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CHAPTER 17

Discovery and Development of Antimalarial Drug-Resistance Reversal Agents

Phanankosi Moyo[1,*], Vinesh Maharaj[1], Sephora M. Mutombo[1], Warren Andrew Andayi[2], Linda Amoah[3,4], Kathithileni M. Kalili[5], Kwame Kumi Asare[6] and **Cynthia Amaning Danquah[7]**

[1] *Department of Chemistry, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria0028, South Africa*

[2] *Department of Physical and Biological Sciences, Murang'a University of TechnologyMurang'a, Kenya*

[3] *Immunology Department, Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana*

[4] *West African Center for Cell Biology of Infectious Pathogens, Department of Biochemistry, Cellular and Molecular Biology, University of Ghana, Accra, Ghana*

[5] *Department of Physics, Chemistry & Material Science, University of Namibia, Private Bag 13301, 340 Mandume Ndemufayo Avenue, Pionierspark, Windhoek, Namibia*

[6] *Department of Biomedical Sciences, School of Allied Health Sciences, College of Allied Health Sciences, University of Cape Coast, Cape Coast, Ghana*

[7] *Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Ghana*

Abstract: Despite the meritorious measures taken to curb the malaria scourge in the last two decades, the drug-resistance phenomenon threatens to reverse the gains made. Evidence of partial resistance against the first-line antimalarial drugs, the artemisinin-based combination therapies, in Africa, a region that accounts for 95% of global malaria cases, has aroused fears that it could spread fast and significantly jeopardise malaria control and eradication efforts. While the antimalarial drug discovery pipeline has several, encouraging candidates emerging through it, unfortunately, the attrition rate is high, with some candidates failing either in preclinical studies or clinical trials. Moreover, the rate of emergence of drug-resistant *Plasmodium* parasite strains far exceeds that of the drug discovery and development process. These challenges demand novel strategies to complement the discovery and development of new therapeutics. One such strategy is the reversal of *Plasmodium falciparum* resistance to old antimalarials by combining these drugs with agents that specifically target drug-resistance mechanisms. This strategy has been successfully used in the antibiotics field, with a classical successful example being the amoxicillin and clavulanic acid combina-

* **Corresponding author Phanankosi Moyo:** Department of Chemistry, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria 0028, South Africa; E-mail: phanankosimoyo@gmail.com; u13386842@tuks.co.za

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tion. In this chapter, we present a case for the need to discover and develop antimalarial drug-resistance reversal agents to prolong the efficacy and use of currently available antimalarial quinoline drugs as well as the re-use of old antimalarial quinoline drugs that have been rendered ineffective by drug-resistance. Furthermore, we provide an overview of noteworthy significant innovations that have been made in the field in search for antimalaria drug-resistance reversal agents. We conclude by providing perspectives on how these efforts can be expedited.

Keywords: 4-aminoquinoline, Chloroquine, Drug-resistance, Malaria, *Plasmodium falciparum*, *P. falciparum* chloroquine resistance transporter (*Pf*CRT), Mefloquine, *P. falciparum* multidrug-resistance-1 (*Pf*MDR1) transporter, Quinine, Quinolines, Resistance breakers, Resistance reversal agents.

1. INTRODUCTION

1.1. Malaria: A Health, Economic and Social Challenge

Malaria, a tropical disease caused by an infection with unicellular parasitic protozoa of the genus *Plasmodium* transmitted by female *Anopheles* mosquitoes, has been a major health challenge to human beings since time immemorial, with documentation from as far back as 2700 BC making reference to the disease [1]. It is presently endemic in 85 countries with half the world's population at risk of getting infected [2]. Despite the concerted intervention measures, malaria remains a serious global public health burden. In 2020, it caused 241 million clinical cases resulting in 627 000 fatalities, with the World Health Organisation (WHO) Africa Region accounting for nearly 95% of those cases (Table **1**) [2]. Children under the age of 5 years are amongst the most vulnerable, along with pregnant women, accounting for 77% of the deaths (Table **1**) [2].

The impact of malaria extends beyond the compromise of the wellbeing of its victims to having a negative impact on the economy [3, 4]. There is concurrent poverty and economic burden on both individuals and the healthcare systems in malaria-endemic countries. For example, it has been reported that Africa is losing close to US$12 billion of its Gross Domestic Product (GDP) per year while also causing stagnation in economic growth by as much as 1.3% [4]. Since 2001, countries in sub-Saharan Africa have been spending on average US$300 million annually on malaria case management, a staggering amount to countries whose meager income resources are already overburdened and stretched by other pressing issues [3, 4]. In addition to the loss of household incomes, malaria also leads to work and school health-related absenteeism [3, 4], with the latter having been reported to range between 17 and 54% in some parts of Kenya [5].

Table 1. The health burden of malaria*

WHO Region	Malaria cases	Malaria deaths	Health burden: Children and pregnant woman
Africa	228 000 000	602 000	▪ 77% deaths were children <5 years ▪ In 33 African countries, 11 600 000 pregnancies were exposed to malaria resulting in 819 000 children with low birth weight
South-East Asia	5 000 000	9 000	
Eastern Mediterranean	5 700 000	12 300	
Western Pacific	1 700 000	3 200	
Americas	650 000	409	

*Source [2]

1.2. Malaria Control: The Perpetual Challenge of Drug-Resistance Emergence and Spread

Along with vector control, chemotherapeutic agents including the quinolines (Fig. **1**) and artemisinin-based combination therapies (ACTs), have been decisive in the control of malaria [6]. Unfortunately, the use of antimalarial drugs has, historically and presently, been undermined by the emergence and spread of drug-resistant *Plasmodium* parasite strains (Table **2**) [7, 8]. This has rendered drugs, such as Chloroquine, less effective, significantly curtailing their role and deployment in malaria control efforts.

Table 2. Antimalarial drug development and resistance emergence*

Drug	Discovered	Launched	Resistance first report	Resistance associated gene mutations
Quinine	1820	19th century	1910	*pfmdr1, pfmrp, pfnhe1*
Chloroquine	1936	1945	1957	*pfcrt, pfmdr1, pfmrp*
Proguanil	1945	1948	1949	*pfdhfr*
Amodiaquine	1940s	1940s	1954	*pfcrt*
S-P$^{\infty}$		1967	1967	*pfdhps*
Artemisinin	1970s	1970s	2009	*kelch13*
Mefloquine	1970s	1970s	1982	*pfmdr1*
Halofantrine	1970s	1988	1992	*pfmdr1*
Atovaquone	1980s	1996	1996	*pfcytb*

*Source [9 - 14]. $^{\infty}$S-P – sulphadoxine-pyrimethamine; *pfmdr1 – P. falciparum* multidrug resistance 1; *pfmrp – P. falciparum* multidrug-resistance-associated protein; *pfnhe – P. falciparum* Na^+/H^+ exchanger; *pfcrt – P. falciparum* chloroquine-resistance transporter; *pfdhfr – P. falciparum* dihydrofolate reductase; *pfdhps – P. falciparum* dihydropteroate synthase; *pfcytb – P. falciparum* cytochrome b

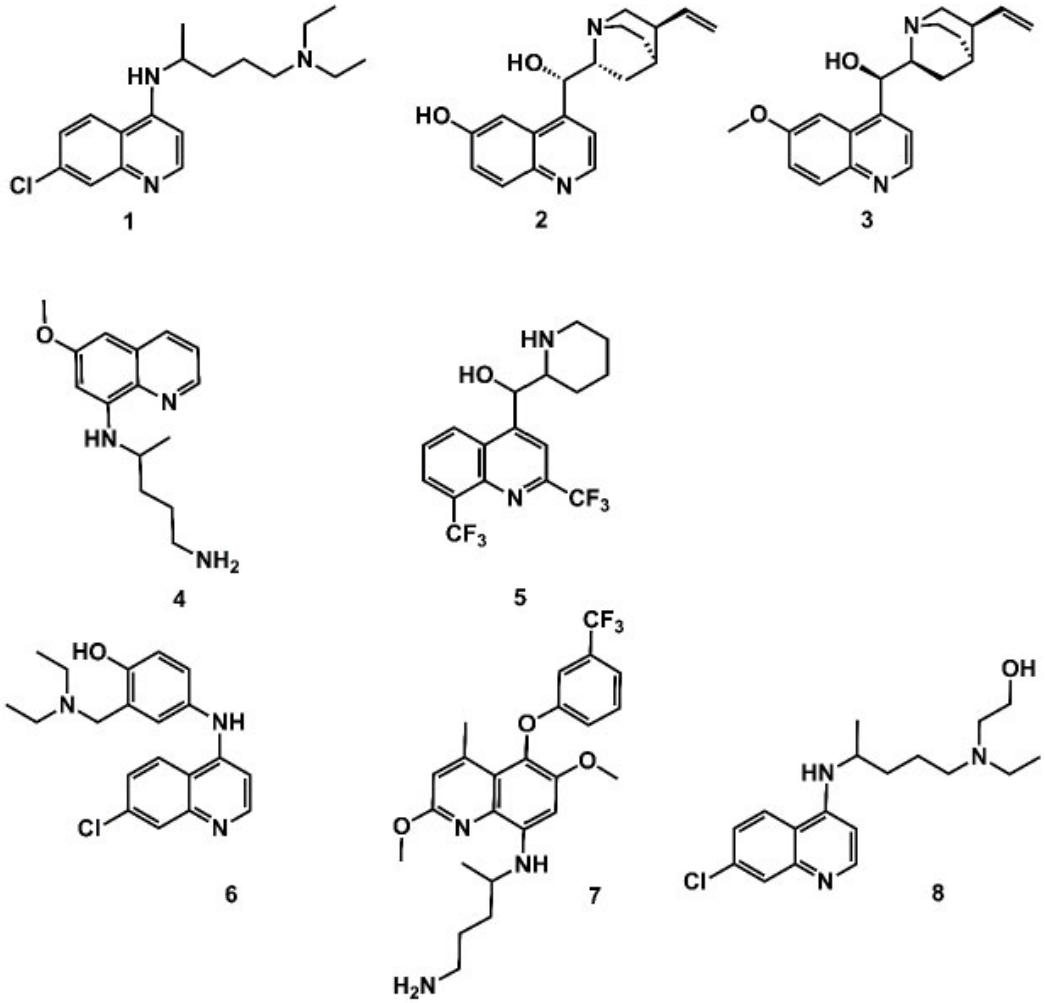


Fig. (1). Structures of common clinically useful quinoline antimalarials (Chloroquine **1**, Quinine **2**, Quinidine **3**, Primaquine **4**, Mefloquine **5**, Amodiaquine **6**, Tafenoquine **7** and Hydroxychloroquine **8**).

Resistance to chloroquine and amodiaquine has been shown to be linked to point mutations on the *P. falciparum* chloroquine-resistance transporter (*pfcrt*) gene which encodes for the PfCRT protein (Fig. **2**) [15, 16]. The PfCRT is a 49-kDa metabolite transporter protein consisting of 10 transmembrane helices located on the membrane of the parasitophorous food vacuole (PFV). Together, these helices form a charged cavity postulated to be the pocket which carries drugs and metabolites during transportation from the PFV into the cytosol. Mutations within this cavity have been resolved to confer chloroquine and amodiaquine-resistance in *P. falciparum* [7, 16]. These mutations lead to the efflux of protonated chloroquine and amodiaquine molecules out of the PFV [15, 16]. Consequently,

chloroquine and amodiaquine fail to accumulate to concentrations which effectively bind to toxic haem species and inhibit their dimerisation to the inert haemozoin crystals [7, 15, 16].

Resistance to mefloquine is associated with point mutations on the *P. falciparum* multidrug-resistance 1 (*pfmdr1*) gene which encodes for PfMDR1, an ATP-binding cassette (ABC) transporter protein homologous to p-glycoprotein (P-gp). In contrast to PfCRT, PfMDR1 transports substrates from the cytosol into the PFV. Mutations on PfMDR1 are thought to result in increased transport of mefloquine into the PFV away from its biological target, the 80S ribosome, in the cytosol (Fig. **2**) [7, 17].

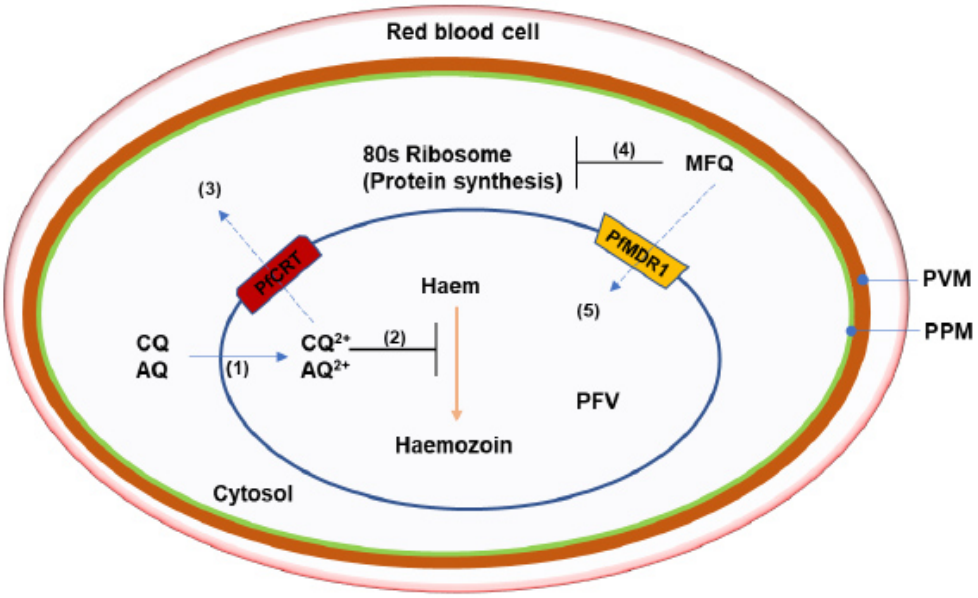


Fig. (2). Resistance mechanisms for chloroquine (chloroquine), amodiaquine (AQ) and mefloquine (MFQ). (1) chloroquine and AQ move into the PFV where they get protonated and can then (2) bind and inhibit dimerisation of haem to the non-toxic haemozoin. (3) However, mutations on PfCRT result in efflux of both chloroquine and AQ from the PFV into the cytosol and so leading to failure in blocking of haemozoin formation. (4) MFQ inhibits protein synthesis by targeting the 80S ribosome in the cytosol. (5) Mutations on the PfMDR1 resulting in the importation of MFQ into the PFV where it is sequestrated away from its target in the cytosol. PVM – parasitophorous vacuole membrane; PPM – parasite plasma membrane.

