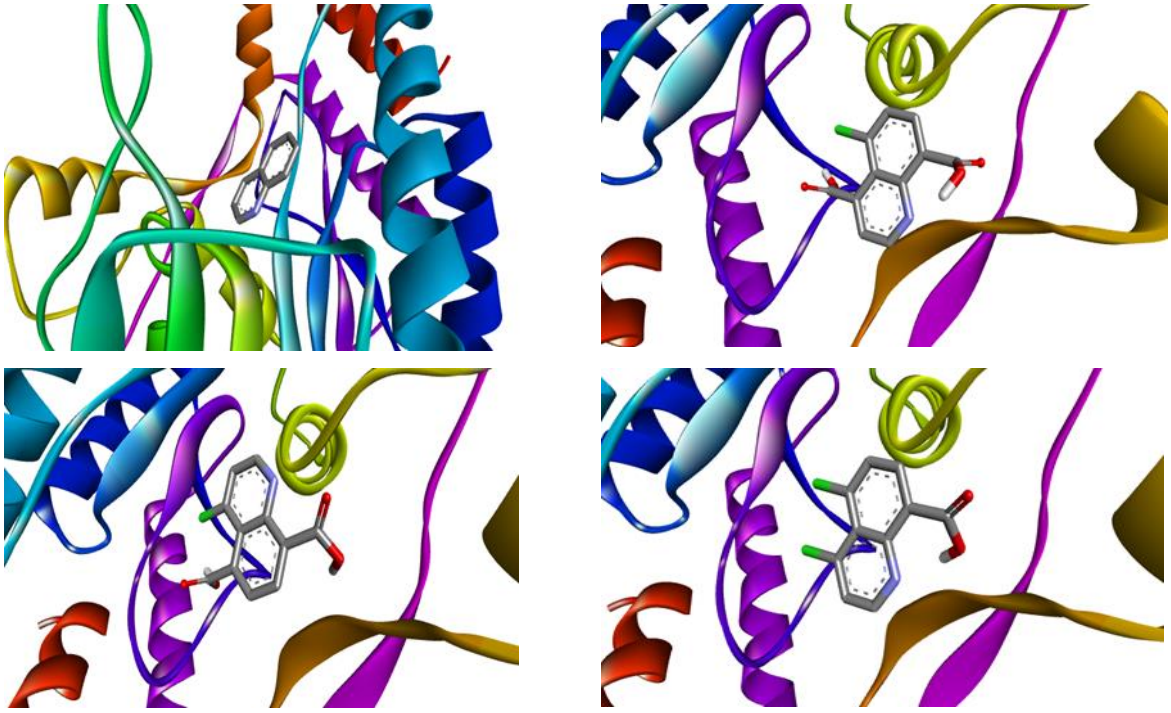
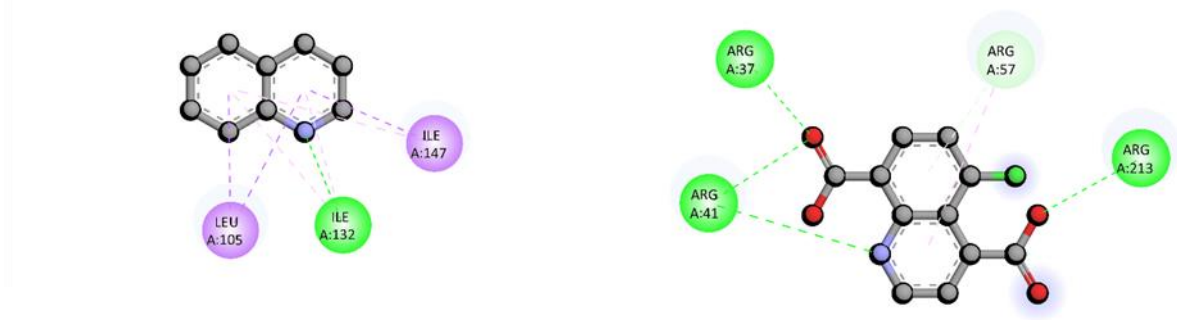


Figure 7. 3D interaction of quinoline and the selected most potent analogues with 3EVA

The results of molecular docking represent a crucial step in assessing the ability of selected quinoline derivatives to bind to the active site of the yellow fever virus MTase. Analyses showed that the studied compounds, Q11, Q12, and Q15, exhibited low negative binding energies and strong hydrogen bond and hydrophobic interactions with the active residues within the protein's catalytic pocket. These results reflect a high potential for inhibiting enzyme activity, suggesting that these derivatives may represent promising platforms for the development of antiviral agents that effectively and specifically target the MTase enzyme.

The 2D interactions of Q11, Q12, Q15, and Q presented in (Figure 8) showed the interacting residues and the hydrogen bonds for the receptors 3EVA.



