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Synthesis and antiplasmodial evaluation of a ciprofloxacin-dihydroartemisinin conjugate

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ABSTRACT

A dihydroartemisinin-ciprofloxacin hybrid was synthesized and its antiplasmodial activity was evaluated against the 3D7 strain of *Plasmodium falciparum*. It was hypothesized that linking the artemisinin pharmacophore (which targets the heme detoxification pathway of the malaria parasite) with the fluoroquinolone scaffold (which targets plasmodial DNA gyrase enzyme) will produce a hybrid antimalarial compound with enhanced potency. The hybrid was synthesized by esterifying the carboxyl group of ciprofloxacin with the hydroxyl group of dihydroartemisinin; it displayed excellent antimalarial activity against the strain of *P. falciparum* tested with between 3- and 4-fold greater activity (IC_{50}: 2.99 nM) compared to the reference drugs chloroquine (IC_{50}: 13.003 nM) and dihydroartemisinin alone (IC_{50}: 9.968 nM) against the parasite. The synthesized compound was also tested for its *in vitro* cytotoxicity and it was found to be relatively non-cytotoxic (LC_{50}: 50.78 µg/ml) as compared to cyclophosphamide (LC_{50}: 1.08 µg/ml). In silico prediction of the Lipinski properties of the hybrid showed that the compound possesses good drug-like properties. The hybrid demonstrated the good activity with minimal toxicity and is, therefore, a potential candidate for further exploration in the quest for desperately needed new antimalarial drugs.

INTRODUCTION

Malaria is a serious tropical disease which accounts for about 216 million cases and up to half a million deaths each year (WHO, 2017). About 90% of the fatalities occur in sub-Saharan Africa, with children under the age of five being the major victims (Vangapandu *et al*., 2007). The disease is caused by protozoan parasites of the *Plasmodium* genus with *Plasmodium falciparum* being the most lethal of the four species responsible for the disease. Progress in the development of vaccines that are effective against the disease has been hindered by the remarkable ability of the parasite to mutate its genome (Fidock *et al*., 2004). However, the RTS/SA01 vaccine is in advanced stages of development and is presently undergoing field trials in three African countries (Ghana, Kenya, and Malawi). Another major obstacle to malaria therapy is the exceptional ability of the parasites to develop resistance to available conventional drug therapy (Plowe, 2003; Trape, 2001; Wellems and Plowe, 2001). There is therefore a pressing need to develop new drugs with greater efficacy, especially those acting on novel targets in order to counter the spread of drug-resistant malaria parasites.

Fluoroquinolones are broad-spectrum antibiotics which are useful in the management of infections due to both Gram-positive and Gram-negative bacteria. They act by inhibition of DNA gyrase or topoisomerase IV in the cytoplasm (Drlica and Zhao, 1997). They have the advantages of being highly efficacious with minimal toxicity and selectivity, together with no cross resistance to other antibiotics (Souza, 2005). They have become very important agents for the treatment of community and hospital-acquired infections of the gastrointestinal and urinary tracts, and respiratory infections (Bartlett *et al*., 2000; Gillespie *et al*., 2001). In addition to their established antibacterial activity, quinolones have also been shown to have moderate antimalarial activity (Pradines *et al*., 2001; Zhang *et al*., 2010).

Artemisinin is a sesquiterpene lactone and was first obtained from a medicinal plant *Artemisia annua* by Chinese

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scientists in the early 1970s (Klayman, 1985). Dihydroartemisinin and other semisynthetic analogs are presently used in combination as the first-line therapy for malaria (Poespoprodjo *et al*., 2014). The most essential structural feature found in the artemisinins is the endoperoxide group in the 1,2,4-trioxane moiety and this group reacts with iron to produce toxic free radicals that go on to damage the parasite.

Hybrid molecules are single chemical entities that possess several structural domains with different pharmacological activities and functions thereby making each component to act as a distinct pharmacophore (Meunier, 2008). They possess a dual mechanism of action due to targeting of different effector mechanisms and therefore act like a "double-edged sword". Hybrid molecules have also been described as "designed multiple ligands" and defined as compounds rationally designed and intended to interact with multiple targets which are of relevance to a disease, with the overarching aim of enhancing safety and improving efficacy (Morphy *et al*., 2004). They may have advantages such as synchronized delivery of drug molecules to multiple target sites, together with the potential to delay or even prevent the development of resistance.