Following the emergence and wide spread of chloroquine-resistant parasites, the WHO has since 2001 recommended the use of ACTs as the first-line treatment drugs for malaria. However, partial resistance against ACTs, which offer the last line of defense against *Plasmodium* parasites has emerged and spread to countries in Asia, South America, and Africa [18 - 22]. This resistance is manifested by delayed parasite clearance associated with point mutations on the propeller

domain of the Kelch13 protein (PfK13) (Table **2**) [7]. The two mechanisms for PfK13 resistance are the inhibition of artemisinin-induced proteinopathy and cell death, and the upregulation of parasite oxidative stress and protein damage pathways via the unfolded protein response [23 - 25].

The never-ending emergence and spread of drug-resistant *Plasmodium* parasite strains have compelled the sustained discovery and development of novel antimalarial chemotypes with unique modes-of-action (MoA). While no new chemotypes have been introduced into the malaria treatment arsenal in the past decade, the current Medicines for Malaria Venture (MMV) portfolio of preclinical and clinical candidate antimalarial drugs does provide optimism for the field (Fig. **3**). However, an err on the side of caution needs to be exercised given the high attrition rate of malaria drugs in clinical trials [26]. This attrition rate is intrinsically higher for novel chemotypes compared to those that are clinically established [26]. Unfortunately, as of now a few preclinical and clinical drugs have dropped out from development due to different reasons including safety [27]. In addition to high failure rate, the inevitable imminent emergence and spread of parasites resistant against these clinical candidates will limit their clinical lifespan. For example, resistance to sulphadoxine and atovaquone emerged 1 and 6 years, respectively, following their introduction into the clinic [10]. Drugs targeting single proteins have been observed to have a higher propensity to select for resistant parasites in a short time frame [10]. Unfortunately, most compounds in the pipeline target single proteins in the parasite [27, 28] raising fears that this could result in them having a short clinical lifespan. In light of these perceived challenges, it is sensible to state that the discovery and development of novel antimalarials cannot on its own guarantee the uninterrupted timeous supply of clinically efficacious antimalarials. Therefore, there is a need to consider alternative strategies to bridge the gap between the clinical need for efficacious chemotherapeutic agents and the drug discovery process. One feasible interim solution worth considering is the preservation of current and old failing antimalarials by the use of drug-resistance reversal agents.

Drug-Resistance Reversal Strategy: A Noble Approach to Complement Drug Discovery and Development Efforts

The drug-resistance reversal strategy entails the use of compounds, with either little or no intrinsic potency, to resensitise either resistant microorganisms or parasites to drugs [30 - 32]. The reversal agents can either be co-administered with or conjugated to drugs rendered ineffective due to resistance. Resistance reversal agents, also referred to as resistance breakers, act by targeting resistance mechanisms including modifying enzyme inhibitors, membrane permeabilisers and efflux pump inhibitors [30 - 32]. The β-lactamase enzyme inhibitors are

arguably the most successful resistance reversal agents that have been used clinically to date. These are compounds that inhibit β-lactamase, an enzyme that hydrolyses β-lactam antibiotics, including penicillins and cephalosporins, hence rendering them inactive. The most prominent example is that of amoxicillin (a penicillin) co-administered with clavulanic acid, a prolific β-lactamase inhibitor compound [30, 32]. This combination therapy was first launched in 1981 and has successfully been used since then and remains relevant to this day [33, 34]. Building on that success, other β-lactam antibiotic and β-lactamase inhibitor combinations have since been successfully developed. These include ceftazidime-avibactam and ceftolozane-tazobactam combination therapies [35]. This shrewd approach has ensured that the β-lactam antibiotics, a class of safe, widely available, and affordable antibiotics, have remained applicable clinically nearly 80 years after they were first introduced. In so doing, it has allowed for continued reasonable management of bacterial infections despite the emergence and spread of drug refractory bacterial strains, a situation exacerbated by the lack of development and deployment of a novel class of antibiotics since 1987.

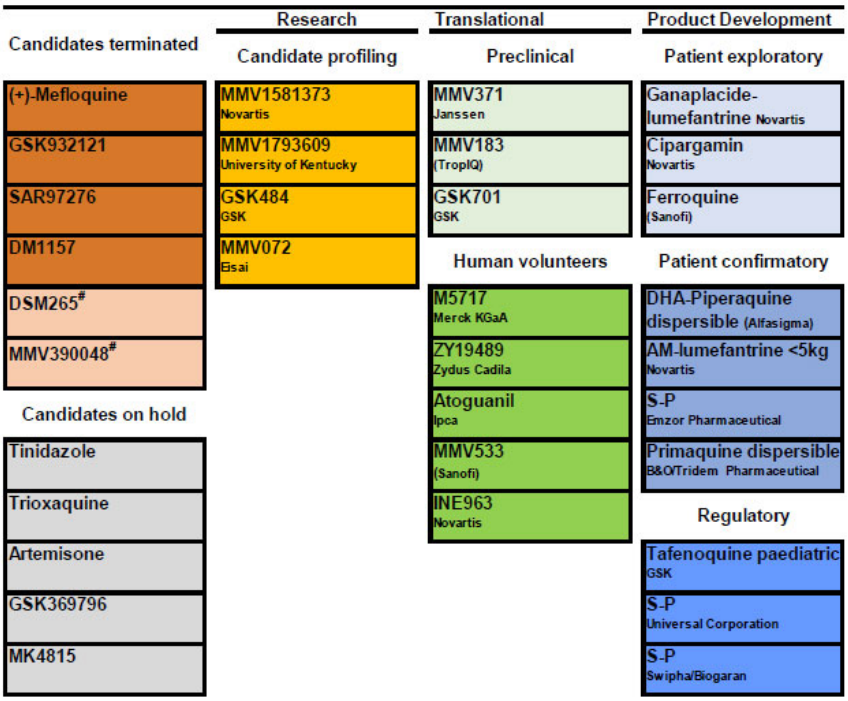


Fig. (3). MMV supported projects drug development portfolio as of November 2021 (https://www.mmv.org/research-development/mmv-supported-projects). The figure shows candidates in the profiling regulatory review phase. Development of some candidates has either been terminated (brown shaded) or placed on hold (grey shade) [27]. DHA – dihydroartemisinin; AM – artemether; S-P – Sulphadoxine-pyrimethamine. # - "Terminated" as a sponsor strategic decision [29].

4-Aminoquinolines, A Class of Antimalarials Worth Preserving

The 4-aminoquinolines, particularly chloroquine, are arguably the most effective therapeutics to have been used clinically in the fight against malaria [6]. This is owed primarily to their efficacy and for being remarkably fast-acting. They have exceptional plasma stability, so they maintain acceptable concentration levels for extended time periods. For example, chloroquine and mefloquine have half-lives of 41 and 14 days, respectively, which is much better compared to that of artemisinin derivatives whose elimination half-life ranges from 0.5 to 5 hours [12, 36, 37]. Furthermore, they have a good safety track record including for use by both pregnant women and infants [38]. In addition to their good pharmacological properties, this class of compounds has the advantage of being widely available and affordable costing under US$0.10 per treatment course [6]. Comparatively, at some point, despite the subsidised pricing, ACTs cost 40 times more than chloroquine [39]. Chloroquine can be stored at room temperature in tropical regions and remains stable for up to 5 years, an advantage it has over the current first-line treatment drugs which have a short shelf-life of 2 years at room temperature [40]. In view of all these advantages, there is a genuine compelling argument to bring 4-aminoquinolines, particularly chloroquine, from the fringes to the forefront in the fight against malaria. Such unparalleled clinical and economic attributes cannot be abandoned. The drug-resistance reversal strategy does offer a practical route for their return.

2. DISCOVERY AND DEVELOPMENT OF RESISTANCE REVERSAL AGENTS FOR 4-AMINOQUINOLINES

Despite its evident potential, the drug-resistance reversal strategy has received subdued interest compared to novel drug discovery in the malaria field as observable from a Scopus database query (Fig. **4**). For example, while publications related to antimalarial drug discovery have drastically increased over the years, in contrast, the number of published papers on malaria drug-resistance reversal stagnated (Fig. **4**). Nonetheless, since the breakthrough study in which verapamil, a weak monoprotic base calcium channel blocker, was shown to clinically reverse chloroquine-resistance in previously refractory *P. falciparum* strains [41], a reasonable number of noteworthy publications on the subject have emerged. Drug-resistance reversal in *P. falciparum* parasites has two defining attributes, namely (i) decrease of chloroquine concentration required to inhibit 50% of parasite proliferation (IC_{50}) and (ii) enhanced uptake and sequestration of chloroquine into the PFV of chloroquine-resistant strains [41, 42]. Here we provide a succinct summary of synthetic and natural compounds, as well as extracts, that have been reported to show the ability to reverse resistance against the 4-aminoquinolines either *in vitro*, *in vivo*, or clinically.

2.1. Synthetic Compounds as Resistance Reversers of 4-Aminoquinolines

2.1.1 Verapamil - A Classical 4-Aminoquinoline Drug-Resistance Reversal Agent

Despite lacking intrinsic antiplasmodial activity, verapamil is able to augment the action of chloroquine in chloroquine-resistant strains [41]. This mechanistically happens independent of calcium channels [43]. Three analogues of verapamil, whereby methoxy groups were varied or exchanged with H, represent the earliest reported synthetic efforts in understanding chloroquine-resistance reversal agents (Fig. **5**). The *R*-(+) isomers of the 3 analogues did not bind Ca^{2+} channels but caused chloroquine-resistance reversal. The *S*-(-) isomers of the three analogues bound to the Ca^{2+} ions in addition to reversing chloroquine-resistance. This confirmed the stereospecificity of verapamil **9** and its analogues to binding cardiovascular calcium channels. It was concluded that the *R*-(+) isomers of verapamil and its analogues, gallopamil **10**, and devopamil **11** are suitable for chloroquine-resistance reversal applications since they will not cause any side effects via blocking of the cardiovascular calcium ion channels.

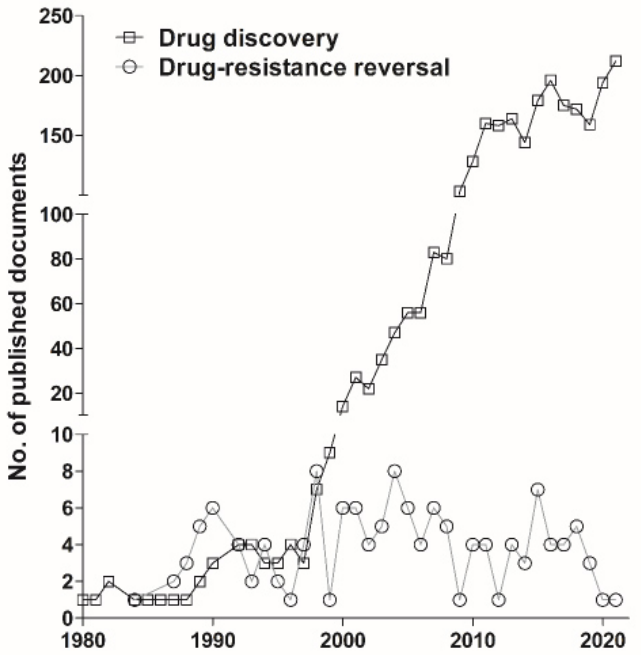


Fig. (4). An illustration showing the number of documents on malaria drug discovery and malaria drug-resistance reversal. It is evident that the number of publications relating to drug discovery has shown a drastic increase over the years (1980-2021). Comparatively, the subject of antimalarial drug-resistance reversal has been subdued as observed by the stagnated publications on the subject. Data used to develop the figure was retrieved from Scopus using the key words “malaria drug discovery” and “malaria drug-resistance reversal” for the database query.

In addition to chloroquine-resistance reversal, verapamil also modulates resistance reversal of quinine and its stereoisomer quinidine. However, this activity is subject to specific mutations on the PfCRT. For example, verapamil can resensitise quinine and quinidine refractory *P. falciparum* strains carrying either the K76T or K76N mutations in PfCRT noticed by a reduction in the IC_{50} of both drugs. However, in *P. falciparum* strains carrying the K76I mutation, interestingly, verapamil increases the IC_{50} of quinine five-fold [44, 45]. In contrast to this, verapamil can reverse resistance against quinidine in all *P. falciparum* strains with a PfCRT mutation on position 76 [44]. Verapamil fails to reverse resistance to mefloquine in *P. falciparum* strains resistant to this drug [46, 47]. It appears that drugs' stereochemistry may or may not influence quinoline-resistance reversal depending on the chemosensitisers' chemistry and binding modes.

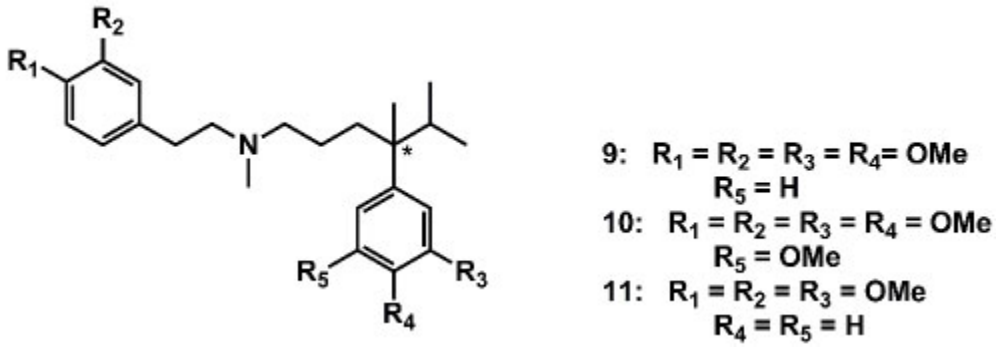


Fig. (5). Structures of Verapamil and its analogues, gallopamil and devopamil. * - denotes stereogenic centre.

Beyond VPL, other structurally and functionally diverse chemosensitising agents with *in vitro* and *in vivo* quinoline reversal activity have been explored. A not so exhaustive list includes calcium channel inhibitors (other than VPL), tricyclic antidepressants such as imipramine, antihistamines, other antimalarials and prostaglandin oligomers. These agents required co-administration with chloroquine. Unfortunately, their *in vivo* and clinical applicability has been limited primarily by their low efficacy, demanding high dosages which are toxic. Furthermore, some agents showed poor pharmacokinetic properties including high plasma binding which severely impacted on their availability and resistance reversal efficacy. On the background of these challenges, a new approach, the 'reversed chloroquine' paradigm, was conceptualised.

2.1.2 Reversed Chloroquine: Chloroquine-Reversal Agent Conjugates or Double Drugs

Apart from toxicity considerations, for a chloroquine-resistance reversal regimen to be therapeutically viable, important pharmacokinetic factors, namely absorption and excretion rates, need to be considered. The chloroquine-resistance reversal agent should ideally have similar absorption rates and half-lives to chloroquine. This ensures that chloroquine activity is maximal as it is absorbed. This can be achieved chemically by coupling the reversing agent with chloroquine into conjugates or double drugs. The conjugation therefore allows simultaneous absorption of the two drugs [38, 48]. This approach has been exemplified by reversed chloroquines.

