CHEMICAL REVIEWS

Review

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"Recycling" Classical Drugs for Malaria

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1. INTRODUCTION

Malaria has been a human health concern ever since the dawn of mankind, but despite the huge struggles made to date to fight this infection, in 2012 over half a million people were killed and a quarter billion got infected, in most cases by the *Plasmodium falciparum* (*P. falciparum*) species.[1] Antimalarial chemotherapy has been based on an endless search for the next weapon to strike *Plasmodium* parasites when they find their way to elude the action of current drugs. New antimalarials should be low-cost ones, or else they will hardly fit a realistic malaria containment scenario. Given that one possible way to lower overall costs in antimalarial drug development is to work on already known therapeutic agents, the present review is focused on efforts that have been made over the past 15 years to find efficient and affordable antimalarials through recycling, rescuing, or repurposing classical drugs.

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The World Health Organization's (WHO) goal of having reduced, by the end of 2015, (i) malaria deaths to near zero and (ii) the number of new infections by 75% (from levels registered in 2000) has triggered a worldwide crusade to fight this disease: according to the WHO's World Malaria Report 2013, "International disbursements to malaria-endemic countries have increased markedly, from less than US$ 100 million in 2000 to US$ 1.6 billion in 2011, and an estimated US$ 1.94 billion in 2012 and 1.97 billion in 2013".[1] This was accompanied by massive adhesion of Medicinal Chemists to this campaign, with consequent proliferation of research groups, and scientific publications, focused on antimalarial chemotherapy. This focus has been such over the past decade that malaria is no longer regarded as a neglected tropical disease (NTD).[2] Still, and again quoting the same WHO's Malaria Report, "This progress is no cause for complacency. The absolute numbers of malaria cases and deaths are not going down as fast as they could. The disease still took an estimated 627 000 lives in 2012, mostly those of children under five years of age in Africa.[1] The fact that so many people are dying from mosquito bites is one of the greatest tragedies of the 21st century".[1] One of the causes for such tragedy is the cost of first-line treatments against chloroquine-resistant *P. falciparum* malaria, such as artemisinin-based combination therapies (ACT), which may be too high for most of the low-income malaria-endemic countries.[3] Such drug cost limitations underlie an even darker perversity: fake and low-quality antimalarial drug pipelines that undermine the recent progress in malaria containment.[4]

The history of antimalarial chemotherapy has been a wavering one: the world has recurrently witnessed the rise and fall of drugs, whose originally thrilling antimalarial properties eventually gave place to disappointing news about previously undisclosed toxicity issues (e.g., quinine, pamaquine, mepacrine, mefloquine) or, predominantly, emergence of parasite resistance (e.g., chloroquine, mefloquine, sulfadoxine-pyrimethamine). The spread of chloroquine-resistant *P. falciparum* strains all around the globe was actually the most devastating drawback of the 20th century in malaria control: earlier regarded as an almost perfect antimalarial, given its efficacy, safety (including for pregnants and newborns), good pharmacokinetics and low cost, chloroquine also became a "fallen angel" in antimalarial chemotherapy.[5] Furthermore, given the recent reports on artemisinin-resistant *P. falciparum* strains that are emerging in Southeast Asia, it seems very likely that the 21st century antimalarial "stars", artemisinin and derivatives, will soon follow the same trail.[6]

Antimalarial drug research has been, and must keep on being, an endless search for the next weapon to strike the parasite when it finds its way to elude the action of currently available drugs. Ideally, the new drugs should be produced at low cost, or else they will hardly fit a realistic malaria containment scenario. In this sense, the "3 R's of the Environment" might prove useful in new antimalarial chemotherapy strategies: "Recycle, Reuse, Reduce". "Recycling" known drugs for malaria may be achieved by (i) performing synthetically affordable chemical modifications on classical antimalarials, now dethroned by ACT, (ii) repurposing drugs originally developed for other diseases, and found to also display antimalarial activity, and (iii) developing new combination therapies based on known drugs. This latter approach benefits from the fact that it provides the antimalarial drug arsenal with new chemical entities that have (i) core structures already known to be active and [at least, reasonably] bioavailable; (ii) already settled companies dedicated to large-scale production of the core structures, for possible "Reuse" with minor adaptations of the manufacture pipeline. This would, in principle, "Reduce" overall drug production costs, as compared to those required for setting up, from scratch, large-scale production of a completely new compound. Reduced costs would also be predictable at the preclinical development level, as synthetic routes targeting the basic core are already known. Recent examples support this view: e.g., a simplified chloroquine analogue, AQ-13 (see section 5.1), reached phase II clinical trials in July 2012.[7] In this connection, this review will mainly focus on efforts that have been made, from 2000 onward, to find alternatives for malaria treatment, through both synthetic and/or computational approaches toward modification of classical antimalarials, and repurposing of known drugs for malaria chemotherapy.

2. CHEMICAL RECYCLING OF QUININE: THE RISE OF EMBLEMATIC 20TH CENTURY ANTIMALARIALS

2.1. Quinine: Past, Present and Future

Much has been said and written about the earliest antimalarial drug recognized as such, the alkaloid quinine (**Q**, Figure 1).

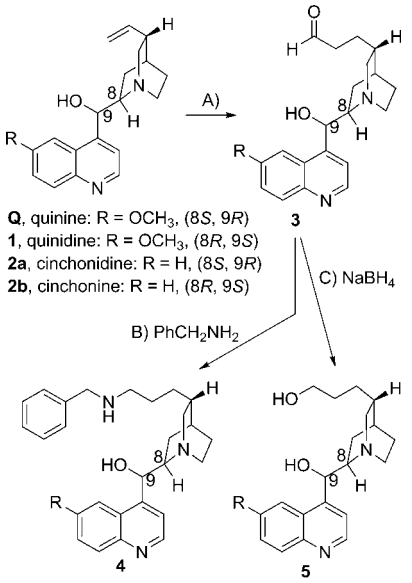

Figure 1. (A) Hydroformylation of the cinchona alkaloids (from **Q**, **1**, **2a** and **2b**); (B) reductive amination; and (C) reduction of aldehyde **3**.[16]

From the mid-19th century to the 1940s, **Q** became the standard therapy for intermittent fever throughout the world, but emergence of safer and more potent/bioavailable antimalarials outdid **Q**'s role as an antimalarial from then on.[8] Still, one of the strengths of **Q** is the fact that malaria parasites are rather slow in developing resistance against this drug. In fact, although **Q**'s use spread in Europe as early as in the beginning of the 17th century, resistance to this drug was first reported only in 1910.[8] In contrast, resistance to proguanil emerged within only one year after its introduction,[9] while for chloroquine resistance appeared after 12 years.[10] A worrying fact is that resistance to the 21st century antimalarial symbol, artemisinin, seems to be also appearing in Southeast Asia.[11] Hence, **Q** is still employed against malaria in the clinics, usually as monotherapy, but also eventually combined with a second

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agent to shorten the duration of therapy and thus minimize **Q**'s adverse effects.[12]

The search for suitable **Q** surrogates through modification of the Cinchona alkaloids (Figure 1) continues to be addressed. The total synthesis of **Q** was only achieved in 1944 by Woodward and Doering,[13] and remains quite laborious as well as economically unviable as compared to its isolation from the bark of Cinchona trees.[14] Still, **Q** has been long the target of numerous synthetic endeavors, including chemical modifications earlier focused on substitutions around the quinoline moiety, as these seemed to be less detrimental for antimalarial activity.[15] Recent examples of chemical recycling of the **Q**'s structure have also involved modification outside the quinoline core; for example, Lambers et al. explored conversion of the vinyl group common to all four naturally occurring cinchona alkaloids (Figure 1) into a functional group that could be used as a linker, such as a carboxylic acid or an aldehyde (**3**, Figure 1); the latter was further modified via either reductive amination (**4**, Figure 1) or reduction to the corresponding alcohol (**5**, Figure 1).[16]

Bhattacharjee et al. have put forth a catalyst-generated binding model that indicates the vinyl group to be eventually important for **Q**'s activity;[17] however, Alumasa et al. have later suggested that such group is nonessential for binding to heme,[15] the putative drug target of **Q** and other quinoline antimalarials.[18] In this connection, Dinio et al. pursued a structure–activity relationship study through modification of the vinyl group by use of the Heck reaction (**6**, Figure 2), which yielded compounds with good antiplasmodial activity in **Q**-resistant and **Q**-sensitive strains.[19]

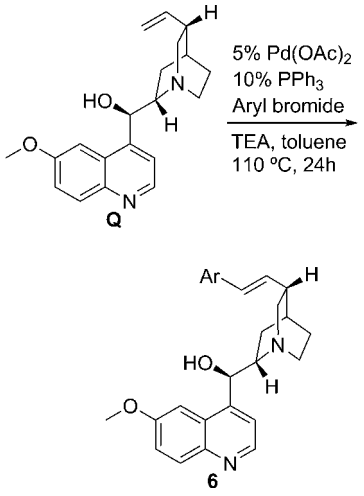

Figure 2. New quinine derivatives (**6**) developed by Dinio et al.[19]

There are other recent works on chemical modifications at the **Q**'s vinyl group to produce novel **Q** analogues (e.g., **7**, Figure 3);[20] however, to the best of our knowledge, their antimalarial properties have not been reported so far.

A newest example of Medicinal Chemistry work around the **Q** scaffold, by Sanders et al., has been inspired in hydroxyethylapoquinine (**8**, Figure 4), which was introduced as an antimalarial in the 1940s, allegedly to overcome cardiotoxicity events associated with **Q** and quinidine. These authors have synthesized and studied four compounds: compound **8**, its novel stereoisomer hydroxyethylapoquinidine (**9**, Figure 4), and two synthetic intermediates, hydroxyethyl-

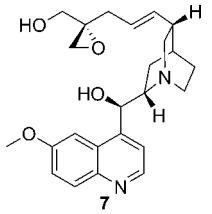

Figure 3. New **Q** derivatives developed by Garner and Koide (**7**).[20b]

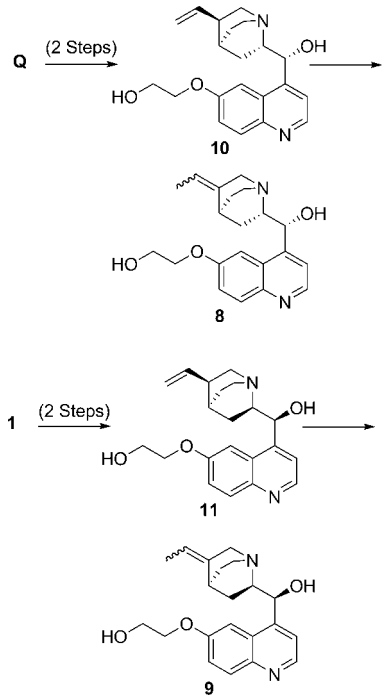

Figure 4. New **Q** and quinidine derivatives developed by Sanders.[21]

quinine (**10**) and hydroxyethylquinidine (**11**). The latter was found to be the most interesting compound of the set, as it inhibits heme crystallization *in vitro*, is comparable to **Q** against human *P. falciparum in vitro* and against mouse *P. berghei* ANKA *in vivo*, and does not appreciably inhibit hERG channels. Hence, compound **11** seems to be an adequate lead for developing a new class of **Q**-based antimalarials, on which further chemical modifications should be pursued.[21]

Finally, an example that emerged in line with two of the major 21st century keywords in antimalarial chemotherapy, artemisinin and covalent bitherapy:[22] Bell and co-workers created an artemisinin/quinine conjugate (**12**, Figure 5)

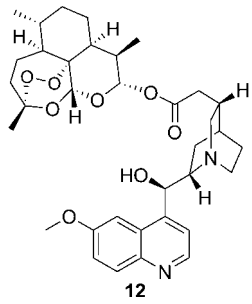

Figure 5. Artemisinin/quinine conjugate (**12**) developed by Bell and co-workers.[23]

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through coupling of dihydroartemisinin to a carboxylic acid derivative of **Q**, via an ester linkage.[23] Hybrid compounds underlying the covalent bitherapy concept consist of a single molecule that joins together, through a covalent bond, two different pharmacological agents.[24] This covalent bitherapy strategy may offer a more effective way to deliver the agent, for instance, diminishing drug−drug adverse interactions.[25] The novel hybrid molecule **12** was active against both sensitive and resistant strains of *P. falciparum* in culture, and its activity was superior to that of artemisinin or **Q** alone, or to a 1:1 mixture of these two drugs.[23]

Despite recent promising findings on **Q** surrogates, none seems to have been taken to clinical trials yet. Still, it is foreseeable that **Q** will continue to play a significant role in the management of malaria in the near future, particularly in resource-limited settings.[14]

2.2. Quinine and the Birth of Major 20th Century Antimalarial Drug Classes

Another, or perhaps the, major contribution of **Q** to the malaria containment scenario has been its role, together with methylene blue (**MB**, Figure 6), as cotemplates on which the most emblematic antimalarial drugs of the 20th century have been built. Following Paul Ehrlich's pioneering use of **MB** to cure malaria in two patients, in the late 19th century, a quest for better antimalarial surrogates of **MB** was headed by the German chemical and pharmaceutical company, Bayer, until the end of World War II (WWII). This led to the discovery, in the first quarter of the 20th century, that **MB**'s antimalarial potency could be raised by replacing one of the dye's methyl groups with a dialkylaminoalkyl chain to give compound **13** (Figure 6).[26] Such findings triggered great efforts that led to the first antimalarial drug of synthetic origin, pamaquine or plasmoquine (**PM**, Figure 6), an 8-aminoquinoline (8-AQ) where the dialkylaminoalkyl chain was combined with the quinoline core of **Q**.[27] This discovery was soon followed by another relevant one in 1931: quinacrine (**QN**, Figure 6), also known as mepacrine or atabrine,[28] which was of chief importance to protect soldiers fighting in tropical regions during WWII.[29] Only three years later, Bayer gave birth to what was going to become the most emblematic antimalarial of the 20th century: the 4-aminoquinoline (4-AQ) resochin, or chloroquine (**CQ**, Figure 6), whose potency, bioavailability and safety outshined those of all antimalarials available by then.[30]

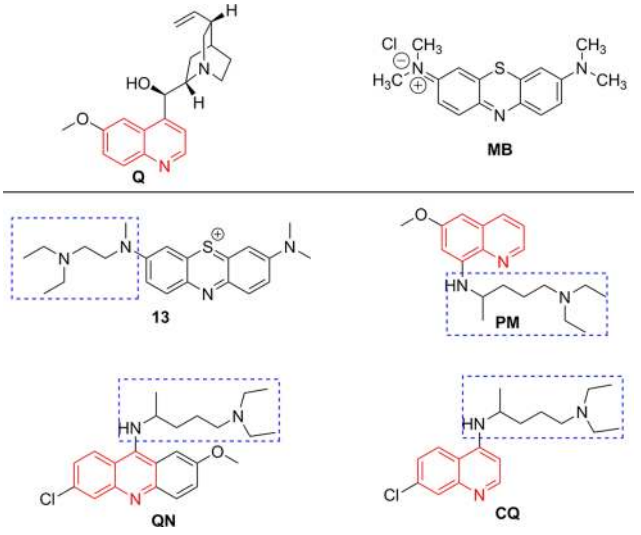

Figure 6. Illustrated role of quinine (**Q**) and methylene blue (**MB**) derivative (**13**) in the rise of the most emblematic classes of antimalarial drugs of the 20th century: 8-aminoquinolines, represented by pamaquine (**PM**), acridines, represented by quinacrine (**QN**), and 4-aminoquinolines, represented by chloroquine (**CQ**).

Those three classical antimalarial drugs from Bayer represented the rise of the three major classes of 20th century antimalarials: 8-aminoquinolines, acridines and 4-aminoquinolines. The relevance of these three families of antimalarials is still felt at present day, since many such compounds are still used in the clinical setting, and also because medicinal chemists worldwide keep up using their scaffolds as templates toward creation of better drugs.

3. ANTIMALARIAL 8-AMINOQUINOLINES: STILL SEEKING FOR A SUITABLE SUBSTITUTE OF PRIMAQUINE

3.1. From Pamaquine to Primaquine—Targeting Parasite's Liver and Sexual Stages

As already mentioned in the previous section, the first synthetic antimalarial drug that emerged from Bayer's effort to find substitutes for **Q** and **MB** was pamaquine (**PM**). This 8-aminoquinoline (8-AQ) was found to be useful in the prevention of infection relapses associated with dormant liver forms (hypnozoites) of *P. vivax* and *P. ovale* species, the first of which being the most prevalent outside Africa. Furthermore, **PM** also acted as a transmission-blocking agent, i.e., was able to impair the parasite's sexual reproductive cycle in *Anopheles* mosquitoes fed on the blood of infected mammals. However, **PM** was quickly abandoned by clinicians due to its high toxicity and limited activity against the prostrating and life-threatening infective phase (blood stage) of *P. falciparum* malaria.[31]

The importance of having a drug in the clinics that targets liver (both active and dormant forms) and sexual stages of malaria parasites, in other words, having complementary action to blood-stage drugs as **QN** or **CQ**, soon became obvious. Such was further reinforced by the fact that, in 1941−45, U.S. soldiers were fighting in *P. vivax*-endemic regions of the Pacific and by the rising menace of war in Korea soon after its division along the 38th parallel, in 1945. Therefore, by the end of WWII, the U.S. Army was deeply engaged in the effort of improving the therapeutic index of PM, participating in academic-military partnerships that led to production of hundreds of other 8-AQ that included pentaquine (**14**, Figure 7),[32] isopentaquine (**15**, Figure 7),[33] and primaquine (**PQ**, Figure 7). It was soon perceived that, among all those 8-AQ, only **PQ**, whose synthesis was reported by Elderfield in 1946,[34] was of real clinical utility. Since 1950, **PQ** remains as the only drug clinically approved worldwide for treatment of relapsing *P. vivax* malaria (30 mg/day/7days).[31]

3.2. Primaquine-Based 8-Aminoquinolines in Specific Clinical Settings or under Clinical Trials

PQ is associated with serious adverse effects as a consequence of its toxic metabolites, such as 5-hydroxy-PQ (**16**, Figure 8) or 6-methoxy-8-AQ (**17**, Figure 8), which have been considered as directly responsible for hematological complications such as methemoglobinemia and hemolytic anemia. Also, **PQ** is rapidly metabolized in mammals to carboxy-PQ (**18**, Figure 8), which is devoid of significant antimalarial activity.[35] Therefore, many efforts were undertaken in the second half of the 20th century

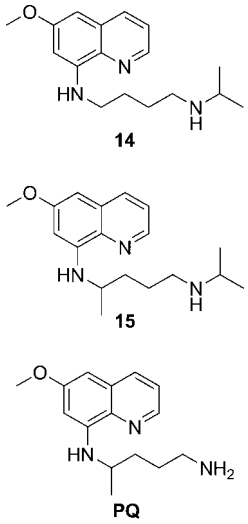

Figure 7. Classical 8-aminoquinolines pentaquine (**14**), isopentaquine (**15**) and primaquine (**PQ**).

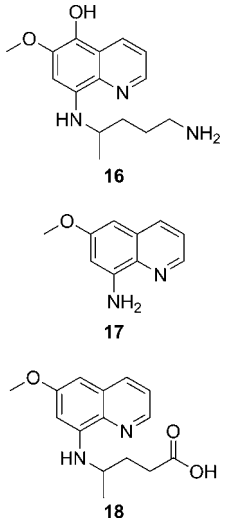

Figure 8. Main metabolites of **PQ**: hiydroxyprimaquine (**16**), 6-methoxy-8-aminoquinoline (**17**) and carboxyprimaquine (**18**).

aimed at improving the therapeutic properties of **PQ**-related compounds.

Some such efforts culminated in **PQ** surrogates which, thus far, have not been accepted for clinical use worldwide, but either have been approved by national entities in some countries or are currently under clinical trials. One such drug is quinocide (**19**, Figure 9), an isomer of **PQ** that was synthesized in the former Soviet Union in the late 1950s but whose use worldwide use was blocked by the fact that it is more toxic than **PQ** itself.[36] India is another country that has been long fostering important efforts toward development of safer **PQ** surrogates to combat *P. vivax* malaria, mainly through its Central Drug Research Institute (CDRI). A series of cyclic enaminone analogues of **PQ** were prepared in CDRI as prodrugs of **PQ**, among which bulaquine, also known as elubaquine or aablaquine (**20**, Figure 9), was believed to be a better alternative to **PQ** against *P. vivax* malaria.[37] Several studies with both **PQ** and bulaquine, in India and Thailand, suggested the latter to be safer and more potent than the former,[38] for both prevention of relapse in *P. vivax* malaria[37b,39]

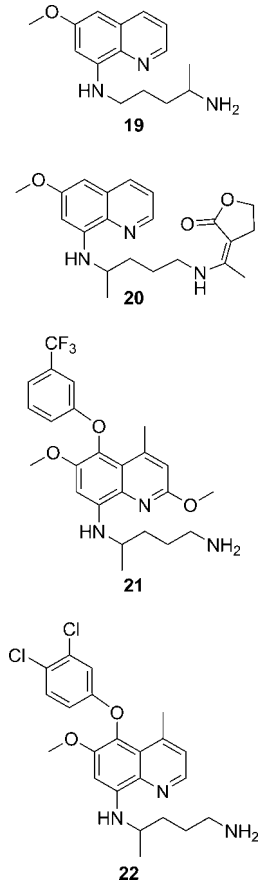

Figure 9. **PQ**-based 8-aminoquinolines in clinical use in some countries, or under clinical trials as antimalarials: quinocide (**19**), bulaquine (**20**), tafenoquine (**21**) and NPC-1161B (**22**).

and as a gametocytocidal agent against *P. falciparum* (25 mg/day/5 days).[40]

One of the most promising **PQ** derivatives, tafenoquine (**21**, Figure 9),[41] has successfully completed phase IIb clinical trials by the end of 2013.[42] Tafenoquine was developed in the U.S. by researchers at the Walter Reed Army Institute of Research (WRAIR) as soon as in 1963, and identified under the code WR238605.[43] Originally, tafenoquine was investigated as a substitute for **PQ** for radical cure of *P. vivax* malaria, but later it was found to be a broad-spectrum antimalarial drug useful for both prophylaxis in nonimmune travelers and treatment of established infections with multidrug-resistant *P. falciparum*.[44] Still, though tafenoquine has a longer half-life *in vivo* than **PQ**, it seems to equally cause hematological disorders, so its use in certain patients will possibly be blocked or limited.[45]

Another encouraging antimalarial 8-AQ, presently in preclinical development sponsored by the Medicines for Malaria Venture, is NPC-1161B (**22**, also referred to as DNS-21-1; Figure 9), which shows a clear-cut enantioselective pharmacological profile and promising antimalarial efficacy for both clinical and radical cure.[43,46] Tafenoquine and NPC-1161B exhibited IC_{50} values in the 500-nM and 50-nM range against *P. falciparum* drug-sensitive (NF54) and drug-resistant (7G8) strains, respectively, and were more potent than **PQ** and elubaquine (IC_{50} = 0.5−2.5 μM) against both strains.[47] Like tafenoquine, NPC-1161B has an *O*-aryl substituent at position 5 of the quinoline ring, and both compounds seem to require metabolic activation by CYP 2D6 as an essential factor to

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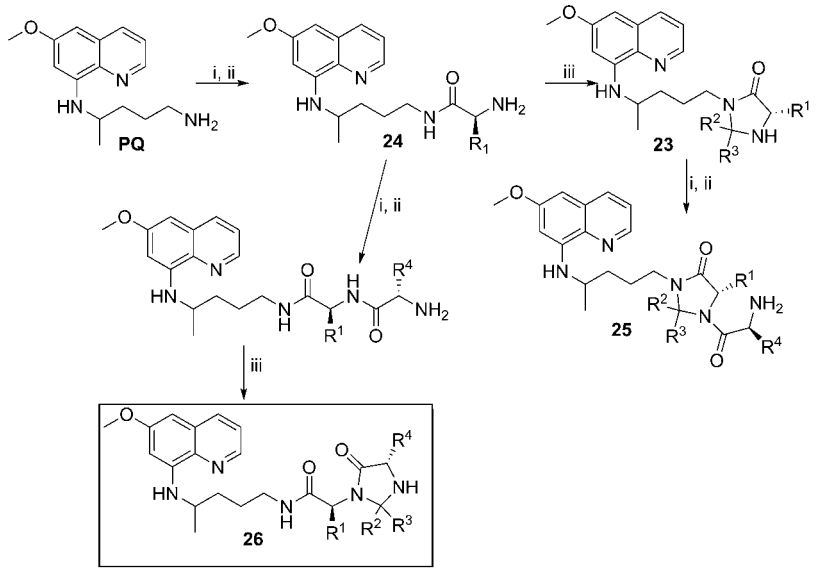

Figure 10. Synthetic route to peptidomimetic derivatives of primaquine (**23**–**26**). General conditions: (i) N^{α}-Boc-protected amino acid, peptide coupling agent, dichloromethane, rt; (ii) neat trifluoroacetic acid, rt; (iii) $R^2(C{=}O)R^3$, refluxing methanol over 3 Å molecular sieves.[57,61]

display antimalarial activity.[47] Interestingly, this finding may provide a possible explanation for patients who do not respond to 8-AQ like **PQ** for treatment of relapsing malaria, as further discussed below.

3.3. Recycling Primaquine: Toward Novel Antimalarial 8-Aminoquinolines

In spite of the hope brought by the promising features of tafenoquine, it is an undeniable fact that **PQ** remains as the only antirelapse and transmission-blocking antimalarial in clinical use all over the world.[42,48] Furthermore, almost seven decades have elapsed since its discovery, and no substantial and clinically relevant resistance against **PQ** has been reported. Yet, **PQ** has limited bioavailability and cannot be safely used in newborns, pregnants, elderly people, or any person bearing glucose-6-phosphate dehydrogenase (6GPD) deficiency.[48,49] Moreover, *vivax* malaria relapses due to failure of **PQ**-based therapies have been identified.[50] Such failures could be indicative of isolated cases of decreased parasite sensitivity to the drug;[51] however, they might instead be related with the recent proposal of a relevant role of CYP 2D6 activity for **PQ** antimalarial action, as **PQ** inefficacy has been associated with patients with decreased CYP 2D6 activity.[52] Such a finding has been supported by recent studies with CYP 2D knockout mice that were not cured from *P. berghei* infection by **PQ**, even when using doses 2-fold higher than those typically efficient in wild-type mice.[53] These results have troubling implications for the use of **PQ** as primary prophylaxis regimen, since, for instance, CYP 2D6 activity is lowered in as many as 5% to 10% of Caucasian travelers; despite the hope that genotyping and "personalized medicine" might eliminate the risk of **PQ**'s lack of efficiency in those individuals, at present CYP 2D6 testing is neither affordable nor widely available.[54] Altogether, these aspects demonstrate the need to go on searching for alternatives to **PQ** as antirelapse and transmission-blocking antimalarial agents. Most of the **PQ** "chemical recycling" efforts made throughout the second half of the 20th century have been extensively revised elsewhere;[47,55] the next examples refer to work from 2000 on.

3.3.1. Modifications Exclusively at the Aliphatic Chain of Primaquine. The simplest way to alter the **PQ**'s scaffold is through chemical modification at the primary amine group that terminates the drug's aliphatic chain. Such has been majorly addressed with the aim of producing **PQ** prodrugs, with the advantage that blocking or masking **PQ**'s primary amine impairs or delays drug's inactivation by oxidative deamination to carboxyPQ.[50,56] With this goal in mind, Gomes and co-workers have carried out the synthesis and biological evaluation of imidazolidin-4-ones prepared from amino acid derivatives of **PQ** (**23**, Figure 10), which exhibited potent gametocytocidal activity *in vivo* against *P. berghei*, hence blocking transmission from infected mice to *Anopheles stephensi* mosquitoes; compounds **23** derived from small amino acids (Gly and L-Ala) were found superior to those containing bulky/hydrophobic amino acid side chains (L-Phe, L-Val, and L-Leu). Interestingly, imidazolidin-4-ones **23** were very stable at physiological pH and *T*, both in aqueous buffer and in human plasma, suggesting that they were active *per se* rather than behaving as **PQ** prodrugs.[57] In agreement with such a hypothesis, the kinetics of hydrolysis of these **PQ** derivatives, investigated at 60 °C in the pH range 0.3–13.5, was quite different from that of imidazolidin-4-one prodrugs of peptides (**24**, Figure 10).[58] Additionally, compounds **23** were found to be active against blood-stages of **CQ**-resistant *P. falciparum* strain W2, although only at modest levels (IC_{50} = 2.42 to >50 μM).[59]

Later on, the same research group developed peptidomimetic derivatives of **PQ**, with general formula **25** (imidazoquines, Figure 10), where the imidazolidin-4-one ring was used as a dipeptide's proline-mimetic building block.[60] The compounds presented IC_{50} values ranging from 5.5 to 12 μM against the *P. falciparum* W2 strain, were chemically and enzymatically stable, and preserved the overall bioactivity pattern of **PQ**, including *in vivo* transmission-blocking activity on the *P. berghei* model of rodent malaria; yet, their activity against liver-stage malaria was not superior to that of the parent drug.[60] Remarkably, compounds **26**, isomers of imidazoquines **25** developed by the same group, were found to behave as **PQ** prodrugs,

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undergoing hydrolysis to the parent dipeptide derivative of **PQ** (**27**, Figure 11) in neutral and basic conditions.[61]

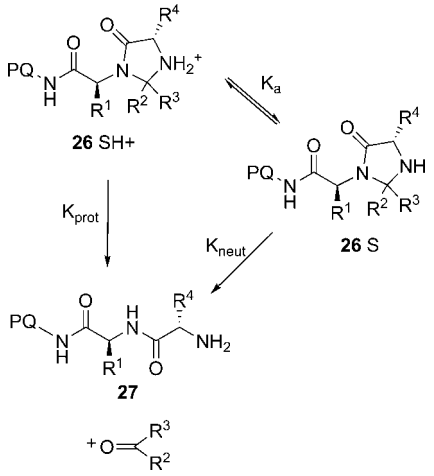

Figure 11. Kinetics of hydrolysis of imidazolidin-4-ones **26** at 37.0 °C originating the dipeptide derivatives of **PQ** (**27**).[61]

Motivated by the inspiring discovery of ferroquine as a promising antimalarial candidate (section 5.1), organometallic derivatives of **PQ** have also been approached by Gomes and co-workers, who synthesized a diversified group of primaquine/ferrocene conjugates (primacenes **28**–**33**, Figure 12). These compounds were tested as liver-stage, blood-stage, and transmission-blocking antimalarial agents, which permitted researchers to conclude that both transmission-blocking and blood-stage activities were preserved only in primacenes bearing a basic aliphatic amine group.[62] In turn, *in vitro* liver-stage activity did not require such a structural feature, and all metallocenes tested were comparable to or better than **PQ** against liver forms of *P. berghei*; remarkably, the replacement of **PQ**'s aliphatic chain by hexylferrocene, as in compound **33**, led to a ∼45-fold higher *in vitro* liver stage activity than that of **PQ** (IC_{50} = 1.25 to >10 μM against *P. falciparum* W2 strain).[62a] Unfortunately, such a promising result was not confirmed later in *in vivo* assays.[63]

Moreira and co-workers have equally addressed **PQ** derivatives that might prevent oxidative deamination of **PQ** to the inactive metabolite carboxy-PQ; to this end, those authors prepared *O*-alkyl and *O*-aryl carbamate derivatives of **PQ** (**34**, Figure 13) as potential **PQ** prodrugs, and studied their degradation kinetics; results obtained were compatible with two alternative pathways: one where compounds **34** undergo direct hydroxide attack at the carbonyl carbon (path A, Figure 13) to produce carbamate **37**, which then readily decarboxylates to **PQ**; alternatively, the conjugate base of **34** (**35**, Figure 13) can suffer E1cB elimination (path B, Figure 13) to an isocyanate (**36**) that rapidly reacts with hydroxide to equally produce **37** followed by decarboxylation to **PQ**. Carbamates **34** were tested *in vivo* for their gametocytocidal activity on *P. berghei*, and the ethyl and *n*-hexyl derivatives were the most active, and proposed as transmission-blocking leads.[64]

Somewhat similar structures have been very recently reported by Zorc and co-workers, who synthesized 1-acyl-4-substituted semicarbazide derivatives of **PQ** (**38**, Figure 14); however, only the compounds' antioxidant, cytotoxic, and antiviral activities were described, and nothing has been yet reported regarding their activity as antimalarials.[65]

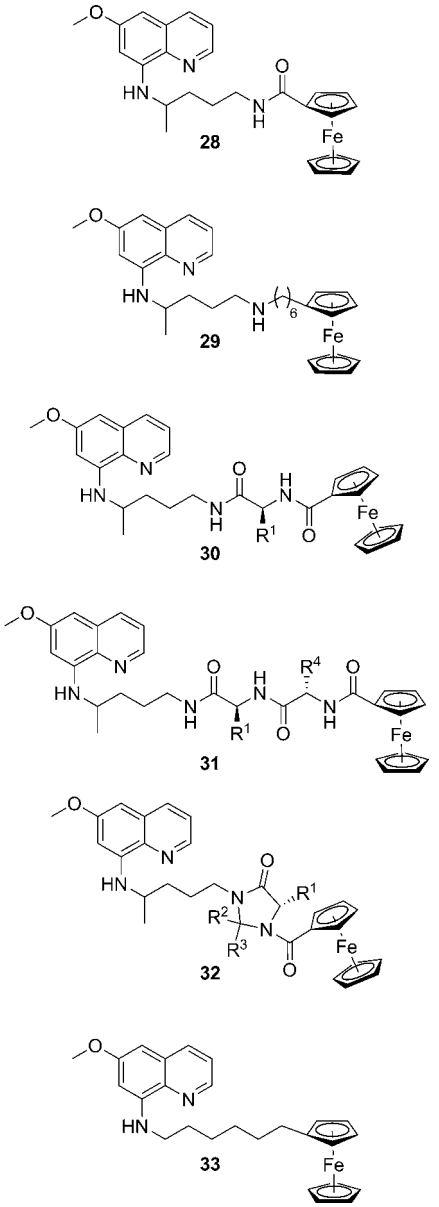

Figure 12. Organometallic derivatives of primaquine (**28**–**33**) developed by Gomes and co-workers.[62]

Modification of the **PQ**'s primary amine to produce prodrugs has also been addressed through more "exotic" approaches, such as conjugation with sugars (**39**, Figure 15) and amino acid-based polymers (**40** and **41**, Figure 15).[66] In 2009, Rajić et al. prepared glucosamine/PQ and polyaspartamide/**PQ** conjugates as potentially useful antimalarial **PQ** prodrugs of increased solubility and prolonged activity; preliminary results showed *in vivo* activity of the polyaspartamide conjugates against *P. berghei* infection in mice, but the potential of these conjugates on pre-erythrocytic stages of parasitemia or as transmission blocking agents was not reported.[66a] Similarly, Tomiya et al. have very recently reported the development of **PQ**/polymer conjugates (**41**, Figure 15), in this case designed to target liver cells; such conjugates were based on poly-L-glutamic acid modified with a glycosidic ligand specific to the hepatocyte asialoglycoprotein receptor, and were found to target and internalize rat hepatocytes, there being extensively

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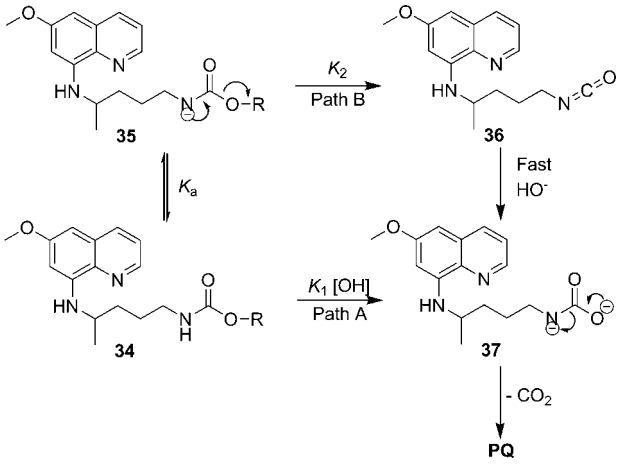

Figure 13. Degradation pathways of **34** to release parent drug **PQ**, according to Moreira and co-workers.[64]

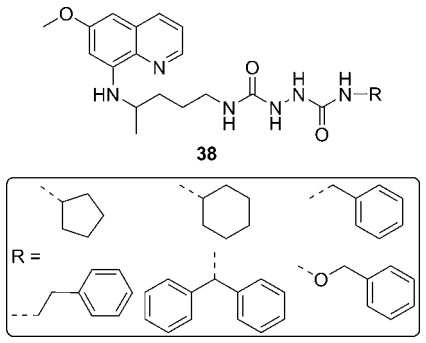

Figure 14. New PQ-semicarbazide derivatives **38** from Zorc's group.[65]

degraded; however, the antimalarial activity of such conjugates was not yet reported.[66b]

3.3.2. Modifications Involving the Quinoline Ring in Primaquine. The fact that major **PQ**'s toxic metabolites arise from modifications in certain positions of the quinoline ring has motivated an intense search for **PQ** surrogates where such positions were blocked by suitable substituents. Hundreds of 8-AQ were thus generated and evaluated in the last quarter of the 20th century, with some positive results that have been conveniently revised elsewhere, and of which tafenoquine is the most prominent example.[42,48,67] Recent efforts have almost invariably included chemical modifications at both the quinoline ring and the aliphatic chain. For instance, Jain and co-workers have developed compounds **42** (Figure 16), with interesting blood-schizontocidal activities *in vivo* against *P. berghei* (drug-sensitive strain) and *P. yoellii nigeriensis* (highly virulent multidrug-resistant strain) in mice; compound **42d** was curative at 5 mg/kg on *P. berghei* malaria, whereas **42e** exhibited curative activity at 50 mg/kg against *P. yoellii nigeriensis*.[68] However, neither blood-stage inhibition of *P. falciparum* nor, more relevantly, liver-stage activity of these **PQ** surrogates was reported.

