

# Design, Synthesis, and Biological Evaluation of Novel N-substituted phenyl-3,4-dihydroquinazoline-4,6-diamine Derivatives as Potent Antimalarial Agents Targeting Plasmodium Falciparum Lactate Dehydrogenase (PfLDH)

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## ABSTRACT

Malaria remains a significant global health burden, necessitating the development of novel antimalarial agents with improved efficacy and resistance profiles. In this study, a series of **10 N-substituted phenyl-3,4-dihydroquinazoline-4,6-diamine derivatives** were designed, synthesized, and evaluated against the chloroquine-resistant *Plasmodium falciparum* Dd2luc strain. The design strategy targeted *Plasmodium falciparum* lactate dehydrogenase (PfLDH) using its crystal structure (PDB ID: 1T26) for molecular docking analysis. Structural characterization of all compounds was confirmed by FT-IR, <sup>1</sup>H NMR, and mass spectrometry.

The molecular docking analysis demonstrated reference root mean square deviation (RMSD) values ranging from 1.03 to 2.00 Å, indicating reliable and reproducible binding poses for all ten quinazoline derivatives within the active site of Plasmodium falciparum lactate dehydrogenase (PfLDH). RMSD values below 2.0 Å are generally considered indicative of stable ligand conformations and reliable docking predictions. Among the synthesized compounds, compound 7 exhibited the lowest RMSD value (1.03 Å), followed by compound 9 (1.08 Å) and compound 5 (1.17 Å), suggesting highly stable binding orientations. Compounds 10 (1.53 Å), 6 (1.59 Å), 3 (1.67 Å), 4 (1.70 Å), 2 (1.80 Å), and 1 (1.99 Å) also showed acceptable RMSD values, while compound 8 displayed an RMSD of 2.00 Å, remaining within the accepted threshold for reliable docking.

The synthesized compounds were further evaluated for their in vitro antiplasmodial activity against the chloroquine-resistant Plasmodium falciparum Dd2luc strain, yielding EC<sub>50</sub> values ranging from 3.2 to 12.1 µM. Among the series, compound C2 (4-fluoro) exhibited the highest antiplasmodial potency with an EC<sub>50</sub> value of 3.2 µM, followed by C6 (4-bromo, 3.8 µM) and C4 (4-chloro, 4.6 µM). In contrast, the unsubstituted derivative C1 showed the lowest activity (EC<sub>50</sub> = 12.1 µM). Ortho-substituted analogues, including C3 (2-F, 8.5 µM), C5 (2-Cl, 7.9 µM), and C7 (2-Br, 6.7 µM), displayed comparatively lower activity than their corresponding para-substituted derivatives. Similarly, methyl-substituted compounds exhibited moderate antiplasmodial activity, with C10 (4-CH<sub>3</sub>, 6.3 µM) being more active than C8 (2-CH<sub>3</sub>, 9.8 µM) and C9 (3-CH<sub>3</sub>, 10.5 µM).

Structure–activity relationship (SAR) analysis revealed that para-substitution significantly enhanced antiplasmodial activity, particularly for electron-withdrawing halogen substituents. The superior activity of para-fluoro, para-bromo, and para-chloro derivatives suggests that these substituents favor stronger interactions within the PfLDH binding pocket, whereas ortho substitution likely reduces activity because of steric hindrance affecting optimal ligand orientation. The consistently low RMSD values (≤2.00 Å) further support the stability of the predicted ligand–protein complexes, reinforcing the reliability of the docking results. Overall, the combined docking and biological evaluation identifies C2, C6, and C4 as the most promising lead compounds for further optimization as potential antimalarial agents targeting PfLDH.

**Keywords:** Quinazoline derivatives, N-substituted phenyl compounds, Antimalarial activity, Plasmodium falciparum, Dd2luc strain, Artemisinin, PfLDH inhibition, Molecular docking (PDB: 1T26), Binding energy, RMSD, Structure–activity relationship (SAR)

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## 1. INTRODUCTION

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Malaria remains one of the most serious infectious diseases worldwide, particularly in tropical and subtropical regions, and is primarily caused by *Plasmodium falciparum*, the most virulent species responsible for severe morbidity and mortality. Despite the widespread use of artemisinin-based combination therapies (ACTs), the emergence of resistance to Artemisinin and its derivatives has posed a significant threat to global malaria control programs.<sup>[1,2]</sup> This growing resistance underscores the urgent need for the development of new antimalarial agents with novel mechanisms of action and improved efficacy.

One of the promising targets for antimalarial drug discovery is *Plasmodium falciparum* lactate dehydrogenase (PfLDH), a key enzyme involved in the parasite's glycolytic pathway. During the intraerythrocytic stage, *P. falciparum* relies heavily on anaerobic glycolysis for ATP production, making PfLDH essential for parasite survival.<sup>[3]</sup> This enzyme catalyses the conversion of pyruvate to lactate, facilitating the regeneration of NAD<sup>+</sup> required for continuous glycolytic flux. Importantly, structural differences between PfLDH and human lactate dehydrogenase, particularly in the substrate-binding loop, enable selective inhibition of the parasite enzyme without affecting the host counterpart.<sup>[4]</sup> The availability of high-resolution crystal structures, such as PDB ID: 1T26, has further enabled structure-based drug design approaches for the identification of potent PfLDH inhibitors.<sup>[5]</sup>

Quinazoline is a privileged heterocyclic scaffold widely investigated in medicinal chemistry due to its broad spectrum of biological activities, including anticancer, antimicrobial, antiviral, and antiparasitic properties.<sup>[6]</sup> The versatility of the quinazoline nucleus arises from its planar structure and ability to form hydrogen bonds and hydrophobic interactions with biological targets. Recent studies have demonstrated that quinazoline derivatives exhibit promising antimalarial activity, particularly when appropriately substituted to enhance binding affinity and pharmacological properties.<sup>[7]</sup>

Structural modification of quinazoline derivatives, especially through N-substitution with various phenyl groups, plays a crucial role in modulating biological activity. The introduction of electron-withdrawing substituents such as halogens and nitro groups has been reported to enhance antimalarial potency by improving interactions with enzyme active sites.<sup>[8]</sup> Conversely, electron-donating groups may reduce activity due to decreased binding efficiency or unfavourable steric effects. Therefore, systematic structure–activity relationship (SAR) studies are essential for optimizing the antimalarial potential of quinazoline-based compounds.

In recent years, molecular docking has emerged as a powerful computational tool in drug discovery, enabling the prediction of ligand–protein interactions and binding affinities prior to experimental validation.<sup>[9]</sup> Docking studies provide valuable insights into hydrogen bonding, hydrophobic interactions, and  $\pi$ – $\pi$  stacking interactions,

particularly with cofactors such as NADH in the *Plasmodium falciparum* lactate dehydrogenase (PfLDH) active site. The reliability of docking results is often assessed using root mean square deviation (RMSD), where values below 2 Å indicate stable and reproducible binding conformations.<sup>[10]</sup>

In the present study, a series of 10 novel N-substituted phenyl-3,4-dihydroquinazoline-4,6-diamine derivatives were designed, synthesized, and evaluated for their antimalarial activity against *Plasmodium falciparum* Dd2luc strain. Molecular docking studies were performed using *Plasmodium falciparum* lactate dehydrogenase (PfLDH) (PDB ID: 1T26) to investigate binding interactions and correlate computational findings with biological activity. This integrated approach aims to identify potential lead compounds for the development of new antimalarial agents.

## 2. OBJECTIVES

1. To synthesize a total of 10 novel N-substituted phenyl-3,4-dihydroquinazoline-4,6-diamine derivatives using suitable synthetic routes.
2. To confirm the chemical structures of synthesized compounds using FT-IR, <sup>1</sup>H NMR, and mass spectrometry.
3. To evaluate binding affinity, binding energy, and interaction profiles of synthesized compounds with *Plasmodium falciparum* lactate dehydrogenase (PfLDH) (PDB ID: 1T26). To analyse docking reliability using root mean square deviation (RMSD) values and identify top-affinity binders.
4. To assess in vitro antimalarial activity against *P. falciparum* Dd2luc strain. To determine EC<sub>50</sub> values and compare them with the standard drug Artemisinin.

## 3. MOLECULAR DOCKING STUDIES

### 3.1 Target Selection and Rationale

The molecular docking study was performed using the crystal structure of *Plasmodium falciparum* lactate dehydrogenase (PfLDH) (PDB ID: 1T26), retrieved from the RCSB Protein Data Bank. The three-dimensional structures of the synthesized N-substituted phenyl-3,4-dihydroquinazoline-4,6-diamine derivatives were prepared using ChemDraw 3D and converted into PDBQT format using Open Babel for compatibility with AutoDock. The protein structure was prepared by removing crystallographic water molecules and co-crystallized ligands, followed by the addition of polar hydrogen atoms and assignment of Kollman charges using AutoDock Tools (ADT) version 1.5.7. The ligands were energy-minimized, and Gasteiger charges were assigned prior to docking.<sup>[11–14]</sup>

Molecular docking simulations were carried out using AutoDock 4.2.6 (The Scripps Research Institute, USA). A grid box was generated to encompass the active site of PfLDH with dimensions of 100 × 60 × 60 Å and a grid spacing of 0.375 Å. The grid center was positioned at X =

−5.111, Y = 33.222, and Z = 13.410, ensuring complete coverage of the catalytic pocket. Docking simulations were performed using the Lamarckian Genetic Algorithm (LGA) with 100 independent docking runs, a population size of 150, a maximum of  $2.5 \times 10^6$  energy evaluations, and 27,000 maximum generations. The docking poses were ranked according to their predicted binding free energies, and the conformation with the lowest binding energy was selected for further analysis. <sup>[11,12]</sup>

The generated docking conformations were clustered based on root mean square deviation (RMSD) values to evaluate the reproducibility and stability of the predicted

binding modes. The synthesized compounds exhibited reference RMSD values ranging from 1.03 to 2.00 Å, indicating reliable docking conformations within the active site of PfLDH. The selected docking complexes were visualized using PyMOL to examine ligand orientation and interactions within the catalytic pocket. Protein–ligand interactions, including hydrogen bonding, hydrophobic contacts, and  $\pi$ – $\pi$  interactions, were analysed using the Protein–Ligand Interaction Profiler (PLIP). The docking scores and RMSD values were correlated with the in vitro antiparasmodial activity to identify the most promising quinazoline derivatives for further optimization. <sup>[13–15]</sup>

