Synthesis of Quinoline-Chemosensitizer Hybrid Molecule for the Management of Plasmodium falciparum

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***Abstract*— Malaria is still counted among the leading causes of death in sub-Saharan Africa. The major setback to treatment of malaria has been the emergence of parasite resistance to antimalarial drugs especially chloroquine (CQ) which was once held as a gold standard for malaria treatment. Research has shown that compounds referred to as chemosensitizers are capable of reversing resistance to CQ and CQ-like molecules. Thus in keeping with the need to save the limited arsenal of antimalarial drugs and to restore the usefulness of CQ and CQ-like molecules, a rational drug design approach to synthesize a novel antimalarial hybrid drug by covalently linking a quinoline pharmacophore to a chemosensitizer pharmacophore, is presented in this study. A linear synthetic route was used to synthesize the quinoline-chemosensitizer molecule making use of ethylene diamine as the linker. This was followed by *in vitro* evaluation of the molecule for antiplasmodial activity using 3D7 (CQ sensitive) and W2 (multidrug resistant) strains of *P. falciparum* and drug cytotoxicity to Vero cells by the MTT assay. The hybrid molecule was then re-evaluated against lumefantrine resistant (LUM-R) *P. berghei* parasites in mice using the 4-day suppressive test. The successfully synthesised hybrid molecule exhibited activity of 0.66 ± 0.06 µg/ml in 3D7 and 0.62 ± 0.01 µg/ml in W2 strain which was higher than that of the individual precursor molecules (4,7-dichloroquinoline, probenecid) and the combination. There was no cytotoxicity exhibited by the compound on Vero cells. Additionally, treatment with the hybrid compound was not effective in the mice infected with lumefantrine (LM) resistant *P. berghei* when compared to mice treated with LM. Our results successfully validate the concept of utilizing a hybrid molecule to combine antiplasmodial activity and resistance reversal activity effective against *P. falciparum* and reflect the likelihood of resistance reversal capability of probenecid *in vitro* as reported previously. However, further structural modification could be considered to improve on the activity of the molecule *in vivo*.**

***Index Terms*— Chloroquine, probenecid, quinoline pharmacophore, chemosensitizer pharmacophore, hybrid molecule**

I. INTRODUCTION

Malaria remains a major public health problem despite it being considered a preventable and treatable infectious disease. It is estimated that 214 million cases and 438,000 deaths – 90% of these occurring in Africa – resulted from malaria in 2015 (WHO, 2015). To date there has been no successful vaccine developed for the prevention of malaria though RTS,S/AS01 vaccine has recently shown promising potential (Olotu *et al*., 2016 and RTS,S Clinical Trials Partnership, 2015), thus chemotherapy remains to be seen as the major means that is largely depended upon to combat malaria. The major setback to treatment of malaria has been the emergence of parasite resistance to antimalarial drugs especially the 4-aminoquinoline class of drugs. In the 4-aminoquinoline class of drugs, chloroquine (CQ) was one of the most extensively used and to some extent abused drug for malaria treatment. It was considered a gold standard and this was attributable to its affordability and Sui-generis target on heme detoxification (Zhang *et al*., 1999).

Several compounds referred to as chemosensitizers have since been studied and have demonstrated the capability of reinstating antiplasmodial activity of chloroquine, in chloroquine resistant (CQR) strains of Plasmodium falciparum. Thus the general assumption is that the chemosensitizer inhibit the efflux of CQ from the digestive vacuole (DV) of *P. falciparum* thus allowing for CQ antimalarial activity to be effected (Henry *et al*., 2006). Verapamil was the first *P. falciparum* CQR chemosensitizer reported by Martin *et al*., (1987) who demonstrated how it reversed CQ resistance at the same concentration i.e. 1 x 10-6M, as that which was used to reverse resistance in MDR cultured neoplastic cells.

