“Recycling” Classical Drugs for Malaria

Cátia Teixeira,*†‡ Nuno Vale,† Bianca Pérez,† Ana Gomes,‡ José R. B. Gomes,‡ and Paula Gomes*†

†Centro de Investigação em Química da Universidade do Porto, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, P-4169-007 Porto, Portugal
‡CICECO, Departamento de Química, Universidade de Aveiro, P-3810-193 Aveiro, Portugal

CONTENTS

1. Introduction 11164
2.1. Quinine: Past, Present and Future 11165
2.2. Quinine and the Birth of Major 20th Century Antimalarial Drug Classes 11167
3. Antimalarial 8-Aminoquinolines: Still Seeking for a Suitable Substitute of Primaquine 11167
3.1. From Pamaquine to Primaquine—Targeting Parasite’s Liver and Sexual Stages 11167
3.2. Primaquine-Based 8-Aminoquinolines in Specific Clinical Settings or under Clinical Trials 11167
3.3. Recycling Primaquine: Toward Novel Antimalarial 8-Aminoquinolines 11169
3.3.1. Modifications Exclusively at the Aliphatic Chain of Primaquine 11169
3.3.2. Modifications Involving the Quinoline Ring in Primaquine 11171
3.3.3. Dual-Core Hybrids 11172
4. Antimalarial Acridines: Revival of Quinacrine, The First Synthetic Blood-Stage Antimalarial 11174
4.1. Acridine-Inspired Antimalarial Leads: Acridones and Related Structures 11175
4.2. Revival of Quinacrine and Pyronaridine: In Pursuit of Next Generation Antimalarial Amino- and Aniline-Acridines 11177
4.2.1. 9-Aminoacridine Leads 11177
4.2.2. 9-Anilinoacridine Leads 11178
4.2.3. Organometallic Leads 11179
4.2.4. Quinacrine Hybrids 11180
5. Antimalarial 4-Aminoquinolines: Keeping Track on 21st Century Chloroquine Surrogates 11181
5.1. Historical Synopsis of Clinically Relevant Antimalarial 4-Aminoquinolines 11181
6. Computational Studies on Antimalarial Classics and Their Analogues 11197
6.1. Targeting Inhibition of Hemozoin Formation 11198
6.2. Aiming at Inhibition of Other Parasitic Targets 11202
6.3. Investigating Possible Mechanisms of Drug Toxicity 11204
7. Rescuing and Repurposing Drugs for Malaria 11205
7.1. Antitumorals 11206
7.2. Antiretrovirals 11210
8. Final Remarks 11211

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 that malaria is no longer regarded as a neglected tropical disease 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.” 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 repeatedly 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 pregnant 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).

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. 9 In contrast, resistance to proguanil emerged within only one year after its introduction, 8 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
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

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-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)
Chemical Reviews

Review

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 (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

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/7 days).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 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).
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 was blocked by the fact that it is more toxic than PQ itself. 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 enamino analogue 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. Several studies with both PQ and bulaquine, in India and Thailand, suggested the latter to be safer and more potent than the former, for both prevention of relapse in P. vivax malaria and as a gametocytocidal agent against P. falciparum (25 mg/day/5 days).

One of the most promising PQ derivatives, tafenoquine (21, Figure 9), has successfully completed phase IIb clinical trials by the end of 2013. 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. 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. Still, though tafenoquine has a longer half-life than PQ, it seems to equally cause hematological disorders, so its use in certain patients will possibly be blocked or limited.

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. Tafenoquine and NPC-1161B exhibited IC50 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 (IC50 = 0.5−2.5 μM) against both strains. Like tafenoquine, NPC-1161B has an O-aryl substituent at position 8 of the quinoline ring, and both compounds seem to require metabolic activation by CYP 2D6 as an essential factor to
display antimalarial activity. 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. 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, pregnant people, elderly people, or any person bearing glucose-6-phosphate dehydrogenase (6GPD) deficiency. Moreover, vivax malaria relapses due to failure of PQ-based therapies have been identified. Such failures could be indicative of isolated cases of decreased parasite sensitivity to the drug; 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. 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. 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. Altogether, these aspects demonstrate the need to go 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; 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. With this goal in mind, Gomes and coworkers 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. 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). 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).

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. 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. Remarkably, compounds 26, isomers of imidazoquines 25 developed by the same group, were found to behave as PQ prodrugs.
undergoing hydrolysis to the parent dipeptide derivative of PQ (27, Figure 11) in neutral and basic conditions.\textsuperscript{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/ferroocene 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.\textsuperscript{62} In turn, \textit{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 \textit{P. berghei}; remarkably, the replacement of PQ’s aliphatic chain by hexylferrocene, as in compound 33, led to a \textasciitilde 45-fold higher \textit{in vitro} liver stage activity than that of PQ (IC\textsubscript{50} = 1.25 to >10 \textmu M against \textit{P. falciparum} W2 strain).\textsuperscript{62a} Unfortunately, such a promising result was not confirmed later in \textit{in vivo} assays.\textsuperscript{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 \textit{in vivo} for their gametocytocidal activity on \textit{P. berghei}, and the ethyl and \textit{n}-hexyl derivatives were the most active, and proposed as transmission-blocking leads.\textsuperscript{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.\textsuperscript{65}  

