Review article

Primaquine derivatives: Modifications of the terminal amino group

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A B S T R A C T

Numerous modifications of the well-known antimalarial drug primaquine, both at the quinoline ring and at the primary amino group, have been reported, mostly to obtain antimalarial agents with improved bioavailability, reduced toxicity and/or prolonged activity. Modifications of the terminal amino group were made with the main idea to prevent the metabolic pathway leading to inactive and toxic carboxyprimaquine (follow-on strategy), but also to get compounds with different activity (repurposing strategy). The modifications undertaken until 2009 were included in a review published in the same year. The present review covers various classes of primaquine N-derivatives with diverse biological profiles, prepared in the last decade by our research group as well as the others. We have summarized the synthetic procedures applied for their preparation and discussed the main biological results. Several hits for the development of novel antiplasmodial, anticancer, antimycobacterial and antibiofilm agents were identified.

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Contents

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1. Introduction

Although the development of first-in-class drugs with a novel mode of action is considered to be truly innovative, there are many other approaches used in drug research and development, like follow-on and analogue-based drug discovery, product-related research and platform drugs [1,2]. These strategies could result in lower costs, lower risk of failure, and shorter time to the market. Drug repositioning or repurposing, i.e. finding new indications for existing/approved drugs, is an increasingly utilized strategy in search of novel medicines [3,4], for example antiparasitic [5,6] and antimycobacterial use of antimalarial drugs [7,8]. Repurposing of antimalarial drugs as anticancer agents is particularly interesting, since different classes of antimalarials display either direct or adjuvant activity against cancer, act as sensitivity reversers of resistant tumour cell lines, inhibitors of drug resistance development, or synergistic agents with known anticancer drugs [9–22]. Hydroxychloroquine, chloroquine and other antimalarial agents (artesunate, quinacrine, atovaquone, doxycycline) were evaluated or are currently under evaluation in numerous clinical trials (approximately 150) against different cancer types, alone or in combination with conventional anticancer drugs/treatments [23]. Derivatization of the registered antimalarial drugs, particularly their hybridization with various scaffolds, is also a popular strategy in search of novel biologically active agents with diverse biological profiles [24–32].

We have focused our research on the antimalarial drug primaquine (PQ) and used it as a starting compound for further derivatization. PQ is an 8-aminoquinoline drug, developed and marketed in the 1940s. Today, after more than 70 years, it is still on the World Health Organization's List of Essential Medicines [33]. Primaquine is active against gametocytes and exoerythrocytic forms of all species of the parasite causing human malaria, including chloroquine-resistant *Plasmodium falciparum* [34]. Until the registration of tafenoquine in 2018, it was the only drug available worldwide against the latent liver forms of relapsing malaria caused by *P. vivax* and *P. ovale*. In general, it is considered as a safe and well-tolerated drug. However, applying primaquine at a broad scale is complicated due to the severe toxicity in a subset of patients with an inherited glucose-6-phosphate dehydrogenase (G6PD) deficiency [35]. The other pitfalls of primaquine are quick metabolism, degradation to inactive carboxyprimaquine and altering the treatment outcome depending on CYP 2D6 enzyme activity [36,37].

In order to get the antimalarial agents with improved bioavailability, reduced toxicity and/or prolonged activity, modifications of the primaquine structure, both at the quinoline heterocycle and at the primary amino group, were performed. The main goal of the terminal amino group modifications was to prevent the metabolic pathway leading to the inactive and toxic carboxyprimaquine [38]. The modifications undertaken before 2009 have been reviewed in detail by Vale et al. [35]. Until 2009, two main sets of compounds were described: primaquine imidazolidin-4-one derivatives

(imidazoquines) [39–41] and amino acid/peptide derivatives [42–45] or their combination − primaquine peptide derivatives bearing the imidazolidin-4-one moiety [46,47]. These compounds were evaluated primarily as antimalarial agents, but some of them were tested for anticancer [48] or antifungal activities [49,50]. However, the present review covers various classes of primaquine *N*-derivatives prepared in the last decade by our research group and others with two goals: to get novel antiplasmodial agents (follow-on strategy) or to get novel compounds with different biological activities (repurposing strategy).

2. *N*-modified primaquine derivatives prepared by our research group

Our research group has prepared various classes of primaquine derivatives: amides, carbamates, ureas, hydroxyureas, acylsemicarbazides, bis-ureas, ureidoamides and dicarboxylic acid derivatives. Their general structures are portrayed in Fig. 1 and the structures of the whole library of compounds are given in SI Tables 1–7. Most of the PQ-derivatives were prepared by common synthetic procedures or by the use of 1*H*-benzo[*d*][1,2,3]triazole (BtH) as a synthetic auxiliary. Versatile use of BtH in synthetic organic chemistry is well documented in the scientific literature [51,52]. Our research group also has a huge experience in the benzotriazole-assisted syntheses [53].

The results have been published in 17 scientific papers. Here we have summarized the synthetic procedures applied for the preparation of various classes of primaquine derivatives and discussed the main biological results.

2.1. Syntheses of N-modified primaquine derivatives

2.1.1. Primaquine amide, carbamate, urea and semicarbazide derivatives

We shall start this review with the hybrid molecules bearing primaquine and cinnamic acid (CA) motifs. Cinnamic acid and its derivatives (CADs) are naturally occurring substances with various pharmacological activities: antimicrobial, antifungal, antiviral, antimycobacterial, antiinflammatory and antioxidative [54–59]. The findings of their anticancer [60–62] and antimalarial activities [63] prompted us to prepare the PQ-CAD hybrids of amide and acylsemicarbazide type [64]. Cinnamic acid and the following CADs were used: α-methylcinnamic acid, methoxy, dimethoxy, trimethoxy, methylenedioxy, chloro, fluoro, trifluoromethyl and bis-trifluoromethyl cinnamic acid. The amides **1–11** were prepared by the simple acylation of PQ with CAD chlorides (CADCl) or CAD-benzotriazolide (CADBt) (Scheme 1). On the other hand, PQ-CAD acylsemicarbazides were obtained by acylation of precursor **61** with the corresponding CAD chlorides.

The amides of primaquine and cinnamic acids with alkyl, halogen or nitro substituents at different positions in the benzene moiety (*N*-cinnamoyl-primaquine conjugates, primacins) were
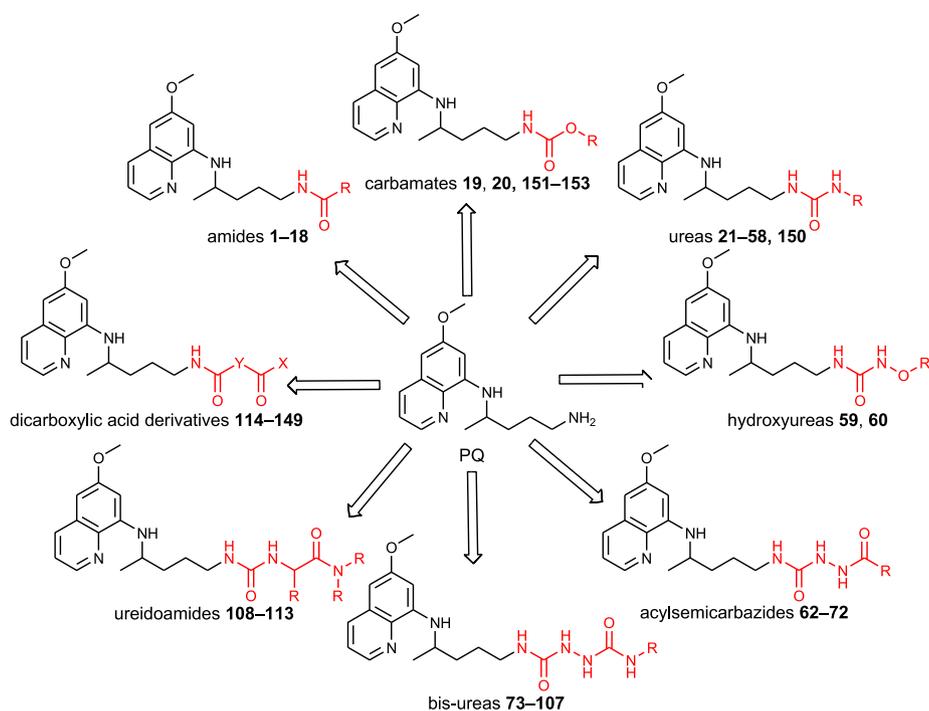
Fig. 1. Chemical classes of the PQ-derivatives prepared by our research group.
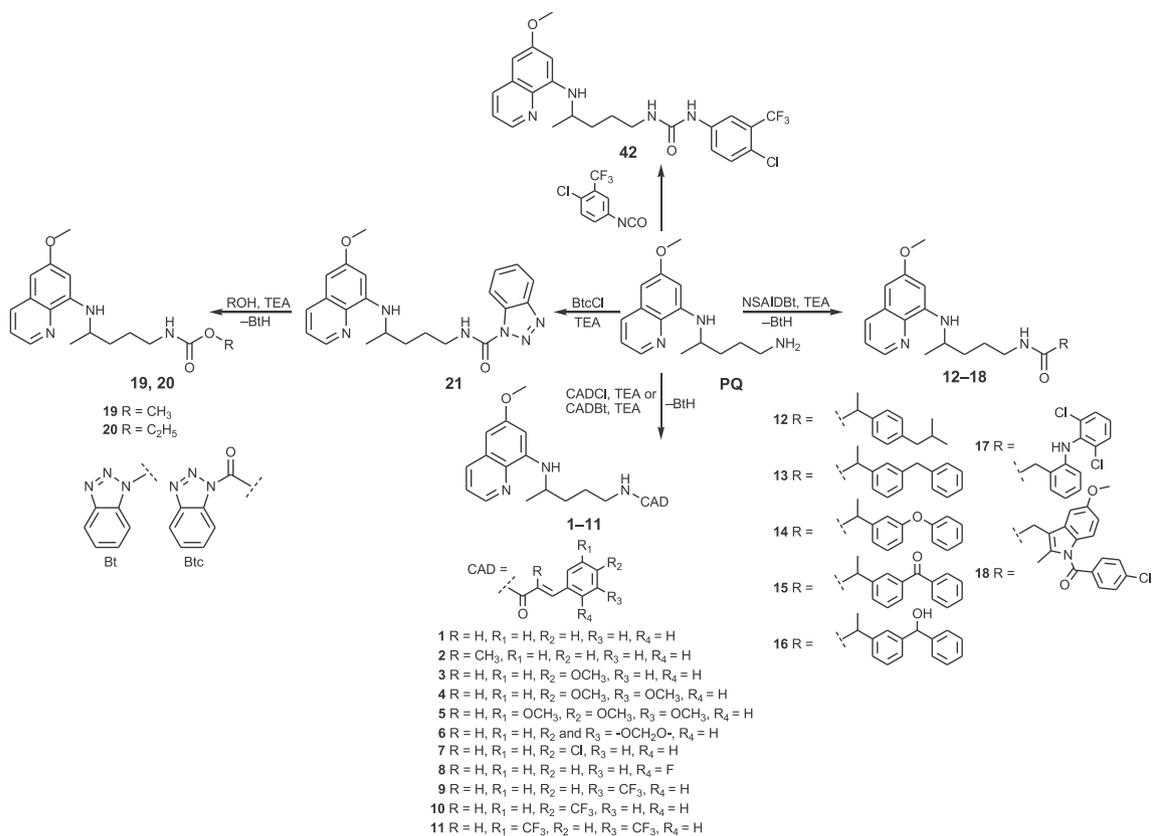
Scheme 1. Synthesis of primaquine amides **1**–**18**, carbamates **19**, **20** and urea **42**.

prepared by Pérez et al. [66]. Primaquine was coupled with the corresponding cinnamic acids in the presence of 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate

(TBTU) and *N,N*-diisopropylethylamine (DIEA). Primacins have shown increased *in vitro* activity against the liver stage of the rodent malaria parasite *P. berghei*, compared to the parent drug

primaquine [66] and only a modest activity against *Leishmania infantum* [67]. They were inactive against erythrocytic stage of *P. falciparum*. In 2017, the same Portuguese research group reported triple-stage antimalarial ionic liquids based on primaquine and cinnamic acids and found them superior to the analogous amide hybrids [68].

In order to obtain potential antioxidative and/or anticancer agents, we have prepared amides **12–18**, conjugates of primaquine with non-steroidal anti-inflammatory drugs (NSAIDs): ibuprofen, ketoprofen, fenoprofen, hydroxy and methylene ketoprofen analogues, diclofenac and indomethacin (Scheme 1) [69]. These derivatives were designed bearing in mind several facts: *i*) inflammation is often accompanied by the excessive formation of reactive oxygen and nitrogen species that are potentially damaging to DNA and cell membranes; *ii*) oxidative stress is a major contributing factor to high mortality rates associated with several diseases, including malaria and cancer; *iii*) long-term use of NSAIDs is associated with a lower risk of some cancer types; *iv*) NSAID derivatives with pronounced cytostatic activity were described in the literature [70–74]. However, the results of biological screening of amides **12–18** were rather disappointing: our compounds exerted moderate activities in the DPPH free radical test and β-carotene-linoleic acid assay and negligible antiproliferative effects, although ketoprofen derivatives **13** and **15** demonstrated a notable Fe^{2+}-chelating ability.

Acylation of primaquine with 1-benzotriazolecarboxylic acid chloride (BtcCl) [75,76] gave derivative **21** (BtcPQ), the common precursor for the synthesis of carbamates **19** and **20** and ureas, respectively (Scheme 1). Alcoholysis of benzotriazolide **21** was successful with methanol and ethanol, but failed with other alcohols [77]. A few years later, several *O*-alkyl and *O*-aryl primaquine carbamates were reported as potential prodrugs that prevent oxidative deamination to the inactive metabolite carboxyprimaquine [78]. The antiplasmodial screening revealed that *O*-alkyl carbamates have the potential to be developed as transmission-blocking antimalarial agents.

Aminolysis of benzotriazolide **21** proceeded under mild conditions, especially in the presence of triethylamine (TEA). More than twenty ureas were prepared (**22–37, 40, 53–58**), including twin derivative **41**, in which two PQ-moieties were linked with carbonyl group (Scheme 2) [77,79–81]. Bis-derivative **41** was previously prepared by Kaur et al. [82]. The reaction of BtcPQ with *O*-benzyl-hydroxylamine gave compound **59**, which afforded *N*-hydroxyurea **60** after catalytic hydrogenation [77]. Finally, compound **21** with hydrazine hydrate gave PQ-semicarbazide **61**, a useful intermediate in the further reaction steps (Scheme 3) [80].

Another set of urea derivatives (**38, 39, 43–52**) was prepared in the opposite way, by the reaction of primaquine with the active ureas BtcNRR₁, obtained from BtcCl and various amines [80,83]. Only urea **42** was synthesized by the classic isocyanate method, e.g. directly from PQ and 4-chloro-3-(fluoromethyl)phenyl isocyanate [80].

