Primaquine homodimers as potential antiplasmodial and anticancer agents

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ABSTRACT

Primaquine homodimers, e.g. symmetric PQ-diamides of dicarboxylic acids containing 4 to 8 carbon atoms, were evaluated against *Plasmodium berghei* hepatic stages and *P. falciparum* blood stages, as well as against three cancer cell lines. Novel PQ-homodimers exerted much higher activity against hepatic stages, but less pronounced activity against blood stages in comparison to the parent drug. The submicromolar activity of succinic, fumaric and maleic derivatives against *P. berghei* was determined (IC_{50} values: 726.2, 198.1 and 358.4 nM, respectively). Our results indicated that the length and type of spacer between two PQ moieties highly modified the antiproliferative activities of PQ-homodimers. The general antiproliferative activity of the adipic and mesaconic derivatives against three cancer cell lines (MCF-7, HCT116, H 460) was observed (GI_{50} = 1.78–13.7 and 2.36–4.31 μM, respectively), but adipic derivative was less toxic to human embryonic kidney cells (HEK 293). High selectivity of fumaric and suberic derivatives against breast adenocarcinoma cell line MCF-7 was detected. These two compounds have shown no antiproliferative activity against other tumor cells and HEK 293.

The dimerization of known antimalarial drugs is an old but still popular strategy to overcome drug resistance and improve therapeutic efficacy. Bis-aminoquinolines and artemisinin-dimer counterparts exhibit strong antimalarial activities and are considered a promising class of antimalarial agents.[1] Various homodimers with two 4-aminoquinoline moieties connected through aliphatic or aromatic linkers of various lengths and chemical natures were developed by Raynes and described in a review article.[2] One of them, WR 268268, consisting of two chloroquine pharmacophores bridged by cyclohexane ring, reached preclinical studies, but its phototoxicity precluded further development.[3] However, the symmetric bisquinoline derivative piperaquine was registered in the 1960s and used in malaria prophylaxis and treatment until the late 1980s.[4,5] Its usage declined when piperaquine-resistant strains of *P. falciparum* arose and artemisinin-based antimalarials became available. Nowadays, piperaquine is used only in fixed-dose combinations with artenimol or arterolane.[6] The clinical studies carried-out with the arterolane-piperaquine combination in *P. falciparum* malaria patients provided evidence of rapid parasite clearance and quick relief of most malaria-related symptoms.[7,8]

Macromolecules of increased rigidity composed of three or four 4-aminoquinolines attached to a cyclam ring were also developed.[9] They showed high activity towards chloroquine-resistant *P. falciparum* strains and decreased toxicity, although their activity towards chloroquine-sensitive strains was not improved (compared to chloroquine).

Dimerization of 8-aminoquinolines is less explored than dimerization of 4-amino analogs. Kaur *et al.* reported the synthesis of three series of bis(8-aminoquinolines) bound by aliphatic, aromatic or amino acid linkers.[1] Several dimers exhibited superior antiplasmodial activities and very low methemoglobin formation compared to the parent drug primaquine (PQ). However, PQ analogs of piperaquine were inactive as blood schizontocides.[10,11] Lödige *et al.* synthesized a number of hybrids consisting of PQ and chloroquine components, which showed activity against all stages of the *Plasmodium* infection in humans as well as against chloroquine-resistant strains.[12,13]