Multi-target drug development strategy has been successfully implemented in the design of various antimalarial hybrids like the hybrid molecule Mefloquine-Artesunate hybrid derived from mefloquine (MQ) and artesunate (AS). It was found to be more effective and has considerably lower toxicity than the combination of MQ and AS, as demonstrated both *in vitro* and also *in vivo* in an animal model (Varotti *et al*., 2008). Grellepois *et al*. (2005) also obtained similar results by combining a fluorinated artemisinin derivative with MQ. Capela *et al*. (2011) synthesized primaquine-artemisinin hybrids as multi-stage antimalarials and one of the hybrids displayed better efficacy as compared to an equimolar combination of the original pharmacophores and this led to better cure rates and improved survival. Jones *et al*., (2009) prepared and evaluated artemisinin-acridine hybrids for their *in vitro* activity against a chloroquine-sensitive strain of *Plasmodium* and tumor cell lines. The compounds showed moderate antimalarial activity in addition to a 4-fold increase in activity against cancer cells. In an unrelated study, another set of artemisinin-acridine hybrids was synthesized and the hybrids displayed good activity against the parasite strains tested and also exhibited greater selectivity against the parasite (Joubert *et al*., 2014). In addition, the hybrid containing ethylenediamine linker turned out to be the most active of all the synthesized compounds. Aminake *et al*. (2012) synthesized drugs for potential combined HIV and Malaria management by covalently fusing azidothymidine (an antiviral) with dihydroartemisinin (an antimalarial tetraoxane). The compounds showed potent antimalarial activity but only moderate anti-HIV action. Furthermore, several ciprofloxacin hybrids have been synthesized and evaluated for improved antibacterial activities. Some of these include ciprofloxacin-triazole hybrid (Kosikowska *et al*., 2016), ciprofloxacin-kanamycin hybrid (Shavit *et al*., 2017), ciprofloxacin-nitroxide hybrids (Verderosa *et al*., 2016), and ciprofloxacin-isatin hybrid (Wang *et al*., 2018).

Based on the foregoing, we are hypothesizing that the potency of dihydroartemisinin analogs against the *Plasmodium* parasite could be improved by linking it to the fluoroquinolone moiety. Also, fluoroquinolones have been shown to be slow-acting as antimalarials (Dahl *et al*., 2007) producing delayed death of the parasite; hence, it may be logical to hybridize them with artemisinins which are quite fast-acting as antimalarials so that they can complement one another. Thus, in the present study, a dihydroartemisinin-ciprofloxacin hybrid was designed, synthesized, and its antimalarial activity evaluated.

MATERIALS AND METHODS

Chemistry: materials

Chloroquine diphosphate was obtained from Greenfield Pharmaceutical Limited, Jiang Su, China, while ciprofloxacin was obtained from Evans Nigeria Limited. Dihydroartemisinin (analytically pure) was procured from Nanjing Zelang Medical Technology Company, (Nanjing, China). Oxalyl Chloride, Triethylamine, Roswell Park Memorial Institute (RPMI)-1640 Medium, Triton X-100, Sodium L-Lactate, Trishydroxymethylamino methane (Trizma base), 3-Acetylpyridine adenine dinucleotide (APAD), Nitro Blue Tetrazolium (NBT), Phenazine Ethosulfate (PES), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), Sodium hydroxide, n-Hexane, methanol, N,N-dimethylformamide, tetrahydrofuran, Diethyl ether, Dichloromethane, ethyl acetate, Dimethylsulfoxide (DMSO), Sodium sulphate, Sodium Bicarbonate, Sodium Chloride, Silica gel 60a,230-400 mesh,40-63u, Silica gel on thin layer chromatography (TLC) aluminium foils silica gel matrix with fluorescent indicator 254nm were all obtained from Sigma-Aldrich (Germany). Albumax (Gibco, Invitrogen, USA) and Dehydrated ethanol (absolute ethanol) were obtained from BDH laboratory reagents (Yorkshire, England). All the chemicals and reagents were of analytical grade and were used without further purification.

