



## Article Info

Received: 18<sup>th</sup> June 2024

Revised: 22<sup>nd</sup> April 2025

Accepted: 3<sup>rd</sup> May 2025

<sup>1</sup>Department of Pure & Industrial Chemistry, Faculty of Physical Science, University of Nigeria, Nsukka, Enugu State.

<sup>2</sup>Department of Chemistry, Faculty of Natural Sciences, Delta State University, Abraka, Delta State.

<sup>3</sup>Department of Pharmaceutical and Medicinal Chemistry, University of Nigeria, Nsukka, Enugu State.

\*Corresponding author's email:

[efeturi.onoabedje@unn.edu.ng](mailto:efeturi.onoabedje@unn.edu.ng)

Cite this: *CaJoST*, 2025, 2, 303-310

## Syntheses and Molecular Docking of New Chloroquine Derivatives as Potential Antiplasmodial and Antioxidant Agents

Chukwuebuka F. Maduechesi<sup>1</sup>, Efeturi A. Onoabedje<sup>1,2,\*</sup>, Benjamin E. Ezema<sup>1</sup>, and Sunday N. Okafor<sup>3</sup>

Quinoline is a privileged pharmacophore in many bioactive compounds possessing antimalarial activity. In this paper, some novel 4-aminoquinoline derivatives were synthesized in good yields in order to investigate their antimalarial and antioxidant properties. This was achieved by combining N'-(7-chloroquinolin-4-yl)propane-1,3-diamine with various Boc-amino acids. Amongst the synthesized derivatives, *tert*-butyl(1-((3-((7-chloroquinolin-4-yl)amino)propyl)amino)-4-methyl-1-oxopenta-2-yl)carbamate (IC<sub>50</sub> = 1.11 mg/mL) (**5c**) and *tert*-butyl2-((3-((7-chloroquinolin-4-yl)amino)propyl)carbamoyl)pyrrolidine-1-carboxylate (IC<sub>50</sub> = 1.30mg/mL) (**5d**) exhibit relatively better antiplasmodial activities compared to chloroquine diphosphate (IC<sub>50</sub> = 0.84mg/mL). In addition, *tert*-butyl2-((3-((7-chloroquinolin-4-yl)amino)propyl)carbamoyl)pyrrolidine-1-carboxylate (**5d**) (IC<sub>50</sub> = 0.48mg/mL) exhibited good antioxidant activity relative to ascorbic acid (IC<sub>50</sub> = 0.41mg/mL).

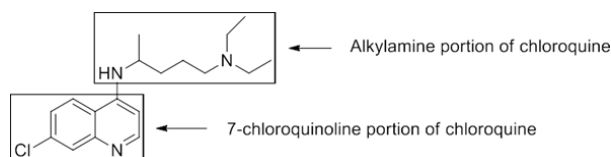
**Keywords:** Antioxidant, Antimalarial, Chloroquine, Malaria, Plasmodium falciparum, Molecular docking

## 1. Introduction

Malaria is an infectious parasite disease that threatens almost half of the world's population (Lai et al., 2019). This parasitic disease is spread by female Anopheles mosquitoes that feed on blood. Children and pregnant people are especially prone to contracting malaria in subtropical areas. In 2021, 247 million cases and 619,000 deaths as a consequence malaria disease were reported, an increase of 33 million cases and 181,000 fatalities than in 2015 (WHO, 2022). This increase is attributed to biological factors such as formation of parasite and mosquito vector resistance to drugs, as well as environmental forces such as temperature change and shifts in vector distribution. While the introduction of the WHO-approved Mosquirix (RTS,S/ASOL) vaccine in 2021 was a significant development in the fight against malaria, none of these drugs provide sterilizing protection (Chandramohan, 2021; Sinnis et al, 2022). There are five parasites in the genus Plasmodium that cause malaria, but Plasmodium falciparum and Plasmodium vivax are the most deadly (Jones, 2015, Murugan, 2015; White, et al., 2014).

Chloroquine is one of the foremost popular antimalarial drug. It is a cheap, possesses rapid onset, long half-life, low toxicity, and high

tolerance characteristics in humans, however, development of parasitic resistance to the drug has restrict its clinical use in treating malaria infection. Thus, functionalizing the chloroquine motif as shown in (figure 1) is a promising approach to malaria prevention in order to overcome chloroquine-resistant parasite strains and create drugs that may have all of the benefits of chloroquine.