Dimers with two quinine molecules are the most potent inhibitors of the chloroquine resistance transporter (PfCRT^{CQR}), which mediates chloroquine resistance by effluxing the drug from the parasite's digestive vacuole, the acidic compartment in which chloroquine exerts its antiplasmodial effect.[14] Quinine dimers accumulate within the digestive vacuole and are not effluxed by PfCRT^{CQR}. It is considered that piperaquine and other bulky bisquinoline compounds also contribute to the inhibition of PfCRT^{CQR} and inhibit parasite hem-digestive pathway.[7] The
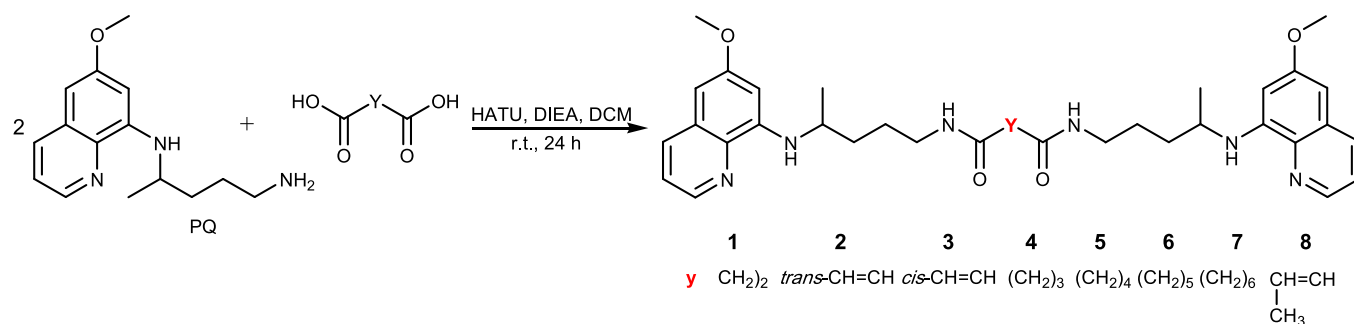

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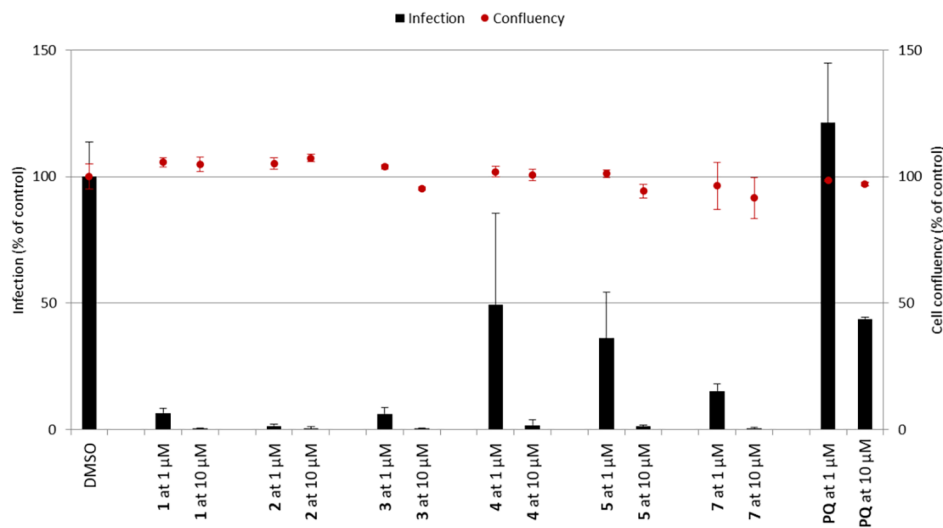**Scheme 1.** Synthesis of PQ-homodimers **1–8**.

Fig. 1. Activity of 1 and 10 μM concentrations of the PQ-homodimers against *P. berghei* liver stages. Parasite load (infection scale, bars) and proliferation of Huh7 cells (cell confluency scale, dots) are shown. PQ and DMSO were included as positive and negative controls, respectively.

higher efficacy of the bis(4-aminoquinolines) against chloroquine-resistant parasites is explained by the fact that they contain a higher number of protonation sites than chloroquine, resulting in their accumulation in the cell compartment with a decreased pH.[1]

The dimerization strategy also became attractive for discovering effective anticancer drugs among antimalarials. A wide variety of artemisinin dimers demonstrated improved antiproliferative properties compared to their parent compounds,[15–23] whereas some promised to potentially circumvent multidrug resistance of cancer cells.[24,25] Heterodimers of various endoperoxides and aminoquinolines were also explored.[26–31] They showed efficient antimalarial activity and/or potent in vitro antitumor activity. Several studies suggested that the nature and length of the linkers were crucial for anticancer activities.[22,32,33]

