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Original Article

# In Silico Analysis, Synthesis and Biological Evaluation of DHFR Inhibitors

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## Abstract

**Introduction:** Malaria is one of the varieties of fatal diseases caused by a protozoan parasite that is now considered to be the greatest global health challenge. A parasite of Plasmodium species triggers it transmitting the disease to humans by the bites of female *Anopheles* mosquitoes.

**Aim:** To screen out designed molecules by molecular docking analysis and assess their pharmacokinetic properties using SwissADME. To synthesize the designed compounds. To characterize the synthesized compounds by TLC, melting point, IR spectroscopy, mass spectrometry, $^{1}$H NMR, and $^{13}$C NMR. To evaluate the synthesized compounds for antimalarial activity.

**Materials and methods:** In silico analysis was performed with SWISSADME, and molecular docking was performed by AutoDock Vina version 4.2. In vitro antimalarial activity study was performed.

**Results:** In-vitro studies of synthesized molecules showed that compounds C2 ($IC_{50}$ 1.23), C6 ($IC_{50}$ 0.48), C10 ($IC_{50}$ 0.79), and C14 ($IC_{50}$ 0.19) possess good antimalarial activity.

**Conclusions:** 7-chloroquinoline-piperazine derivatives exhibited potential antimalarial compounds for *pf*-DHFR inhibitors.

## Keywords

7-chloroquinoline-piperazine, CQ-sensitive 3D7 strain, in-silico study, pf-DHFR, pharmacokinetic study

## INTRODUCTION

Malaria is one of the varieties of fatal diseases caused by a protozoan parasite that is now considered to be the greatest global health challenge.[1] A parasite of *Plasmodium* species triggers it transmitting the disease to humans by the bites of female *Anopheles* mosquitoes.[2] It's furthermost widespread in sub-African, Asian and South American countries, and most distressing to children under the age of 5 and to pregnant women. According to the World Health Organization (WHO) 2019 report, an estimated 219 million cases were reported in 2017, with 435,000 deaths globally. Malaria may be a life-threatening disease.[3] Because of the devastating effects on human population, WHO rates malaria as one of the top three infectious diseases.[4] The disease is caused by any one of the species of *Plasmodium* parasites, namely *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax,* and *P. knowlesi*.[5]

The potential of dihydrofolate reductase (DHFR) enzyme as a therapeutic target in treating infections has been noticed since the middle of the last century.[6,7] DHFR inhibitors are commonly used for fighting malaria and other protozoan diseases, as well as for treating fungal, bac-

terial, and mycobacterial infections.[8] Over the years, several compounds have been discovered, and different drugs have entered the market. Among them, we have to mention pyrimethamine and proguanil as antimalarial drugs.[9,10]

4-aminoquinoline hybridization is now considered as an attractive and viable strategy for preventing and delaying the emergence of drug resistance along with the improvement in efficacy.[11-14] A researcher has reported several substituted 4-aminoquinoline derivatives with antimalarial activity.[15-17] Chalcones and dienones are structurally linked compounds exhibiting notable in vitro and in vivo antimalarial activity[18-21] by acting as inhibitors of either plasmodial aspartate proteases,[22] cysteine proteases or permeability pathways initiated into erythrocyte cell membranes by the malaria parasite[23]. Mallika Pathak et al. have done the design, synthesis and biological evaluation of antimalarial activity of derivatives of 2,4,6-s-triazine.[24] Xue-Qian Bai et al. have reported synthesis, antimicrobial activities, and molecular docking studies of dihydrotriazine derivatives bearing a quinoline moiety.[25]

Additionally, these drugs were characterized by their chemical structure: amino alcohols (quinine, mefloquine, lumefantrine, halofantrine), 4-aminoquinolines (chloroquine, amodiaquine, piperaquine, pyronaridine), 8-aminoquinoline (primaquine), naphthoquinone (atovaquone), antifolates (sulfadoxine-pyrimethamine, proguanil), endoperoxides (artemisinin and its derivatives) (**Fig. 1**). Amongst the currently available clinical antimalarial drugs, the antifolates have the first useful defined molecular targets: the enzymes dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS), functioning within the folate metabolic pathway shown in **Fig. 2** describing the folate metabolism pathway. **Fig. 3** shows the design strategy of DHFR inhibitor.

These two pathways are targeted in both treatment and prophylaxis of the disease. The foremost widely used antifolate antimalarial drugs include pyrimethamine, proguanil, sulfadoxine, and dapsone which have long provided chemotherapy at a low price to the poorer nations. 7-chloroquinoline, the nucleus of chloroquine and piperazine, which is the core of piperaquine when joined together with a linker, meets the whole structural requirement just like the presence of a hydrophobic tail and bond donor head group, respectively, for inhibition of pf-DHFR-TS. Recently, conjugates of 7-chloroquinoline and piperazine are widely studied as novel pf-DHFR-TS inhibitors. As part of our on-going research work to develop hybrid antimalarial molecules, we have designed a replacement series of hybrid 7-chloroquinoline-piperazine derivatives. Supporting the in-silico results, some selected molecules were tested for antimalarial activity against the 3D7 strain of *Plasmodium falciparum*.

## AIM

To screen out designed molecules by molecular docking analysis and assess their pharmacokinetic properties using SwissADME. To synthesize the designed compounds. To characterize the synthesized compounds by TLC, melting point, IR spectroscopy, mass spectrometry, $^{1}$H NMR, and $^{13}$C NMR. To evaluate the synthesized compounds for antimalarial activity.

## MATERIALS AND METHODS

### Materials

All the chemicals and solvents used for synthesis, recrystallization and analysis were of AR grade and used without further purification. The temperature of the synthesized compounds was resolute by temperature apparatus. The FTIR spectra of the synthesized compounds were recorded on Bruker optics alpha FTIR spectrometer. IR spectra of compounds showed transmission, which is characteristic of the expected structure of the synthesized compounds. The $^{1}$H NMR spectra of the synthesized compounds were recorded in $CDCl_3$ at 400 MHz by Bruker 400 MHz NMR spectrometer, and $^{13}$C NMR was also recorded in $CDCl_3$ at 100 MHz by Bruker 400 MHz $^{1}$H NMR spectrometer from Indian Institute of Science, Bangalore. The mass spectra of the synthesized compounds were recorded on empowering software equipped with an Electrospray ionizer as an ionization method from KM Pharma Solution, Ahmedabad, Gujarat.

### Methods

#### *Molecular docking studies*

Molecular docking simulations were conducted on the DHFR inhibitors using the AutoDock Vina 4.5 to get insight into their binding preferences within the active site of the receptor against the wild type of *Plasmodium falciparum* dihydrofolate reductase (PDB entry: 4DPD, resolution = 2.50 **Å)** obtained from the protein data bank (RCSB). The protein structure was prepared using the Discovery Studio Visualizer (version 3.1) and AutoDock Tools (ADT; version 1.5.4) through different steps viz. removal of water molecules and detached co-crystallized ligand, retention of cofactors NADPH, dUMP, the addition of missing hydrogen atoms. Moreover, the file was then saved in pdbqt file format for further analysis. However, Pf-DHFR-TS consists of chain A, chain B, chain C, and chain D in which the DHFR domain is present at chain C and chain D, and TS domain having chain A and chain B.