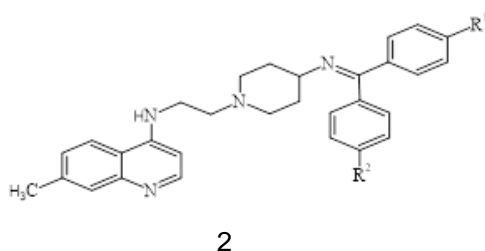


**Figure 1:** Molecular structure of chloroquine

The development of analogues of available drugs, combination therapy, hybrid drug concepts, and the use of drug resistance reversers are some of the strategies used to address the issues of drug resistance. Medicinal chemistry has advanced significantly as a result of the widespread belief that heterocyclic moieties exhibit significant biological activity (Voss et al., 2022). In 21st century, a quinoline bicyclic is an effective moiety among the many heterocyclic compounds used for antimalarial

treatment (Bhagat, 2019; Coban, 2020; Costa, 2021; Matada et al., 2021). Due to its chemistry, ease of synthesis and wide range of biological activities, including antimalarial, antiviral and antioxidant properties, this scaffold is regarded as a biologically vital active material with numerous potentials (Chopra, 2018; Egan, 2020; Yvette, et al., 2018).

Studies have shown that altering the 7-chloroquinoline ring's structure in chloroquine decreases its antimalarial activity, but changing the side chain of chloroquine seems to be more effective (Kaschula, 2002; Stock et al., 2002) as shown in (figure 2). Studies conducted both in-vitro and in-vivo against a strain of *Plasmodium falciparum* that was susceptible to chloroquine (CQS) and resistant to chloroquine (CQR) showed that chloroquine analogs with branched and linear side chains and two or three methylene groups between the amino groups were more effective than chloroquine (Pabon, 2003; Ridley, et al., 1996).



**Figure 2:** designed chloroquine derivative

Increased oxygen free radical production and decreased levels of antioxidant enzymes are linked to the physiopathogenesis of malaria (Clark, 1983; Shankar, et al., 2014). *P. falciparum* breaks down hemoglobin inside the host red blood cells (RBCs), resulting in the creation of heme. This heme then triggers the production of reactive oxygen specie, which causes anemia and oxidative stress-related apoptosis (Guha, 2006; Rahman, et al., 2015). Numerous diseases, including cancer, bacterial infections, and aging, are linked to stress-induced molecular and cellular damage.

Numerous studies have shown that the majority of commercially available drugs cause pathogenic microorganisms to become resistant to them. The problem is aggravated due to the emergence of new pathogenic microorganism. Furthermore, free radicals cause harmful diseases by attacking the body's cells. In order to stop and stabilize the damage brought on by free radicals, antioxidants are compounds that donate their own electrons to these injured cells. It also turns free radicals into waste by-products, which are eliminated from the body (Chioma, et al., 2018)

In our quest to discovering antimalarial agents in the class of 4-aminoquinolines, the syntheses, in vitro antiplasmodial and antioxidant activities of new 4-aminoquinoline analogues were described.

## 2. Materials and Methods

### 2.1 General Information

Every chemical and reagent employed to synthesize the novel chloroquine derivatives was of synthetic quality and came from the Sigma Aldrich chemical business in the United Kingdom. . The syntheses were conducted in the Postgraduate Research Chemistry Laboratory, Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka. Shimadzu FTIR-8400s Fourier Transform Infrared (KBr pellets) was used to record the infrared spectra, which were conducted at Nnamdi Azikiwe University in Awka. Nuclear Magnetic Resonance (<sup>1</sup>H NMR and <sup>13</sup>C NMR) spectra were determined using a Bruker AV-400 spectrometer at Rhodes University, South Africa. The coupling J constant was reported in hertz (Hz), and chemical shifts were recorded on δ-scale (neat). Multiplicity were reported with the following abbreviations: d for doublet, dd for doublet of doublet, t for triplet and m for multiplet. The Department of Pharmacology, Faculty of Pharmacy, University of Nigeria, Nsukka, conducted the antimicrobial evaluation.