The same research group also found that placement of a metabolically stable *tert*-butyl group at the quinolinic C-2 of **PQ** to produce 2-*tert*-butyl-PQ (**43**, Figure 16) results not only in a tremendous enhancement of blood-stage antimalarial activity (IC_{50} = 39.06 ng/mL against *P. falciparum* W2 strain) but also in significant decrease of hematotoxicity.[69] Such a finding was interpreted as possibly arising from a disturbance of the heme catabolism pathway in the malarial parasite.[70]

The same authors also reported bis(8-aminoquinolines) **44** (Figure 17) with promising antimalarial activity *in vitro* against drug-sensitive (D6, IC_{50} = 1.6−4.76 μg/mL) and drug-resistant (W2, IC_{50} = 0.3−3.6 μg/mL) strains of *P. falciparum*, and potent *in vivo* activity in the rodent model of malarial infection.[71] The compounds had also decreased hematotoxicity, as compared to **PQ**, and moderately inhibited β-hematin formation, suggesting this as a plausible pathway of their antimalarial activity.[71] Following these discoveries, the same group reported the design, synthesis, and evaluation of three new series of **PQ**-based 8-AQ modified at the terminal primary amine (**45**, Figure 17).[72] The presence of carboxyl groups seemed disadvantageous, as compounds from series 1 (IC_{50} = 4.76 and 4.2 μg/mL) were less potent than **PQ** (IC_{50} = 2.8 and 2.0 μg/mL) against *P. falciparum* W2 and D6 strains, respectively. However, the presence of basic amino acids L-Arg and L-Lys, as in series 2 (IC_{50} = 0.4−4.76 and 2.0−4.76 μg/mL) and 3 (IC_{50} = 0.26−4.2 and 0.13−3.8 μg/mL), led to encouraging *in vitro* results, again including decreased hematotoxicity as compared to **PQ**. Additionally, when tested for their *in vivo* blood-schizontocidal activity against *P. berghei* infected mice, compounds from series 2 and 3 containing the L-Lys amino acid as substituent exhibited 100% curative activity at an oral daily dose of 100 mg/kg for 4 days.[72] However, the analogue from series 3 displayed a lower selectivity index (SI) (SI > 8.5; SI = IC_{50} VERO/IC_{50} *Pf* W2) compared to its series 2 analogue (SI > 91.5).

The same authors have also studied extended side chain analogues of **PQ** (**46**, Figure 18) that exhibited potent antimalarial activities *in vitro* against both drug-sensitive D6 (IC_{50} = 0.19−2.6 μg/mL; SI > 9.1 to >125) and drug-resistant W2 (IC_{50} = 0.12−1.5 μg/mL; SI > 15.8 to >198) *P. falciparum* strains. The most promising compounds were proven to be 100% curative *in vivo* on *P. berghei*-infected mice after a 4-day treatment (25 mg/kg daily dose). These analogues were also found not to be cytotoxic up to 23.8 μg/mL and to inhibit β-hematin formation (IC_{50} = 9.6−20.8 μM) *in vitro*, underlining the disruption of heme catabolism by the malaria parasite as the potential biochemical pathway for their antimalarial action.[73]

Overall, reports on 8-AQ, such as compounds **42**−**46**, that are devoid of significant hemotoxicity and display appreciable blood-stage activity, emerged as a breakthrough. Should the efficacy of such compounds against liver and sexual (gametocytes) forms of *Plasmodia* been proven, the consequent discovery of triple-action **PQ** surrogates safe for use by 6GPD-deficient patients would have represented a new era in antimalarial chemotherapy. Unfortunately, to the best of our knowledge, neither the liver-stage or gametocytocidal activity of **42**−**46** has been reported up to today, nor have any of these compounds yet stepped forward into clinical development.

Other **PQ** alternates have been obtained by changes in the quinoline core structure itself, such as those reported by Zhu et al., who replaced the quinoline ring by a 1,5-naphthyridine (**47**, Figure 19). The resulting compounds displayed IC_{50} values ranging from 0.021 to 0.11 μM against *P. falciparum* W2 strain and excellent *in vivo* blood-schizontocidal activity against both *P. berghei* (ED_{50} = 0.26−1.8 mg/kg) and *P. yoelii nigeriensis* (ED_{50} = 0.23−2.1 mg/kg), while being over three times less toxic than **PQ**. In that work, the authors further found that introducing a substituent at position 2 (R^1 in **47**) reduced toxicity, while preserving the desired antimalarial activity; in turn, variation of substituent groups at the naphthyridine's position 6 did not have a significant effect in the compounds'

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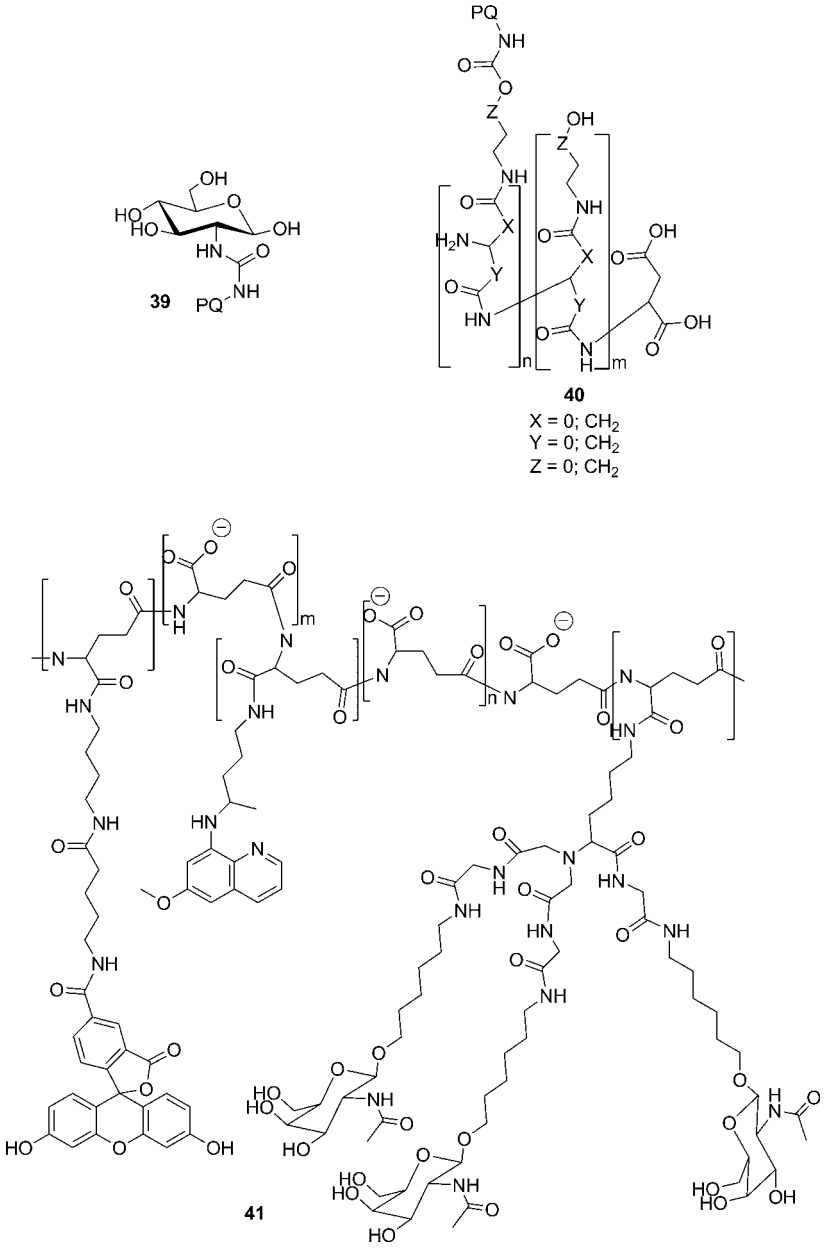
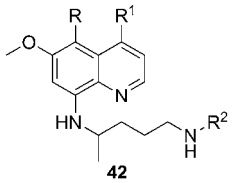


Figure 15. PQ conjugates (**39**–**41**) with sugars and/or amino acid-based polymers.

toxicity.[74] Regrettably, this report also lacked information regarding the compounds' activity against liver and sexual forms of *Plasmodia*, an assessment that should be regarded as mandatory when seeking for worthy **PQ** substitutes.

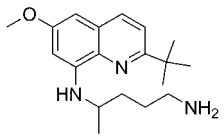
3.3.3. Dual-Core Hybrids. The previous subsections clearly demonstrate that finding a suitable **PQ** surrogate to enter the clinics is revealing itself to be a quite difficult endeavor. Although derivatives with enhanced blood-stage activity and reduced toxicity have been successfully developed in the late few years, their performance as tissue-schozontocidals (i.e., active against liver-stage parasites) and transmission-blockers was not reported or did not supersede that of **PQ** itself. This is a major drawback in current antimalarial chemotherapy, as it is now well-established that malaria eradication will possibly become more than a mirage only when efficient elimination of liver-stage *Plasmodia* and blocking of host-vector transmission are achieved. Quoting 2011's *malERA—a research agenda for malaria eradication*—"drugs will continue to be used to treat acute malaria illness and prevent complications in vulnerable groups, but better drugs are needed for elimination-specific indications, such as mass treatment, curing asymptomatic infections, curing relapsing liver stages, and preventing transmission".[75]

In view of the above, some authors have been using the double-drug approach, by synthesizing molecular constructs where **PQ** is covalently bound to other building blocks [potentially] exhibiting complementary antimalarial properties. In this connection, the most obvious approach is combination of **PQ** with other well-known antimalarial pharmacophores, such as the artemisinin or the chloroquine cores. Accordingly, Moreira and co-workers developed two dual-core hybrids where **PQ** was covalently linked to artemisinin-based moieties (**48**, Figure 20), and they found them to display enhanced *in vitro* activities against liver-stage *P. berghei*, as compared to both parent drugs. Moreover, both molecular constructs were about as potent as **ART** against cultured *P. falciparum* W2 strain (IC_{50}



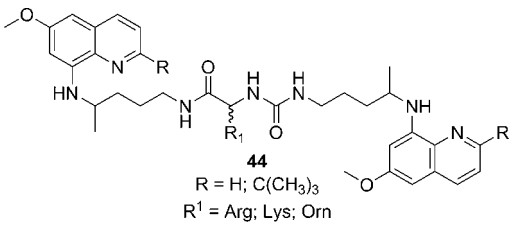
42

a: R = H; R^1 = CH_3; R^2 = Ala
b: R = H; R^1 = CH_3, R^2 = Lys
c: R = OC_6H_{13}; R^1 = CH_3; R^2 = Ala
d: R = OC_4H_9; R^1 = C_2H_5; R^2 = Lys
e: R = OC_5H_{11}; R^1 = C_2H_5; R^2 = Lys

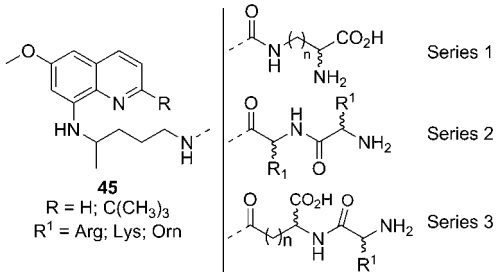


43

Figure 16. Amino acid derivatives of 4-mono- and 4,5-disubstituted-PQ (**42**), and structure of 2-*tert*-butylPQ (**43**) developed by Jain et al.[68,69]



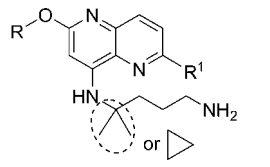
44
R = H; $C(CH_3)_3$
R^1 = Arg; Lys; Orn



45
R = H; $C(CH_3)_3$
R^1 = Arg; Lys; Orn

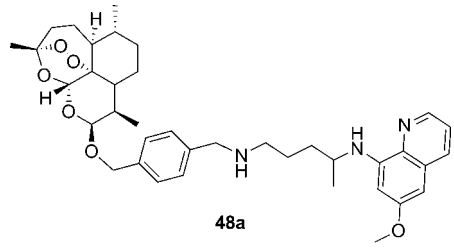
Figure 17. **PQ**-based 8-AQ (**44**–**45**) developed by Jain and co-workers.[71,72]

= 12.5 and 9.1 nM for **48a** and **48b**, respectively), and one of the compounds (**48a**) performed better *in vivo* against murine *P. berghei* infection than an equimolar mixture of the parent pharmacophores.[76] Unfortunately, additional preclinical studies

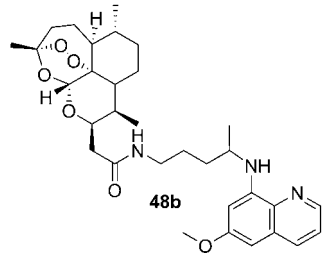


47
R = OCH_3; OCH_2CH_3; $OCH(CH_3)_2$)
R^1 = H; OCH_3; CF_3

Figure 19. Naphthyridine analogues of **PQ** (**47**) developed by Zhu et al.[74]



48a

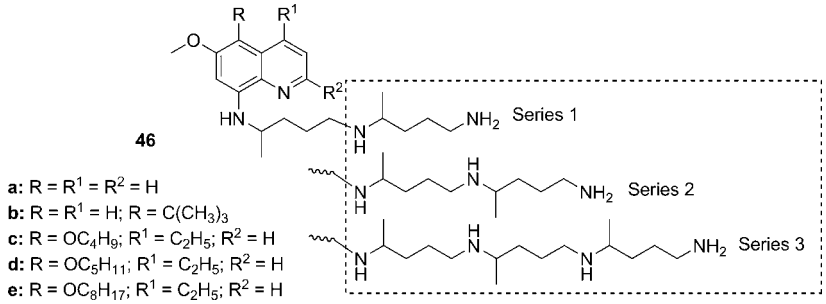


48b

Figure 20. **PQ**/artemisinin hybrids (**48**) as a multistage antimalarial strategy developed by Moreira and co-workers.[76]

on these rather promising hybrids have not been reported up to today.

Subsequent approaches to antimalarial hybrids encompassing the **PQ** core have addressed its conjugation with motifs potentially able to inhibit proteases of chief importance for intraerythrocytic parasites. For instance, Romeo et al. have coupled **PQ** to statine-based inhibitors of plasmepsin II (PLMII) (**49**, Figure 21), as statins are known to inhibit plasmepsins, aspartic proteases that are crucial for the development of blood-stage *Plasmodia*; hybrids **49** were found to exhibit IC_{50} values for PLMII inhibition ranging from 0.59 to 400 nM. The IC50 range for inhibition of *P. falciparum* D10 and W2 growth *in vitro* was 0.4–5.5 and 0.7–4.7 μM. Although these results are not exciting, they represent a remarkable improvement over other statine-based PLMII



46

a: R = R^1 = R^2 = H
b: R = R^1 = H; R = $C(CH_3)_3$
c: R = OC_4H_9; R^1 = C_2H_5; R^2 = H
d: R = OC_5H_{11}; R^1 = C_2H_5; R^2 = H
e: R = OC_8H_{17}; R^1 = C_2H_5; R^2 = H

Figure 18. Structures of double, triple and quadruple extended side chain analogues (**46**) of **PQ**-based compounds developed by Jain and co-workers.[73]

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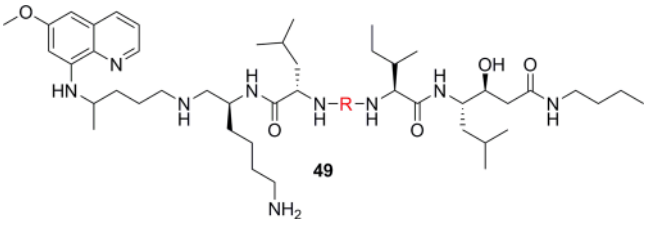
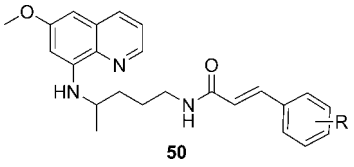


Figure 21. PQ/statin hybrids (**49**) developed by Romeo et al.[77]

inhibitors.[77] Awkwardly, neither the liver-stage nor transmission blocking activity of these **PQ**-statin hybrids was reported.

Following a similar line of thought, a more recent report by Gomes and co-workers described conjugation of **PQ** with different cinnamic acids (**50**, Figure 22), eventually capable of

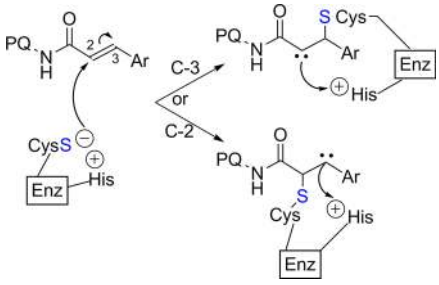


R = H; *p*-Me; *p*-^{i}Pr; *p*-OMe; *m*-F;
p-Cl; *p*-Br; *o*-NO_2; *m*-NO_2; *p*-NO_2

Figure 22. PQ/cinnamic acid conjugates (**50**) reported by Gomes and co-workers.[78]

irreversibly inhibiting falcipains, *P. falciparum* cysteine proteases relevant for the parasites' blood-stage development; the hybrids were designed on the grounds that (i) cinnamic acid derivatives had been reported as displaying interesting antimalarial properties and (ii) the *α,β*-vinylcarbonyl moiety of the cinnamoyl group might participate in a Michael-type addition involving the enzyme's catalytic cysteine thiol (Scheme 1).

Scheme 1. Michael-Type Addition Involving an Enzyme's Catalytic Cysteine Thiol



These hybrids displayed increased *in vitro* activity against liver-stage *P. berghei* parasites (IC_{50} = 1.38−2.39 *μ*M), as compared to the parent **PQ**, and they were nontoxic to human hepatoma cells. However, they were both devoid of significant blood-stage activity (IC_{50} > 4.84 mM against *P. falciparum* W2 strain) and unable to inhibit falcipains *in vitro*.[78]

Surprisingly, conjugation of the 20th century antimalarial stars **PQ** and **CQ** into a single hybrid drug (**51**, Figure 23) was only very recently addressed by Lödige and co-workers. These authors found that such a **PQ**/**CQ** hybrid did not significantly affect sporozoites' motility or ability to invade hepatocytes *in vitro* but was able to perturb development of liver-stage *P. berghei* and of both asexual and sexual (gametocytes) blood-stage *P. falciparum* (IC_{50} = 0.64, 0.58, and 0.08 *μ*M against *P.*

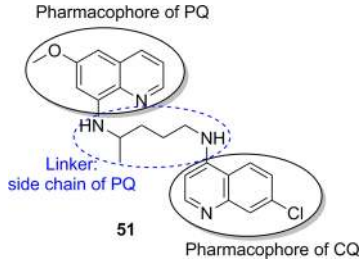


Figure 23. PQ/**CQ** hybrid (**51**) developed by Lödige et al.[79]

falciparum 3D7, Dd2, and K1 strains, respectively). Interestingly, though the *in vitro* performance of hybrid **51** was overall moderate, and generally lower than that of the equimolar mixture of its parent drugs, it performed better than **CQ** alone or its 1:1 mixture with **PQ** against the **CQ**-resistant *P. falciparum* K1 strain. More relevant still, hybrid **51** was active *in vivo* against both liver-stage parasitemia and blood-stage infection, while also being able to prevent blood stage potency and to treat the symptoms of experimental cerebral malaria.[79] It is thoughtful that such a promising lead is now emerging from the recycling of two top antimalarial drugs of the past century.

4. ANTIMALARIAL ACRIDINES: REVIVAL OF QUINACRINE, THE FIRST SYNTHETIC BLOOD-STAGE ANTIMALARIAL

Acridine (**AC**, Figure 24) is a relevant pharmacophore in, e.g., antimalarial, antimicrobial, antitumoral, antiprionic, anti-Alz-

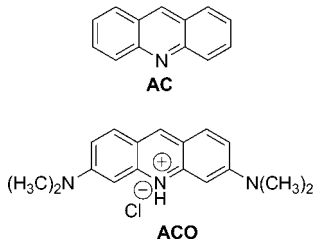


Figure 24. Structures of acridine (**AC**) and acridine orange (**ACO**).

heimer, and antileishmanial agents.[80] The most consensual mechanism of action (MOA) of **AC** derivatives against different diseases is interaction with DNA.[81] However, other MOA have been evoked to explain the antimalarial activity of **AC** derivatives, such as inhibition of parasite's (i) mitochondrial bc_1 complex, (ii) type II topoisomerases, (iii) hemozoin formation, or even chemosensitization. The latter refers to the effect of some compounds, or compound moieties, in reversing *Plasmodium* resistance to classical antimalarial drugs such as chloroquine (**CQ**, Figure 6).[80f]

The interest in **AC**-based structures as antimalarials also emerged from early discoveries on antimicrobial/antiprotozoan activity of the classic synthetic dyes **MB** (Figure 6, as mentioned on section 2) and, later, acridine orange (**ACO**, Figure 24), whose activity against *P. falciparum* 3D7 (IC_{50} = 7.8 and 465.7 for **MB** and **ACO**, respectively) and Dd2 strains (IC_{50} = 14.3 and 166.0 nM for **MB** and **ACO**, respectively) has been recently re-evaluated.[82] Both the toxicity and tendency to induce skin coloration of **MB** soon motivated the search for more adequate antimalarial surrogates, which led to the synthesis of quinacrine (**QN**, Figure 6), in 1932.[83] **QN** was widely used as an antimalarial by soldiers during WWII (Pacific

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War) but was soon superseded by **CQ**, whose efficiency, bioavailability, and safety were considerably superior.[80e,84]

When widespread resistance of *P. falciparum* to **CQ** became indisputable, by the late 1960s, the search for more efficient **QN** derivatives or analogues regained stamina. Such led to the synthesis, in the 1970s, of pyronaridine (**PYR**, Figure 25), an

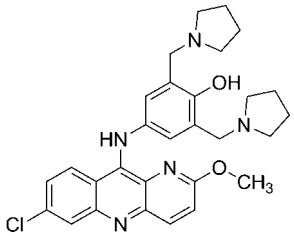

Figure 25. Structure of pyronaridine (**PYR**).

anilinoacridine topoisomerase II inhibitor, active *in vitro* and *in vivo* against drug-resistant *P. falciparum* strains; although this **QN**-based antimalarial has been in clinical use in China since the 1980s, parasite resistance and embryotoxicity issues have prevented its use as monotherapy elsewhere.[85] Still, good results from clinical trials using a **PYR** + artesunate combination in adults led to approval of such a combination (marketed as Pyramax) by the WHO and the European Medicines Agency (EMA) in 2012, for use as a single treatment course against acute, uncomplicated *P. falciparum* or *P. vivax* malaria infection in adults and children above 20 kg, in areas of low transmission with evidence of **ART** resistance.[86]

4.1. Acridine-Inspired Antimalarial Leads: Acridones and Related Structures

The lack of information on **PYR**'s safety regarding (i) children below 20 kg or under 12 years old, (ii) adults over 65 years old, (iii) impairment of renal and hepatic function, and (iv) repeated treatment courses underpinned the continuous effort toward development of novel acridine-inspired antimalarials. In 1994, the WRAIR proposed floxacrine (**52**, Figure 26), an antimalarial dihydroacridinedione.[86a,87] However, daily dosages between 1.25 and 2.5 mg/kg were needed for transient clearance of parasitemia, and *Plasmodia* quickly became resistant to this drug.[88] Moreover, floxacrine displayed suboptimal solubility and was soon associated with chronic periarteritis in animals, which led Raether et al. to investigate new floxacrine derivatives, where four (**53**−**56**, Figure 26; IC_{50} = 0.73−96 nmol against *P. falciparum* FCBR strain) were selected out of nearly two hundred produced. Unfortunately, *in vitro* and *in vivo* assays on the blood schizontocidal activity of such compounds on different drug-resistant strains of *P. berghei* and **CQ**-resistant *P. falciparum* showed that the limitations posed by floxacrine had not been overcome.[89]

In an effort to recycle the floxacrine scaffold toward new antimalarial structures, WRAIR later developed another dihydroacridinedione, WR243251 (**57**, Figure 27), having high blood-schizontocidal activity *in vitro* against a mefloquine-resistant, **CQ**-sensitive strain (D6; IC_{50} = 11 nM), a **CQ**-susceptible strain (NF54; 4.4 nM), and a **CQ**- and pyrimethamine-resistant strain (W2; IC_{50} = 25 nM).[87,90] Preclinical *in vivo* assays with this compound showed it to be 100% curative in mice at a dosage of only 12−20 mg/kg, comparably lower than those for antimalarials in the clinical setting by then. WR243251 was equipotent to **CQ** in inhibiting hemozoin

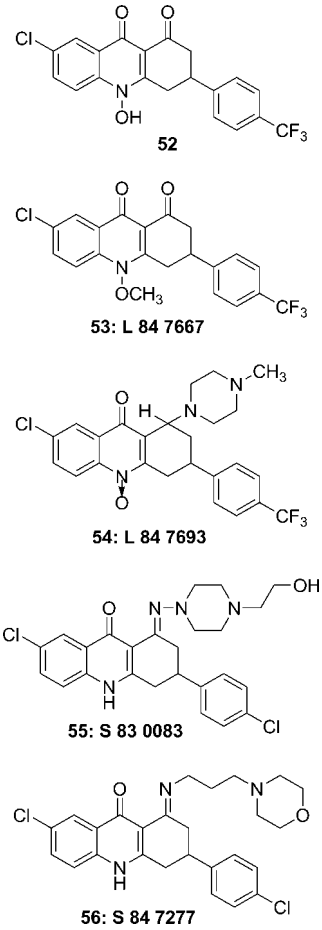

Figure 26. Floxacrine and derivatives studied by Raether et al.[87,89]

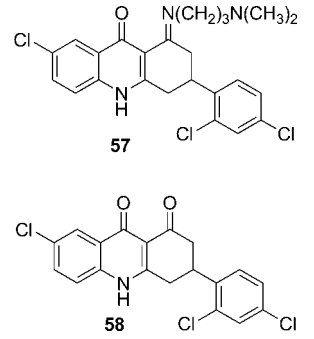

Figure 27. Structures of antimalarial dihydroacridinedione derivatives (WR243251, **57** and WR243246, **58**) developed by WRAIR.[87,90]

formation *in vitro*, and its hydrolysis resulted in a ketone product, WR243246 (**58**, Figure 27; IC_{50} = 6.1 nM against *P. falciparum* NF54 strain), whose *S*-enantiomer (WR249685) also displayed potent *in vitro* antimalarial activity (IC_{50} = 15 nM against *P. falciparum* 3D7 strain) but was unable to inhibit hemozoin formation.[87,90]

Additional computational and experimental studies on these compounds showed their main MOA to be inhibition of the parasite's cytochrome bc_1 complex involving interference with the quinol oxidation site (Q_o).[90] This was a relevant finding, as atovaquone (**59**, Figure 28) is the only bc_1 complex inhibitor in the current antimalarial clinical setting, against which parasite resistance has been identified; relevantly, atovaquone-resistant *Plasmodia* did not present cross-resistance to acridinediones,

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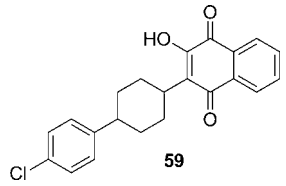

Figure 28. Structure of atovaquone (**59**).

which turns the latter interesting leads into a new generation of antimalarials acting through interference of the plasmodial electron transport chain.[91] Moreover, a bc_1 complex inhibitor, decoquinate, was recently found as a potent multistage antimalarial compound, emphasizing the relevance of including this MOA in future medicines for malaria eradication.[92] Still, despite all the promising features of WR243251, adverse dermatologic, cardiovascular, and neuropsychiatric effects have possibly prevented its clinical development.[87]

Altogether, findings such as those above-described have been fueling research on other floxacrine-based structures as potential antimalarials. Recently, Cross et al. produced a series of acridone analogues of floxacrine (Figure 29) and tested their

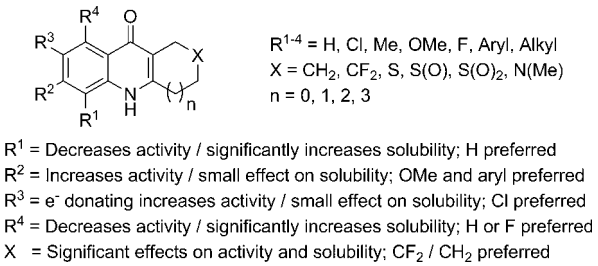

Figure 29. Structure activity relationship of compounds studied by Cross et al.[93]

solubility, antimalarial activity, and permeability. The main structure−activity relationships (SAR) drawn by these authors allowed concluding that acridones with aryl substituents in positions 6 and 7 presented optimal antimalarial activity (IC_{50} = 12.2−58.2 nM against *P. falciparum* W2 strain) and physicochemical properties.[93]

Other acridones have been explored as potential antimalarials, many of which were inspired by known antimalarial xanthones. Ignatushchenko et al., when trying to understand both MOA and SAR of hydroxyxanthones, found that xanthone (**60**, Figure 30) significantly inhibited hemozoin formation.[94] Subsequently, Kelly et al. gathered xanthones' heme-binding ability with the relevance of protonable amines in positions 3 or 6 of the xanthone ring to design compounds **61a** and **61b** (Figure 30); the latter presented IC_{50} values of 0.10 μM and 0.07 μM, respectively, against *P. falciparum* strain D6, being 1000-fold more active than dihydroxyxanthone (**62**, IC_{50} > 60 μM).[95] Based on this knowledge, Winter et al. pursued novel acridone derivatives which might enrol the ability to inhibit hemozoin formation with chemosensitizing properties. To this end, those authors started by synthesizing and evaluating 30 new derivatives of 2-methoxy-6-chloroacridone in order to build some SAR. Haloalkoxyacridones presented potent activity against *P. falciparum in vitro*, with the best compound (**63**, Figure 30) exhibiting an extraordinary picomolar IC_{50} value (1 pM against *P. falciparum* D6 strain) and a low level of cytotoxicity (IC_{50} > 25000 nM against murine splenic lymphocytes); this compound presented a bis(trifluoromethyl)-