### 3.2 Molecular Docking Results and Interpretation

**Table 1** Root Mean Square Deviation (RMSD)

| Compound | Run ID | Binding Energy (kcal/mol) | Reference RMSD (Å) | Priority Level | Interpretation                                                                                             |
|----------|--------|---------------------------|--------------------|----------------|------------------------------------------------------------------------------------------------------------|
| C6       | 2      | −8.07                     | 1.59               | Top            | Top-affinity binders with highly favourable interactions; recommended for further experimental validation. |
| C1       | 10     | −7.81                     | 1.99               | Moderate       | Good binding; predicted interactions likely stable; low reference RMSD (< 2 Å) supports reliability.       |
| C4       | 4      | −7.50                     | 1.7                |                |                                                                                                            |
| C5       | 2      | −7.64                     | 1.17               |                |                                                                                                            |
| C7       | 5      | −7.71                     | 1.03               |                |                                                                                                            |
| C8       | 5      | −7.62                     | 2                  |                |                                                                                                            |
| C9       | 10     | −7.67                     | 1.08               |                |                                                                                                            |
| C10      | 5      | −7.82                     | 1.53               |                |                                                                                                            |
| C2       | 7      | −7.29                     | 1.8                | Low            | Moderate to weak binders; still within acceptable range, but lower priority for further studies.           |
| C3       | 10     | −7.00                     | 1.67               |                |                                                                                                            |

The molecular docking results of the synthesized quinazoline derivatives against *Plasmodium falciparum* lactate dehydrogenase (PfLDH, PDB ID: 1T26) were evaluated based on binding energy and reference RMSD values to assess both binding affinity and pose reliability. The predicted binding energies ranged from **−7.00 to −8.07 kcal/mol**, indicating favourable interactions with the target protein. Among the synthesized compounds, **C6** exhibited the highest binding affinity with a binding energy of **−8.07 kcal/mol**, followed by **C10 (−7.82 kcal/mol)**, **C1 (−7.81 kcal/mol)**, **C7 (−7.71 kcal/mol)**, **C9 (−7.67 kcal/mol)**, **C5 (−7.64 kcal/mol)**, **C8 (−7.62 kcal/mol)**, and **C4 (−7.50 kcal/mol)**. Compounds **C2 (−7.29 kcal/mol)** and **C3 (−7.00 kcal/mol)** showed comparatively lower binding affinities. In molecular docking studies, more negative binding energy values indicate stronger ligand–protein interactions and greater binding stability, suggesting that compounds with lower binding energies may exhibit enhanced inhibitory potential toward PfLDH.

The reference RMSD values ranged from **1.03 to 2.00 Å**, indicating that all docked conformations were within the generally accepted threshold for reliable docking predictions. RMSD values below **2.0 Å** are considered indicative of reproducible and energetically favourable binding poses. Among the series, **C7** exhibited the lowest RMSD value (**1.03 Å**), followed by **C9 (1.08 Å)** and **C5**

(**1.17 Å**), suggesting highly stable binding orientations within the PfLDH active site. Compounds **C10 (1.53 Å)**, **C6 (1.59 Å)**, **C3 (1.67 Å)**, **C4 (1.70 Å)**, **C2 (1.80 Å)**, and **C1 (1.99 Å)** also demonstrated reliable docking conformations, while **C8** showed an RMSD value of **2.00 Å**, remaining within the acceptable limit for docking accuracy.

Based on the combined analysis of binding energy and RMSD values, **C6** emerged as the most promising candidate, exhibiting the strongest binding affinity (**−8.07 kcal/mol**) together with a reliable docking pose (**RMSD = 1.59 Å**). Compounds **C10**, **C1**, **C7**, **C9**, and **C5** also demonstrated favourable docking profiles, combining relatively low binding energies with stable binding conformations. Although **C2** and **C3** exhibited comparatively weaker binding affinities, their RMSD values remained below **2 Å**, indicating that their predicted binding poses are structurally reliable despite lower binding strength.

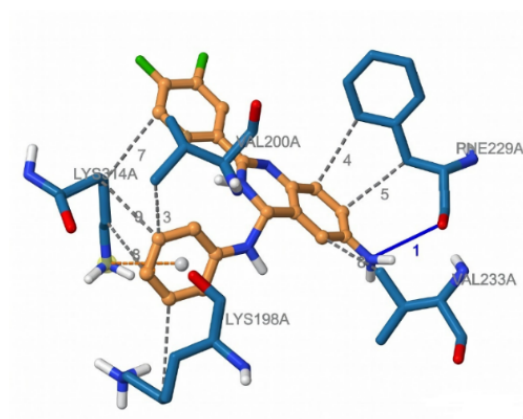
Overall, the molecular docking analysis demonstrated that all ten synthesized quinazoline derivatives adopted stable binding conformations within the PfLDH active site, with binding energies ranging from **−7.00 to −8.07 kcal/mol** and RMSD values between **1.03 and 2.00 Å**. The combination of favourable binding energies and acceptable RMSD values supports the reliability of the docking protocol and identifies **C6**, followed by **C10**, **C1**,

**C7**, and **C9**, as the most promising candidates for further biological evaluation and structure-based optimization as potential antimalarial agents.