The grid maps of the interaction energies of various atom types were pre-calculated using AutoGrid 4.5. In each docking for DHFR inhibitors, a grid box was created using a grid map of 45×45×45 points, 60×60×60 points with a grid spacing of 0.375 **Å** and 0.420 **Å,** respectively. The grid maps were centred on the corresponding ligand binding

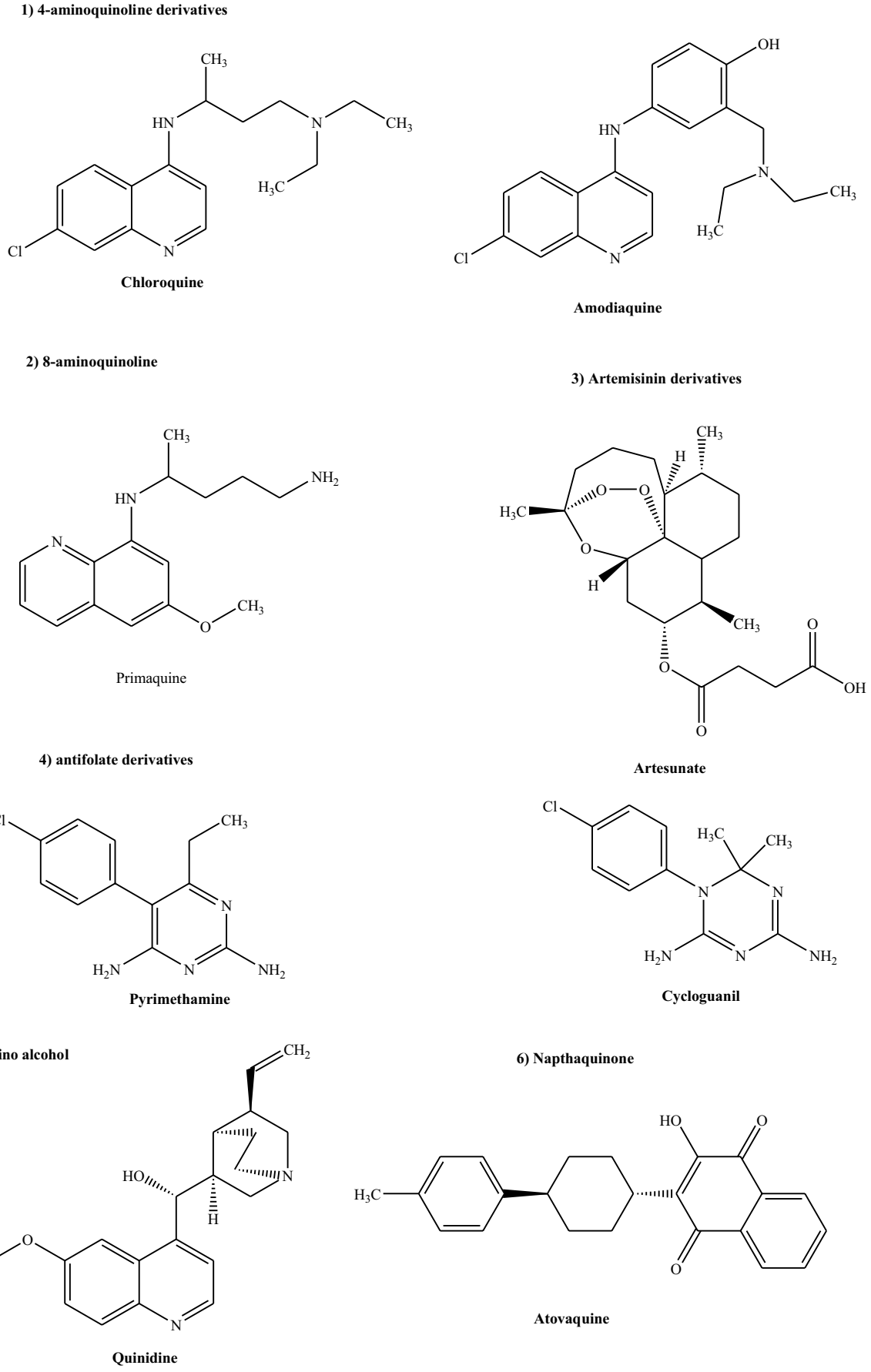


**Figure 1.** Drugs used for the treatment of malaria.



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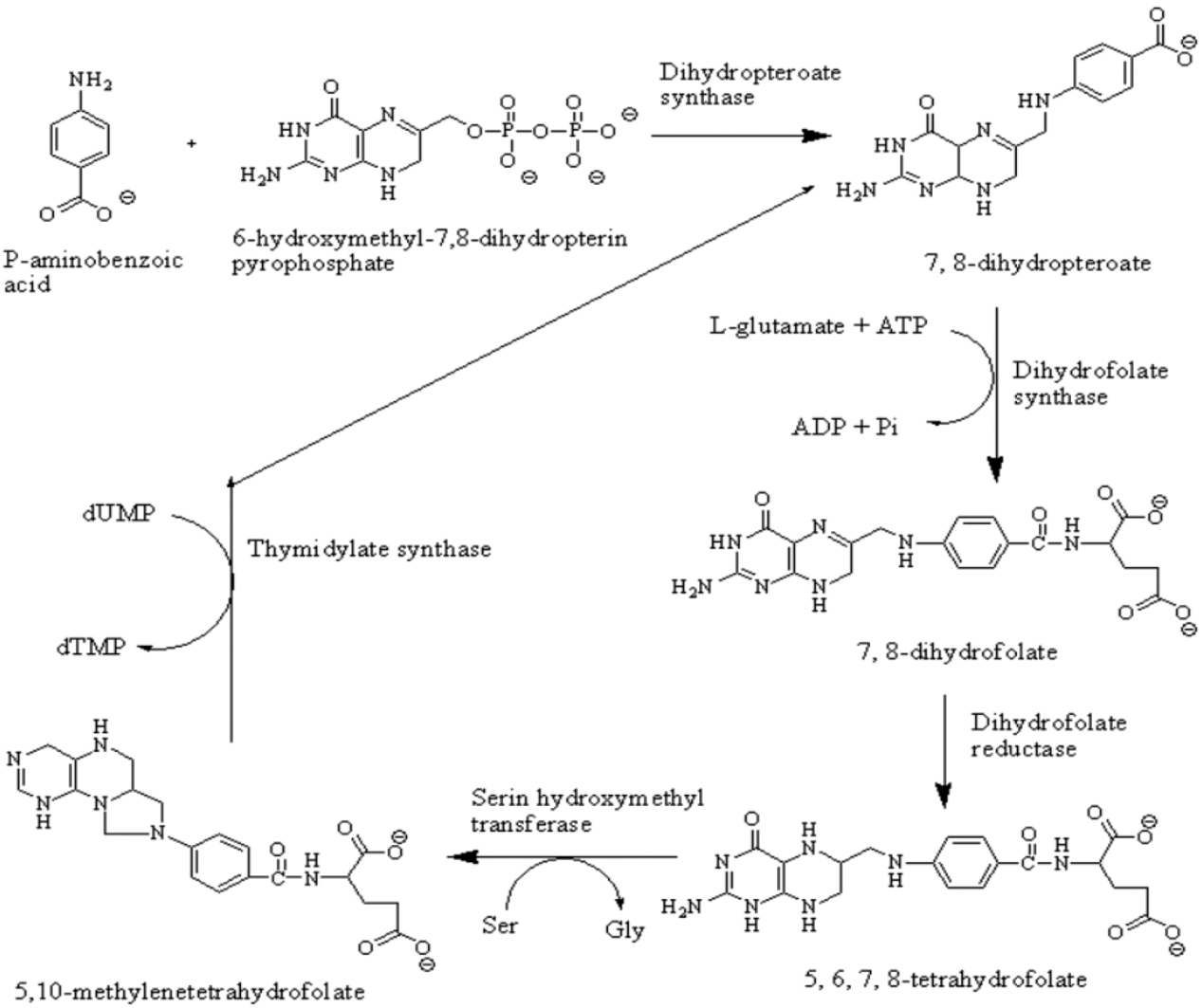