Burgess *et al.* [38] designed reversed chloroquine which consists of chloroquine covalently linked to imipramine, an achiral antidepressant drug that is poorly active (IC_{50} > 140 μM) against *Plasmodium* parasites [49]. They synthesised a prototype N′-(7-chloroquinolin-4-yl)-N-[3-(10,11-dihydrodibenzo[*b,f*]azepin--yl)propyl]-N-methylpropane-1,3-diamine, **12** (Fig. **6**) which was shown to be highly potent against both chloroquine-sensitive (IC_{50} = 0.003 μM) and chloroquine-resistant (IC_{50} = 0.005 μM) strains of *P. falciparum*. This was highly impressive considering that chloroquine had a measured IC_{50} of 0.102 μM against the drug-resistant strain. This prototype compound was further demonstrated to be active and non-toxic in *in vivo* mice studies following oral administration [50]. In the design, the linker modifications did not negatively impact the reversal potency, however a piperazinyl moiety adjacent to the quinoline appeared detrimental [50].

Despite the encouraging activity, prototype compound **12** had an undesirable lipophilic character with a consensus LogP (cLogP) value of approximately 9. To mitigate this, a second-generation series of reversed chloroquine compounds was developed based on conclusions drawn from refined computational tools by Bhattacharjee *et al*. [51]. A batch of compounds was prioritised with minor structural variations, *i.e.* modifying the linker between the quinoline nucleus and the reversal agent; changing the reversal agent aromatic system from diphenylamine to a dibenzylamine or dibenzylamide; and changing the length of the aliphatic appendage (Fig. **6**) [52]. The latter resulted in the incorporation of shorter linkers, *i.e*. from the imipramine propyl in **12** to an ethyl group and to a piperazine. Additionally, the methyl moiety attached to the aliphatic N of the reversal agent aliphatic appendage was removed [52]. Further changes made to the reversing agent moiety were shortening and lengthening this appendage. In each case, the ethyl bridge between the aromatic rings in **12** was removed to give diphenylamino and dibenzylamino groups.

In addition to strong potencies of the compounds against malaria (IC_{50} < 0.010 μM), the cytotoxicity was within acceptable limits. One distinct compound, **10**, showed potent efficacy and low cytotoxicity with a selectivity index (SI) of 12 000 for D6 and 4 800 for Dd2 *P. falciparum* parasite strains. Intriguingly, these SI were a significant improvement relative to those calculated for chloroquine (1 700 for D6 and only 120 for Dd2). High potency, low toxicity and the low cLogP implied that the two compounds from the series, *i.e.*, **13** and **14**, can be leads for further drug development (Fig. **7**).

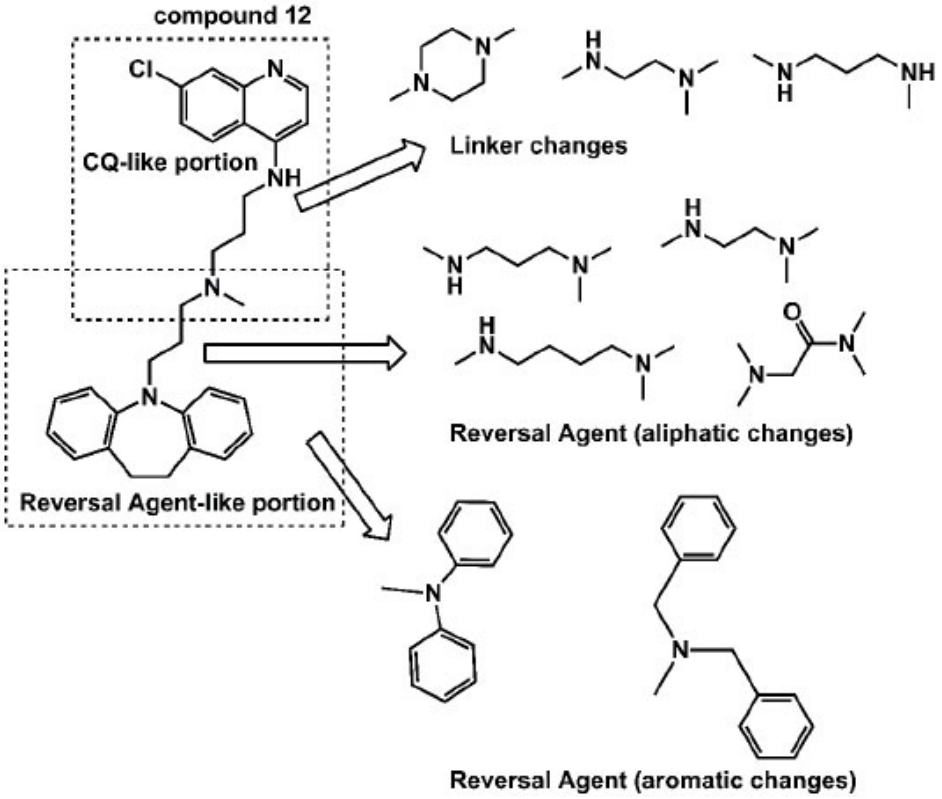


Fig. (6). Structural variations in the reversed chloroquines prepared by Andrews *et al.* [52].

Some advanced synthetic efforts aimed at simplifying the biaryl group of the imipramine-chloroquine based reversed chloroquines have been documented [53, 54]. The study maintained the reversed agent head but modified the biaryls into a single aromatic group in comparison to the original biaryl in imipramine. Data generated from *in vitro* haem binding and β-haematin inhibition assays indicated that the single aromatic reversed chloroquine compounds retain activities similar to those of chloroquine against *P. falciparum*. Such reversed agents head group designs are a deviation from the earlier published pharmacophore [51], but the

main goal was to examine the possibility of reducing the cost of goods, simplifying syntheses, and/or improving the solubility. The evolution of these imipramine-like reversed agents is partially represented in (Fig. **6**)

Compound	cLogP	IC_{50} (μM)		RI#	SI
		D6	Dd2		
13	5.2	0.005	0.013	2.6	ca. 22000
14	5.9	0.014	0.016	1.1	ca. 1375

#RI – Resistance Index = IC_{50} (Dd2)/IC_{50} (D6); ca. - approximately

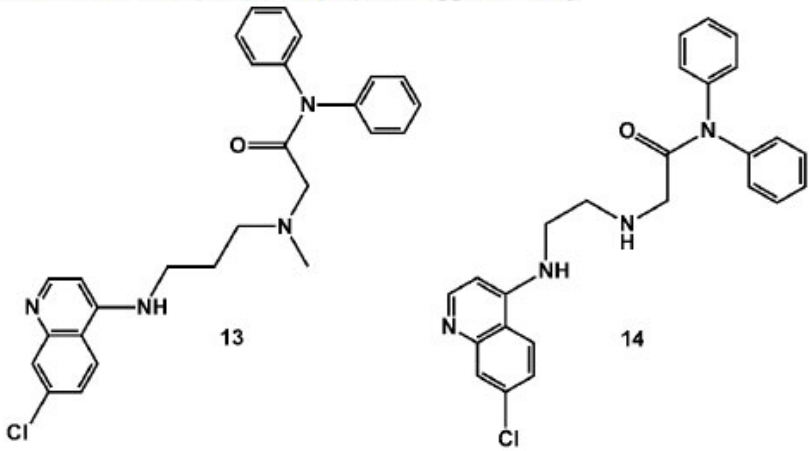


Fig. (7). Potential lead compounds based on imipramine-aminoquinoline reversed chloroquine.

An exemplary mono-aryl reversed agent is compound **15** (Fig. **8**). The deletion of one aryl group in the reversed agent head did not drastically impact the antiplasmodial potency but did lower the cLogP value to a number that is similar to that of chloroquine. Other modifications did not affect the antiplasmodial activity significantly but reduced the cLogP to desirable levels below 5 units. These structural modifications included: introduction of various substituents on the single phenyl ring, insertion of a second linker unit between the phenyl and piperazine rings and replacing the phenyl with naphthyl and heterocyclic ring systems. Of note are the pyridyl and pyrimidine compounds since they formed highly soluble salts that can be assumed to be orally available. Compound **15** remained exemplary when promising compounds from the series were tested *in vivo* against *P. yoelii* in a murine malaria model. Results indicated that **15** had low 50% effective dose (ED_{50} of 2 mg/kg/day) value, indicating oral efficacy. Furthermore, these reversed chloroquines were non-toxic even at doses as high as 150-fold the ED_{50} (for **15**) suggesting a favourable safety margin. The evolution of reversed chloroquine from combinations of two molecules, chloroquine and imipramine, to synthesis of biaryl reversed chloroquines to finally simple mono-aryls is explicit to this point [54]. All through the evolution, the key

antiplasmodial chloroquine reversal agent pharmacophore features have been retained except for the biaryl moiety [22, 51].

In studies involving metal chelators, Andayi *et al.* [55] demonstrated that the iron chelators *N*-alkyl-3,4-hydroxypyridinones (HPOs) have potential to synergise antiplasmodial activity of chloroquine and that reversed chloroquine based on HPOs are potent antiplasmodial compounds. In preliminary studies, the best chloroquine-resistance reversal was achieved by the most lipophilic *N*-alkyl–HPO in the series (**16**) (Fig. **8**), and it exhibited *in vitro* IC_{50} of 3.5 μM on its own but 0.007 μM and 0.009 μM when combined with chloroquine against *P. falciparum* 3D7 and K1 strains, respectively [55]. Further synthetic efforts afforded dual molecule reversed chloroquines in which the aminoquinolines were covalently linked to the HPOs. The exemplary compound **17** (Fig. **8**) was the most potent HPO-aminochloroquinoline with IC_{50} values of 0.004 μM against the chloroquine-sensitive strain 3D7 and IC_{50} = 0.03 μM for chloroquine-resistant strain K1 [55].

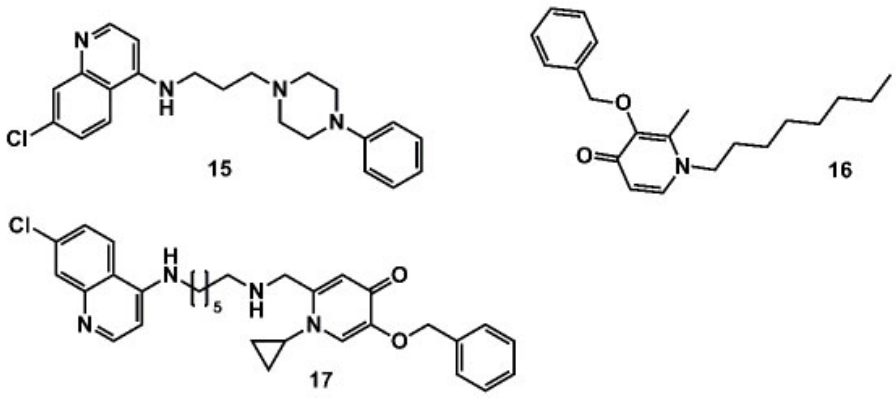


Fig. (8). Structures of a simplified imipramine reversed chloroquine, a N-alkyl-3,4-hydroxypyridinones (HPO) and HPO-AQ conjugate.

2.1.3 3-(Substituted-Phenylmethylene)-Pyrrolidines as Chloroquine-Resistance Reversal Agents

From the lead compound **18** (CDRI 87/207) a series of 1-(3--Diethylaminopropyl)-3-(substituted-phenylmethylene)-pyrrolidines **19** (Fig. **9**) were designed and synthesised [57] Unlike verapamil and desipramine, CDRI 87/209 failed to restore chloroquine accumulation in the resistant parasites despite causing sensitisation, suggesting that apart from the hypothesised inhibition of drug efflux other mechanisms are vulnerable targets for a chemotherapeutic approach towards drug-resistance reversal. To prove this, **18** was found to inhibit enzymes glutathione S-transferase, haem oxygenase and haem polymerase,

notably the latter two are involved in the haem degradation pathway [56]. Similarly, to verapamil and desipramine, CDRI 87/209 showed only weak intrinsic antiplasmodial activity.

Resistance reversal was further observed *in vivo* following the co-administration of chloroquine with CDRI 87/209 in mice infected with either chloroquine-resistant *P. berghei* or *P. yoelii nigeriensis* (multidrug-resistant) strains [57].

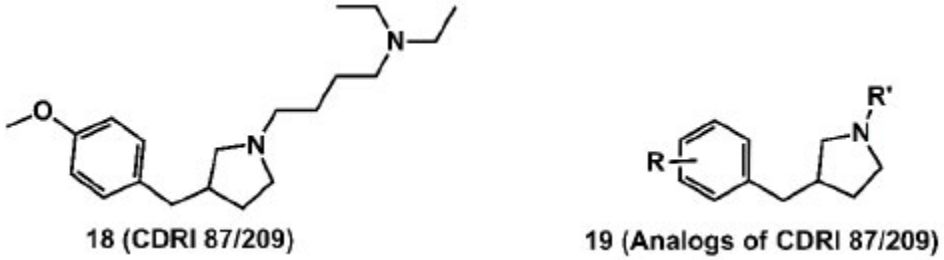


Fig. (9). CDRI 87/209 a substituted methylene pyrolidinone and its analogues.

2.1.4 Adamantane and Pentacycloundecanylamine (PCU)-Chloroquine Conjugates as Reversed Chloroquines

Polycyclics, which include a series of aza-adamantanol and adamantane-imine conjugated to chloroquine, have been reported to have significant antiplasmodial activity (K1 IC_{50}<0.100μM) resulting in improved cross-resistance (RI = 2 - 4.3) relative to chloroquine (RI = 38) [58]. The representative compound **20** showed an 18-fold enhancement of activity against the K1 strain when compared to chloroquine [58]. Prior to these analogues, Yvette *et al.* [58] had demonstrated that the polycyclic amine **21** acted as a chemosensitiser to chloroquine. Additional studies of PCU derivatives led to the discovery of aza-adamantol-aminoquinoline reversed chloroquines as exemplified by **22** (Fig. **10**) [59]. The synthetic routes of the two series commenced from the commercially available 2-adamantanone and its details of these are documented clearly by Yvette *et al.* [58].