Today, artemisinin combined therapy (ACT) is the standard malaria treatment however the notion of covalently joining two or more active agents into a one drug, i.e. the development of "hybrid drugs" is fast gaining recognition as a promising alternative (Meunier 2008). The use of different pharmacophores such as those of quinoline and trioxanes has been incorporated in rational drug design using single hybrid molecules with dual functionality. Due to its advanced mode of action and high selectivity the hybrid molecule based chemotherapy has emerged as a beneficial tool in the contemporary trend of antimalarial drug discovery (Muregi and Ishih, 2010, Muregi *et al*., 2011). Burgess and his colleagues were able to demonstrate the feasibility of this concept when they designed a hybrid molecule that linked a quinoline moiety to a reversal agent (RA) - imipramine and they termed it a reversed chloroquine molecule (RCQ) (Burgess *et al*., 2006).

Due to the significance that haem detoxification presents as a drug target and the allure of restoring quinoline efficacy, we sought to synthesise a quinoline-chemosensitizer hybrid which would in essence incorporate antimalarial activity and restoration of quinoline sensitivity to CQR parasite into one molecule. The compound was then evaluated in vitro, for antiplasmodial activity against CQR (W2) and CQS (3D7) *P. falciparum* strains, cytotoxicity and in vivo for antiplasmodial activity.

II. METHODS

Synthesis of N-{2-[(7-chloroquinolin-4-yl) amino]ethyl} -4- (dipropylsulfamoyl) benzamide

Synthesis of the hybrid molecule was carried out as shown in Fig. 1. A mixture of 4,7-dichloroquinoline (1eq, 1.1883 g, 6 mmol) and ethylene diamine (10eq, 4.00 mL, 60 mmol) was refluxed at 80 °C for 1 hr without stirring and then 100-110 °C for 4 hrs with stirring then cooled and the reaction quenched using 30 mL dichloromethane (CH2Cl2). Extraction of the intermediate, N1-(7-chloroquinolin-4-yl)-ethane-1,2-diamine (2) was done in CH2Cl2 with aqueous NaOH, distilled water and brine solution, dried over anhydrous MgSO4 and concentrated in vacuo to give compound 2 as a yellow solid in 75% yield. Probenecid (1.0 eq, 2 mmol) was dissolved in dichloromethane (5 mL), and then 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (1.5 eq), 1-hydroxy-benzotriazole (HOBt) (1.5 eq) and diisopropylethylamine (DIPEA) (3.0 eq) was added and the mixture stirred at 0°C in a round bottomed flask. The intermediate 2 (5.0 mmol) was dissolved in 5 mL of CH2Cl2 and added dropwise to the reaction mixture which was stirred to room temperature for 24 hrs. The reaction mixture was quenched with saturated NaHCO3 after which the organic phase was separated and the aqueous phase back extracted with CH2Cl2. The combined organic layers were washed with brine, dried using anhydrous MgSO4, filtered and solvent removed in vacuo. Purification was carried out by flash chromatography using silica gel column (ethyl acetate: 10% ammonia in methanol 3.5:0.5) yielding the desired chemosensitizer-quinoline linked molecule N-{2-[(7-chloroquinolin-4-yl)amino]ethyl}-4-(dipropylsulfamoyl) benzamide (3) in 48% yield. Prior to obtaining 48% yield of the hybrid molecule 3, reaction (b) was optimized by altering the reaction solvents i.e. water, xylene and toluene instead of CH2Cl2 and using carbonyl diimidazole (CDI) as the coupling reagent instead of EDC. The compound was characterized by 1H NMR, 13C NMR, IR and MS.