Syntheses of bis-urea derivatives were more complex. Two synthetic methods for the bis-ureas growing from the PQ side were applied: *i*) the reaction of the active ureas BtcNRR₁ with the common precursor **61** (bis-ureas **75, 76**) [80]; *ii*) the reaction of various amines with the active bis-urea intermediate obtained from **61** and BtcCl (bis-ureas **73, 74, 77, 78, 80–94, 100–104**) [80,81,83]. When primaquine was used as an amine, symmetric bis-urea **78** bearing two primaquine moieties was prepared. The invert reactions were applied for the synthesis of bis-ureas **73, 74, 95–99, 105**, i.e., the amine component was introduced in the first and the primaquine moiety in the final reaction step. Bis-urea **79** was obtained by the direct reaction of **61** with 4-chloro-3-(fluoromethyl)phenyl isocyanate. Finally, symmetric bis-urea **107** was prepared by the reaction
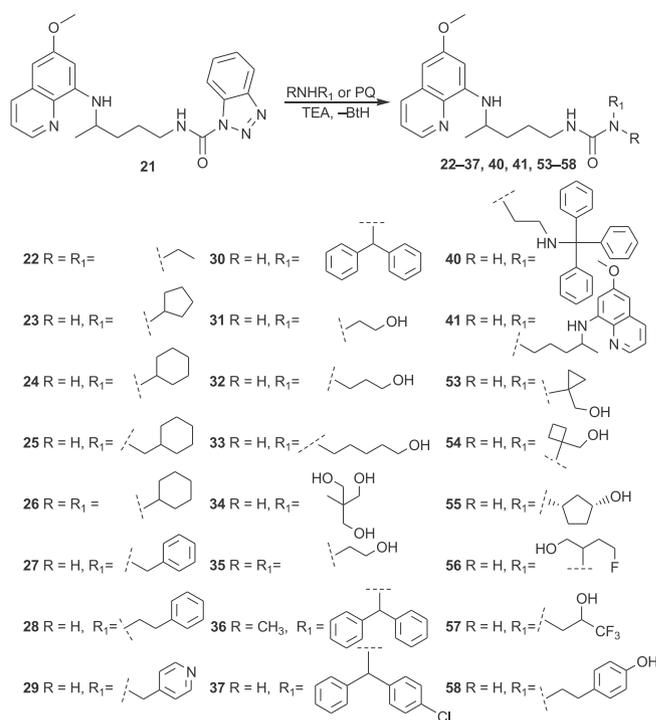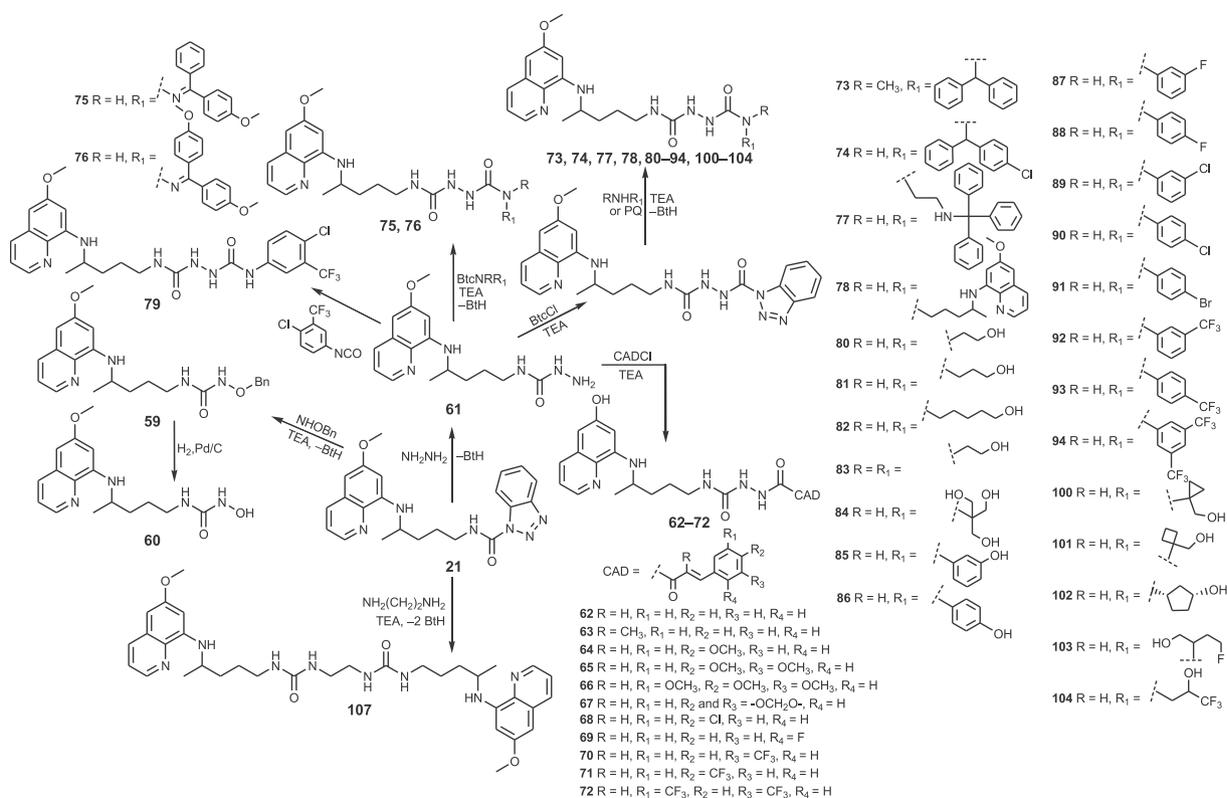

Scheme 2. Synthesis of primaquine ureas.

of BtcPQ **21** and ethylenediamine in a molar ratio 2:1 (Schemes 3 and 4) [77].

In order to obtain urea and bis-urea derivatives of different molecular size, lipophilicity, flexibility, metabolic stability and biological activity, various primary or secondary amines were used: alkyl and cycloalkyl amines [79], amino alcohols of different length and number of hydroxyl groups [77,80], more rigid amino alcohols bearing small cycloalkane moieties, fluoro-substituted amino alcohols [81], amines with one, two or three benzene rings [80,84], aniline derivatives with halogen (F, Cl, CF₃) in *m*- or *p*-position or aminophenols [83]. These modifications were undertaken following common strategies in the drug design and discovery: conformation restrictions might increase selectivity, enhance lipophilicity and bioavailability, while the presence of fluorine atom(s) might increase bioavailability and selectivity [85].

The dimerization of the known antimalarial drugs is an old strategy to overcome drug resistance and improve antimalarial therapy outcomes [86]. One of the symmetric bisquinoline derivatives, piperaquine, was registered in the 1960s and was used in malaria prophylaxis and therapy for more than twenty years [87]. Today, it is approved only in a fixed-dose combination with other antimalarial drugs (dihydroartemisinin or arterolane) [88]. It is considered that bulky bisquinoline compounds accumulate in the cell compartment with a decreased pH gradient, inhibit parasite hem-digestive pathway and contribute to the inhibition of chloroquine efflux transporters [82,89]. The dimerization strategy became attractive in the discovery effective anticancer drugs among antimalarials as well (see for example ref. [90–92]). Having these facts in mind, we have prepared three bis-PQ derivatives (**41, 78** and **107**) with two primaquine moieties connected by linkers bearing one or two urea groups.

2.1.2. Primaquine ureidoamides

A quantitative structure-activity relationship (QSAR) study using the Support Vector Machines learning method yielded a highly accurate statistical model, which was used to prioritize novel

Scheme 3. Synthesis of primaquine hydroxyurea, bis-urea and acylsemicarbazide derivatives.
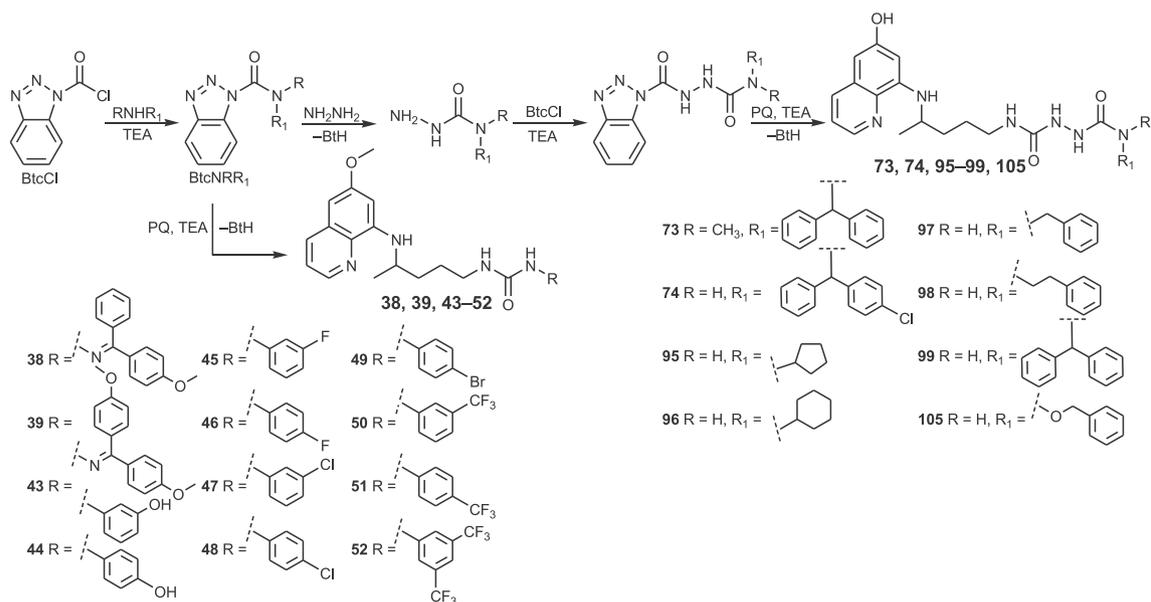
Scheme 4. Synthesis of primaquine urea and bis-urea derivatives.

candidate compounds, PQ-ureidoamides **108**–**113** [93,94]. These compounds were prepared by the reaction of PQ with *N*-Btc-amino acid amides, obtained from *N*-Btc-amino acid chlorides and the corresponding amines or hydrazone (Scheme 5). Three amino acids (L-leucine, D-phenylglycine, D,L-*p*-chlorophenylglycine), four amines (*p*-bromoaniline, diphenylmethanamine, (4-chlorophenyl)(phenyl) methanamine)), *N*-methyl-1,1-diphenylmethanamine and one hydrazone (4-methoxybenzophenone hydrazone) were used.

2.1.3. SAHAquines and related compounds

Our next goal was to prepare SAHAquines **129**–**132**, hybrid molecules that combine moieties of suberoylanilide hydroxamic acid (SAHA), an anticancer agent with weak antiplasmodial activity, and primaquine, an antimalarial drug with low antiproliferative activity (Fig. 2). These compounds were designed as potential histone deacetylase (HDAC) inhibitors and as such, potential cytostatic and/or antiplasmodial agents. Several pan-HDAC inhibitors (SAHA,
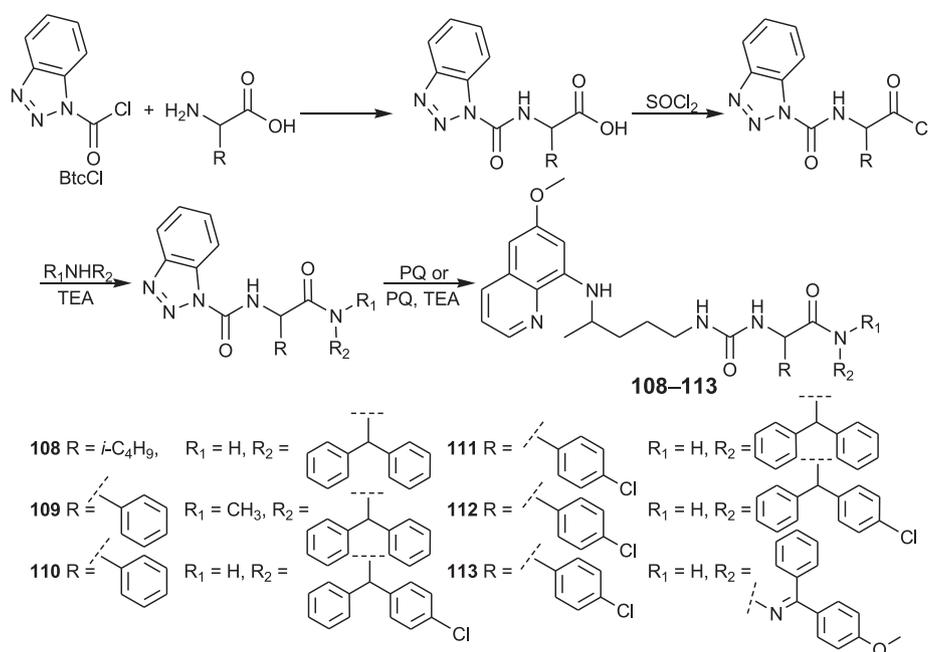
Scheme 5. Synthesis of PQ-ureidoamides **108–113**.

Fig. 2. Dicarboxylic acid derivatives **114–149**.

belinostat, panobinostat), marketed as anticancer drugs, exert antiplasmodial activity as well [95–99]. Their hydroxamic group binds zinc ion in Zn-dependent HDACs, leading to the accumulation of acetylated histones and other proteins [100]. In addition, three Zn-dependent HDACs prone to inhibition by SAHA and related metal-chelating drugs were identified in *P. falciparum* [101,102], thus represent viable targets for drug development [103].
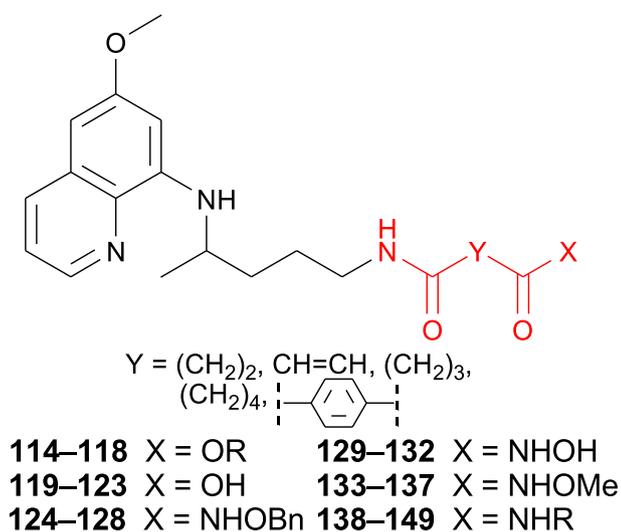
Reaction pathway leading to SAHAquines **129–132** is presented in Scheme 6 [104,105]. In the first reaction step, dicarboxylic acid mono-esters were coupled with primaquine to give products **114–118**, using 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU) as the coupling reagent, along with Hünig's base (DIEA). Basic hydrolysis of esters **114–118** afforded the corresponding acids **119–123**, which were coupled with *O*-benzyl- or *O*-methylhydroxyl amine in the presence of HATU/DIEA and gave the corresponding *O*-

protected hydroxamic acids **124–128** and **133–137**, respectively. SAHAquines **129–132** were obtained by catalytic hydrogenation of *O*-benzyl hydroxamic acids. Derivatives of succinic, fumaric, glutaric, adipic and terephthalic acids were prepared.
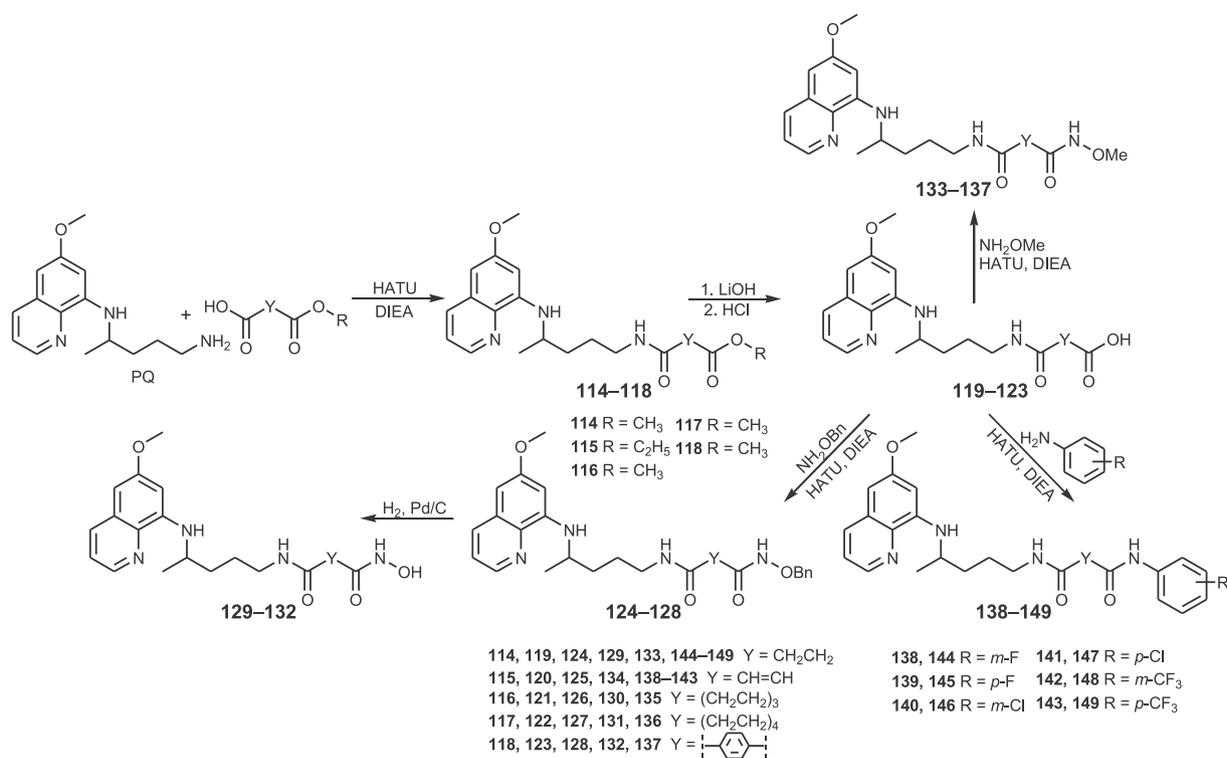
The synthetic procedure shown in Scheme 6 was applied for the synthesis of PQ-halogenaniline asymmetric diamides of fumaric acid (**138–143**) and succinic acid (**144–149**) [106]. Fumardiamides were designed as Michael acceptor analogues of bis-ureas **87–93**, and succindiamides as the saturated counterparts of fumardiamides missing α,β-unsaturated carbonyl group (Michael acceptor moiety). Michel acceptors are often used in the drug design since they might assure the irreversible covalent binding to a cysteine residue of a specific protein [107]. Afatinib, neratinib, osimertinib, ibrutinib and exemestane are examples of registered anticancer drugs with Michael acceptors, while entacapone, dimethyl fumarate, rupintivir and ethacrynic acid are drugs used in the therapy of Parkinson's disease, psoriasis, multiple sclerosis, viral infections or hypertension, respectively. The above mentioned bis-ureas **87–93**, fumardiamides and succindiamides differ only in the central part of the molecule (NHNH, CH=CH or CH_2CH_2), while the PQ-residue and the amine component (3- or 4-fluoroaniline, 3- or 4-chloroaniline, 3- or 4-trifluoromethylaniline) remains unchanged. Structural formulas of bis-ureas, fumardiamides and succindiamides are compared in Fig. 3.