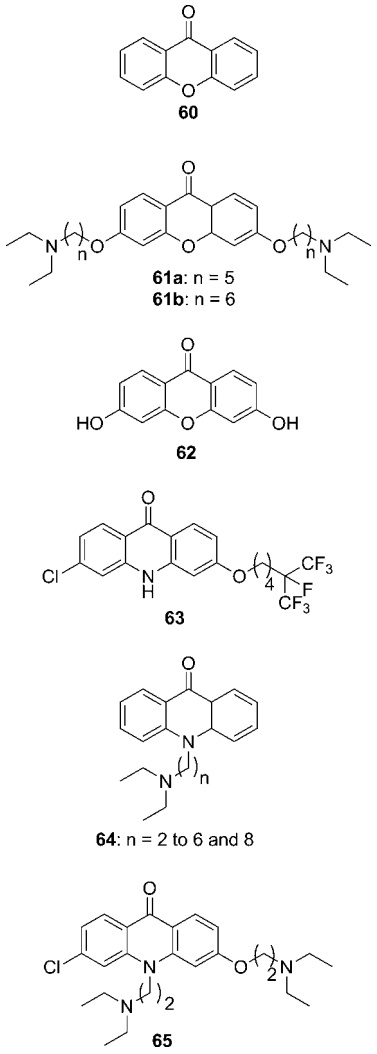

Figure 30. Antimalarial xanthones (**60**−**62**) and acridones (**63**−**65**) developed by Winter and co-workers.[94−98]

fluoroalkoxy group, somewhat evoking the trifluoromethyl substituent in floxacrine.[96] Later on, the same researchers synthesized acridone derivatives with *N*-alkylamine substituents in position 10 (**64**, Figure 30; IC_{50} = 2.6−11.8 and 1.6−10.2 μM against *P. falciparum* D6 and Dd2 strains, respectively), and they found them to display the desired chemosensitization properties and synergy with **CQ** against **CQ**-resistant *P. falciparum*.[97] Further results demonstrated that linking an ionizable side chain to position 6 of the 10-*N*-substituted acridones (**65**, Figure 30; IC_{50} = 44.8 and 77.3 nM against *P. falciparum* D6 and Dd2 strains, respectively) promotes compound's accumulation in the parasite's food vacuole (FV) and interferes with hemozoin formation; moreover, compound **65** showed synergy with quinine, since only one-third of the individual dosage was needed to have the same effect as when given separately.[98]

A similar set of acridones, bearing different 10-*N*-substitutions (allyl, 3-methyl-2-buthenyl, and 1,2-propadienyl) and a chlorine or fluorine atom in positions 1, 2, or 6 of the acridone moiety, was also synthesized and evaluated by Fernandez-Calienes et al. Though the best compound (**66**, Figure 31) was a hit, as it exhibited an IC_{50} (0.16 μg/mL against *P. falciparum* GHA strain) below the 0.2 μg/mL threshold to qualify as such and presented a SI of 112.2 (SI =

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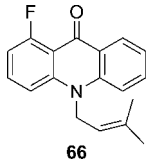


66

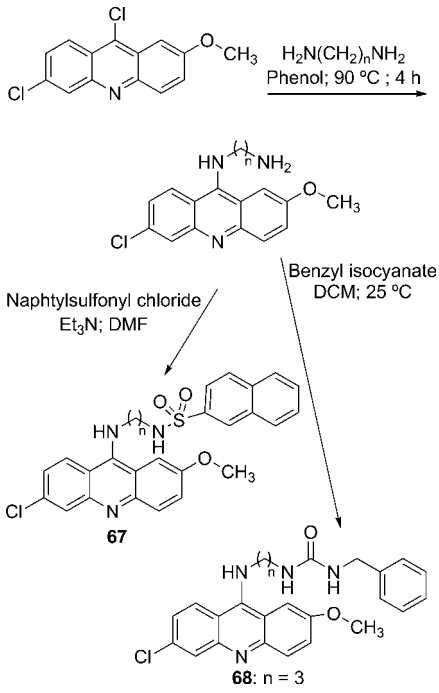
Figure 31. Best antimalarial acridone reported by Fernandez-Calienes et al.[99]

IC_{50} MRC-5/IC_{50} *Pf* GHA), its antimalarial activity was low when compared with acridone **65**.[99] Furthermore, **66** could not inhibit hemozoin formation; instead, it inhibited the bc_1 complex, still not with the same specificity as the aforementioned acridinediones developed in the WRAIR.[90b,99]

4.2. Revival of Quinacrine and Pyronaridine: In Pursuit of Next Generation Antimalarial Amino- and Aniline-Acridines

4.2.1. 9-Aminoacridine Leads. Fair efforts have been done during the first eighth of this century toward rejuvenation of **QN**-inspired antimalarials. In 2001, Chibale and co-workers developed a series of compounds where the acridine core of **QN** has been linked to a second aromatic moiety through sulfonamide (**67**, Scheme 2) or urea groups (**68**, Scheme 2), in order to improve compounds' solubility. The synthetic route employed (Scheme 2) required preactivation of the starting 9-chloroacridine with phenol, to produce a reactive 9-phenoxyacridine intermediate, followed by nucleophilic aromatic substitution with a diamine to yield the desired 9-(*N*-aminoalkyl)aminoacridine; this was then reacted with either naphthylsulfonyl chloride or benzyl isocyanate, to give final products **67** and **68**, respectively. Ureas **68** had the highest antimalarial activities ($0.0005 < IC_{50} < 0.069$ μg/mL) against *P. falciparum* 3D7, some of them being more potent than reference **CQ** (IC_{50} = 0.002 μg/mL). However, cross-resistance with **CQ** may be a feature of these compounds, as **68** was more active against *P. falciparum* **CQ**-sensitive strain 3D7 (IC_{50} = 0.0005 μg/mL) than against the **CQ**-resistant strain K1 (IC_{50} = 0.015 μg/mL). Moreover, compound **68** was found to be toxic against human KB cells.[100]

Scheme 2. Synthetic Route toward Chibale's Sulfonamide (67) and Urea Derivatives (68) of QN[100]



More conservative approaches to structural variation around **QN** have also been reported. Anderson et al. produced compounds **69**−**74** (Figure 32), which translate globally into simple modifications of the original **QN** scaffold. The compounds presented potent *in vitro* activity against *P. falciparum* 3D7 ($1.0 < IC_{50} < 4.1$ nM) and W2 ($1.0 < IC_{50} < 7.6$ nM), and two of them (**69** and **72**) actually exhibited subnanomolar activity against 3D7. Overall, all compounds performed better than **QN** (IC_{50} = 8.1 nM and IC_{50} = 32.1 nM) and **CQ** (IC_{50} = 7.0 nM and IC_{50} = 382.2 nM). Interestingly, results from this work generally suggested some flexibility in modifications at the **QN**'s side chain, as both furyl (**71** and **74**) and alkyl groups (**72** and **73**) were well tolerated. Still, the two best compounds were those whose structure most resembled that of parent **QN**, i.e., compounds **69** and **72**.[101]

Despite the promising features of the **QN** derivatives above, to the best of our knowledge, they were not taken further to clinical development. A possible reason for that may have been related with purity requirements, as the authors mention some difficulty in obtaining good purity degrees due to a persistent acridone impurity.[101]

Parallel efforts by Guetzoyan et al. were built on the knowledge that optimal antimalarial activity was obtained when (a) the 9-aminoacridine core is 6-chloro-2-methoxy-substituted (as in **QN**) and (b) at least two protonable groups, one in the acridine core and the other in the aliphatic side chain, are present. Hence, these authors prepared **QN** analogues where arginine-*N*-terminated oligopeptides were coupled to the primary amine of the aliphatic side chain (Figure 33). These peptidyl derivatives were evaluated *in vitro* against *P. falciparum* 3D7 and found to display only modest micromolar-range activities (IC_{50} = 0.13−42 μM).[102] In view of this, the same authors removed the oligopeptide motif to produce compounds **75** (Figure 33), but the best compound (**75a**) was still less active (IC_{50} = 0.042 μM; SI = 50) than **CQ** (IC_{50} = 0.018 μM) against *P. falciparum* 3D7.[103]

Modification of the **QN**'s aliphatic chain by terminal insertion of heterocyclic motifs has also been approached. Sparatore et al. produced **QN** analogues (**76**, Figure 34) through introduction of a quinolizidinylalkyl moiety in the amino group of the 9-amino-6-chloro-2-methoxyacridine core, aiming at improving the drug's bioavailability and pharmacokinetics; these analogues displayed *in vitro* activities against **CQ**-resistant (W2) and **CQ**-sensitive (D10) *P. falciparum* in the midnanomolar range (IC_{50} = 68−97 and 31−43 nM, respectively), but they were found to be as toxic as **QN** and, consequently, more toxic than reference drug **CQ**.[104] Following a similar line of work, Yu et al. have recently introduced piperazinyl, pyrrolidinyl, imidazolyl, or morpholinyl rings in the side chain of **QN** (Figure 34), as these motifs have been associated with increased bioavailability, metabolic stability, and tolerability in humans. The best compounds were the morpholinyl derivatives (**77**, Figure 34), which displayed (i) moderate capacity to inhibit hemozoin formation, (ii) activity against both **CQ**-resistant (IC_{50} = 30 nM; SI = 3.7−7.2) and **CQ**-sensitive (IC_{50} = 9−10 nM; SI = 12.2−21.5) *P. falciparum* strains similar to that of parent **QN**, (iii) moderate cytotoxicity, and (iv) potent activity on topoisomerase VI-mediated DNA relaxation.[105] This last aspect may be relevant to confer

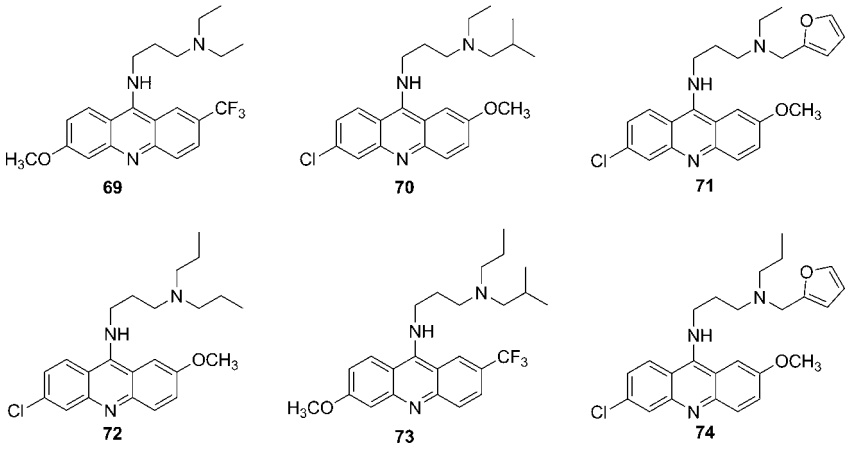


Figure 32. QN analogues (**69**–**74**) developed by Anderson et al.[101]

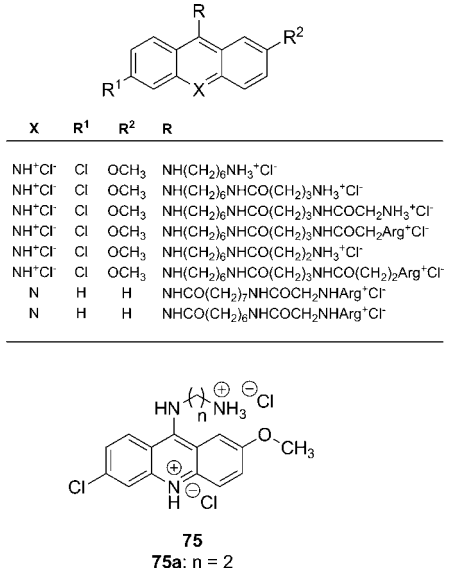


Figure 33. Compounds developed by Guetzoyan et al.[103]

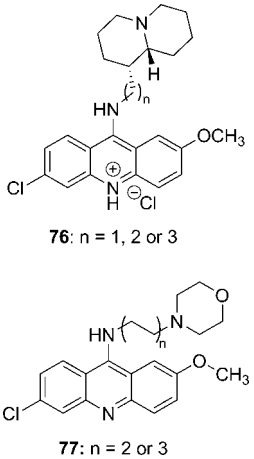


Figure 34. Heterocyclic derivatives of the **QN**'s 9-amino-7-chloro-2-methoxyacridine core of **QN** developed by Sparatore et al. (**76**) and Yu et al. (**77**).[104−106]

selectivity to antimalarial compounds such as **70**, as topoisomerase VI is a unique type of the topoisomerase II family that is almost absent in eukaryotes.[106] According to the authors, most potent compounds would step forward to *in vivo* evaluation on model malaria infection, using *P. berghei* infected mice. To the best of our knowledge, data from such *in vivo* assays has not been published yet.

4.2.2. 9-Anilinoacridine Leads. Structural variations around the 9-anilinoacridine core of **PYR** have also been analyzed over the past few years, based on research conducted in the 1990s, such as that from Figgitt et al. These authors had screened antitumoral anilinoacridine analogues of **PYR** against malaria, observing submicromolar activity (IC_{50} = 0.1 μM) against *P. falciparum* for structures presenting amine groups at positions 6 and 1′ of the acridine and aniline rings, respectively (**78**, Figure 35).[107] The same authors later introduced an additional amino group in position 3 of the acridine ring to yield **78a**, with a concomitant four times increase in the antimalarial activity (IC_{50} = 0.025 μM).[108] Motivated by these findings, Auparakkitanon et al. have studied the effect of different substitutions at both the aniline and acridine moieties of **PYR** (Figure 35) on their ability to block hemozoin formation and inhibit type II topoisomerases in *P. falciparum*; results showed that structures with a 3,6-diamino-substituted acridine core (**79**, Figure 35) were superior to their 3,6-dichloro counterparts *in vitro* against *Plasmodia*, with the best of which (**79a**) displaying nanomolar activity (IC_{50} = 34 nM) against *P. falciparum* K1.[109] The compounds were able to inhibit both

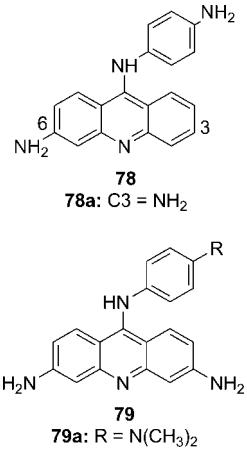


Figure 35. Anilinoacridine derivatives developed by Figgit et al. (**78**) and Auparakkitanon et al. (**79**).[107−110]

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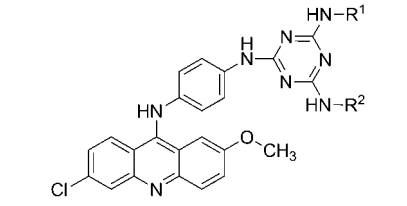
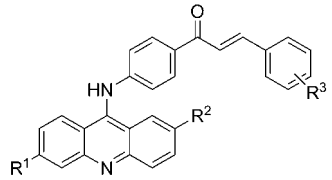
topoisomerase II activity and hemozoin formation, but **79a** was later found not to efficiently penetrate the parasite's FV, thus presenting weak interaction with intracellular hematin.[110]

Addition of a third cyclic motif to 9-anilinoacridines has also been approached. Following earlier work where use of triazine moieties was found beneficial for antimalarial activity,[111] Kumar and co-workers synthesized 9-anilinoacridine triazine derivatives (**80a–c**, Figure 36) that were shown to be almost twice as



80a: R^1 = Aniline; R^2 = *N*,*N*-dimethylpropylenediamine
80b: R^1 = Aniline; R^2 = Hydrazine
80c: R^1 = Aniline; R^2 = 4-(2-aminoethyl)morpholine

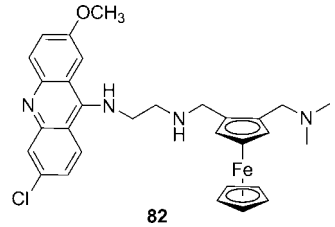


81a: R^1, R^2 = H; R^3 = 4-OCH_3
81b: R^1 = Cl; R^2 = OMe; R^3 = 4-OCH_3

Figure 36. PYR derivatives obtained by insertion of a third cyclic building block, as proposed by Kumar et al. (**80a–c**), Tomar et al. (**81a**) and Prajapati et al. (**81b**).[112−114]

potent *in vitro* (IC_{50} = 4.21–6.97 nM; SI = 295.02–2896.02) as **CQ** (IC_{50} = 8.15 nM; SI = 8983) against *P. falciparum* 3D7 strain.[111,112] The best compounds were tested *in vivo* against **CQ**-resistant *P. yoelii* N-67 infection in mice, but despite being orally active and leading to a suppression >95% at day 4 post-treatment (daily dose of 100 mg/kg), the compounds were unable to provide protection in a 28 day survival assay.[112] Later, Tomar et al. inserted a *p*-cinnamoyl substituent in the 9-anilino moiety of **PYR**, thus producing 9-aminochalcone-acridines such as **81a** (Figure 36). These compounds potently inhibited parasite maturation at concentrations below 10 μg/mL, and groups at the 4′ position of the cinnamoyl ring were found as crucial to such antimalarial activity: the *p*-methoxy-substituted structure **81a** (Figure 36) was the best, leading to 71.4% inhibition at 2 μg/mL.[113] Prajapati et al. proposed similar chalcone derivatives such as **81b**, which simply resulted from replacement of the acridine core in **81a** by its 6-chloro-2-methoxy-substituted congener (Figure 36). Antimalarial activities *in vitro* ranged from IC_{50} = 0.30 to 4.8 μM against *P. falciparum* 3D7 (SI = 13.5–333) and from IC_{50} = 0.15 to 4.5 μM against *P. falciparum* Dd2 (SI = 10–520), still below those of reference **CQ** against both strains (IC_{50} = 0.04 μM and 0.17 μM, respectively). Interestingly, the best compound also displayed a *p*-methoxy-substituted cinnamoyl ring.[114]

4.2.3. Organometallic Leads. The success of the antimalarial drug candidate ferroquine (see section 5.1) prompted the search for organometallic leads derived from other classical antimalarials, such as **QN**.[115] In this connection, Blackie et al. produced a ferrocene derivative of **QN** (**82**, Figure 37) that presented the remarkable IC_{50} values of 1 nM and 8 nM against 3D7 and K1 *P. falciparum* strains, respectively.

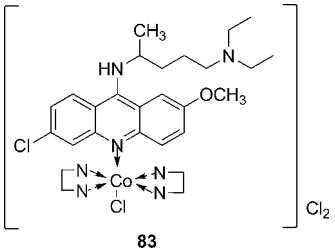


82

Figure 37. Organometallic surrogate of **QN** (**82**) developed by Blackie et al.[116]

These activities were higher than those exhibited by ferroquine itself *in vitro*, a fact that the authors considered as possibly due to simultaneous exertion of different antiplasmodial MOA by compound **82**. Unfortunately, further studies have suggested that undesired toxicity effects might be associated with the ferrocenyl moiety.[116]

A cobalt-based organometallic derivative of **QN** (**83**, Figure 38) has also been developed by Ajibade et al., who found it to

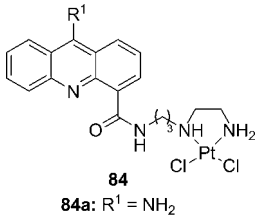


83

Figure 38. Cobalt **QN** derivative (**83**) developed by Ajibade and co-workers.[117]

be more potent (IC_{50} = 0.02 μM) than the same complex with **CQ** (IC_{50} = 4.41 μM) against *P. falciparum* K1.[117] Additionally, **83** was also found to be the least cytotoxic (CC_{50} = 7.26 μM) from a total of five cobalt complexes synthesized by Ajibade and co-workers.

Most recent organometallic derivatives of **QN** have been inspired in antitumoral cisplatins. The fact that 9-amino-acridines such as **QN** interact with DNA and present interesting antiproliferative activities underlies their relevance against malaria as well, since maturation of intraerythrocytic parasites equally relies on fast DNA replication.[118] In view of this, Murray et al. have hypothesized that conjunction of the acridine core with a cisplatin moiety, as in **84a,b** (Figure 39), would probably yield valuable antimalarial hits; such compounds were actually active *in vitro* as antimalarials (IC_{50} = 0.18–0.34 μM) but did not reach nanomolar activities against the *P. falciparum* FCQ-27 strain.[119]



84
84a: R^1 = NH_2
84b: R^1 = H

Figure 39. Cisplatin QN derivatives (**84**) developed by Murray et al.[119]

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4.2.4. Quinacrine Hybrids. Inspired by recent trends with a focus on hybrid drugs, obtained by covalently binding two or more pharmacophores in a single molecular construct, Girault et al. produced bis(9-amino-6-chloro-2-methoxyacridines) by linking two acridine cores to each other through different spacers (alkanediamines, linear polyamines, or branched polyamines); one such dual-core compound (**85**, Figure 40)

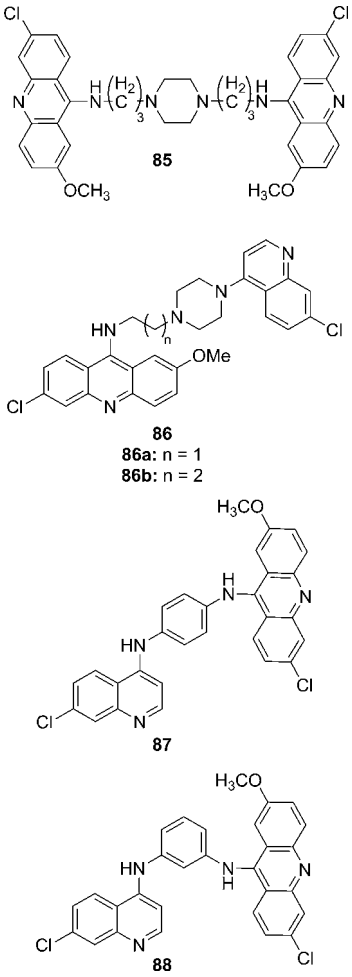

Figure 40. Dual-core bis-acridine and quinoline/acridine hybrids respectively developed by Girault et al. (**85**) and Kumar et al. (**86**–**88**).[120,121]

exhibited potent activity against *P. falciparum* strain FcB1R (IC_{50} = 17 nM) and was nontoxic to MRC-5 cells.[25,120] Based on this finding, Kumar et al. later developed a series of quinoline/acridine hybrids also using different linkers between those two cores (**86**–**88**, Figure 40); the compounds were confirmed to be active *in vitro* against *P. falciparum* strain NF54, and the authors observed that the activity was increased when replacing the *p*-phenylenediamine linker in **87** (MIC = 0.5 μg/mL) by its *m*-phenylenediamine isomer in **88** (MIC = 0.25 μg/mL).[121] Although promising, none of the compounds (0.25 < MIC < 1 μg/mL) performed better than the reference **CQ** (MIC = 0.125 μg/mL).

The relevance of artemisinin-based antimalarials in the current clinical strategies against **CQ**-resistant malaria also led to exploration of several endoperoxides, and derived hybrid constructs, as potential antimalarial leads. Based on the antimalarial activity of **CQ**/trioxolane hybrids against sensitive and resistant *P. falciparum*, reported by Coslédan et al.,[122] O'Neill and co-workers developed hybrid constructs joining the **QN** acridine core with either the artemisinin's endoperoxide core (**89**, Figure 41) or a trioxolane motif (**90**, Figure

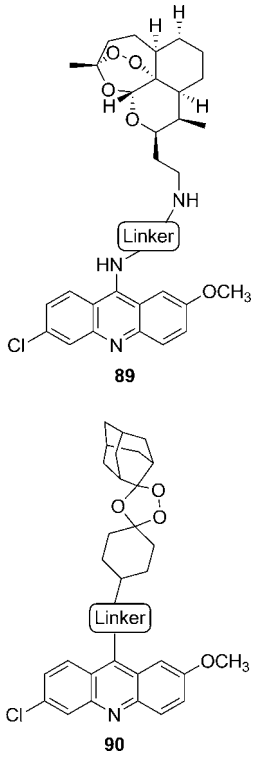

Figure 41. QN-based hybrids developed by O'Neill and co-workers (**89** and **90**).[123]

41).[122,123] The compounds were evaluated against *P. falciparum* **CQ**-sensitive (3D7; IC_{50} = 12.32–16.34 and 9.67–12.52 nM for compounds **89** and **90**, respectively) and **CQ**-resistant (K1; IC_{50} = 14.34–20.22 and 6.76–11.10 nM for compounds **89** and **90**, respectively) strains and were found to display nanomolar activities against both strains, hence presenting no cross-resistance with **CQ**.[123] Many of these hybrids were actually more active than artemisinin, and easily transformed into water-soluble salts, making them suitable for oral and intravenous administration. However, none of them was superior to artemether, suggesting that their expectedly enhanced accumulation within the parasite's FV was not happening and that they might have other targets outside the FV. Finally, the authors used a model trioxolane (Scheme 3) to demonstrate that hybrids **90** could be activated within the ferrous-rich FV to release a **QN**-cyclohexanone structure through a Fe(II)-mediated degradation pathway. Such would mean that the hybrids enclose the ability to be active *per se* and also through release of a hematin-binding antimalarial moiety.[123] Still, despite the fact that a similar quinoline/trioxane hybrid has been selected for development as drug candidate, as far as we know, none of the **QN**/trioxane hybrids has reached that far up to date.[122]

Later, based on promising findings on **CQ**/cinnamic acid conjugates as dual-stage antimalarials (see section 5.2.3), Gomes and co-workers developed new hybrids through combination of the acridine core with a cinnamoyl moiety (**91**, Figure 42). These hybrids were active against both blood- and liver-stage parasites, and were generally less toxic to human

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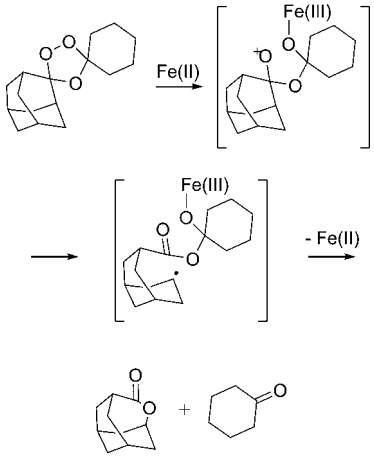
Scheme 3. Possible Mechanism for Trioxane Unit Degradation[a]



[a]Conditions: $FeBr_2$ (1 equiv); THF; rt.[123]

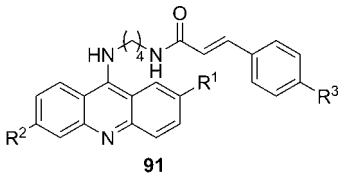


Figure 42. **QN**-based hybrids developed by Gomes and co-workers (**91**).[124]

hepatoma cells than **QN**.[124] These authors confirmed that the presence of 6-chloro and 2-methoxy substituents on the aminoacridine core increased activity against blood-stage parasites; for instance, the best hybrid derived from unsubstituted 9-aminoacridine (**91a**, Figure 42; IC_{50} = 138 nM) was over three times less active than its 6-chloro-2-methoxy-substituted congener (**91b**, Figure 42; IC_{50} = 41 nM; SI = 428) against **CQ**-resistant *P. falciparum* W2.[124] The blood-stage activity of these **QN** surrogates was globally superior to that of (i) parent **QN** against three tested *P. falciparum* strains (**CQ**-sensitive 3D7, resistant Dd2 and W2), and (ii) **CQ** against both **CQ**-resistant strains. The best compound of the series (**91c**, Figure 42) was about four times more efficient against liver forms of *P. berghei* (IC_{50} = 4.9 μM) than the reference drug for liver-stage malaria, **PQ** (IC_{50} = 7.5 μM), and devoid of toxicity to HepG2 cells (CC_{50} = 164.7 mM as compared to 7.4 μM for **QN**). Such findings were reported only very recently, so additional information on *in vivo* performance, and other preclinical assessments, is yet unavailable.[124b]

5. ANTIMALARIAL 4-AMINOQUINOLINES: KEEPING TRACK ON 21ST CENTURY CHLOROQUINE SURROGATES

5.1. Historical Synopsis of Clinically Relevant Antimalarial 4-Aminoquinolines

As mentioned before, one of the earliest synthetic antimalarials to be used in the clinics was quinacrine, which constituted, *ex-aequo* with quinine, first-line antimalarial therapy until 1940. As explained in section 2.2, working from **QN**, Andersag discovered **CQ** in 1934, originally named resochin.[125] However, initial safety studies performed in animals and humans suggested this compound to be too toxic for clinical development; as a result, Andersag concentrated his efforts on the development of a methylated derivative, sontochin (Figure 43), which seemed to present an acceptable safety

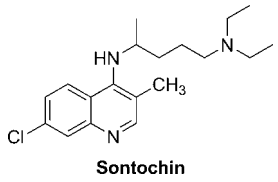


Figure 43. Structure of sontochin.

profile.[125] In the early 1940s, this compound advanced to clinical trials in Tunisia, jointly conducted by German and French researchers.[30] In 1943, French authorities handed over sontochin and accompanying data to the Americans, who sent everything back to the USA for analysis. It was only after this event that resochin was rediscovered and renamed as chloroquine in 1945, by E. K. Marshall.[30] The **CQ**'s superior antimalarial properties were soon recognized, and it was designated the antimalarial drug of choice due to its high efficacy, low cost, and tolerable adverse effects.[5b]

CQ is a weak diprotic base that accumulates in the acidic FV of the parasite and exerts its activity through binding of free heme and inhibition of hematin biocrystallization (hemozoin formation), generating an inhospitable environment for parasite survival.[126] However, abusive use of **CQ** soon led to emergence of **CQ**-resistant parasite strains. Only 15 years after introduction of **CQ** as first-line antimalarial chemotherapy, in the late 1950s, the first cases of *P. falciparum* resistance to **CQ** were reported in Cambodia and Thailand.[125] Ever since, **CQ** resistance has been spreading across the globe, and currently, **CQ**-resistant *P. falciparum* strains prevail in endemic areas, except in the Caribbean and Central America.[127] Recent studies have associated parasite's resistance to **CQ** to genetic changes in transporters, the *P. falciparum* **CQ** resistance transporter (*Pf*CRT), and the *P. falciparum* multidrug resistance protein-1 (*Pf*MDR1), which decrease the accumulation of **CQ** at its site of action, the parasite's acidic FV.[128]

Since the rebirth of resochin as **CQ**, several variations around its 4-aminoquinoline (4-AQ) nucleus and side chain were performed in order to prepare a superior antimalarial drug. This effort was substantially intensified after emergence of parasite resistance to **CQ**, in search for structural modifications that would overcome *Pf*CRT- and *Pf*MDR1-mediated resistance.[129] From these studies, several novel 4-AQ-based antimalarial candidates emerged, among which amodiaquine (**AMQ**) stood out: this phenyl-substituted analogue of **CQ** (Figure 44) was synthesized in 1948 by Burckhalter et al., and found to have an excellent activity/toxicity profile while possibly sharing with **CQ** the mechanism of antimalarial action.[129,130] **AMQ** soon entered into the clinics, but its employment was restricted due to side effects such as agranulocytosis and hepatitis associated with its prophylactic use, which ended up by leading WHO to withdraw its recommendation as monotherapy in the early 1990s.[131] As amodiaquine is effective against several **CQ**-resistant strains, this drug remains an important component of current antimalarial combination therapies, being used together with artesunate.[132] In the early 1960s, another 4-AQ-based

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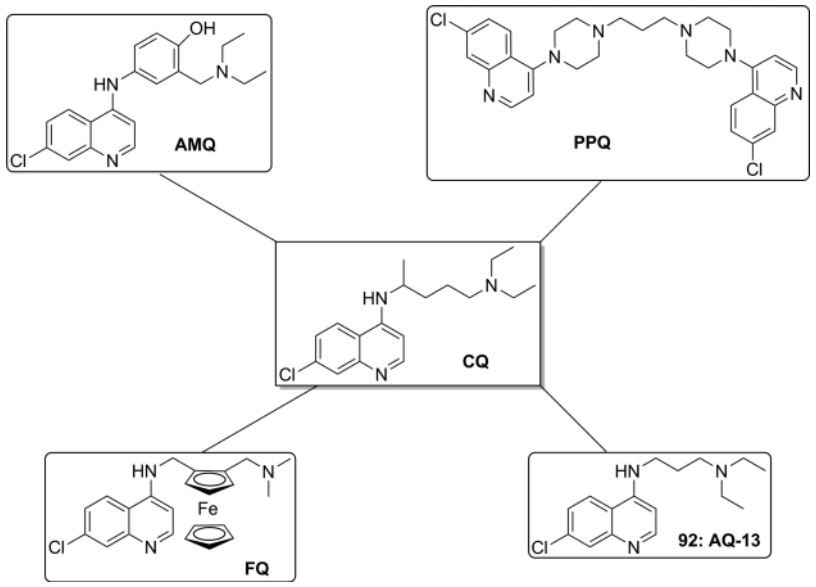


Figure 44. Major antimalarial 4-AQ candidates that emerged from **CQ**.

compound, piperaquine (**PPQ**, Figure 44), was discovered as a promising antimalarial during a drug screening campaign, and developed for clinical use in 1973.[133] Given that **PPQ** is a bis-4-aminoquinoline and was active on **CQ**-resistant strains, it was proposed that compounds with a bulky bisquinoline structure might be less efficiently effluxed by **CQ** resistance transporters, which was later confirmed.[129,133b] Although extensive use of **PPQ** also ended up by translating into considerable parasite resistance to this drug, its combination with dihydroartemisinin was recently approved by EMA.[134] By the end of the 20th century, another encouraging approach to overcome parasite resistance to **CQ** has emerged: coupling the core of **CQ** to an organometallic building block, ferrocene, led to discovery of ferroquine (**FQ**, Figure 44) as a promising antimalarial candidate.[135] The high efficacy of **FQ** as an antimalarial has been shown against both **CQ**-sensitive and -resistant *P. falciparum* strains in preclinical *in vitro* and *in vivo* studies, and currently, this compound is undergoing clinical evaluation as monotherapy and in combination with artesunate.[136] Other encouraging late 20th century development on **CQ**-based antimalarials should be highlighted: after varying the diaminoalkyl side chain at position 4 of the **CQ**'s quinoline core, Krogstad and co-workers demonstrated that compounds with side chains longer than seven and shorter than four carbons were active against **CQ**-sensitive, -resistant, and multidrug resistant *P. falciparum*, with IC_{50} values ranging from 40 to 60 nM against the resistant K1 strain.[137] One such compound, AQ-13 (**92**, Figure 44), has already undergone phase I clinical trials, showing minimal difference in toxicity compared to **CQ**. Though dose adjustment is required, as AQ-13 exhibited increased clearance as compared to **CQ**, efficacy studies are being conducted in Mali.[7,138]

5.2. Current Chloroquine-Inspired Antimalarial Leads

Regardless of the large amount of work performed in the last quarter of the 20th century to improve **CQ** antimalarial efficacy, and the promising results thereof, no new 4-AQ antimalarial has entered the clinics since **PPQ** was brought to the clinical setting. Moreover, none of the current first-line antimalarial therapies in areas where **CQ**-resistant strains prevail was proven to supersede **CQ**'s advantageous safety and cost. Therefore, many antimalarial drug researchers worldwide keep focusing on the development of new **CQ** surrogates, building on the 4-AQ pharmacophore. The main structural modifications carried out today consist on the generation of **CQ**- and **AMQ**-based compounds, bis-4-AQ, organometallic 4-AQ, and, more recently, hybrid constructs embedding the 4-AQ motif. As some reviews on the subject prior to the 21st century can be consulted,[129,139] this section will mainly focus on novel compounds reported after 2000 as displaying potent antimalarial activity.