### 2.2 Synthesis of the intermediate

To 4,7-dichloroquinoline (1.0equiv) was added to 1,3-diaminopropane (12.0equiv). The reaction was run neat at reflux for 10 h. Under vacuum and heat, the majority of the extra propane-1,3-diamine was evaporated. The remaining waxy solid was suspended in 200 mL water, and stirred for 20min. A pure white product was obtained by filtering the mixture. Yield 3.21g (91%), FTIR (KBr, cm<sup>-1</sup>): 2713.724, 2886.18, 3109.68 (C-H, stretch), 3394.78, 3669.28, 3828.42 (N-H, stretch), 1372.41 (C-N). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δppm: 6.39–8.9 (m, 5H, Ar-H), 8.25 (s, 1H, NH), 7.2 (s, 2H, NH), 3.35 (m, 2H, CH<sub>2</sub>), 3.25 (m, 2H, CH<sub>2</sub>), 1.75 (m, 2H, CH<sub>2</sub>). <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>) δ ppm: 140.13, 139.04, 128.15, 127.33, 125.26, 124.50, 120.95, 118.21, 97.59 (Aromatic carbon), 49.16, 36.00, 33.91 (Aliphatic carbon).

### 2.3 General procedure for the synthesis of compound 5a-e

To a stirred solution of Boc-protected amino acids (5 mmol, 1 equiv) in dry DCM (20 mL) was added DCC (7.5 mmol, 1.5 equiv) dissolved in 5 mL of DCM and HOBt (6 mmol, 1.2 equiv) dissolved in 2 mL of DMF at 0 °C. After 5 min, N'-(7-chloroquinolin-4-yl) propane-1,3- diamine was added slowly to the stirred reaction mixture. After the reaction mixture had been stirred for 1 h at room temperature, it was allowed to warm up to room temperature for 30 min.

Following the TLC- indicated completion of the reaction, the precipitated dicyclohexylurea (DCU) was extracted by filtration, and the filtrate was then washed with 10% aqueous  $\text{NaHCO}_3$  (3 X 50 mL) and 10% aqueous citric acid (3 x 50 mL) solution before final wash with a brine solution. The organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and evaporated to a gummy residue. The residue was allowed to cool for 2 h at 0 °C after being dissolved in a minimum amount of THF. The residual DCU was precipitated and filtered at this time. After the filtrate was evaporated, an amide was formed.

**(a). *Tert-butyl(1-((3-((7-chloroquinolin-4-yl)amino)propyl)amino)-1-oxopropan-2-yl)carbamate (5a):***

Yield 0.23g (51%). FTIR (KBr,  $\text{cm}^{-1}$ ): 2718.38, 2824.56, 3077.87 (C-H,  $\text{sp}^3$ ), 1618.72 (C=C, ring), 1735 (C=O), 1372.41 (C-N), 3372.52, 3678.81, 3829.98 (N-H), 816.28 (C-Cl).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 1.3 (s, 9H), 1.5 (d,  $J$  = 6.8 Hz, 3H), 1.75 (m,  $J$  = 7.1 Hz, 2H), 3.25 (t,  $J$  = 7.1 Hz, 2H), 3.35 (t,  $J$  = 7.0 Hz, 2H), 4.35 (m,  $J$  = 6.8 Hz, 1H), 6.39–8.9 (m,  $J$  = 7.5 Hz, 5H), 7.1–8.25 (s, 1H).  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 173.55–174.85 (C=O), 140.13, 139.04, 128.15, 127.33, 125.26, 124.50, 120.95, 118.21, 97.59 (Aromatic carbon), 80.08, 49.16, 36.00, 33.91, 28.28, 27.70 (Aliphatic carbon).

**(b). *Tert-butyl(1-((3-((7-chloroquinolin-4-yl)amino)propyl)amino)-1-oxo-3-phenylpropan-2-yl)glycinate (5b)***

Yield 1.0 g (54%), FTIR (KBr,  $\text{cm}^{-1}$ ): 2610.3, 2849.19, 2954.83 (C-H,  $\text{sp}^3$ ), 1633.33 (C=C, ring), 1715 (C=O), 1396.71 (C-N), 3083.3, 3214.08, 3806.76 (N-H), 761 (C-Cl).  $^1\text{H}$  NMR (400 MHz)  $\delta$  ppm: 1.4 (s, 9H), 1.75 (m,  $J$  = 7.0 Hz, 2H), 3.1 (d,  $J$  = 7.1 Hz, 2H), 3.25 (t,  $J$  = 7.1 Hz, 2H), 3.35 (t,  $J$  = 7.0 Hz, 2H), 4.45 (t,  $J$  = 7.0 Hz, 1H), 7.2 – 7.4 (s, 1H), 7.65–8.6 (m,  $J$  = 7.5 Hz, 10H).  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 171.82–173.04 (C=O), 155.41, 142.38, 139.58, 139.19, 136.71, 129.31, 128.69, 128.61, 128.55, 127.74, 127.08, 126.89, 115.80, 97.50 (Aromatic carbon), 80.16, 49.17, 35.80, 33.91, 28.29, 25.60, 24.94 (Aliphatic carbon).