2.1.4. Primaquine-polymer and glyco-conjugates

Primaquine conjugates **150–153** with glucosamine and two polymers of the polyaspartamide type, poly[α,β-(N-2-hydroxyethyl-D,L-aspartamide)] (PHEA) and poly[α,β-(N-3-hydroxypropyl-D,L-aspartamide)] (PHPA), were synthesized (Scheme 7) [108]. The conjugates differed in the type of covalent bonding (urea bond in **150**, carbamate bond in **151–153**), length of the spacer between the polymeric carrier and the drug, molecular mass and drug-loading.

2.2. Biological evaluation of primaquine derivatives

Our main goal was to find potential anticancer agents derived

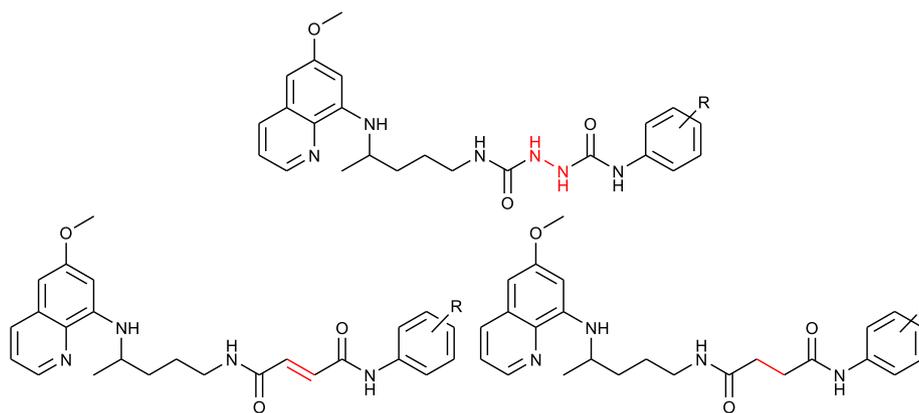**Scheme 6.** Synthesis of dicarboxylic acid derivatives **114**−**149**.

114 R = CH₃ 117 R = CH₃
115 R = C₂H₅ 118 R = CH₃
116 R = CH₃

114, 119, 124, 129, 133, 144–149 Y = CH₂CH₂
115, 120, 125, 134, 138–143 Y = CH=CH
116, 121, 126, 130, 135 Y = (CH₂CH₂)₃
117, 122, 127, 131, 136 Y = (CH₂CH₂)₄
118, 123, 128, 132, 137 Y =

138, 144 R = *m*-F   141, 147 R = *p*-Cl
139, 145 R = *p*-F   142, 148 R = *m*-CF₃
140, 146 R = *m*-Cl 143, 149 R = *p*-CF₃

Fig. 3. Comparison of bis-urea derivatives **87**−**93**, fumardiamides **138**−**143** and succindiamides **144**−**149**.

from primaquine, so the effectiveness of all novel compounds (approximately 150) against various cancer cell lines was evaluated. The second, but not less important goal was to find novel antiplasmodial agents. To get the complete biological profile of novel derivatives, their antimicrobial (antibacterial, antimycobacterial, antifungal, antibiofilm), antiviral, antioxidative potential and, for selected compounds, activity on the central nervous system (CNS) was evaluated as well. In general, bis-ureas and hydroxamic acids were superior in antiproliferative screening, ureidoamides and fumardiamides in antiplasmodial and antibacterial evaluation, ureas and fumardiamides in antimycobacterial, while acylsemicarbazides and fumardiamides in antiviral screening. Several hits, which might present a basis for the discovery of novel drug leads were identified.

2.2.1. Antiproliferative screening

To gain an insight into the antiproliferative profile of the synthesized primaquine derivatives, we evaluated their cytostatic activity *in vitro* against a panel of human cancer cell lines (SI Table 15). The results indicate that scaffold attached to primaquine has a significant impact both on activity and selectivity. Urea and bis-urea derivatives with one or two trifluoromethyl groups, chloro or methoxybenzhydryl moieties or trityl group, and SAHAquines with hydroxamic acid scaffold showed high activity to all tested cell lines, with the highest activity against human breast adenocarcinoma cell line (MCF-7) (for structures and references see Table 1). Urea **33** showed high selectivity towards the colorectal adenocarcinoma (SW620), whereas **29** was active against SW620 and murine lymphocytic leukaemia cell line (L120). However, the majority
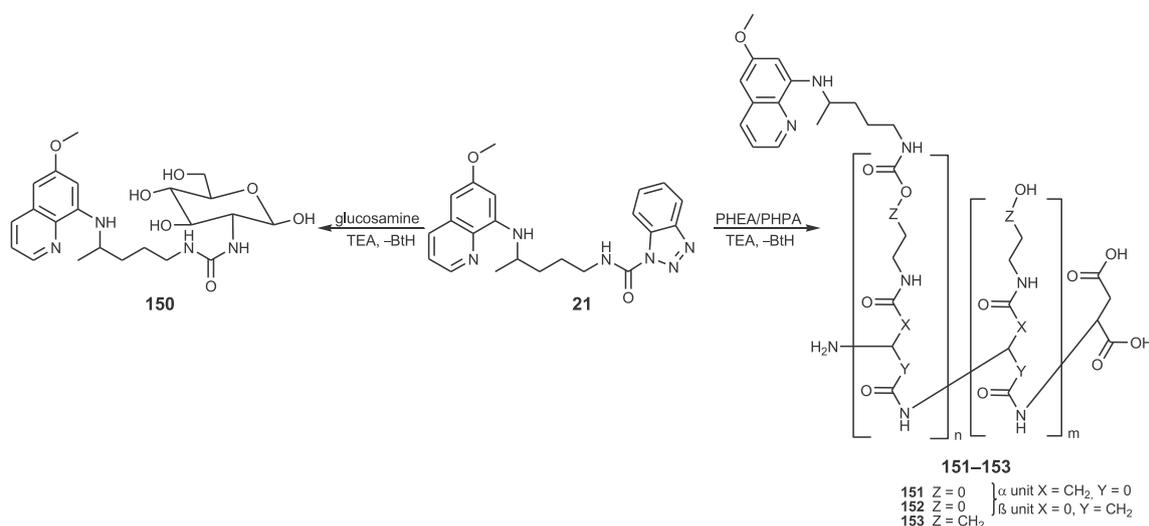
Scheme 7. Synthesis of PQ-glucosamine conjugate **150** and PQ-polymer conjugates **151**−**153**.

of the compounds displayed high selectivity towards MCF-7 cells, e.g. symmetric bis-PQ derivatives **41** and **78** and bis-ureas with hydroxy or halogenphenyl moieties **80**−**82**, **85**−**91**, **106**. Such observation is not surprising since the sensitivity of MCF-7 cell line to primaquine and other antimalarial drugs has been documented earlier [47]. Bis-urea derivatives *p*-F (**88**) and *p*-OH (**86**) substituted benzene or hydroxypentyl (**82**) moieties, and twin primaquine derivative **78** were the most promising compounds in anti-proliferative screening. Due to their high activity against MCF-7 cell line, extreme selectivity and favourable druglike properties, they may be considered as lead compounds in the discovery of breast carcinoma drugs.

In general, ureidoamides **108**−**113** and various dicarboxylic acid derivatives **114**−**128** and **133**−**149** were inactive [93,104], whereas the most active PQ-cinnamic acid derivatives were compounds **8**, **64** and **71**. Amide **8** with *o*-fluorine moiety significantly inhibited the growth of SW620, MCF-7 and cervical carcinoma (HeLa) cells ($IC_{50} = 0.3$−$2.1\,\mu M$), acylsemicarbazides **64** (*p*-methoxy) against MCF-7, L1210, CEM and HeLa ($IC_{50} = 0.4$−$2.4\ 2.4\,\mu M$), whereas **71** (*p*-CF$_3$) showed activity towards MCF-7 cells in the nanomolar scale ($IC_{50} = 0.03 \pm 0.02\,\mu M$) and modest activity towards other tested cell lines [64]. To better understand the underlying mechanisms, the effects of compounds **8**, **64** and **71** on MCF-7 cells were studied [65]. Morphological studies using haematoxylin and eosin staining revealed that compounds **8** and **71** induced morphological changes in cells characteristic for the apoptosis. Cell cycle analysis showed that cells treated with **8** and **71** were in the sub-G1, while cells treated with **64** were mainly in the interphase (G1 phase). The treatment of MCF-7 cells with **8** and **71** resulted in the poly ADP ribose polymerase (PARP) cleavage as well as caspase-9 activation, indicating the induction of apoptotic cell death. Compounds **8** and **71** inhibited the migration and invasion of MCF-7 cells, while compound **64** had marginal effect. Further biological experiments showed that bis-urea **88** induced growth inhibition in MCF-7 cell line through at least two different mechanisms: by induction of the cell cycle arrest in G1 phase and inhibition of the cell cycle progression through transient M phase accumulation and subsequent G2 arrest, and by inducing apoptosis [83]. On the other hand, SAHAquines **129**−**132** containing free hydroxamic acid proved to be HDAC inhibitors. The antibodies against acetylated histone H3K9/H3K14 in MCF-7 cells revealed a significant enhancement following treatment with **130** [104]. We have also shown that SAHAquine **130** selectively inhibits cytoplasmic HDAC6 in nanomolar concentration

without markedly suppressing class I HDACs and suppresses epidermal growth factor receptor and downstream kinase activity, which are prominent therapeutic targets in glioblastoma multiforme [105]. In addition, compound **130** significantly reduces the viability and invasiveness of glioblastoma tumoroids, as well as brain tumour stem cells, which are crucial in the tumour survival and recurrence. These effects are augmented with the combination of SAHAquine **130** with temozolomide, the natural compound quercetin or buthionine sulfoximine, an inhibitor of glutathione biosynthesis.

2.2.2. Antiplasmodial evaluation

Antiplasmodial screening was performed *in vitro* and *in vivo* (only for the selected compounds). Five subsets of PQ derivatives with amide or urea moieties (57 compounds) were evaluated for antiplasmodial activity *in vitro* against the erythrocytic stage of *P. falciparum* NF54 [93,109,110]. Out of them, 33 compounds showed weak activity ($IC_{50} = 10$−$20\,\mu M$) and 7 compounds strong activity in low micromolar range. QSAR analysis of the obtained data yielded a highly accurate statistical model which was used to prioritize novel candidate compounds: PQ-ureidoamides **108**−**113**. These compounds were active against *P. falciparum* while exhibiting a very favourable toxicity profile towards human cells *in vitro*, which was retained even when the glucose-6-phosphate dehydrogenase (G6PD) was inhibited. On the other hand, bis-ureas **90**, **93** and **76** with *p*-chlorobenzene, *p*-CF$_3$-benzene and dimethoxybenzhydryl moieties displayed antiplasmodial activity *in vitro* against liver stage *P. berghei* in the nanomolar scale ($IC_{50} = 42.0$, 74.9 and 82.8 nM) [109]. Compound **93** showed antiproliferative activity in low micromolar concentrations as well. Asymmetric primaquine/halogenaniline fumardiamides **138**−**143** also exhibited high activity against *P. berghei* hepatic stages, higher than the parent drug (IC_{50} values ranging between 0.11 and 0.39 μM; IC_{50} of primaquine $8.4 \pm 3.4\,\mu M$) and moderate activity against *P. falciparum* erythrocytic stages, *Pf*Dd2 and *Pf*3D7 strains ($IC_{50} = 7.74$−$18.43\,\mu M$) [111]. The most active compounds **142** and **143** showed low cytotoxicity towards human cells (MCF-7 and HEK293T), also when G6PD enzyme was inhibited. On the other hand, SAHAquines **129**−**132** showed dual stage antiplasmodial activity which correlated with their cytostatic activity [104]. Finally, blood-schizontocidal activity of primaquine conjugates **150**−**153** with glucosamine and two polymers of polyaspartamide type (PHEA and PHPA) was tested against *P. berghei* infection in Swiss

Table 1
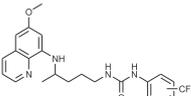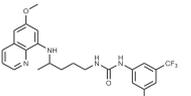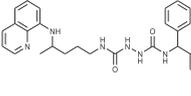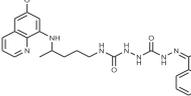The most important outcomes of antiproliferative screening *in vitro*.

Compd.	Structure	IC$_{50}$ (μM)	Ref.
All tested cell lines			
50, 51		1.9−24	[83]
	m- or *p*-CF$_3$		
52		1.6−12	[83]
74		2−9.6	[80]
75		2−18	[80]
77		2−10	[80]
92, 93		0.10−36	[83]
	m- or *p*-CF$_3$		
94		2.4−22	[83]
129−132		1.6−28	[104]
MCF-7			
11		2.6 ± 0.5	[64]
38		5 ± 3	[80]
39		6 ± 2	[80]
41		6 ± 3	[80]
78		0.2 ± 0.1	[80]
80		13 ± 4	[80]
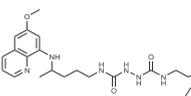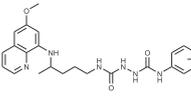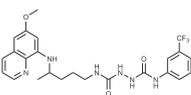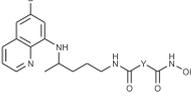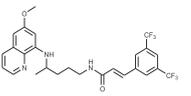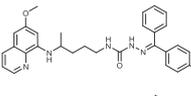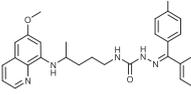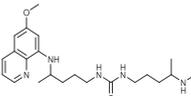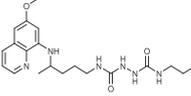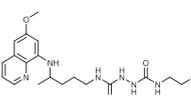
Table 1 (*continued*)

Compd.	Structure	IC$_{50}$ (μM)	Ref.
81		13 ± 0.5	[80]
82		4 ± 0.9	[80]
85−91		0.1−2.6	[83]
	m- or *p*-OH, F or Cl; *p*-Br		
97		1 ± 0.2	[84]
98		4 ± 0.3	[84]
106		3 ± 0.2	[84]
SW620			
29		2 ± 1	[79]
33		0.2 ± 1	[77]
L120			
29		3.0 ± 0.4	[79]
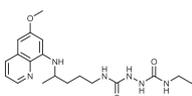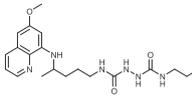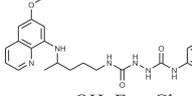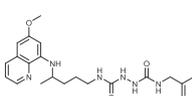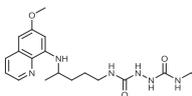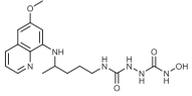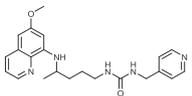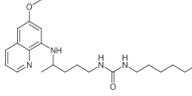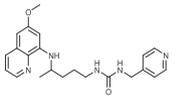
mice [108]. On the day 4, all the mice treated with polymeric conjugates were either aparasitemic or had significantly ($p < 0.001$) lower parasitaemia compared to negative-controls and the glucosamine conjugate. Among polymeric conjugates, compound **152** appeared to be the best with respect to the suppression of parasitaemia.