2.1.5 Quinoline Dimers as Chloroquine-Resistance Reversal Agents

Considering that PfCRT had been predicted to function as a dimer acting as an efflux transporter with the critical K76 residue oriented towards the PFV [60], it was thus postulated that there is a possibility that a dimeric antiplasmodial molecule can bind strongly on the PfCRT given two potential binding sites on this protein. A notable example, related to this theory, the repurposing P-gp efflux pump inhibitors for PfCRT inhibition is the study by Hrycyna and co-workers [61]. A series of dimeric quinine molecules which were rationally designed as second-generation P-gp inhibitors were then evaluated for the potential to reverse

chloroquine-resistance [61, 62]. The dimers antiplasmodial MoA was examined using a *Xenopus laevis* oocyte system, *in vitro* (against drug-sensitive and drug-resistant *P. falciparum* strains) and *in vivo* (against drug-resistant *P. berghei*). The data revealed that the quinine dimers are among the most prolific inhibitors of the PfCRT associated with chloroquine-resistance ($PfCRT^{chloroquineR}$). The lead compounds (**23**, **24**) were not effluxed by $PfCRT^{chloroquineR}$ from the PFV but instead accumulated to very high levels within this organelle. Both **23** and **24** inhibited the formation of synthetic haemozoin showing that the accumulated compounds caused parasite death by haemozoin inhibition. The compounds were orally administered in mice infected with a chloroquine-resistant strain of *P. berghei* and **23** exhibited clearance of parasitaemia in a manner that was comparable to quinine. However, **24** was ineffective and additionally found to be toxic.

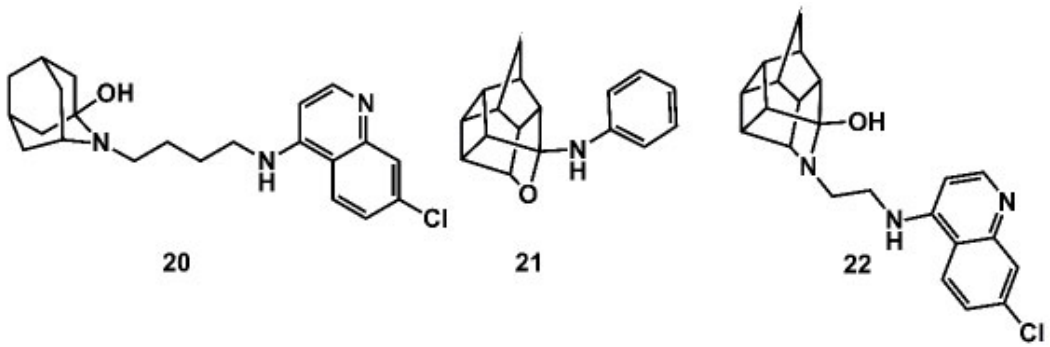


Fig. (10). Adamantane and pentacycloundecanylamine, (PCU)-based reversed chloroquines.

Bivalent quinine dimers, containing an intervening triazole ring prepared by click chemistry (copper (I) catalysed Huisgen 1,3-dipolar cycloaddition reaction between azides and alkynes), and are represented by **25,** are an addition to the antiplasmodial quinoline dimers arsenal (Fig. **11**). The advantage of click chemistry is that it enables rapid synthesis and testing of a variety of tethering moieties. The synthesis involved conversion of the pendant alcohol group of quinine into a series of alkyne and azide-modified quinine analogues of varying tether lengths. These functionalised quinine monomers were then subjected to the click chemistry to synthesise a library of quinine dimers containing a triazole ring within the tether [62].

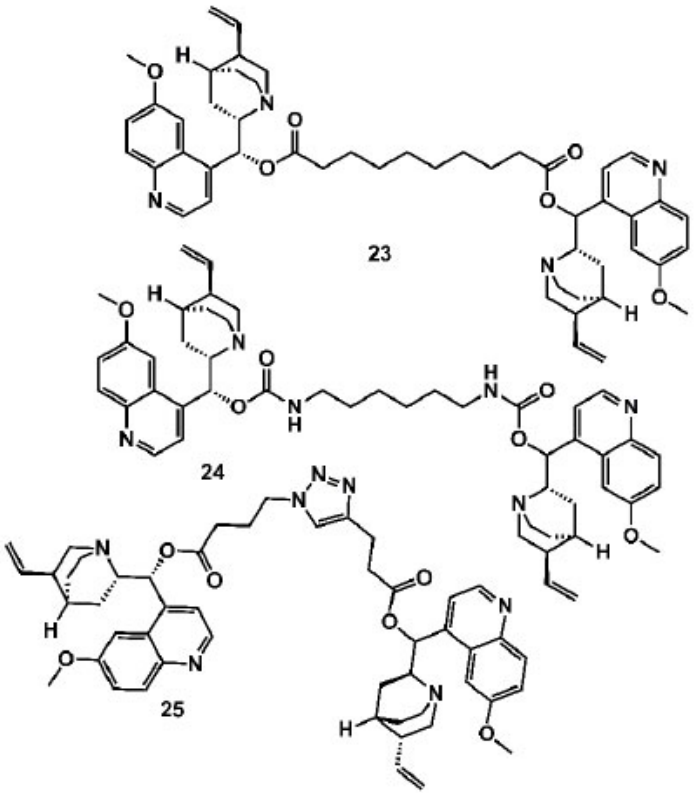


Fig. (11). Structures of quinine dimers as chloroquine-resistance reversal agents.

Compounds related structurally to quinoline dimers, the naturally occurring bisbenzylisoquinolines (BBIQs) have been reported to modulate chloroquine-resistance in *P. falciparum* FcB1 and multidrug-resistant cancer cells [63]. Among these BBIQs, fangchinoline **26** (Fig. **12**) exhibited the best antiplasmodial and cancer potentiating effect. The *in vitro* antiplasmodial activity of fangchinoline **26** was relatively low at 0.93 μM despite its chloroquine potentiating effect. The *in vitro* antiplasmodial IC_{50} of the other BBIQs was also moderate ranging from 0.2 – 4 μM. Semi-synthetic modification of cordobimine **27** to dihydrocordobimine *RR* and *RS* (**28**, **29**) did not improve the *in vitro* activity against the resistant strain (IC_{50} of 0.22, 1.42 and 0.37 μM, respectively) (Fig. **12**) [63].

BBIQs are of several structural types but generally consist of two monomeric units that are linked by one diaryl ether bridge, and the other bridges may be one or two diaryl ether or a C-C linkage.

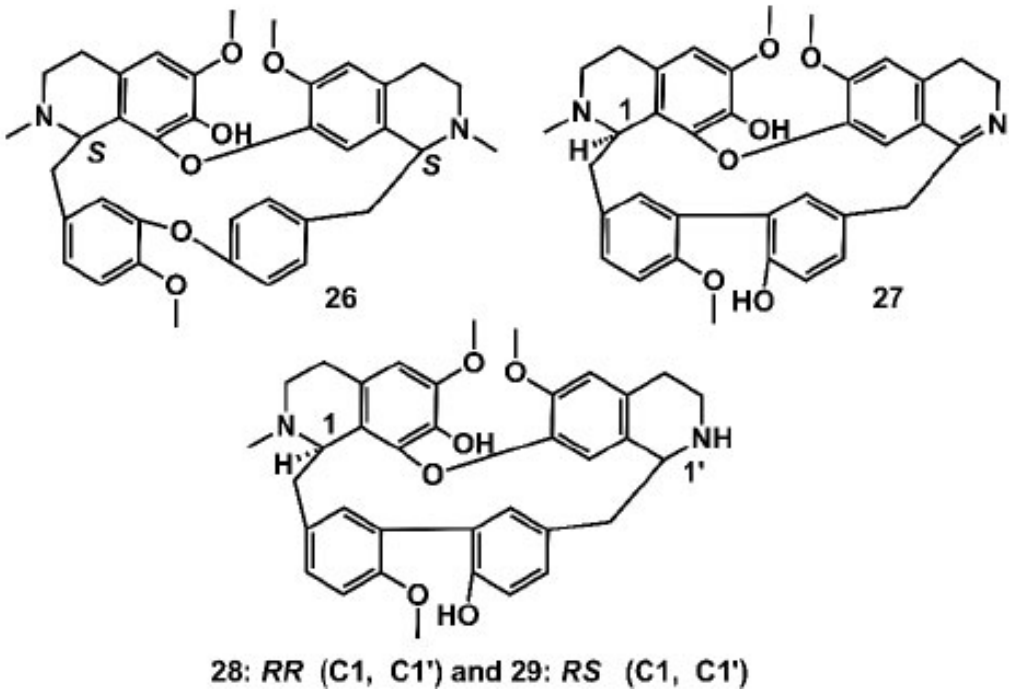
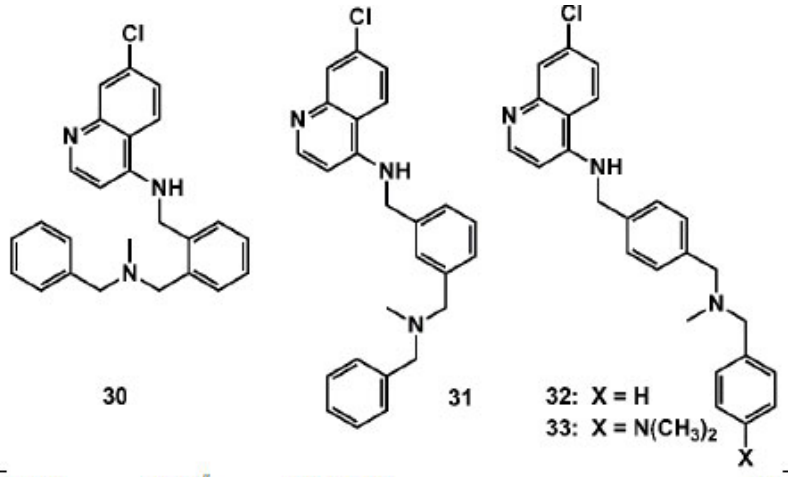


Fig. (12). Structures of bisbenzylisoquinolines (BBIQs) and their semisynthetic analogues as reversed chloroquines.

2.1.6 Dibenzylmethylamine-4-amino-7-chloroquinoline (Dibemethin) as Reversed Chloroquines

Zishiri and co-workers [64, 65] synthesised reversed chloroquines as dual function antimalarials which had the ability to inhibit both hemozoin formation and PfCRT in a *Xenopus* oocyte expression system. The reversed chloroquines consisted of a series of 4-amino-7-chloroquinolines with dibenzylmethylamine (dibemethin) side chains [64, 65] and these compounds were equally active against chloroquine-sensitive (D10) and chloroquine-resistant (K1) *P. falciparum* parasite strains.

The *in vitro* IC_{50} values for the dibemethin derivatives ranged from 0.027 to 178 μM against the *P. falciparum* parasite D10 strain, with the most potent representatives being **30** – **33** (Fig. **13**). Compound **32** emerged as the most prolific inhibitor with an IC_{50} value comparable to that of chloroquine. The compounds were good β-haematin inhibitors to a level comparable or even better than chloroquine. Compounds **30**, **31** and **32** displayed significant *in vivo* potency reducing parasitaemia by 99% in mice after oral administration. The survival of mice at a relatively high dosage regimen (100 mg/kg) indicated the desirable low toxicity of the dibemethin reversed chloroquines.



Compound	BHIA#	IC50 (μM)		RI
		D10	K1	
30	1.1	0.140	0.122	0.9
31	0.69	0.041	0.043	1.0
32	0.32	0.022	0.026	1.2
33	0.4	0.048	0.037	0.9
CQ	1.9	0.023	0.144	6.3

#BHIA - β-hematin inhibition assay

Fig. (13). Structures and *in vitro* antiplasmodial activities of a set of exemplary dibemethin-aminoquinoline reversed chloroquines.

2.1.7 Dibenzosuberanylpiperazine Derivatives as Chloroquine-Resistance Reversal Agents

Studies by Osa *et al.* [66] represent the initial work on dibenzosuberanylpiperazine (DSP) derivatives as chloroquine-resistance reversal agents [66]. Dibenzosuberanylpiperazine (1-(10,11-dihydro-5*H*-dibenzo[a,d--cyclohepten-5-yl)piperazine) and its piperazin-1-yl derivatives are readily synthesised *via* reactions involving the reaction of dibenzosuberanyl chloride with piperazine . On their own, DSP derivatives **34** and **35** were ineffective against *P. chabaudi* in mice but combinations of 50 mg/kg of the DSPs with 3-4 mg/kg of chloroquine gave high reversal activities (0% parasitaemia on the 4th day). The effective compounds had the following R substituents in (Fig. **14**) 2-hydroxybutyl; 2-hydroxyhexen-5-yl; 2-hydroxybuten-3-yl; 4-acetoxybutyn-2-yl; 4-hydroxybutyn-2-yl; and 3-substituted buten-1-yl which correspond to the non-bulky groups of hydroxyalkyl, hydroxyalkenyl, hydroxyalkynyl, or alkenyl [66]. This is a good example of a successful *in vivo* synergistic treatment of malaria involving the combination of chloroquine and a resistance reversal agent.

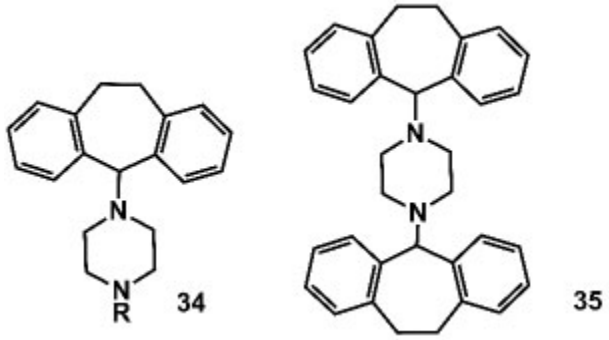


Fig. (14). Structures of dibenzosuberanylpiperazine (DSP) derived chloroquine-resistance reversal agents.

2.1.8 Carboxamide and Aminoquinoline–Carboxamide as Drug-Resistance Reversal Agents Targeting ABCI3

Recently, Murithi and co-workers [67] identified the PFV non-resident ATP-binding cassette (ABC) transporter ABCI3 as both a resistance mediator and a putative drug target opening a way for development of antimalarials that target this protein. The MMV projects' aminoquinoline-carboxamide and carboxamides containing compounds (**36–37**) were identified as drugs that target ABCI3 (Fig. **15**). The classes of antiplasmodial chemotypes represented in the study were: 2,6-disubstituted imidazopyridines (**38**); quinoline tetrazole carboxamide (**39**); thiophene carboxamides (**40**); 8-aminoquinoline pyridine carboxamides (**41**) and pyrazolopyridine carboxamide (**42**) (Fig. **15**). The drug-resistance mechanisms of these chemotypes are associated with ABCI3 amplifications or point mutations [67]. The ABCI3 transporter is widely spread within the parasite among several organelles *i.e*., the cytosol, nucleus, nuclear membrane, the PFV and plasma membrane [67]. Furthermore, the ABC13 confer resistance to broad set of chemotypes including quinolines [67]. The antiplasmodial MoA for the chemotypes represented is not limited to haem inhibition.