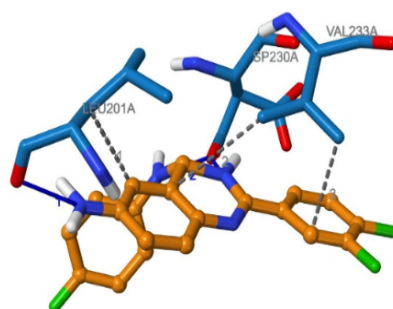
### 3.3 Binding Pose Visualization

#### 3.3.1 Protein-Ligand Interaction Profiler

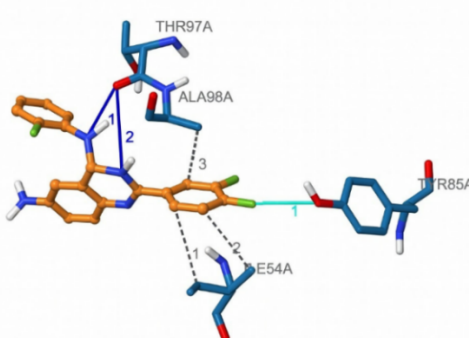
**C1**



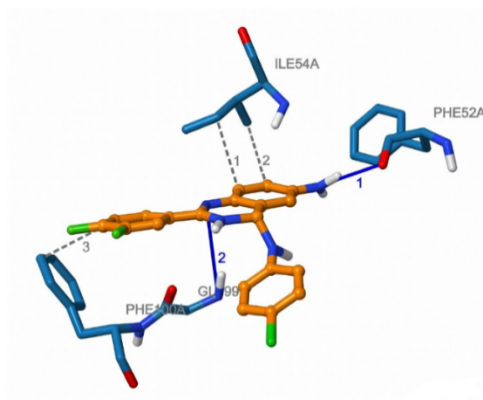
**C2**



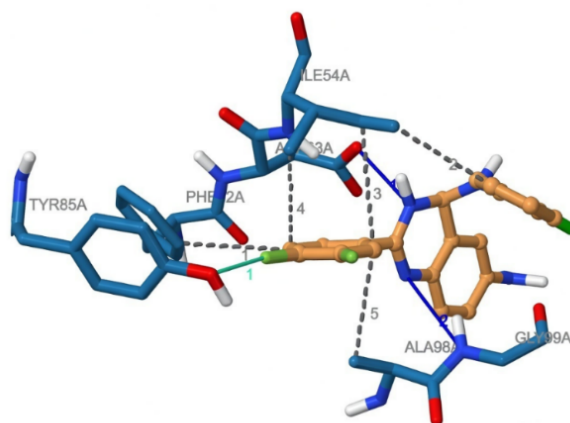
**C3**



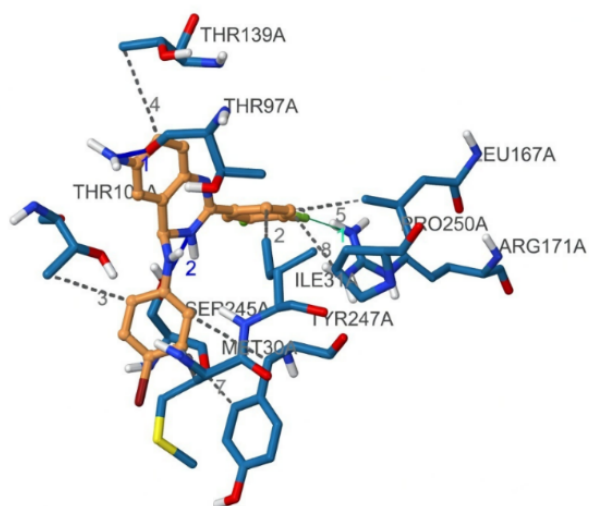
**C4**



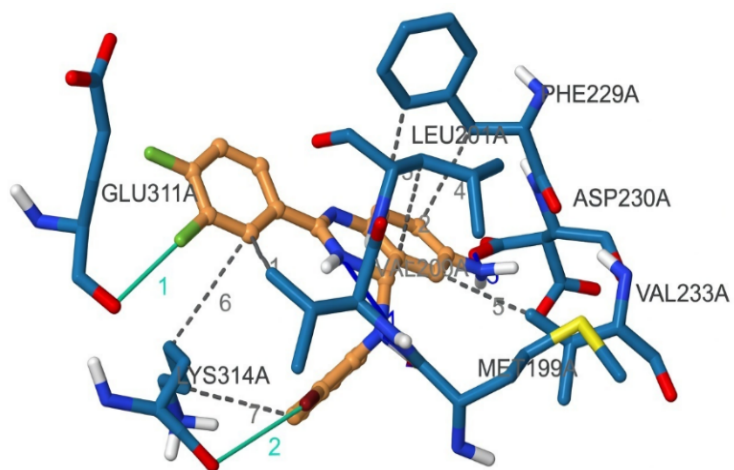
C5



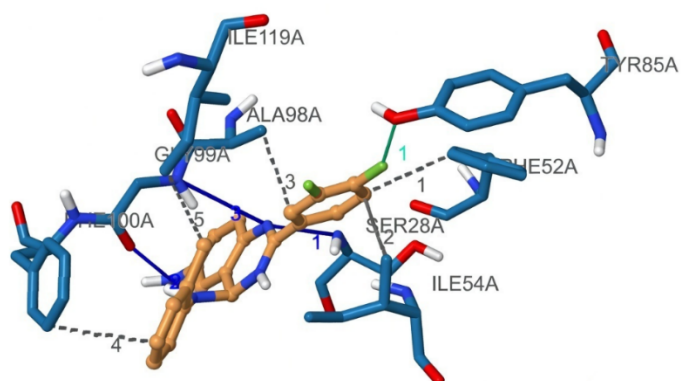
C6



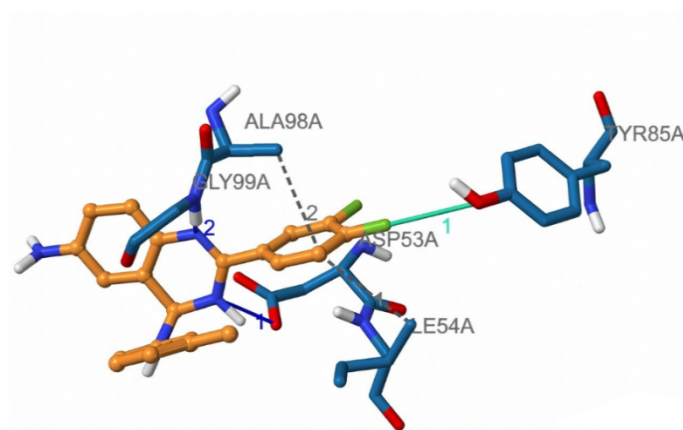
C7



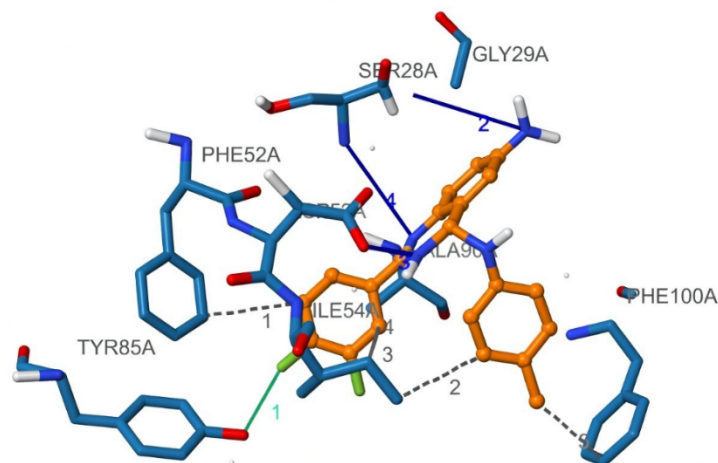
**C8**



**C9**



**C10**



**Table 2.** Comparative analysis of protein–ligand interactions of Compounds 1–10.

| Compound | Major Hydrophobic Interactions | Hydrogen Bonding Residues | Halogen/ $\pi$ Interactions | Key Observations                                                                                                                             |
|----------|--------------------------------|---------------------------|-----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|
| C1       | Lys198, Val200, Phe229, Lys314 | Phe229                    | $\pi$ -Cation: Lys314       | Strong hydrophobic interactions with Val200, Phe229, and Lys314. Unique $\pi$ -cation interaction with Lys314 may enhance binding stability. |
| C2       | Leu201, Val233                 | Leu201, Asp230            | –                           | Stable binding mediated by multiple hydrogen bonds with Asp230 and hydrophobic contacts with Leu201 and Val233.                              |
| C3       | Ile54, Ala98                   | Thr97                     | Halogen: Tyr85              | Classical binding mode involving Ile54–Thr97–Ala98. Halogen bond with Tyr85 may contribute to binding affinity.                              |
| C4       | Ile54, Phe100                  | Phe52, Gly99              | –                           | Hydrophobic interactions with Ile54 and Phe100 accompanied by hydrogen bonding with Gly99 stabilize the ligand.                              |
| C5       | Phe52, Ile54, Ala98            | Asp53, Gly99              | Halogen: Tyr85              | Extensive interaction network involving hydrophobic, hydrogen, and halogen bonds suggests strong ligand stabilization.                       |
| C6       | Met30, Ile31,                  | Thr97, Ser245             | Halogen:                    | Exhibits the most                                                                                                                            |

|            |                                     |                 |                      |                         |                                                                                                                                 |
|------------|-------------------------------------|-----------------|----------------------|-------------------------|---------------------------------------------------------------------------------------------------------------------------------|
|            | Thr101, Leu167, Pro250              | Thr139, Tyr247, |                      | Arg171                  | diverse interaction profile with extensive hydrophobic contacts and multiple hydrogen-bonding residues.                         |
| <b>C7</b>  | Val200, Phe229, Lys314              | Leu201, Val233, | Met199, Asp230       | Halogen: Glu311, Lys314 | Multiple hydrogen and halogen bonds combined with extensive hydrophobic interactions indicate stable binding.                   |
| <b>C8</b>  | Phe52, Ile54, Ala98, Phe100, Ile119 |                 | Ser28, Gly99         | Halogen: Tyr85          | Binding is stabilized through a combination of hydrogen, hydrophobic, and halogen interactions.                                 |
| <b>C9</b>  | Ile54, Ala98                        |                 | Asp53, Gly99         | Halogen: Tyr85          | Conserved interaction pattern similar to compounds 5 and 8, with Asp53 and Gly99 playing key roles.                             |
| <b>C10</b> | Phe52, Ile54, Ala98, Phe100         |                 | Ser28, Asp53, Gly29, | Halogen: Tyr85          | Strong interaction network involving hydrogen bonding with Asp53 and hydrophobic contacts with Phe52, Ile54, Ala98, and Phe100. |

**Table 3.** Frequency of important interacting residues among the docked compounds.

| Residue | Interaction Type                   | Compounds               | Frequency |
|---------|------------------------------------|-------------------------|-----------|
| Ile54   | Hydrophobic                        | C3, C4, C5, C8, C9, C10 | 6         |
| Phe52   | Hydrophobic/H-bond                 | C4, C5, C8, C10         | 4         |
| Asp53   | Hydrogen bond                      | C5, C9, C10             | 3         |
| Thr97   | Hydrogen bond                      | C3, C6                  | 2         |
| Ala98   | Hydrophobic                        | C3, C5, C8, C9, C10     | 5         |
| Gly99   | Hydrogen bond                      | C4, C5, C8, C9          | 4         |
| Tyr85   | Halogen bond                       | C3, C5, C8, C9, C10     | 5         |
| Val200  | Hydrophobic                        | C1, C7                  | 2         |
| Leu201  | Hydrophobic/H-bond                 | C2, C7                  | 2         |
| Phe229  | Hydrophobic/H-bond                 | C1, C7                  | 2         |
| Asp230  | Hydrogen bond                      | C2, C7                  | 2         |
| Val233  | Hydrophobic                        | C1, C2, C7              | 3         |
| Lys314  | Hydrophobic/ $\pi$ -cation/Halogen | C1, C7                  | 2         |

### 3.3.2 Comparative Analysis of Protein–Ligand Interactions

The molecular docking study revealed that all ten compounds were successfully accommodated within the binding pocket of the target protein and established multiple non-covalent interactions, including hydrogen bonds, hydrophobic contacts, halogen bonds, and  $\pi$ -cation interactions. Although the interaction patterns varied among the compounds, several amino acid residues were consistently involved in ligand recognition, suggesting their importance in stabilizing the ligand–protein complexes.

Compounds **C3**, **C4**, **C5**, **C8**, **C9**, and **C10** exhibited highly comparable binding modes, predominantly interacting with residues **ILE54**, **ALA98**, **GLY99**, **ASP53**, **PHE52**, **TYR85**, and **THR97**. Among these, **ILE54** and **ALA98** formed recurrent hydrophobic interactions, indicating their contribution to stabilizing the aromatic scaffold of the ligands within the binding cavity. Hydrogen bonding with **ASP53**, **GLY99**, and **THR97** was frequently observed, suggesting that these residues play a critical role in anchoring the ligands and enhancing binding stability. Furthermore, compounds **C3**, **C5**, **C8**, **C9**, and **C10** formed halogen bonds with **TYR85**,

indicating that halogen substitution contributes favourably to ligand recognition and may improve binding affinity.

Compounds **C1**, **C2**, and **C7** occupied a different region of the binding pocket, interacting primarily with **VAL200**, **LEU201**, **PHE229**, **ASP230**, **VAL233**, and **LYS314**. These compounds displayed extensive hydrophobic interactions with **VAL200**, **PHE229**, and **VAL233**, while **ASP230** participated in one or more hydrogen bonds that likely enhanced ligand stabilization. **C1** was distinguished by the presence of a  $\pi$ -cation interaction with **LYS314**, an interaction absent in the other compounds, suggesting an additional electrostatic contribution to its binding stability. Similarly, **C7** exhibited halogen bonding with **GLU311** and **LYS314**, further strengthening its interaction within this region of the binding pocket.

**C6** displayed a unique interaction profile compared with the remaining compounds. In addition to hydrophobic interactions involving **MET30**, **ILE31**, **THR101**, **THR139**, **LEU167**, **TYR247**, and **PRO250**, it formed hydrogen bonds with **THR97** and **SER245** and a halogen bond with **ARG171**. This broader interaction network suggests that compound **6** occupies an extended binding region and is stabilized through multiple complementary interactions.

Hydrogen bonding was identified as one of the principal contributors to ligand stabilization. The shortest hydrogen-bond distances were observed for **C6** (**THR97**, 1.93 Å), **C7** (**MET199**, 1.78 Å; **ASP230**, 1.90 Å), **C5** (**ASP53**, 2.05 Å), **C9** (**ASP53**, 2.11 Å), and **C10** (**ASP53**, 2.02 Å), indicating strong ligand–protein interactions. Hydrophobic contacts were consistently observed at distances ranging from approximately 3.1 to 4.0 Å, supporting favourable van der Waals interactions throughout the binding cavity. Halogen bonds involving **TYR85**, **ARG171**, **GLU311**, and **LYS314** further contributed to ligand stabilization in halogen-substituted compounds.

Overall, residues **ILE54**, **ASP53**, **THR97**, **ALA98**, **GLY99**, **TYR85**, **PHE52**, **VAL200**, **LEU201**, **PHE229**, **ASP230**, **VAL233**, and **LYS314** emerged as the most

frequently involved amino acids in ligand recognition. The recurrent participation of these residues across multiple docked complexes suggests that they constitute important regions of the binding pocket responsible for ligand stabilization. Among the investigated compounds, **C5**, **C8**, **C9**, and **C10** demonstrated highly similar interaction patterns characterized by a combination of hydrophobic contacts, hydrogen bonding, and halogen bonding, whereas **C1**, **C2**, and **C7** formed a second interaction cluster centered on residues **VAL200**–**LYS314**. **C6** exhibited the most diverse interaction profile, while **C1** was unique in displaying a  $\pi$ -cation interaction with **LYS314**.