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).\textsuperscript{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 \textit{in vivo} activity of the polyaspartamide conjugates against \textit{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.\textsuperscript{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
The same authors also reported bis(8-aminoquinolines) 44 (Figure 17) with promising antimalarial activity in vitro against drug-sensitive (D6, IC50 = 1.6–4.76 μg/mL) and drug-resistant (W2, IC50 = 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 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.42,44,67 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. yoelii 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. yoelii 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 quinolonic 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 (IC50 = 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

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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.42,44,67 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. yoelii 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. yoelii 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 quinolonic 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 (IC50 = 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, IC50 = 1.6–4.76 μg/mL) and drug-resistant (W2, IC50 = 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 (IC50 = 4.76 and 4.2 μg/mL) were less potent than PQ (IC50 = 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 (IC50 = 0.4–4.76 and 2.0–4.76 μg/mL) and 3 (IC50 = 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 = IC50 VERO/IC50 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 (IC50 = 0.19–2.6 μg/mL; SI > 9.1 to >125) and drug-resistant W2 (IC50 = 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 (IC50 = 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 hematotoxicity 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 IC50 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* (ED50 = 0.26–1.8 mg/kg) and *P. yoelii nigeriensis* (ED50 = 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 (R1 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’
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-schizontocidal (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.”

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 (IC50...
= 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. Unfortunately, additional preclinical studies 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 IC50 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.

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

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

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

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

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

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 μM against *P. falciparum* 3D7, Dd2, and K1 strains, respectively). Interest-

ingly, 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. It is thought 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-Alzheimer, and antileishmanial agents. The most consensual mechanism of action (MOA) of AC derivatives against different diseases is interaction with DNA. However, other MOA have been evoked to explain the antimalarial activity of AC derivatives, such as inhibition of parasite’s (i) mitochondrial bc$_2$ 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 24).

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. 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. QN was widely used as an antimalarial by soldiers during WWII (Pacific...
War) but was soon superseded by CQ, whose efficiency, bioavailability, and safety were considerably superior. 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 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. 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.

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. 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. 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; IC50 = 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.

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 melflquine-resistant, CQ-sensitive strain (D6; IC50 = 11 nM), a CQ-sensitive strain (NF54; 4.4 nM), and a CQ- and pyrimethamine-resistant strain (W2; IC50 = 25 nM). 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 formation in vitro, and its hydrolysis resulted in a ketone product, WR243246 (58, Figure 27; IC50 = 6.1 nM against P. falciparum NF54 strain), whose S-enantiomer (WR249685) also displayed potent in vitro antimalarial activity (IC50 = 15 nM against P. falciparum 3D7 strain) but was unable to inhibit hemozoin formation. Additional computational and experimental studies on these compounds showed their main MOA to be inhibition of the parasite’s cytochrome bc1 complex involving interference with the quinol oxidation site (Qo). This was a relevant finding, as atovaquone (59, Figure 28) is the only bc1 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.
which turns the latter interesting leads into a new generation of antimalarials acting through interference of the plasmodial electron transport chain. Moreover, a bc1 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. Still, despite all the promising features of WR243251, adverse dermatologic, cardiovascular, and neuropsychiatric effects have possibly prevented its clinical development.

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 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 (IC50 = 12.2–58.2 nM against P. falciparum W2 strain) and physicochemical properties.

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.

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 IC50 values of 0.10 μM and 0.07 μM, respectively, against P. falciparum strain D6, being 1000-fold more active than dihydroxyxanthone (62, IC50 > 60 μM). 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 IC50 value (1 pM against P. falciparum D6 strain) and a low level of cytotoxicity (IC50 > 25000 nM against murine splenic lymphocytes); this compound presented a bis(trifluoromethyl)-fluoralkoxy group, somewhat evoking the trifluoromethyl substituent in floxacrine. Later on, the same researchers synthesized acridone derivatives with N-alkylamine substituents in position 10 (64, Figure 30; IC50 = 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. Further results demonstrated that linking an ionizable side chain to position 6 of the 10-N-substituted acridones (65, Figure 30; IC50 = 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.

A similar set of acridones, bearing different 10-N-substitutions (allyl, 3-methyl-2-buthenyl, and 1,2-propadienyl) and a chloride 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 IC50 (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 =...
IC$_{50}$ MRC-5/IC$_{50}$ Pf GHA), its antimalarial activity was low when compared with acridone in inhibit hemozoin formation; instead, it inhibited the aminoalkyl)aminoacridine; this was then reacted with either has been linked to a second aromatic moiety through developed a series of compounds where the acridine core of phenoxyacridine intermediate, followed by nucleophilic ar-

4.2. Revival of Quinacrine and Pyronaridine: In Pursuit of

4.2.1. 9-Aminoacridine Leads. Fair e

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Next Generation Antimalarial Amino- and Aniline-Acridines

Aniline-Acridines

4.2. Revival of Quinacrine and Pyronaridine: In Pursuit of

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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$_{50}$ < 0.0069 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.

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.

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.

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). 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.