2.2.3. Antibacterial, antifungal and antibiofilm screening

Numerous quinoline-bearing compounds are well known antibacterial drugs. To mention just the most important ones: 8-hydroxyquinoline, nitroxoline and several generations of quinolone/fluoroquinolone derivatives (norfloxacin, ciprofloxacin, enoxacin, ofloxacin, moxifloxacin, etc.). Based on these facts, the *in vitro* antimicrobial activity of selected PQ derivatives (ureas **43−52**, bis-ureas **85−94**, ureidoamides **108−113**, PQ-fumardiamides **138−143** and succindiamides **144−149**) was carried out against a panel of Gram-positive bacteria, Gram-negative bacteria and fungi [83,94,106]. A list of microorganisms is given in Supplementary Material, SI Table 16. The tested compounds varied in activity (inactive, slightly active or active in low micromolar concentrations, but cytotoxic as well). However, some ureidoamides showed

selective antimicrobial activity without haemolytic and cytotoxic effects (**108** and **111** vs. *Staphylococcus aureus* strain, MIC = 6.5 μg/ml; **110** and **111** vs. *Escherichia coli* and *Pseudomonas aeruginosa*, MIC = 25 μg/ml). In addition, all ureidoamides had high efficiency against the biofilm formation of *E. coli* (the minimum biofilm eradication concentration, MBEC, from 6.25 to 50 μg/ml), and some of them against *P. aeruginosa* and *Candida albicans* [94]. On the other hand, fumardiamides **138**–**143** showed significant antimicrobial activity (e.g. compound **141** vs. *S. aureus, Streptococcus pneumoniae, Acinetobacter baumannii, C. albicans*, MIC = 6.1–12.5 μg/ml). Two microorganisms, namely *E. faecalis* and *S. aureus* showed high susceptibility to all fumardiamides, while *E. coli, S. pneumoniae* and *P. aeruginosa* were susceptible to five out of six fumardiamides. In biofilm eradication assay, majority of the bacteria, particularly *S. aureus*, showed susceptibility to all fumardiamides [106]. The most active compounds were fluoroderivatives **138** and **139** (the lowest MBEC 6.3 μg/ml). A high biofilm eradication potential of fumardiamides might be explained by the reaction of Michael acceptor with cysteine thiol, which could prevent disulfide bond formation. It is a well-known fact that cysteine homeostasis impacts biofilm formation and production of extracellular matrix components, as well as folding and stability of extracytoplasmic proteins [112].

2.2.4. Antimycobacterial evaluation

Taking into account the fact that quinoline or quinolone pharmacophore is present in the antitubercular drugs bedaquiline and ciprofloxacin, antimycobacterial potential of more than seventy primaquine derivatives was evaluated against a number of *Mycobacterium* species (SI Table 16) [81,106,109]. After comparison of their activity and cytotoxicity several hits with MIC values from 2 to 16 μg/ml were identified: *m*-CF$_3$-phenylurea **50**, dimethoxybenzhydryl urea **76**, urea **54** derived from (1-aminocyclobutyl)methanol, urea **58** and bis-urea **86** with phenol moieties [81]. Fumardiamides **139**, **141** and **143** with *p*-fluorine, chlorine or CF$_3$ substituents and bis-CF$_3$ cinnamamide **11** also exerted significant antimycobacterial activity (MIC = 16–64 μg/ml) [106,109].

2.2.5. Antiviral evaluation

PQ-urea derivatives **22**–**30** [79], bis-urea derivatives **95**–**99**, **105**, **106** [84], PQ-cinnamic acid derivatives **1**–**11** and **62**–**72** [64], PQ-fumardiamides **138**–**143** and PQ-succindiamides **144**–**149** [106] were evaluated against a broad variety of viruses (SI Table 17). The ureas with phenylethyl and pyridine moieties (**28** and **29**) showed specific activity against human cytomegalovirus (HCMV) (EC$_{50}$ = 1.2–13.4 μM), while cinnamic acylsemicarbazide derivatives **63**, **67**, **70**–**72** exerted moderate activity against human coronavirus (229E) with EC$_{50}$ values between 7.9 and 15.0 μM. *m*-CF$_3$ and *m*-chloroaniline fumardiamides **142** and **140** showed significant antiviral activity against reovirus-1, sindbis virus and Punta Toro virus (EC$_{50}$ = 3.1–5.5 μM), while **142** was active against coxsackie virus B4 (EC$_{50}$ = 3.1 μM) as well. Bis-urea derivatives, cinnamamides and PQ-succindiamides were inactive towards all tested viruses.

2.2.6. Antioxidative activity

A number of studies have shown the prooxidant effects of primaquine: treatment with primaquine causes an oxidative stress by generating reactive oxygen species within liver, kidney and erythrocytes [113,114]. Therefore, we found it worth to evaluate oxidant/antioxidant ability and lipoxygenase (LOX) inhibition of primaquine derivatives of amide and urea type [64,69,80,83,94]. Two antioxidant assays were used: (*i*) interaction with the stable free 1,1-diphenyl-2-picrylhydrazyl radical (DPPH test) and (*ii*) interaction with the water-soluble azo compound 2,2'-(2-amidinopropane)

dihydrochloride (AAPH) (inhibition of lipid peroxidation, LP). Urea **44** [83], hydroxyurea **60** [77], hydroxy bis-urea **106** [84], semicarbazide **61** [80], PQ-cinnamic acid acylsemicarbazides **65**–**72** [64] and almost all bis-ureas **79**, **85**–**94** [80,83] exerted potent activity, while PQ-NSAIDs **12**–**18** showed moderate antioxidative activity in DPPH test. Ureas **36**–**42** and bis-ureas **73**–**77** with lipophilic substituents showed stronger LP inhibition [80]. PQ-ureidoamides **109**–**113** significantly inhibited lipid peroxidation [94], whereas compound **110**, the primaquine twin drug **107**, cinnamic derivative **65**, methoxy- or chlorobenzhydryl substituted ureas **37**–**39** and bis-ureas **74**–**76** presented higher LOX inhibition [77,80,82,94].

2.2.7. Activity of selected primaquine derivatives on CNS

Semicarbazide/urea PQ-derivatives are possible central nervous system effecting agents as well [115–117]. Selected compounds bearing these features, namely compounds **37**, **74**, **78** and **83** were screened for CNS activity [110]. Several behavioural tests were performed on mice: 'head-twitch' test, nociceptive reactions, influence on body temperature, locomotor activity (spontaneous and amphetamine-induced hyperactivity), motor coordination and pentylenetetrazole-induced convulsions. Compound **78** decreased the body temperature of normothermic mice, inhibited significantly 5-hydroxy-L-tryptophan-induced 'head-twitch' responses and protected mice against clonic seizures. All compounds decreased locomotor activity of mice, while derivatives **74** and **83** increased the hyperactivity caused by the amphetamine administration. All tested compounds showed antinociceptive activity in the 'writhing' test. Compounds **37** and **78** were active in a wide range of doses up to 0.00125 ED$_{50}$ and their antinociceptive effect was reversed by naloxone. In the tail immersion test compound **37** exerted significant antinociceptive effect, which was also reversed by naloxone. None of the compounds caused coordination impairments in the rota-rod and chimney tests.

3. N-modified primaquine derivatives reported by other research groups

Various classes of primaquine derivatives were prepared by other research groups. Their general structures are presented in Fig. 4 and structural formula in SI Tables 8–14. Since these compounds were prepared as potential antiplasmodial agents, their biological outcomes are presented together with their chemical descriptions.

3.1. Primaquine glycosides

Recently, Azad et al. prepared *N*-primaquine glycosides **154**–**161** to overcome the premature oxidative deamination and to increase the life span of the drug in the biological system [118]. The sugar part of the hybrid molecules was thought to direct the drug in the liver, where hypnozoites reside. The title conjugates were prepared by coupling of primaquine with the appropriate monosaccharide: D-glucose (**154**), D-galactose (**155**), D-mannose (**156**), D-arabinose (**157**), L-rhamnose (**158**), D-ribose (**159**), D-xylose (**160**) and *N*-acetyl-D-glucosamine (**161**) (Scheme 8). Compound **155** showed twofold the activity than that of the parent drug in experiments performed on rhesus monkeys.

3.2. Primaquine conjugates with other antimalarial drugs

3.2.1. Primaquine-artemisinin conjugates

Hybrid molecules capable of hitting more than one molecular target are an attractive alternative to currently approved antimalarial drugs. Thus, two antiplasmodial drugs with different modes of action, primaquine and artemisinin, were combined in hybrids
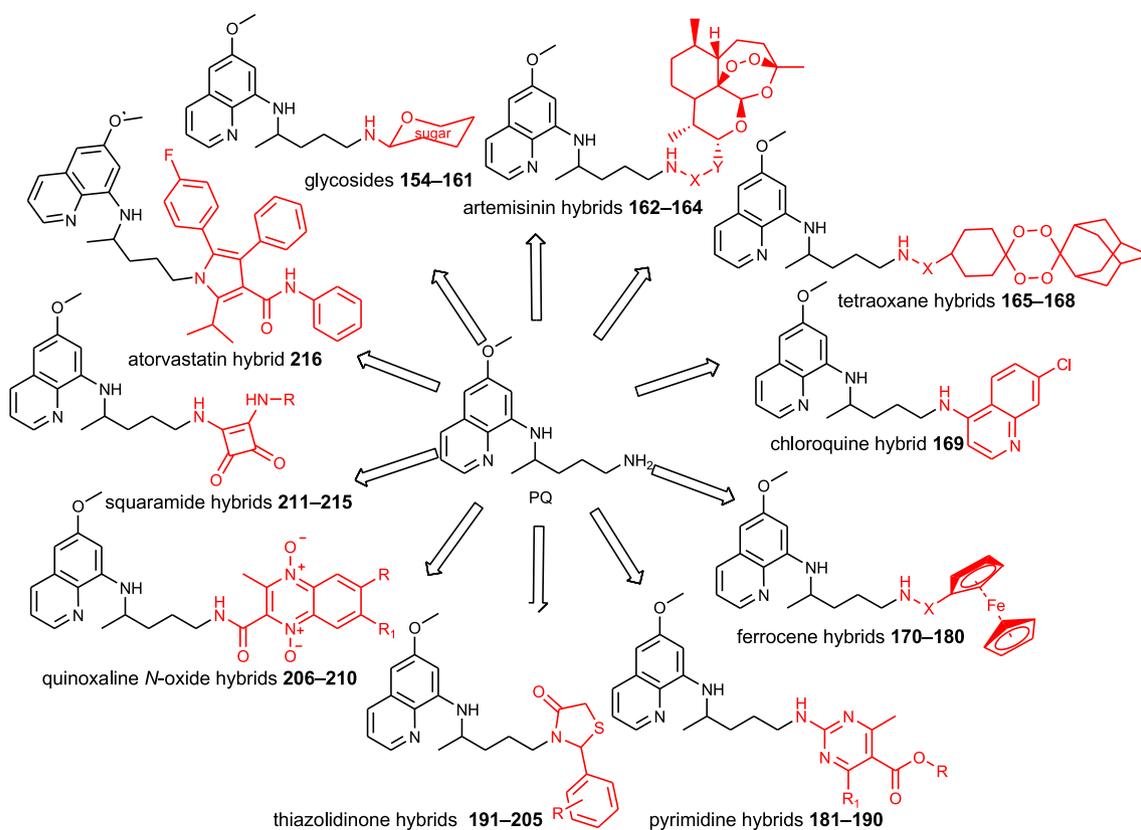
Fig. 4. Chemical classes of PQ-derivatives prepared by other research groups.

154–161

154	D-glucose	**158**	L-rhamnose
155	D-galactose	**159**	D-ribose
156	D-mannose	**160**	D-xylose
157	D-arabinose	**161**	N-acetylglucosamine
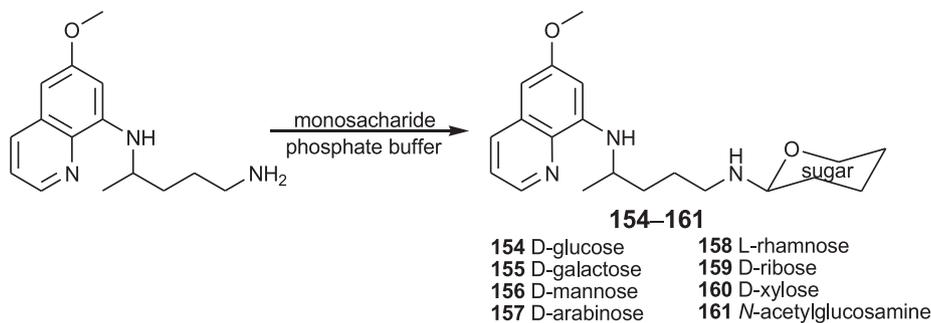
Scheme 8. Synthesis of PQ-glycosides **154**–**161**.

162–**164** [119,120]. Amine and amide covalent bonds between primaquine and artemisinin were achieved according to the procedures presented in Scheme 9.

Hybrid **162** was prepared through several intermediates: artemisinin, dihydroartemisinin, artelinic acid, hydroxamate and corresponding aldehyde, which in reductive amination with primaquine afforded the title hybrid. Hybrid **164** was prepared from 10β-allyldeoxoartemisinin in two reaction steps, involving oxidation of the allyl to corresponding carboxylic acid and coupling of the latter with primaquine. Activities of primaquine-artemisinin conjugates against the liver and blood stages of *Plasmodium* were compared *in vitro* and *in vivo* with those of the parent drugs. Both hybrids displayed enhanced *in vitro* activities against *P. berghei* liver stages and similar activities as artemisinin against *P. falciparum*. In addition, compound **162** showed significant *in vivo* efficacy in controlling parasitaemia.