**Figure 2.** Outline of the folate metabolism pathway.

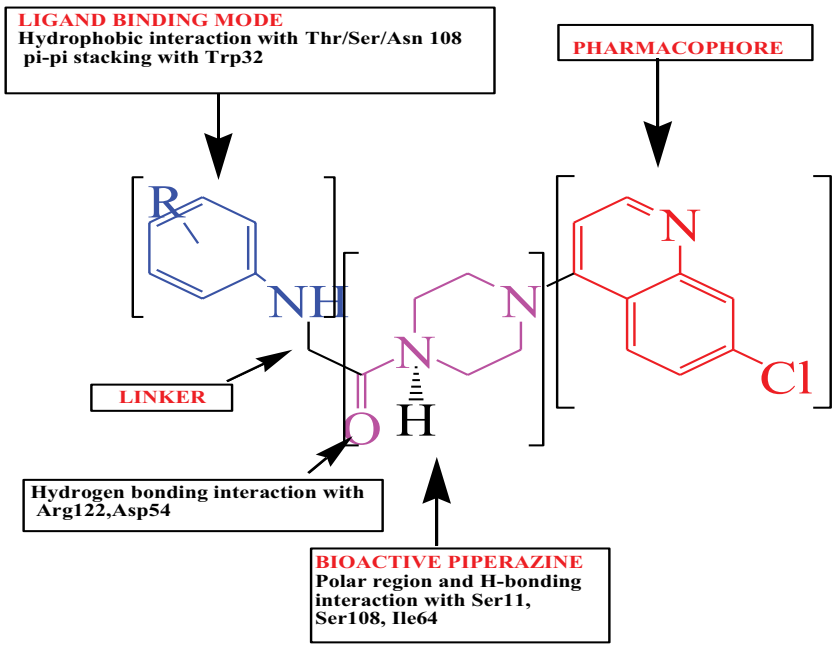
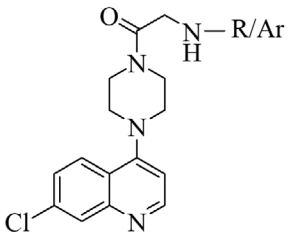



**Figure 3.** Design strategy of DHFR inhibitor.

site within the protein structure.

All computations were carried out on Cygwin and were used to generate both the grid parameter file (.gpf file) and a docking parameter file (.dpf file) for each ligand. The docked conformations of each ligand were ranked into clusters based on the binding energy, and the top-ranked conformations were used for further study. The pose with the lowest ΔG-score was considered the best-fitted one and was further analyzed for ligand-receptor interactions.

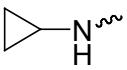
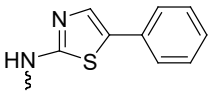
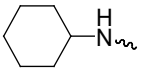
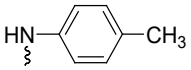
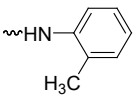
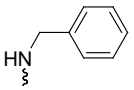
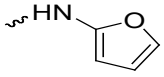
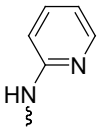
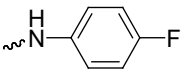
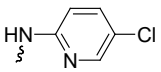
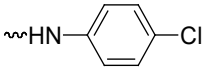
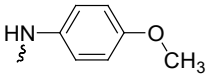
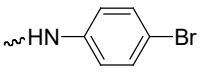
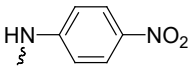
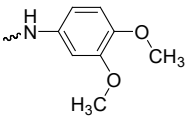
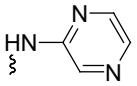



Moreover, a simulated library of hybrid 7-chloroquinoline-piperazine was designed **(Table 1)** by joining with alkyl ketone with different amines for improved H-bonding with the target receptor docked using Auto dock vina 2.5. Moreover, the protocol was validated by calculating the RMSD value, which should be less than 2 **Å** for the best result by taking pyrimethamine as standard.

### *In silico analysis*

Using the SWISSADME program[26,27], and in silico toxicity profile of the designed compounds was carried out to assess the theoretical pharmacokinetic parameters of the ligands to predict the drug-likeness of ligands. Drug development involves assessing absorption, distribution, metabolism and excretion (ADME) increasingly earlier in the discovery process, at a stage when considered compounds are numerous but access to the physical samples is limited.

**Table 1.** Binding affinity of designed compounds

| Code | R/Ar-$NH_2$ | Autodock Binding Score (kcal/mol) | Code | R/Ar-$NH_2$ | Autodock Binding Score (kcal/mol) |
|---|---|---|---|---|---|
| C1 |  | -9.3 | C9 |  | -9.6 |
| C2 |  | -8.4 | C10 |  | -8.9 |
| C3 |  | -9.4 | C11 |  | -9.0 |
| C4 |  | -8.9 | C12 |  | -9.4 |
| C5 |  | -8.9 | C13 |  | -9.0 |
| C6 |  | -9.3 | C14 |  | -9.2 |
| C7 |  | -8.0 | C15 |  | -8.0 |
| C8 |  | -9.5 | C16 |  | -9.4 |



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## Synthesis of Intermediates

### Synthesis of 7-chloro-4-(piperazine-1-yl)quinoline from 4,7- dichloroquinoline (3) [28]

Weigh 1 g (5.04 mmol) of 4,7-dichloroquinoline, 1.3 g (10.08 mmol) piperazine, 0.42 g iodide and 10 volume isopropanol were taken in RBF. The reaction mixture was then refluxed under stirring at 90°C for six hours, and TLC monitored the completion of the reaction. The IPA was evaporated by using downward distillation to obtained yellow residue. DCM and water were added. The pH was adjusted with HCl up-to 3-3.5. The organic and aqueous layer was separated. The aqueous layer was collected, and the pH with $NH_3$ up-to 10-11. From mixture with DCM and the organic layer was collected. The organic layer was evaporated using a Rota evaporator under high vacuum to an obtained yellow solid residue.

*IR (KBr): v($cm^{-1}$); 3257 (N–H str), 1293.11 (C–N str); 2940.10 (C-H, Ar str); 2878 (C-H, str)*

### Synthesis of 2-chloro-1-(4-(7-chloroquinoline-4- yl) piperazine-1-yl) ethenone (4) [29]

Placed 1 g (4.04 mmol) of compound 2 (7-chloro-4-(piperazine-1-yl) quinoline) in RBF and dissolved it in 5 ml of DCM with stirring. To the current solution, $NaHCO_3$ and water were added. Cool the reaction mixture upto 0-5°C, then add 0.3885 ml (4.84 mmol) of chloracetyl chloride. The reaction mixture was stirred under room temperature for 1.5 hours, and TLC monitored the completion of the reaction. After completion of the reaction, take the reaction mixture in separating funnel and extracted with DCM. Both organic and aqueous layer was separated. In the organic layer, $Na_2SO_4$ was added, and the solution was evaporated by using a rotatory evaporator under a high vacuum to obtained precipitate.

*IR (KBr): v($cm^{-1}$) 3398 (N–H str), 1369 (C–N str); 3010 (C-H, Ar str); 2926 (C-H, str); 1757 ( C=O, str); 794 (C-Cl, str) MS: m/z = 324 (M)*

## Synthesis of final compounds

### General procedure for the synthesis of substituted amines, 7-chloroquinoline derivatives [30]

In RBF, add accurately weigh a mix of compound 2-chloro-1-(4-(7- chloroquinolin-4-yl)piperazine-1-yl)ethenone (4) (0.616 mmol), substituted amine (0.739 mmol) and anhydrous $K_2CO_3$ (2.22 mmol) was dissolved in dry DMF. It was stirred and refluxed for 1 hr at 110°C. The reaction mixture was cooled and poured into ice-cold water. The reaction was filtered and dried under a high vacuum to obtained dark brown solid precipitates. The column chromatography purified all the compounds.