Equipment

Double Beam UV-VIS Spectrophotometer (model 1250, Shimadzu, Japan), Fourier-transform infrared spectroscopy (FT-IR) spectrophotometer (Cary 630 model, Agilent Technologies), and Agilent liquid chromatography–mass spectrometry (LC–MS) (model 1260, Infinity HPLC with Agilent 6130 single quadrupole mass spectrometer). The software used is Agilent Chemstation (for

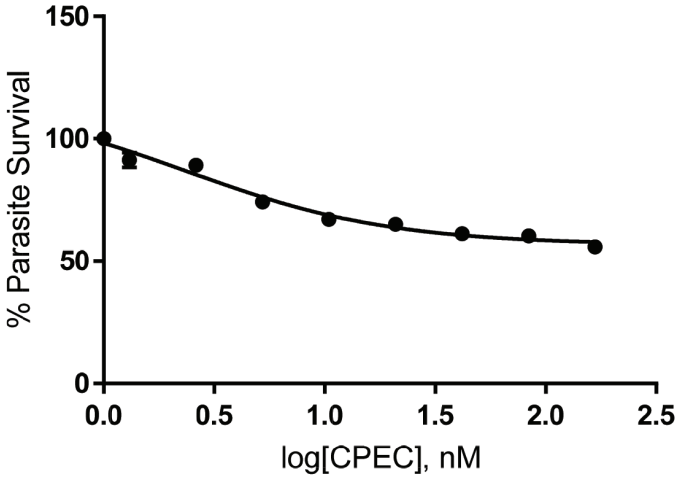


Figure 1. Log dose-response curve of *P. falciparum* (3D7 strain) by DHA-CPEC.

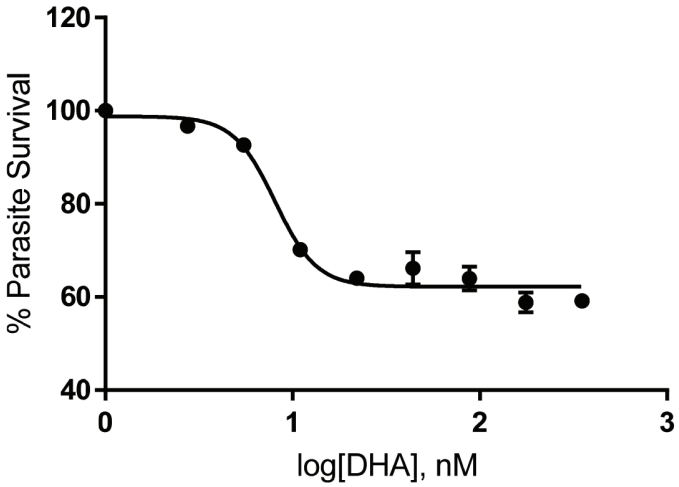


Figure 2. Log dose-response curve of *P. falciparum* (3D7 strain) by DHA.

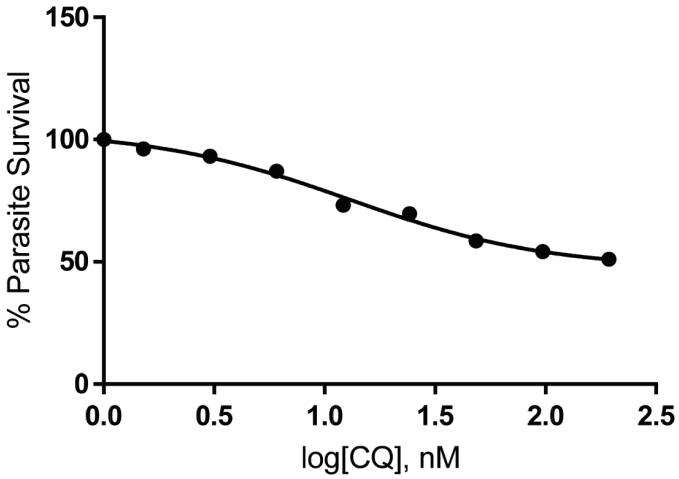


Figure 3. Log dose-response curve of *P. falciparum* (3D7 strain) by CQ.