A small library of hybrid compounds **165**–**168** combining 1,2,4,5-tetraoxane (endoperoxide system similar to 1,3,4-trioxane present in artemisinin) and primaquine or other 8-aminoquinoline moieties was prepared and screened for the antimalarial activity (Scheme 10) [120,121]. These hybrids showed high potency against both exoerythrocytic and erythrocytic forms of malaria parasites and efficiently blocked the development of the sporogonic cycle in the mosquito vector. Compound **165** cleared *P. berghei* infection in mice. Similar hybrids with a trioxane motif (synthetic peroxide that causes damage in the parasites via the production of free radicals) linked to chloroquine and related aminoquinoline entities (which easily penetrate and accumulate within parasites) were also developed and extensively evaluated [122–126].
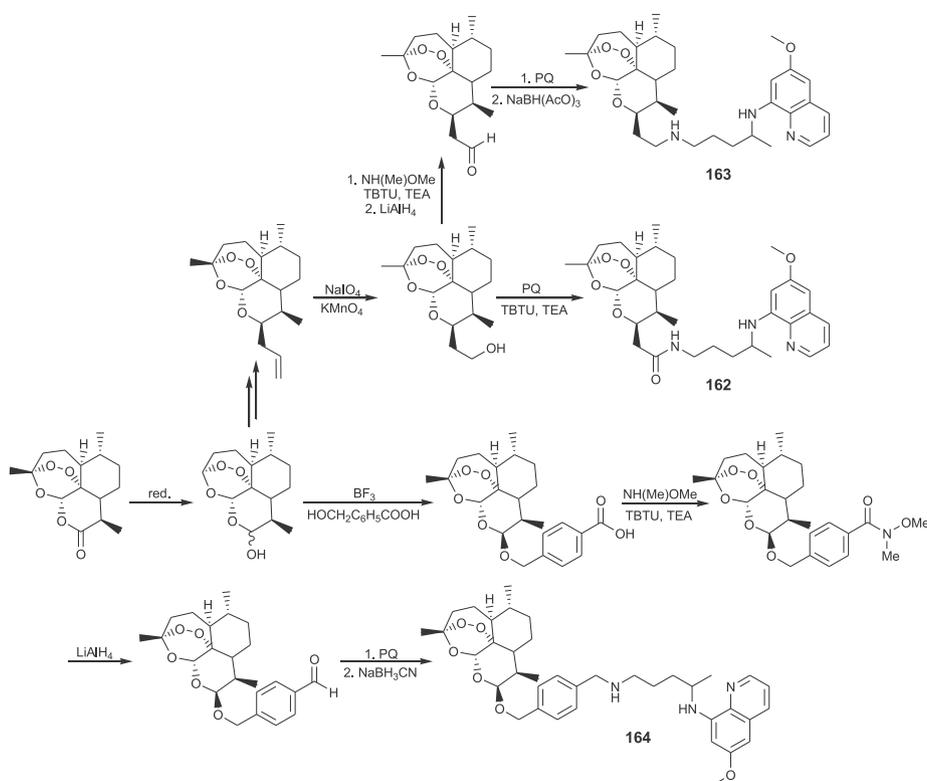
Scheme 9. Synthesis of PQ-artemisinin hybrids **162**–**164**.
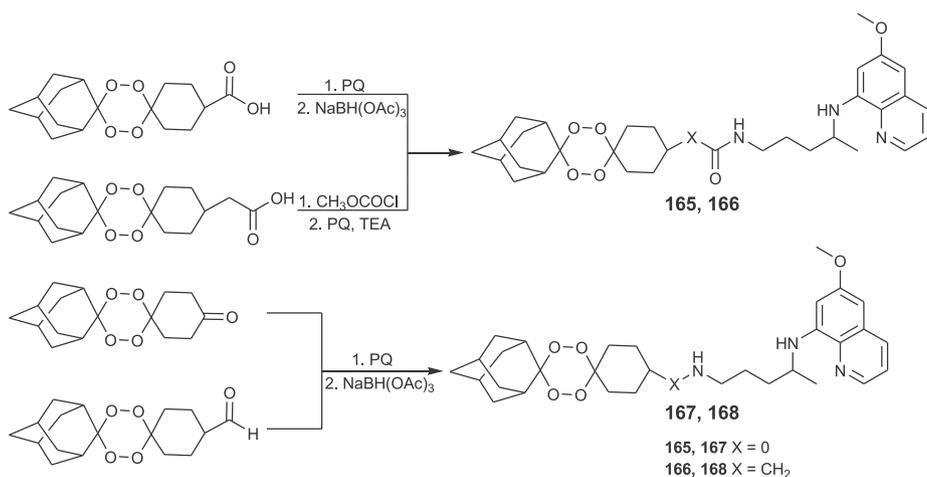
Scheme 10. Synthesis of PQ-1,2,4,5-tetraoxane hybrids **165**–**168**.
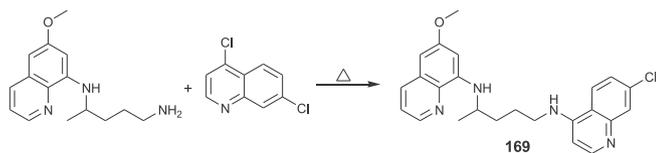
Scheme 11. Synthesis of PQ-chloroquine hybrid **169**.

3.2.2. Primaquine-chloroquine conjugate

Primaquine-chloroquine conjugate **169** was obtained by heating of primaquine with 4,7-dichloroquinoline (Scheme 11) [127].

The conjugate consisting of primaquine and chloroquine components was prepared to validate the concept of utilizing one compound to combine different modes of action that attack different *Plasmodium* stages. Indeed, conjugate **169** showed activity against both *Plasmodium* liver and blood stages, high activity against chloroquine resistant *P. falciparum* strain K1 and was superior to the equimolar combination of the parent drugs.

3.3. Primacenes − primaquine-ferrocene conjugates

Primaquine-ferrocene conjugates (primacenes) **170**−**180** appeared in 2012 as a new class of organometallic compounds. They were synthesized according to the Scheme 12 [128]. Ferrocene moiety was introduced in the molecule using ferrocene-carboxylic acid (FcCOOH) or 6-bromohexylferrocene (BHFc) and amino acid
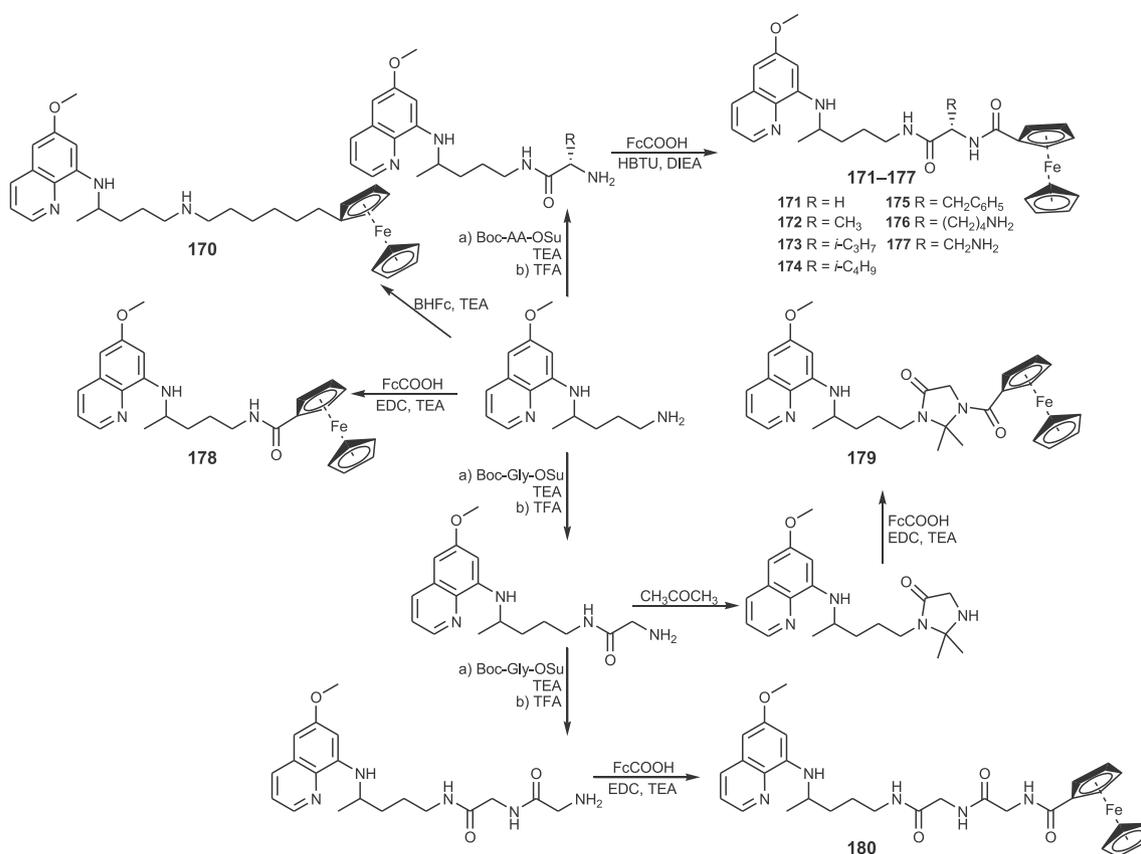
Scheme 12. Synthesis of PQ-ferrocene conjugates (primacenes) **170**–**180**.

moiety with standard methods in peptide chemistry using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) hydrochloride/TEA or 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluoroborate (HBTU)/DIEA as the coupling reagents, *tert*-butoxycarbonyl (Boc) as *N*-protecting and succinimidyl (Su) as C-activating group.

Primacenes were screened for their activity against blood and liver stage of *Plasmodium in vitro* and host-vector transmission *in vivo*. Both transmission-blocking and blood-schizontocidal activity of primaquine was conserved only in primacenes bearing a basic aliphatic amine group. The liver stage activity did not require this structural feature, and all tested metallocenes were comparable to the parent drug or even more active. The replacement of primaquine aliphatic chain by hexylferrocene led to a ~45-fold-higher activity against liver stage parasitaemia in comparison to primaquine. Several primacenes displayed significant activity against *Leishmania infantum* with one of them being active against the clinically relevant intramacrophagic amastigote form of the parasite [129].

3.4. Primaquine derivatives with heterocyclic or carbocyclic scaffold

3.4.1. Primaquine-pyrimidine hybrids

A novel class of hybrids **181**–**190** combining primaquine and pyrimidine-5-carboxylate motif was prepared following Scheme 13 [130].

Three-component Biginelli condensation of various alkyl/aryl acetoacetates, urea and appropriate aldehyde gave 3,4-dihydropyrimidin-2(1H)-ones, which were further oxidized to pyrimidin-2(1H)-ones, chlorinated to 2-chloropyrimidin-2(1H)-ones and condensed with primaquine to give the target compounds. Effectiveness of PQ-pyrimidine hybrids **181**–**190** against blood and liver stages of malaria parasites was evaluated. The title compounds exhibited enhanced liver stage antiplasmodial activity in comparison to primaquine.

3.4.2. Primaquine-thiazolidinone hybrids

Primaquine-thiazolidinones **191**–**205** were recently prepared by multicomponent one-pot reactions from primaquine, mercaptoacetic acid and aromatic aldehyde bearing halogen, nitro, methoxy, methyl or cyano substituents at various positions (Scheme 14) [131].

The title compounds were evaluated in several biological assays, including the ability to block malaria transmission to mosquitoes. All primaquine derivatives exhibited lower cell toxicity than primaquine and none caused haemolysis to normal or G6PD-deficient human erythrocytes *in vitro*. Several conjugates significantly inhibited sporogony in avian (*P. gallinaceum*) and rodent malaria (*P. berghei*). *p*-Methoxy primaquine-thiazolidinone hybrid was the most promising, blocking malaria transmissions and reducing the number of exoerythrocytic forms of *P. berghei* in hepatoma cells *in vitro* and in mice *in vivo*. The same compound also caused a 3-day delay in the malaria pre-patent period.

It is worth to note that the introduction of a similar 5-member heterocyclic ring, i.e. dihydrofuran-2-one with exocyclic C=C bond conjugated with lactone C=O group (Michael acceptor), to primaquine primary amine led to the discovery of antimalarial drug bulaquine (elubaquine) [132]. Since 2000, the bulaquine/chloroquine combination has been marketed in India for the treatment and prevention of *P. vivax*.
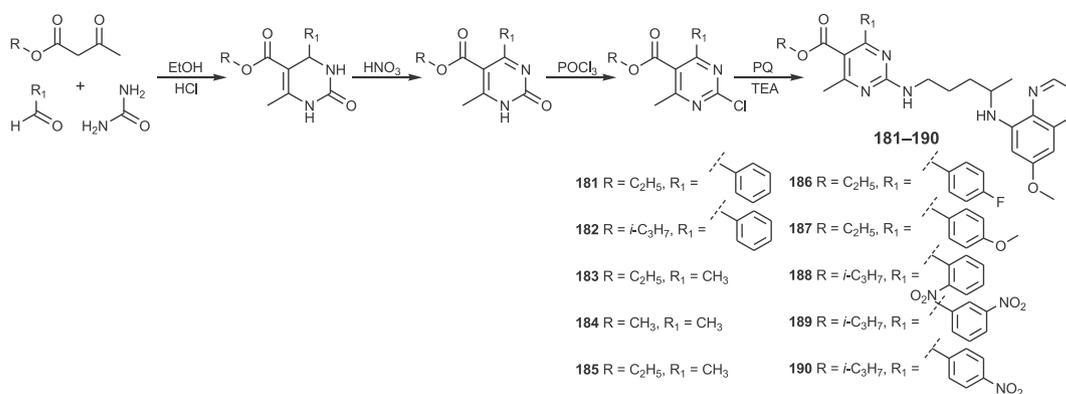
Scheme 13. Synthesis of PQ-pyrimidine hybrids **181**–**190**.
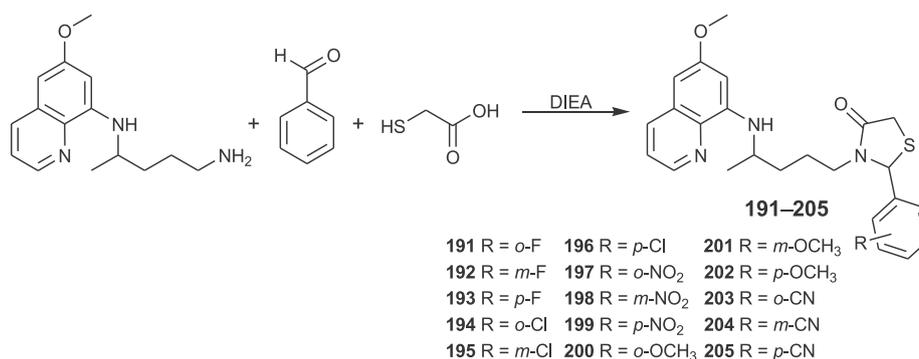
Scheme 14. Synthesis of PQ-thiazolidinone hybrids **191**–**205**.
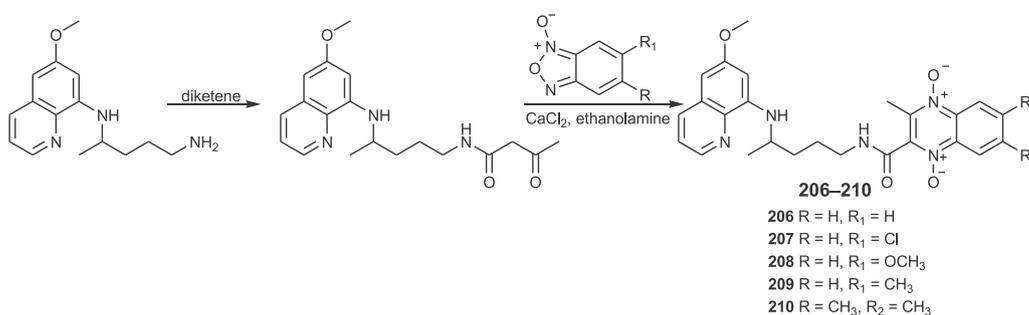
Scheme 15. Synthesis of PQ-quinoxaline 1,4-di-*N*-oxide hybrids **206**–**210**.

3.4.3. Primaquine-quinoxaline 1,4-di-N-oxide hybrids

PQ-quinoxaline hybrids **206**–**210** were recently prepared by a Brasilian research group [133]. The general synthetic approach for the synthesis of the title hybrids is presented in Scheme 15. Reaction of primaquine and diketene provided the β-acetoacetamide derivative, which reacted with benzofuroxans and gave products **206**–**210**.

Compound **206** and **207** were identified as the most active PQ-based hybrids against exoerythrocytic stages, displaying enhanced liver activity against *P. yoelii* and *P. berghei*. They also inhibited sporogony of *P. berghei*. In vivo liver efficacy assays revealed that compound **206** showed causal prophylactic activity affording parasitaemia reduction of up to 95% on day 4. On the other hand, analogous quinoxaline 1,4-di-*N*-oxide hybrids containing chloroquine showed high activity against the erythrocytic stage of the parasite [133].

3.4.4. Primaquine-squaramides

Squaramide is a unique scaffold widely used in medicinal chemistry. Primaquine mono-squaramide derivatives **211**–**213** with different alkoxy side chain were synthesized by reacting primaquine with 3,4-dialkoxy-3-cyclobutene-1,2-dione, whereas derivative **214** was prepared by the reaction of **211** with butylamine [134]. The preparative route leading to squaramides is given in Scheme 16. Compound **213** was 7.3-fold more potent against liver stage parasites than the positive control primaquine. Antiplasmodial activity of this compound, similar 8- and 4-aminoquinoline squaramides and squaramide derivative with two primaquine moieties **215** against *P. falciparum* W2 strain was also reported, illustrating the importance of the squarate moiety for the activity [134,135].
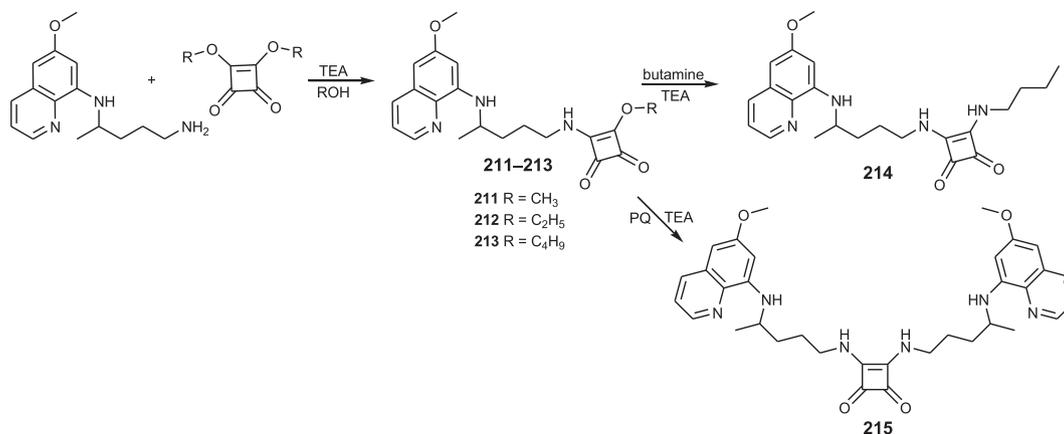
Scheme 16. Synthesis of PQ-squaramides **211**–**215**.

