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Research article

The Antimalarial Effects of Novel Chloroquinoline Acetamide Hybrid Molecules

Kathrada F.[a], Inam A.[c], vanZyl R.[a,b], Azam A.[c]

[a] *Pharmacology Division, Department of Pharmacy and Pharmacology; Faculty of Health Sciences, University of the Witwatersrand, Johannesburg 2193, South Africa.*

[b] *WITS Research Institute for Malaria (WRIM), Faculty of Health Sciences, University of the Witwatersrand, Johannesburg 2193, South Africa.*

[c] *Department of Chemistry, Jamia Millia Islamia, Jamia Nagar, New Delhi 110025, India*

ABSTRACT

The widespread resistance to current antimalarial agents adds to the great burden of malaria. This study evaluated the antimalarial activity of novel compounds against the *Plasmodium falciparum* NF54 chloroquine-sensitive strain individually and combined with quinine using the parasite lactate dehydrogenase (pLDH) assay. Furthermore, toxicity screening was carried out to evaluate the safety profile of the derivatives. All twenty-seven chloroquine acetamide hybrids possessed antimalarial activity (IC50 range: 1.29 – 53.98 µM), while proving to show no toxicity against host red blood cells. The derivatives showed a good safety profile with low toxicity to human embryonic kidney epithelial cells (% inhibition average: 1.93-53.85%) and minimal lethality to brine shrimp (0-4.7%). None of the compounds demonstrated inhibitory effects on the Anopheles arabiensis mosquito larvae. The two most active derivatives displayed favorable ionization properties and synergistic activity in combination with quinine. In morphological studies carried out over a period of 48 hours, the most active derivative showed similar schizonticidal activity as the standard quinine with a lag in progression from the trophozoite stage. The most active derivative CQPA-26 has good antimalarial activity and low toxicity worth being explored with further structural modifications which may increase antimalarial activity.

Keywords: *P. falciparum, antimalarial, hybrid molecules, chloroquinolines*

**Author for correspondence: Email: fatima.kathrada@wits.ac.za; Tel: +27117172171*

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INTRODUCTION

Malaria remains one of the main global health problems, causing 405 000 deaths worldwide in 2018, with the WHO African region accounting for 94% of these deaths (WHO, 2019). Malaria is caused by infection of red blood cells with the protozoan *Plasmodium* parasites and transmitted via the bite of the female *Anopheles* mosquitoes (WHO, 2013). Prompt treatment with the appropriate drugs is vital in reducing morbidity and mortality rates. The first line agents used in the treatment of malaria include artemether-lumefantrine and quinine (SA-DoH, 2017). The biggest threat to successful treatment of malaria is the emergence of resistance by the parasites to the antimalarial agents. In addition to resistance to antimalarial agents, mosquitoes have also shown resistant patterns to current insecticides used in vector control programmes. The extensive spread of resistance has necessitated research for promising antimalarials with novel chemical structures and mechanisms of action to prevent drug resistance.

The recent widespread interest in hybrid molecules over combination molecules have been supported by promising efficacy and toxicity reports (Muregi *et al*., 2010). Hybrid drugs involve linking molecules with individual activity into one single agent. This provides a dual functionality effect such that multiple targets can be achieved (Inam *et al*., 2015). This study evaluated the activity of novel derivatives by employing the covalent bitherapy using novel hybrid molecules that restore the activity of individual drug classes that have become ineffective due to resistance.

The aminoquinoline group found in compounds like chloroquine have shown to have good antimalarial effects with a favourable safety profile (Kumawat *et al*., 2011). Structure-activity relationship studies illustrated that the 4-aminochloroquinoline nucleus is necessary for the antimalarial activity of the group and accumulation within the parasite vacuole (Kumawat *et al*., 2011). The 7

chloroquinoline-4yl-piperazine-1-yl-acetamide hybrid molecules in this study comprised of a 4-aminochloroquinoline group linked via a piperazine group to a substituted acetamide (Figure 1). The rationale behind the design and synthesis of these compounds was that the 4-aminochloroquinoline will stimulate haem binding, the piperazine will act as a linking agent, increasing hydrophilicity and the acetamide group will demonstrate dihydrofolate reductase (DHFR) inhibitory effects (Rastelli *et al*., 2003).

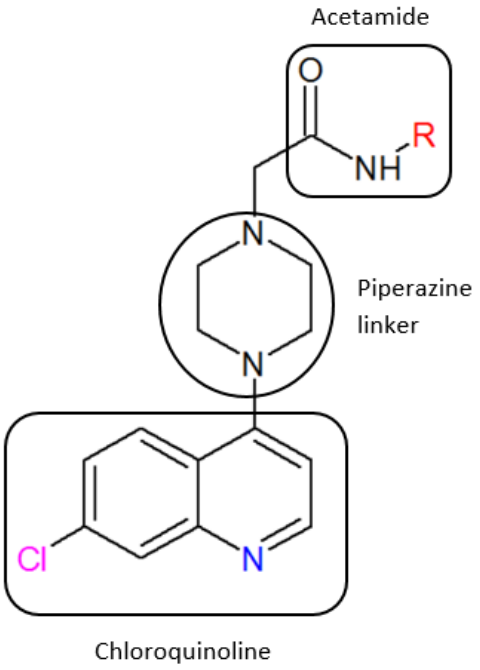

Figure 1:
Hybrid antimalarial with 4 aminochloroquinoline linked to an acetamide via a piperazine linker (R=different aromatic & aliphatic groups)