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 quinolizidylnalkyl moiety in the amino group of the 9-amino-6-chloro-2-methoxacridine core, aiming at improving the drug’s bioavailability and pharmaco-kinetics; 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. 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. This last aspect may be relevant to confer

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

**Scheme 2.** Synthetic Route toward Chibale’s Sulfonamide (67) and Urea Derivatives (68) of QN100
selectivity to antimalarial compounds such as 70, as
topoiso merase VI is a unique type of the topoiso merase 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 (IC50 = 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 (IC50 = 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 (IC50 = 34 nM) against P.
falciparum K1.109 The compounds were able to inhibit both
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 potent in vitro (IC50 = 4.21–6.97 nM; SI = 295.02–2896.02) as CQ (IC50 = 8.15 nM; SI = 8983) against P. falciparum 3D7 strain.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. 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 IC50 = 0.30 to 4.8 µM against P. falciparum 3D7 (SI = 13.5–333) and from IC50 = 0.15 to 4.5 µM against P. falciparum Dd2 (SI = 10–520), still below those of reference CQ against both strains (IC50 = 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 IC50 values of 1 nM and 8 nM against 3D7 and K1 P. falciparum strains, respectively.

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 be more potent (IC50 = 0.02 µM) than the same complex with CQ (IC50 = 4.41 µM) against P. falciparum K1. Additionally, 83 was also found to be the least cytotoxic (CC50 = 7.26 µM) from a total of five cobalt complexes synthesized by Ajibade and co-workers.117

Most recent organometallic derivatives of QN have been inspired in antitumoral cisplatin. The fact that 9-aminoacridines 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. 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 (IC50 = 0.18–0.34 µM) but did not reach nanomolar activities against the P. falciparum FCQ-27 strain.119

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).111–114

![Figure 36](image)

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

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

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

![Figure 37](image)

![Figure 38](image)

![Figure 39](image)
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) exhibited potent activity against \( P. falciparum \) strain \( \text{FcB1R} \) (IC\(_{50} = 17 \text{ nM} \)) 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 \textit{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 \( \mu \text{g/mL} \)) by its \( m \)-phenylenediamine isomer in 88 (MIC = 0.25 \( \mu \text{g/mL} \)).\(^{121} \) Although promising, none of the compounds (0.25 < MIC < 1 \( \mu \text{g/mL} \)) performed better than the reference CQ (MIC = 0.125 \( \mu \text{g/mL} \)).

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 41).\(^{123} \) 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 \textit{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
Scheme 3. Possible Mechanism for Trioxane Unit Degradation

\[
\begin{align*}
&\text{Fe(II)} \\
&\text{O} \\
&\text{O} \\
&\text{O} \\
&\text{Fe(III)} \\
&\text{O} \\
&\text{O} \\
&\text{O} \\
&\text{Fe(II)} \\
&\text{O} \\
&\text{O} \\
&\text{O} \\
&\text{Fe(III)} \\
&\text{O} \\
&\text{O} \\
&\text{O} \\
&\text{Fe(II)} \\

\text{Conditions: FeBr}_2 (1 \text{ equiv}); \text{THF}; \text{rt.}
\end{align*}
\]

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

Figure 43. Structure of sontochin.

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. 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 profile. In the early 1940s, this compound advanced to clinical trials in Tunisia, jointly conducted by German and French researchers. 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. 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.

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 hematia biocrystallization (hemozoin formation), generating an inhospitable environment for parasite survival. 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. 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. Recent studies have associated parasite’s resistance to CQ to genetic changes in transporters, the P. falciparum CQ resistance transporter (PfCRT), and the P. falciparum multidrug resistance protein-1 (PfMDR1), which decrease the accumulation of CQ at its site of action, the parasite’s acidic FV.

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 PfCRT- and PfMDR1-mediated resistance. 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. 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. 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.
compound, piperazine (PPQ, Figure 44), was discovered as a promising antimalarial during a drug screening campaign, and developed for clinical use in 1973. 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. 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. 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. 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. 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. 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.

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, 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. 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). Similarly, Krogstad and co-workers 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

![Figure 44. Major antimalarial 4-AQ candidates that emerged from CQ.](image1)

![Figure 45. Main SAR established for antimalarial 4-AQ.](image2)
They also observed that compounds with a side chain length of $ \leq 3 $ or $ \geq 10 $ displayed better activity against CQ-resistant strains.\textsuperscript{140a} The aforementioned promising results of AQ-13 reported by Krogstad’s group motivated several researchers to pursue short side chain analogues of CQ.\textsuperscript{141} In this context, Guy et al. developed a synthetic pathway to obtain a moderate library of side chain modified 4-AQ (Scheme 4).\textsuperscript{141d} SAR of these derivatives demonstrated that when one of the alkyl functionalities of the terminal amine was held constant as a propyl (such as in 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.\textsuperscript{141c} Compounds were obtained following the multistep procedure (Scheme 4). Initially, the condensation of the appropriate aniline with Meldrum’s acid and trimethylorthoformate 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 nucleophile 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{141b} Particularly, compounds presenting variations of the $ \alpha $-aminocresol moiety (95a–h, Figure 46) displayed an IC$ _{50} $ below 5 nm against P. falciparum 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-substituted-4-AQ using as substituents diaryl ethers, biaryls, and alkylaryl (correspondingly compounds 96–98 in Figure 47).\textsuperscript{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 D7 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
parasites while presenting SI values ranging from 22.7 to 261.9 (SI = EC50 HepG2/EC50 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 efficacy (IC50 = 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 adamantane moiety.143d SAR demonstrated that the use of the amide functionality to link the 4-AQ either to the steroidal or adamantane 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 W2 strain (IC50 = 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 IC50 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 (IC50 = 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
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.\textsuperscript{144a}