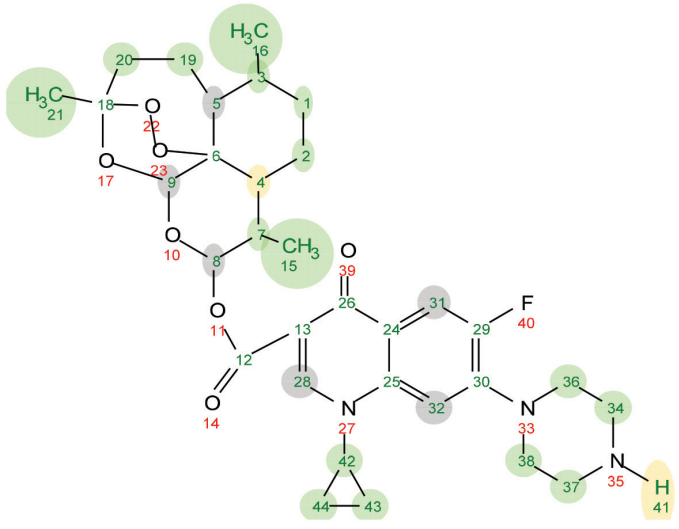


Figure 4. Structure of CPEC.

initial processing) and MassHunter (for the reporting of the results), Bruker Advance III 400 MHz spectrometer with an ultrashield magnet equipped with a Bruker B-ACS-60 autosampler. The software used is Bruker Topspin/ICON nuclear magnetic resonance (NMR) (with the latter controlling the autosampler)—TMS was used as an internal standard. Chemical shifts values are reported in the standard unit of parts per million (ppm). Splitting patterns are described as singlet (s), doublet (d), triplet (t), and multiplet (m). In NMR, atom numbering is presented as indicated in the text.

General methods

The synthetic reactions were monitored using Silica gel pre-coated TLC aluminum plates (Kieselgel 60, 254, E. Sigma-Aldrich, Germany); zones were detected visually under ultraviolet irradiation or by spraying with a solution of 0.5% vanillin in Sulfuric acid for visualization. Silica gel column chromatography was performed using Silica Gel 60 (70–230 mesh).

Synthesis of ciprofloxacin-dihydroartemisinin ester conjugate

Synthesis of ciprofloxacin acid chloride

Oxalyl chloride (0.3 ml, 3.35 mmol) was added dropwise to a solution of ciprofloxacin (1.0 g, 3.01 mmol) in dry CH_2Cl_2 (20 ml). The reaction mixture was stirred at 25°C for 12 hours, and the solvent was removed in vacuo. The crude product was washed with hexane (3 × 25 ml) and dried under vacuum.

Coupling of the acid chloride to dihydroartemisinin

To a solution of dihydroartemisinin (0.77 g, 2.71 mmol) and the acid chloride (1.0 g, 2.73 mmol) dissolved in dry dichloromethane (30 ml) was added triethylamine (0.29 ml, 3.23 mmol) dropwise at 0°C (Singh *et al*., 2008). The mixture was similarly stirred at 0°C for 2 hours. The reaction mixture was then quenched with saturated Na_2CO_3 (25 ml) and extracted with dichloromethane (3 × 25 ml). The organic layer was washed with 10% aqueous HCl solution (2 × 20 ml), then with water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure (Scheme 1).

Purification of conjugate

Accelerated gradient chromatography with gradient elution was used to purify the conjugate after synthesis using an appropriate solvent system.

Characterization of conjugate

The synthesized compound was characterized using ultraviolet-visible absorption spectroscopy, FT-IR spectroscopy, LC-MS, ^{1}H and ^{13}C NMR spectroscopic analysis.