MATERIALS AND METHODS

Chemistry/Synthesis: The 27 7-chloroquinoline-4yl-piperazine-1-yl-acetamide hybrid molecules (CQPA1-CQPA27) were synthesized as illustrated in figure 2. The final compounds were synthesized by the reaction of 7-chloro-4-piperazin-1-yl-quinoline (2) with different chloroacetamides (aliphatic, aromatic and piperzinyl substituted) using potassium carbonate, potassium iodide as catalyst and dimethylformamide (solvent) under refluxing conditions. The compounds were recrystalized in DCM ethanol system and obtained in good yield. The structure of the compounds were confirmed via spectroscopy. The IR spectrum of the final compounds exhibited a characteristic absorption band for the carbonyl group at 1630–1730 cm^{-1}. The structure was further established by 1H NMR spectral data. A broad singlet in the range δ 8.60–11.79 ppm was observed for aromatic -NH and a doublet δ 8.71–8.74 ppm due to aliphatic –NH. The signals due to the aliphatic and aromatic proton appeared in their probable region. The carbonyl peak in 13C NMR of all the compounds appeared at δ 169.80-167.40 ppm. All the structures were further confirmed by mass spectral and the purity of the compounds was confirmed by elemental analysis and data was found in accordance with ±0.3%. The experimental data for the most active compound CQPA26 is as follows: **2-(4-(7-chloroquinolin-4-yl)piperazine-1-yl) 1-(4-hydroxypiperidin-1-yl)- ethanone (CQPA26**): Yield 30% green solid m.pt. 102-105°C ; H1 NMR (CDCl3) δppm: 8.69(d, 1H, J=4.5Hz), 8.03(s, 1H), 7.93(d, 1H, J=9.0Hz), 7.42(d, 1H, J=9.0Hz), 6.82(d, 1H, J=4.8Hz), 4.13(s, 1H), 3.96(s, 2H), 3.48-3.24(m, 8H), 2.91-2.81(m, 4H), 2.63(bs, 1H), 2.14-2.01(m, 2H), 1.72-1.50(m, 2H); 13C NMR (CDCl3) δppm: 167.57, 156.92, 151.75, 149.97, 134.98, 128.67, 126.22, 125.19, 121.84, 108.98, 66.93, 61.08, 52.96, 52.09, 43.01, 39.33, 34.84, 34.17; IR (υmax) cm-1:1720, 1570, ; ESI-MS: m/z 389.09 (M+1); Anal. Calc. for C20H25N4O2Cl; C 61.77, H 6.48 N 14.41, found C 61.48, H 6.47, N 14.30%. The experimental data for the all the other compounds is available as supplementary data.

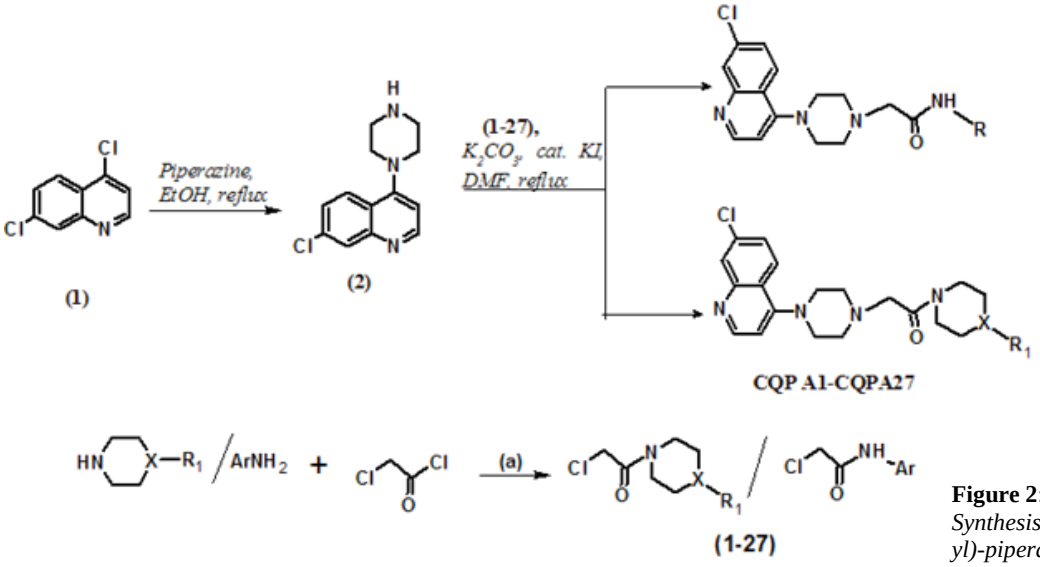

Figure 2:
Synthesis of 2-[4-(7-chloro-quinolin-4-yl)-piperazin-1-yl] acetamides hybrid molecules (CQPA1-CQPA27).