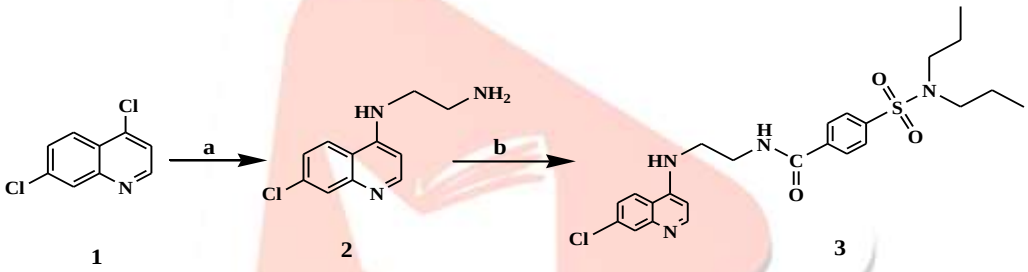

Figure 1: Scheme of quinoline-probenecid hybrid synthesis. a) 10 eq ethylene diamine, 80°C 1hr then 100-110oC for 4hrs, yield 75%; b) 1.0 eq, 2 mmol probenecid, 1.5 eq EDC, 1.5 eq HOBt, 3.0 eq DIPEA, 2.5 eq of 2, CH_2Cl_2, 0°C to room temperature stirring for 24 hrs, yield 48%.

Cytotoxicity tests *in vitro*

The cell viability was determined by the MTT (3-(4,5-dimethyltrazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay method according to Mosmann (1983). Vero cells were grown in Eagle's minimum essential medium (MEM) (GIBCO, Grand Island, N.Y.) supplemented with 5% fetal bovine serum (FBS) in 25 ml cell culture flasks and incubated at 37˚C in 5% CO_2. Upon attainment of confluence, the cells were seeded with 5×10^4 cells/well in 96-well plates and incubated under the same environment. The drug samples were solubilized in MEM and DMSO (0.02%, v/v) and after 24 h, culture medium was replaced by the solution of fresh MEM containing the drugs at different concentrations ie 1000 µg/mL for the hybrid molecule and 100 µg/mL for CQ, followed by 48 hours of further incubation. The MTT (2.0 mg/mL) was added, followed by incubation at 37˚C in an atmosphere of 5% CO_2 for 4 hours. The cells in the micro-culture were detached by trypsinization and the number of viable cells determined by MTT bioassay.The optical density was determined at 570 nm and 630 nm to measure the signal and background, respectively. The cell viability was expressed as a percentage of the control absorbance in the untreated cells after subtracting the appropriate background. Inhibition data was plotted as dose-response curves, from which CC_{50} (concentration required to cause visible alterations in 50% of intact cells) was determined by linear regression.

In vitro antiplasmodial activity assay

Laboratory-adapted *P. falciparum* cultures of the international reference isolates 3D7 and W2 which were cultured and maintained at the Malaria Laboratories at the Kenya Medical Research Institute (KEMRI) were used in this study. Culture medium was a variation of that described by Trager and Jensen (Trager and Jensen, 1976), consisting of RPMI 1640 supplemented with 10% human serum, 25 mM N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES) and 25 mM $NaHCO_3$. Human type O^+ erythrocytes (< 28 days old) served as host cells and the cultures were incubated at 37˚C in an atmosphere of 3% CO_2, 5% O_2 and 92% N_2. Quantitative assessment of antiplasmodial activity *in vitro* was determined via the semi-automated micro-dilution assay technique that measures the ability of the drug molecules to inhibit the incorporation of (G-^{3}H) hypoxanthine into the malaria parasite (Desjardins *et al*., 1979; Le Bras and Deloron, 1983). The fresh test samples were prepared to a 10 mg/ml stock solution in 100% DMSO for the hybrid molecule, 4,7-dichloroquinoline and probenecid and sterilized distilled water for CQ which was used

as the reference drug in all experiments. To compare whether or not the hybrid drug was more potent than a combination of its individual pharmacophores, wells of the combination of CQ pharmacophore and chemosensitizer pharmacophore were included in the test. The contents of each well were harvested onto glass fiber filters, washed thoroughly with distilled water, dried, and the radioactivity in counts per minute (cpm) was measured by liquid scintillation counter. The concentration causing 50% inhibition of radioisotope incorporation (IC_{50}) was determined by interpolation after logarithmic transformation of both concentration and cpm values.