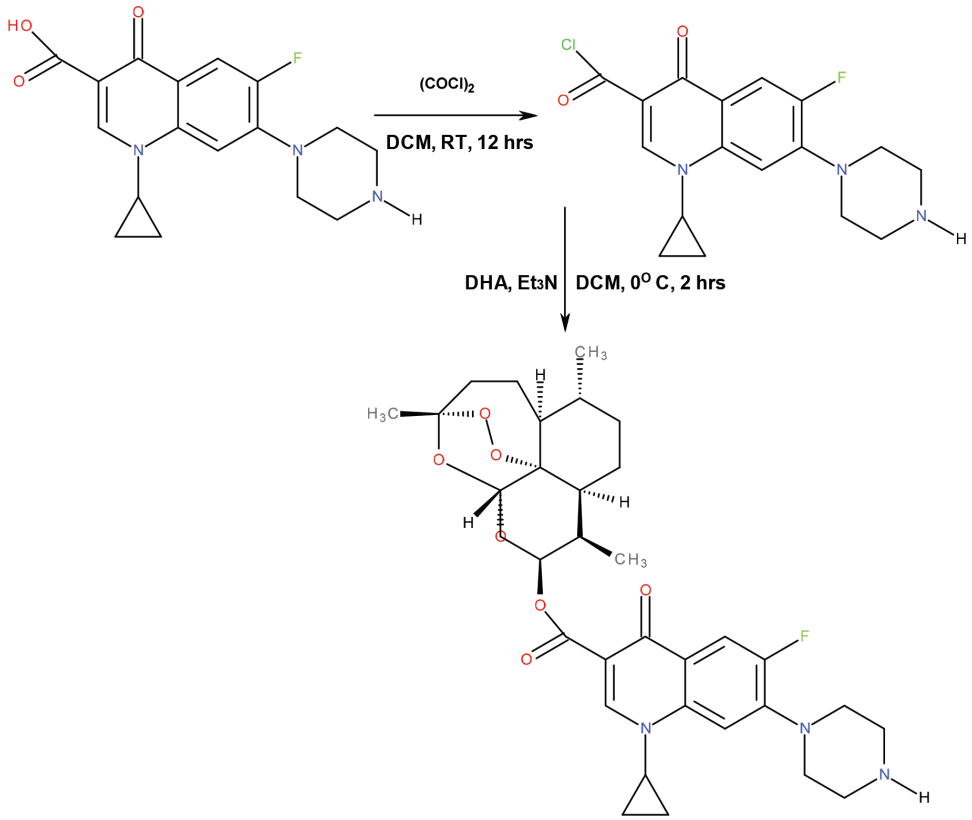
Biological evaluation: ethical consideration

Prior to the commencement of the study, ethical clearance was sought and obtained from the Institutional Health Research Ethics Committee of the Bingham University Teaching Hospital (BHUTH) for this work.

In vitro antimalarial screening (parasite Lactate dehydrogenase (pLDH) assay)

Plasmodium falciparum culture and maintenance

The *in vitro* cell culture experiments were carried out using the 3D7 clone of the chloroquine-sensitive strain of *P.*



Scheme 1. Synthesis of ciprofloxacin-dihydroartemisinin ester conjugate.

falciparum which were grown and maintained in culture using an earlier reported method (Trager and Jensen, 1976) with some modifications (Andrade-Neto *et al*., 2007). Cultures consisted of a 4 % hematocrit suspension of O+ human erythrocytes in RPMI-1640 medium supplemented with Gentamicin solution at 0.01 mg/ml, 25 mM HEPES buffer, 25 mM $NaHCO_3$, and 0.5 % Albumax I. The parasites were cultured at 37°C under a low-oxygen atmosphere (5% oxygen, 5% carbon dioxide, and the 90 % nitrogen). The level of parasitemia was estimated before incubation with the aid of normal light (Giemsa stain) microscopy.

Determination of in vitro antiplasmodial activity

Stock solutions (1 mg/ml) of the test drug was prepared by dissolving 10 mg of drug in 1 ml of DMSO and the volume made up to 10 ml with distilled water. All stocks were then serially diluted with distilled water to achieve the concentrations required. The eight final concentrations obtained were 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, and 0.195 ng/ml. The commercial drug standards, chloroquine (CQ) diphosphate (Greenfield Pharmaceutical LTD, Jiang Su, China), and dihydroartemisinin (Nanjing Zelang Medical Technology Co Ltd, China) were used as positive controls in the antimalarial assay. In all cases, except CQ, the final solution contained less than 0.01 % DMSO, which is not toxic to the parasite. (Chloroquine stock solution was prepared in distilled water). The drug stock solutions were stored at −20° C until required. Dilutions were freshly prepared on the day of the assay.

Each sample of diluted compounds was tested in triplicate in 96-well microtiter plates containing 100 µl of RPMI culture medium with 50 µl of *P. falciparum* culture with fresh red blood cells (pRBCs), with a hematocrit of 4%, and 2% initial parasitemia and finally 100 µl of the drug solution. Wells containing parasitized red blood cells (pRBCs) in culture medium without drug were the negative control for the assay and 0.01% DMSO solution was also used as a control to confirm that the solvent did not have any effect on parasite growth.