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Antimalarial activity: The chloroquine-sensitive *P. faliparum* (NF54) strain was maintained *in vitro* in supplemented RPMI-1640 culture media at 37 ˚C before being gassed with a mixture of 5% CO_2, 3% O_2 and 92% N_2 (Jensen *et al*., 1977). The culture was synchronised, using 5% D-sorbitol, in the ring stage (Lambro *et al*., 1979). To determine the antimalarial activity of various compounds, the synchronised ring stage parasites were adjusted to 2% parasitaemia and 2% haematocrit, to which serial dilutions of the derivatives and positive control (quinine) were added after a 24 h incubation. Negative controls included uninfected erythrocytes and drug-free parasitized erythrocytes. Following a 48 h incubation period, the plates were frozen at -70˚C for 1 h and thawed for 2 h. To quantify the parasite lactate dehydrogenase (pLDH) activity, 25 µl of lysate was transferred to a non-sterile plate, to which 100 µl Malstat™ and 20 µl nitroblue tetrazolium and phenazine ethosulphate (1:1) mixture was added to each well and incubated for 40 min at 37 ˚C (Makler *et al*., 1993). Thereafter, as an indicator of the viability of the parasite, 5% acetic acid was added to each well and the absorbance of the formazan products read at 620 nm. The percentage parasite growth, taking the appropriate controls into account were calculated and used to determine the concentration required to inhibit parasite growth by 50% (IC_{50} value) from dose response curves (log sigmoid) using the GraphPad Prism® 5.0 software. The experiment was carried out in triplicate.

Combination studies: The experiment was carried out using the parasite lactate dehydrogenase assay. The most active compound was combined in multiple ratios with quinne and the serial dilutions of each ratio used to generate a dose response curve. Positive controls containing the individual compound were prepared such that IC_{50} values for the individual drug and the drug in combination were obtained. To determine the interaction between the two compounds, an isobologram was constructed (GraphPad® Prism) using the Fractional Inhibitory Concentration (FIC). The sum FIC was calculated to evaluate the degree of interaction where ≤0.75 was considered synergistic, >0.75 to ≤ 1.25 additive and >1.25 antagonistic. Each experiment was repeated in duplicate (Berenbaum *et al*., 1989).

Stage-determination: The most active compound (CQPA-26) and quinine were tested at their respective IC_{50} and IC_{90} values on synchronised parasites in the ring stage (2% parasitaemia, 2% haematocrit) at 37˚C. Blood smears were prepared every 6 hours for 72 hours into the next cycle and stained before being microscopically analysed to determine the overall total parasitaemia number, the % parasitaemia at every stage and observed morphological conversions (Moseley, 2012).

Drug permeability: The drug-likeness of the most active derivatives ability in permeation of the acidic digestive vacuole of the *P. falciparum* parasite, was measured using the physicochemical properties of the derivatives in comparison to quinine as the reference drug (Lipinski *et al*., 1997). The properties of the derivatives were predicted using the Lipinski's rule of five (Ro5) predictions to determine the bioavailability of the derivatives with solubility and permeability predictions performed using ACD/iLab version 2.

Toxicity assays

Cell viability assay: Human embryonic kidney epithelial (HEK-293) cells were housed as a single layer in Dulbecco's Modified Eagle's Medium (DMEM) which was supplemented with fetal bovine serum (10%), 100 IU ml^{-1} penicillin and 100 µg ml^{-1} streptomycin, at 37 ˚C in a humidified environment with CO_2 (5%). A cell suspension (10 000 cells per well) was incubated at 37 ˚C for 48 h with serial dilutions of compound agents/positive control. A final concentration of less than 1% DMSO had no effect on the viability of the cells. Thereafter, 40 µl of (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT; 5mg ml^{-1} in phosphate buffer saline (pH 7.3)) was added to each well and incubated for an additional 2 h. DMSO was used to dissolve the formazan crystals, and the absorbance read at 540 nm (reference wavelength:690 nm) (Labsystems Multiskan RC) (Mosmann *et al*., 1983). Percent cellular viability was determined using the controls from which IC_{50} values were calculated and compared to the positive control, camptothecin. The experiment was carried out in triplicate and repeated 3 times.

Haemolysis assay: An adjusted 1% haematocrit in RPMI-1640 culture media suspension of fresh human red blood cells was incubated for 48 h with 25 µl test compound/control (50 µM) (Hayat *et al*., 2011). The absorbance of the supernatant was read at 412 nm. The % haemolysis was calculated using a 0.2% Triton X-100 solution (100% haemolytic control) and chloroquine (reference agent).