## 4. MATERIALS AND METHODS

### 4.1 Experimental Section

All chemicals and reagents employed for synthesizing intermediates and target compounds were of analytical grade and procured from commercial suppliers. Melting points were determined using a digital melting point apparatus and are reported as uncorrected values.<sup>[16]</sup>

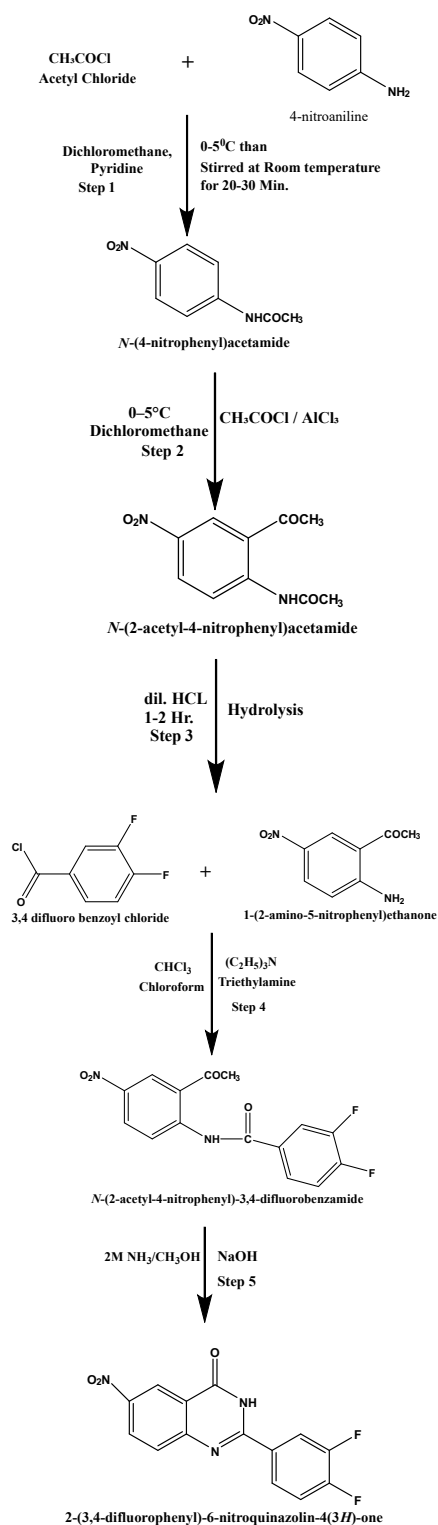
FT-IR spectra were recorded on a PerkinElmer FTIR spectrometer using KBr pellets. <sup>1</sup>H NMR spectra were acquired on a Bruker Avance II 400 MHz spectrometer, with chemical shifts ( $\delta$ ) expressed in parts per million (ppm) relative to tetramethylsilane (TMS) as the internal standard. Mass spectra were obtained using an LC-MSD-Trap-XL 2010A (Shimadzu) mass spectrometer with CDCl<sub>3</sub> as the solvent.<sup>[17,18]</sup>

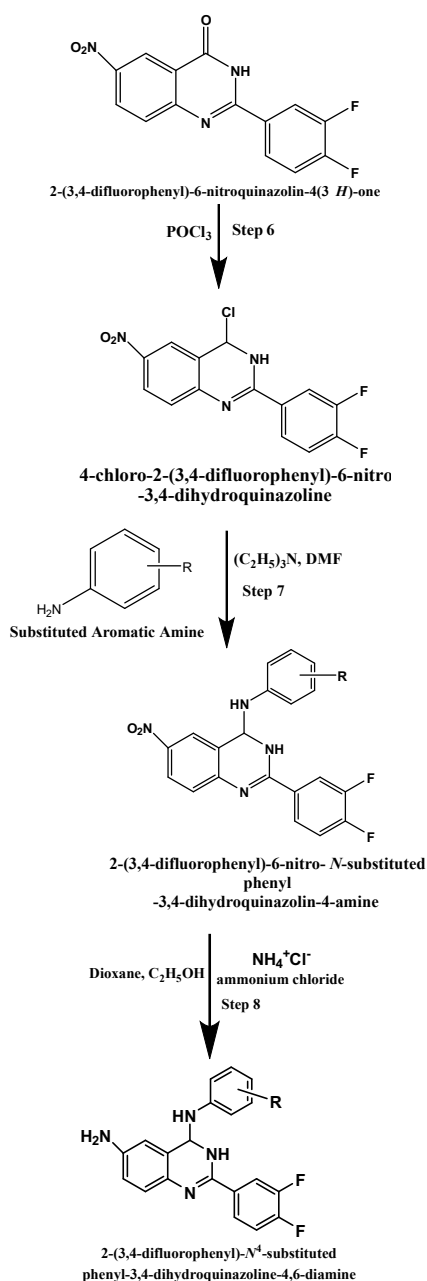
The purity of the synthesized compounds was monitored by thin-layer chromatography (TLC) on precoated silica gel G plates using **Benzene:Acetone (9:1)** and **Toluene:Ethyl acetate:Formic acid (5:4:1)** as the mobile phases. The chromatograms were visualized under UV light at 254 nm. Whatman No. 1 filter paper was used for vacuum filtration during the experimental procedures. Crude products were purified by recrystallization from ethanol (or ethanol/DMF, as appropriate). The purity of the final compounds was confirmed by TLC, which showed a single spot for each compound.<sup>[19,20]</sup>

**Table- 4:** Physicochemical data of 2-(3,4-difluorophenyl)-N4-substituted phenyl-3,4-dihydroquinazoline-4,6-diamine

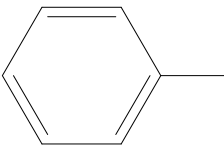
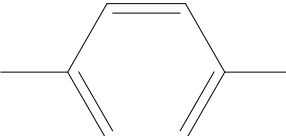
| Compound Code | R                 | Molecular Formula                                               | Molecular Weight (g/mol) | Melting Point (°C) | % Yield | Rf Value |
|---------------|-------------------|-----------------------------------------------------------------|--------------------------|--------------------|---------|----------|
| C1            | -H                | C <sub>20</sub> H <sub>16</sub> F <sub>2</sub> N <sub>4</sub>   | 350.37                   | 210–212            | 78%     | 0.67     |
| C2            | 4-F               | C <sub>20</sub> H <sub>15</sub> F <sub>3</sub> N <sub>4</sub>   | 368.36                   | 198–200            | 75%     | 0.71     |
| C3            | 2-F               | C <sub>20</sub> H <sub>15</sub> F <sub>3</sub> N <sub>4</sub>   | 368.36                   | 202–204            | 73%     | 0.54     |
| C4            | 4-Cl              | C <sub>20</sub> H <sub>15</sub> ClF <sub>2</sub> N <sub>4</sub> | 384.81                   | 215–217            | 70%     | 0.61     |
| C5            | 2-Cl              | C <sub>20</sub> H <sub>15</sub> ClF <sub>2</sub> N <sub>4</sub> | 384.81                   | 208–210            | 72%     | 0.51     |
| C6            | 4-Br              | C <sub>20</sub> H <sub>15</sub> BrF <sub>2</sub> N <sub>4</sub> | 429.27                   | 230–232            | 68%     | 0.59     |
| C7            | 2-Br              | C <sub>20</sub> H <sub>15</sub> BrF <sub>2</sub> N <sub>4</sub> | 429.27                   | 227–229            | 69%     | 0.69     |
| C8            | 2-CH <sub>3</sub> | C <sub>21</sub> H <sub>18</sub> F <sub>2</sub> N <sub>4</sub>   | 364.4                    | 195–197            | 76%     | 0.62     |
| C9            | 3-CH <sub>3</sub> | C <sub>21</sub> H <sub>18</sub> F <sub>2</sub> N <sub>4</sub>   | 364.4                    | 193–195            | 77%     | 0.75     |
| C10           | 4-CH <sub>3</sub> | C <sub>21</sub> H <sub>18</sub> F <sub>2</sub> N <sub>4</sub>   | 364.4                    | 190–192            | 78%     | 0.83     |

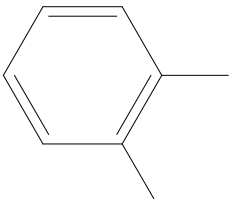
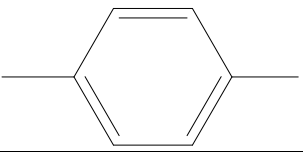
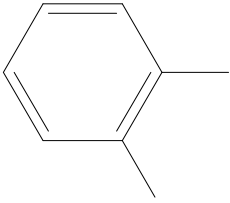
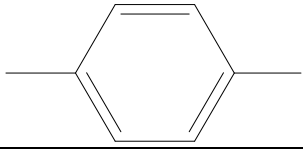
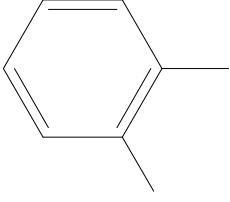
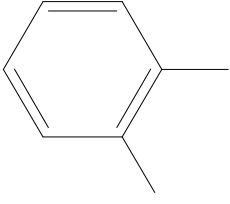
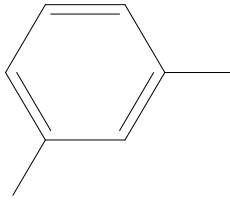
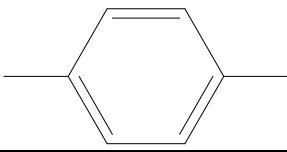
## 4.2 Synthesis Scheme





**Table 5:** Different Aromatic amine to be used in the synthesis of proposed product

| S. No. | Name of aromatic amine | Structure                                                                            | R   |
|--------|------------------------|--------------------------------------------------------------------------------------|-----|
| 1      | Aniline                |  | -H  |
| 2      | 4-fluorobenzenamine    |  | 4-F |

|    |                     |                                                                                      |                   |
|----|---------------------|--------------------------------------------------------------------------------------|-------------------|
| 3  | 2-fluorobenzenamine |    | 2-F               |
| 4  | 4-chlorobenzenamine |    | 4-Cl              |
| 5  | 2-chlorobenzenamine |    | 2-Cl              |
| 6  | 4-bromobenzenamine  |    | 4-Br              |
| 7  | 2-bromobenzenamine  |   | 2-Br              |
| 8  | o-toluidine         |  | 2-CH <sub>3</sub> |
| 9  | m-toluidine         |  | 3-CH <sub>3</sub> |
| 10 | p-toluidine         |  | 4-CH <sub>3</sub> |

### 4.3 Procedure:

#### 4.3.1 Step 1: (Acetylation Using Acetyl Chloride): Synthesis of N-(4-nitrophenyl)acetamide

2-Amino-5-nitrobenzene was subjected to acetylation to protect the amino group by converting it into the corresponding amide derivative. In a dry round-bottom

flask, the compound (2–3 g) was dissolved in dry dichloromethane, and an equimolar amount of pyridine was added to act as a base. The reaction mixture was cooled in an ice bath (0–5°C), and acetyl chloride was added dropwise with continuous stirring to control the exothermic nature of the reaction. After complete addition,

the reaction mixture was stirred at room temperature for 30–60 minutes to ensure completion of acetylation. The mixture was then poured into cold water and transferred to a separating funnel. The organic layer was washed successively with dilute hydrochloric acid to remove excess pyridine, followed by water, and then dried over anhydrous sodium sulphate. The solvent was evaporated under reduced pressure to obtain the crude product, which was subsequently purified by recrystallization from ethanol to yield 2-acetamido-5-nitrobenzene as a purified product in good yield.