3.4.5. Primaquine-atorvastatin hybrid

Atorvastatin is HMG CoA reductase inhibitor used in treatment of hypercholesterolemia. It also exerts antiplasmodial activity [136] and has synergistic effect with dihydroartemisinin [137,138]. These facts inspired Carvalho et al. to prepare PQ-atorvastatin hybrid **216** and several related aminoquinoline-atorvastatin conjugates [139]. Their preparation was achieved through Paal_Knorr reaction between 1,4-diketone derivative and corresponding aminoquinoline. Synthesis of compounds **210** is outlined in Scheme 17.

Antiplasmodial activity *in vitro* against *P. falciparum* W2 clone (chloroquine resistant strain) and cytotoxicity assay against a monkey kidney cell line (BGM) showed that PQ-derivative was more active and less toxic than the parent drug.

3.5. Primaquine-amino acid/peptide conjugates

Numerous primaquine conjugates with amino acids, oligopeptides or pseudopeptides were reported before 2009 [34,42,45,47,140] and in the last decade [141,142]. Primaquine was conjugated to amino acids containing: *i*) a free terminal amino group, *ii*) a side-chain amino group of lysine or ornithine, *iii*) a carboxylic group of C-terminus or *iv*) a side-chain carboxylic group of aspartic or glutamic acid. Standard synthetic methods common in the peptide synthesis were applied for their preparation using adequate protecting groups and coupling reagents.

Antiplasmodial screening revealed that the conjugates containing cationic amino acids exhibit higher activities as compared to those containing anionic and lipophilic acids. For example, Lys-Lys-PQ and Arg-Arg-PQ were among the most active peptide conjugates against chloroquine-sensitive (D6) and chloroquine-resistant (W2) *P. falciparum* strains [141]. The same conjugates

produced suppressive activity and partial cure in *P. berghei* mouse malaria model. On the other hand, Phe-Ala-PQ showed high activity against *P. vivax* and a more favourable pharmacokinetic profile compared to the parent drug [142], while Val-Leu-Lys-PQ expressed reduced toxicity and increased activity [41]. Despite the improved activity/toxicity ratio, most of these derivatives are rapidly hydrolysed to primaquine. However, introduction of imidazolidin-4-one moiety protects the *N*-terminal amino acid residue of di-, tri- and pentapeptides against aminopeptidase-catalysed hydrolysis [46].

Binding of primaquine to various carriers (lysosomotropic carriers, gum arabic microspheres) is an old idea [143,144]. Gomes and collaborators made this approach interesting again. They have recently reported synthesis of primaquine conjugates with cell-penetrating peptides transportan and transportan 10 [145]. Generally, the conjugates were more active against liver stage *P. berghei* parasites than the parent peptides and, in some cases, than the parent drug.

4. Conclusions

Derivatizations of the primaquine structure were primarily undertaken in search of novel antiplasmodial agents. Although the modification of the quinoline part of primaquine is not covered in this review, we want to stress that such efforts led to the discovery of a novel antimalarial drug tafenoquine. On the other hand, modification of the primaquine primary amino group was awarded by the registration of bulaquine (elubaquine). However, increasing resistance to the currently available antimalarial drugs, including artemisinin-based combination therapies, urges the need to develop novel and more efficient agents.
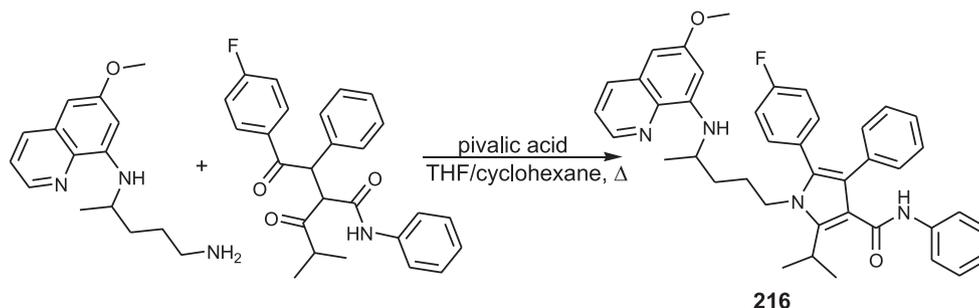
Scheme 17. Synthesis of PQ-atorvastatin hybrid **216**.

The literature survey revealed that the modification of primaquine molecule was quite explored in the last decade and successful in finding novel pharmacologically active agents. Highly reactive terminal amino group allowed modification to various derivatives (amide, carbamate, urea, semicarbazide, ureidoamide) or introduction of glycoside, peptide or heterocyclic (tetraoxane, pyrimidine, thiazolidone, quinoxaline, squaramide) moieties. Hybrids consisting of primaquine and another antiplasmodial drug (artemisinin, chloroquine) or drug from different therapeutic group (atorvastatin) As a result, high diversity of molecules with broad spectrum of activity and selectivity was achieved. Most of the prepared primaquine hybrids displayed more pronounced effect on the *Plasmodium* liver stages or better activity/toxicity ratio than the parent drug. On the other hand, some hybrids exerted dual activity against both stages of malaria parasite and were capable of hitting more than one molecular target. These compounds offer possibility for development of novel antiplasmodial agents active against resistant *Plasmodium* strains.

The molecular hybridization strategy was also successful in search of other biologically active agents outside the antiplasmodial field. Several compounds with encouraging antimycobacterial or antibiofilm activities were identified. Especially important are findings of novel antiproliferative agents displaying high potency to several cancer cell lines or high selectivity towards MCF-7 cells. A number of primaquine derivatives may be considered as lead compounds in the discovery of breast carcinoma drugs. In our opinion, this research area is still not exhausted and has a bright perspective with a lot of work that could be done in the future.

Acknowledgments

Abbreviations

AAPH	2,2'-(2-amidinopropane) dihydrochloride
BHFc	6-bromohexylferrocene
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Bt	benzotriazolyl
Btc	1-benzotriazolecarbonyl
BtH	1*H*-benzo[*d*][1,2,3]triazole
CA	cinnamic acid
CAD	cinnamic acid derivative
DIEA	*N,N*-diisopropylethylamine
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
Fc	ferrocene
G6PD	glucose-6-phosphate dehydrogenase
HATU	1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate
HBTU	2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethylaminium hexafluoroborate
HDAC	histone deacetylase
IC$_{50}$	the concentration that causes 50% growth inhibition
LP	lipid peroxidation
LOX	soybean lipoxygenase
MBEC	minimum biofilm eradication concentration
MIC	minimum inhibitory concentration
MTT	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NSAID	non-steroidal anti-inflammatory drug
PARP	poly ADP ribose polymerase
PHEA	poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]
PHPA	poly[α,β-(*N*-3-hydroxypropyl-DL-aspartamide)]
PQ	primaquine
QSAR	quantitative structure-activity relationship
SAHA	suberoylanilide hydroxamic acid
Su	succinmidyl
TBTU	2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate
TEA	trimethylamine.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2019.111640.

References

[1] F. Giordanetto, J. Bostrom, C. Tyrchan, Follow-on drugs: how far should chemists look? Drug Discov. Today 16 (2011) 722−732.

[2] H. Zhao, Z. Guo, Medicinal chemistry strategies in follow-on drug discovery, Drug Discov. Today 14 (2009) 516−522.

[3] T.I. Oprea, J. Mestres, Drug repurposing: far beyond new targets for old drugs, AAPS. J 14 (2012) 759−763.

[4] T.T. Ashburn, K.B. Thor, Drug repositioning: identifying and developing new uses for existing drugs, Nat. Rev. Drug Discov. 3 (2004) 673−683.

[5] R. Rufener, D. Ritler, J. Zielinski, L. Dick, E.T. da Silva, A. da Silva Araujo, D.E. Joekel, D. Czock, C. Goepfert, A.M. Moraes, M.V.N. de Souza, J. Müller, M. Mevissen, A. Hemphill, B. Lundström-Stadelmann, Activity of mefloquine and mefloquine derivatives against *Echinococcus multilocularis*, Int. J. Parasitol. Drugs Drug Resist. 8 (2018) 331−340.

[6] R. Rufener, D. Ritler, J. Zielinski, L. Dick, E.T. da Silva, A. da Silva Araujo, D.E. Joekel, D. Czock, C. Goepfert, A.M. Moraes, M.V.N. de Souza, J. Müller, M. Mevissen, A. Hemphill, B. Lundström-Stadelmann, Activity of mefloquine and mefloquine derivatives against *Echinococcus multilocularis*, Int. J. Parasitol. Drugs Drug Resist. 8 (2018) 331−340.

[7] D. Krieger, S. Vesenbeckh, N. Schönfeld, G. Bettermann, T.T. Bauer, H. Russmann, H. Mauch, Mefloquine as a potential drug against multidrug-resistant tuberculosis, Eur. Respir. J. 46 (2015) 1503−1505.

[8] L.E. Bermudez, L. Meek, Mefloquine and its enantiomers are active against *Mycobacterium tuberculosis* in vitro and in macrophages, Tuberc. Res. Treat. 2014 (5) (2014) 530815.

[9] R. Duffy, C. Wade, R. Chang, Discovery of anticancer drugs from antimalarial natural products: a MEDLINE literature review, Drug Discov. Today 17 (2012) 942−953.

[10] V.R. Solomon, H. Lee, Chloroquine and its analogs: a new promise of an old drug for effective and safe cancer therapies, Eur. J. Pharmacol. 625 (2009) 220−233.

[11] T. Kimura, Y. Takabatake, A. Takahashi, Y. Isaka, Chloroquine in cancer therapy: a double-edged sword of autophagy, Cancer Res. 73 (2013) 3−7.

[12] A.K. Abdel-Aziz, S. Shouman, E. El-Demerdash, M. Elgendy, A.B. Abdel-Naim, Chloroquine as a promising adjuvant chemotherapy together with sunitinib, Sci. Proc. 1 (2014). Article ID e384.

[13] F. Liu, Y. Shang, S.-Z. Chen, Chloroquine potentiates the anti-cancer effect of lidamycin on non-small cell lung cancer cells in vitro, Acta Pharmacol. Sin. 35 (2014) 645−652.

[14] A. Ganguli, D. Choudhury, S. Datta, S. Bhattacharya, G. Chakrabarti, Inhibition of autophagy by chloroquine potentiates synergistically anti-cancer property of artemisinin by promoting ROS dependent apoptosis, Biochimie 107 (2014) 338−349.

[15] A.R. Choi, J.H. Kim, Y.H. Woo, H.S. Kim, S. Yoon, Anti-malarial drugs primaquine and chloroquine have different sensitization effects with anti-mitotic drugs in resistant cancer cells, Anticancer Res. 36 (2016) 1641−1648.

[16] C. Verbaanderd, H. Maes, M.B. Schaaf, V.P. Sukhatme, P. Pantziarka, V. Sukhatme, P. Agostinis, G. Bouche, Repurposing drugs in oncology (ReDO) − chloroquine and hydroxychloroquine as anti-cancer agents, eCancer 11 (2017). Article ID 781.

[17] L. Liu, C. Han, H. Yu, W. Zhu, H. Cui, L. Zheng, C. Zhang, L. Yue, Chloroquine inhibits cell growth in human A549 lung cancer cells by blocking autophagy and inducing mitochondrial-mediated apoptosis, Oncol. Rep. 39 (2018) 2807−2816.

[18] F. Wang, J. Tang, P. Li, S. Si, H. Yu, X. Yang, J. Tao, Q. Lv, M. Gu, H. Yang, Z. Wang, Chloroquine enhances the radiosensitity of bladder cancer cells by inhibiting autophagy and activating apoptosis, Cell. Physiol. Biochem. 45 (2018) 54−66.

[19] R.H. van Huijsduijnen, R. Kiplin Guy, K. Chibale, R.K. Haynes, I. Peitz, G. Kelter, M.A. Phillips, J.L. Vennerstrom, Y. Yuthavong, T.N.C. Wells, Anti-cancer properties of distinct antimalarial drug classes, PLoS One 8 (2013) e82962.

[20] A. Kamal, A. Aziz, S. Shouman, E. El-Demerdash, M. Elgendy, A.B. Abdel-Naim, Chloroquine synergizes sunitinib cytotoxicity via modulating autophagic, apoptotic and angiogenic machineries, Chem. Biol. Interact. 217 (2014) 28−40.

[21] G.W. Soo, J.H. Law, E. Kan, S.Y. Tan, W.Y. Lim, G. Chay, N.I. Bukhari, I. Segarra, Differential effects of ketoconazole and primaquine on the pharmacokinetics and tissue distribution of imatinib in mice, Anti Canccer Drugs 21 (2010) 695−703.

[22] Y.K. Wong, C. Xu, K.A. Kalesh, Y. He, Q. Lin, W.S.F. Wong, H.M. Shen, J. Wang, Artemisinin as an anticancer drug: recent advances in target profiling and mechanisms of action, Med. Res. Rev. 37 (2017) 1492−1517.

[23] https://clinicaltrials.gov/ct2/home. (Accessed 15 March 2019).

[24] R.S. Gonçalves, C.R. Kaiser, M.C. Lourenço, F.A. Bezerra, M.V. de Souza, J.L. Wardell, S.M. Wardell, M.D. Henriques, T. Costa, Mefloquine-oxazolidine derivatives, derived from mefloquine and arenecarbaldehydes: in vitro activity including against the multidrug-resistant tuberculosis strain T113, Bioorg. Med. Chem. 20 (2012) 243−248.

[25] J. Mao, H. Yuan, Y. Wang, B. Wan, D. Pak, R. He, S.G. Franzblau, Synthesis and antituberculosis activity of novel mefloquine-isoxazole carboxylic esters as prodrugs, Bioorg. Med. Chem. Lett 20 (2010) 1263−1268.

[26] S. Eswaran, A.V. Adhikari, I.H. Chowdhury, N.K. Pal, K.D. Thomas, New quinoline derivatives: synthesis and investigation of antibacterial and anti-tuberculosis properties, Eur. J. Med. Chem. 45 (2010) 3374−3383.

[27] B.C. Pérez, I. Fernandes, N. Mateus, C. Teixeira, P. Gomes, Recycling antimalarial leads for cancer: antiproliferative properties of *N*-cinnamoyl chloroquine analogues, Bioorg. Med. Chem. Lett 23 (2013) 6769−6772.

[28] S. Slezakova, J. Ruda-Kucerova, Anticancer activity of artemisinin and its derivatives, Anticancer Res. 37 (2017) 5995−6003.

[29] Q. Sun, J. Wang, Y. Li, J. Zhuang, Q. Zhang, X. Sun, D. Sun, Synthesis and evaluation of cytotoxic activities of artemisinin derivatives, Chem. Biol. Drug Des. 90 (2017) 1019−1028.

[30] A. Gomes, I. Fernandes, C. Teixeira, N. Mateus, M.J. Sottomayor, P. Gomes, A quinacrine analogue selective against gastric cancer cells: insight from biochemical and biophysical studies, ChemMedChem 11 (2016) 2703−2712.

[31] F.F. Zhang, J. Li, Inhibitory effect of chloroquine derivatives on presenilin 1 and ubiquilin 1 expression in Alzheimer's disease, Int. J. Clin. Exp. Pathol. 8 (2015) 7640−7643.

[32] Y. Zhang, G. Xu, S. Zhang, D. Wang, P.S. Prabha, Z. Zuo, Antitumor research on artemisinin and its bioactive derivatives, Nat. Prod. Bioprospect. 8 (2018) 303−319.

[33] https://en.wikipedia.org/wiki/WHO_Model_List_of_Essential_Medicines. (Accessed 8 March 2019).