**(c). *Tert-butyl(1-((3-((7-chloroquinolin-4-yl)amino)propyl)amino)-4-methyl-1-oxopenta-2-yl)carbamate (5c)***

Yield 1.08 g (56%), FT-IR (KBr,  $\text{cm}^{-1}$ ): 2629.17, 2951.19, 3230.72 (C-H,  $\text{sp}^3$ ), 1620.91 (C=C, ring), 1670 (C=O), 1434.306 (C-N), 3424.23, 3541.06, 3826.63 (N-H), 758.98 (C-Cl).  $^1\text{H}$ NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 0.85 (m,  $J$  = 6.8 Hz, 6H), 1.15 (s, 9H), 1.3 (m,  $J$  = 6.8 Hz 1H), 1.6 (t,  $J$  = 7.0 Hz, 2H), 1.7 (m,  $J$  = 7.1 Hz, 2H), 3.0 (t,  $J$  = 7.1 Hz, 2H), 3.4 (t,  $J$  = 7.0 Hz, 2H), 4.3 (t,  $J$  = 7.0 Hz, 1H), 7.35 – 7.7 (s, 1H), 7.8–9.4 (m,  $J$  = 7.5 Hz, 5H).  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 173.51–174.77

(C=O), 155.90, 155.65, 142.02, 139.74, 138.80, 127.81, 125.17, 119.78, 97.55 (Aromatic carbon), 80.01, 53.54, 41.08, 40.06, 35.87, 28.32, 28.30, 24.61, (Aliphatic carbon).

**(d). *Tert-butyl2-((3-((7-chloroquinolin-4-yl)amino)propyl)carbamoyl)pyrrolidine-1-carboxylate (5d)***

Yield 1.10 g (55%), FT-IR (KBr,  $\text{cm}^{-1}$ ): 2718.38, 2824.56, 3077 (C-H,  $\text{sp}^3$ ), 1619.72 (C=C, ring), 1720 (C=O), 1388.76 (C-N), 3372.52, 3676.8, 3829.98 (N-H), 816 (C-Cl).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 1.1 (s, 9H), 1.6 (m,  $J$  = -12.4 Hz, 6H), 1.75 (m,  $J$  = 7.1 Hz, 2H), 3.25 (t,  $J$  = 7.1 Hz, 1H), 3.35 (t,  $J$  = 7.0 Hz, 2H), 3.4 (m,  $J$  = 7.0 Hz, 2H), 7.3 -7.8 (s, 1H), 8.1–9.35 (m,  $J$  = 7.5 Hz, 5H),  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 173.51–174.77 (C=O), 155.53, 139.71, 138.52, 136.7, 133.6, 127.78, 125.32, 119.96, 96.5 (Aromatic carbon), 80.54, 49.24, 33.83, 28.41, 25.56, 24.90 (Aliphatic carbon).

**(e). *Tert-butyl(2-((3-((7-chloroquinolin-4-yl)amino)propyl)amino)-2-oxoethyl)carbamate (5e)***

Yield 0.85 g (50%), FT-IR (KBr,  $\text{cm}^{-1}$ ): 3018 (C-H,  $\text{sp}^3$ ), 1485 (C=C, ring), 1740 (C=O), 1245 (C-N), 3280 (N-H), 752 (C-Cl).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 1.1(s, 9H), 1.8 (m,  $J$  = 7.1 Hz, 2H), 3.2 (t,  $J$  = 7.1 Hz, 2H), 3.35 (t,  $J$  = 7. Hz, 2H), 3.95 (s, 2H), 6.9–7.9 (m,  $J$  = 7.5 Hz, 5H), 7.2 -7.6 (s, 1H),  $^{13}\text{C}$  NMR (400MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 173.51– 174.77 (C=O), 155.90, 155.65, 142.02, 139.74, 138.80, 127.81, 125.17, 119.78, 97.55 (Aromatic carbon), 80.1, 49.35, 33.82, 30.94, 28.32, 25.56 (Aliphatic carbon).