### The procedure of column chromatography

A column was fixed in a stand. Silica gel G was added with mesh 100-200. The minimum amount of compound was added in a solvent (DCM). The solvent was evaporated in a rota evaporator at low temperature. Dry powder was transferred to the top of the column into the funnel. Sodium sulphate was added. The separation procedure was started by adding hexane. The stopcock was opened, and the fraction of the solvent was collected in a beaker.

Spectral data of synthesized derivatives are given in **Table 2**.

*1-(4-(7-chloroquinolin-4-yl) piperazine-1-yl)-2-(cyclopropylamino)ethanone (C1):* yield: 60.72%; m.p.:122-124°C

*1-(4-(7-chloroquinolin-4-yl)piperazine-1-yl)-2(cyclohexylamino)ethanone (C2):* yield: 65.32%; m.p.: 128-130°C

*2-(o-tolylamino)-1-(4-(7-chloroquinolin-4-yl)piperazine-1-yl)ethanone (C3)*: yield: 75.89%; m.p.: 142- 144°C

*1-(4-(7-chloroquinolin-4-yl)piperazine-1-yl)-2-(furan-2-ylamino)ethanone (C4)*: yield: 55.55%; m.p.: 140-142°C

*2-(4-fluorophenylamino)-1-(4-(7-chloroquinolin-4-yl) piperazine-1-yl)ethanone (C5)*: yield: 68.89%; m.p.: 140-142°C

*2-(4-chlorophenylamino)-1-(4-(7-chloroquinolin-4-yl) piperazine-1-yl)ethanone (C6)*: yield: 67.89%; m.p.: 134-136°C

*2-(4-bromophenylamino)-1-(4-(7-chloroquinolin-4-yl)piperazine-1-yl)ethanone (C7)*: yield: 62.89%; m.p: 142-144°C

*2-(3,4-dimethoxyphenylamino)-1-(4-(7-chloroquinolin-4-yl)piperazine-1-yl)ethanone (C8)*: yield: 62.89%; m.p: 140-142°C

*2-(p-tolylamino)-1-(4-(7-chloroquinolin-4-yl)piperazine-1-yl)ethanone (C10)*: yield: 72.77%; m.p: 134-136°C

*2-(benzylamino)-1-(4-(7-chloroquinolin-4-yl)piperazine-1-yl)ethanone (C11)*: yield: 70.77%; m.p: 134-136°C

*2-(4-methoxyphenylamino)-1-(4-(7-chloroquinolin-4-yl) piperazine-1-yl)ethanone (C14)*: yield: 70.87%; m.p: 140-142°C

*2-(p-nitrophenylamino)- 1-(4-(7-chloroquinolin-4-yl)piperazine1-yl)ethanone (C15)*: yield: 69.87%; m.p: 120-122°C

## Antimalarial activity[31,32]

The in vitro antimalarial assay was scattered in 96 well small title plates to keep with the microassay protocol of Rieckmann and associates with minor modifications. The cultures of *P. Falciparum* strain were maintained in medium RPMI1640 supplemented with 25 millimetres HEPES, 1 Chronicles D-glucose, 0.23% bicarbonate and 100%

**Table 2.** Spectral data of all the compounds

| Compound code | IR ($cm^{1}$) Wavenumber | MASS | Proton NMR (δ ppm) | $^{13}C$ NMR (δ ppm) |
|---|---|---|---|---|
| C1 | 3245 (N-H, str), 3031 (C-H, Ar str), 2975 (C-H, Ar str), 1657 (C=O, str), 1550, 1388 (C=C, Ar str), 1629 (C=N, str); | ---- | ------ | ----- |
| C2 | 3374 (N-H, str), 3091(C-H, Ar str), 2924 (C-H, Ali str), 1639 (C=O, str), 1607 (C=N, str), 1575, 1379 (C=C, Ar str); | 387.2 (M+1) | 8.64 (d, 1H, quinoline), 6.49 (d, 1H, quinoline), 8.0 (s, 1H, quinoline), 7.43 (d, 1H, quinoline), 7.61-7.62 (d, 1H, quinoline), 3.32-3.34 (t, 4H, piperazine), 3.56-3.58 (t, 4H, piperazine), 3.44 (s, 2H, $CH_2$), 1.39-1.64 (t, 4H, cyclohexyl), 1.25-1.27 (m, 4H, cyclohexyl), 1.49-1.52 (m, 2H, cyclohexyl); | ---- |
| C3 | 3398 (N-H, str), 3068 (C-H, Ar str), 2981 (C-H, Ali str), 1629 (C=O, str), 1606 (C=N, str), 1574, 1379 (C=C, Ar str); | 395.4 (M+1) | 3.24 (t, 4H, piperazine), 3.96 (t, 4H, piperazine), 3.52 (s, 2H, $CH_2$), 3.76 (s, NH), 6.85-6.86 (d, 2H, ArH), 7.010-7.031 (d, 2H, ArH), 7.46-7.47 (d, 1H, quinoline), 7.48-7.49 (d, 1H, quinoline), 7.951-7.973 (d, 1H, quinoline), 6.57-6.59 (d, 1H, quinoline), 8.083-8.088 (s, 1H, quinoline); | ----- |
| C4 | 3398 (N-H, str), 3068 (-C-H, Ar str), 2981 (CH, Ali str), 1629 (C=O, str), 1606 (C=N, str), 1574, 1379 (C=C, Ar str); | -------- | -------- | -------- |
| C5 | 3358 (N-H, str), 3065 (C-H, Ar str), 2982 (CH, Ali str), 1655 (C=O, str), 1608(C=N, str), 1576, 1380 (C=C, Ar str); | 399.4(M+1) | 3.9 (d, 2H, $CH_2$), 3.23-3.26 (t, 4H, piperazine), 3.74-3.77 (t, 4H, piperazine), 3.98 (s, NH), 8.087 (d, 1H, quinoline), 6.55-6.58 (d, 1H, quinoline), 8.092 (s, quinoline),7.47 (d, 1H, quinoline), 7.49 (d, 1H, quinoline), 6.55-6.57 (d, 2H, ArH), 7.351-7.356 (d, 2H, ArH); | --------- |
| C6 | 3349 (N-H, str), 3097 (C-H, Ar str), 2919 (C-H, Ali str), 1650 (C=O, str), 1604 (C=N, str), 1574, 1379 (C=C, Ar str); | 415.6 (M+1), 416.56 (M+2) | 3.9 (d, 2H, $CH_2$), 3.23-3.26 (t, 4H, piperazine), 3.74-3.77 (t, 4H, piperazine), 3.98 (s, NH), 8.087 (d, 1H, quinoline), 6.55-6.58 (d, 1H, quinoline), 8.092 (s, H, quinoline), 7.47 (d, 1H, quinoline), 7.49 (d, 1H, quinoline), 6.55-6.57 (d, 2H, ArH), 7.351-7.356 (d, 2H, ArH); | ------ |
| C7 | 3344 (N-H, str), 3064 (C-H, Ar str), 2917 (CH, Ali str), 1648 (C=O, str), 1606 (C=N, str), 1574, 1379 (C=C, Ar str); | 459.7 (M+1) | 3.9 (d, 2H, $CH_2$), 3.23-3.26 (t, 4H, piperazine), 3.74-3.77 (t, 4H, piperazine), 3.98 (s, NH), 8.087 (d, 1H, quinoline), 6.55-6.58 (d, 1H, quinoline), 8.092 (s, H, quinoline), 7.47 (d, 1H, quinoline), 7.49 (d, 1H, quinoline), 6.55-6.57 (d, 2H, ArH), 7.351-7.356 (d, 2H, ArH); | -------- |
| C8 | 3365 (N-H, str), 3065 (C-H, Ar str), 2925 (C-H, Ar str), 1657 (C=O, str), 1607 (C=N, str), 1574,1379 (C=C, Ar str); | ----- | 3.9 (d, 2H, $CH_2$), 3.46-3.71 (t, 4H, piperazine), 3.74-3.77 (t, 4H, piperazine), 3.98 (s, NH), 8.1 (d, 1H, quinoline), 6.55-6.58 (d, 1H, quinoline), 8.092 (s, H, quinoline), 7.47 (d, 1H, quinoline), 7.49 (d, 1H, quinoline), 6.52-6.58 (d, 4H, ArH), 7.15-7.65 (d, 2H, ArH), 3.52 (s,6H, $OCH_3$); | -------- |