The plates were then incubated for 72 hours at 37°C under a low-oxygen atmosphere (Incubator Memmert GmbH, Germany). The culture medium was replenished on a daily basis during the incubation period. While incubation of the plates was going on, the two reagents for the detection and measurement of LDH enzyme were then prepared. First, Malstat reagent was prepared by dissolving 400 µl of Triton X-100 in 80 ml of deionized water, together with 4 g of L-lactate, 1.32 g of Tris buffer, and 0.022 g of APAD. The pH of the solution was then adjusted to nine with hydrochloric acid, and the volume made up to 200 ml with deionized water. Next, the NBT/PES solution was prepared by dissolving 0.160 g of nitro blue tetrazolium salt and 0.008 g of phenazine ethosulfate in 100 ml of deionized water. The solution was protected from light and stored in the refrigerator until required for use.

When incubation was complete, plates were harvested and next, 100 µl of Malstat reagent was added to each well of a 96-well microtiter plate in triplicate. Subsequently, the content of each well was mixed and 20 µl taken and added to the corresponding well of the Malstat plate followed by addition of 25 µl of the NBT/PES solution to initiate the lactate dehydrogenase reaction. Absorbance readings were then taken at 650 nm using the

microplate reader after an hour of incubation in the dark. (Emax-Molecular Devices Corporation, California, USA)

Analysis of test results from the LDH assay

The LDH assay generates optical density (OD) values at various concentrations of the drug as raw data. OD values from negative control wells (containing parasitized but untreated RBC) represent the maximum amount of LDH (100%) produced by the parasites. The growth value at each concentration of the drug was obtained by expressing the OD value as a percentage of the 100% growth value and plotted against the Logarithm of the corresponding molar concentrations of the drug using GraphPad Prism Software to generate log dose–response curves from which IC_{50} values were obtained.

$$\% \text{ Parasite Growth} = \frac{(\text{Average OD (no drug : negative control} - \text{Average OD (test)})}{\text{Average OD (no drug : negative control)}} \times 100$$

Brine shrimp lethality assay

Brine shrimp lethality assay is a widely used bioassay for the screening of bioactive compounds. The assay was carried out in accordance with a previously described protocol with slight modifications (Krishnaraju *et al*., 2005). Cyclophosphamide was used as positive control. The concentration-mortality data obtained were analyzed using probit analysis for the determination of LC_{50} values.

Statistical analysis

Numerical data (IC_{50} values) obtained from the assay are expressed as the mean value ± standard error of the mean. Statistical analyses were performed using the analysis of variance (ANOVA) followed by Tukey post test with the aid of IBM Statistical Package for Social Scientist (SPSS 20) software. Statistical significance was defined at the 5% level ($p < 0.05$).

RESULTS

Results of the synthetic reaction

The results obtained following the synthetic procedures for the preparation of the conjugates are presented in Table 1. It includes the molecular formula of the compounds, their physical appearance, melting point, and the percentage yield obtained.

Table 1. Physicochemical properties of the synthesized conjugates.

Compound code	Molecular formula	Appearance	Melting point (°C)	% Yield
CPEC	$C_{32}H_{40}FN_3O_7$	Light Brown granules	134–135	64.91

CPEC = Ciprofloxacin-dihydroartemisinin ester conjugate.

Table 2. *In silico* prediction of Lipinski drug-like properties for the conjugates.

Compound	Molecular weight (g/mol)	Clog*	Hydrogen bond acceptors	Hydrogen bond donors	Violations
CPEC	597.674	3.74	10	1	2

*Calculated using ACD iLabs.