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

Following the same line of thought, Chauhan et al. recently reported a series of tetrazole derivatives of CQ.\textsuperscript{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 multi-component 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.\textsuperscript{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.\textsuperscript{145} Gemma et al. synthesized a series of hydrazone derivatives 109 (Figure 51) and tested their antimalarial properties.\textsuperscript{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
quinoxine 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, 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 functionality led to a decrease in the antiplasmodial activity. Amino group in the 4-position of the quinoline ring by alkylthio or alkoxy substituents (113, Figure S2) increases selectivity against CQ-resistant over CQ-susceptible parasites, it decreases the antiplasmodial activity of the compounds. According to pK_d determinations, the introduction of these two moieties into the 4-position of the quinoxine 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 can explain the generally better inhibitory activities displayed by these analogues when compared to the CQ derivatives 113 with X = O or X = S (Figure S2).

Wolf et al. synthesized and evaluated different 4-amino-7-chloroquinoxinolyl-derived amides, sulfonamides, ureas, and thio-ureas 114–118 (Figure S3) against CQ-sensitive (HB3) and CQ-resistant (Dd2) strains. 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. For example, Sparatore and co-workers obtained potent antimalarial CQ derivatives by incorporating a heteroaromatic group in the side chain (119–120 in Figure S4). 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–

**Figure 52.** 4-AQ derivatives 111–113 developed by Roepe et al. A 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 quinoxine ring by alkylthio or alkoxyl substituents (113, Figure S2) increases selectivity against CQ-resistant over CQ-susceptible parasites, it decreases the antiplasmodial activity of the compounds.
13.4 nM against D10 strain and IC50 = 20.9–26.5 nM against W2 strain) than CQ and had moderate cytotoxicity (IC50 = 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 IC50 values ranging from 19 to 50 nM.149c Results showed than none of the tested compounds 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 (IC50 = 0.9 nM on D6 strain) and multidrug-resistant strains (IC50 = 1.4 nM on Dd2 strain) and in vivo efficacy in P. yoelii 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 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

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

13.4 nM against D10 strain and IC50 = 20.9–26.5 nM against W2 strain) than CQ and had moderate cytotoxicity (IC50 = 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 IC50 values ranging from 19 to 50 nM.149c Results showed than none of the tested compounds 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.

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

**Figure 56.** AMQ derivatives 123–125 developed by Sergheraert and co-workers.152
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 IC50 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 cytotoxicity 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 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 IC50 value of 17.63 nM, and displayed an excellent oral in vivo ED50 and ED90 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 (IC50 = 22 nM against P. falciparum K1 strain), low toxicity in in vitro studies (IC50 = 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 against the multidrug resistant Dd2 strain (IC50 = 15–31 nM) higher or comparable to the parent drug (IC50 = 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 (IC50 = 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...
comparable to their parent compound against CQ-sensitive (Dd6) and CQ-resistant (W2) strains, with IC50 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 IC50 values ranging from 0.75 to 11.6 ng/mL. Despite the potent in vitro antimalarial activity displayed by the most active derivative 129a (IC50 = 0.3 and 0.4 ng/mL against Dd6 and W2 strains, respectively), the compound did not show significant in vivo activity against 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.

Melnky 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](image)

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 (IC50 = 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 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 IC50 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 reviewed165 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 (CH2)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 IC50 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
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. Most compounds (Figure 62) were as potent as CQ against a CQ-susceptible (D10; IC50 = 37.38−128.59 nM) strain, while displaying significantly superior antimalarial activity against multidrug resistant (Dd2; IC50 = 35.49−72.88 nM) parasites. Derivatives exhibited cytotoxicity upon Chinese hamster ovarian cell line, with IC50 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.

Bavari and colleagues recently reported a very different family of bis-4-AQ (compounds 137, Figure 63). 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. Also, introduction of morpholine moieties further improved antiplasmodial activity. The most potent antimalarials, compounds 137j and 137l−m, displayed IC50 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 IC50 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.