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| | | | | |
|---|---|---|---|---|
| C10 | 3351 (N-H, str), 3064 (C-H, Ar str), 2910 (C-H, Ali str), 1654 (C=O, str), 1606 (C=N, str), 1574, 1379 (C=C, Ar str); | 395.4 (M+1) | 6.15 (d, 1H, quinoline), 8. 3.24 (t, 4H, piperazine), 3.96 (t, 4H, piperazine), 3.52 (s, 2H, CH2), 3.76 (s, NH), 6.856.86 (d, 2H, ArH), 7.010-7.031 (d, 2H, ArH), 7.46-7.47 (d, 1H, quinoline), 7.48-7.49 (d, 1H, quinoline), 7.951-7.973 (d, 1H, quinoline), 6.57-6.59083-8.088 (s, 1H, quinoline); | 150.2 (quinoline C-2, C4), 52.27 (piperazine C-2, C-6), 44.56 (piperazine C-3), 45.99 (piperazine C-5), 145.26 (Ar C1), 113.4 (Ar C-2, C-6), 129.18 (Ar C-3, C-5), 127.3 (Ar C-4), 168.35 (C=O), 52.39 ($CH_2$), 22.89 (Ar-$CH_3$); |
| C11 | 3397 (NH, str), 3062 (C-H, str), 2922 (-C-H, Ali str), 1641 (C=O, str), 1606 (C=N, str), 1573, 1379 (-C=C, Ar str); | ---- | 6.78 (d, 1H, quinoline), 8. 3.14 (t, 4H, piperazine), 3.36 (t, 4H, piperazine), 3.54 (s, 2H, CH2), 3.78 (s,NH), 7.15-7.26 (d, 2H, ArH), 7.010-7.031 (d, 2H, ArH), 7.46-7.47 (d, 1H, quinoline), 7.48-7.49 (d, 1H, quinoline), 7.953-7.979 (d, 1H, quinoline); | --- |
| C14 | 3386 (N-H, str), 3067 (C-H, Ar str), 2922 (-C-H Ali str), 1647 (C=O, str), 1607 (C=N, str), 1575, 1380 (-C=C, Ar str); | 395.4 (M+1) | 3.24 (t, 4H, piperazine), 3.96 (t, 4H, piperazine), 3.52 (s, 2H, CH2), 3.76 (s, NH), 6.856.86 (d, 2H, ArH), 7.010-7.031 (d, 2H, ArH), 7.46-7.47 (d, 1H, quinoline), 7.48-7.49 (d, 1H, quinoline), 7.951-7.973 (d, 1H, quinoline), 6.57-6.59 (d, 1H, quinoline), 8.083-8.088 (s, 2H, quinoline); | 150.2 (quinoline C-2, C4), 52.27 (piperazine C-2, C-6), 44.56 (piperazine C-3), 45.99 (piperazine C-5), 145.26 (ArC1), 113.4 (ArC-2, C-6), 129.18 (ArC-3, C-5), 127.3 (ArC-4), 168.35 (C=O), 52.39 ($CH_2$), 22.89 (Ar-$CH_3$); |
| C15 | 3341 (N-H, str), 3077 (-C-H, Ar str), 2921 (-C-H Ali str), 1632 (C=O, str), 1599 (C=N, str), 1574, 1380 (-C=C, Ar str); | ---- | 3.9 (d, 2H, -$CH_2$), 3.23-3.26 (t, 4H, piperazine), 3.74-3.77 (t, 4H, piperazine), 3.98 (s, NH), 8.087 (d, 1H, quinoline), 6.55-6.58 (d, 1H, quinoline), 8.092 (s, 1H, quinoline), 7.47 (d, 1H, quinoline), 7.49 (d, 1H, quinoline), 6.55-6.57 (d, 2H, ArH), 7.351-7.356 (d, 2H, ArH); | ----- |

heat-inactivated human blood serum. The asynchronous parasites of P. falciparum were synchronous once five-hitter D-sorbitol treatment to urge the ring stage parasitized cells solely. The 8 to 1.5% at 3-D hematocrit in associate degree extremely total volume of µl of medium RPMI-1640 created up our minds staining to assess the % parasitemia (rings) and uniformly maintained with five hundredth RBCs (O+). A stock resolution of 5 mg/ml of each of the check samples was ready in DMSO, and resulting dilutions were ready with the matter. The diluted samples in 20 µl volume were side to the check wells thus, on getting final concentrations (at multiple dilutions), go between 0.4 µg/ml to 100 µg/ml in duplicate well containing parasitized cell preparation.

The culture plates were incubated at 37°C in associate degree extremely candle jar. Once 36 to 40 h incubation, skinny blood smears from every well were ready and stained with JSB stain. The slides were microscopically ascertained to record the maturation of ring-stage parasites into trophozoites and schizonts in the presence of various concentrations of the check agents. The check concentration that suppressed the full maturation into schizonts was recorded due to the minimum repressing concentrations (MIC). Antimalarial was used as a result of the reference drug. The mean variety of rings, trophozoites and schizonts recorded per a hundred parasites from duplicate wells once incubation for 38 hours and % maturation inhibition with relevance management cluster.

## RESULTS AND DISCUSSION

### Molecular docking[33] and pharmacokinetic studies[34,35]

The binding affinity of the designed compounds is given in **Table 1**. Docked poses of all the compounds having bind-

ing energy between -10 to +10. Most of the compounds having a binding affinity between -8.0 to -9.6 compare to pyrimethamine. These compounds were selected for synthesis, which was having good interactions with receptor showing in **Figs 4, 5, 6**. Among these compounds, C1, C2, C4, and C10 have shown good interaction with the receptor-like Phe 116, Pro 113, Ile 112, Leu 46, Lys 49. The presence of tertiary amine in piperazine showing pi-pi interaction with aminoalkanoic acid residue Phe 58. **Table 3** describes the prediction of the Lipinski rule of designed 7-chloroquinoline derivatives. Different pharmacokinetic parameters of the most active compounds were calculated using ADME/T predictions by SWISS ADME online tools. All 7-chloroquinoline derivatives are fully in agreement with the Lipinski rule of five for prospective small molecular drugs: MW ≤ 500, log P ≤ 5, number of H-bond donors ≤ 5, number of H-bond acceptors ≤ 10, molecular polar surface area (PSA) < 140 Å. It shows that all the compounds hold the potential of flattering an orally active drug. The pharmacokinetic properties of designed compounds were mentioned in **Table 4**. In silico pharmacokinetics, results specify that all the molecules possess high GI absorption and no blood-brain barrier (BBB) permeation.