In a flame-dried round-bottom flask, 2–3 g of **2-amino-5-nitrobenzene** was dissolved in anhydrous **dichloromethane (DCM)**. An equimolar amount of **pyridine** was added to the solution to serve as both a nucleophilic catalyst and a base for neutralizing the generated HCl. The flask was immersed in an ice bath to maintain a temperature of 0–5°C. **Acetyl chloride** was then introduced dropwise with continuous stirring to control the exothermic nature of the acylation. Once addition was finalized, the mixture was stirred at room temperature for 30–60 minutes to ensure full conversion. The reaction was quenched by pouring the contents into cold water, and the resulting mixture was transferred to a separating funnel. The organic layer was isolated and washed sequentially with **dilute hydrochloric acid** to extract residual pyridine as a water-soluble salt. This was followed by a water wash and subsequent drying over **anhydrous sodium sulphate** to remove trace moisture before further processing.<sup>[21]</sup>

#### 4.3.2- Step 2: (Friedel–Crafts Acylation): Synthesis of N-(2-acetyl-4-nitrophenyl)acetamide

Under moisture-free conditions, 2-acetamido-5-nitrobenzene was dissolved in anhydrous dichloromethane (DCM) within a dry, nitrogen-purged round-bottom flask. The solution was cooled to 0–5°C in an ice bath, and a stoichiometric excess (typically >1 equivalent) of anhydrous  $\text{AlCl}_3$  was added slowly under constant stirring to minimize premature reaction. Acetyl chloride was added dropwise, ensuring the exothermic reaction was controlled, and the temperature remained low. The mixture was allowed to reach room temperature and stirred for several hours, with reaction completion monitored using Thin-Layer Chromatography (TLC). The reaction mixture was quenched by careful pouring onto crushed ice containing dilute HCl to decompose the  $\text{AlCl}_3$ -ketone complex. The organic layer was separated, washed, dried over anhydrous sodium sulfate, and evaporated. The resulting crude acetylated derivative was purified via recrystallization from ethanol.

#### 4.3.3 - Step 3: (Deprotection of Acetamide Group): Synthesis of 1-(2-amino-5-nitrophenyl)ethanone

The acetyl-protected intermediate underwent hydrolytic deprotection to regenerate the free amino functionality. The compound was refluxed in dilute hydrochloric acid (alternatively, aqueous sodium hydroxide may be utilized) for 1–2 hours to achieve amide linkage ( $-\text{NHCOCH}_3$ ) cleavage, yielding the corresponding primary amine ( $-\text{NH}_2$ ).

Reaction completion was monitored via thin-layer chromatography (TLC).<sup>[22]</sup>

Upon completion, the reaction mixture was cooled to room temperature and, in the case of acid hydrolysis, neutralized with dilute sodium hydroxide to a neutral pH. The product was extracted using an organic solvent such as ethyl acetate. The organic phase was separated, washed with water to remove residual inorganic impurities, and dried over anhydrous sodium sulphate. Subsequent evaporation of the solvent under reduced pressure yielded the crude product. The final compound, 1-(2-amino-5-nitrophenyl)ethanone, was purified by recrystallization from ethanol, resulting in the desired product in high purity and yield.<sup>[23]</sup>

#### 4.3.4 - Step 4: Synthesis of N-(2-Acetyl-4-nitrophenyl)-3,4-difluorobenzamide

A solution of **1-(2-amino-5-nitrophenyl)ethanone** (X g) and **triethylamine** (as base) in **chloroform** (50 mL) was treated dropwise with **3,4-difluorobenzoyl chloride** at room temperature. The reaction mixture was stirred for 20 hours to ensure complete consumption of the amine. The mixture was then processed by washing the organic layer with 10%  $\text{NaHCO}_3$  and 5% HCl to remove acidic and basic impurities, respectively. The resulting product was isolated by precipitation using an **ethyl acetate/hexane** solvent system, followed by drying to yield the target amide.<sup>[24]</sup>

#### 4.3.5 - Step 5: Synthesis of 2-(3,4-Difluorophenyl)-6-nitroquinazolin-4(3H)-one

The synthesis of quinazolinone derivatives can be achieved by a two-stage process involving ammonolysis followed by alkaline-mediated cyclization. Specifically, an appropriate amide derivative (X g) is dissolved in methanol (10 mL) and treated with ammonium hydroxide under reflux for 5 hours. Subsequently, an aqueous sodium hydroxide solution (40 mL) is added to the mixture, followed by prolonged reflux for 12 hours to complete the cyclization. The target quinazolinone derivative is isolated by filtration of the precipitate, followed by washing and drying.<sup>[25]</sup>

#### 4.3.6- Step 6: Synthesis of 4-chloro-2-(3,4-difluorophenyl)-6-nitro-3,4-dihydroquinazoline

The substituted 4(3H)-quinazolinone was subjected to reflux in phosphorus oxychloride  $\text{POCl}_3$  for 5 hours. Following completion, the excess  $\text{POCl}_3$  was removed under reduced pressure, and the residue was carefully quenched by pouring into a stirring mixture of ice-water. The crude product was extracted into chloroform, washed with saturated  $\text{NaHCO}_3$  (aqueous) and brine, and purified via column chromatography to afford the corresponding 4-chloroquinazoline derivative.<sup>[26]</sup>

#### 4.3.7 - Step 7: Synthesis of 2-(3,4-Difluorophenyl)-6-nitro-N-substituted phenyl-3,4-dihydroquinazolin-4-amine

A 4-chloroquinazoline derivative (X g) was subjected to nucleophilic aromatic substitution  $\text{S}_{\text{N}}\text{Ar}$  by reacting it with various aromatic amines in anhydrous N,N-

dimethylformamide (DMF, 4 mL) in the presence of N,N-diisopropylamine 90  $\mu$ L as a base. The reaction mixture was refluxed for 3–6 h, followed by cooling, filtration, and purification via column chromatography to yield the corresponding N-substituted 4-aminoquinazoline derivatives.

#### 4.3.8 - Step 8: Synthesis of 2-(3,4-Difluorophenyl)-6-nitro-N-substituted phenyl-3,4-dihydroquinazoline-4,6-diamine (C1-C10)

The N-substituted nitro-quinazoline derivative (X g) was dissolved in a solvent mixture of ethanol, dioxane, and water. Ammonium chloride ( $\text{NH}_4\text{Cl}$ ) and iron (Fe) powder were added, and the resulting suspension was refluxed at 100°C for 5 hours. Upon completion of the reaction (monitored by TLC), the mixture was cooled to room temperature, filtered through a Celite bed to remove iron sludge, and the residue was washed with 10% methanol or dichloromethane. The combined filtrate was concentrated under reduced pressure and subsequently extracted using a mixture of 10% isopropanol in chloroform. The combined organic layers were dried over anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), filtered, concentrated, and crystallized to yield the desired diamine derivative.<sup>[27-28]</sup>

#### 4.3.8 - 2-(3,4-difluorophenyl)-N<sup>4</sup>-phenyl-3,4-dihydroquinazoline-4,6-diamine (C1)

Molecular Formula:  $\text{C}_{20}\text{H}_{16}\text{F}_2\text{N}_4$  (MW 350): Yield 78%; mp 210–212 °C;  $R_f = 0.67$ ; FT-IR (KBr,  $\text{cm}^{-1}$ ): 3420, 3300 (br, N–H), 3075 (Ar–H), 2955 (CH), 1620 (C=N/Ar C=C), 1250, 1180 (C–F);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.40 (d,  $J = 8.5$  Hz, 1H), 7.95 (d,  $J = 8.5$  Hz, 1H), 7.65 (dd,  $J = 8.5, 2.5$  Hz, 2H), 7.40 (m, 4H), 7.20 (m, 2H), 6.95 (t,  $J = 7.5$  Hz, 1H), 5.80 (br s, 2H,  $\text{NH}_2$ ), 4.20 (s, 2H,  $\text{CH}_2$ ); MS:  $m/z$  350.13  $[\text{M}+\text{H}]^+$ .

#### 4.3.9 - 2-(3,4-difluorophenyl)-N<sup>4</sup>-(4-fluorophenyl)-3,4-dihydroquinazoline-4,6-diamine (C2)

Molecular Formula:  $\text{C}_{20}\text{H}_{15}\text{F}_3\text{N}_4$  (MW 368.36): Yield 75%; mp 198–200 °C;  $R_f = 0.71$ ; FT-IR (KBr,  $\text{cm}^{-1}$ ): 3312 (N–H), 3260 (br, N–H), 3050 (Ar–H), 2930 (CH), 1718 (C=N), 1622 (Ar C=C), 1582, 1512, 1485, 1338 (C–N), 1218 (C–F), 1123, 1028, 752, 710 (Ar C–H oop);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.65 (br s, 1H, NH), 8.42 (d,  $J = 8.5$  Hz, 1H), 7.98 (d,  $J = 8.5$  Hz, 1H), 7.75 (dd,  $J = 8.8, 2.6$  Hz, 1H), 7.60 (dd,  $J = 8.8, 2.6$  Hz, 1H), 7.40 (m, 4H), 7.20 (m, 1H), 7.05 (m, 1H), 5.85 (br s, 2H,  $\text{NH}_2$ ), 4.18 (s, 2H); MS:  $m/z$  368.1249  $[\text{M}+\text{H}]^+$ .