Antiplasmodial tests against Lumefantrine Resistant *P. berghei* in mice

A *P. berghei* ANKA lumefantrine (LM) resistant strain, selected by increasing doses of LM, maintained in mice under constant drug pressure (LM 63.55 mg/kg) (Kiboi *et al*., 2009) and stored at −80°C was defrosted and used to inoculate mice. The mice were inoculated with 1 x 10^7 parasitized RBCs using blood parasites from a donor with parasites resistant to 63.55 mg/kg of LM. The 4-day suppressive test was performed as described by Peters *et al*., (1975) using the LM-resistant *P. berghei* ANKA blood parasites, with some modifications. Briefly, the blood parasites were maintained through weekly blood passages in mice. For the experiments, groups of 20–30 mice were inoculated with 1 × 10^7 infected erythrocytes. Approximately three hours later, they were randomly distributed into groups of five mice per cage, which were treated daily orally using cannula for four consecutive days. All the compounds were freshly diluted in a stock solution of the drug vehicle, 70% Tween-80 and 30% ethanol (the drug vehicle) that was subsequently diluted 10-fold with double distilled water (to result in a solution of 7% Tween-80 and 3% ethanol concentration) and were administered orally at doses of 64 mg/kg for LM, 50 mg/kg for hybrid and 114 mg/kg for the combination of LM and hybrid. The control mice received the drug vehicle. On days 4, 7 and 9 after parasite inoculation, blood was taken from the tail of each mouse and used to prepare thin smears, which were methanol-fixed, Giemsa-stained and examined microscopically to determine parasitaemia. The inhibition of parasite growth was evaluated in relation to parasitaemia in the untreated mice, which were considered to have 100% parasite growth.

III. Results and Discussion

Structural Characterization

N-{2-[(7-chloroquinolin-4-yl)amino]ethyl}-4-(dipropylsulfamoyl) benzamide (**3**), was isolated as a pale white powder, 0.4663g, 48% yield, Rf 0.5:SiO_2, 7 EtOAc:1 10% NH_3 in MeOH. ^{1}H NMR (400 MHz, DMSO-d6): δ 0.80 (t, J = 7.4 Hz, 6H, 2×CH_2), 1.46 (sextet, J = 7.4 Hz, 4H, 2×CH_2), 3.04 (t, J = 7.6 Hz, 4H, 2×CH_2), 3.52 (t, J = 5.2 Hz, 2H, CH_2), 3.57 (t, J = 6.3 Hz, 2H, CH_2), 6.63 (d, J = 5.5 Hz, 1H, ArH), 7.45 (dd, J = 8.9,2.1 Hz, 1H, ArH), 7.79 (d, J = 2.1 Hz, 1H, ArH), 7.90 (dd, J = 6.7,1.9 Hz, 2H, 2×ArH), 5.02 (dd, J = 6.7,1.9 Hz, 2H, 2×ArH), 8.21 (d, J = 9.0 Hz, 1H, ArH), 8.42 (d, J = 5.4 Hz, 1H, ArH). ^{13}C NMR (125 MHz, DMSO-d): δ 10.916, 21.503, 37.992, 41.742, 49.515, 98.609, 117.433, 123.889, 124.140, 126.799, 127.467, 128.169, 133.403, 137.810, 141.758, 149.011, 150.045, 151.831, 165.562. IR (KBr): υ_{max} 1643.35 (C=O stretch), 3348.42 (Secondary amide N-H stretch), 1610.56 (N-H bend). MS/FAB: m/z $[M-H]^+$ calculated 489.03, found 489.2.