## 2.4 Antimalarial activity evaluation

Compounds 1–5 were incubated with 3 mM hematin, 10 mM oleic acid, and 1 M HCl at varying doses (0–2 mg/mL in DMSO). Using sodium acetate buffer at pH 5, the final volume was brought down to 1 mL. The positive control was chloroquine diphosphate. As the reaction mixtures were incubated at 37 °C for the whole night, they were constantly shaken gently. Following incubation, the samples were centrifuged at 14,000 rpm for 10 minutes at 21 °C. Following that, the hemozoin pellet was repeatedly shaken while being washed in 2.5% (w/v) SDS with phosphate buffered saline for 15 minutes at 37 °C. Finally, the supernatant was washed in 0.1 M sodium bicarbonate until it was clear. One milliliter of 0.1 M NaOH was used to dissolve the pellets after the last wash, and the supernatant was disposed of. The absorbance at 400 nm was then measured using a Beckman DU640 spectrophotometer to determine the amount of hemozoin present. The results were recorded as %inhibition of heme crystallization compared to negative control (DMSO) using the following equation:

Percentage inhibition =  $[(AN-AS)/AN] \times 100$ ,

where AN: absorbance of negative control and AS is absorbance of test samples.

## 2.5 Antioxidant evaluation

The Liyana-Pathiranan and Shahidi (2005) approach was used to calculate the DPPH Radical Scavenging Activity. Samples D1–D5 were produced in methanol with 5–0.625 mg/mL of the samples and the standard drugs (ascorbic acid) separately, and 1.0 mL of this solution was combined with 0.135 mL of DPPH in methanol. After a thorough shake, the reaction mixture was allowed to sit at room temperature for half an hour in the dark. At 517 nm, the mixture's absorbance was determined using spectrophotometry. The sulphonamide's capacity to scavenge DPPH was determined using the following formula:

$$\text{DPPH radical scavenging activity} = \frac{(\text{Abs}_{\text{control}}) - (\text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100$$

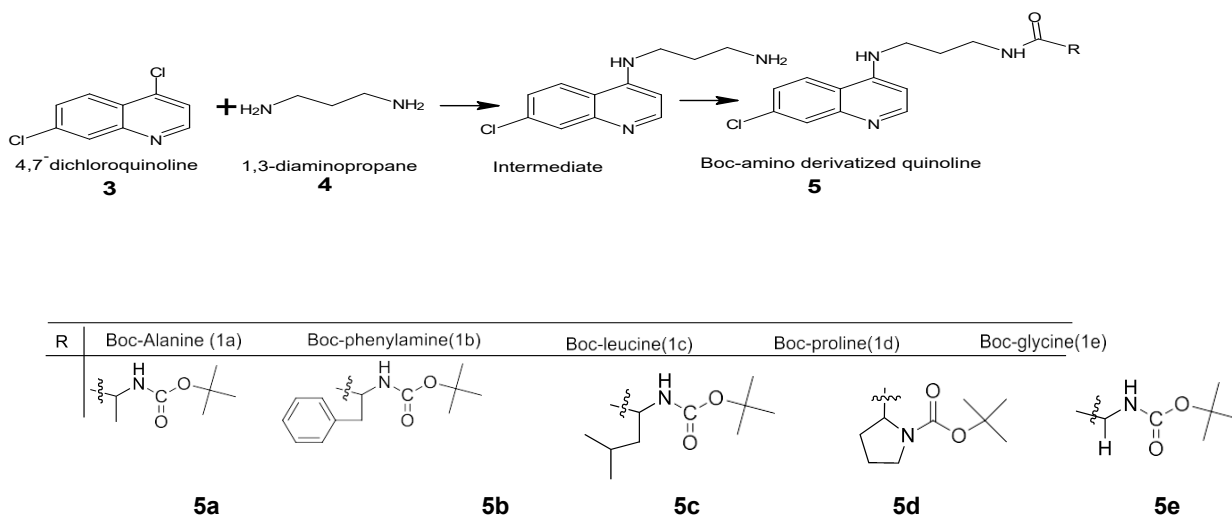
## 3. Results and Discussion

Refluxing of 4,7-dichloroquinoline with excess 1,3-diaminopropane under neat condition for 10 h gave N'-(7-chloroquinolin-4-yl)propane-1,3-diamine as shown in (figure 3) as intermediate product after work-up, melting at 96 – 98 °C. The reaction occurred by nucleophilic aromatic substitution of amino group with chloro group of 4,7-dichloroquinoline to afford the intermediate as yellow product. The N'-(7-chloroquinolin-4-yl)propane-1,3-diamine was further coupled with various Boc-amino