## Chemistry

The synthesis of the intermediates and the final compounds was completed by the procedure shown in **Fig. 7**. 7-chloro-4-(piperazine-1-yl)quinolone (3) was synthesized by substituting the chlorine in the fourth position of the 4,7-di-

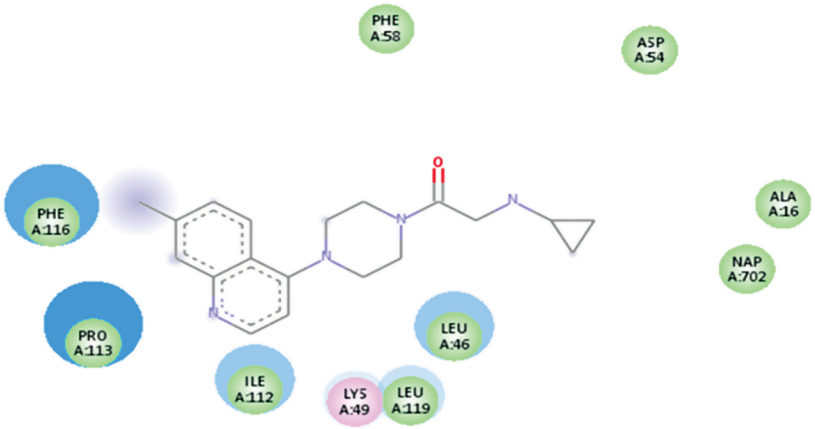


**Figure 4.** Structural screenshot of superimposed DHFR inhibitor C1 docked into the binding pocket *P. falciparum* dihydrofolate reductase (PDB ID: 4DPD). It has shown good interaction with the receptor like Phe 116 (green), Pro 113 (green), Ile 112 (green), Leu 46 (green), Lys 49 (pink).

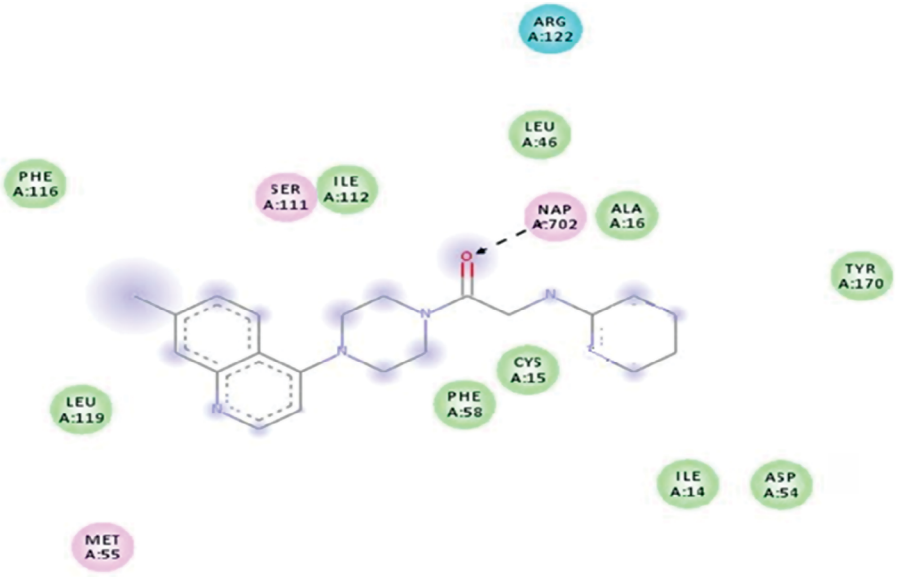


**Figure 5.** Structural screenshot of superimposed DHFR inhibitor C2 docked into the binding pocket *P. falciparum* dihydrofolate reductase (PDB ID: 4DPD). It has shown good interaction with the receptor like Phe 58 (green), Pro 113 (green), Ile 112 (green), Leu 119 (green), Lys 49 (pink), Phe 116 (green), NAP 702 (pink), ser 111 (pink), Met (pink).

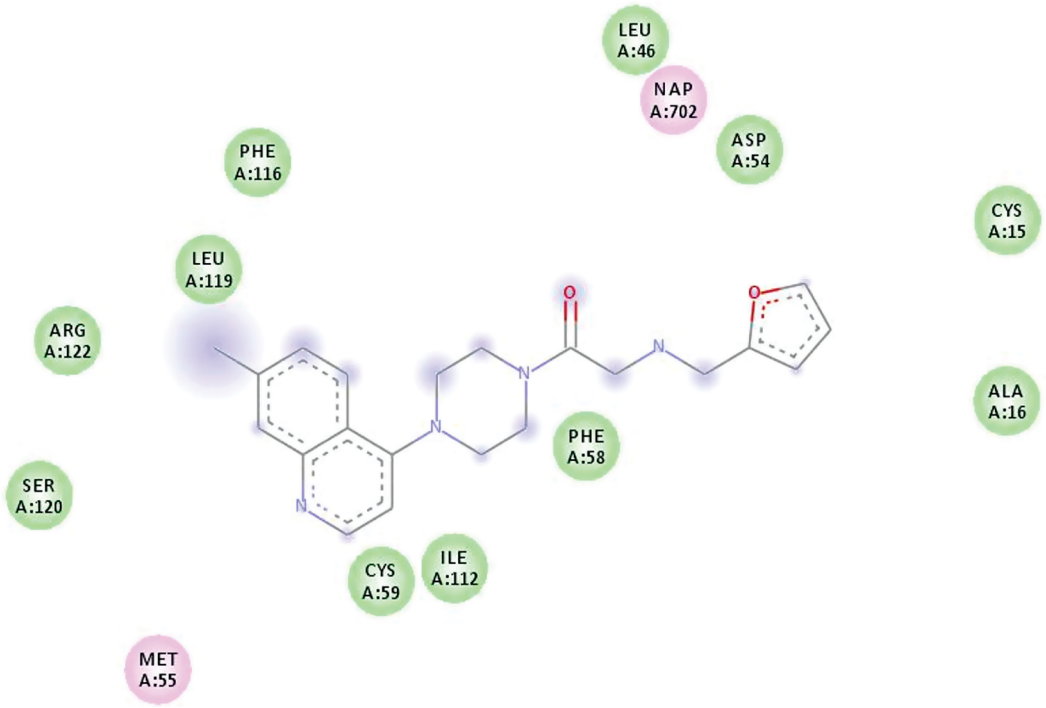


**Figure 6.** Structural screenshot of superimposed DHFR inhibitor C4 docked into the binding pocket P. falciparum dihydrofolate reductase (PDB ID: 4DPD). It has shown good interaction with the receptor like Phe 58 (green), Leu 46 (green), Pro 113 (green), Ile 112 (green), Leu 119 (green), Phe 116 (green), Arg 122 (green), NAP 702 ( pink), Ser 111 (pink), Met 55 (pink), Phe 58 (green).