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

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. Although most of the compounds 138 exhibited low IC50 (below 100 nM) against CQ-sensitive (HB3) and CQ-resistant (Dd2 and W2) strains, they presented high IC50 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 IC50 and IC90 (<40 nM) values against all tested parasite strains. This study demonstrated that compounds with the ferrocene moiety...
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 evaluated against CQ-sensitive (NF54) and CQ-resistant (K1) strains, and it was found that compounds 140 were the most active in vitro, with IC₅₀ 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 antimalarial activity was slightly lower compared to parent FQ. Biot et al. hypothesized that this decrease in antimalarial 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 IC₅₀ and IC₉₀ values in the low nanomolar range (<100 nM). In addition, these derivatives were also found to present high cytotoxicity (IC₅₀ = 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 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 assess the influence of the metal center on the antiprotozoal activity, they included a cyrhetrene analogue in the study. Compound 145 displayed good activity (IC₅₀ = 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 IC₅₀ values of 0.27 and 0.37 μM, respectively. The in vitro cytotoxicity of both compounds was assessed upon human macrophage cells with IC₅₀ 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.25,123 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 alkylation 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 IC₅₀ 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 dicarboxyl 146d (IC₅₀ = 8–21 nM) and its base analogue 146b (IC₅₀ = 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
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 (IC50 = 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 (IC50 = 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 (IC50 = 69 nM) or mature gametocytes (IC50 = 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 (IC50 = 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), 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; IC50 = 12.18−201.38 nM) and resistant (Dd2; IC50 = 17.12−275.99 nM) strains than CQ, with hybrid 149d displaying the best antimalarial activity (IC50 = 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 IC50 values. The in vitro cytotoxicity of derivatives 149 was also assessed against the Chinese hamster ovary cell line, presenting IC50 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 IC50 values ranging from 19 to 35 nM. Compound 150 was also found to share the same MOA with both artesinim and CQ, as the tested...
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 PfCRT. Based on the identification of structurally diverse molecules as reversal agents (RA), known to inhibit PfCRT, 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 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 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...
indicated that these compounds seem to act similarly to 
CQ. This suggested that the RA motif contributes to 
the activity by increasing their accumulation in parasites. Some of the 
compounds were tested in vivo, and those results pointed to 
hybrid 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 
(Figure 75), conjugating the 4-AQ core and a clotrimazole- 
like moiety.182 The synthesis and evaluation of were 
motivated by promising antimalarial 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 were 
found to inhibit several P. falciparum strains with IC50 
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 
like the C7 position generally displayed better activities than their 
analogues. Conjugate was identified as the most active 
compound of the series, displaying activity in the nanomolar 
range (IC50 = 12–65 nM) against CQ-sensitive (3D7 and D10) 
and CQ-resistant (W2 and Dd2) parasites, moderate in vitro 
toxicity (IC50 = 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 hetero- 
cycle in the benzhydryl functionality.184 Such led to compound 
(Figure 76), which showed (i) better activity against β- 
hematin formation compared to CQ, (ii) potent activity against 
CQ-sensitive (IC50 = 17–62 nM) and CQ-resistant (IC50 = 
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.

The development of novel 4-AQ-based dual-action com- 
pounds has also been addressed by functionalizing the lateral 
chain of CQ with a triazine group, the core of cycloguanil that 
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 (Figure 77), on which 
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 resulting in 
nanomolar activities (IC50 = 4.43–256 ng/mL) against the 
CQ-sensitive 3D7 strain, although none of the compounds 
surpassed CQ activity (IC50 = 2.6 ng/mL).111c The most active 
compounds of the series, 155a–b, presented an IC50 of 7.15 
and 4.43 ng/mL in vitro, respectively, and SI values of 328.61 
and 481.48 (SI = IC50 VERO/IC50 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 (Figure 78) with antimalarial activity (IC50 = 5.2– 
164.0 ng/mL) against CQ-sensitive P. falciparum 3D7 strain 
and CQ-resistant (IC50 = 17–62 nM) parasites, and promising pharmacokinetic properties.

Further optimization studies on the 4-AQ/clotrimazole 
hybrids were performed by modifying the protonable hetero-
cycle in the benzhydryl functionality.184 Such led to compound 
(Figure 76), which showed (i) better activity against β-
hematin formation compared to CQ, (ii) potent activity against 
CQ-sensitive (IC50 = 17–62 nM) and CQ-resistant (IC50 = 
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 
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The development of novel 4-AQ-based dual-action com- 
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Figure 75. Structure of CQ/clotrimazole hybrid compounds developed by Gemma and co-workers.182

Figure 76. Structure of CQ/clotrimazole derivative developed by Gemma et al.184

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

Figure 78. Structure of CQ/triazine hybrid compounds developed by Chauhan and co-workers.185
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. 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) 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. 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, Kaiser and colleagues engaged in the synthesis of CQ/astemizole hybrids 158–161 (Figure 80). 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). 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
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.\textsuperscript{189a} Moreover, the replacement of the diaminoalkyl side chain by a less basic alkoxy linker resulted in lower antimalarial activity.\textsuperscript{189a} The lead compound of this series was 162a, as it showed the lowest IC\text{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, \textit{P. falciparum} dihydrofolate reductase, and parasite DNA.\textsuperscript{189b}

Similarly, N’Da et al. have recently reported the development of pyrimidine/4-AQ conjugates, 163–164 (Figure 82), varying the linker joining the two pharmacophores.\textsuperscript{190} The most active hybrid 164 displayed IC\text{50} values of 0.070 and 0.157 \mu M 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\text{50} CHO/IC\text{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 \textit{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.\textsuperscript{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\text{50} values for \textit{P. falciparum} growth \textit{in vitro} ranging from 0.051 to 1.51 \mu M.\textsuperscript{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 strong correlation between the compound activities against the parasites and falcipain-2 inhibition was observed, as hybrids 165–166 only exhibited modest IC\text{50} values (>6 \mu M) 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.\textsuperscript{193} The most promising compound 167a displayed IC\text{50} values ranging from 40 to 90 nM against the...
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. 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.