## 2.6 Molecular docking studies

The drug targets used for antioxidant and antimicrobial activities of our compounds were cytochrome C peroxidase (PDBcode: 2X08) and plasmepsin II (PMI) from *Plasmodium falciparum* (PDB code: 3QS1) respectively. Protein data was used to obtain the 3D structures of these drugs (<http://www.rcsb.org>). The protein was prepared in Discovery Studio and the unwanted multiple chains and water of crystallization was removed. The prepared proteins and the synthesized compounds were loaded into Molecular Operating Environment and energy minimized using Force field MMFF94X. Using the MOE docking program, the binding of the ligand molecule to the protein molecule was examined to determine the proper conformation.

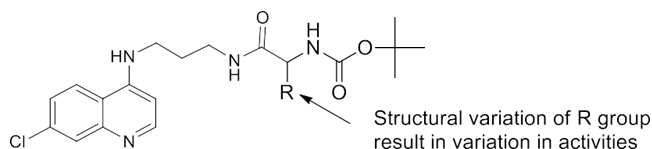
acids in the presence of N,N'-Dicyclohexylcarbodiimide/hydroxybenzotriazole (DCC/HOBt) as coupling reagent to afford variety of Boc amino substituted 7-chloroquinoline analogs. The reaction was simply carried out by stirring the Boc-amino acid and the intermediate in dichloromethane (DCM) at room temperature for 3 h until the reaction reaches completion, excess dicyclohexylurea (DCU) was eliminated by washing with 10% NaHCO<sub>3</sub> (3x50ml) and brine solution.



**Figure 3:** Reagent and conditions: (a) Reflux for 10 h, 120 °C ; (b) Room temperature, Boc-amino acid (5.0 mmol), dichloromethane (DCM), N, N'-dicyclohexylcarbodiimide (DCC) (1.5 equiv), hydroxybenzotriazole (HOBt) (1.2 equiv in 2 mL of dimethylformaldehyde (DMF)

DCC can also be substituted with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), as the latter is soluble in water and the resultant product is readily separated by solvent extraction. In this work, DCC was used to activate the amino acid's carboxyl group rather than EDC.HCl since it is less expensive, despite the fact that it can result in an inactive N-acylurea. At low temperatures, however, the protonation of O-acylurea into N-acylurea was reduced by the use of DCC and 1-hydroxybenzotriazole (HOBT).

employed as reference standards respectively. The structural variation in R group in (figure 4) lead to changes in the activities of the analogs. Table 1 and 2 shows the results of the biological activity investigations.



R: hydrogen, methyl, isopropyl, phenyl

**Figure 4:** Boc-amino acids derivatized quinoline

### 3.1 Biological studies

The synthesized compounds were evaluated for antiplasmodial and antioxidant activities with chloroquine diphosphate and ascorbic acid

### 3.2 *In Vitro* antiplasmodial activity

The antiplasmodial properties of the five compounds were evaluated *in-vitro* using chloroquine diphosphate as the reference. The half maximum inhibitory concentration (IC<sub>50</sub>) values for the synthesized analog were given in table 1. However, none of the synthetic 7-chloroquine derivatives were

as effective against parasites as the conventional chloroquine diphosphate. The compounds **5a** and **5e** containing Boc-alanine and Boc-glycine moieties exhibited poor activities. The derivatives incorporating Boc-leucine (**5d**), Boc-proline (**5c**) and Boc-phenylalanine (**5b**) in their molecular structures showed enhanced activities.