Lipid peroxidation assay: Compounds (50 µM) were plated out and reacted with linoleic emulsion and sodium phosphate buffer in a 96 well non-sterile plate and incubated for 24 h at 37˚C (Gulcin, 2007). Thereafter the latter suspension was reacted with 70% ethanol (150 µl), 3.94 M ammonium thiocyanate and 5 mM ferrous chloride and compared to the positive control, Trolox™. The absorbance was read at 492 nm and the Trolox equivalent anti-oxidant concentration (TEAC) was calculated from a standard curve of Trolox™,

Brine shrimp lethality assay: *Artemia fransiscana* eggs were hatched and 20-40 nauplii plated out into a 48 well plate with salt water and compound (0.5 µM) in triplicate. The compounds were constituted in DMSO ensuring it equates to < 1%. Potassium dichromate (1.6 mg/ml) served as the positive control and salt water served as the drug-free negative control. Prior to incubation of the plates, the nauplii were viewed under a microscope to report any dead nauplii. The plates were incubated for 24 hours at 25˚C afterwhich the number of dead nauplii in each well was counted using a microscope . Thereafter, 100 µl of acetic acid (5%) was added to all wells to kill the nauplii, to allow counting of the total number of nauplii in each well under a microscope. The percentage mortality was reported along with morphological

features and abnormalities (Ruebhart *et al*., 2009). The data was analysed using the IBM Statistics®22 probit analysis.

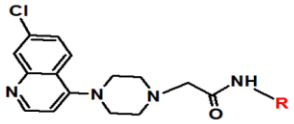
Larvicidal activity: A permanent colony of *Anopheles arabiensis* (KGB) mosquito larvae were sourced from the National Institute for Communicable Diseases Botha de Meillon Insectary, and fed according to WHO protocols (WHO, 2005). The compounds (0.5 µM) were incubated with cohorts of 25 fourth instar larvae in single distilled water for 24 h at 25-28˚C. Thereafter, percentage mortality was recorded in comparison to DDT, the positive control. The morphological features of the untreated larvae were microscopically compared to those treated with DDT and **CQPA** derivatives. The experiments were repeated in quadruplicate.

Data analysis: All experiments were carried out in triplicate to produce a mean ± standard deviation (SD) and each individual experiment repeated at least three times. To determine if there was a statistically significant difference between a test compound and control, an unpaired students T-test was performed using GraphPad® Prism Version 5.02 software, with a p-value of < 0.05 considered to be significant

RESULTS

Antimalarial activity: All 27 compounds inhibited malaria growth (IC_{50} values range: 1.29 – 53.98 µM), compound **26** proved to be the most active (IC_{50}: 1.29 ± 3.35 µM), followed by compound **25** with an IC_{50} of 1.72 µM compared to quinine (IC_{50}: 0.18 ±0.05 µM). Compound **6** demonstrated the least antimalarial activity (IC_{50}: 53.98 ±3.01 µM) (Table 1). The most active compound **26** demonstrated a synergistic interaction in combination with quinine with a ΣFIC of 0.29 ±0.0014. (Graph 1)

Table 1a:
In vitro antimalarial activity of compounds (CQPA1-7)



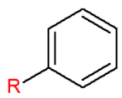
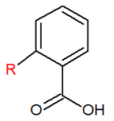
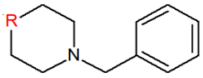
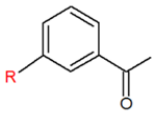
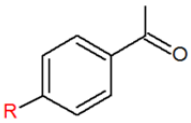
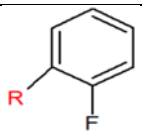
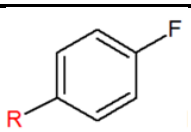
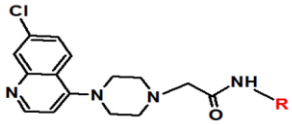
		Antimalarial activity	*Cytotoxicity*	
Derivative	***Structure***	**IC_{50} ± S.D. (µM)**	**% Cell inhibition ± S.D. (at 50 µM)**	% RBC lysis ± S.D. (at 50 µM)
CQPA1		34.19 ± 1.07	34.91±0.21	0.95±0.37
CQPA2		42.16 ± 3.42	44.08±4.89	1.32±0.59
CQPA3		21.85 ± 3.66	32.65±5.06	1.43±0.39
CQPA4		35.35 ± 7.33	40.50±8.44	1.42±0.41
CQPA5		30.13 ± 7.40	51.68±8.48	0.10±0.01
CQPA6		53.98 ± 3.01	43.05±3.72	0.10±0.01
CQPA7		18.75 ± 3.32	46.49±3.61	0.10±0.01

Table 1b:
In vitro antimalarial activity of compounds (CQPA8-18)