5.2.1. Chloroquine-Based Compounds. Many research groups have worked on the design of **CQ** derivatives, leading to well-established SAR of their parent compound.[140] For example, studies performed by Egan et al. suggested that (i) the aminoalkyl side chain is necessary for strong antiplasmodial activity; (ii) the 7-chlorine substituent is crucial for efficient inhibition of hemozoin formation; and (iii) the 4-AQ core is responsible for complexing ferriprotoporphyrin IX, Fe(III)-PPIX (Figure 45).[140c,d] Similarly, Krogstad and co-workers

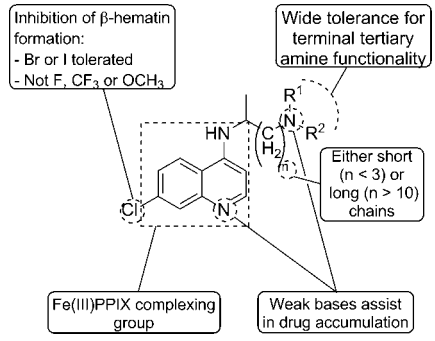
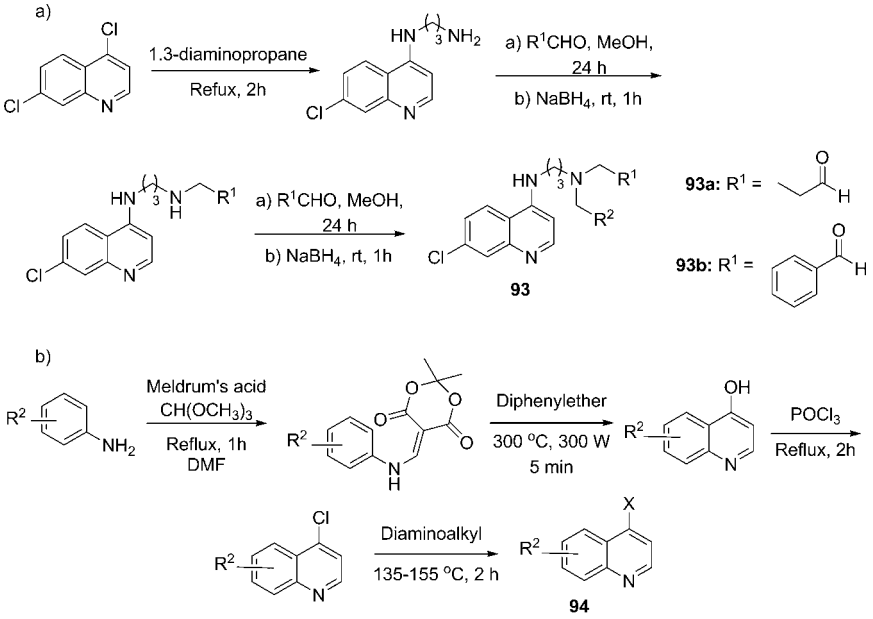


Figure 45. Main SAR established for antimalarial 4-AQ.

identified four structural features related to activity against both **CQ**-sensitive and -resistant parasites (Figure 45): (i) a protonable nitrogen at position 1 and at the end of the side chain; (ii) a 4-AQ core without alkyl substituents; (iii) a halogen atom at position 7 (Cl preferred, Br or I tolerated, but not F); and (iv) a wide tolerance for terminal tertiary amine

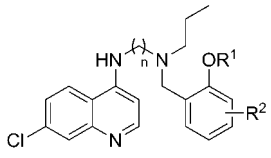
Scheme 4. Synthetic Pathway Developed by Guy *et al.* To Obtain (a) Side Chain Modified 4-Aminoquinoline 93 and (b) Quinoline Modified 4-Aminoquinoline Derivatives 94[141c,d]



functionality.[140a] They also observed that compounds with a side chain length of ≤3 or ≥10 displayed better activity against **CQ**-resistant strains.[140a]

The aforementioned promising results of AQ-13 reported by Krogstad's group motivated several researchers to pursue short side chain analogues of **CQ**.[141] In this context, Guy et al. developed a synthetic pathway to obtain a moderate library of side chain modified 4-AQ **93** (Scheme 4).[141d] SAR of these derivatives demonstrated that when one of the alkyl functionalities of the terminal amine was held constant as a propyl (such **93a** series), compounds displayed activity at least comparable to their parent compound against **CQ**-sensitive strain 3D7, and superior against **CQ**-resistant strains W2 and Dd2. However, no significant activities were observed when a benzyl group was held constant (such as in **93b** series). Guy et al. also worked on the synthesis and evaluation of 4-AQ analogues with diverse substitutions at the C-5, C-6, C-7, and C-8 positions of the quinoline ring **94**.[141c] Compounds were obtained following the multistep procedure (Scheme 4). Initially, the condensation of the appropriate aniline with Meldrum's acid and trimethyl-*ortho*-formate was carried out. Subsequently, the ene-amines were subject to microwave irradiation, and the resulting hydroxyquinolines were transformed into 4-chloroquinolines using phosphorus oxychloride in reflux. Compounds **94** were finally obtained by a nucleophilic addition of the respective side chain. The side chains used were (*N*,*N*-diethyl)-1,4-diaminopentane and (*N*,*N*-diethyl)-1,3-diaminopropane, with the latter known to restore activity against **CQ**-resistant parasites.[141c] All compounds displayed better activities against 3D7 (EC_{50} = 9−115 nM) than W2 (EC_{50} = 50−309 nM) *P. falciparum*, while compounds presenting the shorter side chain were more active, especially against the **CQ**-resistant W2 strain. Derivatives **94** with substituents located at the 6- and/or 7-position of the quinoline heterocycle performed generally better against both strains. Active substitutions tended to be small electron withdrawing groups such as 7-CF_3; 6-Me,7-Cl; 6-CF_3; and 6-OCF_3. In general, compared to their parent compound, none of the derivatives with the side chain of **CQ** presented better activities against both **CQ**-sensitive and **CQ**-resistant strains.[141c]

Further work carried out by Guy et al. demonstrated that the incorporation of an intramolecular hydrogen bond in the side chain promotes the antimalarial potency of the compounds against the **CQ**-resistant W2 strain.[141b] Particularly, compounds presenting variations of the α-aminocresol moiety (**95a−h**, Figure 46) displayed an IC_{50} below 5 nm against *P. Falciparum*



95

95a-h:

n = 3; R^1 = H; R^2 = H; 3-F; 3-OMe; 5-OMe
n =3; R^1,R^2 = -OCH_2O-
n = 4; R^1 = H; R^2 = 3-F; 3-OMe
n =4; R^1 = Me; R^2 = 5-F

Figure 46. Side chain modified 4-aminoquinoline derivatives **95** developed by Guy et al.[141b]

W2. Additionally, SAR demonstrated that compounds containing the propylalkyl and butylalkyl linkers were generally more potent than those with cyclic linkers.

Later, the same researchers synthesized a set of 7-substitued-4-AQ using as substituents diarylethers, biaryls, and alkylaryls (correspondingly compounds **96−98** in Figure 47).[141a] A fixed propyl spacer between the distal end of the side chain and the quinoline core was chosen by Guy et al., as it was previously found to be active in **CQ**-resistant parasites. Results obtained showed good antimalarial activity against the **CQ**-sensitive 3D7 strain, with EC_{50} values ranging from 1 to 1363, 8 to 1154, and 4 to 720 nM for diaryl ether, biaryl, and alkylaryl families, respectively. However, the biaryl series was the only one displaying EC_{50} values below 50 nM against drug resistant K1

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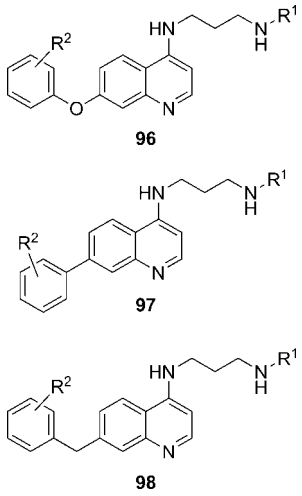

Figure 47. General structure of 7-diarylether- (**96**), 7-biaryl- (**97**), and 7-alkylaryl-4-AQ (**98**) derivatives developed by Guy et al.[141a]

parasites while presenting SI values ranging from 22.7 to 261.9 (SI = EC_{50} HepG2/EC_{50} K1).[141a]

Most of the compounds developed by Guy et al. were further used in *in silico*, *in vitro*, and *in vivo* absorption−distribution−metabolism−excretion−toxicity (ADMET) profiling in order to select leads for further development into antimalarial candidates.[142] *In vitro* assays suggested that converting the **CQ** side chain to a secondary amine increased the antimalarial activity of the compounds and improved their pharmacokinetic (PK) properties. *In vivo* studies identified two lead molecules, **99** and **100** (Figure 48), already found to display potent *in vitro*

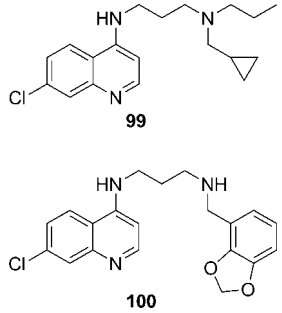

Figure 48. Structure of two lead 4-AQ derivatives (**99** and **100**) developed by Guy et al.[142]

efficacy (IC_{50} = 5.6 nM and 17.3 nM, respectively for the **CQ**-resistant W2 strain), which presented low risk for drug−drug interactions, improved ADMET properties, and PK profiles suitable to pursue these compounds in future clinical trials.[142]

Another strategy used to enhance activity against parasite resistance is the incorporation of a biologically interesting motif in the side chain of 4-AQ derivatives.[104,143] Bavari et al. identified highly potent derivatives **101** and **102** (Figure 49) against malaria by synthesizing and evaluating a series of **CQ** analogues containing a steroidal or adamantine moiety.[143d] SAR demonstrated that the use of the amide functionality to link the 4-AQ either to the steroidal or adamantine motif did not favor the antiplasmodial activity of the derivatives, while derivatives with two ionizable nitrogens presented the most potent activities. Two of the most active derivatives of series **101** (**101a,b**) displayed highly potent activity against **CQ**-resistant

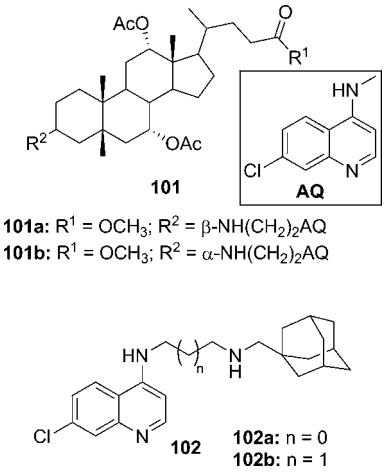

Figure 49. 4-AQ derivatives containing a steroidal (**101**) or adamantine (**102**) moiety.[143d]

W2 strain (IC_{50} = 3.38 and 5.74 nM, respectively). Although not as active, two **102** derivatives (**102a−b**) inhibited development of **CQ**-resistant *P. falciparum* W2 parasites with IC_{50} values of 8.40 and 12.10 nM, respectively.

Katti et al. have also worked on the development of **CQ** analogues with biologically relevant motifs as potential antimalarials.[141g,144] They investigated the antimalarial activity of **CQ** derivatives presenting a thiazolidin-4-one nucleus at the distal end of the side chain.[144a] Unlike what was proposed by Krogstad et al.,[140a] these results suggest that the basicity of the side chain nitrogen is not essential for the antimalarial activity of the compounds, since derivatives **103−105** (Figure 50) presented moderate to good activity (IC_{50} = 0.013−7.153 μM) against the **CQ**-sensitive NF54 strain. Two of the most active compounds, **103a** and **105a**, were tested *in vivo* (daily

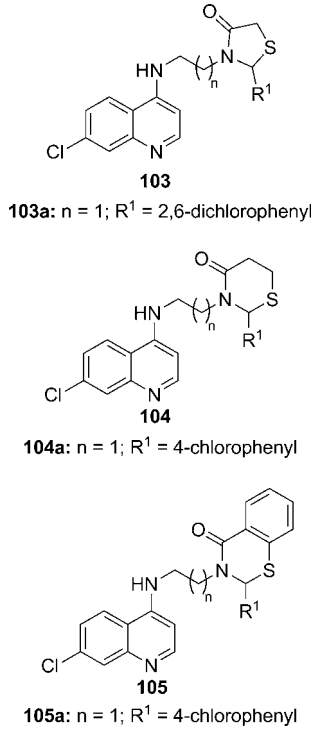

Figure 50. 4-AQ analogues (**103−105**) developed by Katti et al.[144a]

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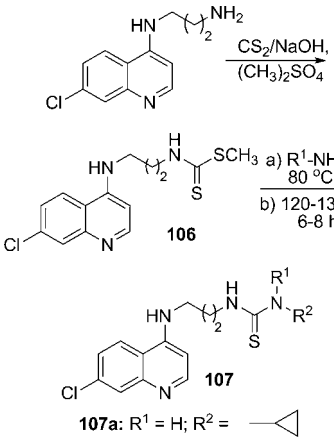
intraperitoneal dose of 30 mg/kg during 4 days) in *P. yoelli* infected mice. The compounds suppressed 76 and 81% parasitemia on day 4, respectively, and both presented a mean survival time of 15 days.[144a]

Based on these results, Katti and co-workers decided to synthesize a series of thiourea derivatives **107**.[141g] Initially, the desired [3-(7-chloroquinolin-4-ylamino)propyl]dithiocarbamic acid methyl ester (**106**) was obtained through a one pot reaction of *N*-(7-chloroquinolin-4-yl)propane-1,3-diamine and carbon disulfide followed by the respective methylation using dimethyl sulfate (Scheme 5). Finally, the resulted product was

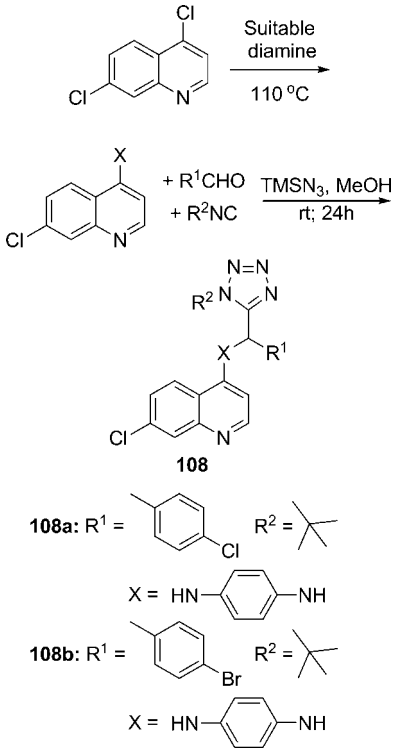
Scheme 5. Synthetic Pathway To Obtain 4-AQ Derivatives 107 Developed by Katti *et al.*[141g]



subjected to a nucleophilic substitution reaction with the appropriate amine (Scheme 5). All derivatives inhibited *β*-hematin formation. The most active compound **107a** displayed superior activity than **CQ** with an IC_{50} of 23.9 and 14.1 nM against the *P. falciparum* **CQ**-sensitive (D6) and **CQ**-resistant strain (Dd2), respectively. These results were in accordance with the previous findings of the group that the basicity of the nitrogen of the distal end of the side chain of **CQ** derivatives is not essential for antimalarial activity.[141g]

Following the same line of thought, Chauhan et al. recently reported a series of tetrazole derivatives of **CQ**.[143c] Compounds were synthesized in two reaction steps (Scheme 6). Initially, the appropriate amine was obtained through the nucleophilic reaction of the corresponding diaminoalkane, piperazine, or *p*-phenylenediamine with 4,7-dichloroquinoline. The amine obtained was then subjected to a $TMSN_3$−Ugi multicomponent reaction. SAR on these compounds demonstrated that their activity was greatly influenced by the linker, as substitution of the flexible aliphatic linker by an aromatic ring led to significant increase in the activity of the derivatives. Two of the most active compounds (**108a** and **108b**) displayed promising *in vitro* activities against the **CQ**-sensitive 3D7 strain (IC_{50} = 10.66 and 11.78 nM, respectively) and the resistant K1 strain (IC_{50} = 142.9 and 233.7 nM, respectively), performing better than the parent drug and presenting high SI values (4616.44 and 2332.43, respectively). Furthermore, after the oral administration of a daily dose (100 mg/kg) during 4 days to *P. yoelli* infected mice, these two compounds exhibited 99.99% parasite suppression on day 4, and 60% of survival on day 28. Preliminary *in vivo* PK studies performed by the same group suggested that compound **108b** could be a better candidate drug than **108a**.[143c]

Scheme 6. Synthetic Pathway To Obtain Tetrazole Derivatives of CQ (108) Developed by Chahan *et al.*[143c]



Several efforts have been made to obtain **CQ** derivatives capable of overcoming drug resistance and to identify structural features to take into account in the design and optimization of potent antimalarial agents.[145] Gemma et al. synthesized a series of hydrazone derivatives **109** (Figure 51) and tested their antimalarial properties.[145a,146] The results showed that **CQ** derivatives presenting a methoxy in the 8-position (**109a**−**b**) or a methylenedioxy moiety in the 6,7-position (**109c**) of the

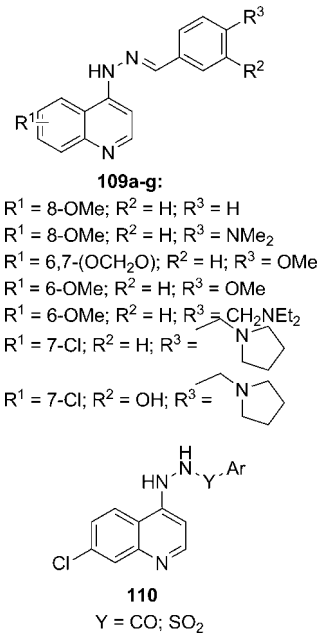


Figure 51. 4-AQ/hydrazone derivatives **109** and their analogues **110** developed by Gemma et al.[146]

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quinoline ring displayed lower activity (IC_{50} values ranging from 163 to 356 nM against *P. falciparum* W2) than those compounds with a methoxyl or a chlorine group (**109d−g**) in the 6- and 7-position (IC_{50} = 58.3−128 nM against *P. falciparum* W2), respectively. SAR studies demonstrated that the introduction of electron-donating moieties such as alkoxy or alkylamino groups at the *para* position in the arylidene motif did not significantly influence the antimalarial activity,[146] while the replacement of the arylidene moiety by a heteroarylidene functionality led to a decrease in the antiplasmodial activity. Later, Gemma and co-workers showed that the hydrazine moiety is critical for antimalarial activity, as derivatives **110** (Figure 51), bearing either an acylhydrazino or sulfonylhydrazino linker, presented a complete loss of activity.[146] Two of the compounds displaying the best activity *in vitro* (**109f−g** with IC_{50} values of 16.4 and 39.6 nM against *P. falciparum* K1; IC_{50} = 260 nM for **CQ**) and moderate toxicity (ED_{50} values of 19.5 and 152 μM against KB cells) presented promising *in vivo* activity (daily intraperitoneal dose of 30 mg/kg) by suppressing 83 and 71% of parasitemia at day 4.[146] β-Hematin inhibitory assays carried out suggest the existence of an alternative MOA for the antimalarial activity of these **CQ** derivatives.[146]

Roepe et al. have also worked on the development of side chain modified **CQ** analogues to overcome parasite resistance, by synthesizing and evaluating compounds **111−113** (Figure 52) against **CQ**-sensitive and **CQ**-resistant parasites.[141f,147] A

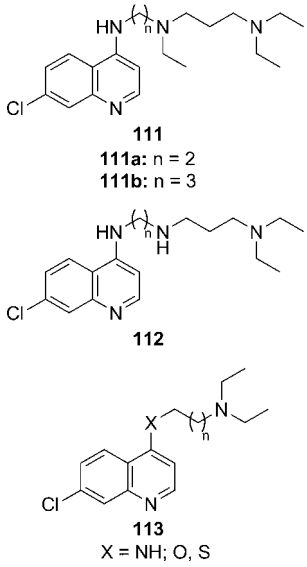

Figure 52. 4-AQ derivatives **111−113** developed by Roepe et al.[141f,147]

better performance of compounds **111** over compounds **112** against the *P. falciparum* Dd2 parasites suggested that the presence of a tertiary central amino group in series **111** is essential for the activity against the **CQ**-resistant strain. In particular, compounds **111a−b** are potent antiplasmodials and equally effective against both **CQ**-sensitive (HB3; IC_{50} = 27.3 and 21.2 nM, respectively) and **CQ**-resistant (Dd2; IC_{50} = 31.2 and 28.1 nM, respectively) strains. Further studies performed by the same group showed that, although the substitution of the amino group in the 4-position of the quinoline ring by alkylthio or alkoxy substituents (**113**, Figure 52) increases selectivity against **CQ**-resistant over **CQ**-susceptible parasites, it decreases the antiplasmodial activity of the compounds.[141f] According to pK_a determinations, the introduction of these two moieties into the 4-position of the quinoline ring affords effective monoprotic weak bases at physiological pH. Thus, Roepe et al. hypothesized that since **CQ** analogues **113** with X = NH are diprotic bases, they probably accumulate better in the FV, which could explain the generally better inhibitory activities displayed by these analogues when compared to the **CQ** derivatives **113** with X = O or X = S (Figure 52).

Wolf et al. synthesized and evaluated different 4-amino-7-chloroquinolyl-derived amides, sulfonamides, ureas, and thioureas **114−118** (Figure 53) against **CQ**-sensitive (HB3) and

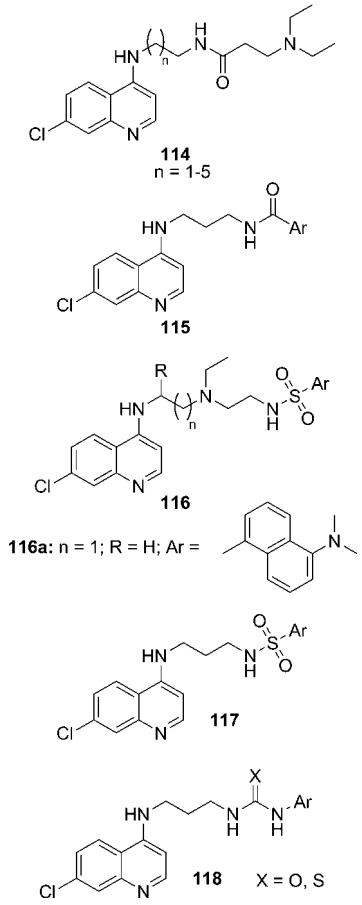

Figure 53. General structure of **CQ**-based amides (**114−115**), sulfonamides (**116−117**), ureas and thioureas (**118**) developed by Wolf and co-workers.[148]

CQ-resistant (Dd2) strains.[148] Most of the derivatives presented activity in the submicromolar range and low resistance indices [IC_{50}(HB3)/IC_{50}(Dd2)]. Although none of the derivatives was as active as their parent compound, the sulfonamide analogue **116a** displayed improved activity against Dd2 strain parasites (IC_{50} = 23 nM).

Recent studies keep demonstrating that modification of **CQ** side chain continues to be a validated strategy to obtain potent antimalarials.[149] For example, Sparatore and co-workers obtained potent antimalarial **CQ** derivatives by incorporating a heteroaromatic group in the side chain (**119−120** in Figure 54).[149b] Most synthesized compounds **119−120** displayed activity in the nanomolar range against **CQ**-susceptible D10 (IC_{50} = 5.5−2656.6 nM) and **CQ**-resistant W2 (IC_{50} = 20.9−4219.9 nM) *P. falciparum* parasites. The most interesting compounds, **119a−b** and **120a**, were more active (IC_{50}= 11.8−

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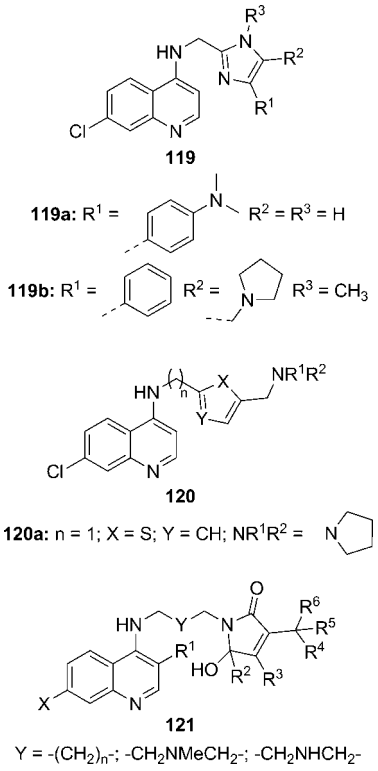

Figure 54. **CQ** derivatives containing a heteroaromatic (**119**–**120**) and γ-lactam (**121**) motif in the side chain.[149b,c]

13.4 nM against D10 strain and IC_{50}= 20.9–26.5 nM against W2 strain) than **CQ** and had moderate cytotoxicity (IC_{50} = 6.7–28.6 μM against HMEC-1 cell line). Further studies carried out by Médebielle et al. demonstrated that the introduction of a γ-lactam motif into the side chain of **CQ** resulted in potent compounds **121** (Figure 54) against **CQ**-sensitive (3D7) and **CQ**-resistant (W2) parasites, with IC_{50} values ranging from 19 to 50 nM.[149c] Results showed than none of the tested compounds **121** presented cytotoxicity when evaluated against human umbilical vein endothelial cells at a concentration up to 100 μM. SAR revealed that the length of the spacer has a significant effect on the antiplasmodial activity; generally, a propyl spacer was preferred over butyl or hexyl spacers. According to the authors, additional studies are being performed, such as the evaluation against multiple *P. falciparum* strains, *in vivo* efficacy of the derivatives, and definition of the MOA of the compounds.

After Riscoe and co-workers discovered that the early **CQ** surrogate, sontochin, retains *in vitro* activity against *P. falciparum* **CQ**-resistant strains, they synthesized derivatives **122** (Figure 55), with variations of the side chain and with alkyl or aryl substituents at C-3 of the quinoline ring to enhance activity against drug-resistant parasites.[150] Results showed that the introduction of a lipophilic aromatic group at the 3-position of the quinoline ring leads to a significant increase in *in vitro* and *in vivo* activity against multidrug-resistant *P. falciparum* strains and murine *P. yoelii* infections, respectively. Indeed, compound **122a** exhibited low nanomolar activities against **CQ**-sensitive (IC_{50} = 0.9 nM on D6 strain) and multidrug-resistant strains (IC_{50} = 1.4 nM on Dd2 strain) and *in vivo* efficacy in *P. yoelli* infected mice that were superior to **CQ**. Compound **122a** presented 30-day cures in all animals at 16 and 64 mg/kg (oral dose during 4 days) without signs of

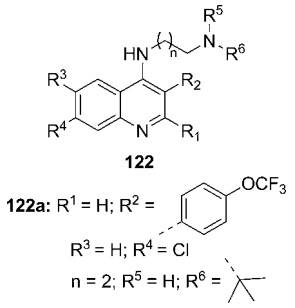

Figure 55. General structure of sontochin derivatives **122** developed by Riscoe et al.[150]

distress or toxicity. Riscoe et al. are currently investigating possible explanations to the enhanced performance of compound **122a** over **CQ** in the murine model, such as its conversion into active metabolites *in vivo* or enhanced metabolic stability and PK in mice.

5.2.2. Amodiaquine-Based Compounds. The side chain of **AMQ** contains a 4-aminophenol group that is oxidized by one or more cytochrome P450 enzymes to a quinoneimine, which underlies **AMQ**'s toxicity.[151] Consequently, several **AMQ** analogues have been studied in order to avoid quinoneimine formation.[129]

Sergheraert et al. worked on the synthesis and evaluation of the antimalarial activity of 4-anilinoquinoline analogues lacking the phenolic hydroxyl group (compounds **123**–**125** in Figure 56).[152] These analogues contained two basic side chains at the 3′- and 5′-positions which hindered the 4′-site from impeding a possible nucleophilic addition even if 4′-hydroxylation occurred *in vivo*. The compounds presented, in general, lower activities compared to **AMQ**. Also, the replacement of the terminal

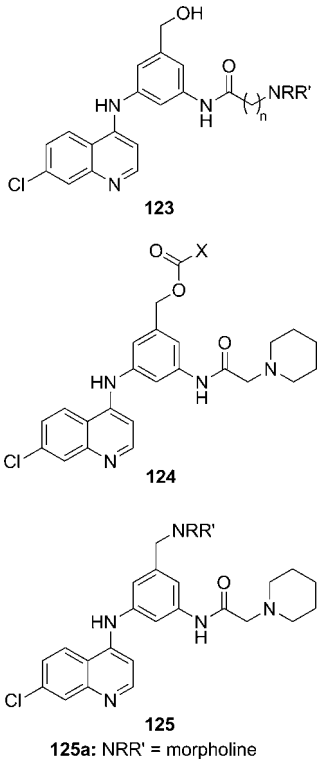

Figure 56. **AMQ** derivatives **123**–**125** developed by Sergheraert and co-workers.[152]

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methylpiperidine ring at the 3′-position of series **123** by a chlorine, a hydroxyl, or an aromatic ring led to the decrease of antimalarial activity. The most promising compound of the series (**125a**) displayed an IC_{50} value of 9.5 nM *in vitro* against the *P. falciparum* **CQ**-resistant FcB1R strain, and presented excellent performance *in vivo* on *P. berghei* infected mice (daily intraperitoneal dose of 40 mg/kg for 4 days), by suppressing 100% parasitemia at day 4 and showing decreased cytoxicity upon mouse macrophage as compared to **AMQ**. However, to the best of our knowledge, no further studies on this promising compound were reported yet.

Equally aiming at avoiding oxidation of **AMQ**-based compounds to quinoneimines, O'Neill and colleagues synthesized a series of analogues **126a–j** (Figure 57) which possess

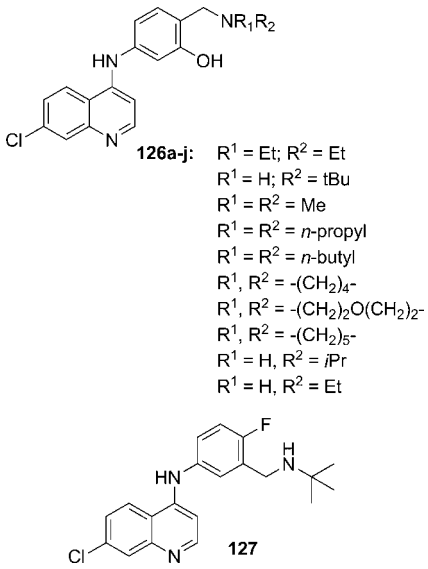
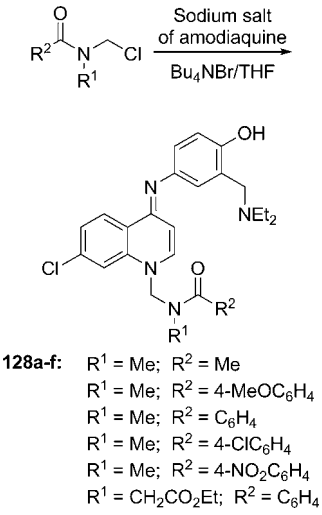


Figure 57. AMQ derivatives **126** and **127** developed by O'Neill and co-workers.[153,156]

the 3′ hydroxyl and the 4′ Mannich side-chain group of **AMQ** interchanged.[153] Several derivatives presented potent antimalarial activity (low nanomolar range) against both sensitive (HB3) and multidrug resistant (K1) *P. falciparum* strains. One of the most active compounds, named isoquine (**126a**), inhibited *in vitro* development of K1 parasites with an IC_{50} value of 17.63 nM, and displayed an excellent oral *in vivo* ED_{50} and ED_{90} activity of 1.6 and 3.7 mg/kg, respectively, on *P. yoelii* infected mice.[153] Unfortunately, the development of isoquine as an antimalarial was compromised due to the metabolic cleavage of the *N*-diethylamino group originating dealkylated metabolites.[154] Still, O'Neill and colleagues demonstrated the superior PK and pharmacodynamics (PD) profile of the most metabolically stable compound of the series **126b**, as compared to **126a**, in four animal species.[154] In spite of the excellent exposures and near quantitative oral bioavailabilities in animal models, development of compound **126b** was discontinued due to insufficient demonstration of drug safety superior to **CQ**.[155] Those authors kept on searching for a back-up compound for **126b**, through the study of a series of **AMQ** analogues where the 4′-hydroxyl functionality was replaced by either fluorine or chlorine, and demonstrated that such substitution confers metabolic stability to the compounds.[156] Accordingly, compound **127** was identified as a candidate for further clinical studies, since it presented potent activity against **CQ**-resistant parasites (IC_{50} = 22 nM against *P. falciparum* K1 strain), low toxicity in *in vitro* studies (IC_{50} = 385 μM against rat hepatocytes), moderate to excellent oral bioavailability, and acceptable safety profiles.[156] To the best of our knowledge, no further reports on this promising compound have emerged up to today.