[34] K. Kaur, M. Jain, S.I. Khan, M.R. Jacob, B.L. Tekwani, S. Singh, P.P. Singh, R. Jain, Amino acid, dipeptide and pseudopeptide conjugates of ring-substituted 8-aminoquinolines: synthesis and evaluation of anti-infective, β-haematin inhibition and cytotoxic activities, Eur. J. Med. Chem. 52 (2012) 230−241.

[35] N. Vale, R. Moreira, P. Gomes, Primaquine revisited six decades after its discovery, Eur. J. Med. Chem. 44 (2009) 937−953.

[36] B.S. Pybus, S.R. Marcsisin, X. Jin, G. Deye, J.C. Sousa, Q. Li, D. Caridha, Q. Zeng, G.A. Reichard, C. Ockenhouse, J. Bennett, L.A. Walker, C. Ohrt, V. Melendez, The metabolism of primaquine to its active metabolite is dependent on CYP 2D6, Malar. J. 12 (2013) e212.

[37] S.R. Marcsisin, G. Reichard, B.S. Pybus, Primaquine pharmacology in the context of CYP 2D6 pharmacogenomics: current state of the art, Pharmacol. Ther. 161 (2016) 1−10.

[38] K. Kaur, M. Jain, S.I. Khan, M.R. Jacob, B.L. Tekwani, S. Singh, P. Pal Singh, R. Jain, Extended side chain analogues of 8-aminoquinolines: synthesis and evaluation of antiprotozoal, antimicrobial, β-hematin inhibition, and cytotoxic activities, MedChemComm 2 (2011) 300−307.

[39] P. Gomes, M.R. Araújo, M. Rodrigues, N. Vale, Z. Azevedo, J. Iley, P. Chambel, J. Morais, R. Moreira, Synthesis of imidazolidin-4-one and 1*H*-imidazo[2,1-*a*]isoindole-2,5(3*H*,9b*H*)-dione derivatives of primaquine: scope and limitations, Tetrahedron 60 (2004) 5551−5562.

[40] M.J. Araújo, J. Bom, R. Capela, C. Casimiro, P. Chambel, P. Gomes, J. Iley, F. Lopes, J. Morais, R. Moreira, E. de Oliveira, V. do Rosário, N. Vale, Imidazolidin-4-one derivatives of primaquine as novel transmission-blocking antimalarials, J. Med. Chem. 48 (2005) 888−892.

[41] N. Vale, J. Matos, J. Gut, F. Nogueira, V. do Rosário, P.J. Rosenthal, R. Moreira, P. Gomes, Imidazolidin-4-one peptidomimetic derivatives of primaquine: synthesis and antimalarial activity, Bioorg. Med. Chem. Lett 18 (2008) 4150−4153.

[42] A. Philip, J.A. Kepler, B.H. Johnson, F.I. Carroll, Peptide derivates of primaquine as potential antimalarial agents, J. Med. Chem. 31 (1988) 870−874.

[43] R. Jain, S. Jain, R.C. Gupta, N. Anand, G.P. Dutta, S.K. Puri, Synthesis of amino acid derivatives of 8-[(4-amino-1-methylbutyl)amino]-6-methoxy-4-substituted/4,5-disubstituted-quinolines as potential antimalarial agents, Indian J. Chem. 33B (1994) 251−254.

[44] M.J. Portela, R. Moreira, E. Valente, L. Constantino, J. Iley, J. Pinto, R. Rosa, P. Cravo, V.E. do Rosário, Dipeptide derivatives of primaquine as transmission-blocking antimalarials: effect of aliphatic side-chain acylation on the gametocytocidal and on the formation of carboxyprimaquine in rat

[45] S. Vangapandu, S. Sachdeva, M. Jain, S. Singh, P.P. Singh, C.L. Kaul, R. Jain, 8-Quinolinamines conjugated with amino acids are exhibiting potent blood-schizontocidal antimalarial activities, Bioorg. Med. Chem. 12 (2004) 239−247.

[46] N. Vale, J. Matos, R. Moreira, P. Gomes, Amino acids as selective acylating agents: regioselective N^1-acylation of imidazolidin-4-one derivatives of the antimalarial drug primaquine, Tetrahedron 64 (2008) 11144−11149.

[47] N. Vale, F. Nogueira, V.E. do Rosário, P. Gomes, R. Moreira, Primaquine dipeptide derivatives bearing an imidazolidin-4-one moiety at the *N*-terminus as potential antimalarial prodrugs, Eur. J. Med. Chem. 44 (2009) 2506−2516.

[48] I. Fernandes, N. Vale, V. de Freitas, R. Moreira, N. Mateus, P. Gomes, Anti-tumoral activity of imidazoquines, a new class of antimalarials derived from primaquine, Bioorg. Med. Chem. Lett 19 (2009) 6914−6917.

[49] N. Vale, M.S. Collins, J. Gut, R. Ferraz, P.J. Rosenthal, M.T. Cushion, R. Moreira, P. Gomes, Anti-*Pneumocystis carinii* and antiplasmodial activities of primaquine-derived imidazolidin-4-ones, Bioorg. Med. Chem. Lett 18 (2008) 485−488.

[50] N. Vale, M. Prudêncio, C.A. Marques, M.S. Collins, J. Gut, F. Nogueira, J. Matos, P.J. Rosenthal, M.T. Cushion, V.E. do Rosário, M.M. Mota, R. Moreira, P. Gomes, Imidazoquines as antimalarial and anti-pneumocystis agents, J. Med. Chem. 52 (2009) 7800−7807.

[51] A.R. Katritzky, X. Lan, J.Z. Yang, O.V. Denisko, Properties and synthetic utility of *N*-substituted benzotriazoles, Chem. Rev. 98 (1998) 409−548.

[52] J.-C.M. Monbaliu (Ed.), The Chemistry of Benzotriazole Derivatives: A Tribute to Alan Roy Katritzky (Topics in Heterocyclic Chemistry), Springer, New York, 2016.

[53] B. Zorc, Z. Rajić Džolić, I. Butula, Benzotriazole as a synthetic auxiliary, Croat. Chem. Acta 85 (2012) 595−602.

[54] S.A. Carvalho, E.F. da Silva, M.V. de Souza, M.C. Lourenco, F.R. Vicente, Synthesis and antimycobacterial evaluation of new *trans*-cinnamic acid hydrazide derivatives, Bioorg. Med. Chem. Lett 18 (2008) 538−541.

[55] R. Bairwa, M. Kakwani, N.R. Tawari, J. Lalchandani, M.K. Ray, M.G.R. Rajan, M.S. Degani, Novel molecular hybrids of cinnamic acids and guanylhydrazones as potential antitubercular agents, Bioorg. Med. Chem. 20 (2010) 1623−1625.

[56] M.D. Kakwani, P. Suryavanshi, M. Ray, M.G.R. Rajan, S. Majee, A. Samad, P. Devarajan, M.S. Degani, Design, synthesis and antimycobacterial activity of cinnamide derivatives: a molecular hybridization approach, Bioorg. Med. Chem. Lett 21 (2011) 1997−1999.

[57] R. Lone, R. Shuab, K.K. Koul, Role of cinnamate and cinnamate derivatives in pharmacology, Glob. J. Pharmacol. 8 (2014) 328−335.

[58] G.K. Yoya, F. Bedos-Belval, P. Constant, H. Duran, M. Daffé, M. Baltas, Synthesis and evaluation of a novel series of pseudo-cinnamic derivatives as antituberculosis agents, Bioorg. Med. Chem. Lett 19 (2009) 341−343.

[59] P.J. Sharma, Cinnamic acid derivatives: a new chapter of various pharmacological activities, Chem. Pharm. Res. 3 (2011) 403−423.

[60] E. Pontiki, D. Hadjipavlou-Litina, K. Litinas, G. Geromichalos, Novel cinnamic acid derivatives as antioxidant and anticancer agents: design, synthesis and modeling studies, Molecules 19 (2014) 9655−9674.

[61] M. De, P. Baltas, F. Bedos-Belval, Cinnamic acid derivatives as anticancer agents − a review, Curr. Med. Chem. 18 (2011) 1672−1703.

[62] B.C. Pérez, I. Fernandes, N. Mateus, C. Teixeira, P. Gomes, Recycling antimalarial leads for cancer: antiproliferative properties of *N*-cinnamoyl chloroquine analogues, Bioorg. Med. Chem. Lett 23 (2013) 6769−6772.

[63] J. Kanaani, H. Ginsburg, Effects of cinnamic acid derivatives on in vitro growth of *Plasmodium falciparum* and on the permeability of the membrane of malaria-infected erythrocytes, Antimicrob. Agents Chemother. 36 (1992) 1102−1108.

[64] K. Pavić, I. Perković, P. Gilja, F. Kozlina, K. Ester, M. Kralj, D. Schols, D. Hadjipavlou-Litina, E. Pontiki, B. Zorc, Design, synthesis and biological evaluation of novel primaquine-cinnamic acid conjugates of amide and acylsemicarbazide type, Molecules 21 (2016) 1629−1653.

[65] P. Mabeta, K. Pavić, B. Zorc, Insights into mechanism of antiproliferative effect of primaquine-cinnamic acid conjugates on MCF-7, Acta Pharm. 68 (2018) 337−348.

[66] B. Pérez, C. Teixeira, I.S. Albuquerque, J. Gut, P.J. Rosenthal, M. Prudencio, P. Gomes, PRIMACINS, *N*-cinnamoyl-primaquine conjugates, with improved liver-stage antimalarial activity, MedChemComm 3 (2012) 1170−1172.

[67] S. Vale-Costa, J. Costa-Gouveia, B. Pérez, T. Silva, C. Teixeira, P. Gomes, M.S. Gomes, *N*-cinnamoylated aminoquinolines as promising antileishmanial agents, Antimicrob. Agents Chemother. 57 (2013) 5112−5115.

[68] R. Ferraz, M. Pinheiro, A. Gomes, C. Teixeira, C. Prudêncio, S. Reis, P. Gomes, Effects of novel triple-stage antimalarial ionic liquids on lipid membrane models, Bioorg. Med. Chem. Lett 27 (2017) 4190−4193.

[69] Z. Rajić, M. Zovko Končić, K. Miloloža, I. Perković, I. Butula, F. Bucar, B. Zorc, Primaquine-NSAID twin drugs: synthesis, radical scavenging, antioxidant and Fe^{2+} chelating activity, Acta Pharm. 60 (2010) 325−337.

[70] P. Andrews, X. Zhao, J. Allen, F. Li, M. Chang, A comparison of the effectiveness of selected non-steroidal anti-inflammatory drugs and their derivatives against cancer cells in vitro, Cancer Chemother, Pharmacol 61 (2008) 203−214.

[71] E.R. Rayburn, S.J. Ezell, R. Zhang, Anti-inflammatory agents for cancer therapy, Mol. Cell. Pharmacol. 1 (2009) 29−43.

[72] D. Wang, R.N. DuBois, Eicosanoids and cancer, Nat. Rev. Cancer 10 (2010) 181—193.

[73] B. Zawidlak-Węgrzyńska, M. Kawalec, I. Bosek, M. Łuczyk-Juzwa, G. Adamus, A. Rusin, P. Filipczak, M. Głowala-Kosińska, K. Wolańska, Z. Krawczyk, P. Kurcok, Synthesis and antiproliferative properties of ibuprofen-oligo(3-hydroxybutyrate) conjugates, Eur. J. Med. Chem. 45 (2010) 1833—1842.

[74] S. Fogli, I. Banti, F. Stefanelli, L. Picchianti, M. Digiacomo, M. Macchia, M.C. Breschi, A. Lapucci, Therapeutic potential of sulindac hydroxamic acid against human pancreatic and colonic cancer cells, Eur. J. Med. Chem. 45 (2010) 5100—5107.

[75] I. Butula, M.V. Proštenik, V. Vela, Reactions with 1-benzotriazole carboxylic acid chloride. I. Synthesis of the 2,6-bis(hydroxymethyl)pyridine dicarbamates, Croat. Chem. Acta 49 (1977) 837—842.

[76] I. Kalčić, M. Zovko, M. Jadrijević-Mladar Takač, B. Zorc, I. Butula, Synthesis and reactions of some azolecarboxylic acid derivatives, Croat. Chem. Acta 76 (2003) 217—228.

[77] M. Šimunović, I. Perković, B. Zorc, K. Ester, M. Kralj, D. Hadjipavlou-Litina, E. Pontiki, Urea and carbamate derivatives of primaquine: synthesis, cytostatic and antioxidant activities, Bioorg. Med. Chem. 17 (2009) 5605—5613.

[78] G. Mata, V.E. do Rosário, J. Iley, L. Constantino, R. Moreira, A carbamate-based approach to primaquine prodrugs: antimalarial activity, chemical stability and activation, Bioorg. Med. Chem. 20 (2012) 886—892.

[79] G. Džimbeg, B. Zorc, M. Kralj, K. Ester, K. Pavelić, J. Balzarini, E. De Clercq, M. Mintas, The novel primaquine derivatives of N-alkyl, cycloalkyl or aryl urea: synthesis, cytostatic and antiviral activity evaluations, Eur. J. Med. Chem. 43 (2008) 1180—1187.

[80] K. Pavić, I. Perković, M. Cindrić, M. Pranjić, I. Martin-Kleiner, M. Kralj, D. Schols, D. Hadjipavlou-Litina, A.-M. Katsori, B. Zorc, Novel semicarbazides and ureas of primaquine with bulky aryl or hydroxyalkyl substituents: synthesis, cytostatic and antioxidative activity, Eur. J. Med. Chem. 86 (2014) 502—514.

[81] K. Pavić, Z. Rajić, H. Michnová, J. Jampílek, I. Perković, B. Zorc, Second generation of primaquine ureas and bis-ureas as potential antimycobacterial agents, Mol. Divers. 23 (2019) 657—667.

[82] K. Kaur, M. Jain, S.I. Khan, M.R. Jacob, B.L. Tekwani, S. Singh, P.P. Singh, R. Jain, Synthesis, antiprotozoal, antimicrobial, β-hematin inhibition, cytotoxicity and methemoglobin (MetHb) formation activities of bis(8-aminoquinolines), Bioorg. Med. Chem. 19 (2011) 197—210.

[83] I. Perković, M. Antunović, I. Marijanović, K. Pavić, K. Ester, M. Kralj, J. Vlainić, I. Kosalec, D. Schols, D. Hadjipavlou-Litina, E. Pontiki, B. Zorc, Novel urea and bis-urea primaquine derivatives with hydroxyphenyl and halogenphenyl substituents: synthesis and biological evaluation, Eur. J. Med. Chem. 124 (2016) 622—636.

[84] I. Perković, S. Tršinar, J. Žanetić, M. Kralj, I. Martin-Kleiner, J. Balzarini, D. Hadjipavlou-Litina, A.M. Katsori, Novel 1-acyl-4-substituted semicarbazide derivatives of primaquine — synthesis, cytostatic, antiviral and antioxidative studies, J. Enzym. Inhib. Med. Chem. 28 (2013) 601—610.

[85] V. Reddy, Organofluorine Compounds in Biology and Medicine, first ed., Elsevier, Amsterdam, 2015.

[86] K. Raynes, Bisquinoline antimalarials: their role in malaria chemotherapy, Int. J. Parasitol. 29 (1999) 367—379.

[87] T.M. Davis, T.Y. Hung, I.K. Sim, H.A. Karunajeewa, K.F. Ilett, Piperaquine: a resurgent antimalarial drug, Drugs 65 (2005) 75—87.

[88] http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/001199/WC500118113.pdf. (Accessed 14 February 2019).

[89] C.Y. Patil, S.S. Katare, M.S. Baig, S.M. Doifode, Fixed dose combination of arterolane and piperaquine: a newer prospect in antimalarial therapy, Ann. Med. Health Sci. Res. 4 (2014) 466—471.

[90] P. Buragohain, B. Saikia, N. Surineni, N.C. Barua, A.K. Saxena, N. Suri, Synthesis of a novel series of artemisinin dimers with potent anticancer activity involving Sonogashira cross-coupling reaction, Bioorg. Med. Chem. Lett 24 (2014) 237—239.

[91] T. Fröhlich, A.Ç. Karagöz, C. Reiter, S.B. Tsogoeva, Artemisinin-derived dimers: potent antimalarial and anticancer agents, J. Med. Chem. 59 (2016) 7360—7388.

[92] H.C. Lai, N.P. Singh, T. Sasaki, Development of artemisinin compounds for cancer treatment, Investig. New Drugs 31 (2013) 230—246.