#### 4.3.10 - 2-(3,4-difluorophenyl)-N<sup>4</sup>-(2-fluorophenyl)-3,4-dihydroquinazoline-4,6-diamine (C3)

Molecular Formula:  $\text{C}_{20}\text{H}_{15}\text{F}_3\text{N}_4$  (MW 368.36): Yield 75%; mp 202–204 °C;  $R_f = 0.54$ ; FT-IR (KBr,  $\text{cm}^{-1}$ ): 3340 (N–H), 3220 (br, N–H), 3050 (Ar–H), 2955, 2870 (CH), 1670 (C=N), 1605 (Ar C=C), 1508, 1450 (C–N/Ar), 1375 (C–N), 1255 (C–F), 1115, 1030, 760, 700 (Ar C–H oop);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.65 (br s, 1H, NH), 8.42 (d,  $J = 8.5$  Hz, 1H), 7.98 (d,  $J = 8.5$  Hz, 1H), 7.75 (dd,  $J = 8.8, 2.6$  Hz, 1H), 7.60 (dd,  $J = 8.8, 2.6$  Hz, 1H), 7.45 (dd,  $J = 8.2, 5.0$  Hz, 1H), 7.30 (td,  $J = 8.0, 2.0$  Hz, 1H), 7.20 (m, 1H), 7.05 (dd,  $J = 8.2, 2.0$  Hz, 1H), 6.95 (td,  $J = 8.0, 1.5$

Hz, 1H), 5.85 (br s, 2H,  $\text{NH}_2$ ), 4.18 (s, 2H,  $\text{CH}_2$ ); MS:  $m/z$  368.12  $[\text{M}+\text{H}]^+$ .

#### 4.3.11 - N<sup>4</sup>-(4-chlorophenyl)-2-(3,4-difluorophenyl)-3,4-dihydroquinazoline-4,6-diamine (C4)

Molecular Formula:  $\text{C}_{20}\text{H}_{15}\text{ClF}_2\text{N}_4$  (MW 384.81): Yield 70%; mp 215–217 °C;  $R_f = 0.61$ ; FT-IR (KBr,  $\text{cm}^{-1}$ ): 3321 (br, N–H), 3212 (N–H), 3078 (Ar–H), 2951 (CH), 1662 (C=N), 1615 (Ar C=C), 1572, 1516 (C–F), 1474, 1326 (C–N), 1248 (C–F), 1135, 1016, 833, 756, 612 (C–Cl), 520;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.60 (br s, 1H, NH), 8.42 (d,  $J = 8.5$  Hz, 1H), 7.95 (d,  $J = 8.5$  Hz, 1H), 7.75 (dd,  $J \approx 8.8, 2.6$  Hz, 1H), 7.60 (dd,  $J \approx 8.8, 2.6$  Hz, 1H), 7.40 (m, 4H), 7.20 (m, 2H), 7.05 (m, 1H), 5.85 (br s, 2H,  $\text{NH}_2$ ), 4.20 (s, 2H,  $\text{CH}_2$ ); MS:  $m/z$  384.09  $[\text{M}+\text{H}]^+$ .

#### 4.3.12 - N<sup>4</sup>-(2-chlorophenyl)-2-(3,4-difluorophenyl)-3,4-dihydroquinazoline-4,6-diamine (C5)

Molecular Formula:  $\text{C}_{20}\text{H}_{15}\text{ClF}_2\text{N}_4$  (MW 384.81): Yield 72%; mp 208–210 °C;  $R_f = 0.51$ ; FT-IR (KBr,  $\text{cm}^{-1}$ ): 3345, 3205 (N–H), 3075 (Ar–H), 2960 (CH), 1670 (C=N), 1620 (Ar C=C), 1515 (C–F), 1480, 1350 (C–N/C–F), 1250 (C–F), 1140, 1020, 840, 760, 690 (C–Cl), 540;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.60 (br s, 1H, NH), 8.42 (d,  $J = 8.5$  Hz, 1H), 7.95 (d,  $J = 8.5$  Hz, 1H), 7.75 (dd,  $J \approx 8.8, 2.6$  Hz, 1H), 7.60 (dd,  $J \approx 8.8, 2.6$  Hz, 1H), 7.40 (m, 4H), 7.20 (m, 2H), 7.05 (m, 1H), 5.85 (br s, 2H,  $\text{NH}_2$ ), 4.20 (s, 2H,  $\text{CH}_2$ ); MS:  $m/z$  384.09  $[\text{M}+\text{H}]^+$ .

#### 4.3.13 - N<sup>4</sup>-(4-bromophenyl)-2-(3,4-difluorophenyl)-3,4-dihydroquinazoline-4,6-diamine (C6)

Molecular Formula:  $\text{C}_{20}\text{H}_{15}\text{BrF}_2\text{N}_4$  (MW 429.27): Yield 68%; mp 230–232 °C;  $R_f = 0.59$ ; FT-IR (KBr,  $\text{cm}^{-1}$ ): 3440, 3330 (N–H), 3070 (Ar–H), 2960–2870 (CH), 1660 (C=N), 1600–1585 (Ar C=C/N–H), 1505, 1375 (C–N), 1250, 1180 (C–F), 1030, 840, 720–690, 640 (C–Br), 560;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.62 (br s, 1H, NH), 8.42 (d,  $J = 8.5$  Hz, 1H), 7.98 (d,  $J = 8.5$  Hz, 1H), 7.76 (dd,  $J \approx 8.8, 2.6$  Hz, 1H), 7.61 (dd,  $J \approx 8.8, 2.6$  Hz, 1H), 7.40 (m, 4H), 7.20 (m, 2H), 7.05 (m, 1H), 5.85 (br s, 2H,  $\text{NH}_2$ ), 4.20 (s, 2H,  $\text{CH}_2$ ); MS:  $m/z$  429  $[\text{M}+\text{H}]^+$ .

#### 4.3.14 - N<sup>4</sup>-(2-bromophenyl)-2-(3,4-difluorophenyl)-3,4-dihydroquinazoline-4,6-diamine (C7)

Molecular Formula:  $\text{C}_{20}\text{H}_{15}\text{BrF}_2\text{N}_4$  (MW 429.27): Yield 69%; mp 227–229 °C;  $R_f = 0.69$ ; FT-IR (KBr,  $\text{cm}^{-1}$ ): 3340, 3210 (N–H), 3075 (Ar–H), 2950 (CH), 1675 (C=N), 1610 (Ar C=C), 1515, 1485, 1355 (C–N), 1260, 1145 (C–F), 1025, 840, 760, 700, 540 (C–Br);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.64 (br s, 1H, NH), 8.44 (d,  $J = 8.5$  Hz, 1H), 7.99 (d,  $J = 8.5$  Hz, 1H), 7.78 (dd,  $J \approx 8.8, 2.6$  Hz, 1H), 7.63 (dd,  $J \approx 8.8, 2.6$  Hz, 1H), 7.45–7.05 (m, 4H, Ar–H), 5.87 (br s, 2H,  $\text{NH}_2$ ), 4.21 (s, 2H,  $\text{CH}_2$ ); MS:  $m/z$  428.0448  $[\text{M}+\text{H}]^+$ .

#### 4.3.15 - 2-(3,4-difluorophenyl)-N<sup>4</sup>-(o-tolyl)-3,4-dihydroquinazoline-4,6-diamine (C8)

Molecular Formula:  $\text{C}_{20}\text{H}_{18}\text{F}_2\text{N}_4$  (MW 364.40): Yield 76%; mp 195–197 °C;  $R_f = 0.62$ ; FT-IR (KBr,  $\text{cm}^{-1}$ ): 3352 (N–H), 3248 (N–H), 3064 (Ar–H), 2952 (CH), 1716 (C=N), 1614 (Ar C=C), 1522 (C–F), 1476, 1324 (C–N), 1212 (C–F), 1118, 1032, 872, 798, 746, 682;  $^1\text{H}$  NMR (400 MHz,

CDCl<sub>3</sub>):  $\delta$  9.60 (br s, 1H, NH), 8.42 (d,  $J$  = 8.5 Hz, 1H), 7.95 (d,  $J$  = 8.5 Hz, 1H), 7.70 (dd,  $J$   $\approx$  8.8, 2.5 Hz, 1H), 7.55 (dd,  $J$   $\approx$  8.8, 2.5 Hz, 1H), 7.30–7.10 (m, 4H, Ar-H), 7.05 (m, 1H), 5.85 (br s, 2H, NH<sub>2</sub>), 4.20 (s, 2H, CH<sub>2</sub>), 2.35 (s, 3H, CH<sub>3</sub>); MS:  $m/z$  364 [M+H]<sup>+</sup>.

#### 4.3.16 - 2-(3,4-difluorophenyl)-N<sup>4</sup>-(*m*-tolyl)-3,4-dihydroquinazoline-4,6-diamine (C9)

Molecular Formula: C<sub>20</sub>H<sub>18</sub>F<sub>2</sub>N<sub>4</sub> (MW 364.40): Yield 77%; mp 193–195 °C;  $R_f$  = 0.75; FT-IR (KBr, cm<sup>-1</sup>): 3345 (N-H), 3220 (N-H), 3075 (Ar-H), 2955, 2875 (CH), 1675 (C=N), 1615 (Ar C=C), 1516 (C-F), 1475, 1378 (CH<sub>3</sub>), 1348 (C-N/C-F), 1255 (C-F), 1140, 1020, 845, 760, 690, 540; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  9.60 (br s, 1H, NH), 8.42 (d,  $J$  = 8.5 Hz, 1H), 7.95 (d,  $J$  = 8.5 Hz, 1H), 7.70 (dd,  $J$   $\approx$  8.8, 2.5 Hz, 1H), 7.55 (dd,  $J$   $\approx$  8.8, 2.5 Hz, 1H), 7.30–7.15 (m, 4H, Ar-H), 7.05 (m, 1H), 5.85 (br s, 2H, NH<sub>2</sub>), 4.20 (s, 2H, CH<sub>2</sub>), 2.33 (s, 3H, CH<sub>3</sub>); MS:  $m/z$  364 [M+H]<sup>+</sup>.