**Table 1:** Half maximal inhibitory concentration of compounds 5a-5e for antiplasmodial activities

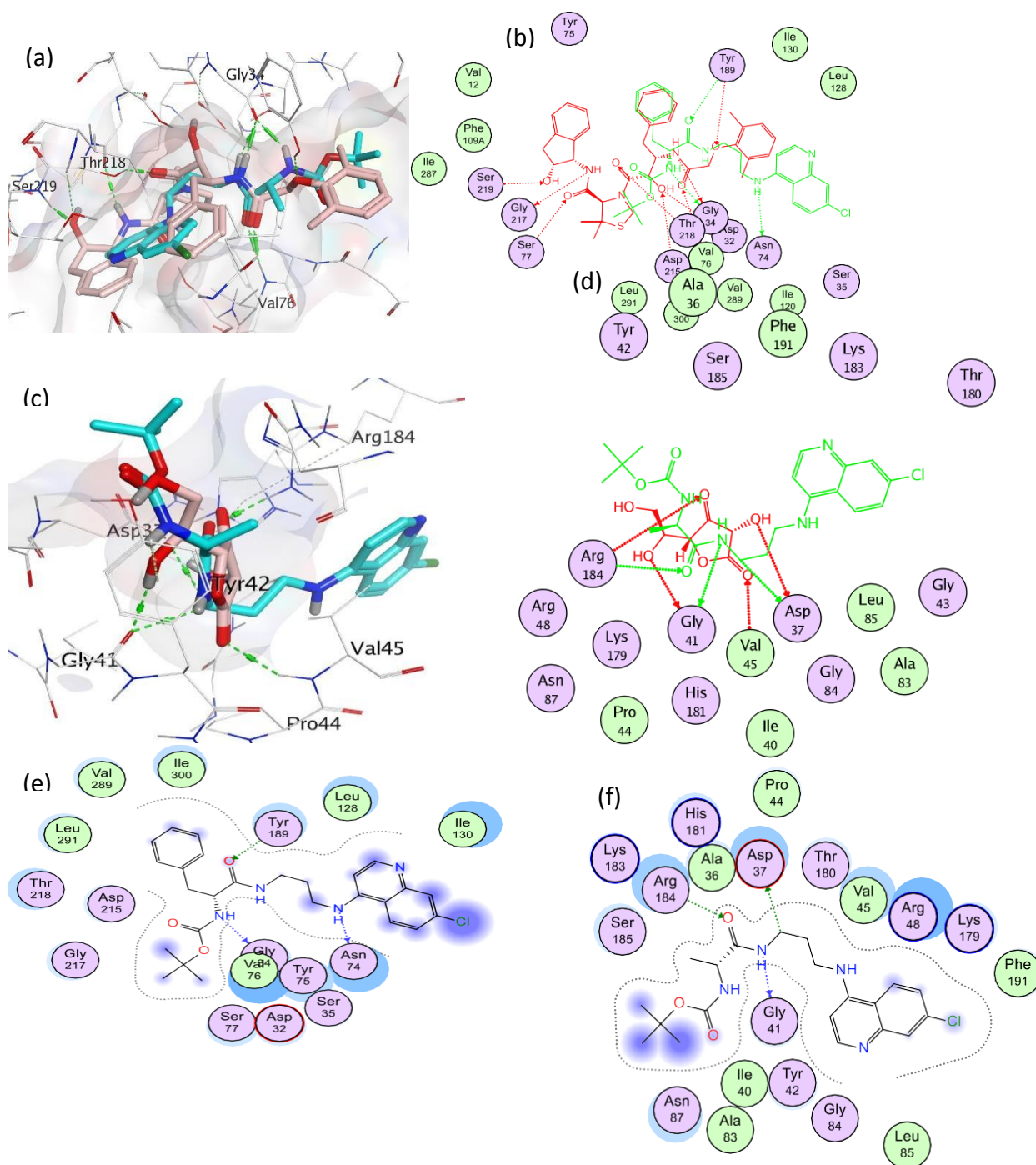
| Concentration    | Chloroquine diphosphate | Compound 5a | Compound 5b | Compound 5c | Compound 5d | Compound 5e |
|------------------|-------------------------|-------------|-------------|-------------|-------------|-------------|
| 5mg/mL           | 87                      | 51          | 11.9        | 35          | 20.6        | 58          |
| 2mg/mL           | 79                      | 48          | 12          | 34          | 19.1        | 47          |
| 1.25mg/mL        | 80                      | 37          | 11          | 33          | 30.1        | 46          |
| 0.625mg/mL       | 75                      | 39          | 8.5         | 28          | 16.2        | 51          |
| IC <sub>50</sub> | 0.84                    | 2.35        | 1.72        | 1.11        | 1.30        | 5.68        |

**Table 2:** Half maximal inhibitory concentration of compounds 5a-5e for antioxidant activities

| Concentration    | Ascorbic acid | Compound 5a | Compound 5b | Compound 5c | Compound 5d | Compound 5e |
|------------------|---------------|-------------|-------------|-------------|-------------|-------------|
| 5mg/mL           | 80.6          | 79          | 38          | 12.64       | 67.9        | 75          |
| 2mg/mL           | 82.4          | 82          | 46          | 41.33       | 74          | 81          |
| 1.25mg/mL        | 79.1          | 77          | 31          | 41.21       | 66          | 80          |
| 0.625mg/mL       | 81.9          | 80.34       | 72          | 57.7        | 81          | 87.5        |
| IC <sub>50</sub> | 0.41          | 1.774       | 0.545       | 2.980       | 0.484       | 1.25        |