Following a similar line of thought, Gomes and co-workers later reported 4-AQ-based hybrids 170 (Figure 85) linking through a dipeptide spacer the CQ core to a trans-cinnamic acid motif, capable of inhibiting catalytic Cys residues. 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-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. 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 linker replaced the dipeptide linker. These compounds displayed potent *in 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. 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.

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 degradation process. For example, dipeptides and bis-quinoline compounds were shown to inhibit and target hemoglobin degradation inside infected red blood cells.

Figure 86. Structure of CQ/cinnamic acid hybrid compounds 172 developed by Gomes et al. [195b](#xref195b).
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 the stability of a very large number of possible binding poses. 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.

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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, PQ, 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 14N 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 14N 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 formation203 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 14N and 2H nuclei of isolated 2- and 4-AQ and of their complexes with 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 (logK) measured in aqueous DMSO, the logarithm of the 50% β-hematin inhibitory activity (logBHIA50), 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

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.
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 \( \log K \beta \)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 charge 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 \( \log K \beta \)Mulliken, the results from Nsumiwa et al. suggest that the extension of the atomic charges as introduced by Nsumiwa et al. show that the extension of the quinoline ring is key for the inhibition of \( \beta \)-hematin formation, and that withdrawing groups (NO\(_2\), CN, CI and CF\(_3\)) at position 7 are crucial.\(^{205c} \) Analogues with electron donating groups at position 7 were found to be poor inhibitors of \( \beta \)-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 \( \log K \beta \)Mulliken. In the case of the R = H series, no experimental correlation was found between \( \log K \) and \( \log K \beta \)Mulliken, which suggests that inhibition of \( \beta \)-hematin formation is not directly related to the association between 4-AQ and ferriprotoporphyrin IX in solution. Thus, inhibition of \( \beta \)-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 \( \log K \beta \)Mulliken 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 \( \beta \)-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 hemohin crystal (\( \beta \)-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 \( \beta \)-hematin.\(^{200,207} \) The docked configurations in the work of Buller et al. show that CQ and AMQ stereoelectronically cap onto the \{0,0,1\} face of the \( \beta \)-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–C 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.\(^{206} \)

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

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 parasites. 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.
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 ferrirprotoporphyrin 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* t-lactate dehydrogenase (PfLDH). The model for PfLDH was obtained from a 3D structure of PfLDH complexed with NADH and the oxamate substrate available at the Protein Data Bank (PDB, code 1LDG). From the calculated interaction energies, 173 and 174 are suggested to be weak inhibitors of PfLDH, as happens with CQ. Nevertheless, as found in docking studies to dimeric hematin, the interaction of protonated forms of 174 with PfLDH 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-amino pyrimidine moiety, due to the role of 2,4-diaminopyrimidine drugs in the inhibition of *P. falciparum* dihydrofolate reductase (PfDHFR). These authors considered molecular docking for understanding whether the antiplasmodial activity of the synthesized compounds could or not be attributed to inhibition of PfDHFR. They considered the PDB structure of wild-type *P. falciparum* DHFR-thymidylate synthase (PfDHFR-TS) complexed with WR99210 (lead compound shown in Figure 94), between the ligands and residues of wild type (PDB code: 1J3I) and quadruple mutant (PDB code: 3QG2) PfDHFR. Despite identification of the contacts made by each compound with PfDHFR, 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 directly or through a retro-enantio dipeptide spacer (170–171, Figure 85 in section 5.2.5). 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-NO2), 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.

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. 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). The latter compounds were found to be more active (nanomolar range, *in vitro* assays) against CQ-resistant *P. falciparum* W2 strain.
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).

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 Qo 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 Qo site residues with ligand-host interactions predominantly hydrophobic, but a backbone hydrogen bond from Ser241 to the...
aromatic secondary amine of the ligand was also seen (Figure 97).

![Figure 96. Structural alignment of selected regions from the Q 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.](image)

![Figure 97. Compound 58 (white cylinders) docked at the Q 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.](image)

Importantly, a putative association between the inhibitor and residues 236–241 constituting the E-f 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 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. These authors hypothesized that, in the process of converting O₂ to H₂O₂, 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. 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, S-hydroxy-PQ (16) is known to cause methemoglobinemia and to form H₂O₂ 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 2DSX. Molecular docking shows that compound 16 interacts with the carboxylic side chain of heme through the terminal ammonium group.

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 O₂...hemoglobin--PQ 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 −H₂N−HOOC− local configuration. In the case of the unprotonated complex, and for all possible spin states, a single electron is transferred to O₂ and, since the spin density in compound 16 is zero, it is contributed by Fe(II) forming a local Fe(III)...O₂...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 O₂ comes from 16. 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&rr) relies on the fact that many drug targets are eventually shared by more than one physiological process. This drug “promiscuity” has motivated several groups to pursue drug r&rr with promising results. In addition, the high costs and failure rates to bring drugs to market have led to more and more interest in drug r&rr.