In our previous papers, we have described PQ-homodimers in which two PQ moieties were linked with one urea group (**I**), two subsequent urea groups (**II**) or two urea groups separated by two methylene groups (**III**).[34,35] The bis-urea derivative **II** showed extreme selectivity against breast adenocarcinoma cell line MCF-7 and negligible activity against the other seven tested cancer cell lines. Derivative **I** was less active, but also selective towards MCF-7, while dimer **III** showed low activity towards all tested cancer cell lines. Compounds **I** and **II** also showed activities against the erythrocytic stage of the *P. falciparum* NF54 strain in micromolar concentrations and low cytotoxicity towards L6 cells.[36,37]

Considering that the potential of bis(8-aminoquinolines) is not fully explored, we now report the synthesis of eight PQ-homodimers **1–8** varying in length and type of spacer between two PQ scaffolds, with the aim to increase their antiplasmodial and/or anticancer activities. The amide bonds between PQ and succinic, fumaric, maleic, glutaric, adipic,

pimelic, suberic or itaconic acid were achieved using (1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU) as a coupling reagent and Hünig's base *N,N*-diisopropylethylamine (DIEA) (Scheme 1). The synthetic method employed is simple, requires short reaction times and mild reaction conditions. To check how the distance between two PQ pharmacophores reflects on the activity, linkers containing between four and eight carbon atoms were introduced. Four different C-4 linkers were designed, one without double bonds (succinic derivative **1**) and three with double bonds (fumaric derivative **2** with *trans* configuration, maleic derivative **3** with *cis*-configuration and itaconic acid derivative **8** with the exomethylene group, which isomerized to mesaconic isomer).

Antiplasmodial activity of novel compounds was evaluated in two models. *P. berghei* model was employed to assess compound activity against hepatic infection in vitro, the *P. falciparum* study addressed the activity of the compounds against the blood stage of infection, also in vitro. We started by evaluating activity of 1 and 10 μM concentrations of the synthesized PQ-homodimers against the hepatic stages of the rodent *P. berghei* parasite (Fig. 1). Compounds **1**, **2** and **3**, all derived from C-4 dicarboxylic acids (succinic, fumaric or maleic), displayed the highest activity in this assay, much higher than the parent drug.

We then proceeded to determine the IC_{50} values of these three compounds, by evaluating the dose-dependent activity of 7 concentrations of each compound against *P. berghei* infection of Huh7 cells (Fig. 2). Our data show that all three compounds display sub-micromolar activity against *P. berghei* hepatic infection in vitro, with IC_{50} values of 726.2, 198.1 and 358.4 nM, for compounds **1**, **2** and **3**, respectively.