#### 4.3.17 - 2-(3,4-difluorophenyl)-N<sup>4</sup>-(*p*-tolyl)-3,4-dihydroquinazoline-4,6-diamine (C10)

Molecular Formula: C<sub>20</sub>H<sub>18</sub>F<sub>2</sub>N<sub>4</sub> (MW 364.40): Yield 78%; mp 190–192 °C;  $R_f$  = 0.83; FT-IR (KBr, cm<sup>-1</sup>): 3330 (N-H), 3060 (Ar-H), 2955 (CH), 1620 (C=N), 1505 (Ar C=C), 1375 (C-N), 1250 (C-F), 1180, 1105, 1020, 760, 695; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  9.60 (br s, 1H, NH), 8.42 (d,  $J$  = 8.5 Hz, 1H), 7.95 (d,  $J$  = 8.5 Hz, 1H), 7.70 (dd,  $J$   $\approx$  8.8, 2.5 Hz, 1H), 7.55 (dd,  $J$   $\approx$  8.8, 2.5 Hz, 1H), 7.35 (d,  $J$   $\approx$  8.5 Hz, 2H), 7.08 (d,  $J$   $\approx$  8.5 Hz, 2H), 7.20 (m, 1H), 5.85 (br s, 2H, NH<sub>2</sub>), 4.20 (s, 2H, CH<sub>2</sub>), 2.34 (s, 3H, CH<sub>3</sub>); MS:  $m/z$  364 [M+H]<sup>+</sup>.

### 5.1 In vitro Antiplasmodial Activity of Quinazoline Derivatives

A library of 10 quinazoline derivatives (C1–C10), synthesized using various substituted aromatic amines, was evaluated for their antimalarial efficacy against the chloroquine-resistant *Plasmodium falciparum* Dd2luc strain. The assay utilized a two-tiered screening strategy: an initial four-point SYBR Green I fluorescence-based screen to estimate relative growth inhibition and a follow-up luciferase-based bioluminescence assay to determine EC<sub>50</sub> values.

### 5.2 Growth Inhibition Profile and EC<sub>50</sub> Determination

The antiplasmodial activity of the synthesized quinazoline derivatives (C1–C10) was evaluated against *Plasmodium falciparum* using a concentration-dependent growth inhibition assay. The compounds exhibited varying degrees of inhibition, indicating a clear structure–activity relationship among the synthesized derivatives. The EC<sub>50</sub> values were determined from the concentration–response curves and are presented as mean  $\pm$  SD.

Among the tested compounds, C6 (4-Br) showed the highest antiplasmodial activity with an EC<sub>50</sub> of  $3.8 \pm 0.34$   $\mu$ M, followed by C1 (–H) with  $4.1 \pm 0.32$   $\mu$ M and C4 (4-Cl) with  $4.6 \pm 0.34$   $\mu$ M. Compounds C5 (2-Cl) and C7 (2-Br) exhibited moderate activity, with EC<sub>50</sub> values of  $5.3 \pm 0.39$  and  $5.7 \pm 0.42$   $\mu$ M, respectively. The methyl-substituted derivatives C8 and C9 showed comparatively

lower activity, with EC<sub>50</sub> values of  $6.8 \pm 0.63$  and  $7.5 \pm 0.59$   $\mu$ M, respectively. C3 (2-F) displayed the lowest activity among the synthesized compounds (EC<sub>50</sub> =  $8.5 \pm 0.56$   $\mu$ M). The reference drug artemisinin exhibited the highest activity, with an EC<sub>50</sub> of  $2.5 \pm 0.24$   $\mu$ M.

Overall, the results indicate that halogen substitution, particularly bromine at the para position, favorably influenced antiplasmodial activity, whereas ortho substitution and methyl groups generally resulted in reduced potency. The concentration-dependent inhibition profile and EC<sub>50</sub> values provide evidence that structural modifications of the quinazoline scaffold significantly influence antiplasmodial activity.

### 5.5 Structure–Activity Relationship (SAR) Insights

The antiplasmodial activity of the synthesized quinazoline derivatives revealed several important structure–activity relationship (SAR) trends. The nature and position of the substituent on the aromatic ring markedly influenced the EC<sub>50</sub> values.

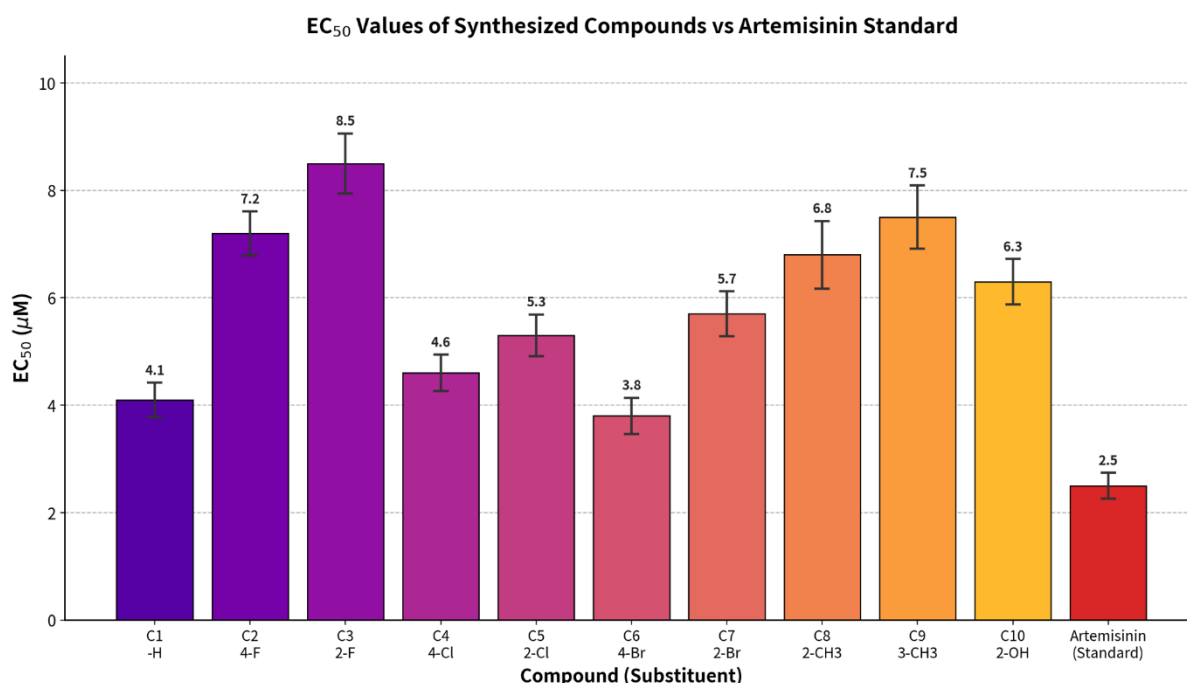
- **Halogen substitution:** Halogen-containing derivatives generally showed improved activity, particularly the para-substituted compounds. C6 (4-Br) was the most potent synthesized derivative (EC<sub>50</sub> =  $3.8 \pm 0.34$   $\mu$ M), followed by C4 (4-Cl) ( $4.6 \pm 0.34$   $\mu$ M). This suggests that electron-withdrawing halogens at the para position may favorably contribute to antiplasmodial activity.
- **Positional effect:** The para-substituted halogen derivatives were more active than their corresponding ortho analogues. For example, C2 (4-F) showed better activity than C3 (2-F), while C4 (4-Cl) and C6 (4-Br) were more potent than C5 (2-Cl) and C7 (2-Br), respectively. This indicates that the position of substitution is an important determinant of activity, possibly due to differences in steric effects and molecular orientation.
- **Fluoro substitution:** The fluoro derivatives displayed moderate activity, with C2 (4-F) showing an EC<sub>50</sub> of  $7.2 \pm 0.41$   $\mu$ M and C3 (2-F) showing  $8.5 \pm 0.56$   $\mu$ M. The lower activity of the ortho analogue may be associated with steric effects affecting the preferred conformation or target interactions.
- **Methyl substitution:** Introduction of a methyl group resulted in comparatively reduced activity. C8 (2-CH<sub>3</sub>) and C9 (3-CH<sub>3</sub>) showed EC<sub>50</sub> values of  $6.8 \pm 0.63$  and  $7.5 \pm 0.59$   $\mu$ M, respectively, suggesting that the electron-donating and hydrophobic character of the methyl group did not provide the same activity enhancement observed with selected halogen substituents.
- **Hydroxyl substitution:** The ortho-hydroxy derivative C10 (2-OH) showed moderate activity (EC<sub>50</sub> =  $6.3 \pm 0.42$   $\mu$ M). Although the hydroxyl group can provide additional hydrogen-bonding capacity, its ortho position may introduce steric constraints and alter the orientation of the aromatic ring.

Overall, the SAR data suggest that para-halogen substitution, particularly bromine, is favorable for antiplasmodial activity, whereas ortho substitution generally resulted in reduced potency. These findings

provide a useful basis for further structural optimization of the quinazoline scaffold toward more potent antiplasmodial candidates.

**Table 6:** EC<sub>50</sub> Values of Quinazoline Derivatives Against *P. falciparum* Dd2luc Strain

| Compound    | Substituent       | EC <sub>50</sub> (μM) | SD   |
|-------------|-------------------|-----------------------|------|
| C1          | –H                | 4.1                   | 0.32 |
| C2          | 4-F               | 7.2                   | 0.41 |
| C3          | 2-F               | 8.5                   | 0.56 |
| C4          | 4-Cl              | 4.6                   | 0.34 |
| C5          | 2-Cl              | 5.3                   | 0.39 |
| C6          | 4-Br              | 3.8                   | 0.34 |
| C7          | 2-Br              | 5.7                   | 0.42 |
| C8          | 2-CH <sub>3</sub> | 6.8                   | 0.63 |
| C9          | 3-CH <sub>3</sub> | 7.5                   | 0.59 |
| C10         | 2-OH              | 6.3                   | 0.42 |
| Artemisinin | Standard          | 2.5                   | 0.24 |



**Fig 1:** Graph showing comparative analysis between EC<sub>50</sub> of C1 – C10 compared to standard artemisinin.

### 5.6 Relevance to Drug Resistance

The antiplasmodial activity of the synthesized quinazoline derivatives demonstrates their potential relevance in addressing drug resistance in *Plasmodium falciparum*. Among the tested compounds, **C6 (4-Br)** exhibited the highest activity (EC<sub>50</sub> = 3.8 ± 0.34 μM), followed by **C4 (4-Cl)** (4.6 ± 0.34 μM) and the parent compound **C1 (–H)** (4.1 ± 0.32 μM). Notably, para-halogen substitution, particularly bromine and chlorine, enhanced activity compared with the corresponding ortho-substituted analogues. In contrast, compounds bearing ortho-fluoro, ortho-chloro, ortho-bromo, or methyl substituents showed comparatively reduced activity.

The retention of measurable antiplasmodial activity across structurally diverse derivatives suggests that the **quinazoline scaffold may provide a useful framework for developing new antimalarial candidates with potential activity against drug-resistant parasites**. The improved activity observed with electron-withdrawing para-halogen substituents, especially C6, provides an important structure–activity relationship for further optimization. However, the present EC<sub>50</sub> data alone do not establish activity against a drug-resistant strain or confirm a resistance-breaking mechanism. Further evaluation against resistant *P. falciparum* strains and studies involving established resistance markers are required to validate this potential.