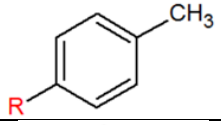
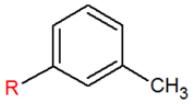
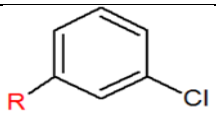
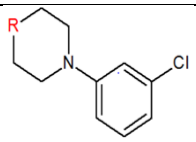
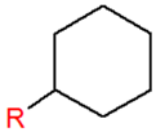
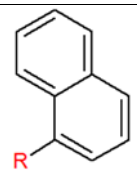
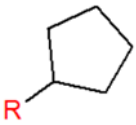
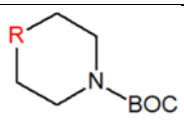
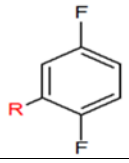
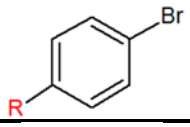
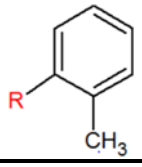
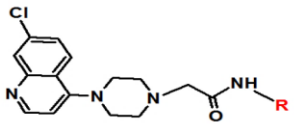
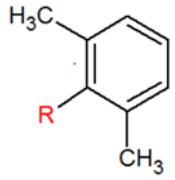
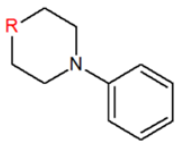
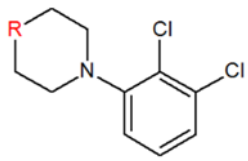
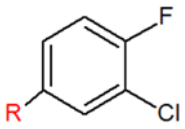
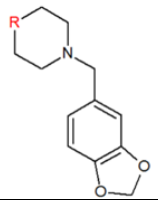
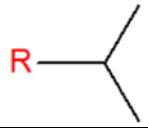
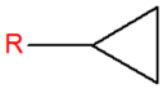
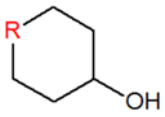
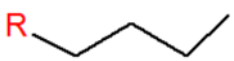
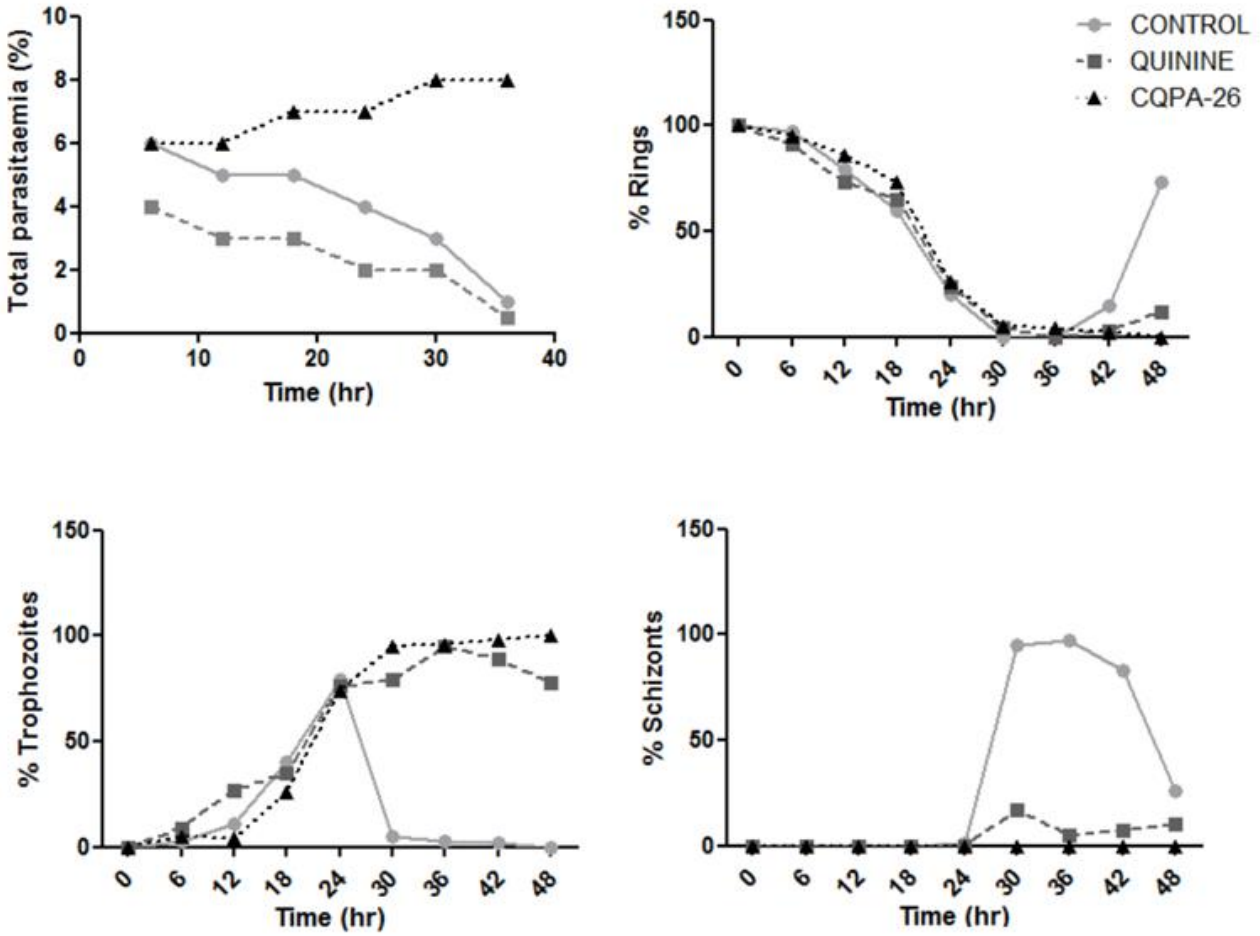
Derivative	***Structure***	***Antimalarial activity*** **IC_{50} ± S.D. (µM)**	*Cytotoxicity* **% Cell inhibition ± S.D. (at 50 µM)**	% RBC lysis ± S.D. (at 50 µM)
CQPA8		29.88 ± 5.73	43.47±3.73	0.08±0.04
CQPA9		23.76 ± 3.11	33.62±3.52	0.20±0.11
CQPA10		18.99 ± 3.04	53.85±0.88	0.25±0.25
CQPA11		24.48 ± 5.78	43.23±5.78	1.18±0.55
CQPA12		31.57 ± 2.91	43.86±1.08	0.30±0.17
CQPA13		19.07 ± 1.42	43.43±2.44	0.37±0.29
CQPA14		17.08 ± 2.31	51.38±4.62	0.68±0.41
CQPA15		11.06 ± 1.97	59.94±6.01	0.42±0.28
CQPA16		35.92 ± 3.11	**19.45±9.55**	0.67±0.31
CQPA17		24.16 ± 5.73	55.25±2.94	0.79±0.38
CQPA18		13.10 ± 2.89	38.25±4.84	0.51±0.24