Further works have been carried to obtain **AMQ** surrogates unable to undergo P450 oxidation to produce quinoneimine metabolites.[157] Moreira et al. synthesized a series of *N*-Mannich base derivatives **128** (Scheme 7) which displayed activity

Scheme 7. Synthetic Procedure Used by Moreira and Co-Workers To Obtain AMQ Analogues 128[157]



against the multidrug resistant Dd2 strain (IC_{50} = 15–31 nM) higher or comparable to the parent drug (IC_{50} = 30 nM).[157] Compounds **128a–f** were obtained by reacting the convenient tertiary *N*-chloromethylamide with the sodium salt of **AMQ** (Scheme 7). The most active compound was the *p*-nitrophenyl-substituted derivative **128e** (IC_{50} = 15 nM against Dd2 strain), which was also very stable in human plasma. Results showed that the antiplasmodial activity is not significantly influenced by the physicochemical properties of the amide functionality. Further preclinical or clinical studies on these *N*-Mannich base derivatives of amodiaquine have not been yet reported.

Additional work carried out by Lin and co-workers originated **AMQ** analogues with general formula **129** (Figure 58).[158] The results showed that the new analogues were generally better or

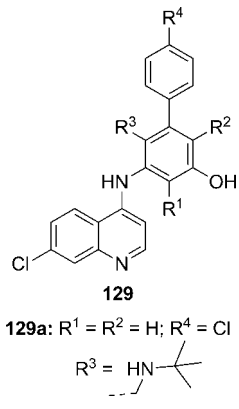


Figure 58. AMQ analogues **129** developed by Lin and co-workers.[158]

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comparable to their parent compound against **CQ**-sensitive (Dd6) and **CQ**-resistant (W2) strains, with IC_{50} values in the range of 0.3−130 ng/mL. The *in vitro* cytotoxicity of derivatives **129** was also assessed against macrophage line J774, obtaining IC_{50} values ranging from 0.75 to 11.6 μg/mL. Despite the potent *in vitro* antimalarial activity displayed by the most active derivative **129a** (IC_{50} = 0.3 and 0.4 ng/mL against Dd6 and W2 strains, respectively), the compound did not show significant *in vivo* activity against *P. berghei* at doses up to 192 mg/kg, which could be explained by its poor solubility in polar organic solvents and water.

Melnyk et al. synthesized and evaluated carbamate, amide, ester, and amine analogues of **AMQ** (**130**−**133**, Figure 59).[159]

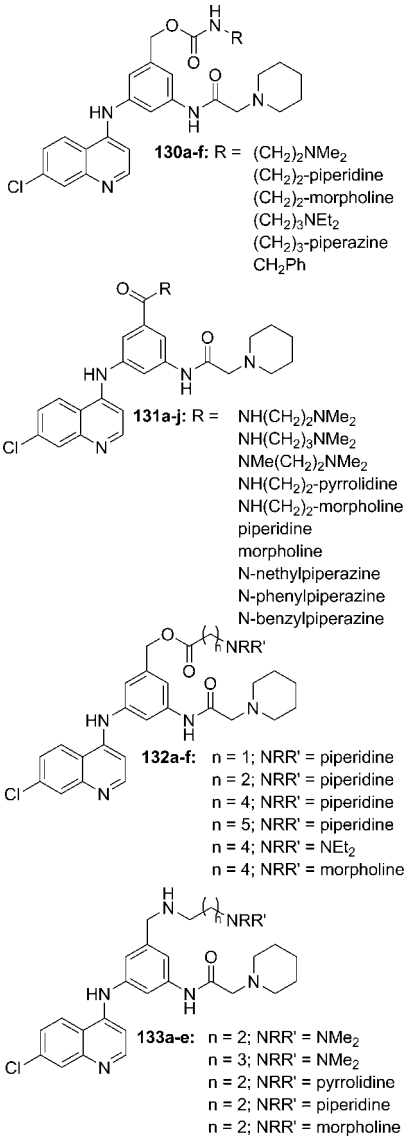

Figure 59. **AMQ**-based carbamate (**130**), amide (**131**), ester (**132**) and amine (**133**) derivatives developed by Melnyk et al.[159]

All the derivatives presented comparable or superior activity compared to the parent drug. Accordingly, in the carbamate series **130**, the authors devised that (i) the presence of a terminal tertiary amine improves the antimalarial activity of the compounds, (ii) cyclic or acyclic terminal amines present the same range of activity, and (iii) the presence of an additional electron-withdrawing atom in the side chain decreases activity; similar SAR were observed in the amide series **131**. In addition, Melnyk et al. found that tertiary amines are preferred over secondary amines. Contrary to results found in the carbamate series, more than two methylene groups clearly decrease activity in the amide derivatives. In the ester series, cyclic amine **132** seems to be preferable, but such was not observed in the amine series **133**. Compound **131h** was the most active overall, as it was highly potent against the three *P. falciparum* strains tested (IC_{50} = 38.4, 19.2, and 51.2 nM against Thai, FcB1R, and K1, respectively), presented a high SI (833), and displayed reasonable *in vivo* activity.[159]

More recent works have been carried out to also circumvent the formation of toxic **AMQ** metabolites while retaining/improving their antiplasmodial activity.[160] Carvalho et al. reported a series of compounds where the **AMQ** core was conjugated either with furoxan or nitrooxy NO-donors (**134**, Figure 60) based on the idea that NO seems to play an

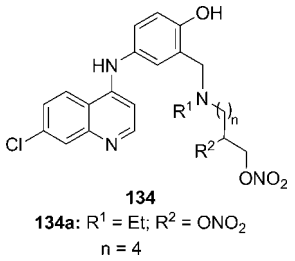

Figure 60. General structure of **AMQ** derivatives **134** developed by Carvalho et al.[160a]

important role in the pathogenesis of the malaria parasite.[160a] Most of the derivatives presented antimalarial activities comparable to that of the parent compound. The most active compound, **134a**, displayed an IC_{50} of 21 nM against **CQ**-resistant parasites (W2) and total cleared parasitemia *in vivo* against *P. berghei* ANKA after 72 h. However, results also demonstrated that NO-donor properties did not significantly contribute to the antiplasmodial activities exhibited by the compounds.

5.2.3. Piperaquine-Inspired Compounds. Bis-4-aminoquinolines are compounds that, like **PPQ**, contain two 4-AQ cores. As mentioned earlier, **PPQ**'s use declined in the 1980s after the emergence of *P. falciparum* strains resistant to this drug.[161] However, **PPQ**'s relevance in antimalarial chemotherapy was rediscovered in the following decade, as it was found suitable to be combined with artemisinin derivatives.[161] This discovery triggered the search for novel bis-4-AQ in the past decade of the 20th century. While the antimalarial activity of many such novel compounds has been reported earlier, and their role in malaria chemotherapy has been extensively reviewed,[162] only a few cases have been reported in the past decade, as next reviewed in this section.

Deady et al. synthesized a series of bis-4-AQ that contained a $(CH_2)_n$ linker in the 2-position of the 4-AQ ring joining the two heteroaromatic rings (**135**, Figure 61).[163] The results showed that the linker influenced antimalarial activity, which increased with the increase of spacer length. The most active compound **135c** displayed IC_{50} values of 43 and 17 nM against **CQ**-sensitive (D10) and **CQ**-resistant (K1) strains. As compared to **CQ**, the activity of **135c** was identical, or 12-fold higher, against *P. falciparum* D10 or K1, correspondingly. Compound **135c** was demonstrated to be an efficient inhibitor

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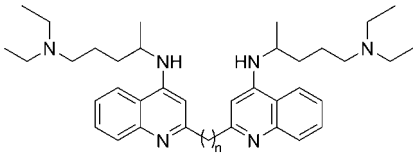
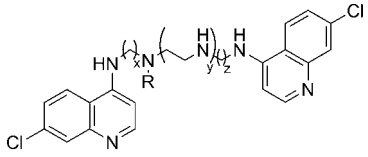


135a-c: n = 8; 10; 12

Figure 61. General structure of bis-4-AQ derivatives **135** developed by Deady and co-workers.[163]

of β-hematin formation, similar to **CQ**, suggesting this could be the mechanism responsible for its antimalarial activity.

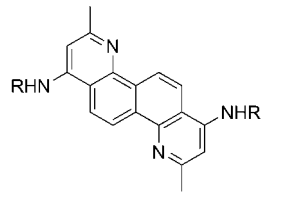
More recently, N'Da et al. reported a series of bis-4-AQ containing polyamine linkers.[164] Most compounds **136** (Figure 62) were as potent as **CQ** against a **CQ**-susceptible (D10; IC_{50} = 37.38−128.59 nM) strain, while displaying significantly superior activity against multidrug resistant (Dd2; IC_{50} = 35.49−72.88 nM) parasites. Derivatives **136** exhibited cytotoxicity upon Chinese hamster ovarian cell line, with IC_{50} values ranging from 2.61 to 12.97 μM. The authors hypothesized that the increased antimalarial activity against resistant *P. falciparum* strains might be due to the increased number of protonation sites, which in turn could induce a higher accumulation inside the parasite's acidic FV.



136a-f: R = H; x = 2; y = 0; z = 2
R = H; x = 3; y = 0; z = 2
R = H; x = 3; y = 0; z = 3
R = CH_3; x = 3; y = 1; z = 3
R = H; x = 2; y = 1; z = 2
R = H; x = 3; y = 1; z = 3

Figure 62. General structure of bis-4-AQ derivatives **136** developed by N'Da and co-workers.[164]

Bavari and colleagues recently reported a very different family of bis-4-AQ (compounds **137**, Figure 63).[165] These compounds were first discovered as inhibitors of the botulinum neurotoxin serotype A light chain. However, the structural similarities of these molecules with **CQ** and derivatives, motivated Bavari et al. to evaluate them against **CQ**-sensitive and **CQ**-resistant strains D6 and W2, respectively. Results showed that all compounds inhibited parasite growth at nanomolar concentrations. Compounds whose side chains had a primary amino group were the least effective, with antimalarial activity increasing with increased substitution on the basic side chain nitrogens.[165] Also, introduction of morpholine moieties further improved antiplasmodial activity. The most potent antimalarials, compounds **137j** and **137l−m**, displayed IC_{50} values of 6.01, 5.23, and 5.93 nM against D6 strain and 3.48, 2.00, and 6.12 nM against W2 strain, respectively, being more potent than **CQ**. The cytotoxicity of the compounds was evaluated in a rat macrophage cell line, obtaining IC_{50} values in a range of 7−10 μM. Furthermore, derivative **137j** was also effective when administered orally in a rodent malaria model infection, but additional studies are needed to fully assess the potential of these compounds as antimalarials.

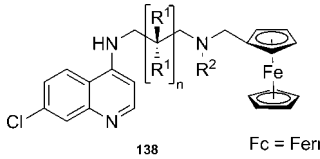


137a-m: R = $(CH_2)_4NH_2$
R = $(CH_2)_3NH_2$
R = $(CH_2)_2NH_2$
R = $(CH_2)_3NMe_2$
R = $(CH_2)_2NMe_2$
R = $(CH_2)_3NEt_2$
R = $(CH_2)_2NEt_2$
R = $(CH_2)_3N(CH_2)_4$
R = $(CH_2)_2N(CH_2)_4$
R = $(CH_2)_3N(CH_2)_5$
R = $(CH_2)_2N(CH_2)_5$
R = $(CH_2)_3N(CH_2CH_2)_2O$
R = $(CH_2)_2N(CH_2CH_2)_2O$

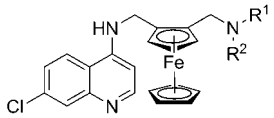
Figure 63. General structure of novel bis-4-AQ derivatives, **137**, developed by Bavari and colleagues.[165]

5.2.4. Organometallic 4-Aminoquinolines. Metal complexes have found therapeutic application against several distinct pathologies.[166] As mentioned earlier, ferroquine (**FQ**) was the first organometallic compound to enter clinical trials as a potential antimalarial drug candidate.[136] This exciting breakthrough encouraged an intense interest in the design and synthesis of other organometallic 4-AQ derivatives.[167]

Biot and co-workers synthesized compounds **138−139** (Figure 64) in order to evaluate the best position at which to place the ferrocene moiety: (i) as a substituent of the amino group at the distal end of the **CQ** side chain in series **138** or (ii) as a part of the linker between the two amino groups of the **CQ** side chain in series **139**.[168] Although most of the compounds **138** exhibited low IC_{50} (below 100 nM) against **CQ**-sensitive (HB3) and **CQ**-resistant (Dd2 and W2) strains, they presented high IC_{90} values (>500 nM), suggesting they will possibly be ineffective to strongly inhibit *P. falciparum* parasites. In series **139**, only compounds with alkyl substituents at the amino group at the distal end of the side chain presented low IC_{50} and IC_{90} (<40 nM) values against all tested parasite strains. This study demonstrated that compounds with the ferrocene moiety



138 Fc = Ferrocenyl
n = 0, 1, 2 or 4
R^1 = H or CH_3
R^2 = H, CH_3, C_2H_5 or $FcCH_2$



139
R^1 = CH_3, C_2H_5 or $FcCH_2$
R^2 = H, CH_3 or C_2H_5

Figure 64. General structure of **FQ** derivatives **138** and **139** developed by Biot and co-workers.[168]

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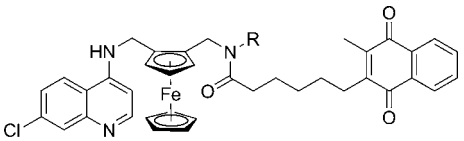
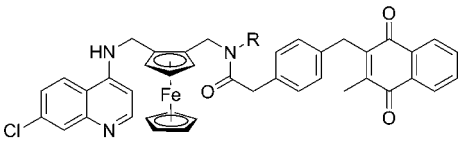
covalently flanked by a 4-AQ and an alkylamine group are more prone to display potent antimalarial activity.

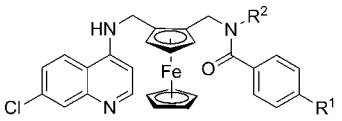
Based on the fact that glutathione reductase inhibitors were developed to reverse **CQ** resistance and to combat malaria,[169] a few years later, the same group reported a series of compounds that linked together an **FQ** analogue with a glutathione reductase inhibitor.[170] Derivatives **140**–**142** (Figure 65) were



140a-f: R = Me; Et; Pr; ^{i}Pr; Bu; ^{i}Bu



141a-f: R= Me; Et; Pr; ^{i}Pr; Bu; ^{i}Bu

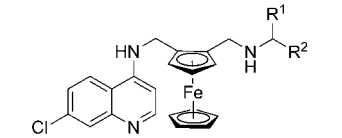


142a-l: R^1 = Br; R^2 = Me; Et; Pr; ^{i}Pr; Bu; ^{i}Bu
R^1 = OMe; R^2 = Me; Et; Pr; ^{i}Pr; Bu; ^{i}Bu

Figure 65. General structure of **FQ** derivatives linked to glutathione reductase inhibitor, **140**–**142**, developed by Biot and co-workers.[170]

evaluated against **CQ**-sensitive (NF54) and **CQ**-resistant (K1) strains, and it was found that compounds **140** were the most active *in vitro*, with IC_{50} values ranging from 26.7 to 104.2 nM. Regardless of the choice of the alkyl substituent on the amino group at the distal end of **CQ** side chain, compounds **140** were more active on both strains than their parent glutathione reductase inhibitor. However, their antiplasmodial activity was slightly lower compared to parent **FQ**. Biot et al. hypothesized that this decrease in antiplasmodial activity might be due to the fact that both the side chain and amide bond in **FQ** are cleaved in the course of oxidative metabolism in the parasite's FV.[170]

Further **FQ** analogues have been reported, such as those by Davioud-Charvet and co-workers.[171] Compounds **143** (Figure 66) displayed potent antimalarial activity, with IC_{50} and IC_{90} values in the low nanomolar range (<100 nM). In addition, these derivatives were also found to present high cytotoxicity (IC_{50} = 1–64 μM) against the human lung cell line MRC-5. The authors then observed that compounds **143** show high DNA binding properties, thus suggesting that these **FQ**

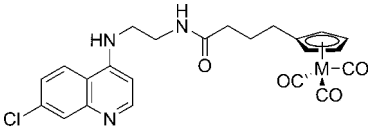


143a-b: $R^1 = R^2$ = Me
R^1 = Ph; R^2 = $(CH_2)_2$-piperidine

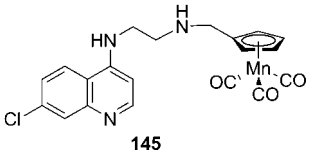
Figure 66. General structure of **FQ** derivatives **143** developed by Davioud-Charvet and co-workers.[171]

derivatives may be more appropriate for further development as antiproliferative agents.[171]

Further recent reports on 4-AQ organometallic derivatives include Nordlander's work.[172] Based on the idea that cymantrene is stable in water and air, Nordlander et al. conjugated cymantrene with **CQ** analogues (**144**–**145**, Figure 67) and evaluated their activity against malaria. In addition, to



144a-b: M = Mn; Re



145

Figure 67. General structure of **CQ** conjugates with cymantrene (**144**) and cyrhetrene (**145**) developed by Nordlander and co-workers.[172]

assess the influence of the metal center on the antiprotozoal activity, they included a cyrhetrene analogue in the study. Compound **145** displayed good activity (IC_{50} = 0.16 μM) against the **CQ**-susceptible (D10) strain but did not present any activity against the multidrug resistant Dd2 strain up to 2 μM. On the contrary, the cymantrene derivative **144a** presenting an amide linker was active against both strains with IC_{50} values of 0.27 and 0.37 μM, respectively. The *in vitro* cytotoxicity of both compounds was assessed upon human macrophage cells with IC_{50} values of 7.4 and 4.6 μM for **144a** and **145**, respectively. No significant effect in antimalarial activity was observed when replacing manganese for rhenium; still, none of the compounds presented better antiprotozoal activity than **CQ**.

5.2.5. 4-Aminoquinoline Hybrids. As mentioned before, a recently proposed strategy to combat parasite resistance is the development of hybrid compounds also known as dual-action drugs.[22,123] This, in addition to artemisinin-based combination therapies as first-line treatment in malaria chemotherapy, led Meunier's group to develop the novel hybrid compounds trioxaquines (compounds **146a**–**d**, Figure 68), obtained by covalently joining a trioxane moiety, meant to mimic the alkylating ability of artemisinin, with the 4-AQ core of **CQ**, known to easily penetrate within infected red blood cells.[173] All trioxaquines were tested against FcM29 and FcB1 **CQ**-resistant strains and the Nigerian **CQ**-sensitive *P. falciparum* strain, presenting IC_{50} values ranging from 2 to 86 nM.[173b] The authors observed that the biological results obtained for the **CQ**-sensitive strain were dependent on the length of the spacer between the 4-AQ and trioxane motifs, with a shorter chain (**146b**) being preferred over the longer ones (**146a,c**). Among the tested trioxaquines, the dicitrate **146d** (IC_{50} = 8–21 nM) and its base analogue **146b** (IC_{50} = 2–18 nM) were the most active, presenting better antimalarial activities than each of the parent compounds.

Encouraged by the high activity displayed by the hybrid **146d**, Meunier and co-workers synthesized and evaluated the antimalarial activity of a new series of trioxaquines (**146e,f and 147**, Figure 68).[174] This study aimed at understanding the

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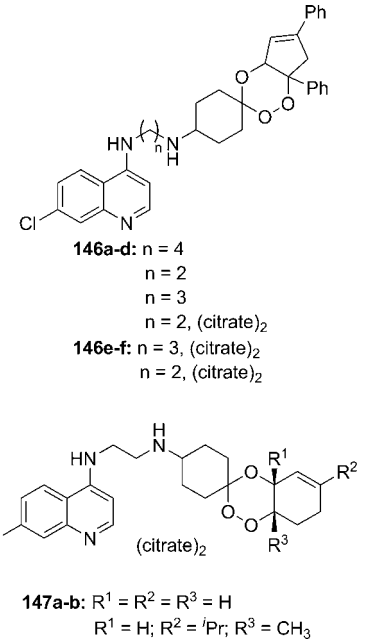

Figure 68. Trioxaquine hybrid compounds **146a–f** and **147** developed by Meunier et al.[173]

influence of different structural parameters, namely the length of the linker between the trioxane and 4-AQ motifs (**146e,f**) and the nature of the starting diene (**147**). These molecules presented antimalarial activities in the low nanomolar range (IC_{50} = 5–181 nM) against both **CQ**-sensitive and resistant strains. Results indicated that no significant influence was observed in compounds activity when varying the size of the linker, except for activities on Nigerian **CQ**-sensitive strain, as already reported for compounds **146a–d**.[173b,174] Similarly, no significant change was detected regarding the nature of the starting diene. Still, compound **147b** presented the best activities (IC_{50} = 5–19 nM), and was thus selected for *in vivo* tests, which revealed that a daily intraperitoneal dose of 20 mg/kg for 4 days led to parasitemia clearance without recrudescence.[174] It was later confirmed that compound **147b** was also active against young (IC_{50} = 69 nM) or mature gametocytes (IC_{50} = 67 nM) and presented the absence of toxicity for both human cell lines and mice.[175] However, despite its promising profile, compound **147b** was not considered for further development due to its high number of chiral centers. In view of this, a joint project between PALUMED, Sanofi-Aventis, and the French National Center for Scientific Research focused on the synthesis of simpler third-generation trioxanes (**148**, Figure 69) and their *in vitro* evaluation against **CQ**-sensitive and **CQ**-resistant *P. falciparum* strains.[122] Among the ∼120 tested compounds, the hybrid **148a** was selected for preclinical development and found to (i) present high *in vitro* antimalarial activity against several *P. falciparum* strains (IC_{50} = 7–24 nM); (ii) be curative for infected mice, by the oral route (26–32 mg/kg); (iii) be highly efficient in humanized infected mice; and (iv) present a good ADMET profile. However, it appears that further development of **148a** was halted in 2010 due to restructuration of Sanofi-Aventis.[176]

Lategan and colleagues have equally developed 4-AQ/artemisinin hybrids by joining the dihydroartemisin motif to different 4-AQ via an ether/amine bond (**149** in Figure 70),

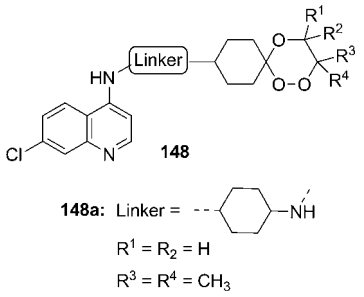

Figure 69. Trioxaquine hybrid compounds **148** developed as a joint project between PALUMED, Sanofi-Aventis and the French National Center for Scientific Research.[122]

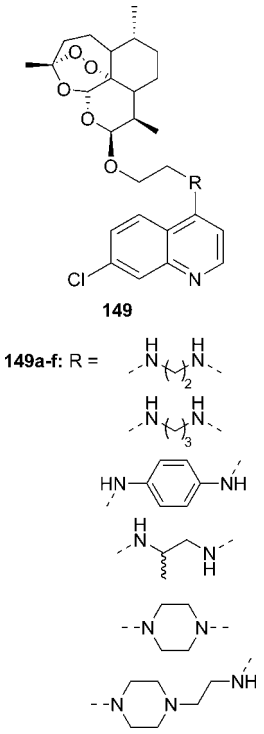

Figure 70. 4-AQ/artemisinin hybrids **149** developed by Lategan and colleagues.[177]

with the aim to increase the half-life of dihydroartemisin.[177] Most of the tested compounds presented higher or comparable potency against both **CQ**-sensitive (D10; IC_{50} = 12.18–201.38 nM) and resistant (Dd2; IC_{50} = 17.12–275.99 nM) strains than **CQ**, with hybrid **149d** displaying the best antimalarial activity (IC_{50} = 12.18 and 17.12 nM against D10 and Dd2, respectively). Cyclic linkers seemed to be detrimental to antimalarial activity, as compounds with an alkyl chain generally possessed lower IC_{50} values. The *in vitro* cytotoxicity of derivatives **149** was also assessed against the Chinese hamster ovarian cell line, presenting IC_{50} values within the range of 0.17–37.34 μM.

Somewhat similar hybrids were reported a few months later by Chibale and co-workers, who synthesized hybrids **150** (Figure 71) by coupling dihydroartemisinin and several 4-AQ moieties through an ether/amide bond.[178] All the compounds displayed excellent *in vitro* activities against **CQ**-sensitive (D10) and resistant (K1) *P. falciparum* strains with IC_{50} values ranging from 19 to 35 nM. Compound **150** was also found to share the same MOA with both artemisinin and **CQ**, as the tested

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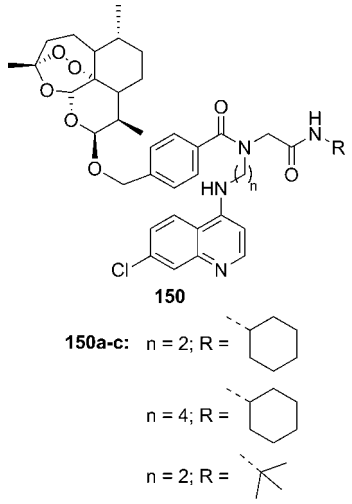

Figure 71. 4-AQ/artemisinin hybrids **150** developed by Chibale and co-workers.[178]

compounds displayed potent activity against β-hematin formation and contributed to an increase in accumulation of hemoglobin within the parasites. Nevertheless, despite the potent biological results, hybrids **150** were found to exhibit cytotoxicity against a human cervical cancer cell-line (HeLa), presenting lower SI values (8−26) compared to their parent compound **CQ** (39).

As mentioned at the beginning of section 5.1, **CQ** resistance has been associated with the membrane protein *Pf*CRT. Based on the identification of structurally diverse molecules as reversal agents (RA), known to inhibit *Pf*CRT, Peyton et al. developed a hybrid compound linking the **CQ**-like moiety to a reversal agent (**151** in Figure 72).[179] **151** presented low nanomolar

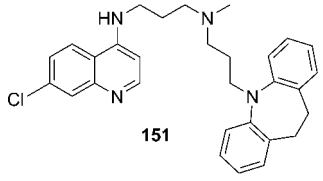

Figure 72. Structure of hybrid compound **151** developed by Peyton and co-workers.[179]

antimalarial activity against **CQ**-sensitive (D6; IC_{50} = 2.9 nM) and **CQ**-resistant (Dd2; IC_{50} = 5.3 nM) *P. falciparum* strains and suppressed more than 99% parasitemia in *P. chabaudi* infected mice via the oral route (daily dose of 64 mg/kg during 4 days).[179]

Encouraged by the excellent performance of **151**, the same team further searched to identify the structural factors required for good antimalarial activity by introducing structural modifications in **151**, as depicted in Figure 73.[180] All the compounds displayed significant activity against D6 and Dd2 *P. falciparum* strains (IC_{50} < 125 nM). The *in vitro* cytotoxicity was also assessed against mouse spleen lymphocytes, obtaining IC_{50} values ranging from 0.7 to 62 μM. Although compounds with a piperazinyl linker presented the lowest activities, the overall good results indicated that there is enough freedom to design improved **151** analogues.

In this context, Peyton and co-workers performed a more extensive SAR study with further changes to both the linker and the aromatic headgroup of the RA motif originating hybrids

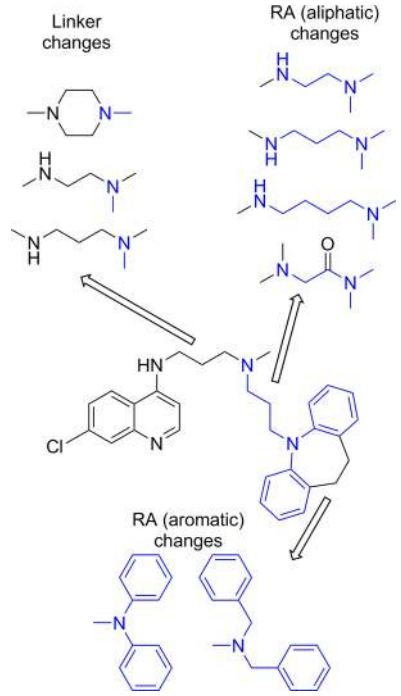

Figure 73. Structural variations introduced in structure **151**.

152 (Figure 74).[181] *In vitro* results showed that these compounds have high efficacy against **CQ**-sensitive and resistant *P. falciparum* strains (IC_{50} = 0.9−56 nM) and that their SI values (132−69400) are greater than the value for **CQ** (122). The MOA by which **152** could exert their activity was also assessed, and although not fully elucidated, the results

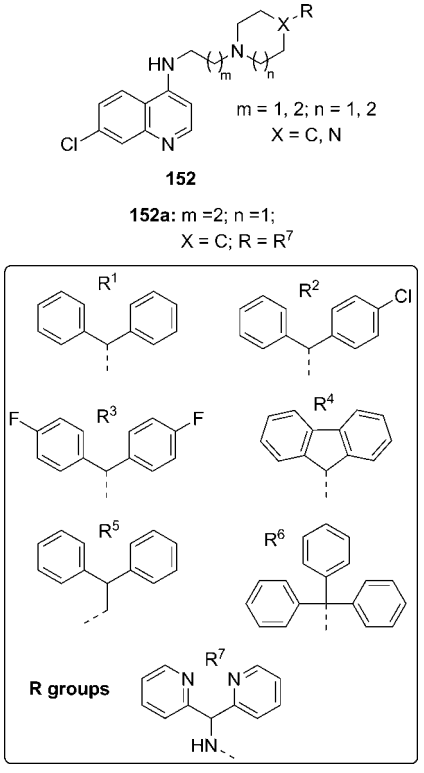

Figure 74. Structure of hybrid compounds **152** developed by Peyton and co-workers.[181]

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indicated that these compounds seem to act similarly to **CQ**. This suggested that the RA motif contributes to **152** activity by increasing their accumulation in parasites FV. Some of the compounds were tested *in vivo*, and those results pointed to hybrid **152a** as a promising lead for preclinical development, as it presented (i) low clogP value; (ii) good oral activity, and (iii) no evident signs of toxicity. As far as we know, no further results on these promising hybrids were reported so far.

Other potent hybrid antimalarials constituted the ones developed by Gemma et al., who synthesized compounds **153** (Figure 75), conjugating the 4-AQ core and a clotrimazole-

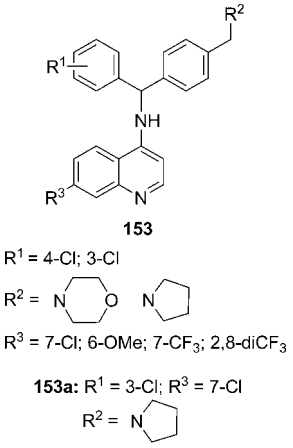

Figure 75. Structure of **CQ**/clotrimazole hybrid compounds **153** developed by Gemma and co-workers.[182]

like moiety.[182] The synthesis and evaluation of **153** were motivated by promising antiplasmodial activities previously found for clotrimazole derivatives. The latter had been synthesized based on the hypothesis that the imidazole ring would be able to coordinate with free heme and, consequently, to generate trityl radicals toxic to the parasite.[183] Hybrids **153** were found to inhibit several *P. falciparum* strains with IC_{50} values ranging from 3.9 to 1371 nM. The results indicated that the 4-AQ ring substituents play an important role on the antimalarial activity, as the compounds bearing a chlorine atom at the C7 position generally displayed better activities than their analogues. Conjugate **153a** was identified as the most active compound of the series, displaying activity in the nanomolar range (IC_{50} = 12−65 nM) against **CQ**-sensitive (3D7 and D10) and **CQ**-resistant (W2 and Dd2) parasites, moderate *in vitro* toxicity (IC_{50} = 213 μM against KB cells), *in vivo* activity (oral administration) against infections produced by *P. chabaudi* and *P. berghei* parasites, and promising pharmacokinetic properties.

Further optimization studies on the 4-AQ/clotrimazole hybrids were performed by modifying the protonable heterocycle in the benzhydryl functionality.[184] Such led to compound **154** (Figure 76), which showed (i) better activity against β-hematin formation compared to **CQ**, (ii) potent activity against **CQ**-sensitive (IC_{50} = 17−62 nM) and **CQ**-resistant (IC_{50} = 22−58 nM) parasites, and (iii) an optimal half-life in mice. Despite the good preliminary results, this compound was found to cause negative effects in mice when evaluated at higher concentrations. The Gemma group is currently working on the optimization of this novel family of hybrids.[184]

The development of novel 4-AQ-based dual-action compounds has also been addressed by functionalizing the lateral chain of **CQ** with a triazine group, the core of cycloguanil that

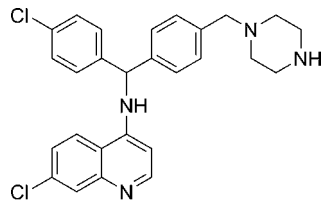

Figure 76. Structure of **CQ**/clotrimazole derivative **154** developed by Gemma et al.[184]

is a potent inhibitor of *P. falciparum* dihydrofolate reductase, an essential enzyme in the folate pathway. In 2008, Chauhan et al. reported the synthesis of hybrids **155** (Figure 77), on which

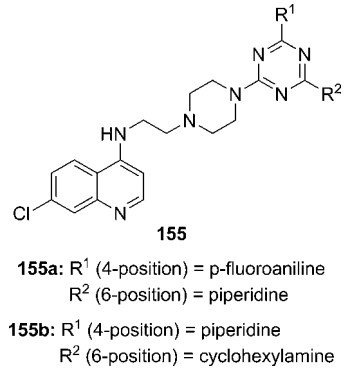

Figure 77. Structure of **CQ**/triazine hybrid compounds **155** developed by Chauhan and co-workers.[111c]

SAR studies were conducted.[111c] It was found that piperidine, cyclohexylamine, *p*-fluoroaniline, aniline, and morpholine substituents in the triazine functionality are well tolerated for the antimalarial activity of compounds **155** resulting in nanomolar activities (IC_{50} = 4.43−256 ng/mL) against the **CQ**-sensitive 3D7 strain, although none of the compounds surpassed **CQ** activity (IC_{50} = 2.6 ng/mL).[111c] The most active compounds of the series, **155a−b**, presented an IC_{50} of 7.15 and 4.43 ng/mL *in vitro*, respectively, and SI values of 328.61 and 481.48 (SI = IC_{50} VERO/IC_{50} 3D7). Furthermore, derivatives **155a−b** were also found to suppress *in vivo* (daily intraperitoneal dose of 50 mg/kg) 99.11% of parasitemia in mice infected with *P. yoelli* **CQ**-resistant strain N-67.