## 6. RESULTS AND DISCUSSION

The present study involved the design, synthesis, and biological evaluation of a series of **ten N-substituted phenyl-3,4-dihydroquinazoline-4,6-diamine derivatives (C1–C10)** targeting *Plasmodium falciparum* lactate dehydrogenase (PfLDH). All synthesized compounds were successfully characterized by FT-IR, <sup>1</sup>H NMR, and mass spectrometry, confirming the formation of the desired quinazoline framework. The biological potential of these derivatives was assessed through molecular docking studies and *in vitro* antiparasmodial evaluation against the chloroquine-resistant *Plasmodium falciparum* Dd2luc strain.<sup>[29,30]</sup>

Molecular docking analysis revealed binding energies ranging from **−8.07 to −7.00 kcal/mol**, indicating favourable interactions between the synthesized ligands and the PfLDH active site. The reference RMSD values ranged from **1.03 to 2.00 Å**, confirming the reliability and stability of the predicted binding conformations. Among the synthesized compounds, **C6** exhibited the strongest binding affinity (−8.07 kcal/mol), followed by **C10** (−7.82 kcal/mol), **C1** (−7.81 kcal/mol), **C7** (−7.71 kcal/mol), and **C9** (−7.67 kcal/mol). The favourable docking scores suggest that these compounds interact effectively with the catalytic pocket of PfLDH through hydrogen bonding, hydrophobic interactions, and  $\pi$ – $\pi$  stacking, thereby contributing to enzyme inhibition.<sup>[31–33]</sup>

The EC<sub>50</sub> values of compounds C1–C10 against *P. falciparum* Dd2luc strain ranged from **3.8 to 8.5  $\mu$ M**, whereas the reference drug **artemisinin** exhibited an **EC<sub>50</sub> of 2.5  $\pm$  0.24  $\mu$ M**. Lower EC<sub>50</sub> values indicate greater antiparasmodial potency.

Among the synthesized derivatives, **C6 (4-Br)** showed the highest activity with an EC<sub>50</sub> of **3.8  $\pm$  0.34  $\mu$ M**, followed by **C1 (–H)** with **4.1  $\pm$  0.32  $\mu$ M** and **C4 (4-Cl)** with **4.6  $\pm$  0.34  $\mu$ M**. The **ortho-substituted halogen derivatives C5 (2-Cl), C7 (2-Br), and C3 (2-F)** displayed comparatively lower activity, with EC<sub>50</sub> values of **5.3  $\pm$  0.39**, **5.7  $\pm$  0.42**, and **8.5  $\pm$  0.56  $\mu$ M**, respectively.

The methyl-substituted compounds **C8 (2-CH<sub>3</sub>)** and **C9 (3-CH<sub>3</sub>)** exhibited moderate activity, with EC<sub>50</sub> values of **6.8  $\pm$  0.63** and **7.5  $\pm$  0.59  $\mu$ M**, respectively. The hydroxyl-substituted derivative **C10 (2-OH)** showed an EC<sub>50</sub> of **6.3  $\pm$  0.42  $\mu$ M**.

Overall, the data suggest that **halogen substitution, particularly para-bromine (4-Br), favored antiparasmodial activity**, while ortho substitution generally resulted in reduced potency. Among the halogen derivatives, the observed activity trend was **4-Br > 4-Cl > 2-Cl > 2-Br > 4-F > 2-F**. However, the contribution of substituent position, electronic effects, and lipophilicity should be interpreted cautiously, as the differences in activity may arise from multiple structural factors.

Although none of the synthesized compounds was more potent than artemisinin, **C6 (4-Br) showed the highest activity among the synthesized derivatives and**

**approached the activity of the reference drug**, identifying it as a promising lead for further structural optimization and biological investigation.<sup>[34,35]</sup>

A clear structure–activity relationship (SAR) was observed among the synthesized quinazoline derivatives. **The nature and position of substitution on the phenyl ring markedly influenced antiparasmodial activity**, with para-substituted derivatives generally exhibiting superior activity compared with their corresponding ortho-substituted analogues. This trend was particularly evident among the halogen derivatives, where **4-Br (C6) > 4-Cl (C4)** and the para-substituted analogues showed better activity than their ortho counterparts. The enhanced activity of para-substituted derivatives may be attributed to reduced steric hindrance and a more favourable orientation within the PfLDH binding pocket.

Furthermore, **electron-withdrawing halogen substituents (F, Cl, and Br)** generally enhanced biological activity, with **4-Br substitution producing the highest potency** among the synthesized compounds. This may reflect favourable hydrophobic and electronic interactions with the target binding site. In contrast, methyl-substituted derivatives exhibited comparatively lower activity, suggesting that **electron-donating and sterically less favourable substituents may be less advantageous** for antiparasmodial activity. The moderate activity observed with the 2-OH derivative further indicates that hydrogen-bonding capability alone may not be sufficient to enhance potency when accompanied by an unfavourable substitution position.

Overall, the SAR findings indicate that **para-halogen substitution, particularly 4-Br, represents a favourable structural modification of the quinazoline scaffold** and provides a useful direction for further optimization of potent antiparasmodial candidates.<sup>[36,37]</sup>

A good correlation was observed between the molecular docking results and the biological activity of the synthesized quinazoline derivatives. Compounds showing **more favourable binding energies and acceptable RMSD values** generally exhibited enhanced antiparasmodial activity, suggesting that stronger and more stable interactions within the PfLDH binding pocket may contribute to improved biological potency.

In particular, **C6 (4-Br)** demonstrated the highest antiparasmodial activity among the synthesized derivatives (EC<sub>50</sub> = **3.8  $\pm$  0.34  $\mu$ M**) and showed favourable molecular docking interactions with PfLDH. **C4 (4-Cl)** also exhibited good *in vitro* activity (EC<sub>50</sub> = **4.6  $\pm$  0.34  $\mu$ M**), supporting the contribution of favourable target interactions to its biological response. Although **C2 (4-F)** showed comparatively weaker activity (EC<sub>50</sub> = **7.2  $\pm$  0.41  $\mu$ M**), its docking profile indicated favourable binding interactions, suggesting that factors beyond docking affinity may also influence cellular antiparasmodial activity.

Overall, the combined computational and experimental findings support the use of **molecular docking as a useful**

**preliminary predictive tool for prioritizing potential PfLDH inhibitors.** However, docking scores alone cannot establish PfLDH inhibition, and the proposed target relationship should be further validated through **recombinant PfLDH enzyme inhibition studies.** [38,39,40]

Overall, the synthesized quinazoline derivatives demonstrated **promising antiplasmodial activity against the chloroquine-resistant *P. falciparum* Dd2luc strain.** The combined biological evaluation and molecular docking studies highlighted the importance of **halogen substitution, particularly at the para position,** in enhancing antiplasmodial activity. Among the synthesized series, **C6 (4-Br), C4 (4-Cl), and C1 (-H)** emerged as the most promising derivatives based on their in vitro EC<sub>50</sub> values, with C6 exhibiting the highest activity ( $3.8 \pm 0.34 \mu\text{M}$ ). These compounds may serve as useful lead structures for further **structural optimization, PfLDH enzyme inhibition studies, mechanistic investigations, cytotoxicity assessment, and pharmacological evaluation** toward the development of novel antimalarial agents. [41–42]

## 7. CONCLUSION

The present study successfully designed, synthesized, and evaluated a series of ten N-substituted phenyl-3,4-dihydroquinazoline-4,6-diamine derivatives (C1–C10) as potential antimalarial agents against the chloroquine-resistant Plasmodium falciparum Dd2luc strain. Structural characterization by FT-IR, <sup>1</sup>H NMR, and mass spectrometry confirmed the successful synthesis of the target compounds. Molecular docking studies against Plasmodium falciparum lactate dehydrogenase (PfLDH, PDB ID: 1T26) revealed favourable binding energies ranging from  $-8.07$  to  $-7.00$  kcal/mol with RMSD values between 1.03 and 2.00 Å, indicating reliable binding conformations and stable ligand–protein interactions. [43,44]

The structure–activity relationship (SAR) analysis demonstrated that both the nature and position of the substituent significantly influenced antiplasmodial activity. Para-halogenated derivatives exhibited superior activity compared with the corresponding ortho-substituted analogues, indicating that para substitution provides a more favourable orientation within the PfLDH binding pocket. Among the electron-withdrawing substituents, 4-fluoro (C2), 4-bromo (C6), and 4-chloro (C4) derivatives showed the greatest antiplasmodial potency, whereas the unsubstituted derivative (C1) and methyl-substituted analogues displayed comparatively lower activity. These findings suggest that electron-withdrawing groups enhance ligand–protein interactions, while ortho substitution may reduce activity because of steric hindrance [45,46].

A good correlation between molecular docking and biological activity was observed throughout the study. Compounds exhibiting **favourable binding energies and acceptable RMSD values** generally demonstrated appreciable in vitro antiplasmodial activity, suggesting that favourable ligand–PfLDH interactions may contribute to the observed biological response. Among the synthesized

derivatives, **C6 (4-Br)** exhibited the highest antiplasmodial potency with an EC<sub>50</sub> of  $3.8 \pm 0.34 \mu\text{M}$ , followed by **C1 (-H)** with  $4.1 \pm 0.32 \mu\text{M}$  and **C4 (4-Cl)** with  $4.6 \pm 0.34 \mu\text{M}$  against the chloroquine-resistant *P. falciparum* Dd2luc strain. These compounds therefore emerged as the most promising derivatives of the series based on their experimental activity.

The enhanced activity of C6 and C4 is consistent with the favourable contribution of **para-halogen substitution**, which may promote appropriate molecular orientation and hydrophobic or other non-covalent interactions within the PfLDH binding pocket. However, the relationship between docking scores and biological activity was not absolute, as compounds such as C2 (4-F) did not show correspondingly high in vitro potency despite favourable computational interactions. This indicates that factors such as cellular uptake, molecular physicochemical properties, target accessibility, and parasite-specific effects may also influence the observed antiplasmodial activity.

Overall, the agreement between the computational predictions and experimental findings supports **molecular docking as a useful preliminary tool for identifying and prioritizing potential lead compounds** during antimalarial drug discovery. Nevertheless, docking results should be considered predictive rather than definitive, and the proposed PfLDH-targeting mechanism requires further confirmation through **recombinant PfLDH enzyme inhibition studies.** [41,44]

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## 9. CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this study.

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