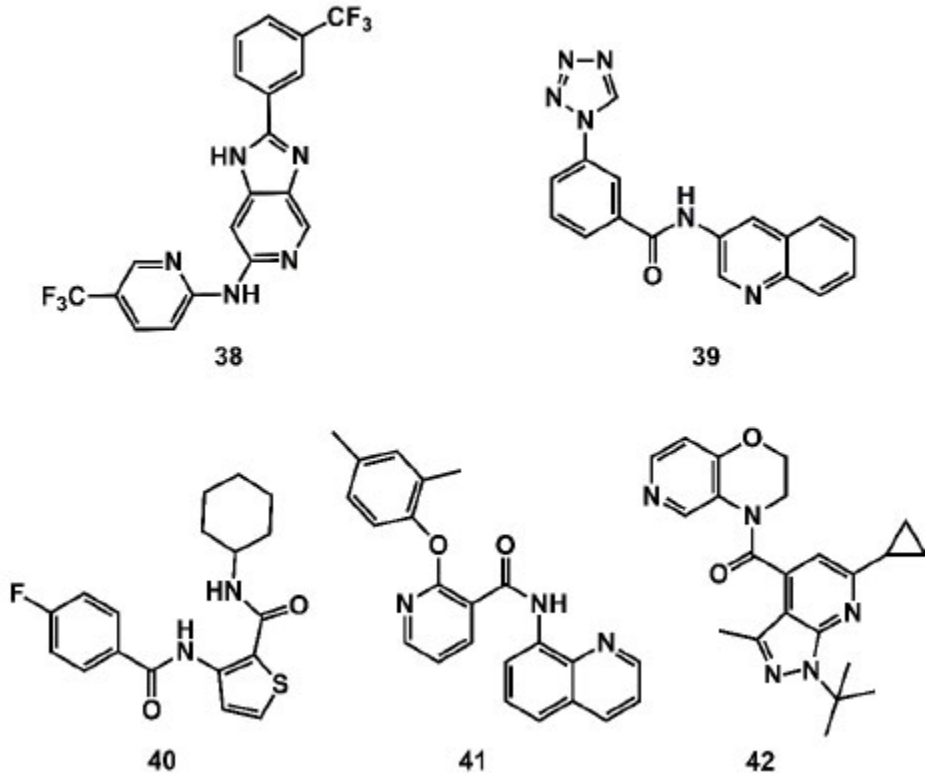


Fig. (15). Structures of some novel ABCI3 inhibitors.

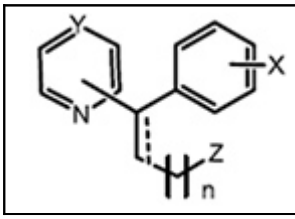
2.1.9 Chlorpheniramine and its Analogues as Chloroquine-Resistance Reversal Agents

Chlorpheniramine (CP, **45**), an antihistamine, partially resensitizes resistant parasites but possesses little intrinsic antiplasmodial activity. Deane *et al.* [68] synthesised novel analogues of CP (Table **3**). They demonstrated that these compounds alongside other chloroquine-resistance reversal agents can inhibit the transport of chloroquine *via* a resistance conferring form of PfCRT expressed in *Xenopus* oocytes. The level of PfCRT inhibition correlates well with both the restoration of chloroquine accumulation and the level of chloroquine resensitisation in resistant parasites. Synthesis of the CP analogues is *via* Wittig olefination whereby the amine sidechain from pyridyl or pyrazinyl chlorophenyl ketones is incorporated [68].

All the 24 CP analogues prepared inhibited PfCRT chloroquine-resistance mediated transport at 100 μM, however they were less potent than verapamil [68]. None of the analogues affected the diffusion of chloroquine into oocytes expressing $PfCRT^{chloroquineS}$. The structural changes resulted in reduced

PfCRT^{chloroquineR} inhibition relative to CP, with the exception of compound **43** (the 3-chlorophenyl derivative of CP) and **44** (containing 3-chlorophenyl and 3-pyridyl groups). Some general trends were derived from the data *i.e*. (1) the reduced compounds which were also racemates were more potent relative to their olefinic counterparts, (2) lengthening the alkyl sidechain was not beneficial, (3) the 3-chlorophenyl substituent is preferable to 2-chloro, 4-chloro, or unsubstituted phenyl groups [68].

Table 3. Some analogues of chlorpheniramine (CP) (52).



Compound	Config	Subst	Y	X	n	Z
43	rac	2	CH	3-Cl	1	NMe_2
44	rac	3	CH	3-Cl	2	NMe_2
45 (CP)	rac	2	CH	4-Cl	1	NMe_2
46	(+)	2	CH	4-Cl	1	NMe_2

Rac – racemic; Config – configuration; Subst – substitute

2.1.10 Xanthene Derivatives as Chloroquine-Resistance Reversal Agents

The initial studies of xanthenes as potential trypanothione reductase (TryR) inhibitors and chloroquine-resistance reversal agents were reported by Chibale *et al.* [69]. The rationale of the synthesis and biological evaluation was to seek chemical structures of agents that enhance chloroquine accumulation and/or reverse chloroquine-resistance. The presence of a hydrophobic group and a protonatable nitrogen was revealed to be important for resistance reversal and chloroquine accumulation for these molecules [69]. The two structural features are also important for potency and selectivity (against the human homologue glutathione reductase) in other TryR inhibitors. The features are exemplified in the antimalarial acridine quinacrine **46** and tricyclic antidepressants promazine **47** and clomipramine (Fig. **16**). Thus, these tricyclics were envisaged as potential leads for future development as dual acting compounds for chloroquine-resistance reversal and TryR inhibition.

Further synthetic efforts were based on polyamine **48**, (Fig. **17**) previously synthesised by Chibale *et al.* [70]. Even though **48** was reported to be a weak inhibitor of TryR it was used as the template to explore the parallel synthesis of novel series of 9,9-dimethyl-xanthene as represented by **49** [71]. The synthetic strategy utilised the commercially available diiodo-9,9-dimethylxanthene as the starting material. Dimethyl-9,9-xanthene derivatives as exemplified by **48** showed a 5 to 6-fold increase in chloroquine accumulation in the resistant K1 strain compared to that of verapamil (3.5-fold) [71]. Most of these derivatives had weak intrinsic antiplasmodial activities against the sensitive strain compared to

chloroquine with the exception of the sulfonamides **50** and **51** (Fig. **17**). *In vitro* data showed a direct relationship between chloroquine accumulation and sensitisation for most derivatives.

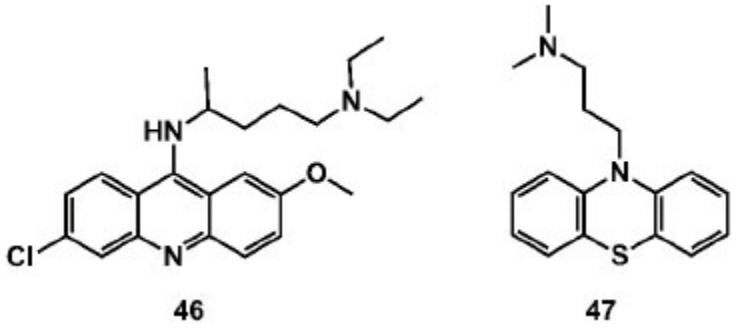


Fig. (16). Tricyclic compounds that act as TryR inhibitors in *Trypanosoma* and chemosensitisers of chloroquine.

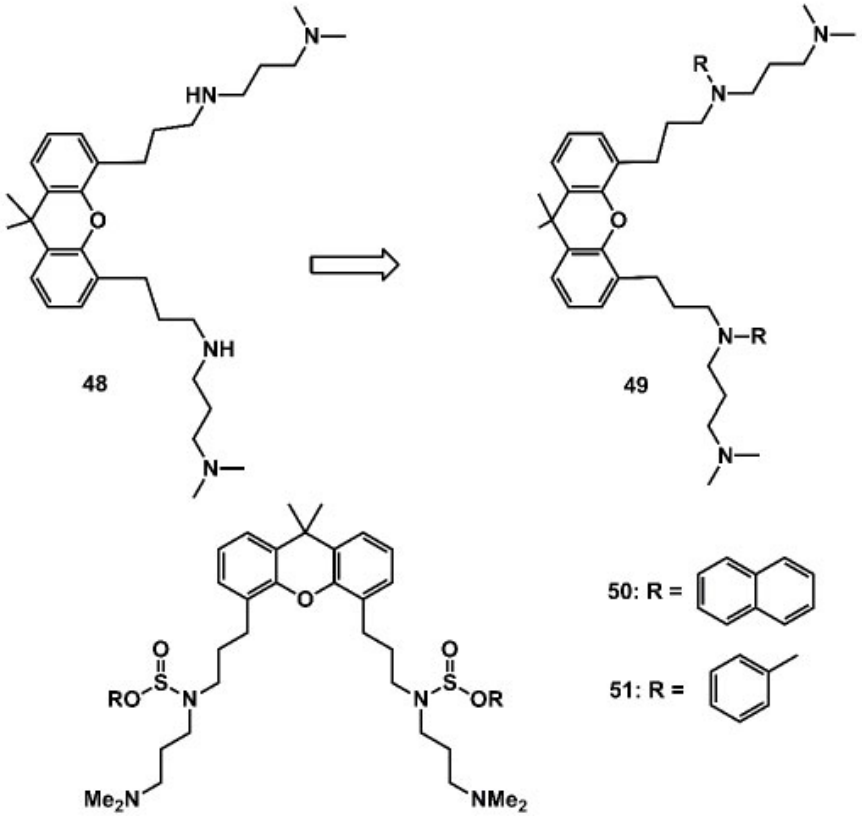


Fig. (17). Chloroquine-resistance reversal agents derived from 9,9-dimethylxanthene.

Wu *et al.* [72] prepared structurally different xanthenes, the 9*H*-xanthene derivatives, with compound **52** from the series showing relatively high intrinsic antimalarial activity with an IC_{50} value of 0.78 μM (Fig. **18**). Its MoA differed from that of chloroquine since the enhanced activity was seen in the chloroquine-resistant strain only. In combination with chloroquine, **52** exhibited the highest synergy, with a response modification index of 0.36 alongside a 4-fold increase in chloroquine accumulation in the resistant strain [72]. Among the 9*H*-xanthenes, **53** (Fig. **18**) exhibited the highest selective therapeutic index thus indicating more toxicity to the malaria parasite than it is to the mammalian cell. The low toxicity was up to 100 μM tolerance in different cell lines [72].

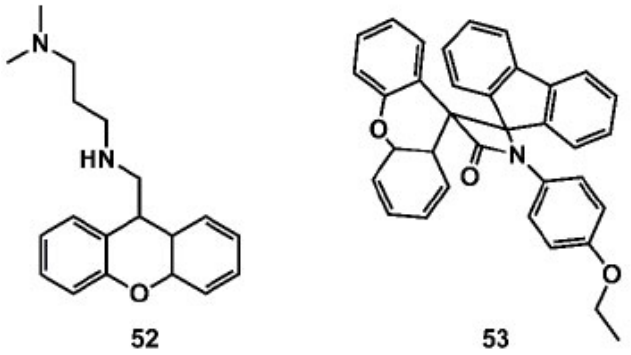


Fig. (18). Chloroquine-resistance reversal agents derived from 9*H*-xanthene.

Apart from pharmacophore features and organic synthetic methodologies, the xanthene sub-structure has served as a model to medicinal chemistry beyond the field of drug discovery. In the study of fluorescent probes, xanthenes are being explored as "turn on" activatable compounds that can be turned on in response to enzymatic transformations and thus useable as intracellular probes. Thus, it is possible to track the site of drug action for xanthene based antimalarials. Alternatively, xanthene moieties can be tagged along experimental antimalarials to enable tracking them in the cell.

2.1.11 Nonylphenolethoxylates (NPEs) as Chloroquine-Resistance Reversal Agents

Nonylphenolethoxylates (NPEs) are inexpensive polymers whose structure consists of hydrophobic tails with a polymeric hydrophilic head made up of repeating units of ethoxylate (EO) (Fig. **19**). NPEs are synthesised *via* co-polymerisation of ethylene oxide with nonylphenol (NP). They are already used for medicinal purposes *i.e.*, as wetting agents and as intestinal permeability enhancers to improve oral drug delivery [73, 74].

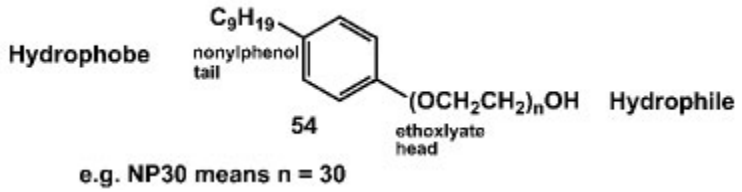


Fig. (19). General structure of nonylphenolethoxylates (NPEs), a class of surfactants.

The presence of less than 10 μM of NPEs causes the IC_{50} values in chloroquine-resistant lines to drop to levels observed for sensitive lines *i.e.*, below 0.012 μM. NPEs with n>30 (Fig. **19**) were found to be most prolific in reversing chloroquine-resistance in *P. falciparum* [73]. In terms of structural requirements for activity, the head and tail parts of the molecule are necessary for chloroquine-resistance reversal activity of the NPEs.

The IC_{50} of these type of surfactants in *P. falciparum* was reported to be significantly lower than the concentrations at which they form micelles (100 μM) thus their MoA is not due to the disruption of membrane integrity. Therefore, this implies that the NPEs interact with a cellular component(s) of the parasite to exert sensitisation. Chinese hamster ovarian cultures were unaffected by chloroquine-NP30 combinations, even when concentrations of up to 25 mM chloroquine were used in the presence of NPE concentrations of up to 0.01%, proving the NPEs to be non-toxic. NPEs seem to target a resistance mechanism that is specific to quinolines since they are able to sensitise *P. falciparum* to chloroquine, quinine and quinidine but not artemisinin [73].

2.1.12 Combination of Prochlorperazine and Chloroquine as a Chloroquine-Resistance Reversal Strategy

A combination of chloroquine and prochlorperazine in *Aotus* monkeys infected by *P. vivax* represents another major preclinical study. The oral administration (chloroquine 10 mg/kg and prochlorperazine 20 mg/kg) achieved complete chloroquine-resistance reversal in the infected animals thus curing them. Neither prochlorperazine nor chloroquine on their own could cure the *Aotus* monkeys. In initial studies, this molecule reversed chloroquine-resistance in *P. falciparum* thus additional efficacy against *P. vivax* is a significant leap in the search for clinically relevant chloroquine-resistance reversal agents [75].

2.1.13 Structure Activity Relationship Studies of Quinoline Drug-Resistance Reversal Agents

Despite the diversity in the function and structure, different compounds can reverse chloroquine-resistance in *P. falciparum*. These reversal agents appear to have specific structural characteristics that are responsible for chemosensitisation in chloroquine-resistant *Plasmodium* strains. These characteristics are important in guiding synthetic efforts in the discovery or optimisation of chloroquine-resistance reversal agents.