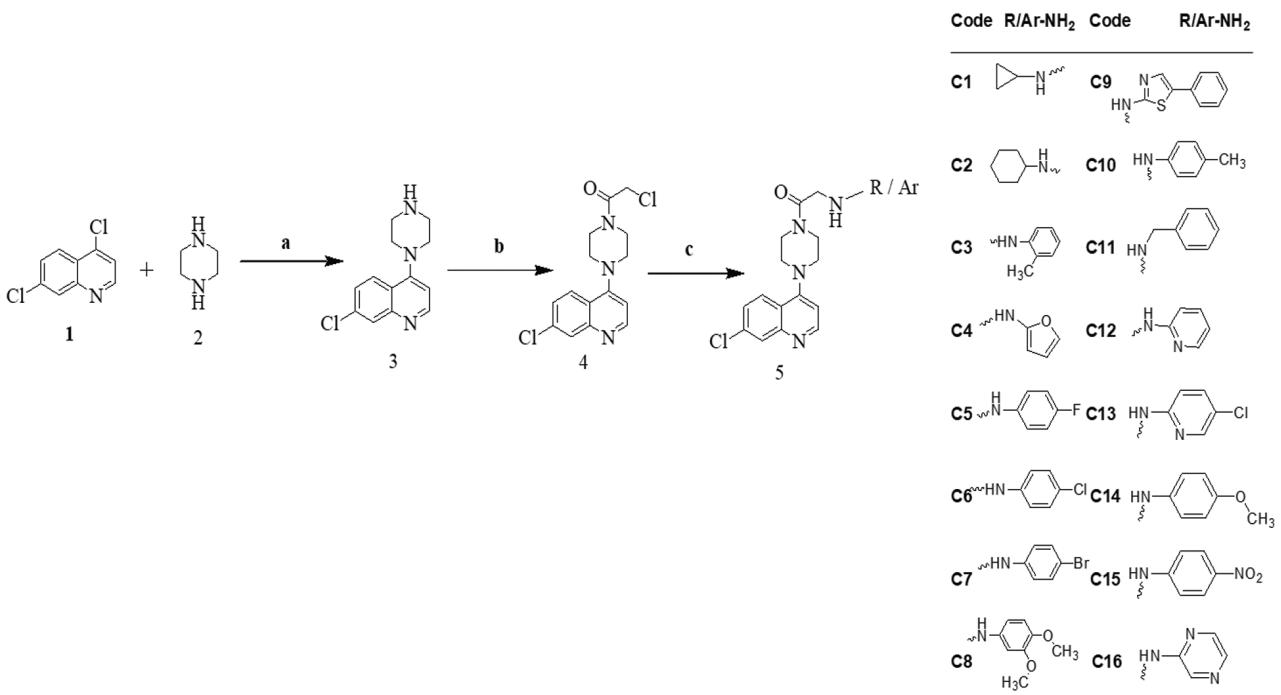


**Figure 7.** Chemistry scheme. Reagents and conditions: **a**) KI, Isopropyl alcohol (IPA), reflux at 90°C for 6 hours.; **b**) Chloroacetylchloride, DCM, $NaHCO_3$, $H_2O$, 0-5°C for 2 hours; **c**) Substituted aromatic/aliphatic amines, dry DMF, anhydrous $K_2CO_3$, reflux at 110°C for 1 hour.



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**Table 3.** Prediction of Lipinski rule of synthesised compounds

| Code | Molecular weight (g/mol) | H-bond acceptor | H-bond donor | LogP | Lipinski Rule |
|---|---|---|---|---|---|
| Standard value | <500 | <10 | <5 | <5 | Yes/No |
| C1 | 344.84 | 3 | 1 | 1.85 | Yes |
| C2 | 386.92 | 3 | 1 | 2.52 | Yes |
| C3 | 394.9 | 2 | 1 | 2.77 | Yes |
| C4 | 384.86 | 4 | 1 | 1.29 | Yes |
| C5 | 415.32 | 2 | 1 | 3.04 | Yes |
| C6 | 398.86 | 3 | 1 | 2.93 | Yes |
| C7 | 459.77 | 2 | 1 | 3.15 | Yes |
| C8 | 440.92 | 4 | 1 | 1.91 | Yes |
| C9 | 463.98 | 3 | 1 | 2.6 | Yes |
| C10 | 394.9 | 2 | 1 | 2.77 | Yes |
| C11 | 394.9 | 3 | 1 | 2.5 | Yes |
| C12 | 381.86 | 3 | 1 | 1.93 | Yes |
| C13 | 416.3 | 3 | 1 | 2.42 | Yes |
| C14 | 410.9 | 3 | 1 | 2.23 | Yes |
| C15 | 426.88 | 4 | 2 | 2.45 | Yes |
| C16 | 382.85 | 4 | 1 | 0.91 | Yes |

**Table 4.** Pharmacokinetic properties of designed compounds

| Code | GI absorption | BBB permeation | Pgp substrate | CYP1A2 | CYP2C19 | CYP2C9 | CYP2D6 | CYP3A4 |
|---|---|---|---|---|---|---|---|---|
| C1 | High | No | Yes | Yes | Yes | No | Yes | No |
| C2 | High | No | Yes | Yes | Yes | No | Yes | No |
| C3 | High | No | No | Yes | Yes | Yes | Yes | Yes |
| C4 | High | No | Yes | Yes | Yes | Yes | Yes | Yes |
| C5 | High | No | No | Yes | Yes | Yes | Yes | Yes |
| C6 | High | No | No | Yes | Yes | Yes | Yes | Yes |
| C7 | High | No | No | Yes | Yes | Yes | Yes | Yes |
| C8 | High | No | No | No | Yes | Yes | Yes | Yes |
| C9 | High | No | Yes | Yes | Yes | Yes | Yes | Yes |
| C10 | High | No | No | Yes | Yes | Yes | Yes | Yes |
| C11 | High | No | Yes | Yes | Yes | Yes | Yes | Yes |
| C12 | High | No | Yes | Yes | No | Yes | Yes | Yes |
| C13 | High | No | No | Yes | Yes | Yes | Yes | Yes |
| C14 | High | No | No | Yes | Yes | Yes | Yes | Yes |
| C15 | High | No | Yes | Yes | No | No | No | No |
| C16 | High | No | Yes | Yes | No | Yes | Yes | Yes |

choloroquinoline ring by piperazine with KI under a reflux condition.2-chloro-1-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)ethanone (4) was obtained by chloroacetylchloride under the cooling condition. Final compounds were obtained by chloro-amine coupling reaction using anhydrous $K_2CO_3$, under reflux at 110°C. Reaction was monitored by thin-layer chromatography, the mobile phase was ethyl acetate and hexane (7:3). All the final compounds were purified by recrystallization and column chromatography.

## Spectral interpretation

All new compounds were fully characterized by the usual spectroscopic methods (IR, $^{1}$H NMR, $^{13}$C NMR, MASS). In mass spectroscopy, for cyclohexyl derivative (C2) M+1 peak was obtained m/z at 387.2, o-tolylamino derivative (C3) m/z at 395.4 (M+1), p-fluorophenylamino derivative (C5) m/z at 399.4 (M+1), 4-chlorophenylamino derivatives (C6) m/z at 415.6 (M+1), 416.56 (M+2), 4-bromophenylamino (C7) m/z at 459.7 (M+1), p-tolylamino derivatives (C10) m/z at 395.4 (M+1), 4-methoxyphenylaminoderivative (C14) m/z at 395.4 (M+1). In $^{1}$H NMR, singlet was obtained forquinoline hydrogen (CH-8) at δ 8.0 (C2), 8.08 (C3, C10, C14), and 8.092 (C5, C6, C7, C15) ppm values. Besides, the doublet was observed for quinoline hydrogen (CH2, CH3, CH5, CH6) at δ ppm between 6.49-8.64 (C2), 6.57-7.97 (C3), 6.55-8.08 (C5, C6, C7), 6.57-7.49 (C10), and 6.57-7.97 (C14). In the same way, singlet of NH was obtained at 3.76 (C3, C10, C14), and 3.98 (C5, C15) § ppm. The results of $^{13}$C NMR of 2-(4-methoxyphenylamino)-1-(4-(7-chloroquinolin-4-yl)piperazine-1-yl)ethanone (C14) describes the chemical shift (ppm) of C in aromatic rings at 150.2 (quinoline C-2, C-4), 52.27 (piperazine C-2, C-6), 44.56 (piperazine C-3), 45.99 (piperazine C-5), 145.26 (ArC-1), 113.4 (ArC-2, C-6), 129.18 (ArC-3, C-5), 127.3 (ArC-4), 168.35 (C=O), 52.39 ($CH_2$), 22.89 (Ar-$CH_3$).

## Antimalarial activity

All synthesized compounds were evaluated for their in-vitro antimalarial activity against *Plasmodium falciparum* 3D7 chloroquine-sensitive strain at Microcare laboratory & TRC, Surat, Gujarat. All experiments were performed in the mean number of rings, trophozoites, and schizonts recorded per 100 parasites from duplicate wells after incubation for 38 hours, and percentage maturation inhibition concerning control group. **Table 5** shows the experimental $IC_{50}$ values (μg/ml) ($IC_{50}$) of synthesized compounds. **Table 6** shows the in-vitro antimalarial activity ($IC_{50}$ value). Their graphical representation is shown in **Fig. 8**. In-vitro antimalarial evaluation of synthesized compounds by taking chloroquine and quinine was taken as a standard.