The same authors further developed additional **CQ**/triazine hybrids **156** (Figure 78) with antimalarial activity (IC_{50} = 5.2−164.0 ng/mL) against **CQ**-sensitive *P. falciparum* 3D7 strain

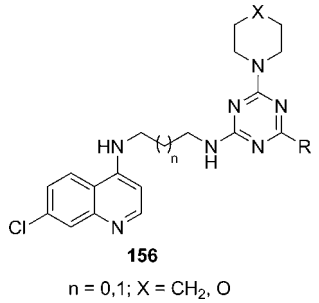

Figure 78. Structure of **CQ**/triazine hybrid compounds **156** developed by Chauhan and co-workers.[185]

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comparable to that of **CQ** (IC_{50} = 5.2 ng/mL) and presenting SI values (SI = IC_{50} VERO/IC_{50} 3D7) ranging from 65.21 to 4692.08.[185] The results demonstrated that the piperidine substitution on the triazine ring enhances the antimalarial activity of the compounds, contrary to what was observed for compounds bearing a morpholine substituent. Unlike the hybrids previously reported by the group, **156** did not show *in vivo* activity.

Rawat et al. have also worked on the development of similar triazine/4-AQ hybrids, such as compounds **157** (Figure 79),[186]

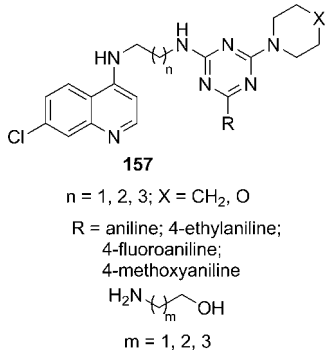

Figure 79. Structure of **CQ**/triazine hybrid compounds **157** developed by Rawat et al.[186c]

with nanomolar activities against **CQ**-susceptible D6 (IC_{50} = 0.06−0.67 μM) and **CQ**-resistant W2 (IC_{50} = 0.11−1.70 μM) *P. falciparum* strains and SI values (SI = IC_{50} VERO/IC_{50} W2) ranging from 14.7 to 227.2.[186c] SAR demonstrated that the linker between the 4-AQ ring and the 1,3,5-triazine nucleus seems to influence the antimalarial activity of the hybrids, as it was generally observed that increasing the number of methylene groups from two to four improves antimalarial activity. In addition, aromatic substitution on the 1,3,5-triazine seems to be preferred over aliphatic substitution, and the incorporation of amino alcohol substituents with a terminal hydroxyl functionality in the triazine core was also found to enhance antimalarial activity.

Based on the report that astemizole is a potent inhibitor of *P. falciparum* parasites both *in vitro* and *in vivo*,[187] Kaiser and colleagues engaged in the synthesis of **CQ**/astemizole hybrids **158**−**161** (Figure 80).[188] With the exception of hybrid **158** bearing the conformationally constrained piperazine linker, all the compounds were 3- to 10-fold more active than **CQ** and with SI values (SI = IC_{50} rat L6 myoblasts/IC_{50} K1) higher than 100. The best activity was obtained for **159** with an IC_{50} of 23 nM against **CQ**-resistant *P. falciparum* K1 strain. However, only hybrids **160**−**161** (IC_{50} = 64 and 37 nM, respectively) presented *in vivo* activity against *P. berghei* infected mice, with **160** reducing parasitemia comparable to **CQ** after the administration of daily doses of 50 mg/kg.

Other 4-AQ-based dual-action compounds have been obtained by joining the **CQ** core with another antimalarial moiety such as aminopyrimidine, such as those reported by Singh and co-workers (**162**, Figure 81).[189] Most of the compounds displayed antimalarial activity in the nanomolar range against the **CQ**-resistant K1 *P. falciparum* strain, and SI values (CC_{50} VERO/IC_{50} K1) ranging from 0.48 to 638. The authors noticed that compounds with linear alkyl linkers presented enhanced activity with the increase in length of the linker up to 4 carbons, while longer spacers resulted in a

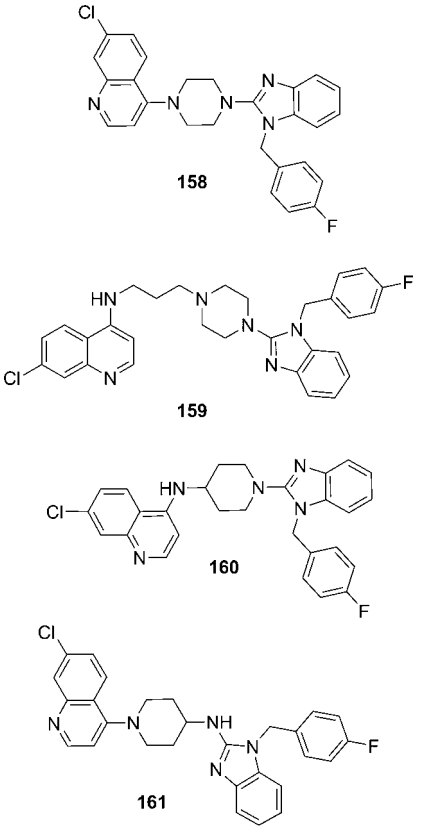

Figure 80. Structure of **CQ**/astemizole hybrid compounds **158**−**161** developed by Kaiser and co-workers.[188]

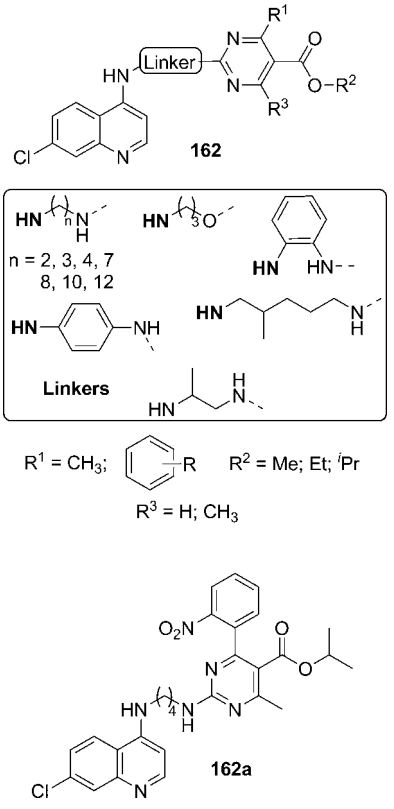

Figure 81. Structure of hybrid compounds **162** developed by Singh and co-workers.[189]

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significant reduction of antimalarial activity. It was also observed that the substitution of the methyl or ethyl ester by the *iso*-propyl ester resulted in an increase of the activity against the **CQ**-resistant strain, while the reverse trend was obtained for the **CQ**-sensitive strain.[189b] Moreover, the replacement of the diaminoalkyl side chain by a less basic alkoxy linker resulted in lower antimalarial activity.[189a] The lead compound of this series was **162a**, as it showed the lowest IC_{50} values (18.2 and 3.6 2 nM) against **CQ**-sensitive 3D7 and resistant K1 strains, respectively, and the highest SI (638). The authors did also conduct MOA studies, finding that lead compound **162a** could act on multiple targets, such as binding to heme, *P. falciparum* dihydrofolate reductase, and parasite DNA.[189b]

Similarly, N'Da et al. have recently reported the development of pyrimidine/4-AQ conjugates, **163**−**164** (Figure 82), varying

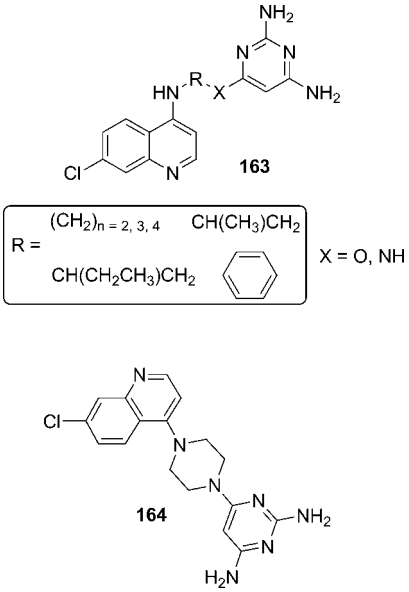

Figure 82. Structure of hybrid compounds **163**−**164** developed by N'Da et al.[190]

the linker joining the two pharmacophores.[190] The most active hybrid **164** displayed IC_{50} values of 0.070 and 0.157 μM against **CQ**-sensitive (D10) and **CQ**-resistant (Dd2) strains, comparable to those of the parent compounds alone and the equimolar combination of pyrimethamine with **CQ**. Additionally, derivative **164** exhibited a high SI (SI = IC_{50} CHO/IC_{50} K1) value of 2073.54. The authors found **164** worthy of being further investigated in order to assess if the antimalarial activity is retained in *in vivo* tests; however, no additional results were reported so far.

Subsequent approaches to dual-action antimalarials bearing the **CQ** core included its conjugation with moieties containing an electrophilic warhead prone to alkylate the cysteine residue of falcipain, an enzyme that participates in the hemoglobin degradation process essential for the parasite survival.[191] For instance, Chibale et al. have coupled the core of **CQ** to the isatin's scaffold (**165**−**166**, Figure 83), in which the ketone and thiosemicarbazone moieties could serve as electrophilic warheads; hybrids **165**−**166** were found to display IC_{50} values for *P. falciparum* growth *in vitro* ranging from 0.051 to 1.51 μM.[192] According to the results, the most active compounds were those with an ethylene linker, and generally, thiosemicarbazones were superior to their ketone analogues. However, no

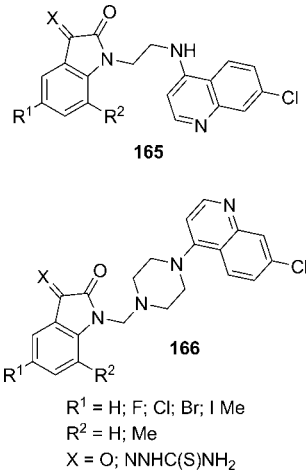

Figure 83. Structure of hybrid compounds **165**−**166** developed by Chibale et al.[192]

strong correlation between the compound activities against the parasites and falcipain-2 inhibition was observed, as hybrids **165**−**166** only exhibited modest IC_{50} values (>6 μM) against the cysteine protease. Therefore, these compounds probably exert their antimalarial activity through a different MOA.

The same authors later reported the synthesis of **CQ**-based hybrids **167**−**168** (Figure 84) by replacing the ketone and thiosemicarbazone motifs by another electrophilic warhead, the chalcone moiety.[193] The most promising compound **167a** displayed IC_{50} values ranging from 40 to 90 nM against the

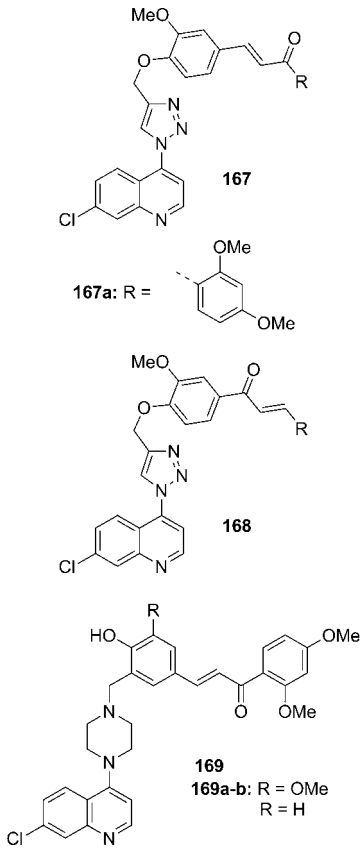

Figure 84. Structure of hybrid compounds **167**−**169** developed by Chibale and co-workers.[193]

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three tested *P. falciparum* strains. And, although **167a** only showed activity in the micromolar range against falcipain (IC_{50} = 10.8 μM), it was highly active as inhibitor of hemozoin formation, which suggests this could be its primary MOA. In an effort to improve the solubility of hybrid **167a** while its retaining antimalarial activity, the same group later synthesized new **CQ**/chalcone derivatives through replacing the triazole motif by either an aminoethoxy or piperazine linker.[193] The most active compounds of the series, **169a−b** (Figure 84, IC_{50} of 300−600 nM against *P. falciparum* D10, Dd2, and W2) showed improved solubility but did not exhibit better antimalarial activities than **167a**. No cytotoxicity was observed when compounds **169a−b** were tested at a concentration of 100 μM against the Chinese hamster ovarian cell line. The authors further confirmed that **169a−b** also seem to owe their activity to inhibition of hemozoin formation.[193]

Following a similar line of thought, Gomes and co-workers later reported 4-AQ-based hybrids **170** (Figure 85) linking

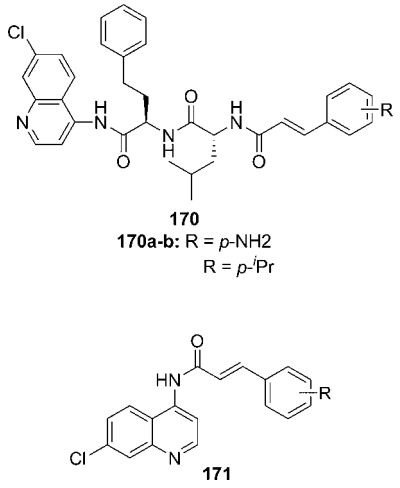
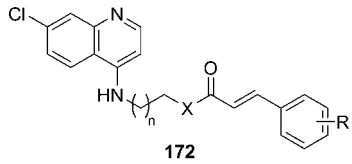


Figure 85. Structure of **CQ**/cinnamic acid hybrid compounds **170**−**171** developed by Gomes et al.[194]

through a dipeptide spacer the **CQ** core to a *trans*-cinnamic acid motif, capable of inhibiting catalytic Cys residues.[194] The hybrid that best reached the goal to exert antimalarial activity by inhibiting the two expected targets, i.e. hemozoin formation conferred by the **CQ** pharmacophore and *P. falciparum* cysteine protease through the electrophilic warhead of the cinnamic moiety, was **170a**. This compound was highly active against β-hematin formation and displayed IC_{50} values of 20.3 μM and 3.18 μM against falcipain-2 and **CQ**-resistant *P. falciparum* W2, respectively. Still, the *p*-^{i}Pr derivative **170b**, which was unable to inhibit β-hematin formation and falcipain action, presented the lowest IC_{50} of the series against *P. falciparum* W2 parasite (IC_{50}= 0.83 μM), suggesting the existence of alternative mechanism(s) through which these compounds inhibited *in vitro* parasite growth. In order to evaluate the influence of the dipeptide spacer, Gomes and co-workers also synthesized a series of molecules (**171**, Figure 85) without any linker between the quinoline ring and the cinnamic moiety.[194] These compounds were not active against **CQ**-resistant *P. falciparum* W2 at a concentration of 10 μM, showing the relevant role for the linker joining the 4-AQ and the cinnamoyl moieties in a single molecule.

Based on these results, Gomes and co-workers then reported a next generation of compounds with general structure **172** (Figure 86), where a more hydrophobic alkyl spacer replaced the dipeptide linker.[195] These compounds displayed potent *in*



172a-d: n = 3; X = NH; R = *p*-^{i}Pr
n = 3; X = NH; R = *p*-Cl
n = 3; X = O; R = *p*-OMe
n = 3; X = NH; R = *p*-OMe

Figure 86. Structure of **CQ**/cinnamic acid hybrid compounds **172** developed by Gomes et al.[195b]

vitro activity against both blood-stage *P. falciparum* strains (IC_{50} = 15.63−137.95 and 11.0−110.8 nM against 3D7 and W2, respectively) and liver-stage *P. berghei* (IC_{50} = 1.06−4.05 μM). All of them were more active than **CQ** on both stages, and the best hybrids, **172a−b** (IC_{50} = 11.0 and 11.6 nM against *P. falciparum* W2 strain, respectively), were equipotent to artemisinin on blood-stage parasites. With the exception of **172c**, all the hybrids were also better than **PQ** on liver-stage parasites. SAR studies carried out demonstrated that the **CQ** core, the butyl linker, and the amide bond between this linker and the cinnamoyl group were optimal for the dual-stage activity of the compounds. Furthermore, two of the most promising compounds (**172b,d**) were confirmed to be active against the murine model of malarial infection, although with modest *in vivo* performances compared to *in vitro* ones. This result could be due to bioavailability issues demanding future structural optimization of the reported dual-stage leads. As none of the hybrids **172** presented activity against falcipains and as the activity presented by the compounds against hemozoin formation did not fully account for the potent antimalarial activities observed, Gomes and co-workers are currently investigating possible MOA underlying dual-stage activities of hybrids **172**.

6. COMPUTATIONAL STUDIES ON ANTIMALARIAL CLASSICS AND THEIR ANALOGUES

Computational modeling studies have been extremely useful to elucidate the underlying nature of interactions in many different chemical and biochemical systems.[196] Despite the enormous successes in several fields, the number of computational-based studies toward antimalarial drug development is rather low when compared with the literature arising from the consideration of experimental techniques. This is essentially due to the complex life cycle of the malaria parasite and to the difficulties in the determination of putative receptors and MOA of compounds with proven antimalarial activity. This picture is further aggravated by mutations that malaria parasites undergo to escape antimalarial drugs action. Quite encouraging, the number of potential targets in the malaria parasite found a significant increase after the unveiling of the *P. falciparum* genome in 2002, which is very beneficial for detailed studies of the mode of interaction between drugs and receptors of interest in this field.[191a]

Most of the computational studies developed so far in the field of antimalarial chemotherapy concerned hemoglobin degradation inside parasitized red blood cells, focused either on inhibition of the parasitic enzymes involved in the globin

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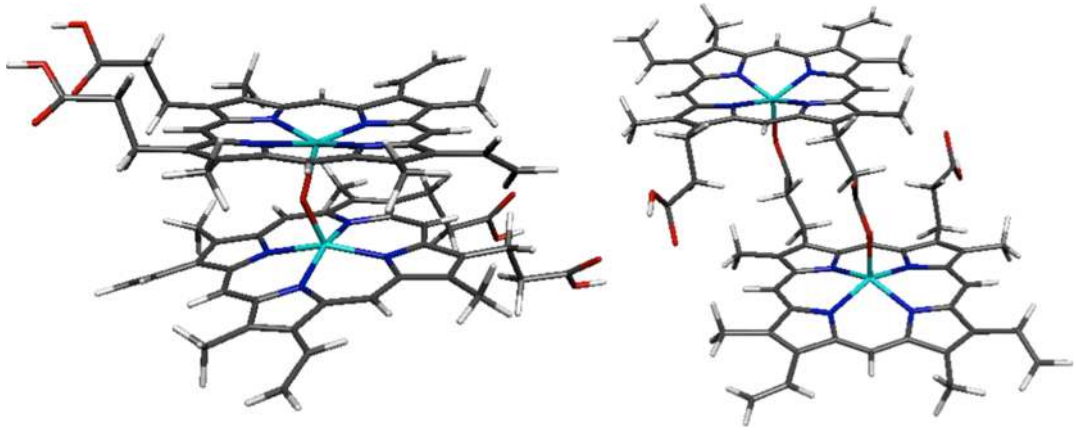

Figure 87. Molecular models of the μ-oxo-dimer of hematin (left) and of the hemozoin dimer (right). Reprinted with permission of the Federation of the European Biochemical Societies from ref 198. Copyright 2003 Elsevier.

degradation or on the hematin aggregation process.[191b] The latter has been traditionally associated with the MOA of antimalarial 4-AQ, as **CQ**, whose putative targets have been investigated for obtaining information on the inhibition processes at the atomic level. Such knowledge has been often used to develop quantitative structure−activity relationship (QSAR) models thought to be useful for the recycling of the 4-AQ scaffold toward improved antimalarials. Here, relevant computational approaches can be divided into two large families, molecular mechanics and *ab initio* electronic structure methods, depending on whether classical or quantum mechanics is used to calculate the interactions between atoms, groups of atoms, molecules, or even molecular clusters. The energy of a particular system is calculated as a function of only the nuclear positions, in the case of the molecular mechanics (MM) methods, while the nuclear positions and the electronic structure are considered in the case of the quantum mechanics (QM) methods. Thus, the choice of one or other family is usually highly dependent on the size of the molecular models used to represent the systems of interest and on the properties to be calculated. The MM methods are computationally less expensive than the QM methods, but for instance, they cannot be used to study chemical reactions and to locate transition state structures or to analyze the electronic charge distribution in a molecule. The two families of methods include approaches of different complexity and different degrees of parametrization, and the rule of thumb for practical applications is the larger the system, the more approximate the approach.

Among the vast computational modeling machinery, molecular docking is employed to predict the preferred orientations of molecules (drugs) around macromolecular receptors based on a score function to discriminate and rank the stability of a very large number of possible binding poses. The most sophisticated docking algorithms can tackle flexible systems and simulate the solvent effects. Thus, if the structure of a putative drug receptor is known, docking methods can be used for structural elucidation or for virtual high throughput screening (vHTS) purposes, i.e., for screening compound libraries in order to rank their abilities to bind the target receptor. The ligand−receptor structures obtained with molecular docking can be further refined by MM, by QM, or by a combination of both approaches (QM/MM). In the latter, QM is used to model the region of contact while MM is used for the remaining system and to provide a correct embedding of the QM region, i.e., to mimic the full system and the effects of the solvent. The most used QM methods are based on the density functional theory (DFT), which relies on the calculation of the energy of the system from the electron density through an appropriate density functional. This is due to the fact that, for an N-electron system, the computational efforts in the case of DFT methods scale with N^3 while wave function based methods scale with N^4 (or even more). The computational burden may be alleviated by the consideration of additional approximations and of empirical parameters giving rise to the semiempirical approaches. Both for QM and MM methods, it is possible to calculate the time dependent behavior of a molecular system, in the so-called molecular dynamics (MD) simulations. For additional details, the interested reader may consult textbooks on the subject of computational chemistry.[197]

6.1. Targeting Inhibition of Hemozoin Formation

Some of the computational approaches introduced above were used by Portela et al. to investigate the interaction of several 4-AQ antimalarials, including **AMQ** and **CQ**, with two putative targets involved in the hematin aggregation process, namely, the μ-oxo-dimer of hematin and the hemozoin dimer (Figure 87).[198] Their main idea was to obtain further knowledge about the molecular mechanisms underlying the antimalarial action of 4-AQ, which is very relevant both for the synthesis of new compounds and for the chemical recycling, i.e., structural optimization of existing antimalarials. Due to the absence of an experimental structure for the μ-oxo-dimer of hematin, Portela et al. used primarily a classical approach (CHARMM force field) for a conformational search of the most likely position of the porphyrin subunits, which was followed by an optimization of the structure with the AM1(d) semiempirical method.[199] In turn, the structure of the hemozoin dimer used was that reported by Pagola and co-workers.[200] The optimized and crystallographic structures of the μ-oxo-dimer of hematin and of the hemozoin dimer (Figure 87), respectively, were considered in subsequent single-point calculations with the B3LYP functional (a popular DFT-based hybrid approach) for calculation of the electrostatic properties.[201] In the case of the former species, the molecular electrostatic potential (MEP) surface shows the concentration of the negative potential at the iron atom, the tetrapyrrole system and, to a smaller extent, at the carboxyl oxygen atoms, while the positive potential is at the other regions.[198] The hemozoin dimer shows negative potential at the iron-carboxylate region and positive potential in the

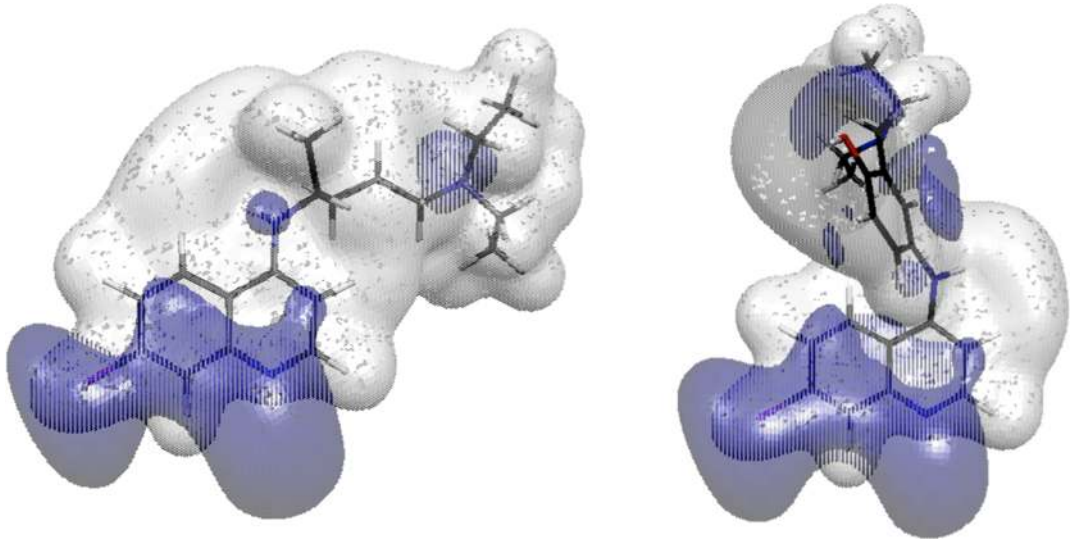

Figure 88. Electrostatic potential isosurfaces for chloroquine (left) and amodiaquine (right). Negative and positive potential regions are shown in blue and white, respectively. Reprinted with permission of the Federation of the European Biochemical Societies from ref 198. Copyright 2003 Elsevier.

peripheral molecular volume. The concentration of negative potential at the central region of the two putative targets surrounded by a region of positive potential suggests that the antimalarial activity would be enhanced for compounds presenting a region with positive potential surrounded by a region of negative potential; that is, the compounds should present an electrostatic potential profile complementary to those found for the targets.

Curiously, the calculations performed by Portela et al. show that the electrostatic profiles of several inhibitors of hematin aggregation, e.g. **AMQ**, **CQ**, **Q**, and derivatives, are in agreement with the complementarity-based hypothesis of drug/receptor association. Further docking studies show that the association of 4-AQ with the deprotonated form of hematin μ-oxo-dimer occurs through contacts of the amino group at the quinoline's C-4 and the central region of the hematin dimer and also of the negative potential aromatic regions of the ligand and the peripheral positive regions of the receptor (Figure 88). In the case of the hemozoin target, the association of 4-AQ occurred also between the negative area at the iron-carboxylate moiety and the area of positive potential of the ligands.[198]

Rafiee et al. analyzed the relationship between the electronic structures of several aminoquinolines (AQ, Figure 89) and their ability to interact with hematin.[202] Such analyses were based on calculated data for the nuclear quadrupole coupling constants (NQCC) obtained from the components of the electric field gradient (EFG) tensor in the principal axis system calculated at the B3LYP/6-31G* level of theory. The NQCC for ^{14}N in the quinoline rings of 3-, 5-, 6-, and 8-AQ substantially differ from those of 2- and 4-AQ; that is, the NQCC for ^{14}N in the latter compounds are larger than in the former. In other words, the charge density on the nitrogen from the quinoline ring is larger for 2- and 4-AQ than for the other AQ considered, which is in agreement both with the fact that 8-AQ, like **PQ**, does not owe its antimalarial action to inhibition of hemozoin formation[203] and with observation of stronger complexes with hematin in aqueous dimethyl sulfoxide (DMSO) solution in the former than in the latter AQ.[140c,204] Additionally, Rafiee et al. compared the variation of the NQCC in the ^{14}N and ^{2}H nuclei of isolated 2- and 4-AQ and of their complexes with

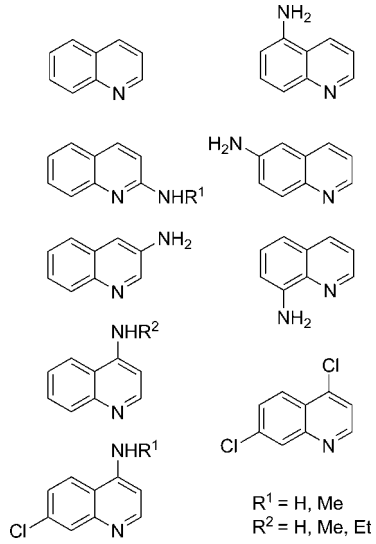

Figure 89. Structures of the AQ considered for the calculations of the NQCC by Rafiee et al.[202]

Fe(III) and concluded that the change of charge density on nitrogen is greater than that of deuteriums, which suggests that binding to hematin is indeed related with the charge density in the nitrogen atom of the quinoline ring.[202] But, as presented below, the relationship between charge density profiles at the quinoline ring, the binding to hematin, and the inhibition of β-hematin formation is not that simple.

Nsumiwa et al. calculated, also at the B3LYP level of theory, Mulliken and CHelpG atomic charges for neutral and quinolinium forms of several 7-substituted 4-AQ (Figure 90), in vacuum or under implicit water solvent conditions, which were correlated with either the logarithm of the constant of association to hematin (log$K$) measured in aqueous DMSO, the logarithm of the 50% β-hematin inhibitory activity (log$BHIA_{50}$), or both.[205] The experimental data were also correlated with empirical properties, such as the hydrophobicity and substituent Hammett constants, which suggested that the inhibition of β-hematin formation appears to be favored by

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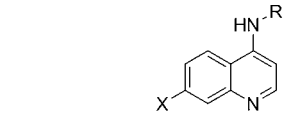


R = H and X = Me, CF_3, Cl, $CONH_2$, CN, H, NH_2, NO_2, OMe
R = Me and X = Me, CF_3, Cl, $CONH_2$, CN, H, NH_2, NO_2, OMe
R = $EtNH_2$ and X = Me, CF_3, Cl, CN, H, NO_2, OMe
R = $EtN(Et)_2$ and X = Me, CF_3, Cl, H, NH_2, NO_2, OMe, F, Br, I

Figure 90. General chemical structures of the four different series of 7-X-4-AQ studied by Nsumiwa et al.[205c]

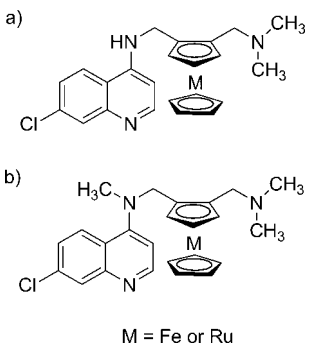
stronger association with ferriprotoporphyrin IX (hematin) and by more electron withdrawing substituents at position 7 of the quinoline ring. In the case of the charges calculated with the Mulliken approximation for the R = H series (Figure 90), eight statistically significant ($P < 0.05$) correlations of log*K* and single atomic charges were found and two additional correlations were obtained from multiple linear correlation analysis (MLCA). The best correlation was found between log*K* and the charges on carbon atoms 6 and 8a (cf. Figure 90) for the neutral forms in water.[205c] The association is predicted to be favored by greater negative charge at C8a and reduced negative charge at C6, but in the case of the other series, the log*K* values estimated with the relationship obtained for the R = H series were found not to correlate with the experimental results.[205c] Many correlations were found between the $logBHIA_{50}$ and Mulliken atomic charges, but the strongest one was based on the charges at the N1 and H8 atoms of the quinolinium forms, with increased activity favored by a less negative charge on N1 and a more positive charge on H8.[205c]

In the case of the CHelpG charges, the strongest correlation with the log*K* for compounds with R = H was obtained for the quinolinium species in vacuum and involved atoms X and H8, with the association with hematin favored by a more negative charge on X (withdrawing groups are benefic) and a less positive change on H8. Interestingly, by contrast to the results obtained with the Mulliken charges, log*K* values calculated for the other series with the relationship derived for R = H based on the CHelpG charges produced a statistically significant linear correlation with the experimental log*K* values.[205c] In the case of $logBHIA_{50}$, the results from Nsumiwa et al. suggest that the charges in the N1 and C8 or N1 and C7 (neutral species in vacuum) provided very good correlations and that the β-hematin inhibitory activity is improved with decreasing negative charge on N1, decreasing negative charge on C8, and decreasing positive charge on C7.[205c] Notice that a comparative analysis is difficult, since correlations obtained for log*K* and for $logBHIA_{50}$ based on Mulliken or CHelpG charges involve different species, namely, quinolinium and neutral forms, in vacuum or in implicit solvent.[205c]

In summary, the correlations based on Mulliken and CHelpG atomic charges as introduced by Nsumiwa et al. show that the quinoline ring is key for the inhibition of β-hematin formation and that withdrawing groups (NO_2, CN, Cl and CF_3) at position 7 are crucial.[205c] Analogues with electron donating groups at position 7 were found to be poor inhibitors of β-hematin formation. Importantly, it was found that specific atoms yielding statistically meaningful correlations for log*K* are for the most part not the same as those that yield interesting correlations for $logBHIA_{50}$. In the case of the R = H series, no experimental correlation was found between log*K* and $logBHIA_{50}$, which suggests that inhibition of β-hematin formation is not directly related to the association between 4-AQ and ferriprotoporphyrin IX in solution. Thus, inhibition of β-hematin formation seems to occur preferentially by interaction of the inhibitor with the growing crystal surface, but since some correlation was found between log*K* and $logBHIA_{50}$ as the lateral side chain is lengthened, a role for solution association cannot be ruled out for all 4-AQ.[205c] Quite interestingly, Nsumiwa et al. suggest that the extension of the lateral side-chain, despite not having a visible effect on β-hematin inhibition, modulates the influence of the substituent at position 7 in the quinoline ring.[205c]

In another combined experimental and computational study, Dubar et al. docked the interaction of **FQ** (cf. Figure 44) with a synthetic hemozoin crystal (β-hematin), and found that the ligand can interact specifically with the {0,0,1} and {1,0,0} faces of hemozoin, blocking crystal growth.[200,206] Notably, intercalation of the quinoline rings between the heme rings in the {0,0,1} face of hemozoin was found, along with the formation of hydrogen bonds with the heme surface, while the formation of N−H···O contacts between deprotonated **FQ** and charged carboxylate groups of hemozoin was important when the interaction occurs with the {1,0,0} face of the crystal.[206] These findings agree well with previous results obtained by Buller et al. for the interaction of 4-AQ (4-amino-7-chloroquinoline, **CQ**, 4-hydroxyanilino-7-chloroquinoline, and **AMQ**) and **PQ** with synthetic β-hematin.[200,207] The docked configurations in the work of Buller et al. show that **CQ** and **AMQ** stereochemically cap onto the {0,0,1} face of the β-hematin crystal via (porphyrin)acid−(quinoline)amine salt bridges and by intercalation of the quinoline rings between the aromatic groups of the crystal. Additionally, these two compounds form (aromatic)N···HC═C(vinyl) and CCl···H_3C interactions which aid the host to anchor the guest within the crevice.[207] While the formation of the salt bridge and ring intercalation are still possible in the case of **PQ**, the latter hydrogen interactions are not possible in the case of this 8-AQ. This is one of the reasons for the lower drug−hematin binding energy of **PQ** as compared with **CQ** and **AMQ**, which agrees with the fact that **PQ** is known as a poor hemozoin growth inhibitor. Notably, quinoline drugs would not be able to dock effectively into position at the {0,0,1} face when protonated at the aromatic nitrogen (N1) atom.[207]

Dubar et al. also compared the influence of the structures of **FQ**, and other metallo-4-AQ, namely, ruthenoquine (**RQ**), methylferroquine (**Me-FQ**), and methylruthenoquine (**Me-RQ**) (Figure 91) on properties considered to be relevant for antimalarial activity, namely, lipophilicity, heme binding, and noncovalent interactions with hemozoin.[206]



M = Fe or Ru

Figure 91. Chemical structures of (a) metallo-**CQ** derivatives **FQ** and **RQ**, and (b) *N*-methylmetallo-**CQ** derivatives **Me-FQ** and **Me-RQ**.