Drug r&rr 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. Another classic example is thalidomide by Celgene, first marketed for morning sickness, and then approved for leprosy and recently for multiple myeloma. 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. 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&rr are particularly valuable for development of medicines against diseases endemic to low-income countries, such as malaria.

Drug r&rr 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. 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. 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. 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. Unfortunately, GlaxoSmithKline withdrew Lapdap in 2008 due to significant reductions in hemoglobin levels of G6PD-deficient patients. 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. The dapsone example highlights how drug repurposing can be derailed by predictable side effects. Thus, although drug r&rr 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. The nonsedating antihistamine astemizole (175, Figure 98) was one of the most promising compounds 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. The two compounds showed efficacy in P. vinckei-infected mice, reducing parasitemia by 80 and 81% when treated with astemizole (intraperitoneal daily dose of 30 mg/m³ for 4 days) and desmethylastemizole (intraperitoneal daily dose of 15 mg/m³) for 4 days), respectively. The same researchers also determined that these two compounds concentrate inside the parasite’s FV and, like antimalarial 4-AQ, inhibit heme crystallization. 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...
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. Since 2008, nearly six million molecules have been screened against blood-stage *P. falciparum* parasites. 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. 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. 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 potent antimalarial activity and favorable pharmacological profile. 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 Primate Research Center, and the Swiss Tropical Institute. After evaluation of approximately 200 analogues, the optimized spiroindolone NITD609 (178, Figure 99) was obtained. This compound is a potent antimalarial candidate that (i) inhibits 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. 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. 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. 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.

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. 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. 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. In a recent study, two antitumoral compounds, Bay 43-9006 and SU-11274 (179 and 180 respectively, Figure 100), were shown to present potent activities against the *P. falciparum* W2 strain with IC$_{50}$’s of 384 and 320 nM, respectively. 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. 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. SU-11274 was designed as an antitumoral that acts as a human ATP competitive inhibitor of the MET receptor tyrosine kinase activity. 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. 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.

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. Paclitaxel (181, Figure 101) is an antitumoral agent used to treat different types of cancers, and it is also known to cause eryptosis. 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*.
injected mice. Results showed that paclitaxel actually increased eryptosis of infected red blood cells and, consequently, decreased in vitro growth of \textit{P. falciparum} at concentrations higher than 0.01 \(\mu\text{M}\). In vivo, a significant decrease in parasitemia was observed on \textit{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. New drugs that inhibit this class of proteins are reaching market approval to treat cancer. In the specific case of \textit{P. falciparum}, heat shock protein 90 (Hsp90) has also been found essential for parasite development during the intraerythrocytic cycle. Thus, in addition to their antitumoral potential, HSP inhibitors have also been evaluated as competitive inhibitors of the ATP-binding domain of \textit{P. falciparum} Hsp90 and for their potential as antimalarials. By screening about 4000 compounds, issued from three different libraries, Shahinas et al. have identified 46 inhibitors of the \textit{P. falciparum} Hsp90 ATP-binding domain.

Harmine (182, Figure 102), a naturally occurring \(\beta\)-carboline alkaloid with antitumoral activity, specifically inhibited the \textit{P. falciparum} Hsp90 ATP-binding domain compared to the human Hsp90 ATP-binding domain. In addition, this molecule also inhibited both sensitive (3D7) and resistant (W2) \textit{P. falciparum} strains with IC\(_{50}\) values of 50.1 and 28.0 nM, respectively, and demonstrated synergistic activity with CQ. 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. The importance of proteasome inhibition as a possible way to also treat parasitic diseases was highlighted by several studies. Following this line of thought, Lindenthal and co-workers found that MLN-273 (183, Figure 103), a dipeptidyl boronic acid proteasome inhibitor, arrests the \textit{P. falciparum} erythrocytic cycle with IC\(_{50}\) values in the low nanomolar range in both sensitive and drug-resistant strains. Two other boronic acid dipeptides, bortezomib (184, Figure 103), approved to treat multiple myeloma, and its analogue ZL3B (185, Figure 103), were then reported as potent inhibitors of four \textit{P. falciparum} strains (3D7, HB3, W2 and Dd2) with IC\(_{50}\) values ranging between 31 and 45 nM. 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) \textit{P. falciparum} strains. 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 \textit{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. 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 \textit{Plasmodium} proteasome complex.