***In vitro* antiplasmodial and cytotoxicity test**

Antiplasmodial activity was evaluated using the 3D7-CQS and W2-CQR strains of *P. falciparum* according to the procedure reported by Trager and Jensen (1976). The IC50 values were calculated from experiments carried out in triplicate and the results are presented in Table 1. The in vitro activity data suggests that linking the 4,7-DCQ to probenecid via an ethylene diamine linker caused the hybrid molecule (CQProb-H) to have antiplasmodial activity (0.66 ± 0.06 - 3D7 and 0.61 ± 0.01 - W2) albeit less than the reference drug CQ in both strains. However in comparison to the CQProb-H precursor molecules i.e. 4,7-DCQ, probenecid and the combination, CQProb-H showed better antiplasmodial activity. The 4,7-dichloroquinoline was used as the starting material in the synthesis of the hybrid molecule 3 however it is not an active antimalarial drug thus the reason why in both 3D7 and W2 strains the antiplasmodial activity was >100µg/ml. Probenecid which is a uricosuric drug that increases uric acid excretion in the urine is primarily used for the treatment of gout and hypertension. Therefore not surprising was the antiplasmodial activity >100µg/ml in 3D7 strain. Nonetheless probenecid when combined with 4,7-dichloroquinoline showed activity of 67.446 ± 6.32 µg/ml whereas activity of 4,7-dichloroquinoline was found to be >100 µg/ml. This could imply the possibility of a potentiating effect of probenecid on 4,7-DCQ. Probenecid has previously been shown to increase the sensitivity of chloroquine-resistant isolate V1/S (Nzila *et al*., 2003), and so a similar mechanism could be inferred in this case as 7-chloro-4-alkylamino-quinoline present in the hybrid 3 does form part of the CQ pharmacophore.

Probenecid is not a typical chemosensitizer like the calcium channel blockers, antidepressant drugs or antihistaminic drugs however it does have the essential components in its structure pointed out by Bhattacharjee *et al*., (2002) required to serve as a chemosensitizer. Thus in the case of compound 3, the 7-chloro-4-aminoquinoline nucleus which is obligatory for antimalarial activity, particularly, inhibition of β-hematin formation and accumulation of the drug at the target site (Kaschula *et al*., 2002, Cheruku *et al*., 2003 and Egan, 2003), coupled with the presence ethylene diamine link essential for maintained activity against CQR strains of *P. falciparum* (Ridley *et al*., 1996 and De *et al*., 1998) that was linked to probenecid could be said to be the contributing factor for increased antiplasmodial activity observed. Cytotoxicity of compound 3 was tested on Vero cell line (Table 1) and it was not cytotoxic at maximum concentration used (1000 µg/mL).

Table 1 : Antiplasmodial activity and cytotoxicity of quinoline-probenecid hybrid compound and its precursor molecules.[a]

Molecule	Antiplasmodial Activity (IC_{50} µg/ml)		Cytotoxicity (CC_{50} µg/ml)
	3D7	W2	
4,7-DCQ	> 100	> 100	NT

Probenecid	> 100	53.178	NT
4.7-DCQ+ Probenecid	> 100	67.446	NT
CQProb-H	0.66	0.61	NC
CQ[b]	0.005	0.078	57.46

DCQ – Dichloroquinoline, CQ – Chloroquine, (IC_{50} – maximum concentration tested 100µg/ml for 4,7-DCQ, Probenecid and 4.7-DCQ+ Probenecid, 10µg/ml for hybrid, 0.05µg/ml for CQ-3D7 and 0.2µg/ml for CQ-W2), NT – Not Tested, NC – Not Cytotoxic (maximum concentration tested 1000µg/ml for hybrid and 100µg/ml for CQ).

[a] Values are means of triplicate samples

[b] Reference drug

Antiplasmodial tests against Lumefantrine Resistant *P. berghei* in mice

The efficacy of the hybrid molecule 3 was measured using the 4-day suppressive test (Peters *et al*., 1975) using a model of LM–resistant P. berghei infection. For this study, Swiss albino mice were infected on day 0 and treated once daily for 4 days. Parasitaemia was measured on day 4 and compared to those of control non-treated mice. As seen in Table 2, 50mg/kg of the hybrid alone suppressed day 4 parasitaemia by 8.69% whereas 64 mg/kg of LM and a combination of 64 mg/kg LM and 50 mg/kg hybrid molecule (114 mg/kg) gave a parasite suppression of 82.27% and 62.06% respectively. The hybrid showed no suppression activity when compared to LM and even further combination of the two drugs i.e. LM and the hybrid showed no improved suppression.