[93] J. Levatić, K. Pavić, I. Perković, K. Uzelac, K. Ester, M. Kralj, M. Kaiser, M. Rottmann, F. Supek, B. Zorc, Machine learning prioritizes synthesis of primaquine ureidoamides with high antimalarial activity and attenuated cytotoxicity, Eur. J. Med. Chem. 146 (2018) 651—667.

[94] J. Vlainić, I. Kosalec, K. Pavić, D. Hadjipavlou-Litina, E. Pontiki, B. Zorc, Insights into biological activity of ureidoamides with primaquine and amino acid moieties, J. Enzym. Inhib. Med. Chem. 33 (2018) 376—382.

[95] D. Shahinas, A. Folefoc, D.R. Pillai, Targeting Plasmodium falciparum Hsp90: towards reversing antimalarial resistance, Pathogens 2 (2013) 33—54.

[96] S.O. Tcherniuk, O. Chesnokova, I.V. Oleinikov, A.I. Potopalsky, A.V. Oleinikov, Anti-malarial effect of semi-synthetic drug amitozyn, Malar. J. 14 (10) (2015) 425.

[97] A. Kreidenweiss, P.G. Kremsner, B. Mordmüller, Comprehensive study of proteasome inhibitors against Plasmodium falciparum laboratory strains and field isolates from Gabon, Malar. J. 7 (8) (2008) 187.

[98] J.A. Engel, A.J. Jones, V.M. Avery, S.D. Sumanadasa, S.S. Ng, D.P. Fairlie, T. Skinner-Adams, K.T. Andrews, Profiling the anti-protozoal activity of anti-cancer HDAC inhibitors against Plasmodium and Trypanosoma parasites, Int. J. Parasitol. Drugs Drug Resist. 5 (2015) 117—126.

[99] K. Trenholme, L. Marek, S. Duffy, G. Pradel, G. Fisher, F.K. Hansen, T.S. Skinner-Adams, A. Butterworth, C.J. Ngwa, J. Moecking, C.D. Goodman, G.I. McFadden, S.D.M. Sumanadasa, D.P. Fairlie, V.M. Avery, T. Kurz, K.T. Andrews, Lysine acetylation in sexual stage malaria parasites is a target for antimalarial small molecules, Antimicrob. Agents Chemother. 58 (2014) 3666—3678.

[100] K.T. Andrews, A. Haque, M.K. Jones, HDAC inhibitors in parasitic diseases, Immunol. Cell Biol. 90 (2012) 66—77.

[101] B.K. Chaal, A.P. Gupta, B.D. Wastuwidyaningtyas, Y.H. Luah, Z. Bozdech, Histone deacetylases play a major role in the transcriptional regulation of the Plasmodium falciparum life cycle, PLoS Pathog. 6 (13) (2010) e1000737.

[102] H. Hu, A. Cabrera, M. Kono, S. Mok, B.K. Chaal, S. Haase, K. Engelberg, S. Cheemadan, T. Spielmann, P.R. Preiser, T.-W. Gillberger, Z. Bozdech, Transcriptional profiling of growth perturbations of the human malaria parasite Plasmodium falciparum, Nat. Biotechnol. 28 (2010) 91—98.

[103] K.T. Andrews, T.N. Tran, N.C. Wheatley, D.P. Fairlie, Targeting histone deacetylase inhibitors for anti-malarial therapy, Curr. Top. Med. Chem. 9 (2009) 292—308.

[104] M. Beus, Z. Rajić, D. Maysinger, Z. Mlinarić, M. Antunović, I. Marijanović, D. Fontinha, M. Prudêncio, J. Held, S. Olgen, B. Zorc, SAHAquines, novel hybrids based on SAHA and primaquine motifs, as potential anticancer and antiplasmodial agents, ChemistryOpen 7 (2018) 624—638.

[105] I. Zhang, M. Beus, U. Stochaj, P.U. Le, B. Zorc, Z. Rajić, K. Petrecca, D. Maysinger, Inhibition of glioblastoma cell proliferation, invasion, and mechanism of action of a novel hydroxamic acid hybrid molecule, Cell Death Dis. 5 (14) (2019) 41.

[106] Z. Rajić, M. Beus, H. Michnova, J. Vlainić, L. Persoons, I. Kosalec, J. Jampilek, D. Schols, T. Keser, B. Zorc, Asymmetric primaquine and halogenaniline fumardiamides as novel biologically active Michael acceptors, Molecules 23 (18) (2018) 1724.

[107] P.A. Jackson, J.C. Widen, D.A. Harki, K.M. Brummond, Covalent modifiers: a chemical perspective on the reactivity of α,β-unsaturated carbonyls with thiols via hetero-Michael addition reactions, J. Med. Chem. 60 (2017) 839—885.

[108] Z. Rajić, G. Kos, B. Zorc, P.P. Singh, S. Singh, Macromolecular prodrugs. XII. Primaquine conjugates: synthesis and antimalarial evaluation, Acta Pharm. 59 (2009) 107—115.

[109] K. Pavić, I. Perković, Š. Pospíšilová, M. Machado, D. Fontinha, M. Prudêncio, J. Jampilek, A. Coffey, L. Endersen, H. Rimac, B. Zorc, Primaquine hybrids as promising antimycobacterial and antimalarial agents, Eur. J. Med. Chem. 143 (2018) 769—779.

[110] E. Kedzierska, J. Orzelska, I. Perković, D. Knežević, S. Fidecka, M. Kaiser, B. Zorc, Pharmacological activity of primaquine ureas and semicarbazides on central nervous system in mice and antimalarial activity in vitro, Fundam. Clin. Pharmacol. 30 (2016) 58—69.

[111] M. Beus, D. Fontinha, J. Held, Z. Rajić, L. Uzelac, M. Kralj, M. Prudêncio, B. Zorc, Primaquine and chloroquine fumardiamides as promising antiplasmodial agents, Molecules (2019). https://doi.org/10.3390/molecules24152812.

[112] D.A. Hufnagel, J.E. Price, R.E. Stephenson, J. Kelley, M.F. Benoit, M.R. Chapman, Thiol starvation induces redox-mediated dysregulation of Escherichia coli biofilm components, J. Bacteriol. 200 (2018) e00389-17 (15 pages).

[113] J. Vásquez-Vivar, O. Augusto, Oxidative activity of primaquine metabolites on rat erythrocytes in vitro and in vivo, Biochem. Pharmacol. 47 (1994) 309—316.

[114] L.N. Grinberg, A. Samuni, Nitroxide stable radical prevents primaquine-induced lysis of red blood cells, Biochim. Biophys. Acta 1201 (1994) 284—288.

[115] S.K. Kashaw, V. Kashaw, P. Mishra, N.K. Jain, J.P. Stables, Synthesis, anticonvulsant and CNS depressant activity of some new bioactive 1-(4-substituted-phenyl)-3-(4-oxo-2-phenyl/ethyl-4H-quinazolin-3-yl)-urea, Eur. J. Med. Chem. 44 (2009) 4335—4343.

[116] A. Jain, S. Patil, A. Gajbhiye, Synthesis and anticonvulsant activity of some 1-cyclohexylidine/cycloheptylidine-4-substituted semicarbazide derivatives, Int. J. Pharmacol. Pharm. Technol. 1 (2012) 51—56.

[117] G. Saravanan, V. Alagarsamy, C.R. Prakash, Design, synthesis and anticonvulsant activities of novel 1-(substituted/unsubstituted benzylidene)-4-(6,8-dibromo-2-(methyl/phenyl)-4-oxoquinazolin-3(4H)-yl)phenyl) semicarbazide derivatives, Bioorg. Med. Chem. Lett 22 (2012) 3072—3078.

[118] C.S. Azad, M. Saxena, A.J. Siddiqui, J. Bhardwai, S.K. Puri, G.P. Dutta, N. Anand, A.K. Saxena, Synthesis of primaquine glyco-conjugates as potential tissue schizontocidal antimalarial agents, Chem. Biol. Drug Des. 90 (2017) 254—261.

[119] R. Capela, G.G. Cabal, P.J. Rosenthal, J. Gut, M.M. Mota, R. Moreira, F. Lopes, M. Prudêncio, Design and evaluation of primaquine-artemisinin hybrids as a multistage antimalarial strategy, Antimicrob. Agents Chemother. 55 (2011) 4698—4706.

[120] D. Miranda, R. Capela, I.S. Albuquerque, P. Meireles, I. Paiva, F. Nogueira, R. Amewu, J. Gut, P.J. Rosenthal, R. Oliveira, M.M. Mota, R. Moreira, F. Marti, M. Prudêncio, P.M. O'Neill, F. Lopes, Novel endoperoxide-based transmission-blocking antimalarials with liver- and blood-schizontocidal activities, ACS Med. Chem. Lett. 5 (2014) 108—112.

[121] R. Capela, J. Magalhães, D. Miranda, M. Machado, M. Sanches-Vaz, I.S. Albuquerque, M. Sharma, J. Gut, P.J. Rosenthal, R. Frade, M.J. Perry, R. Moreira, M. Prudêncio, Endoperoxide-8-aminoquinoline hybrids as dual-stage antimalarial agents with enhanced metabolic stability, Eur. J. Med. Chem. 149 (2018) 69−78.

[122] O. Dechy-Cabaret, F. Benoit-Vical, C. Loup, A. Robert, H. Gornitzka, A. Bonhoure, H. Vial, J.F. Maagnaval, J.P. Séguéla, B. Meunier, Synthesis and antimalarial activity of trioxaquine derivatives, Chemistry 10 (2004) 1625−1636.

[123] F. Benoit-Vical, J. Lelièvre, A. Berry, C. Deymier, O. Dechy-Cabaret, J. Cazelles, C. Loup, A. Robert, J.F. Magnaval, B. Meunier, Trioxaquines are new anti-malarial agents active on all erythrocytic forms, including gametocytes, Antimicrob. Agents Chemother. 51 (2007) 1463−1472.

[124] S.S. Chauhan, M. Sharma, P.M. Chauhan, Trioxaquines: hybrid molecules for the treatment of malaria, Drug News Perspect. 23 (2010) 632−646.

[125] A.E. Kiszewski, Blocking *Plasmodium falciparum* malaria transmission with drugs: the gametocytocidal and sporontocidal properties of current and prospective antimalarials, Pharmaceuticals (Basel) 4 (2011) 44−68.

[126] O.C. Odhiambo, H.N. Wamakima, G.N. Magoma, P.G. Kirira, B.J. Malala, F.T. Kimani, F.W. Muregi, Efficacy and safety evaluation of a novel triox-aquine in the management of cerebral malaria in a mouse model, Malar. J. 16 (9) (2017) 268.

[127] M. Lödige, M.D. Lewis, E.S. Paulsen, H.L. Esch, G. Pradel, L. Lehmann, R. Brun, G. Bringmann, A.K. Mueller, A primaquine-chloroquine hybrid with dual activity against Plasmodium liver and blood stages, Int. J. Med. Microbiol. 303 (2013) 539−547.

[128] J. Matos, F.P. da Cruz, É. Cabrita, J. Gut, F. Nogueira, V.E. do Rosário, R. Moreira, P.J. Rosenthal, M. Prudêncio, P. Gomes, Novel potent metal-locenes against liver stage malaria, Antimicrob. Agents Chemother. 56 (2012) 1564−1570.

[129] S. Vale-Costa, N. Vale, J. Matos, A. Tomás, R. Moreira, P. Gomes, M. Salomé Gomes, Peptidomimetic and organometallic derivatives of primaquine active against *Leishmania infantum*, Antimicrob. Agents Chemother. 56 (2012) 5774−5781.

[130] H. Kaur, M. Machado, C. de Kock, P. Smith, K. Chibale, M. Prudêncio, K. Singh, Primaquine-pyrimidine hybrids: synthesis and dual-stage antiplasmodial activity, Eur. J. Med. Chem. 101 (2015) 266−273.

[131] A.C.C. Aguiar, B. Figueiredo Jr., P.D. Neuenfeldt, T.H. Katsuragawa, B.B. Drawanz, W. Cunico, P. Sinnis, F. Zavala, A.U. Krettli, Primaquine-thia-zolidinones block malaria transmission and development of the liver exoerythrocytic forms, Malar. J. 16 (11) (2017) 110.

[132] https://newdrugapprovals.org/2014/05/01/bulaquine/. (Accessed 15 April 2019).

[133] L. Bonilla-Ramírez, A. Rios, M. Quiliano, G. Ramírez-Calderon, I. Beltrán-Hortelano, J.F. Franetich, L. Corcuera, M. Bordessoulles, A. Vettorazzi, A. López de Cerain, I. Aldana, D. Mazier, A. Pablón, S. Galiano, Novel antimalarial chloroquine- and primaquine-quinoxaline 1,4-di-*N*-oxide hybrids: design,

[134] C.J.A. Ribeiro, M. Espadinha, M. Machado, J. Gut, L.M. Gonçalves, P.J. Rosenthal, M. Prudêncio, R. Moreira, M.M. Santos, Novel squaramides with in vitro liver stage antiplasmodial activity, Bioorg. Med. Chem. 24 (2016) 1786−1792.

[135] C.J.A. Ribeiro, S.P. Kumar, J. Gut, L.M. Goncalves, P.J. Rosenthal, R. Moreira, M.M.M. Santos, Squaric acid/4-aminoquinoline conjugates: novel potent antiplasmodial agents, Eur. J. Med. Chem. 69 (2013) 365−372.

[136] B. Pradines, M. Torrentino-Madamet, A. Fontaine, M. Henry, E. Baret, J. Mosnier, S. Briolant, T. Fusai, C. Rogier, Atorvastatin is 10-fold more active in vitro than other statins against *Plasmodium falciparum*, Antimicrob. Agents Chemother 51 (2007) 2654−2655.

[137] H. Savini, J.B. Souraud, S. Briolant, E. Baret, R. Amalvict, C. Rogier, B. Pradines, Atorvastatin as a potential antimalarial drug: in vitro synergy in combina-tional therapy with dihydroartemisinin, Antimicrob. Agents Chemother. 54 (2010) 966−967.

[138] J. Dormoi, S. Briolant, A. Pascual, C. Desgrouas, C. Travaillé, B. Pradines, Improvement of the efficacy of dihydroartemisinin with atorvastatin in an experimental cerebral malaria murine model, Malar. J. 12 (9) (2013) 302.

[139] R.C.C. Carvalho, W.A. Martins, T.P. Silva, C.R. Kaiser, M.M. Bastos, L.C.S. Pinheiro, A.U. Krettli, N. Boechat, New pentasubstituted pyrrole hybrid atorvastatin-quinoline derivatives with antiplasmodial activity, Bioorg. Med. Chem. Lett 26 (2016) 1881−1884.

[140] M. Jain, S. Vangapandu, S. Sachdeva, R. Jain, Synthesis and blood-schozontocidal antimalarial activities of 2-substituted/2,5-disubstituted-8-quinolinamines and some of their amino acid conjugates, Bioorg. Med. Chem. 12 (2004) 1003−1010.

[141] K. Kaur, M. Jain, S.I. Khan, M.R. Jacob, B.L. Tekwani, S. Singh, P.P. Singh, R. Jain, Amino acid, dipeptide and pseudodipeptide conjugates of ring-substituted 8-aminoquinolines: synthesis and evaluation of anti-infective, β-haematin inhibition and cytotoxic activities, Eur. J. Med. Chem. 52 (2012) 230−241.

[142] M.G. Davanço, A.C. Campos Aguiar, L.A. dos Santos, E.C. Padilha, M.L. Campos, C.R. de Andrade, L.M. da Fonseca, J.L. Dos Santos, C.M. Chin, A.U. Krettli, R.G. Peccinini, Evaluation of antimalarial activity and toxicity of a new pri-maquine prodrug, PLoS One 9 (2014) e105217.

[143] K.K. Nishi, A. Jayakrishnan, Preparation and in vitro evaluation of primaquine-conjugated gum Arabic microspheres, Trends Biomater. Artif. Organs 18 (2) (2005) 191−197.

[144] A. Trouet, P. Pirson, R. Steiger, M. Masquelier, R. Baurain, J. Gillet, Develop-ment of new derivatives of primaquine by association with lysosomotropic carriers, Bull. World Health Organ. 59 (1981) 449−458.

[145] L. Aguiar, M. Machado, M. Sanches-Vaz, M. Prudêncio, N. Vale, P. Gomes, Coupling the cell-penetrating peptides transportan and transportan 10 to primaquine enhanced its activity against liver-stage malaria parasites, MedChemComm 10 (2019) 221−226.