Spectroscopic characteristics of the synthesized conjugate

(**CPEC**) 3,6,9-trimethyldecahydro-12H-3,12-epoxypyrano[4,3-j][1,2]benzodioxepin-10-yl 1-cyclopropyl6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate;

UV: 285, 245 nm; **IR (Neat):** 3,449 cm^{-1} (N-Hstr 2° amine), 2,922 cm^{-1} (C-Hstr. aliphatic), 1,733 cm^{-1} (C=Ostr ester), 1,602 cm^{-1} (C=Cstr unsaturated), 1,267 cm^{-1} (C-Cstr)

^{1}H NMR (400 MHz, in $CDCl_3$) δ 8.74 (s, 1H, 28), 8.01 (d, J = 8.0 Hz, 1H, 31), 7.57 (d, J = 5.0 Hz, 1H, 32), 5.88 (dq, J = 7.0, 1.5 Hz, 1H, 8), 5.42 (s, 1H, 9), 3.85 (p, J = 7.1 Hz, 1H, 42), 3.42 – 3.33 (m, 4H, 36, 38), 3.08 (td, J = 7.0, 4.9 Hz, 4H, 34, 37), 2.26 (h, J = 6.9 Hz, 1H, 4), 2.10 – 1.94 (m, 2H, 3, 20″), 1.93 – 1.33 (m, 13H, 1, 2, 5, 7, 19′, 41, 43, 44), 1.44 (s, 3H, 21), 1.26 – 1.10 (m, 2H, 19″, 20′), 0.95 (dd, J = 6.8, 1.5 Hz, 3H, 16), 0.88 (dt, J = 6.8, 1.5 Hz, 3H, 15).

^{13}C NMR (100 MHz in $CDCl_3$) δ 175.34 (C − 26), 168.60 (C – 12), 151.86 (C − 29), 149.20 (C – 28), 144.98 (C − 30), 136.18 (C − 25), 121.65 (C − 24), 111.51 – 110.40 (C − 13, C − 31), 107.54 (C − 32), 105.63 (C – 18), 97.21 (C – 9), 93.88 (C – 8), 81.76 (C – 6), 51.45 (C − 36, C − 38), 50.60 (C – 5), 46.03 (C – 34, C – 37), 44.33 (C – 4), 40.24 (C – 42), 37.95 (C – 20), 34.61 (C − 1, C − 3), 32.46 (C – 7), 24.13 (C − 2, C − 16, C − 19), 21.62 (C – 21), 20.15 (C – 15), 13.61 (C − 43, C − 44).

HRMS (ESI): m/z $[M + H]^+$ 598.8

Antimalarial activity of the conjugate

The *in vitro* antimalarial activity of the synthesized compound against the 3D7 strain of *P. falciparum* determined using parasite lactate dehydrogenase assay is given in Table 3.

Brine shrimp lethality assay

Cytotoxic effects as illustrated by LC_{50} values of the compounds and the standard drug cyclophosphamide are summarized in Table 4.

DISCUSSION

The ciprofloxacin-Dihydroartemisinin (DHA) ester conjugate (CPEC) was prepared in a reasonable yield of 64.91%

Table 3. *In vitro* antimalarial activity of the synthesized compounds against the 3D7 strain of *P. falciparum* determined using parasite lactate dehydrogenase assay.

S/n	Compound	IC_{50} (nM)
1.	CPEC	2.925*# ± 0.436
2.	DHA alone	9.968 ± 0.114
3.	CQ alone	13.003 ± 0.758

The IC_{50} values are expressed as mean ± SEM, $n = 3$ in each group.
*Indicates a significant difference compared to DHA, while # indicates a significant difference compared to CQ (ANOVA followed by Tukey's post-hoc test, $p < 0.05$).

Table 4. Brine shrimp lethality assay of the synthesized compound.

S/n	Compound	LC_{50} (µg/ml)
1.	CPEC	50.78* ± 6.53
2.	Cyclophosphamide (Standard)	1.08 ± 0.20

The LC_{50} values are expressed as mean ± SEM, $n = 3$ in each group.
*Indicates a significant difference compared to cyclophosphamide (ANOVA followed by Tukey's post-hoc test, $p < 0.05$).