In general, the synthesized compounds displayed good to moderate activity profiles in the *in vitro* anti-malarial assay, with $IC_{50}$ values ranging from 0.10 μM (compound C8) to 1.43 μM (compound C15). Modifications in the steric and electronic characteristics of the test compounds (C1–C16) afforded by the introduction of various monosubstitutions (viz. Cl, F, Br, $OCH_3$, $CH_3$, and $NO_2$) at the para-position on the phenyl ring and attachment to piperazinyl-7-chloroquinoline scaffold through ketonic linkage significantly influenced the inhibitory profile of these compounds.

**Table 6.** In-vitro antimalarial activity ($IC_{50}$ value)

| Code | Experimental $IC_{50}$ values (μg/ml) |
|---|---|
| Chloroquine | 0.020 |
| Quinine | 0.268 |
| C1 | 0.34 |
| C2 | 1.23 |
| C3 | 0.21 |
| C5 | 0.70 |
| C6 | 0.48 |
| C7 | 1.42 |
| C8 | 0.17 |
| C10 | 0.79 |
| C11 | 0.57 |
| C14 | 0.19 |
| C15 | 1.53 |

Interestingly, compounds possessing 3,4- $OCH_3$ or $OCH_3$ aniline (C8, C14) showed better PTP1B inhibition followed by those with halogen substitution like 4-F or 4-Cl substitution with phenyl ring aniline (C3, C6). Moreover, 2-(3,4-dimethoxyphenylamino)-1-(4-(7-chloroquinolin-4-yl)piperazine-1-yl)ethenone (C8) was found to be the most promising DHFR inhibitor amongst other compounds with IC50 value of 0.10 μM.

Surprisingly, all these inhibitors (compounds C8, C14) possessed $OCH_3$ group at meta or para position of the phenyl ring thus indicating that $OCH_3$ group seemed to play an important role for DHFR inhibition. Further, compounds bearing halogens like F or Cl (compounds C3, C6) substitutions on phenyl ring and cyclopropyl amino attachment showed moderate anti-malarial acidity. however, the presence of electron-withdrawing $NO_2$ groups on phenyl ring further reduced the anti-malarial activity with $IC_{50}$ value 1.43 μM (compound C15).

## Comparison of experimental and computational results

The ranking order of inhibitors C1–C16 for potency against DHFR as per the experimental and computational data is

**Table 5.** Comparison of experimental and computational activity data for compounds C1–C16

| Activity | Experimental results ($IC_{50}$ value) | Computational results (ΔG value) |
|---|---|---|
| DHFR | C8>C14>C3>C1>C6>C11>C4>C5>C10>C2>C7>C15 | C9>C8>C3>C12>C16>C1>C6>C14>C11>C13>C4>C5>C10>C2>C7>C15 |

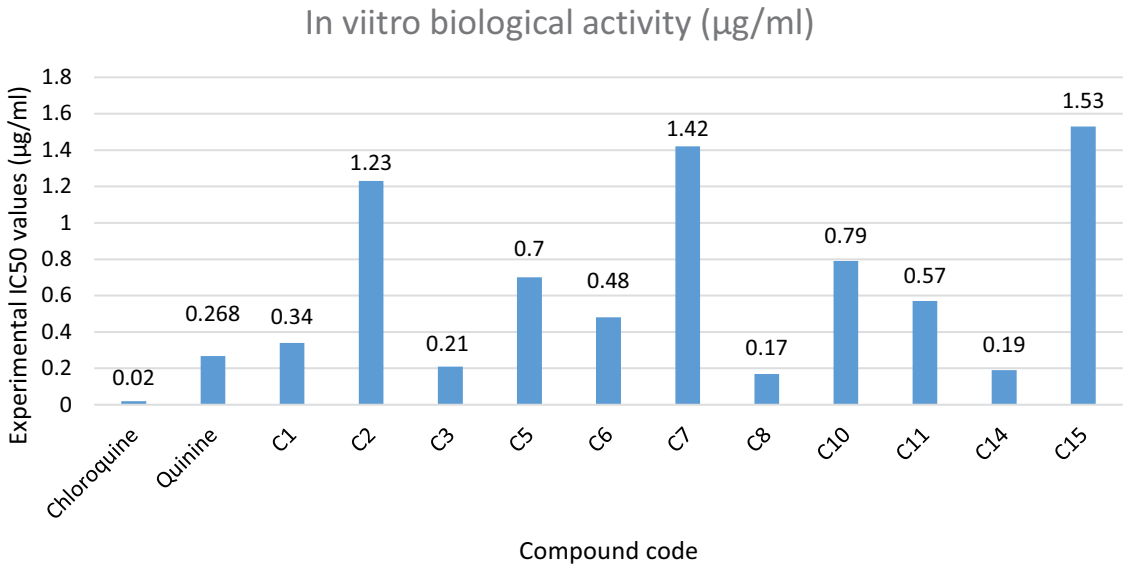


**Figure 8.** Graphical representation of some synthesized compounds with Inhibitory concentration $IC_{50}$ (µg/ml).

presented in **Table 5**. These data indicated almost a good-to-better correlation between the experimental (in-vitro) and computational data for DHFR inhibition, thus supporting our hypothesis.

## CONCLUSIONS

In summary, we have reported the in-silico studies, synthesis and antimalarial activity of a series of N-substituted 7-chloroquinoline-piperazine derivatives. The in vitro antimalarial activity of these molecules against plasmodium falciparum 3D7 chloroquine-sensitive strain is shown in µg/ml. The molecular docking studies of designed molecules were performed and among these compounds shows good interaction with the binding site of pf-DHFR. The ADME studies show excellent pharmacokinetics properties of designed compounds. The promising antimalarial activity displayed by the N-substituted 7-chloroquinoline-piperazine derivatives, molecular docking studies and pharmacokinetic properties defined in the current study approves their potential for upcoming development as antimalarial molecules.

## Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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# Анализ in Silico, синтез и биологическая оценка ингибиторов DHFR

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## Резюме

**Введение:** Малярия – это тип смертельного заболевания, вызываемого простейшими паразитами, которое в настоящее время считается самой большой проблемой для здоровья в мире. Паразит вида *Plasmodium* открывает его, передавая болезнь людям через укусы самок комаров *Anopheles*.

**Цель:** Скрининг дизайнерских молекул с помощью молекулярного стыковочного анализа и оценка их фармакокинетических свойств с помощью SwissADME. Синтезируйте соответствующие соединения. Охарактеризуйте синтезированные соединения по данным ТСХ, точки плавления, ИК-спектроскопии, масс-спектроскопии, 1-ЯМР водорода ($^1$H NMR) и 13-углеродного ЯМР ($^{13}$C NMR). Оценить противомалярийную активность синтезированных соединений.

**Материалы и методы:** *In silico*-анализ был выполнен с помощью SWISSADME, а молекулярный докинг – с помощью AutoDock Vina версии 4.2. Было проведено исследование противомалярийной активности *in vitro*.

**Результаты:** Исследование синтезированных молекул *in vitro* показало, что соединения C2 ($IC_{50}$ 1.23), C6 ($IC_{50}$ 0.48), C10 ($IC_{50}$ 0.79) и C14 ($IC_{50}$ 0.19) обладают хорошей противомалярийной активностью.

**Заключение:** Производные 7-хлорхинолин-пиперазина выявили потенциальные противомалярийные соединения для ингибиторов *pf*-DHFR.

## Ключевые слова

7-хлорхинолин-пиперазин, CQ-чувствительный штамм 3D7, исследование *in-silico*, *pf*-DHFR, фармакокинетическое исследование