Salinosporamide A (186, Figure 104), a proteasome inhibitor extracted from the marine actinomycete \textit{Salinospora tropica} and currently advancing through clinical trials as an antimiymeloma agent, was found to have an IC\(_{50}\) of 11.4 nM against \textit{P. falciparum} 3D7 strain. The effectiveness of this compound against malaria parasites was further tested in vivo using the \textit{P. yoelii} mouse model. In agreement with in vitro data, infected mice showed a significant decrease in parasitemia when treated with salinosporamide A (130 \(\mu\text{g/kg}\)). Also, biochemical and structural-based analyses validate the hypothesis of the parasite’s 20S proteasome being the primary target of salinosporamide A. Though no human clinical trials were performed to confirm the effectiveness of proteasome inhibitors against human malaria, targeting \textit{P. falciparum}’s protein degradation pathways has undeniably shown great promise.
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. 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. HDAC has also been shown as a crucial transcription regulator in apicomplexan parasites. Since then, several HDAC inhibitors have been found active against Plasmodium parasites, as reported by reviews on the subject. 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-resistant (W2) P. falciparum strains. SAHA, clinically approved to treat persistent or refractory T-cell lymphoma, inhibits growth of P. falciparum D6 and W2 strains with IC₅₀ values of 247 and 161 nM, respectively. One of SAHA’s derivatives (188, Figure 105) was found to be about 14- and 5-fold more active than its parent compound, with IC₅₀ values of 17 and 32 nM for P. falciparum D6 and W2, respectively.
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. The IC_{50} values for compounds 2-ASA-9 (189, Figure 106) and 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 reported by Sumanadasa and co-workers. 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. 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. 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. 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 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. 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. These 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. 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. Some mTOR inhibitors are being used in the treatment of cancer. 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. The results revealed that torin-2 (193, Figure 109) presented 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
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 repurposing 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. 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. It is known that mature-stage parasitized erythrocytes adhere to endothelial cells in order to accumulate in the microvasculature. 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. 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). 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). 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

![Figure 110. Structures of ritonavir (194), saquinavir (195), indinavir (196), lopinavir (197), atazanavir (198) and nelfinavir (199).](https://dx.doi.org/10.1021/cr500123g)
and W2, correspondingly. In addition, they also found that lopinavir inhibits the \textit{P. falciparum}’s aspartyl protease plasmepsin II at a concentration (IC$_{50}$ = 2.7 $\mu$M) near the ones observed for disruption of cultured malaria parasites growth. Although not as potent as lopinavir, atazanavir (IC$_{50}$ = 3.3 and 13 $\mu$M) and nelfinavir (IC$_{50}$ = 6.5 and 12.1 $\mu$M) were also active against proliferation of \textit{P. falciparum} W2 and 3D7.

Andrews et al. further investigated the effect of combining some of the HIV-1 protease inhibitors mentioned above, in a murine model of malaria. When \textit{P. chabaudi}-infected mice were treated orally twice daily for 8 days with ritonavir (10 mg/kg) or atazanavir (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. However, Parikh et al. reported that, in contrast to what is observed with pepstatin (known aspartyl protease inhibitor), HIV-1 protease inhibitors were more active against \textit{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. 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. To gain an understanding of how these compounds impair parasite development, Peatey et al. investigated their effects on individual stages of asexual growth. 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.

More recently, Grimberg et al. found that TMC-125 and R278474 (correspondingly 200–201 in Figure 111), two HIV-1 protease inhibitors, were more active against \textit{P. falciparum} parasites than the ones observed for disruption of cultured malaria parasites growth. Although not as potent as lopinavir, nelfinavir (IC$_{50}$ = 6.5 and 12.1 $\mu$M) were also active against proliferation of \textit{P. falciparum} W2 and 3D7.

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

1 non-nucleoside reverse transcriptase inhibitors, presented potent activity against \textit{P. falciparum} W2 (IC$_{50}$ of 0.5 and 1.1 $\mu$M, respectively). 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. 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.

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?

AUTHOR INFORMATION

Corresponding Authors
*(C.T.) E-mail: ca.teixeira@ua.pt.
*(P.G.) E-mail: pgomes@ic.up.pt.

Notes
The authors declare no competing financial interest.
Biographies

Cátia A. S. Teixeira is a Postdoctoral Research Fellow at the University of Aveiro, Portugal, who works in close collaboration with José R. B. Gomes and Paula A. C. Gomes. She received her Chemistry degree at the University of Porto, Portugal, in 2003, and her M.Sc. in Chemistry at the same University, in 2005. She then moved to France, to carry out her Ph.D. thesis in Computational Medicinal Chemistry at the ITODYS research center of the Université Paris 7 - Paris Diderot. In 2008, she was awarded the L’Oreal France - Unesco 2008 in recognition of the excellence of her doctoral research work. She is now responsible for all computational studies of Gomes’s team, which are aimed at the development of novel anti-infective agents.

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.
José R. B. Gomes is a Principal Researcher (“Investigator FCT”) at the University of Aveiro, Portugal. He graduated in Chemistry at the University of Porto (Portugal), from where he also received his Ph.D. in 2000 on the topic of computational heterogeneous catalysis under the supervision of José A. N. F. Gomes. He performed postdoctoral work in the groups of Francesc Illas and Manuel A. V. Ribeiro da Silva at the Universities of Barcelona (Spain) and Porto. He is the recipient of the Vicente de Seabra medal (2010) from the Portuguese Chemical Society (SPQ). His interests include computational studies of interactions and reactions of molecules at interfaces, and of structural and spectroscopic properties of molecular systems.

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, acride; ACT, artemisinin-based combination therapies; AMDMET, absorption-distribution-metabolism-excretion-toxicity; AM1, Austin Model 1 semiempirical method; AMQ, amodiaquine; AOC, acridine orange; AQ, aminquinoline; 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; MRC5, human lung cell line; mTOR, mammalian kinase target of rapamycin; 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, pamaque; 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, ruthenenoque; 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

REFERENCES