Table 2: Antiplasmodial activity of LM and hybrid molecule against P. berghei evaluated as percentage reduction of parasitaemia 4 days after innoculation with LM-resistant P berghei

Drug	**Dosage mg/kg**	**Percentage reduction of parasitaemia (%)***		
		Day 4	**Day 7**	**Day 9**
Lumefantrine	64	82.27	58.11	47.61
Hybrid	50	8.69	8.55	14.46
LM + Hybrid	114	62.06	59.00	60.24

*Percentage reduction of parasitaemia calculated in relation to control non-treated mice (100% of parasite growth)

The lack of parasite suppression by the hybrid molecule could be attributed possibly to high lipophilicity of the hybrid molecule 3 which although not tested for could have contributed to poor bioavailability of the drug. Burgess *et al*., (2006) reported a similar limitation in the reversed chloroquine molecule which they synthesised and although it displayed high antiplasmodial activity, (3 nM in CQS D6 and 5 nM in CQR –Dd2 *P. falciparum* strains) it was highly lipophilic as such could not be retained for further drug development. Secondly, there is a possibility that the molecule could be metabolically unstable in vivo, as 4,7-dichloroquinoline and probenecid were tethered via an ethylene diamine link. Consequently, metabolism of the molecule which was administered orally could have led to failed delivery of the hybrid molecule as a whole entity to the site of action – the parasite digestive vacuole. Additionally, unpublished in vivo data on the activity of 4,7-dichloroquinoline against P. berghei at a dose of 50 mg/kg resulted in percentage parasite suppression of 52.4%, further supporting the argument of minimal antiplasmodial activity from the precursor molecule – 4,7-dichloroquinoline. Probenecid was envisioned as inhibiting the *P. falciparum* chloroquine resistant transporter (CQRT) associated with CQ export from the DV in CQR parasite. On its own however, it possesses minimal antiplasmodial activity in vitro 53.178 µg/ml which did not translate in vivo as observed when the hybrid was combined with LM. Assuming that in this case (combination of LM with hybrid) there was delivery of the hybrid molecule to the DV one would expect a potentiating effect which was not observed on the contrary the percentage parasite suppression reduced.

In the present study a quinoline-chemosensitizer hybrid consisting of quinoline and probenecid scaffold was synthesized. Ethylene diamine was used as the linker to facilitate hybridization via an amide bond. The synthesis was carried out in a 2-step process producing the hybrid molecule which was then evaluated in vitro and in vivo for antiplasmodial activity. In vitro, the hybrid molecule showed antiplasmodial activity of 0.66 ± 0.06 µg/ml in CQS -3D7 strain and 0.61 ± 0.01 µg/ml in CQR –W2 strain which was less active than the reference drug CQ (0.005 µg/ml, 0.078 µg/ml –W2). Further evaluation of antiplasmodial activity in vivo, went to show that the synthesized hybrid molecule could not counter CQ resistance as had earlier been hypothesized. In the LM-resistant P. berghei mouse malaria model, the hybrid molecule displayed no significant activity on its own (8.9% parasite suppression) and when combined with LM (62.06% parasite suppression) compared to the 82.27% parasite suppression obtained from LM.

Despite having no activity in vitro and in vivo proof of concept of covalently linking two structural motifs into one molecule was achieved. However more can still be done to improve on the molecule. Variation of both the linker (De *et al*., 1996, Hocart *et al*., 2011 and Ridely *et al*., 1996) and possibly the reversal agent moiety (Carradori *et al*., 2015 and Salahuddin *et al*., 2013) could be considered with the aim of making the compound more stable to metabolic cleavage and possibly improving the overall activity of the compound. More importantly, alternative reversal agents/ chemosensitizers could be sought that preferably possess considerably good antiplasmodial activity so as to give not only resistance reversal activity but also dual antiplasmodial activity to the hybrid molecule.