Table 1c: *In vitro* antimalarial activity of compounds (CQPA19-27)



Derivative	***Structure***	***Antimalarial activity*** **IC_{50} ± S.D. (µM)**	*Cytotoxicity* **% Cell inhibition ± S.D. (at 50 µM)**	% RBC lysis ± S.D. (at 50 µM)
CQPA19		1.85 ± 0.52	50.20±5.25	0.28±0.16
CQPA20		18.95 ± 7.53	**22.47±3.79**	0.09±0.02
CQPA21		9.70 ± 1.51	51.93±4.89	0.15±0.09
CQPA22		51.41 ± 4.96	48.90±10.30	0.21±0.10
CQPA23		10.81 ± 1.60	44.32±2.89	0.14±0.07
CQPA24		13.11 ± 2.59	39.88±0.72	0.49±0.16
CQPA25		1.72 ± 0.17	34.93±2.81	0.59±0.46
CQPA26		**1.29 ± 3.35**	54.88±0.48	0.40±0.28
CQPA27		21.79 ± 2.06	42.05±5.44	0.09±0.02
Quinine		0.18 ± 0.05	23.46±3.78	0.10±0.01
Camptothecin		n.t.	78.11±6.60	

The most active compound **26** showed stage-specific morphological changes in the parasite whereby the parasites appeared to be smaller (pyknotic) in size compared to the untreated control (Figure 3). Compound **26** had no effect on the ring stage of the parasite but caused a lag in the progression of parasites from the trophozoite to the schizont stage. Majority of the parasites (95%) remained in the trophozoite stage in comparison to 95% of the untreated parasites progressing to the schizont stage. Compound **26** prevented the parasite life-cycle from completing, with only 10% of the parasites eventually progressing to the schizont stage at the time the untreated control had begun a new cycle as early rings (73%) (Graph 2).



Graph 2:
Effect of CQPA-26 on total parasitaemia and each stage (rings, trophozoites, schizonts) in the parasite lifecycle

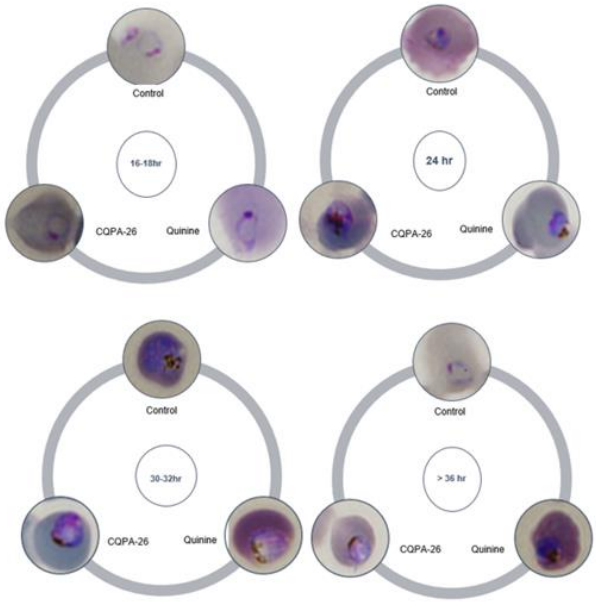
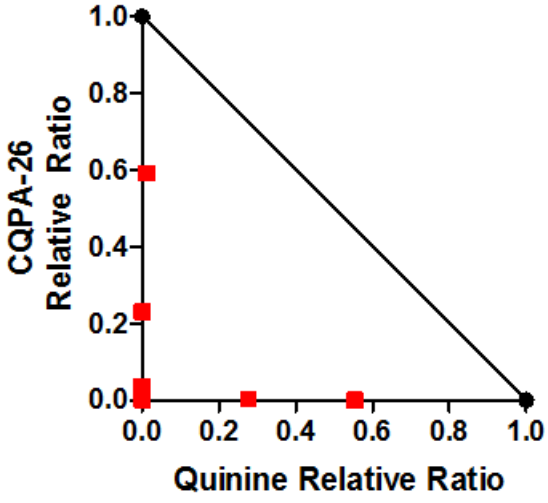