We then evaluated the in vitro activity of the PQ-homodimers

Compound 1
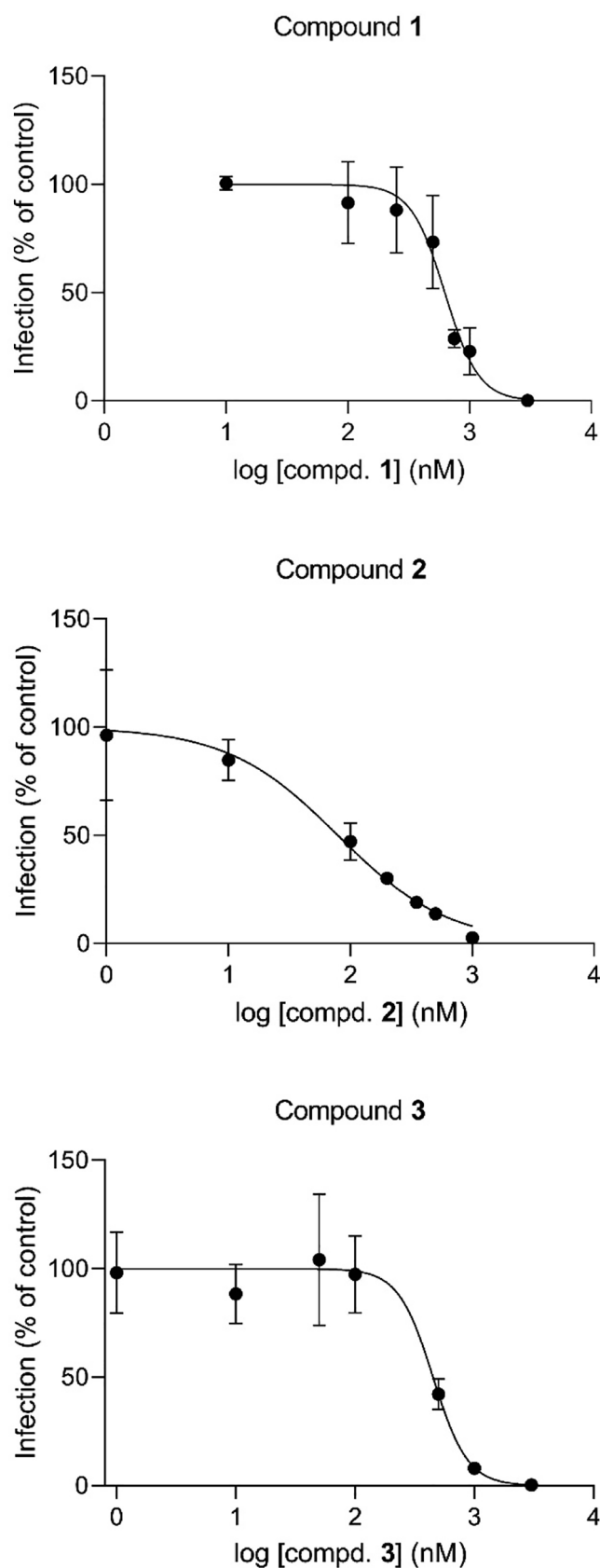

Compound 2

Compound 3

Fig. 2. Dose-dependent activity of the most active PQ-homodimers against *P. berghei* hepatic stages. Infection is represented as a percentage of the negative control, DMSO.

Table 1
IC_{50} values of PQ-homodimers against the erythrocytic stages of chloroquine sensitive (*Pf*3D7) and chloroquine resistant (*Pf*Dd2) *P. falciparum* strains.

Compd.	IC_{50} (μM)	
	*Pf*3D7	*Pf*Dd2
1	17.2	> 27.7
2	14.6	> 27.7
4	16.5	> 27.7
5	> 27.7	> 27.7
7	23.1	12.7
PQ[a]	1.30	1.9

[a] PQ – primaquine
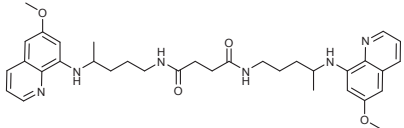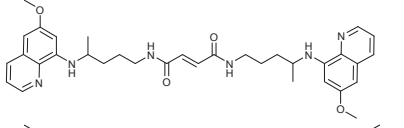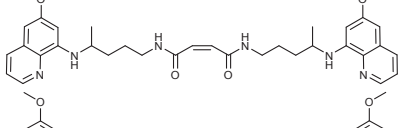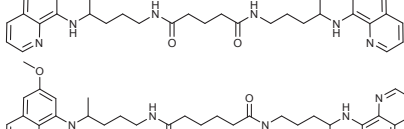
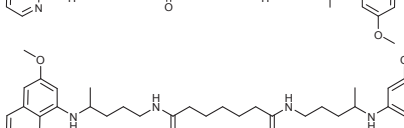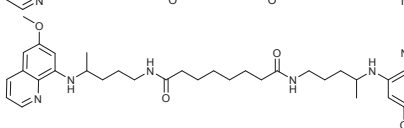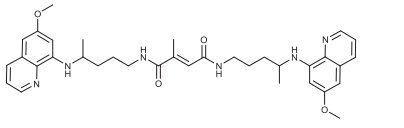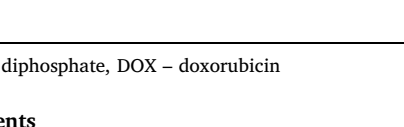against the erythrocytic stages of the chloroquine-sensitive 3D7 and chloroquine-resistant Dd2 strains of the human *P. falciparum* parasite. Our results have shown that all compounds exerted only weak activity against either parasite strain, lower than the parent drug, indicating that the improvement against chloroquine-resistant strain was not achieved (Table 1). In conclusion, the liver stage-specific activity is significantly more pronounced than the blood stage activity, which is agreement with the fact that the parental compound PQ is the reference compound against the liver stage of mammalian infection by *Plasmodium*.