ACKNOWLEDGEMENT

We wish to acknowledge the financial support from the Association of African Universities (Small Grants for Theses and Dissertations Ref: PC/6), Kenya Medical Research Institute and the Directorate of Research Grants & Endowments – Mount Kenya Universities for provision of facilities to carry out the project.

The synthesised molecule is patented under Kenya Industrial Property Institute, Patent Application Number: KE/P/2017/2759.

REFERENCES

[1] Bhattacharjee, A. K., Kyle, D. E., Vennerstrom, J. L. and Milhous, W. K. A 3D QSAR pharmacophore model and quantum chemical structure-activity analysis of chloroquine(CQ)-resistance reversal. J. Chem. Inf. Comp. Sci., vol. 42, pp. 1212–1220, 2002.

[2] Burgess, S.J., Selzer, A., Kelly, J.X., Smilkstein, M.J., Riscoe, M.K. and Peyton, D.H. A Chloroquine-like Molecule Designed to Reverse Resistance in Plasmodium falciparum. J. Med. Chem., vol. 49, pp. 5623–5625, 2006.

[3] Cheruku, S. R., Maiti, S., Dorn, A., Scorneaux, B., Bhattacharjee, A. K., Ellis, W. Y. and Vennerstrom, J. L. Carbon isosteres of the 4-aminopyridine substructure of chloroquine: Effects on pKa, hematin binding, inhibition of hemozoin formation, and parasite growth. J. Med. Chem., vol. 46(14), pp. 3166-3169, 2003.

[4] Carradori, S., Mollica, A., Ceruso, M., D'Ascenzio, M., De Monte, C., Chimenti, P., Sabia, R., Akdemir, A.and Supuran, C. T. New amide derivatives of Probenecid as selective inhibitors of carbonic anhydrase IX and XII: Biological evaluation and molecular modelling studies. Bioorg. Med. Chem., vol. 23, pp. 2975–2981, 2015.

[5] De, D., Krogstad, F.M., Cogswell, F.B. and Krogstad, D.J. Aminoquinolines that circumvent resistance in Plasmodium falciparum in vitro. Am. J. Trop. Med. Hyg., vol. 55(6), pp. 579–583, 1996.

[6] De, D., Krogstad, F. M., Byers, L. D. and Krogstad, D. J. Structure-activity relationships for antiplasmodial activity among 7-substituted 4-aminoquinolines. J. Med. Chem., vol. 41(25), pp.4918-4926, 1998.

[7] Desjardins, R.E., Canfield, C.J., Haynes, J.D. and Chulay, J.D. Quantitative assessment of antimalarial activity in vitro by semi-automated micro dilution technique. Antimicrob. Agents Chemother. vol. 16, pp. 710-718, 1979.

[8] Egan, T. J. Haemozoin (malaria pigment): A unique crystalline drug target. Targets, vol. 2(3), pp. 115-124, 2003.

[9] Henry, M., Alibert, S., Orlandi-Pradines, E., Bogreau, H., Fusai, T., Rogier, C., Barbe, J. and Pradines, B. Chloroquine resistance reversal agents as promising antimalarial drugs. Curr. Drug Targets, vol. 7(8), pp. 935-948, 2006.

[10] Hocart, S.J., Liu, H., Deng, H., De, D., Krogstad, F.M. and Krogstad, D.J. 4-aminoquinolines active against chloroquine-resistant Plasmodium falciparum: basis of antiparasite activity and quantitative structure-activity relationship analyses. Antimicrob. Agents Chemother., vol. 55(5), pp.2233–2244, 2011.

[11] Kaschula, C. H., Egan, T. J., Hunter, R., Basilico, N., Parapini, S., Taramelli, D., Pasini, E. and Monti D. Structure-activity relationships in 4-aminoquinoline antiplasmodials. The role of the group at the 7-position. J. Med. Chem., vol. 45(16), pp. 3531-3539, 2002.