These attributes are unique for chloroquine-resistance only and are not applicable to other reversal agents like multidrug-resistance reversal in human cancer cells. These characteristics were initially proposed by Gerena *et al.* [76] and refined on using computational tools by Bhatacharjee *et al.* [51]. The basic requisite features proposed include a cationic charge at digestive vacuole pH, two planar rings, significant lipophilicity and a nitrogen atom, often tertiary.

Using computational derived dipole moments, it was found that the direction and not the magnitude of the dipole moment vector of the reversal agent was critical for chloroquine-resistance reversal [77]. Furthermore, it was found that a monoaryl moiety would replace the previously recommended biaryl without significantly compromising the reversal agent potency [51, 54].

2.2. Natural Products Derived Drug-Resistance Reversal Agents

Plants remain an indispensable source of new drugs and a key informant in drug research and development against various diseases including malaria. A large number of studies have investigated the antimalarial activity of numerous medicinal plants and many plants have shown promise as potential sources of new antimalarial agents (some good reviews on this have appeared over the years, such as Rasoanaivo *et al.* [78], Adebayo and Krettli [79], Muthaura *et al.* [80], Ibrahim *et al.* [81], Onguene *et al.* 2013, Ntie-Kang *et al.* [82], Memvanga *et al.* [83], Benneli *et al.* [84]). In the same vein, various researchers have focused on the evaluation of plants as potential resistance reversal agents for new and old antimalarial drugs. This section presents an updated overview of some of these studies. Special emphasis is placed on the use of whole plant extracts or plant-derived compounds in the reversal of resistance against chloroquine-based antimalarial drugs. This includes studies in which either extracts and/or plant-derived compounds either demonstrate synergy with chloroquine or potentiate its activity against *P. falciparum* parasites.

2.2.1 Natural Products from Artemisia annua as Chloroquine-Resistance Reversal Agents

In one of the earlier studies in this research area, the interaction between chloroquine and qinghaosu (QHS, artemisinin) and two methoxylated flavones (casticin and artemetin, isolated from the bark of *Artemisia annua*) was evaluated using an *in vitro* growth assay based on [^{3}H]-hypoxanthine incorporation in synchronised cultures of a cloned line of *P. falciparum* which phenotypically was shown to be resistant to chloroquine [85]. In this work, the antimalarial activity of QHS was found to be significantly enhanced by the presence of casticin, and to a lesser extent, artemetin, which showed no antimalarial activity in their individual capacities at the studied concentrations. However, the two flavones had little effect on the activity of chloroquine.

Druihle *et al.* [86] evaluated the effect of different combinations of three cinchona bark alkaloids (quinidine, cinchonine and cinchonidine) on the antimalarial activity of quinine against several strains of *P. falciparum*. The most notable finding of this study was that a combination of equal parts of quinine, quinidine and cinchonine exhibited activity that was comparable to that of quinine alone when tested against the quinine-susceptible strains, however the activity increased by factors of 2 to 10 for the quinine-resistant strains. The potentiation effect observed in this study was believed to be as a result of the synergistic effects of the alkaloids against the quinine-resistant strains, and interestingly, it was found to be largely dependent on the presence of cinchonine in the mixture.

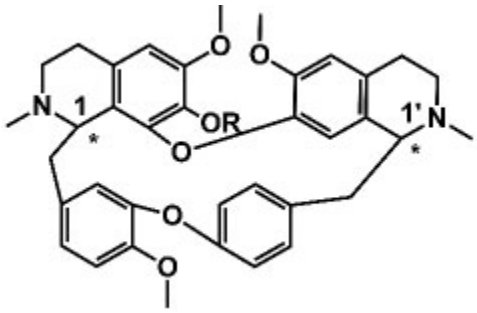
2.2.2 Bisbenzylisoquinoline (BBIQ) Alkaloids from Plants as Chloroquine-Resistance Reversal Agents

In one of their earlier studies, Ye and Dyke [87] evaluated the *in vitro* antimalarial activity of tetrandrine (a bisbenzylisoquinoline (BBIQ) alkaloid first discovered in a Japanese medicinal plant, *Stephania tetrandra* [88]) and its potentiation effect on chloroquine using chloroquine-sensitive and resistant strains of *P. falciparum*. Tetrandrine **55** was found to have strong antimalarial activity against both the sensitive and resistant strains, however it was more selective towards the resistant strain than the sensitive strain. It was further established that tetrandrine does not possess any potentiation effect on the antimalarial potency of chloroquine despite the fact that it has verapamil-like calcium channel blocking activity. In a subsequent study, Ye *et al.* [87] assessed the *in vitro* potentiating effect of tetrandrine on chloroquine and artemisinin against the chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum* using the isobologram method. Tetrandrine **55** was found to exhibit a synergistic effect to both chloroquine and artemisinin against the chloroquine-resistant strain, and it lowered the IC_{50} dose of

chloroquine by a factor of up to 40. The enhancement effect of tetrandrine on the antimalarial potency of chloroquine against the chloroquine-resistant strain of *P. falciparum* was further confirmed in an *in vivo* study conducted on *Aotus* monkeys [89]. In the study, five *Aotus* monkeys were treated with different combined doses of tetrandrine (15 to 60 mg/kg) and chloroquine (20 mg/kg), five monkeys were treated with 20 mg/kg of chloroquine alone while one monkey received no treatment. Parasitaemia was cleared much faster (7-16 days after initial treatment) in monkeys that were treated with the tetrandrine/chloroquine combination, however no parasitaemia reduction (or only a slight reduction) was noted in monkeys that were treated with chloroquine alone. Recrudescence was observed when the tetrandrine/chloroquine combination therapy was administered for shorter periods (one week), however this was resolved when the treatment period was extended to two weeks. None of the monkeys that received treatment died, however the untreated monkey died within two weeks of infection.

Ratsimamanga-Urverg *et al.* [90] evaluated the *in vitro* antimalarial activity and chloroquine-resistance countering action of two BBIQ alkaloids, fangchinoline **26** and limacine **56**, isolated from *Strychnopsis thouarsii* and *Spirospermum penduliflorum*, against the chloroquine-resistant *P. falciparum* FCM29 from Cameroon. Both fangchinoline and limacine displayed notable antiplasmodial activity with similar IC_{50} values of 0.740 and 0.789 μM, respectively (IC_{50} for chloroquine was 0.214 μM). A considerable reduction in the IC_{50} values was noted when the two compounds were used in combination with chloroquine, suggesting synergistic interactions. Overall, the potentiation action of fangchinoline was stronger than that of limacine.

In a separate study [91], the same group of researchers studied the ability of ten naturally-occurring BBIQs (isolated from various plants belonging to the *Annonaceae* and *Menispermaceae* families) and two dihydro derivatives to inhibit the growth of *P. falciparum* and reverse the resistance of the strain FcB1 to chloroquine. Three of the 10 compounds showed remarkable antimalarial activity, with IC_{50} values less than 1 μM, and cocsoline proved to be the most potent antimalarial compound with an IC_{50} of 0.22 μM. Fangchinoline **26** exerted the most significant potentiating activity on chloroquine, while other compounds displayed either little synergistic or antagonistic effects [91]. Based on these observations, the enhancement action of BBIQs on chloroquine is believed to be directly linked to the stereochemistry at C-1 and C-1' centres (see Fig. **20**).



26: R = H, 1S 1'S Fangchinoline
55: R = Me, 1S, 1'S Tetrandrine
56: R = H, 1R 1'R Limacine

Fig. (20). Chemical structures for bisbenzylisoquinolines. BBIQS of Tetrandine **56**.

2.2.3 Pavine-Benzyltetrahydroisoquinolines (pavine-BTIQ) Dimers from Hernandia Voyronii as Chloroquine-Resistance Reversal Agents

Various researchers have conducted extensive research on a large number of medicinal plants used traditionally in Madagascar to treat malaria. This research was motivated by the indigenous knowledge of some local communities in Madagascar that use plant concoctions in combination with low doses of chloroquine to enhance the activity of chloroquine against chronic malaria, which was found to counteract resistance and collateral effects [92].

Hernandia voyronii is a plant species which is endemic to Madagascar whose stem bark is characterised by a repulsive and stinky odour, and it is widely used in Madagascan traditional medicine against various ailments [92]. Ratsimamanga-Urverg *et al.* [92] investigated the cytotoxicity as well as the *in vitro* antimalarial activity and chloroquine potentiating effect of the alkaloids of *H. voyronii* against the *P. falciparum* strain FCM29. Initially, a crude ethanol extract was prepared and fractionated into two fractions (neutral and basic alkaloidal fractions) by means of column chromatography before testing for antimalarial activity, chloroquine potentiating effect and cytotoxicity. The antimalarial activity and chloroquine potentiation effect were found to be contained in the polar alkaloidal fraction and the extract displayed low cytotoxicity to HeLa cells and L929 fibroblasts.

In a subsequent study, the same authors [93] isolated three new isoquinoline alkaloid dimers with pavine and benzyl-tetrahydroisoquinoline moieties (named hervelines A **57**, B **58** and C **59**) through bioassay-guided fractionation of the alkaloidal extract of *H. voyronii* (Fig. **21**). The three compounds were examined for *in vitro* antiplasmodial activity, cytotoxicity and chloroquine-potentiating activity against the chloroquine-resistant strain of *P. falciparum* FCM29. All three isoquinoline alkaloid dimers displayed moderate inherent antiplasmodial activity, however only hervelines B and C increased the antimalarial efficacy of chloroquine in a synergistic manner. Although the IC_{50} values for herveline A

were also decreasing with increase in dosage when combined with chloroquine, the chloroquine-potentiation activity was deemed absent as the activity enhancement indices (AEI) remained below 1.5 for dosages up to 2.3 μM. The same observation was made from the isobolograms: hervelines B and C exhibited concave curves, which signify synergism while herveline A had a near-straight diagonal line, which is indicative of a simple additive effect. Moreover, hervelines A and C showed no cytotoxic effect on HeLa cells while herveline B displayed moderate cytotoxicity.

Further investigation into the active alkaloidal fraction of *H. voyronii* led to the identification of a new pavine-benzyltetrahydroisoquinoline (pavine-BTIQ) dimer, herveline D **60**, together with herveline A, five aporphine alkaloids, two morphinane alkaloids, and their bio-synthetic precursor, BTIQ (*S*)-reticuline [94]. It is worth mentioning here that the extract used in this study was prepared from a stem bark of a young *H. voyronii* tree relative to the one used in the preceding study and it was only found to contain the less methylated hervelines A and D while the more methylated hervelines B and C were absent, which indicates that methylation occurs at a later stage in the biosynthesis of these compounds. Their biosynthetic precursor, (*S*)-reticuline, was also found to be present in a higher amount. In this study, reticuline **61**, laudanosine **62** and all four hervelines (A to D) were tested for *in vitro* intrinsic antiplasmodial activity and chloroquine-potentiating action against the chloroquine-resistant strain of *P. falciparum* FCM29. In addition, laudanosine, and hervelines A and B were investigated for *in vivo* antiplasmodial and chloroquine-potentiation activity against a chloroquine-resistant strain of *P. yoelii* N67 in mice. Reticuline and laudanosine exhibited insignificant intrinsic antiplasmodial activity while all the hervelines (A to D) were characterised by moderate *in vitro* inherent antiplasmodial activity. In the drug combination, laudanosine exhibited a dose-dependent synergistic enhancement on chloroquine activity, but reticuline had a weak synergistic effect. The combined use of the hervelines with chloroquine confirmed synergistic kinds of interactions for hervelines B and C, and an additive effect for herveline A, which were reported in a previous study. On the contrary, herveline D exerted an antagonistic kind of interaction.

Moreover, herveline C enhanced the *in vitro* antiplasmodial activity of herveline D to a lesser extent. The activity enhancement effects of laudanosine and herveline B as well as the activity diminution effect of herveline A on chloroquine were further demonstrated in the *in vivo* study against the chloroquine-resistant *P. yoelii* malaria in mice. The biological activity of hervelines is assumed to be centred around the BTIQ moiety, although the pavine unit seems to also have a to play. It is further believed that methylation of the hydroxyl group at C12' on the pavine is necessary for chloroquine potentiation action in the hervelines [94].

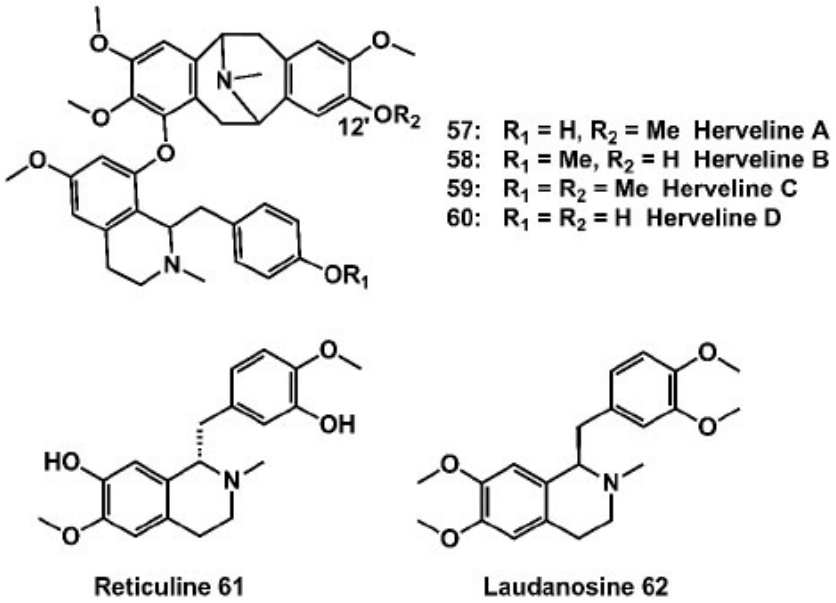


Fig. (21). Chemical structures of pavine-benzyltetrahydroisoquinoline (pavine-BTIQ) dimers and isoquinolines [84].

2.2.4 Compounds from the Strychnos Genus as Chloroquine-Resistance Reversal Agents

Plants of the *Strychnos* genus are also some of the medicinal plants that are used as adjuvants to chloroquine by the Malagasy people of Madagascar in the traditional treatment of malaria. Research conducted on these plant species revealed some rather interesting findings. For example, a screening study [94] of the intrinsic *in vitro* and *in vivo* antimalarial activity of a crude alkaloid extract of the stem bark of *Strychnos myrtoides* and its two major constituents, strychnobrasiline and malagashanine **63**, showed virtually no activity (Fig. **22**). However, when used in combination with chloroquine at dose levels much lower than their IC_{50} values, they significantly enhanced the *in vitro* activity of chloroquine against a chloroquine-resistant strain of *P. falciparum* in a dose-dependent manner. A similar observation was made for the crude alkaloid extract in the *in vivo* chloroquine-potentiating activity against a resistant strain of *P. yoelii* in mice. Furthermore, the crude extract showed no apparent cytotoxicity when it was administered orally to mice at a dose of a 100 mg/kg.