In order to investigate the antiproliferative potential of PQ-homodimers, an in vitro assay on three human cancer cell lines (breast adenocarcinoma MCF-7, colon carcinoma HCT116 and lung carcinoma H 460) and human embryonic kidney cells (HEK 293) were performed. Our results pointed that the length and type of spacer between two PQ moieties highly modified the antiproliferative activity. All compounds were active against the MCF-7 cell line at single-digit micromolar concentrations (IC_{50} = 2–9 μM) (Table 2). Activities towards the other two cancer cell lines were in general lower, and similar to those of PQ (IC_{50} = 10–30 μM). Two compounds, namely fumaric and suberic derivatives **2** and **7**, showed high selectivity towards MCF-7 cell line and practically no activity against the other cancer cell lines and HEK 293. Compound **5** showed high activity against all cancer cells and no antiproliferative effect against HEK 293 (selectivity indices from 7.3 to 56). Compounds **3**, **4** and **6** were also more active against cancer cell than against HEK 293, with selectivity ratio from 1.6 to 31, depending on the cancer cell type. However, mesaconic derivative **8**, containing the carbon–carbon double bond conjugated with a carbonyl group (Michael acceptor), exerted high antiproliferative activity towards all three cell lines employed in these assays and 2–6 times less activity against HEK 293. Only succinic derivative **1** was equally active against cancer and noncancerous cells tested.

We have demonstrated that activity of PQ-homodimers against *P. berghei* hepatic stages increased and activity against *P. falciparum* blood stages (both chloroquine-sensitive and chloroquine-resistant strains) decreased, in comparison to the parent drug. These facts indicate that PQ-homodimers target different molecular systems in these two stages of the parasite's life cycle. The chain length of four heavy atoms between two PQ scaffolds is optimal for PQ-homodimer activity against *P. berghei* hepatic stages.

We further confirmed the high selectivity of fumaric and suberic derivatives **2** and **7** towards the MCF-7 cancer cell line and the general antiproliferative activity of adipic and mesaconic derivatives **5** and **8**. Compounds **2**, **5** and **7** showed no antiproliferative activity against non-cancer cells HEK 293. Our results suggest that compound **2** could serve as a lead for the development of novel antiplasmodial and compound **5** for the development of anticancer agents. On the other hand, derivatives **2** and **7** show promising results for the targeted therapy of breast adenocarcinoma.

Table 2
Growth inhibition of selected tumour cell lines (MCF-7, HCT116, H 460) and human embryonic kidney cells (HEK 293) in vitro.

Compd.	Structural formula	IC$_{50}$ (µM)[a]			
		MCF-7	HCT116	H 460	HEK 293
1		2.82 ± 1.19	11.27 ± 2.04	18.49 ± 9.85	2.25 ± 0.4
2		9.30 ± 5.44	≥100	≥100	≥100
3		3.14 ± 1.80	9.19 ± 1.40	14.11 ± 0.81	17.47 ± 7.5
4		3.37 ± 0.55	16.94 ± 1.19	10.35 ± 8.62	26.38 ± 18.93
5		1.78 ± 0.71	13.70 ± 0.86	2.49 ± 1.73	≥100
6		3.04 ± 1.20	26.07 ± 1.01	26.69 ± 4.64	94.9 ± 0.4
7		1.85 ± 1.06	≥100	≥100	≥100
8		2.36 ± 0.47	4.31 ± 2.88	1.63 ± 1.13	10.0 ± 5.1
PQ		8.73 ± 3.55	13.99 ± 5.35	19.84 ± 10.71	10.96 ± 1.23
DOX		0.01 ± 0.001	0.01 ± 0.006	0.003 ± 0.002	0.018 ± 0.0004

PQ – primaquine diphosphate, DOX – doxorubicin

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.08.018.

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