Figure 3:
The effects of CQPA-26 and quinine on parasite morphology and development over 48 hours



Graph 1:
Isobologram showing the drug interaction between the most active CQPA-26 compound with quinine (ΣFIC = 0.29 ±0.0014)

DISCUSSION

The derivatives demonstrated favourable effects in reducing the emergence of resistance as combination therapy. Resistance to chloroquine is mainly through the alteration of the accumulation mechanism of a drug in the digestive vacuole of the parasite. Mutations in the *P. falciparum* chloroquine resistance transporter (pfCRT), a protein found in the parasite digestive vacuole, aids efflux of the positively charged chloroquine from the digestive vacuole. This leads to decreased accumulation of chloroquine in the parasite to levels which are safe for the parasite (Boudhar *et al*., 2016). With the decline in chloroquine use, the drug pressure has been removed resulting in the loss or down-regulation of the resistance gene, making chloroquine and its mechanism of action viable again (Boudhar *et al*., 2016). This highlights the rationale in maintaining the chloroquinoline core structure in the design of the derivatives in this study with aditional acetamide and pyrrolidine side chains to broaden the target sites against the parasite, while maintaining the core structure with efficacious antimalarial activity.

The food vacuole of the malaria parasite is the target of quinolone agents. The weak basic properties of these drugs allow them to accumulate in the acidic vacuole of the *P. falciparum* parasite via transmembrane pH gradients (Yayon *et al*., 1985). The presence of the 7-chloroquinoline moiety in antimalarial compounds has been shown to be necessary in binding to haematin in the parasites' acidic food vacuole, thereby inhibiting haemozoin formation (Gupta *et al*., 2008). The aromatic quinoline nucleus forming a part of the derivatives is required to intercalate on the surface of the FPIX to form a drug-haem complex (Gupta *et al*., 2008). The drug-haem complex is incorporated into the growing dimer chains preventing further sequestration of toxic haem and disrupting membrane function (O'Neill *et al*., 2012).

The chloroquinoline class has been identified as drugs with potent antimalarial activity with minimum inhibitory concentrations in the range of 0.05 to 0.11 µM (Gupta *et al*., 2008). The 7-chloroquinolin-4-yl piperazine-1-yl acetamide derivatives in this study inhibited the NF54 strain of the *P. falciparum* at varying degrees with 50% minimum inhibitory concentrations between 1.29 to 53.98 µM (Table 1). Evidence that the 27 derivatives activity was directed towards the intra-erythrocytic parasite is evident in the unaltered host red blood cell membrane in response to the drugs (0.1-1.74% lysis) (Table 1). This highlights a favourable property for the use of these derivatives as antimalarials. Considering the pathology of malaria which includes red blood cell haemolysis during the parasite lifecycle, the antimalarial drug should not further compromise the patients uninfected red blood cells.

Compounds **26,25,29** showed five times higher antimalarial activity compared to compound **21**. This could be due to the introduction of the piperazine group in compound **21**. The addition of a Fluorine group in the *ortho* position of the aromatic amine decreased the antimalarial activity significantly to produce the least active compound **6**. However, positioning the Fluorine group in the *para* positon in the aromatic amine increased the antimalarial activity 3 fold (Compound **7** IC_{50}: 18.75 µM). This could be due to the effect of the *ortho* substituents which decrease the basic properties of the compound and decreasing its antimalarial effect. The addition of the strong electron donating group (-OH) leads to an increased activity of compound **26** by increasing its basic properties and activity against the parasite, particularly its accumulation capability in the parasite digestive vacuole.

The presence of the 7-chloroquinoline moiety in antimalarial compounds has proven to confer haematin binding capacity in the parasites' acidic food vacuole, resulting in haemozoin formation inhibition (Gupta *et al*., 2008). The presence of the 7-chloroquinoline moiety in the derivatives could confer its possible mechanism of action as backed by a study which showed that the aminochloroquinoline moiety of the chloroqunoline-acetamide hybrids inhibited β-haematin formation (Inam *et al*., 2015). The **CQPA-26** derivative remained in the trophozoite stage with no progression to the schizont stage, with evidence of swelling of the vacuole and diminished haemozoin formation which supports the proposed mechanism in haemozoin formation inhibition.

In the treatment of microbial infections, combination therapy is preferred to monotherapy to delay the emergence of resistance (Bell, 2005). The most active derivative from the 7-chloroquinolin-4-yl piperazine-1-yl acetamide derivatives showed a synergistic effect in combination with quinine (Graph 1). This proves to be favourable as the combination therapy additionally allows for dose reduction of individual agents when used in combination and minimizing adverse effects, reduction in costing and improves patient compliance (Bell, 2005).

In conclusion, the derivatives provided an ideal scaffold with numerous proposed mechanisms of actions that makes further investigation worthwhile. The most active derivative **CQPA-26** evidently demonstrated good antimalarial activity and low toxicity and is worthy to be explored with further structural modifications which may increase antimalarial activity.