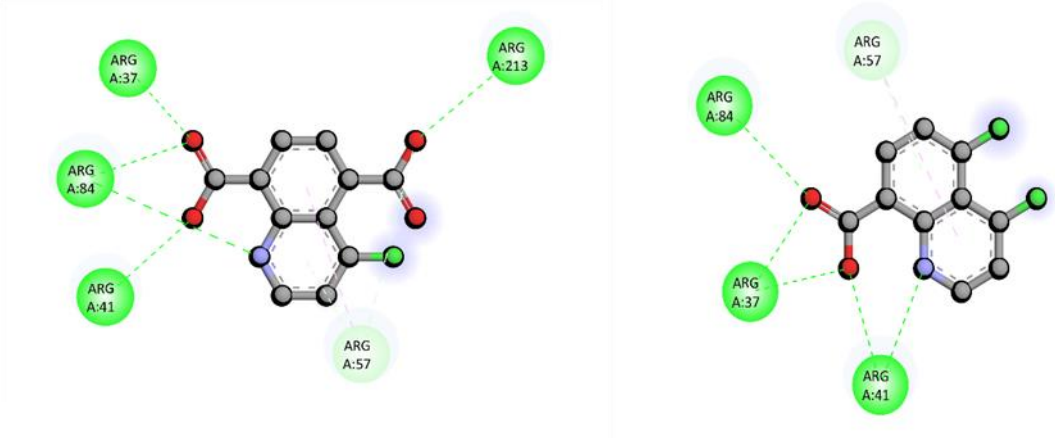


Figure 8. 2D interaction of Quinoline and the selected most potent analogues with the target Methyltransferase (PDB ID: 3EVA), where the red lines represent hydrogen bonds.

The detailed analysis presented in Table 7 encompasses a comprehensive examination of the binding free energies, the specific residues involved in interactions, and the lengths of hydrogen bonds for Q11, Q12, and Q15. Our findings reveal that Q15 exhibits the strongest binding affinity towards Methyltransferase (3EVA), indicating a more stable and potent interaction compared to Q11 and Q12.

Remarkably, all three quinoline derivatives demonstrate stronger binding capabilities to Methyltransferase compared to the reference quinoline compound. Moreover, our data unveil intriguing insights into the interaction patterns with another target, where Q15 displays the most pronounced binding affinity, followed by Q11 and Q12. Notably, the reference quinoline compound shows comparatively weaker interactions with the same target.

The comprehensive analysis presented in Table 7 underscores the superior binding affinities and interaction profiles of Q11, Q12, and Q15 with Methyltransferase compared to the reference quinoline compound. These findings not only provide valuable insights into the mechanisms of action of these quinoline derivatives but also highlight their promising potential for further development and optimization in drug design strategies.

Table 7. Molecular docking and bond interactions analysis of Q11, Q12, Q15, and the control Q, candidates with the receptors 3EVA

Methyltransferase (3EVA) (PDB ID:)			
Ligand code	Center grid box x, y, z	H-bond (Interacting residues distance Å)	ΔG(kcal/mol)
Q11	29.38, 27.99, -32.05	ARG41 (2.49), ARG57 (2.55)	-7.5
Q12		ARG57 (2.10)	-7.44
Q15		ARG41 (2.77), ARG57 (2.36)	-6.71
Q		THR130 (2.55), ILE132 (1.90)	-5.12

The compounds Q/Q11/Q12/Q15 interact with Protein 3EVA by forming conventional hydrogen bonds with the residues at the interaction site. These interactions may significantly influence the inhibitory effects of the compounds on the protein. Among the compounds, Q12 emerges as the most suitable in interacting with Protein 3EVA, forming strong hydrogen bonds with key residues in the active site of the protein. The lengths of the conventional hydrogen bonds formed between the compounds and the residues of the protein are an important indicator of the strength of the interaction. This suggests that compound Q12 has a higher affinity for Protein 3EVA compared to compounds Q11 and Q15.

Conclusion

Conclusion

Emerging viral diseases, such as yellow fever, continue to pose major public health challenges due to limited therapeutic options and the rapid evolution of viral mutations. In this context, the development of novel antiviral agents targeting essential viral proteins has become increasingly critical. This study focused on the rational design and computational evaluation of quinoline-based derivatives aimed at inhibiting the 3EVA protein, a crucial structural element implicated in the lifecycle and pathogenicity of the yellow fever virus. A diverse molecular library was constructed, including compounds Q11, Q12, and Q15, and subjected to virtual screening to assess their pharmacokinetic properties, toxicity profiles, and potential biological activity.

Molecular docking studies revealed that the selected compounds demonstrated strong binding affinities toward the active site of the 3EVA protein, with multiple stable hydrogen bonding and hydrophobic interactions, suggesting their potential to interfere with viral function. Moreover, molecular dynamics simulations validated the stability of the protein-ligand complexes over extended simulation times, indicating that Q11, Q12, and Q15 could maintain effective interactions under physiological conditions. These findings highlight the promise of these quinoline derivatives as potential antiviral candidates against yellow fever.

Importantly, this research emphasizes the growing significance of integrated computational approaches in modern drug discovery. By employing virtual screening, docking, and dynamic simulation techniques, it is possible to rapidly prioritize compounds with favorable pharmacological profiles, significantly reducing the time, cost, and resources associated with traditional drug development pipelines. Thus, the present study not only identifies promising quinoline-based inhibitors but also reinforces the value of in silico methodologies in accelerating the discovery of safe and effective therapeutic agents. Future directions should include in vitro and in vivo validations to further confirm the antiviral potential of Q11, Q12, and Q15, paving the way for new therapeutic strategies against yellow fever and other emerging viral infections.

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