which indicates that the synthetic pathway used for preparing the conjugate is suitable for the synthesis of esters of this kind. The Lipinski's rule of five is a rule that predicts the likelihood of a molecule being orally bioavailable. It requires that for a compound to be orally active, it should not have more than a single violation of the following criteria: not more than 10 and 5 hydrogen bond acceptors and donors, respectively, a molecular weight of less than 500 atomic mass units (amu), and a calculated octanol-water partition coefficient (clogP) of less than five. The conjugate showed only one violation of the Lipinski's rule of five (Table 2) with a molecular weight of 597.674 g/mol which exceeds the threshold of 500 g/mol and is therefore likely to be orally bioavailable. The conjugate showed maximum absorption (λmax) at 285 nm which can be attributed to the conjugated system of the quinolone ring which is the chromophore. In the FTIR spectrum of the compound, the OH stretch band has also disappeared while the carbonyl stretch of the ester link can be found at 1,733 cm^{-1}. The N-H stretch of the secondary amine can also be observed at 3,449 cm^{-1} and the C=C stretch unsaturated can be found at 1,602 cm^{-1}. In the 1H-NMR, a signal at 8.74 ppm which appeared as a singlet belonging to the –CH=C-proton at position 28 is the most downfield signal due to its close positional stereochemically to the oxygen atom of the ester which exerts some deshielding effect. In the 13C-NMR, a signal at 175.34 ppm belonging to C=O of the ketone at position 26 of the quinolone nucleus together with the resonance at 168.60 ppm belonging to the ester is indicative of the formation of the compound. Liquid chromatographic analysis of the compound showed one main peak in the Chromatogram with retention time of 1.693 minutes while ESI-MS of the compound also displayed a peak in the mass spectrum with m/z at 598.8 (M^+ + H) which corresponds to the molecular ion peak (M) with addition of hydrogen and this agrees with its molecular formula $C_{32}H_{40}FN_3O_7$.

The antimalarial action of fluoroquinolones can be enhanced by as much as between 10–100 fold via derivatization as reported by Dubar *et al*. (2009) who combined the organometallic and prodrug approach (Dubar *et al*., 2011) in synthesizing ciprofloxacin derivatives. Based on this concept, therefore, the design and synthesis of a new hybrid of DHA and ciprofloxacin; a representative quinolone is a viable direction for the development of new antimalarials. DHA is one of the most important artemisinin derivatives and possesses greater bioavailability, solubility, and antimalarial activity compared to artemisinin. Furthermore, it also possesses a hemiacetal hydroxyl group that provides a crucial reactive site for additional modification without damaging the pharmacophore of the parent structure. The DHA-Ciprofloxacin conjugate (structure shown in Fig. 4) was found to be highly active and showed about 3- and 4-fold increase in activity compared to dihydroartemisinin and chloroquine alone (Figs. 1–3). The enhanced activity of the ciprofloxacin-DHA conjugate may be linked to two factors. First, esterification of ciprofloxacin is expected to make the original compound more lipophilic; thus, favoring its diffusion across multiple membranes of the *plasmodium* parasite including those of the apicoplast, which harbors the probable target of the ciprofloxacin derivative. Second, upon possible hydrolysis of the ester linkage *in vivo*, the DHA and ciprofloxacin molecules would be free to attack the parasite via their different mechanisms and this attack would occur in a synergistic manner which will be lethal to the parasite and this might account for the result obtained. The result obtained also aligns with that obtained by Martins-Duarte *et al*. (2015) who synthesized and tested ciprofloxacin esters against another parasitic organism *Toxoplasma gondii*. Furthermore, the result also agrees with that obtained by Agarwal *et al*. (2015) who had evaluated the *in vitro* antimalarial effects of fixed-ratio combinations of some fluoroquinolone analogs with artemisinin and found the combinations to have synergistic effects. The conjugate obtained in this study has the advantage of being a single compound with a markedly better activity compared to the standard drugs. An additional benefit of this hybrid is the observation that quinolones have been shown to have *cidal* action on multiple stages of the *Plasmodium* parasite (Sáenz *et al*., 2013) and this could be beneficial in interrupting transmission of the parasite in addition to clearing the parasite in an acute case of malaria infection. The hybrid also had low cytotoxicity (LC_{50} value: 50.78 ± 6.53 µg/ml) as compared to the standard drug cyclophosphamide (LC_{50} value: 1.08 ± 0.20 µg/ml) as shown in Table 4 and it might, therefore, be sufficiently safe for further development.

CONCLUSION

In this study, we have synthesized and characterized a new dihydroartemisinin-ciprofloxacin conjugate. The antimalarial activity and cytotoxicity of the compound were also evaluated *in vitro*. The results show that the antiplasmodial activity of the hybrid was significantly enhanced as compared to the standard drugs dihydroartemisinin and chloroquine but the cytotoxicity did not increase concurrently.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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