[12] Kiboi, D.M., Irungu, B.N., Langat, B. Wittlin, S., Brun, R., Chollet, J., Abiodun, O., Nganga, J.K., Nyambati, C.S., Rukunga, G.M., Bell, A., Nzila, A. Plasmodium berghei ANKA: selection of resistance to piperaquine and lumefantrine in a mouse model. Exp. Parasit., vol. 122, pp. 196-202, 2009.

[13] Le Bras, J and Deloron, P. In vitro Study of Drug Sensitivity of Plasmodium falciparum: An evaluation of a new semi-microtest. Am. J. Trop. Med. Hyg., vol. 32, pp. 447- 451, 1983.

[14] Martin, S.K., Oduola, A.M. and Milhous, W.K. Reversal chloroquine resistance in Plasmodium falciparum by verapamil. Sci., vol. 235, pp. 899-901, 1987.

[15] Meunier, B. Hybrid Molecules with Dual Mode of Action: Dream or Reality? Acc. Chem. Res., vol. 41(1), pp. 69-77, 2008.

[16] Mossmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Meth., vol. 65, pp. 55–63, 1983.

[17] Muregi, F.W. and Ishih, A. Next-generation antimalarial drugs: hybrid molecules as a new strategy in drug design. Drug Dev. Res., vol. 71, pp. 20–32, 2010.

[18] Muregi, F.W., Kirira, P.G. and Ishih, A. Novel rational drug design strategies with potential to revolutionalize malaria chemotherapy. Curr. Med. Chem., vol. 18, pp.113-143, 2011.

[19] Olotu, A., Fegan, G., Wambua, J., Nyangweso, G., Leach, A., Lievens, M., Kaslow, D. C., Njuguna, P., Marsh, K., and Bejon P. Seven-Year Efficacy of RTS,S/AS01 Malaria Vaccine among Young African Children. N. Engl. J Med., vol. 374, pp.26, 2016.

[20] Peters, W., Portus J.H. and Robinson B.L. The chemotherapy of rodent malaria XXII. The value of drug-resistant strains of Plasmodium berghei in screening for schizontocidal activity. Ann. Trop. Med. Parasitol., vol. 69, pp. 155-171, 1975.

[21] Ridley, R. G., Hofheinz, H., Matile, H., Jaquet, C., Dorn, A., Masciadri, R., Jolidon, S., Richter, W. F., Guenzi, A., Girometta, M. A., Urwyler, H., Huber, W., Thaithong, S. and Peters, W. 4-Aminoquinoline analogs of chloroquine with shortened side chains retain activity against chloroquine-resistant Plasmodium falciparum. Antimicrob. Agents Chemother., vol. 40(8), pp. 1846-1854, 1996.

[22] RTS,S Clinical Trials Partnership. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomized, controlled trial. Lanc., vol. 386, pp. 31-45, 2015.

[23] Salahuddin, A., Inam, A., van Zyl, R. L., Heslop, D. C., Chen, Chien-Teng , Avecilla, F., Agarwal, S. M. and Azam, A. Synthesis and evaluation of 7-chloro-4-(piperazin-1-yl)quinoline-sulfonamide as hybrid antiprotozoal agents. Bioorg. Med. Chem., vol. 21, pp. 3080–3089, 2013.

[24] Trager, W. and Jensen, J.B. Human Malaria Parasites in Continuous Culture. Sci., vol. 193, pp. 673-675, 1976.

[25] World Health Organization. World Malaria Report 2015. Geneva, Switzerland. World Health Organization, 2015.

[26] Zhang, J., Krugliak, M. and Ginsburg, H. The fate of ferriprotorphyrin IX in malaria infected erythrocytes in conjunction with the mode of action of antimalarial drugs. Mol. Biochem. Parasitol., vol. 99, pp. 129–141, 1999.