In a subsequent study, the same research team further investigated the *in vivo* potentiating action of malagashanine on quinolines (chloroquine, quinine and mefloquine), aminoacridines (quinacrine and pyronaridine), and a structurally unrelated drug (halofantrine) against sensitive, susceptible and resistant strains of

P. falciparum [95]. Interestingly, malagashanine interacted differently with compounds belonging to different classes despite the fact that they are all thought to exert their antimalarial activity through a haematin-binding mechanism. Malagashanine was found to exhibit a significant enhancement effect on chloroquine against the resistant strain W2 and the previously resistant strain FcB1 but had no effect on the susceptible strain 3D7 and two sensitive isolates. It exhibited a strong synergistic effect on the quinolines (mefloquine and quinine), aminoacridines (pyronaridine and quinacrine) as well as a phenanthrenemethanol (halofantrine). Furthermore, it exerted a simple additive effect on artemisinin (sesquiterpene lactone) and a strong antagonistic action on burasaine (isoquinoline alkaloid) **64** against the chloroquine-resistant *P. falciparum* FcM29 strain (Fig. **22**). Malagashanine was further found to have a selective inhibitory effect on the trophozoite developmental stage of *P. falciparum*.

2.2.5 Indole Alkaloids as Chloroquine-Resistance Reversal Agents

In another study, eight monoindole alkaloids isolated from different *Strychnos* plant species were evaluated for the *in vitro* antiplasmodial activity and resistance reversal of chloroquine against a chloroquine-resistant strain of *P. falciparum* W2 [96]. All the compounds were devoid of significant intrinsic antiplasmodial activity, *i.e.*, their IC_{50} were all greater than 10 μM or 5 μg/mL. Three compounds, icajine **65**, isoretuline **66** (Fig. **22**) and strychnobrasiline, synergistically enhanced the antimalarial efficacy of chloroquine, while holstiine displayed an additive effect with chloroquine. Icajine and isoretuline were further evaluated for *in vitro* mefloquine-potentiating activity against the mefloquine-resistant strain of *P. falciparum* F32. Icajine showed a synergistic enhancement effect with mefloquine while isoretuline had no activity.

A number of studies have also been conducted in other parts of the world on the potentiation effect of different plant extracts or plant-derived compounds on the antiplasmodial activity of chloroquine. For instance, Haruki *et al.* [97] conducted a comparative study on the *in vitro* chloroquine-resistance reversal action of cepharanthin **67** (Fig. **22**) and verapamil against the chloroquine-resistant *P. falciparum* K1 and chloroquine-susceptible HB3 strains. Cepharanthin, an extract obtained from the root tubers of *Stephania cepharantha* and widely used in Japan in liquid and tablet forms for the treatment of inflammatory diseases, demonstrated strong chloroquine-resistance reversal activity on the K1 strain. At a concentration of 1.2 ng/mL, cepharanthin produced a 5-fold increase in chloroquine accumulation, which enhanced the activity of chloroquine against K1 *P. falciparum* by a factor of 15. The main components of cepharanthin are BBIQ alkaloids isotetrandrine, cepharanthine, berbamine, homoaromoline, cepharanoline and cycleanine. Except for cycleanine, all alkaloids, cepharanthine,

cepharanoline, homoaromoline, berbamine and isotetrandrine, enhanced the activity of chloroquine by maximum factors of 3, 4, 4.2, 6.8 and 9.7, respectively, and these were lower than that of the extract [98]. Cepharanthin was 50 times more active than verapamil in increasing sensitivity of *P. falciparum* K1 strain to chloroquine. For the HB3 strain, cepharanthin improved chloroquine accumulation by a maximum factor of 2.5 and enhanced the antimalarial activity by 3-fold, while verapamil was devoid of any activity.

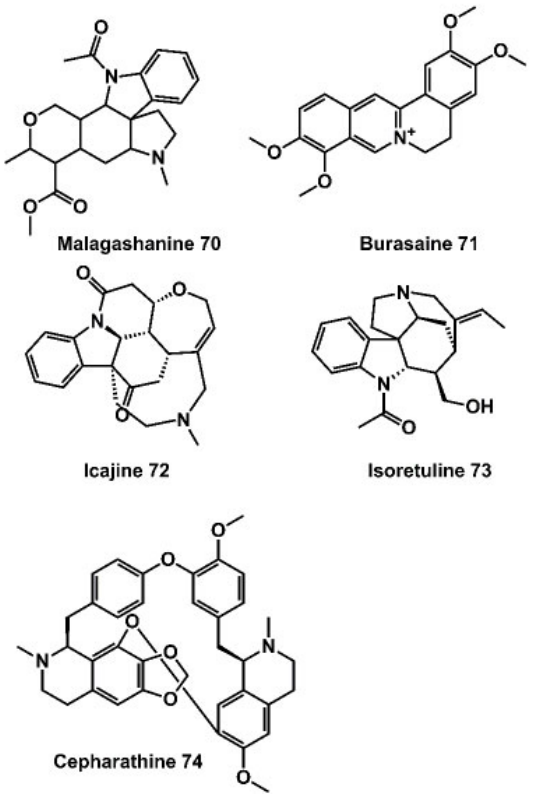


Fig. (22). Indole alkaloids and some isoquinoline alkaloids that potentiate chloroquine.

2.2.6 Pregnanes as Natural Products with Chloroquine-Resistance Reversal Agents

Another Nigerian research team recently reported the *in vitro* antiplasmodial and chloroquine-resistance reversal activities of one pregnane and three pregnane glycosides isolated from the leaves of G*ongronema latifolium* (compounds reported to have anti-leukemic activities [99] using the chloroquine-sensitive *P. falciparum* 3D7 strain and chloroquine-resistant *P. falciparum* W2 strain [100]. Compounds **68** and **69** (Fig. **23**) demonstrated significant antiplasmodial activity against both the sensitive and resistant strains (IC_{50} values less than 20 μg/mL) in

their individual capacities, however only compound **68** (iloneoside) showed remarkable activity (potentiated chloroquine activity by up to 3200%) when co-administered with chloroquine in concentrations greater than 0.625 μg/mL against *P. falciparum* W2. Strangely, compound **71** was only active at concentrations ≥20 μg/mL but interacted antagonistically with chloroquine at concentrations ≤2.5 μg/mL. On the contrary, compounds **69** and **70** (Fig. **23**) showed moderate activity when used individually (IC_{50}>20 μg/mL) against both strains, but synergistically enhanced the antimalarial potency of chloroquine (IC_{50} < 11 μg/mL) when used in combination with chloroquine against the sensitive strain. Compounds **69** and **70** proved inactive against the resistant W2 strain at all tested concentrations.

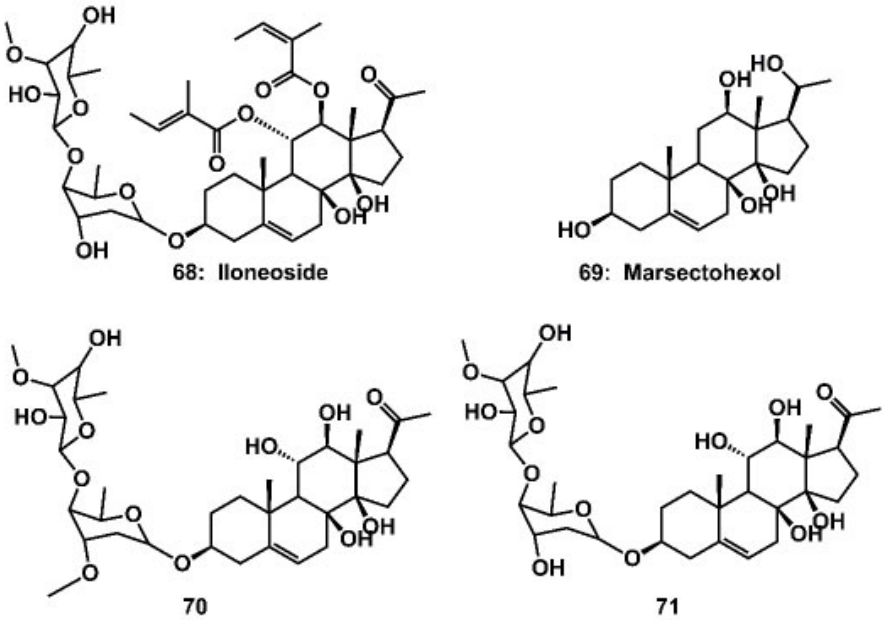


Fig. (23). Chemical structures for iloneoside (a pregnane) and pregnane glycosides from the leaves of G*ongronema latifolium* [100].

2.2.7 Reversal Activities of Coumarins, Diterpenoids and Xanthones from Plants

Zaid *et al.* [101] investigated the cytotoxicity, inhibitory activity as well as the chloroquine-resistance reversal effect of five coumarin derivatives (umbeliferon, esculetin, scopoletine, herniarin and 3-aminocoumarine) against *P. falciparum* K1 and 3D7 strains. Individual compounds were found to have weak to moderate *in vitro* antiplasmodial activity, with IC_{50} values in the micro-molar range, which compared poorly with that of chloroquine which was in the nano-molar range. Isobologram analysis revealed a synergistic interaction between chloroquine and

umbeliferon, 3-aminocoumarin and esculetin. Moreover, coumarins proved safe to human cells.

In later studies, the same research group assessed the *in vitro* antiplasmodial and chloroquine enhancement activities of andrographolide (a diterpenoid lactone found in *Andrographis paniculata*) [102] and mangostin (a naturally occurring xanthone) [103] using the SYBR Green-1-based drug sensitivity assay and isobologram method. Andrographolide displayed a weak synergistic action with chloroquine against K1 strain [102], while mangostin showed a moderate synergistic effect with chloroquine against the K1 strain when combined in a ratio of 3/7 (mangostin/chloroquine) [103].

2.2.8 Resistance Reversal Activities of Crude Plant Extracts

Muregi *et al.* [104] investigated the *in vitro* antiplasmodial activity and the chloroquine-potentiation effects of organic and aqueous extracts of 11 plants used in malaria treatment in Kisii District in Kenya. The extracts were screened against chloroquine-sensitive and resistant strains of *P. falciparum*. Three out of the eleven plants, *Vernonia lasiopus*, *Rhamnus prinoides* and *Ficus sur*, displayed notable antiplasmodial activities (with IC_{50} values less than 30 μg/mL), however, the chloroform, methanol and ethyl acetate extracts of *V. lasiopus* proved to be more potent, giving IC_{50} values below 4 μg/mL against both sensitive and resistant strains. The extracts of *V. lasiopus* which had IC_{50} values less than 5 μg/mL were combined with chloroquine in different proportions and tested against the multidrug-resistant *P. falciparum* V1/S strain. All extracts were found to potentiate the efficacy of chloroquine in a synergistic manner, except for a few combinations which contained at least 80% of the extracts which displayed either additive or antagonistic effects.

In a subsequent study, Muregi *et al.* [105] studied the *in vitro* antiplasmodial activity of eleven medicinal plants used in Kenyan traditional medicine using chloroquine-resistant and sensitive strains of *P. falciparum*. Hexane, chloroform, ethyl acetate, methanol and water extracts were prepared from the relevant part(s) of each plant. Only extracts of four plants, *Ekebergia capensis, Clerodendrum myricoides*, *Stephania abyssinica* and *Ajuga remota* exhibited some form of activity, with IC_{50} values ranging between 3.9 and 26 μg/mL. All the other extracts were devoid of activity. From these results, only the extracts that had IC_{50} values less than 20 μg/mL were investigated further for antiplasmodial activity enhancement effect on chloroquine against the resistant and sensitive strains of *P. falciparum*. In the extract-chloroquine interaction studies, ethyl acetate and methanol extracts of the stem bark of *E. capensis* were found to exert more antagonistic effects on chloroquine, more especially at lower extract

concentrations, while the chloroform and water extracts had mild synergistic effects at all concentrations studied. Conversely, the methanol extract of the root bark of *C. myricoides* proved to have the best enhancement effect on chloroquine activity despite the fact that it showed only moderately good intrinsic antiplasmodial activity against different *Plasmodium* strains. This exemplifies the fact that plant extracts may lack intrinsic activity when used alone, however they could exert stronger enhancement effects when used in association with drugs, as was observed in the study of Rasoanaivo *et al*. [78]. It is therefore imperative that intrinsic antiplasmodial activity should not be used as the deciding factor when screening for potentiation activities of extracts or compounds on antimalarial drugs.

In other studies, Muregi *et al.* screened hot water extracts of eight medicinal plants [106] and methanolic extracts of 15 medicinal plants [107] used in the traditional treatment of malaria in Kenya for *in vivo* antimalarial activity and chloroquine-resistance reversal effect against a chloroquine-resistant strain of *P. berghei* NK65 using mice. The *in vivo* antimalarial activity was evaluated on the basis of the mouse survival time and parasitaemia suppression percentage over a 30-day period. For the aqueous extracts, the most significant parasite reduction (51-75%) was noted for the root bark extract of *Toddalia asiatica*, leaf and root bark of *Rhamnus prinoides*, and root bark of *Vernonia lasiopus*, while the root bark extract of *Withania somnifera* had the lowest parasitaemia clearance of 21%. For the methanolic extracts, leaves, stems and/or root barks for seven plants (*C. myricoides*, *Ficus sur*, *Maytenus acuminata*, *Rhamnus prinoides*, *Rhamnus staddo*, *Toddalia asiatica* and *Vernonia lasiopus*) showed marked parasitaemia suppression (31.7–59.3%). In all instances, notable improvement in parasitaemia reduction (up to 85% in some cases) was observed when plant extracts were used in association with chloroquine, suggesting synergistic effects. This was substantiated by prolonged mice survival rates noted for the extracts alone and extract-chloroquine combinations when compared to the controls (untreated and chloroquine-treated mice). All untreated mice died within 9 days and all chloroquine-treated mice died within 14 days of infection, however, some of the mice that were treated either with the extracts alone or extracts in combination with chloroquine survived for an extended period of more than 2 weeks after the controls have died.

A study conducted in Côte d'Ivoire examined the *in vitro* intrinsic antiplasmodial activity and chloroquine-potentiating effect of the methanol extract of *Mitragyna ciliata* leaves against a chloroquine-resistant strain of *P. falciparum* FcM29 as well as chloroquine-resistant and sensitive isolates of *P. falciparum* using the isobologram method [108]. The extract displayed moderately good antimalarial activity against both sensitive and resistant strains, with IC_{50} values ranging

between 14 and 18 µg/mL after a 72 h incubation period. A five-fold reduction in the IC_{50} values was noted when the extract was used in combination with chloroquine, suggesting a strong potentiation action of the extract on chloroquine against the resistant strains. A concave curve was also noted in the isobologram, which is suggestive of a synergistic effect.