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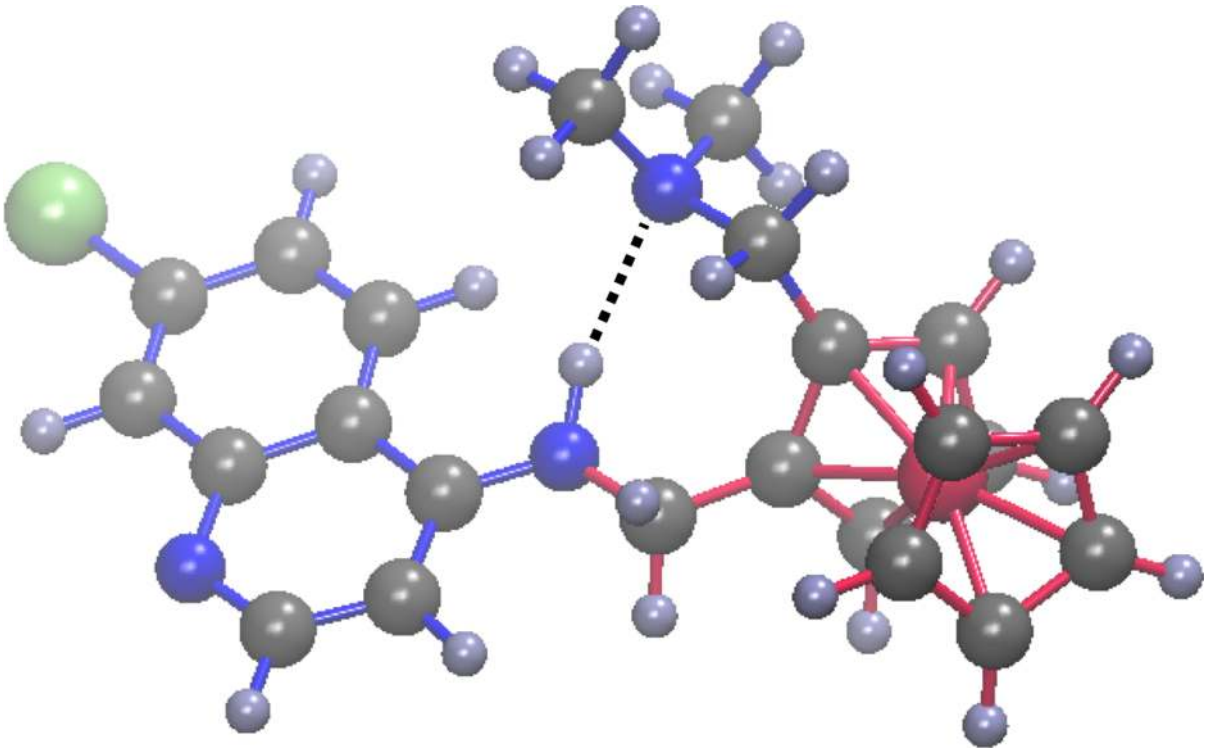

Figure 92. Optimized structure for **FQ** obtained at the RPBE/TZVP level of theory.[206] The internal hydrogen bond is highlighted by a dotted line and has a length of 2.229 Å. In the similar **RQ** compound, the length of the intramolecular hydrogen bond is 2.236 Å.

The methylated derivatives were considered since a possible explanation for the enhanced activity of **FQ** when compared with that of **CQ** is that its folded conformation through the establishment of an intramolecular hydrogen bond (Figure 92) improves its lipophilic character, which, expectedly, enhances its membrane permeation. The formation of the intramolecular hydrogen bond is hindered in the methylated compounds. Dubar et al. considered the RPBE approach (another DFT functional) and the TZVP basis set to optimize the structures of the **FQ**, **RQ**, and their methylated congeners in vacuum.[206,208] They found that the optimal structures of **FQ** and **RQ** (Figure 92) present intramolecular hydrogen bonds between the unprotonated terminal tertiary amino group and the 4-amino group of the quinoline ring; the bond in **FQ** was calculated to be 1.4 kcal·mol^{-1} stronger than in **RQ**. The polar surface areas estimated from the MEP surfaces for **FQ** and **Me-FQ**, with values 14 Å^2 and 10 Å^2, respectively, suggest that, at least in low dielectric media, the nonmethylated derivatives are likely to exhibit an intramolecular hydrogen bond. Experimental nuclear magnetic resonance (NMR) studies for the compounds in $CDCl_3$ demonstrate the existence of such intramolecular bonds and folded configurations for **FQ** and **RQ**, but also in **CQ**, and their absence in **Me-FQ** and **Me-RQ**. In the case of water solvent, combined computational studies and NMR experiments show that intramolecular hydrogen bonds in the lateral side chains of **FQ** and **RQ** compounds are not formed, which suggests that the hydrogen bonds are susceptible to the dielectricity of the medium. This is very relevant for the crossing of membranes, as the observation of a subtle balance between neutral, monoprotonated, and deprotonated forms of the **FQ** and **RQ** compounds is equally important.[206] The methylation of the 4-amino group of **FQ** and **RQ** was found to reduce their biological activities but also their toxicity by almost an order of magnitude.[206] The ability of **FQ**, **RQ**, and their methylated derivatives to generate hydroxyl radicals was also evaluated from a combination of experimental and computational approaches. Calculations were very relevant in this respect for compounds with ruthenium, since cyclic voltammetry experiments were not possible due to electroprecipitation issues. It was suggested that **FQ** and **Me-FQ** can generate hydroxyl radicals while such is not possible with their ruthenium counterparts. These results probably explain why only **FQ** and **Me-FQ** break down the parasite's FV membrane.[206]

In another study reviving the 4-AQ pharmacophore, Aguiar et al. used experimental and computational techniques to examine the antimalarial activity and mechanisms of action of compounds **173** and **174** (Figure 93) against **CQ**-resistant

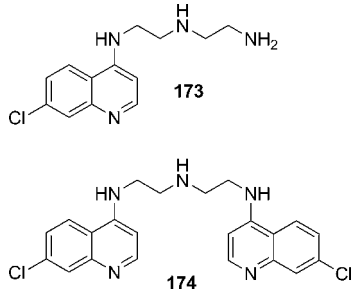

Figure 93. Structures of compounds **173** and **174**.

parasites.[149a] These compounds were tested against *P. falciparum in vitro* and against *P. berghei* in mice, and they were also evaluated *in vitro* for their cytotoxicity and ability to inhibit hemozoin formation. **173**, **174**, and **CQ** showed activity in the nanomolar range against **CQ**-resistant/mefloquine-sensitive (W2) and **CQ**-sensitive (3D7) *P. falciparum* parasites,

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and they were also found to be active when evaluated in mice. Additionally, **173** and **174** did not display toxicity against human hepatoma (HepG2) or kidney (BGM) cell lines. The two novel compounds inhibit heme polymerization, and docking studies, in the presence of water and considering molecular flexibility, show that they interact with dimeric hematin in similar fashion to **CQ**. In the highest ranked conformations, the aromatic rings of all protonated forms of **173** and **174** are parallel to the ferriprotoporphyrin group, and the interaction also involves weak hydrogen bonding, as happens with **CQ**. The presence of two quinoline moieties in **174** was found to increase the probability of hydrophobic interactions when compared with the compound with just a quinoline ring. In fact, the docking energies for the protonated forms of **174** were found to be larger (more negative) than those corresponding to the **CQ** and **173** compounds. In order to analyze if **173** and **174** were NADH competitors, Aguiar et al. also docked these compounds to *P. falciparum* L-lactate dehydrogenase (*Pf*LDH). The model for *Pf*LDH was obtained from a 3D structure of *Pf*LDH complexed with NADH and the oxamate substrate available at the Protein Data Bank (PDB, code 1LDG).[209] From the calculated interaction energies, **173** and **174** are suggested to be weak inhibitors of *Pf*LDH, as happens with **CQ**.[149a] Nevertheless, as found in docking studies to dimeric hematin, the interaction of protonated forms of **174** with *Pf*LDH is energetically more favorable than protonated forms of **CQ** and **173**, which suggests that the **174** scaffold can be a very interesting target for additional synthetic modifications.

6.2. Aiming at Inhibition of Other Parasitic Targets

Singh et al. combined the best of two worlds in a series of hybrid compounds (**162**, Figure 81 in section 5.2.5) featuring the 4-amino-7-chloroquinoline moiety, due to the characteristics associated with this molecular entity in blocking heme polymerization, and the 2-aminopyrimidine moiety, due to the role of 2,4-diaminopyrimidine drugs in the inhibition of *P. falciparum* dihydrofolate reductase (*Pf*DHFR).[189b] These authors considered molecular docking for understanding whether the antiplasmodial activity of the synthesized compounds could or not be attributed to inhibition of *Pf*DHFR. They considered the PDB structure of wild-type *P. falciparum* DHFR-thymidylate synthase (*Pf*DHFR-TS) complexed with WR99210 (lead compound shown in Figure 94),

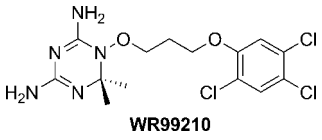

Figure 94. Chemical structure of lead compound WR99210.

NADPH, and dUMP with code 1J3I. Close inspection of the top scored configurations for compound **162a** (Figure 81 in section 5.2.5) and WR99210 shows that the former adopts a slightly different pose when compared to the latter. Importantly, **162a** was found to establish more hydrogen contacts with *Pf*DHFR than WR99210, which originated a better top score for the former compound. Such improved interaction seems to be the relevant factor for the significant antiplasmodial activity evidenced by **162a**.[189b] Very recently, Bhat et al. docked a series of 4-AQ-1,3,5-triazines with *P. falciparum* DHFR to correlate activity and specific interactions between the ligands and residues of wild type (PDB code: 1J3I) and quadruple mutant (PDB code: 3QG2) *Pf*DHFR.[210] Despite identification of the contacts made by each compound with *Pf*DHFR, which can be relevant for future synthetic studies, unfortunately, a clear correlation between antimalarial potency and the strength of the interaction was not established.

In a similar approach to that of Singh et al., Gomes and co-workers reported in the same year and journal a series of novel compounds based on the **CQ** core and on the cinnamoyl moiety, linked to each other either directly or through a retro-enantio dipeptide spacer (**170**−**171**, Figure 85 in section 5.2.5).[189b,194] As already mentioned in the previous section, it was found that the compounds with the dipeptide spacer (**170**) inhibited *in vitro* both hemozoin formation and development of blood-stage *P. falciparum*, while compounds without the spacer (**171**) were better falcipain-2 (FP2) inhibitors; none of the compounds was a falcipain-3 (FP3) inhibitor. In order to obtain additional knowledge about the FP2 inhibitory capacity of the latter compounds, molecular docking and MD simulations were performed to predict the structures of the complexes between the compounds with or without the dipeptide linker and FP2 (PDB code: 3BPF) or FP3 (PDB code: 3BWK). The calculations suggest that both families of compounds cannot fit into the catalytic site of FP3 as efficiently as into that of FP2. In fact, the docked configurations for the most active inhibitor with the dipeptide linker (**170** with R = H) show that its vinyl group is located in the S2 subsite of FP2 but with the vinyl bond at ∼4.5 Å of the enzyme's Cys thiolate (A), while in FP3 it appears at the S2′ well (Figure 95B). In the case of the most active compound without the dipeptide spacer (**171** with R = *m*-NO_2), the vinyl group is also located in the S2 subsite of FP2 with the vinyl bond at ∼3 Å of the enzyme's Cys thiolate (Figure 95C), but in the case of FP3 the compound is outside the binding pocket (Figure 95D). All compounds with and without the spacer were found to not fit entirely in the binding pocket of FP3, which seems to be a convincing explanation for their lack of FP3 inhibitory activity. In the case of FP2, the shorter distance between the vinyl bond of the compounds without the linker and the Cys thiolate of the enzyme than for the compounds with the spacer is in line with the experimental observation that the former were better FP2 inhibitors.[194]

While molecular docking combined with realistic molecular models is able to provide static information about the most favorable host−guest configurations, MD simulations can be used to gain knowledge on the evolution of the docked configurations with time. In fact, Gomes and co-workers showed that FP2 inhibitory activities measured experimentally could be correlated with the time evolution of the distance between the vinyl bond and the enzyme's Cys thiolate determined from classical MD simulation. It was found that compounds manifesting FP2 inhibitory activity kept their vinyl bond within 3.5−4 Å of the thiolate, while the distance increased to larger values in those compounds shown to be weak FP2 inhibitors. Note that this information is based just on the noncovalent interactions since the electronic structure is not taken into account in the classical approaches considered by those authors.[194] Nevertheless, this strategy was proven to be quite robust again in a separate study where the same authors synthesized a novel family of cinnamic acid/chloroquinoline conjugates but linked by an alkyl spacer group (**172**, Figure 86 in section 5.2.5).[195a] The latter compounds were found to be more active (nanomolar range, *in vitro* assays) against **CQ**-resistant *P. falciparum* W2 strain

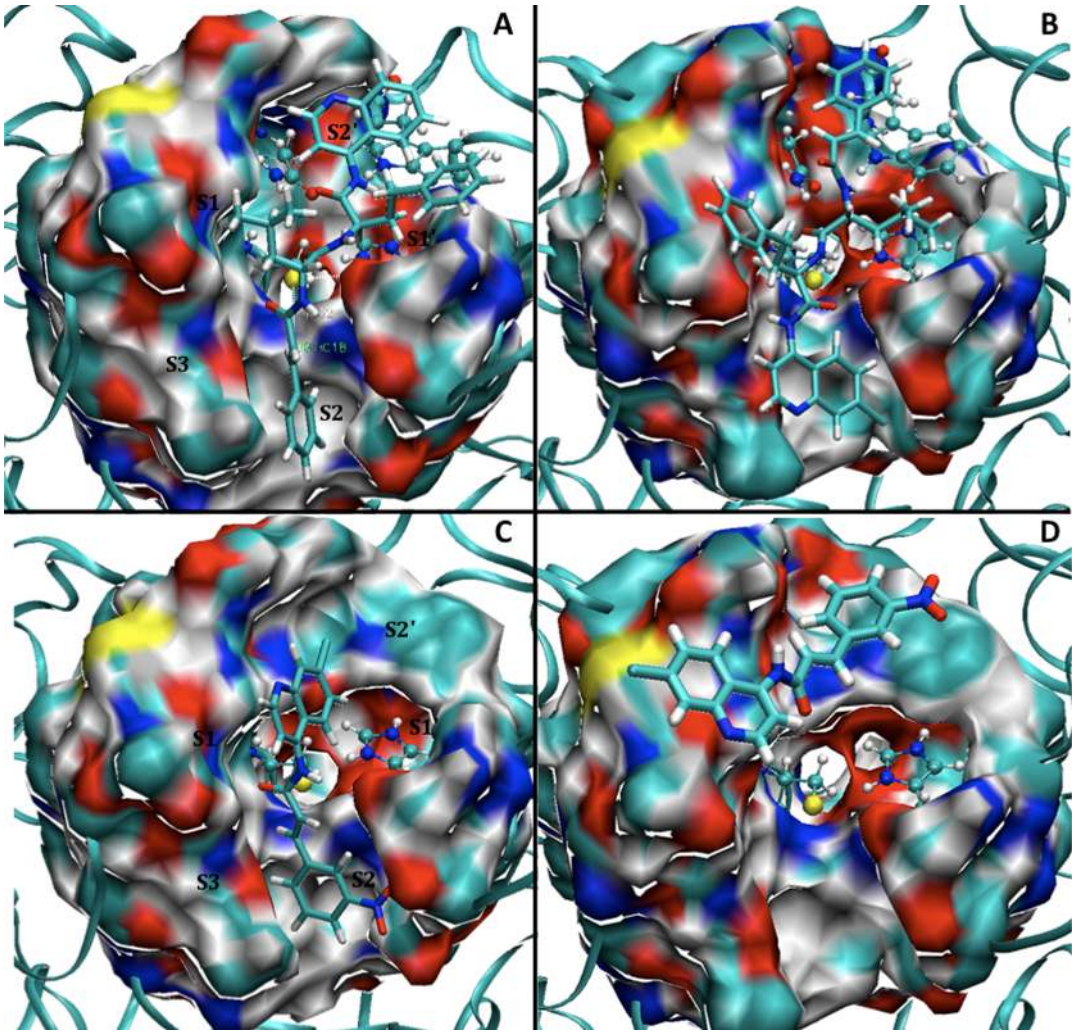

Figure 95. Preferred docked binding mode of the most active cinnamic acid/chloroquinoline conjugates with (top) and without (bottom) a dipeptide linker into FP2 (left) and FP3 (right) binding sites. The inhibitors are shown in ball and stick representation while residues forming the "oxyanion hole" of parasitic cysteine proteases are in CPK representation. Reproduced with permission from ref 194. Copyright 2012 Elsevier Masson SAS. All rights reserved.

than those without spacer (**171**) or those with a dipeptide linker (**170**). Experimental *in vitro* data suggest that their potent antiplasmodial activity was not exerted through FP2 inhibition. Such experimental findings were corroborated with results from MD simulations on the docked configurations showing that the putative site of nucleophilic attack, i.e., the vinyl group, also moves away from the enzyme's Cys thiolate in the compounds with the alkyl linker (**172**).[195a]

Acridine-inspired derivatives have also been the focus of computational studies, as their mechanisms of antimalarial action remain unclear. Biagini et al. investigated by experimental and computational approaches the MOA of two dihydroacridinediones, namely, compounds **52** and **58** (cf. Figures 26 and 27, respectively), shown to have antimalarial activity.[90b] Thermodynamic analysis of heme-binding showed that both compounds could bind to heme but to a lesser extent than **CQ** and **AMQ** (**CQ** > **AMQ** > **52** ≫ **58**). The low heme binding affinity of floxacrine (**52**), IC_{50} = 140 nM, is in line with its lower *in vitro* antimalarial activity when compared with **CQ** and **AMQ** with IC_{50}s of 7.4 nM and 4.5 nM, respectively. However, IC_{50} of 15 nM for **58** contrasts with its very poor affinity for heme, and therefore, the MOA of dihydroacridinediones cannot be explained by interaction with heme. Hence, the ability of the dihydroacridinediones to inhibit bc_1 complex activity was compared with those of known inhibitors atovaquone, sitgmatellin and myxothiazol. Compound **58** was shown to inhibit selectively and exclusively *P. falciparum* bc_1 complex with IC_{50} = 3 nM and K_i = 0.3 nM, values identical to those for atovaquone but with the latter having superior selectivity (much smaller IC_{50}s for bc_1 complex inhibition in bovine heart, rat liver and human liver than those of **58**). Compound **52** displayed moderate inhibitory activity against cross-species bc_1 activities but without selectivity for *P. falciparum* bc_1. Further experiments with wild-type yeast and mutants showed that these compounds target the Q_o site of the bc_1 complex. For better understanding the inhibitory process, Biagini et al. constructed a homology model of the *P. falciparum* cytochrome *b* using as structural template the atomic coordinates from its bovine counterpart (Figure 96), which was used to dock compounds **52** and **58**.[90b]

These compounds were found to bind favorably the *P. falciparum* bc_1 model. Compound **58** was within 4 Å of Q_o site residues with ligand–host interactions predominantly hydrophobic, but a backbone hydrogen bond from Ser241 to the

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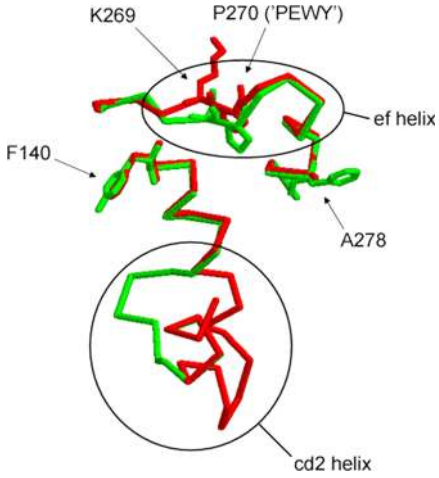

Figure 96. Structural alignment of selected regions from the Q_o site regions of the bovine (red, PDB code: 1SQX) and of the *P. falciparum* homology model (green) cytochrome *b* complexes. Relevant residues, including the catalytically essential PEWY motif, are highlighted. Adapted with permission from ref 90b. Copyright 2008 American Society for Pharmacology and Experimental Therapeutics.

aromatic secondary amine of the ligand was also seen (Figure 97).

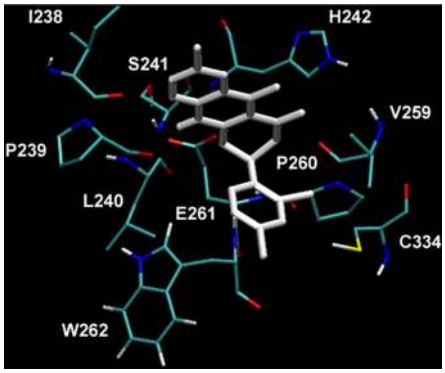

Figure 97. Compound **58** (white cylinders) docked at the Q_o site of the *P. falciparum* homology model of cytochrome *b*. Only cytochrome *b* residues within 4 Å of the inhibitor are shown (CPK). Adapted with permission from ref 90b. Copyright 2008 American Society for Pharmacology and Experimental Therapeutics.

Importantly, a putative association between the inhibitor and residues 236−241 constituting the E-ef linker region of the cytochrome was observed; this region possesses low sequence identity between *P. falciparum* and mammalian cytochrome *b*. Despite the absence of water molecules in the Q_o site of the model, with possible influence in the binding energy and pose obtained by molecular docking, the computational results explain the very high selectivity of compound **58** highlighted above, which is very encouraging for other investigations in the field.

6.3. Investigating Possible Mechanisms of Drug Toxicity

Another relevant piece of information that may be obtained from the consideration of computational approaches regards the understanding of biochemical processes associated with drug toxicity. An example of such an approach is the work of Liu et al. focused on the possible molecular mechanism underlying methemoglobinemia caused by 8-AQ, particularly in G6PD-deficient patients.[211] These authors hypothesized that, in the process of converting O_2 to H_2O_2, one electron is provided by oxidation of the Fe(II) from hemoglobin, which is concomitantly converted into methemoglobin, and the second electron comes from an 8-AQ metabolite.[211] **PQ** metabolites considered were 5-hydroxy-PQ, 6-methoxy-8-AQ, and carboxy-PQ, respectively, structures **16**, **17**, and **18** in Figure 8, section 3.2. While **PQ** requires metabolic activation before leading to the formation of methemoglobin, 5-hydroxy-PQ (**16**) is known to cause methemoglobinemia and to form H_2O_2 and hence was chosen to test their working hypothesis. As in the studies reviewed above (section 6.1), due to pH conditions, the protonated 8-aminoalkylammonium form of **16** was considered for the calculations. The structure of hemoglobin was taken from the crystal structure of horse carbonmonoxyhemoglobin complexed with 2-[4-(3,5-dichlorophenylureido)phenoxy]-2-methylpropionic acid, with PDB code 2D5X. Molecular docking shows that compound **16** interacts with the carboxylic side chain of heme through the terminal ammonium group.[211] The pose derived in the previous step was used as input for the DFT calculations (B3LYP approach considering an unrestricted formalism) in which the structures of unprotonated, singly protonated, and doubly protonated forms of O_2···hemoglobin··· **16** complexes were fully optimized. It was found for all protonation states that a proton is transferred from the terminal ammonium group to the carboxylic group of heme, resulting in a $-H_2N\cdots HOOC-$ local configuration. In the case of the unprotonated complex, and for all possible spin states, a single electron is transferred to O_2 and, since the spin density in compound **16** is zero, it is contributed by Fe(II) forming a local Fe(III)$\cdots O_2^{\bullet-}$ moiety. In the case of the single-protonated complex, it is found for different states that ∼0.6 electrons are contributed by **16**. In the case of the double protonated complex, the **16** species presents a spin density around 1.0, which confirms the hypothesis that the second electron transferred to the π^* orbital of O_2 comes from **16**.[211] Liu et al. end by suggesting that the mechanism unveiled by the DFT calculations may be generalized to other aromatic compounds that interact with the carboxyl arm of heme via hydrogen bonding either through amine or hydroxyl groups. This suggestion is supported by the observation that aniline and its metabolites catalyze the production of methemoglobin *in vivo*.

The studies reviewed above demonstrate that important atomic level information on the role of drugs and their interaction mechanisms on defined targets can be obtained at low cost by the consideration of computational chemistry techniques. The quality of the results will be dramatically improved if electronic structure methods and more realistic models are used, which has to be envisaged in the near future due to significant progresses in the field (e.g., computer architectures, computational algorithms, and theories) and to the unveiling of targets important to the life cycle of the malaria parasite. Moreover, computational methods are crucial in the screening of bioactive compound libraries aimed at rescuing or repurposing (see next section) known structures to the field of malaria chemotherapy. In summary, no less than a bright future is to be expected for application of computational methods to the search for new antimalarial agents.

7. RESCUING AND REPURPOSING DRUGS FOR MALARIA

As shown by previous sections, one main approach to accelerate the development of novel antimalarials is to start from the chemical framework of known ones to produce new drugs. However, as developing new drug products is a costly and time-consuming process, a cost-effective reduced-risk strategy is to identify new therapeutic uses for molecules that were already synthesized but did not find clinical application (rescuing) or that were already approved to treat a specific disease or group of diseases but might be relevant against other therapeutic targets (repurposing). The basis for drug rescuing/repurposing (r&r) relies on the fact that many drug targets are eventually shared by more than one physiological process. Such a paradigm shift is enhanced by the increasing awareness that many drugs may have more than one biological target.[212] This drug "promiscuity" has motivated several groups to pursue drug r&r with promising results.[212b,213] In addition, the high costs and failure rates to bring drugs to market have led to more and more interest in drug r&r.[214]

Drug r&r is primarily driven by serendipitous observations in clinical and preclinical *in vivo* settings, of which the high profile drug sildenafil by Pfizer, first developed for angina but later approved for erectile dysfunction, is an emblematic example.[215] Another classic example is thalidomide by Celgene, first marketed for morning sickness, and then approved for leprosy and recently for multiple myeloma.[216] Genomic screens have also contributed to find new uses for known drugs. For example, the antileishmanial properties of amphotericin, originally developed to treat fungal infections by disrupting the pathogen's plasma membrane through binding to sterols, were identified based on the discovery of homologous 24-substituted sterols in leishmanial cells.[217] Rescued/repurposed drugs have also been discovered through cell-based screenings directly against living organisms, which has been the major thrust area for the chemotherapy of tropical parasitic diseases. The undeniable advantages of drug r&r are particularly valuable for development of medicines against diseases endemic to low-income countries, such as malaria.[218]

Drug r&r in malaria is not as novel as one might think. The sulfur-based antibacterial drugs developed as dyes in the early 1900s are a classical example of compounds that were rescued/repurposed to treat malaria and to serve as starting points to develop new active compounds. Examples are sulfonamide drugs such as sulfadiazine and sulfanilamide, as well as the sulfone dapsone, which were a landmark of early synthetic antibacterial agents later found to exhibit antimalarial properties.[219] Dapsone's relevance as an antimalarial was established in the 1940s, but the efficacy of the already in use quinine outshined these findings; it was only during the Vietnam War that the interest to pursue the development of dapsone as an antimalarial was renewed.[220] This interest was further confirmed when it was demonstrated that dapsone synergized with inhibitors of dihydrofolate reductase, which led to the development of Lapdap (a combination of dapsone and chlorproguanil) by a public–private partnership between the WHO, the British Government, the University of Liverpool, and GlaxoSmithKline.[221] Clinical trials of Lapdap were performed in Africa and, although more cases of anemia appeared in patients treated with dapsone–chlorproguanil, the drug was licensed in the U.K. in 2003.[222] Unfortunately, GlaxoSmithKline withdrew Lapdap in 2008 due to significant reductions in hemoglobin levels of G6PD-deficient patients.[222b] As dapsone causes hemolytic anemia, this potential side effect was to be expected; still, no link between Lapdap, anemia aggravation, and G6PD deficiency was observed because no G6PD testing was done during a key study.[222b] The dapsone example highlights how drug repurposing can be derailed by predictable side effects. Thus, although drug r&r is a cost-effective reduced-risk strategy, it is important to bear in mind that clinical trials to detect known complications of existing drugs are needed in order to ensure safety.

In the dawn of the 21st century, several groups at pharmaceutical companies and academic institutions have performed cell-based screens of their chemical libraries, containing approved, discontinued and/or "shelved" drugs, against tropical parasitic diseases. For example, Chong, Sullivan and co-workers have created a library of 2,687 existing drugs, called the Johns Hopkins Clinical Compound Library (JHCCL), and have screened it for inhibition of *P. falciparum* growth.[187] The nonsedating antihistamine astemizole (**175**, Figure 98) was one of the most promising compounds

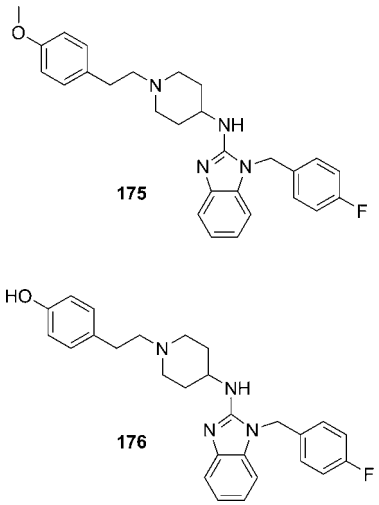

Figure 98. Structures of astemizole (**175**) and desmethylastemizole (**176**).

identified, by inhibiting both **CQ**-sensitive (3D7) and **CQ**-resistant (Dd2) *P. falciparum* strains with IC_{50} values of 227 and 457 nM, respectively. Remarkably, desmethylastemizole (**176**, Figure 98), the principal human metabolite of astemizole, was approximately two to four times more potent than its parent compound, having IC_{50} = 117 nM or 106 nM against 3D7 or Dd2 *P. falciparum* strains, respectively.[187] The two compounds showed efficacy in *P. vinkey*-infected mice, reducing parasitemia by 80 and 81% when treated with astemizole (intraperitoneal daily dose of 30 mg/m^2 for 4 days) and desmethylastemizole (intraperitoneal daily dose of 15 mg/m^2 for 4 days), respectively.[187] The same researchers also determined that these two compounds concentrate inside the parasite's FV and, like antimalarial 4-AQ, inhibit heme crystallization.[187] Although astemizole and its main metabolite did not present enough potency as to immediately enter clinical trials, their scaffold is a starting point for further development. As a long-term goal, the JHCCL initiative is intended to add to its collection the approximately 11,000 drugs ever used in medicine, aiming at their rescuing/repurposing, and to make such a set available to

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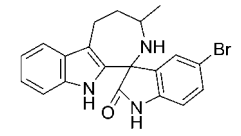
any researcher willing to screen it for rare and/or neglected diseases.

A few years later, a similar but even more ambitious screening project took place.[223] Since 2008, nearly six million molecules have been screened against blood-stage *P. falciparum* parasites.[224] This generated a plethora of hits, approximately 0.5% of which presented an EC_{50} below 1 μM, hence serving as promising leads for candidates with novel mechanisms of antimalarial action.[223] The pharmaceutical companies involved in this campaign, Novartis and GlaxoSmithKline, and the St. Jude Children's Research Hospital, have made the most of these drug discovery data, including chemical structures, freely available and fully searchable through the European Bioinformatics Institute's ChEMBL database.[225] Among the active compounds identified through this screening campaign, a molecule belonging to the spiroazepineindole family (**177**, Figure 99) entered into the lead optimization phase due to its



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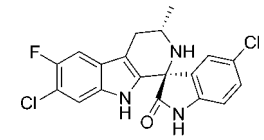

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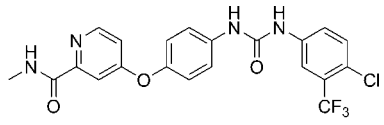
Figure 99. Structures of spiroazepineindole lead (**177**) and NITD609 (**178**).

potent antimalarial activity and favorable pharmacological profile.[226] This project was developed by the NGBS consortium, a collaboration between the Novartis Institute for Tropical Diseases, the Genomic Institute of the Novartis Research Foundation, The Biomedical Primates Research Center, and the Swiss Tropical Institute.[227] After evaluation of approximately 200 analogues, the optimized spiroindolone NITD609 (**178**, Figure 99) was obtained.[226] This compound is a potent antimalarial candidate that (i) kills the blood stages of *P. falciparum* (IC_{50} = 0.7 and 0.9 nM for 3D7 and W2 strains, respectively) and *P. vivax* (IC_{50} < 10 nM) by rapidly inhibiting a parasite's protein biosynthesis and (ii) presents a single oral dose cure at 100 mg/kg in the *P. berghei* mouse model.[226] In the same study, results from NITD609's target identification efforts, through drug pressure to a cultured clone of *P. falciparum* Dd2, suggested this compound might act on the P-type cation-transporter ATPase 4.[226] Later, it was also found that NITD609 (i) inhibits the early and late development of *P. falciparum* gametocytes in a dose-dependent manner (5−500 nM) and (ii) is very effective in decreasing transmission to the *Anopheles stephensi* mosquito vector.[228] Five years after the screening campaign, NITD609 entered phase IIa clinical studies in 2012, representing a remarkable achievement for a new class of molecules and reinforcing the tremendous potential of drug rescuing and/or repurposing.[227]

Similar screens have been performed leading to other molecules that have entered into lead optimization development, as was recently reviewed by two papers on the subject.[136b,229] For the sake of simplicity, the remainder of this section will mainly focus on compounds belonging to two major drug families, antitumorals and antiretrovirals, which have been rescued and/or repurposed for malaria since 2000 and reported as displaying potent antiparasitic activity.