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REFERENCES

World Health Organization WHO (2019): 'World Malaria Report 2019'https://www.who.int/publications/i/item/world-malaria-report-2019 (accessed December 2019)

World Health Organization WHO (2013): World Malaria Report', Geneva https://www.who.int/malaria/publications/world_malaria_report_2013/en/ (accessed June 2018)

South African National Department of Health (SA-DOH), 2017, 'Guidelines for the treatment of malaria in South Africa 2017', *National Department of Health*

Muregi, F.W., Ishih, A., 2010, 'Next-generation antimalarial

drugs: hybrid molecules as a new strategy in drug design', *Drug Development Research* 71, 20-32

Inam, A., Van Zyl, R.L., van Vuuren, N.J., Chen, C.T., Avecilla, F., Agarwal, S.M. and Azam, A., (2015): 'Chloroquinoline–acetamide hybrids: a promising series of potential antiprotozoal agents', *RSC Advances* 5 (60), 48368-48381

Kumawat, M.K., Singh, U.P., Singh, B., Prakash, P., Chetia, D., (2011): 'Synthesis and antimalarial activity evaluation of 3-(3-(chloroquinolin-4-ylamino)propyl)-1,3-thiazinin-4-one derivatives', *Arabian Journal Of Chemistry* 9 (1), 643-647

Rastelli, G., Pacchioni, S., Sirawaraporn, W., Sirawaraporn, R., Parenti, M.D., Ferrari, A.M., (2003): 'Docking and database screening reveal new classes of *Plasmodium falciparum* dihydrofolate reductase inhibitors', *Journal of Medicinal Chemistry* 46 (14), 2834-2845

Jensen, J.B., Trager, W., (1977): '*Plasmodium falciparum* in culture: use of outdated erythrocytes and description of candle jar method', *The Journal of Parasitology* 63 (5), 883-886

Lambros, C., Vanderberg, J.P., (1979): 'Synchronization of *Plasmodium falciparum* erythrocytic stages in culture', *The Journal of Parasitology* 65 (6), 418-420

Makler, M.T., Ries, J.M., Williams, J.A., Bancroft, J.E., Piper, R.C., Gibbins, B.L., Hinrichs, D.J., (1993): 'Parasite lactate dehydrogenase as an assay for *Plasmodium falciparum* drug sensitivity', *The American Society of Tropical Medicine and Hygiene* 48 (6), 739-741

Berenbaum, M.C., (1989):'What is synergy?', *Pharmacological Reviews* 41 (2), 93-141

Moseley, E., (2012): 'The antimalarial properties of thiosemicarbazone, chalcone, and nucleoside phosphonate derivatives*', WITS* MSc Dissertation

Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J., (1997): 'Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings', *Advanced Drug Delivery Reviews* 23 (1-3), 3-25

Mosmann, T., (1983): 'Rapid calorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays', *Journal of Immunological Methods* 65, 55-63

Hayat, F., Moseley, E., Salahuddin, A., Van Zyl, R.L., Azam, A., 2011, 'Antiprotozoal activity of chloroquinoline based chalcones', *European Journal of Medicinal Chemistry* 46 (5), 1897-1905

Gülçin, I., 2007, 'Comparison of in vitro antioxidant and antiradical activities of L-tyrosine and L-Dopa', *Amino acids* 32 (3), 431-438

Ruebhart, D.R., Wickramasinghe, W., Cock, I.E., (2009): 'Protective efficacy of the antioxidants vitamin E and Trolox against Microcystis aeruginosa and microcystin-LR in *Artemia franciscana* nauplii', *Journal of Toxicology and Environmental Health Part A* 72 (24), 1567-1575

World Health Organisation, (2005), 'Guidelines for laboratory and field testing of mosquito larvicides'

Boudhar, A., Ng, X.W., Loh, C.Y. *et al*., 2016, 'Overcoming chloroquine resistance in malaria: design, synthesis, and structure-activity relationships of novel hybrid compounds', Antimicrobial agents and Chemotherapy 60 (5), 3076-3089

Yayon, A., Cabantchik, Z.I., Ginsburg, H., (1985), 'Susceptibility of human malaria parasites to chloroquine is pH dependent', Proceedings of the National Academy of Sciences 82 (9), 2784-2788

Gupta, L., Srivastava, K., Singh, S., Puri, S.K., Chauhan, P.M.S., (2008), 'Synthesis of 2-[3-(7-chloro-quinolin-4-ylamino)-alkyl]-1-(substituted phenyl)-2,3,4,9-tetrahydro-1H-β-carbolines as a new class of antimalarial agents', Bioorganic & Medicinal Chemistry Letters 18 (11), 3306-3309

O'Neill, P.M., Barton, V.E., Ward, S.A., Chadwick, J., (2012): '4-Aminoquinolines: chloroquine, amiodaquine and next generation analogues', Treatment and Prevention of Malaria, 19-44

Bell, A., (2005): 'Antimalarial drug synergism and antagonism: mechanistic and clinical significance', FEMS Microbiology Letters 253, 171-184