Iwalokun [109] investigated the *in vivo* enhancement effect of the Nigerian *Vernonia amygdalina* leaf extract on the prophylactic and potency of chloroquine against sensitive and resistant *P. berghei* malaria in mice. In this study, mice were injected intraperitoneally with a standard dose of a 10^6 parasitized erythrocyte suspension in phosphate buffered saline (PBS, pH 7.2). In the prophylactic experiment, a dose-dependent (57.2 – 72.7%) suppression of parasitaemia was obtained for the combination of chloroquine (5 mg/kg) with different doses of the extract, for both chloroquine sensitive and resistant *P. berghei* strains. In the treatment setup, administration of the combined dose of chloroquine at 30 mg/kg and different doses of the Vernonia extract (31.25, 62.5 and 125 mg/kg) for 3 days was found to reduce the parasite clearance times, extend the recrudescent times and improve day 14 cure rate (in a dose-dependent manner) in mice that were infected with the sensitive strain of *P. berghei* compared to those treated with chloroquine alone. Furthermore, combining chloroquine with chlorpheniramine (0.25 mg/kg, 12 h, 7 days) cleared parasitaemia and achieved a 100% cure rate by day 14 in both sensitive and resistant strains of *P. berghei* malaria, indicating resistance reversal of chloroquine by chlorpheniramine. These results were comparable to those of the combination of chloroquine with 125 mg/kg of the *Vernonia* extract.

Another study conducted in Nigeria assessed the *in vivo* antiplasmodial activity of the leaf extract of *Ageratum conyzoides* in combination with chloroquine and artesunate against the sensitive strain of *P. berghei* NK65 using Swiss albino mice [110]. Results for the parasitaemia suppression tests revealed significant, dose-dependent parasitaemia reduction for the extract-drug combinations compared to the individual drugs alone. Moreover, the suppressive effect of the extract-chloroquine combination proved to be better than the extract-artesunate combination at similar dose combinations. Although parasitaemia was not completely cleared 7 days after treatment, parasitaemia count was markedly reduced, more especially for the extract-drug combinations compared to the individual drugs alone, signifying a potent synergistic interaction between the extract and the drugs. No apparent recrudescence was noted with all the extract-drug combinations in this study, parasitaemia was consistently cleared until the 14^{th} day of treatment.

Adepiti *et al.* [111] have also investigated the antimalarial activities and interactions of various plant extracts (used in malaria therapy in Nigeria) and antimalarial drugs against chloroquine-sensitive and resistant *P. berghei* strains. In one study, Agbedahunsi *et al.* [111] assessed the antimalarial activity of the ethanolic extracts of the leaves of *Morinda lucida* and *Alstonia boonei* as well as the rhizome extract of *Curcuma longa* against the chloroquine-sensitive *P. berghei* NK 65 strain. The effects of the combined use of the extracts with sulphadoxine-pyrimethamine (*M. lucida* and *A. boonei* extracts only) and quinine (*C. longa* extract only) on the antimalarial activity were also investigated. Notable antimalarial activities were observed for the extract-drug combinations compared to either drug or extract alone, and the effects were dose-dependent. In the extract-drug combination, the efficacies of the drugs were enhanced by factors up to 2.5 in relation to the individual drug/extract, indicating either additive or synergetic interactions.

In another study, Adepiti *et al.* [112] evaluated the *in vivo* effect of co-administration of amodiaquine with MAMA decoction (a herbal antimalarial drug prepared from equal parts of the leaves of *Mangifera indica*, *Alstonia boonei*, *Morinda lucida* and *Azadirachta indica* used in the treatment of malaria in Nigeria) on the antimalarial efficacy of amodiaquine against the chloroquine-sensitive *P. berghei* NK65 and chloroquine-resistant *P. berghei* ANKA strains. In this study, a noteworthy potentiation effect was seen on the antimalarial activity of amodiaquine when the herbal concoction was administered in combination with amodiaquine at therapeutic doses, suggesting either an additive or synergistic interaction. A remarkable reduction in parasitaemia was observed for the MAMA-amodiaquine combination, reaching 100% on day 5 after treatment for the chloroquine-sensitive *P. berghei* NK65-infected mice, however no favourable interaction was noted in the activity of amodiaquine when therapeutic doses of both drugs were administered on chloroquine-resistant *P. berghei* ANKA-infected mice.

Adepiti *et al.* [111] also investigated the antimalarial activities and amodiaquine-potentiation effect of the methanolic extracts of the leaf and fruit of *Uvaria chamae*, a plant used in the traditional antimalarial formulations in Nigeria. Extracts were administered orally in a prophylactic and curative manner at different doses and time intervals in mice using the four-day test. Both the chloroquine-sensitive *P. berghei* NK65 and chloroquine-resistant *P. berghei* ANKA strains were studied. Results showed moderate antimalarial activity in the suppressive and curative tests, however the activity was much more pronounced in the prophylactic mode. A slight enhancement in activity was observed when the leaf extract was administered together with amodiaquine in lower doses for the sensitive strain, but it was not significantly different from that of amodiaquine

alone, which is indicative of an additive effect. No significant improvement in activity was recorded for co-administration of the leaf extract and drug in the mice infected with the chloroquine-resistant strain. Moreover, repetitive co-administration of the fruit extract and amodiaquine at therapeutic doses proved to be of no benefit and resulted in high mortality, despite the fact that the fruit is widely eaten by both wild animals and humans.

More recently, Adepiti *et al.* [113] studied the *in vivo* antimalarial activities and chloroquine potentiation effects of the aqueous extracts of the roots of *Chasmanthera dependens* and *Vernonia amygdalina* as well as the methanol extract of the leaves of *Sphenocentrum jollyanum* using chloroquine-sensitive and resistant *P. berghei* strains. Aqueous root extracts of *C. dependens* and *V. amygdalina* exhibited potent antimalarial activity against the chloroquine-sensitive strain, however *S. jollyanum* showed poor activity against the chloroquine-resistant strain. No notable improvement in activity was observed in the co-administration of the root extracts of *C. dependens* and *V. amygdalina* in comparison with the individual extracts. The combined use of the root extract of *C. dependens* and chloroquine in mice infected with chloroquine-resistant *P. berghei* revealed an additive interaction.

Chenniappan and Kadarkarai [114] also conducted an ethno pharmacological study on Western Ghats plants traditionally used to treat malaria or malaria-like symptoms in India. A total of 50 out of 372 plants were shortlisted for the screening of *in vitro* antiplasmodial activity, both individually and in combination with chloroquine, against a chloroquine-resistant *P. falciparum* strain MRC-Pf-43. Two hundred (200) extracts were prepared from 54 parts of the 50 plants using 80% ethanol, dichloromethane, cyclohexane and methanol. From the 200 extracts, 29 extracts (14.5%) exhibited significant antiplasmodial activity (IC_{50} values ranged between 3.96 and 4.85 µg/mL), 81 extracts (40.5%) showed relatively good antiplasmodial activity (IC_{50} values ranged between 5.02 and 14 µg/mL), while the rest of the extracts were devoid of significant antiplasmodial activity. When the extracts were used in combination with chloroquine, 103 extracts (or 51.5%) displayed significant *in vitro* antiplasmodial activity, suggesting a synergistic interaction between the extracts and chloroquine. The synergistic factors ranged between 1.03 and 1.92.

Another study conducted in Malaysia evaluated the effect of a dietary combination of *Nigella sativa* seeds and seed oil with chloroquine on the survival rates of mice infected with the chloroquine-sensitive *P. berghei* NK65 strain [115]. In general, longer parasitaemia clearance durations (20 to 26 days) were noted in mice supplemented with the *N. sativa* seed and oil alone, however parasitaemia suppression was greatly enhanced (86.0 – 99.9% suppression) when

the seed and oil were used in combination with chloroquine. Parasitaemia cleared within 12 and 15 days for the *N. sativa* oil and seed, respectively. Parasitaemia cleared within 12 days when chloroquine was administered alone. It was interesting to note that all the mice (100% survival rate) that were treated with the seed or oil alone as well as seed/oil-chloroquine combination survived for the duration of the experiments (45 days), but only 60.0% of the mice treated with chloroquine alone survived until the end of the study, indicating an enhancement effect of *N. sativa* seeds and oil on the efficacy of chloroquine.

Sibhat and Hiben [116] studied the effect of oral co-administration of the hydroethanolic fruit extract of *Balanites aegyptiaca* and leaf latex of *Aloe camperi* and chloroquine on the mean survival time of mice infected with the *P. berghei* strain using Peter's 4-day suppressive test. *Balanites aegyptiaca* and *A. camperi* represent two of the plants used in the preparation of herbal concoctions administered concurrently with chloroquine in the traditional treatment of malaria in Ethiopia. Acute toxicity and *in vitro* antiplasmodial activity were tested on the extracts alone and also on extracts combined with chloroquine. The fruit extract of *B. aegyptiaca* displayed significant antiplasmodial activity when tested alone, but the effect was much higher when it was co-administered with chloroquine. In contrast, the leaf latex extract of *A. camperi* exhibited poor antiplasmodial activity when administered alone, however the activity was greatly enhanced when it was administered in combination with chloroquine, signifying a synergistic interaction. Percentage parasitaemia suppression was significantly lower (<58%) for the extracts alone compared to chloroquine alone (93.3%) and the extract-chloroquine combinations (>95%), however it was considerably higher than the control group that received only distilled water. The mean survival times for the mice that were treated with extracts were shorter relative to the ones treated with the extract-chloroquine combinations, however none of the mice in these treatments survived until the end of the experiment (28 days). Interestingly, no death was recorded in the group that was treated with chloroquine only for the duration of the experiment despite the fact that all the groups received the same dose of 12.5 mg/kg chloroquine/day, which could imply that the extracts interfered with the pharmacokinetics of chloroquine, thus impairing its bioavailability and rendering it ineffective.

Hiben and colleagues [117] also examined the *in vivo* antimalarial activity and chloroquine-potentiating effect of the leaf extract of *Senna singueana* against the chloroquine-sensitive *P. berghei* ANKA strain. The hydroethanolic extract showed a dose-dependent *in vivo* antimalarial activity against *P. berghei*. The combined use of the extract and chloroquine resulted in an enhanced antimalarial activity, suggesting some form of interaction between the extract and chloroquine. The extract reduced the percent parasitaemia, however all the mice died for all the

tested doses with the exception of the combination of 10 mg/kg of chloroquine and 500 mg/kg of the extract.

3. FUTURE PERSPECTIVES AND CONCLUDING REMARKS

The future of quinoline-resistance reversal agents looks promising, and to ensure fruition, a number of strategies may be useful and are hereby proposed. Continuous use of the reversed-chloroquines is one of the most recommended strategies which is key to correcting pharmacokinetics, absorption, metabolism, and excretion and toxicity shortfalls of synthetic and natural chemosensitisers. This is in the backdrop of earlier studies identifying several chloroquine-resistance reversal compounds, however, their clinical use for malaria has been restrained by issues such as low efficacy *in vivo*, poor pharmacokinetic properties, and/or toxicity. These liabilities were partly corrected by the synthetic analogues of these reversal agents. This approach was followed by the reversed chloroquine molecule concept in which one covalently links a chloroquine-like quinoline ring with moieties designed to inhibit efflux, thus the double drug approach. In this vein, dimers of quinolines can be pursued since it can be assumed that one monomer acts as the reversal agent while the other one acts as the drug. It is also proposed that dual reversal agents consisting of two or more reversal agents heads' linked by a covalent bond can be considered for future studies. This can be considered as an off-shoot from the concept of using reversal agent cocktails in chloroquine reversal agents in sensitisation of resistant plasmodia.

For fast-track identification of drugs targeting the PfCRT, one of the best approaches is utilising molecular modelling whereby compound libraries can be screened *in silico* against drug-sensitive and drug-resistant isoforms. PfCRT has less similarity to human proteins and is essential for parasite proliferation, which are favourable attributes for a drug target. With modern computational and synthetic tools, medicinal chemists can design and test highly specific PfCRT inhibitors with fewer off target affinities that contribute to toxicity [118]. A study by Antony and co-workers in 2019 developed an *in silico* model of PfCRT protein and its interactions with chloroquine [119]. The study used molecular docking and ligand binding studies alongside biochemical experiments to confirm that the Transmembrane Domain (TMD) 1 of the PfCRT protein is involved in the substrate selectivity and chloroquine efflux. In the study, chloroquine was found to bind strongly to the mutant PfCRT when compared to the wild PfCRT protein. This could imply that chloroquine resistance reversal agents with chloroquine substructures present a preferable substrate for the drug efflux protein. Therefore, *in silico* explorations of the TMD 1 binding pocket are likely to open

a new frontier for design and development of more potent chloroquine resistance reversal agents.

Repurposing of other resistance reversal compounds meant for combating antimicrobial resistance and multidrug-resistant cancer can be employed as a strategy for identifying novel chloroquine-resistant chemosensitisers. For example, novel P-gp inhibitors aimed at multidrug-resistance in cancer have been reported and have progressed to clinical trials. Such P-gp inhibitors can be repurposed for malaria [120]. A specific example is the quinolines which have elicited great interest in cancer research as potential multidrug-resistance reversal agents [121].

There is a need to explore compounds that can target other resistance inducing proteins, for example the *P. falciparum* Multidrug Resistance-associated Protein 1 (PfMRP1; PF3D7_0112200), which is an ABCI transporter localised to the parasite plasma membrane. It has been demonstrated by Silva *et al.* [122] that parasites with disrupted PfMRP1 are resistant to antimalarial folate analogues, methotrexate and aminopterin. Furthermore, recent studies revealed the interconnectedness of *P. falciparum* transporters (*e.g.* PfMDRs and PfCRTs) in overcoming drug pressure in different parasite strains allowing concurrent development of resistance of antimalarials that do not belong to the same chemical space [67].

Utilisation of affordable high-throughput screening (HTS) technologies for *in vitro* and *in vivo* assaying is important for speedy drug discovery and development. Whole cell pathway screening is one of the rapid screening assays that can be upgraded to HTS to enable faster identification of potent quinoline-resistance reversal agents [123]. Given the evident potential of plants as a source of chloroquine-resistance reversal compounds, the value of HTS will be key in accelerating efforts to unearth these agents. Moreover, biochemometrics tools can be of great use in this regard [124].

Safety, efficacy and cost-effectiveness of chloroquine were undisputed in the past, maintaining these advantageous characteristics remains key in the pursuit of reviving chloroquine as an antimalarial *via* resistance reversers. Usage of most quinoline reversal agents in their optimal *in vitro* concentrations in humans shows potential toxicities. Thus, the need exists to optimise the existing quinoline reversal agents in order to achieve inexpensive, safe reversal agents that are potent at lower doses.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

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

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