7.1. Antitumorals

Several intracellular pathogens ensure survival by tailoring their host environment to their specific need through interference with cellular programs such as cell proliferation, differentiation and death.[230] These findings led to the exploration of antitumoral drugs toward antimalarial drug development, an approach that has been further enhanced by the recent discovery that artesunate is effective against some cancer types.[231] In a recent study, two antitumoral compounds, Bay 43-9006 and SU-11274 (**179** and **180** respectively, Figure 100),



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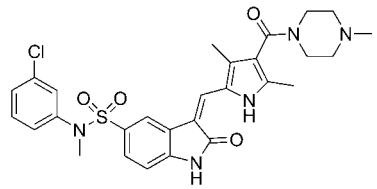

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Figure 100. Structures of Bay 43-9006 (**179**) and SU-11274 (**180**).

were shown to present potent activities against the *P. falciparum* W2 strain with IC_{50}'s of 384 and 320 nM, respectively.[232] In humans, the known targets responsible for the antitumoral properties of Bay 43-9006 are the serine−threonine kinase B-Raf, the Raf/MEK/Erk pathway, and several receptor tyrosine kinases.[233] However, no homologous MEK/Erk kinase cascade was observed nor have tyrosine kinases been identified in malaria parasites. Still, the potent antimalarial activity of this compound should motivate researchers to further screen Bay 43-9006 derivatives and analogues as parasite growth inhibitors.[234] SU-11274 was designed as an antitumoral that acts as a human ATP competitive inhibitor of the MET receptor tyrosine kinase activity.[235] Recently, it was shown that the inhibition of the hepatocyte growth factor/MET kinase (HFG/MET) signaling pathway leads to an increase in apoptosis of *P. falciparum*-infected cells, consequently inducing a considerable reduction of infection.[236] However, it was not confirmed that this could be the possible target for SU-11274 activity against *P. falciparum*. Although the specific mechanisms of SU-11274 and Bay 43-9006 antimalarial action remain unclear, the *Plasmodium* kinome, which is emerging as a major antimalarial strategy, comprises highly promising targets for such compounds.[237]

During *P. falciparum* proliferation, clearance of infected red blood cells preceding the development of trophozoites with the ability to intoxicate macrophages can occur as a result of stimulation of suicidal erythrocyte death, eryptosis.[238] Paclitaxel (**181**, Figure 101) is an antitumoral agent used to treat different types of cancers, and it is also known to cause eryptosis.[239] In this context, Koka et al. investigated whether this compound influences eryptosis of *P. falciparum*-infected human erythrocytes, *in vitro* parasite growth, and survival of *P. berghei*-

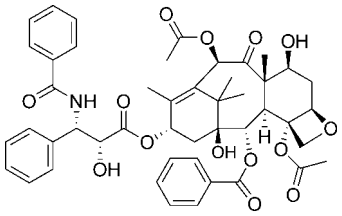

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Figure 101. Structure of paclitaxel (**181**).

infected mice.[240] Results showed that paclitaxel actually increased eryptosis of infected red blood cells and, consequently, decreased *in vitro* growth of *P. falciparum* at concentrations higher than 0.01 μM. *In vivo*, a significant decrease in parasitemia was observed on *P. berghei*-infected mice treated with the compound (intraperitoneal dose of 8.5 mg/kg during 12 days). Paclitaxel treatment increased the survival of treated animals, with 69% of the infected mice surviving the infection for more than 30 days. Despite the encouraging results, further studies are needed to investigate if paclitaxel could be an effective antimalarial in humans.

Heat shock proteins (HSP) are a class of highly conserved molecular chaperones that facilitate protein folding.[241] New drugs that inhibit this class of proteins are reaching market approval to treat cancer.[242] In the specific case of *P. falciparum*, heat shock protein 90 (Hsp90) has also been found essential for parasite development during the intraerythrocytic cycle.[243] Thus, in addition to their antitumoral potential, HSP inhibitors have also been evaluated as competitive inhibitors of the ATP-binding domain of *P. falciparum* Hsp90 and for their potential as antimalarials.[244] By screening about 4000 compounds, issued from three different libraries, Shahinas et al. have identified 46 inhibitors of the *P. falciparum* Hsp90 ATP-binding domain.[244] Harmine (**182**, Figure 102), a naturally occurring β-carboline

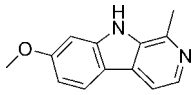

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Figure 102. Structure of harmine (**182**).

alkaloid with antitumoral activity, specifically inhibited the *P. falciparum* Hsp90 ATP-binding domain compared to the human Hsp90 ATP-binding domain.[244,245] In addition, this molecule also inhibited both sensitive (3D7) and resistant (W2) *P. falciparum* strains with IC_{50} values of 50.1 and 28.0 nM, respectively, and demonstrated synergistic activity with **CQ**.[244] The previous use of harmine as antitumoral agent and its potent antimalarial effect make this compound attractive for further clinical development.

The inhibition of proteasome is reported to interrupt the degradation of intracellular proteins that, in fast-proliferating cancer cells, can lead to inhibition of cell cycle regulators and cause apoptosis.[246] The importance of proteasome inhibition as a possible way to also treat parasitic diseases was highlighted by several studies.[247] Following this line of thought, Lindenthal and co-workers found that MLN-273 (**183**, Figure 103), a dipeptidyl boronic acid proteasome inhibitor, arrests the *P. falciparum* erythrocytic cycle with IC_{50} values in the low nanomolar range in both sensitive and drug-resistant strains.[247b] Two other boronic acid dipeptides, bortezomib

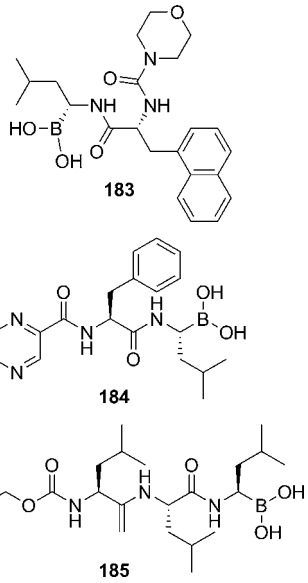

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Figure 103. Structures of dipeptidyl boronic acids MLN-273 (**183**), bortezomib (**184**) and ZL3B (**185**).

(**184**, Figure 103), approved to treat multiple myeloma, and its analogue ZL3B (**185**, Figure 103), were then reported as potent inhibitors of four *P. falciparum* strains (3D7, HB3, W2 and Dd2) with IC_{50} values ranging between 31 and 45 nM.[248] Results showed that these boronate protease inhibitors disrupted the cell cycle prior to DNA synthesis but had no effect on parasite egress at the late schizont stage or subsequent erythrocyte invasion.

In order to evaluate the antimalarial activity of some known proteasome inhibitors, Kreidenweiss and co-workers tested a few of these specific inhibitors, YU101, MG132, Ada-Ahx_3-L_3-VS, Z-L_3-VS and epoxomicin (Table 1), against **CQ**-resistant (Dd2) and **CQ**-sensitive (3D7 and D10) *P. falciparum* strains.[249] The results showed that most of the inhibitors presented antimalarial activity, with values of IC_{50} ranging from 1.7 to 4000 nM, with epoxomicin as the most effective against the three *P. falciparum* strains (IC_{50} = 6.8 nM, 1.7 nM, and 10.4 nM for strains 3D7, D10 and Dd2, respectively). Later, it was further demonstrated that the nanomolar concentrations of epoxomicin effectively kill all stages of intraerythrocytic parasites and block oocyst production in the mosquito midgut.[250] Although the MOA of these inhibitors is not clear yet, the increased detection of ubiquitin conjugates after drugs incubation suggests these compounds might target the *Plasmodium* proteasome complex.[247b]

Salinosporamide A (**186**, Figure 104), a proteasome inhibitor extracted from the marine actinomycete *Salinospora tropica* and currently advancing through clinical trials as an antimyeloma agent, was found to have an IC_{50} of 11.4 nM against *P. falciparum* 3D7 strain.[251] The effectiveness of this compound against malaria parasites was further tested *in vivo* using the *P. yoelii* mouse model. In agreement with *in vitro* data, infected mice showed a significant decrease in parasitemia when treated with salinosporamide A (130 μg/kg).[251b] Also, biochemical and structural-based analyses validate the hypothesis of the parasite's 20S proteasome being the primary target of salinosporamide A.[251b] Though no human clinical trials were performed to confirm the effectiveness of proteasome inhibitors against human malaria, targeting *P. falciparum*'s protein degradation pathways has undeniably shown great promise,

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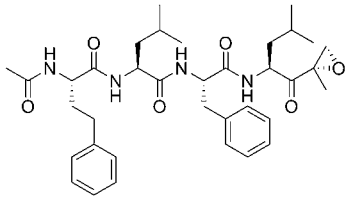
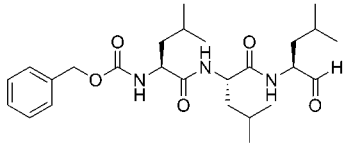
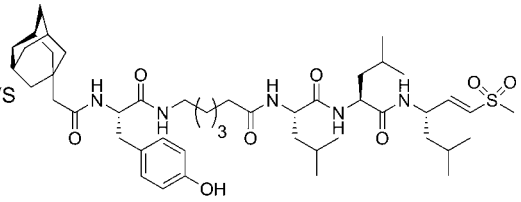
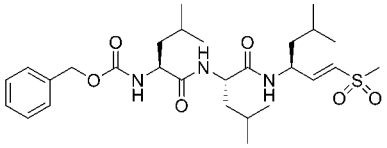
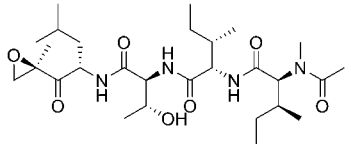
Table 1. IC_{50} Values of Some Proteasome Inhibitors against *P. falciparum* CQ-Sensitive (3D7 and D10) and CQ-Resistant Strains (Dd2)

Drug	Formula	IC_{50} (nM)
YU101		24.5 (3D7) 17.0 (D10) 13.8 (Dd2)
MG132		22.4 (3D7) 33.2 (D10) 17.3 (Dd2)
Ada-Ahx$_3$-L$_3$-VS		300 (3D7) 210 (Dd2)
Z-L$_3$-VS		16.4 (3D7) 4.9 (Dd2)
Epoxomicin		6.8 (3D7) 1.7 (D10) 10.4 Dd2

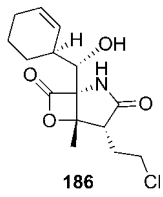


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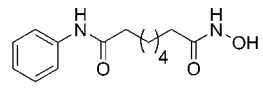
Figure 104. Structure of salinosporamide A (**186**).

suggesting the importance of the parasite's proteasome in DNA synthesis, cell cycle and parasite development.

In eukaryotes, histone acetyltransferases catalyze the transfer of an acetyl group from acetyl-coenzyme A to the lysines' side chain ϵ-nitrogen, while histone deacetylases (HDAC) catalyze the reverse reaction.[252] Because these two processes have been found to play critical roles in a variety of vital cellular functions, such as DNA replication and repair, transcription, cell cycle regulation and differentiation, and cell signaling, HDAC enzymes are validated therapeutic targets for some types of cancers.[252,253] HDAC has also been shown as a crucial transcription regulator in apicomplexan parasites.[254] Since then, several HDAC inhibitors have been found active against *Plasmodium* parasites, as reported by reviews on the subject.[255] For example, Chen et al. have discovered that suberoylanilide hydroxamic acid, SAHA (**187**, Figure 105) and derivatives present potent *in vitro* activity against sensitive (D6) and drug-



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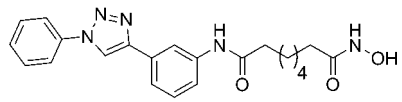

188

Figure 105. Structures of SAHA (**187**) and its optimized triazole derivative (**188**).

resistant (W2) *P. falciparum* strains.[256] SAHA, clinically approved to treat persistent or refractory T-cell lymphoma, inhibits growth of *P. falciparum* D6 and W2 strains with IC_{50} values of 247 and 161 nM, respectively.[256] One of SAHA's derivatives (**188**, Figure 105) was found to be about 14- and 5-fold more active than its parent compound, with IC_{50} values of 17 and 32 nM for *P. falciparum* D6 and W2, respectively.

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Similarly, Marfurt et al. have disclosed that 2-aminosuberic-based HDAC inhibitors present potent *in vitro* activity against sensitive (3D7) and drug-resistant (K1) *P. falciparum* strains.[257] The IC_{50} values for compounds 2-ASA-9 (**189**, Figure 106) and

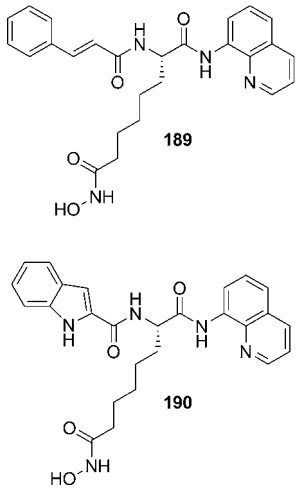

Figure 106. Structures of 2-ASA-9 (**189**) and 2-ASA-14 (**190**).

2-ASA-14 (**190**, Figure 106) are, respectively, 15 nM and 13 nM against the 3D7 strain and 39 nM and 33 nM in the case of the K1 strain. In the same study, the authors have also discovered that these compounds highly inhibit maturation of *P. vivax* schizonts, with IC_{50} values of 503 and 278 nM for 2-ASA-9 and 2-ASA-14, respectively. These results greatly encourage the development of potential candidates to treat malaria in geographical regions where both *P. falciparum* and *P. vivax* are endemic.

SB939 (**191**, Figure 107) is another example of hydroxamate-based HDAC inhibitors with potent antimalarial activity, as

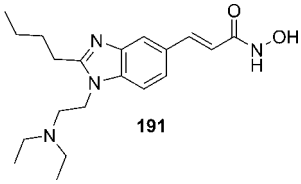

Figure 107. Structure of SB939 (**191**).

reported by Sumanadasa and co-workers.[258] According to these authors, this compound exhibited IC_{50} values of 80 and 150 nM against 3D7 and Dd2 *P. falciparum* strains, respectively. In addition, they also found that SB939 inhibited the growth of *P. berghei* parasites within HepG2 liver cells with an IC_{50} of 150 nM. *In vivo*, when given orally (25 mg/kg twice a day for 3 days) to C57BL/6J mice, the compound significantly disrupted parasite growth, protecting against experimental cerebral malaria-like symptoms.[258] Altogether, these results encourage the development of HDAC inhibitors as a promising new class of antimalarial agents.

Committed to better understand how malaria parasites force the liver cells into submission on a molecular level, Kaushansky and co-workers found that most of the changes caused by *Plasmodia* in infected liver cells highly resemble changes observed when normal cells convert into cancer cells.[259] Using protein lysate microarrays, they found that the pro-death protein p53 was significantly decreased in infected hepatocytes, meaning that the parasite could target this protein to foster proliferation.[259] Thus, the authors hypothesized that boosting p53 activity could counteract its suppression, impeding parasite survival. Nutlin-3 (**192**, Figure 108), a small molecule in clinical

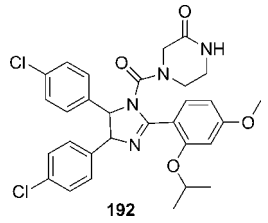

Figure 108. Structure of nutlin-3 (**192**).

development to treat several cancer types, induces apoptosis and growth arrest in several cancer cell lines by selectively binding to the p53-binding region of E3-ubiquitin ligase MDM-2, thus preventing p53 degradation and, consequently, increasing p53 levels.[260] In this context, the authors have confirmed that p53 levels were increased when HepG2 cells were treated with nutlin-3 (20 μM) and that, once the treated cell line was infected with *P. berghei*, liver-stage burden was dramatically reduced.[259] The *in vitro* results were further supported by *in vivo* experiments; a dramatically lower liver-stage parasite burden in BALB/cJ mice treated with nutlin-3 (daily dose of 50 mg/kg for 2 days) was observed .[259] These findings suggest that host pathways might constitute promising targets for antimalarial prophylaxis.

The mammalian kinase target of rampamycin (mTOR) is responsible for altering cellular lipid and protein synthesis as well as autophagy, as a response to integrated signals from oxygen, growth factors, energy, amino acids and stress.[261] Some mTOR inhibitors are being used in the treatment of cancer.[262] In order to evaluate how inhibition of host mTOR signaling would affect *Plasmodium* development, Hanson et al. have tested torins, a structural class of mTOR inhibitors, against liver- and blood-stage malaria parasites *in vitro* and *in vivo*.[263] The results revealed that torin-2 (**193**, Figure 109) presented

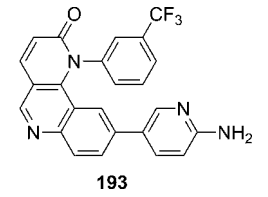

Figure 109. Structure of torin-2 (**193**).

an EC_{50} for asexual blood stage of 1.4 and 0.7 nM against *P. falciparum* 3D7 and Dd2 strains. This compound was also highly potent against *in vitro* development of liver stage *P. berghei* (EC_{50} = 1.1 nM) and early *P. falciparum* gametocytes (EC_{50} = 6.62 nM). Moreover, mice treated with a single oral dose of torin-2 (10 mg/kg) presented a high reduction of *Plasmodium* liver load. The same was observed when mice infected with *P. berghei*-GFP sporozoites were treated with the same dose of torin-2, while control mice became blood-stage positive. It is noteworthy that torin-2 appears to have a novel mode of action against *Plasmodium* parasites, distinct from its ability to target human mTOR. Although the actual target of torin-2 in the parasite is currently unknown, Hanson and co-workers have demonstrated that its antimalarial activity is

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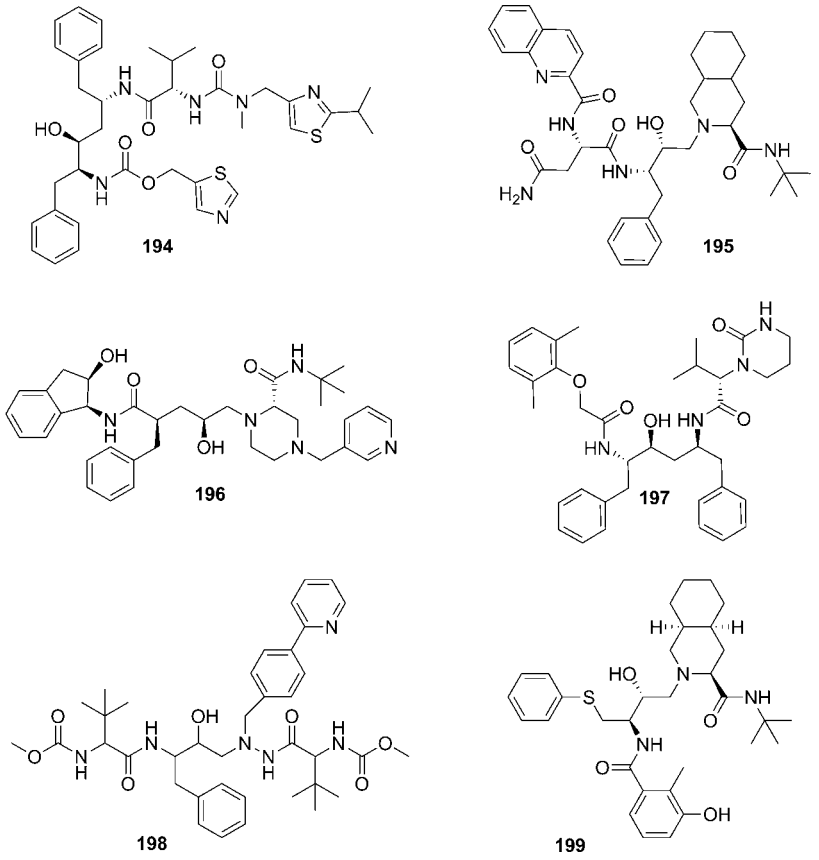

Figure 110. Structures of ritonavir (**194**), saquinavir (**195**), indinavir (**196**), lopinavir (**197**), atazanavir (**198**) and nalfinavir (**199**).

conferred by disrupting the dynamic trafficking of parasite proteins, named upregulated in sporozoites 4 (UIS4) and exported protein 1 (EXP1), to the parasitophorous vacuole membrane inside the infected liver cell, inducing *Plasmodium* elimination by the hepatocyte.

In summary, the sequencing of the *P. falciparum*'s genome permitted researchers to access vital information such as parasite's putative proteins and what types of cellular functions are important to the parasite's biology. Subsequently, several studies indicated that most of such crucial proteins and/or cellular functions were well-established targets in other pathologies, such as cancer, which resulted in the discovery that many antitumoral agents are also able to impair malaria parasite growth. These findings should stimulate both industry and academia to further explore, at low cost, the potential of r&r antitumoral drugs for malaria chemotherapy. However, it is worth noting that although these reports are quite promising regarding the development of antitumoral agents as antimalarials, additional safety studies must be undertaken. Indeed, antitumoral agents are generally considered to be toxic as, at the concentration they are used, in addition to blocking cancer cells, they also impair normal development of healthy cells. But, according to Paracelsus' law, "*sola dosis facit venenum*"; that is, only the dose makes the poison. Therefore, after obtaining potent *in vitro* activities against *Plasmodia* strains, it might be possible that the antitumoral agents could be safely used at the therapeutic window required for antimalarial action. Still, it is imperative to confirm this possibility.

7.2. Antiretrovirals

In response to the HIV/AIDS pandemic in Africa, treatment with antiretroviral agents started to be implemented in sub-Saharan countries where HIV-1 is coendemic with malaria.[264] A short time later, evidence started to suggest that each disease was affecting the outcome of the other, and following, it was found that antiretrovirals decreased CD36 surface concentrations *in vivo*.[265] It is known that mature-stage parasitized erythrocytes adhere to endothelial cells in order to accumulate in the microvasculature.[266] Such adhesion is mediated by interactions between various host receptors, such as CD36, and *P. falciparum* erythrocyte membrane protein 1. In order to test the hypothesis that impairment of CD36 function could directly affect *Plasmodium* parasites and host interactions, Nathoo and co-workers evaluated antiretroviral drugs on nonopsonic phagocytosis by human macrophages and CD36-mediated cytoadherence of parasitized erythrocytes.[265b] The results showed that the protease-inhibitor class of antiretrovirals particularly impaired both processes. Since then, several studies were performed to test antiretrovirals against malaria, with the hope of a possible repurposing of those drugs against this parasitic disease.

For example, Skinner-Adams and co-workers reported that the HIV-1 protease inhibitors ritonavir, saquinavir and indinavir (correspondingly, **194**−**196** in Figure 110) were effective against *P. falciparum* Dd2 *in vitro* at clinically relevant concentrations (EC_{50} = 0.6, 0.4, and 1 μM, respectively).[267] Subsequently, other antiretroviral protease inhibitors were also found to display potent antimalarial activity, such as lopinavir, atazanavir and nelfinavir (correspondingly **197**−**199** in Figure 110).[268] Parikh et al. reported that this latter compound inhibited *P. falciparum* **CQ**-sensitive strains HB3 and D6 with IC_{50} values of 1.4 and 2.0 μM, respectively, while IC_{50} values of 2.1 and 0.9 μM were observed for drug-resistant strains Dd2

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and W2, correspondingly.[268b] In addition, they also found that lopinavir inhibits the *P. falciparum*'s aspartyl protease plasmepsin II at a concentration (IC_{50} = 2.7 μM) near the ones observed for disruption of cultured malaria parasites growth. Although not as potent as lopinavir, atazanavir (IC_{50} = 3.3 and 13 μM) and nalfinavir (IC_{50} = 6.5 and 12.1 μM) were also active against proliferation of *P. falciparum* W2 and 3D7.[268a]

Andrews et al. further investigated the effect of combining some of the HIV-1 protease inhibitors mentioned above, in a murine model of malaria.[269] When *P. chabaudi*-infected mice were treated orally twice daily for 8 days with ritonavir (10 mg/kg)−lopinavir (40 mg/kg) or ritonavir−saquinavir (10 mg/kg each), a significant attenuation of parasitemia and a delay in potency were observed. The mechanism of antimalarial action of these compounds was hypothesized to be inhibition of parasite's aspartyl proteases, plasmepsins.[269] However, Parikh et al. reported that, in contrast to what is observed with pepstatin (known aspartic protease inhibitor), HIV-1 protease inhibitors were more active against *P. falciparum* parasites knocked out regarding their cysteine protease falcipain-2 than against wild-type parasites, and not synergistic with E-64, a cysteine protease inhibitor.[270] In addition, the antiretroviral compounds were equally active against parasites with knocked out plasmepsins and wild-type parasites, suggesting that the antimalarial mechanism of HIV-1 protease inhibitors differs from that of pepstatin.[270] To gain an understanding of how these compounds impair parasite development, Peatey et al. investigated their effects on individual stages of asexual growth.[271] Results showed that schizonts and trophozoites were more sensitive to the compounds than earlier ring-stage parasites. Taken together with the fact that all the drugs inhibited gametocytogenesis, these findings suggest that the primary target of these HIV-1 protease inhibitors is likely to be expressed in both mature intraerythrocytic parasites and early gametocytes.[271]

More recently, Grimberg et al. found that TMC-125 and R278474 (correspondingly **200**−**201** in Figure 111), two HIV-1 non-nucleoside reverse transcriptase inhibitors, presented potent activity against *P. falciparum* W2 (IC_{50}s of 0.5 and 1.1 μM, respectively).[232] They hypothesized that the possible target of these compounds could be a catalytic reverse transcriptase component of the parasite's telomerase, found to be expressed in asexual blood-stages that have started DNA synthesis.[232] As telomerase activity seems to be necessary during blood-stage parasite development, designing specific antitelomerase molecules or screening other reverse transcriptase inhibitors could lead to new antimalarial agents with potent inhibition activity against proliferation of blood-stage malaria parasites.

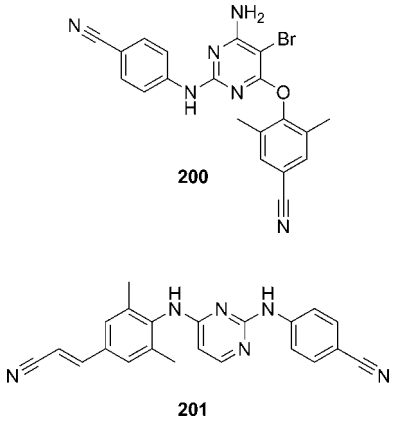

Figure 111. Structures of TMC-125 (**200**) and R278474 (**201**).

Overall, the fact that some antiretroviral agents also inhibit malaria parasite growth suggests that HIV-infected individuals being treated with antiretroviral drugs may also profit from an antimalarial effect. Notice that most countries where malaria is endemic also bear the burden of the HIV pandemic and have increasing access to antiretroviral chemotherapy, making a potential crossover between HIV and malaria treatments undoubtedly important. However, further studies should be performed in order to better understand the potential interactions between therapies for these two infections.

8. FINAL REMARKS

Herein were reviewed representative works that, over the past sesquidecade, have been focused on the discovery of new antimalarial agents by building on already known molecular scaffolds. Three main approaches were distinguished, (i) the "chemical recycling" of classical drugs once considered as first-line antimalarials but which became "fallen angels" of malaria chemotherapy, (ii) the rescuing, for malaria, of known bioactive molecules currently lacking clinical application, and (iii) the repurposing, for malaria, of therapeutic agents in clinical use or development against other diseases. Despite the time span and subject restrictions imposed for the sake of conciseness, the panoply of antimalarial hits, leads, and even candidates reported as emerging from such approaches is quite remarkable. This poses the question of how justified is the design, from scratch, of novel drugs for a disease that majorly affects low-income countries and some remote areas of the globe. Possibly the time has come for a paradigm shift in antimalarial chemotherapy, taking advantage of the huge plethora of known bioactive compounds and active pharmaceutical ingredients (API), either shelved or in clinical use, not necessarily related to malaria; combining such chemical entities with already available high-throughput whole-cell assays for malaria may provide a fast and low-cost way to find antimalarial candidates whose synthesis routes and physicochemical and pharmacokinetic properties have been previously established. Hence, in analogy to the three R's of the environment, perhaps progress in antimalarial chemotherapy should prioritize its own three R's: recycle, rescue, repurpose. After all, have not current first-line artemisinin-based therapies emerged from the recycling of a millenary antimalarial medicine?

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Notes

The authors declare no competing financial interest.

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Nuno F. S. Vale is a Postdoctoral Research Fellow on Gomes's team, at the Faculty of Sciences of the University of Porto, Portugal. He received his Chemistry degree at the University of Porto, Portugal, in 2003, after which he joined the Portuguese Pharma company, BIAL. In 2004, he rejoined Gomes's group to carry out his Ph.D. thesis, devoted to the synthesis, characterization and biological evaluation of peptidomimetic derivatives of the antimalarial drug primaquine. Nuno's research interests are focused on the development of new agents against priority infectious diseases such as malaria, tuberculosis and AIDS. He is the member of the team responsible for training young researchers in solid-phase peptide synthesis and for the technical management of the group's peptide synthesis facility.

Bianca C. Pérez de Lucani is a Postdoctoral Researcher at the Department of Engineering at Aarhus University, Denmark. She received her B.S. in Chemistry degree at Universidad Simón Bolívar, Venezuela, in 2007. She continued her studies at Northeastern University, USA, and then moved to the University of Porto, Portugal, where she recently finished her Ph.D. in Chemistry under Gomes's and Teixeira's guidance. Currently, she is carrying out postdoctoral research studies in lipid chemistry. Her research focus lies in the design and synthesis of compounds of interest in Medicinal Chemistry.

Ana S. M. Gomes is an Assistant Researcher on Gomes's team at the Faculty of Sciences of the University of Porto, Portugal. She graduated in Chemistry in 2011 at the same University, where she also obtained her M.Sc. degree in Chemistry in 2013. After a curricular internship in Gomes's lab, she remained there to carry out an extracurricular internship devoted to the chemical synthesis of potential antimalarial drugs derived from chloroquine. Her M.Sc. thesis, taken under Gomes's and Teixeira's guidance, was focused on the development of chloroquine and quinacrine derivatives as dual-action antimalarials. Her current work follows this same line of research.

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Paula A. C. Gomes is an Associate Professor with Habilitation at the Department of Chemistry and Biochemistry in the Faculty of Sciences of the University of Porto, Portugal. She graduated in Chemistry at the same University, where she also carried out her M.Sc. in Chemistry, under the guidance of Professors Maria Joaquina Amaral-Trigo and Maria Isabel Oliveira Santos. She then pursued her Ph.D. in Chemistry at the University of Barcelona (1997–2000), on the topics of solid phase peptide synthesis and use of surface plasmon resonance sensors for the study of peptide–receptor interactions, under the guidance of Professor David Andreu. She now leads her own team, whose main research interests include antiparasitic agents and membrane-active peptides.

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ABBREVIATIONS

AC, acridine; ACT, artemisinin-based combination therapies; ADMET, absorption-distribution-metabolism-excretion-toxicity; AM1, Austin Model 1 semiempirical method; AMQ, amodiaquine; AOC, acridine orange; AQ, aminoquinoline; B3LYP, Becke's three-parameter hybrid functional; CCL2, CC chemokine ligand 2; CCR2, CC chemokine receptor 2; CCR5, CC chemokine receptor 5; CDRI, central drug research institute; CQ, chloroquine; CRT, chloroquine resistance transporter; DFT, density functional theory; DHFR, dihydrofolate reductase; EXP1, exported protein 1; FQ, ferroquine; EFG, electric field gradient; FQ, ferroquine; EMA, European Medicines Agency; FV, food vacuole; G6PD, glucose 6-phosphate dehydrogenase; HDAC, histone deacetylases; HFG/MET, hepatocyte growth factor/MET kinase; HMEC-1, human microvascular endothelial cell line; HSP, heat shock proteins; Hsp90, heat shock protein 90; JHCCL, Johns Hopkins Clinical Compound Library; MB, methylene blue; MD, molecular dynamics; MDR1, multidrug resistance protein-1; MEP, molecular electrostatic potential; MLCA, multiple linear correlation analysis; MM, molecular mechanics; MOA, mechanism of action; MRC-5, human lung cell line; mTOR, mammalian kinase target of rampamycin; NMR, nuclear magnetic resonance; NQCC, nuclear quadrupole coupling constants; NTD, neglected tropical disease; PDB, Protein Data Bank; PD, pharmacodynamics; PK, pharmacokinetic; PLMII, plasmepsins II; PM, pamaquine; PPQ, piperaquine; PQ, primaquine; PYR, pyronaridine; Q, quinine; QM, quantum mechanics; QN, quinacrine; QSAR, quantitative structure–activity relationships; RA, reversal agent; RPBE, revised Perdew–Burke–Ernzerhof functional based on the generalized gradient approximation; RQ, ruthenoquine; rt, room temperature; r&r, rescuing/repurposing; SAR, structure–activity relationship; SI, selectivity index; UIS4, upregulated in sporozoites 4; vHTS, virtual high throughput screening; WHO, World Health Organization; WWII, World War II

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