**Table 3:** Binding energy of the compounds

| Target | Native ligand | Compound 5a | Compound 5b | Compound 5c | Compound 5d | Compound 5e |
|--------|---------------|-------------|-------------|-------------|-------------|-------------|
| 3qs1   | -9.46         | -7.02       | -7.55       | -7.35       | -6.64       | -6.76       |
| 2x08   | -4.57         | -7.70       | -7.12       | -6.10       | -7.01       | -6.98       |



**Figure 5:** (a) **5b** and native ligand in the active binding site of 3QS1 (b) 2D representations showing the binding interactions of **5b** and the native ligand (c) **5a** and ascorbic acid in the active binding site of **2X08** (d) 2D representations showing the binding interactions of **1a** and ascorbic acid. 2D representation of the binding interactions of (e) **5b** with **3QS1** and (f) **5a** with **2X08**

Compound **5b** and **5c** with branch and alicyclic R groups respectively lead to increase in antimalarial activities than compound **5a** and **5e** where R groups are  $-\text{CH}_3$  and  $-\text{H}$ . Compound **5b** where  $\text{R} = -\text{CH}_2\text{C}_6\text{H}_5$  showed moderate activity compare to the other analogs. Overall, it was observed that compound **5c** (1.11 mg/mL) demonstrated higher

activity compared to other compounds and this may be attributed to the branched carbon chain.

### 3.3. In Vitro antioxidant activity:

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability of the newly synthesized chloroquine derivatives as shown in Table 2 were relatively moderate. Compounds **5b**, **5c** and **5d** gave



maximal inhibitory concentration (IC<sub>50</sub>) range 0.4100 – 2.984 mg/mL. The percentage free radical inhibition of compound 5a where R = -CH<sub>3</sub> and 5e where R = H were generally low compared to other derivatives with R being a group with higher molecular masses. Compound 5b where R = phenyl group and 5d where R = pyrrolidine group exhibit enhanced and comparable activity compared to ascorbic acid (0.41mg/mL).

It is worthy to note that compound 5d elicited the highest activity and this may be attributed to the presence of heterocyclic pyrrolidine ring in the molecule. In general compound 5b and 5d recorded both good antiparasmodial and antioxidant activities.

Figure 5: (a) 5b and native ligand in the active binding site of 3QS1 (b) 2D representations showing the binding interactions of 5b and the native ligand (c) 5a and ascorbic acid in the active binding site of 2X08 (d) 2D representations showing the binding interactions of 1a and ascorbic acid. 2D representation of the binding interactions of (e) 5b with 3QS1 and (f) 5a with 2X08

Molecular docking studies were performed to gain further insight into the interactions of the synthesized compounds with antioxidant and antimalarial drug targets. The results in Table 3 show that the synthesized compounds had strong binding affinity and interacted favorably with the active binding sites of the proteins. Inhibiting the biochemical activities of these proteins is possible according to our compound's strong binding affinities 16. The maximum antioxidant efficacy was provided by compound 5a (−7.70 kcal/mol). Figure 2 illustrates the 2D interactions between compounds 5a and 5b and the drug targets 2X08 and 3QS1, respectively, as well as their binding modes with the targets' amino acid residues. These compounds showed good fitting into the active binding sites of the targets. Compound 5a (green) demonstrated an effective interaction with the same essential amino acids as the ascorbic acid (native ligand, red) in Figure 2(d). GLY41, ASP37 and ARG184 played key roles. This could explain the high binding affinity that compound 5a has with 2X08. 5a could be a potential anti-oxidant agent.

#### 4. Conclusion

A number of chloroquine derivatives with of Boc-amino acids were successfully synthesized in good yields. The in vitro antiparasmodial and antioxidant investigation indicated that compounds 5b and 5d incorporating benzyl and pyrrolidine moieties respectively showed good activities. The molecular docking studies confirmed the compounds were inhibitors of 3QS1 and 2X08 protein drug targets. Since compounds 5b and 5d have unique dual antiparasmodial and antioxidant properties, they merit more research.

#### Acknowledgements

The authors are grateful to the staff of the Department of Pure & Industrial Chemistry for their invaluable supports throughout the research work.

#### Conflict of Interest

The authors declare no conflict of interest.

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