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1,2-Diphenoxiethane salts as potent antiplasmodial agents

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ABSTRACT

In this article we present a series of non-cytotoxic potent human choline kinase (CK) inhibitors that exhibit nanomolar antiplasmodial activity *in vitro*. The most active antiplasmodial compounds, **10a–b**, bearing a pyridinium cationic head were inactive against CK, while compounds **10g** and **10j** with a quinolinium moiety exhibit moderate inhibition of both the parasite and the enzyme. The results point towards an additional mechanism of action unrelated to CK inhibition that remains to be established.

Malaria is an important health problem and according to the World Malaria Report published in 2017, 445000 deaths occur annually. Approximately 80% of the cases and 90% of the deaths are estimated to occur in sub-Saharan Africa where children, young people and pregnant women are most severely affected.[1]

Resistance and acquired immunity are major elements influencing malaria transmission and symptoms intensity. A major obstacle to the eradication of this disease is the emerging resistance of the malaria-causing parasite *Plasmodium falciparum* to most marketed antimalarial drugs. As such, new compounds acting through novel mechanisms of action are needed.[2–4] Choline kinase (CK) has been proposed as a specific drug target for malaria. This enzyme is the first player in the Kennedy pathway for the biosynthesis of phosphatidylcholine, (PC) although it can also participate in the biosynthesis of phosphatidylethanolamine (PE), which are both essential phospholipids in *P. falciparum* representing 40–50% of the total lipid content.[5,6]

The proliferation of the parasite within erythrocytes is concomitant with a massive increase of PC biosynthesis. It has been demonstrated that the inhibition of *P. falciparum* choline kinase (*Pf*CK) disrupts the Kennedy pathway, which results in parasite death.[7–10]

Recently we reported similarities among the CK binding sites from *Pf*CK, *Cryptosporidium parvum* choline kinase (*Cp*CK) and human choline kinase α1 (*Hs*CKα1), highlighting the feasibility of designing novel inhibitors based on the choline-binding pocket. The active sites of *Hs*CKα1, *Pf*CK, *P. knowlesi* (*Pk*CK) and *Cp*CK are highly conserved (69% in *Plasmodium* sp and 80% in *Cp*CK with respect to the human enzyme).[9] Moreover, these proteins use similar mechanisms of ligand recognition both exhibiting conserved interactions.[9]

As in humans, *Pf*CK catalyzes the phosphorylation of choline or ethanolamine in the presence of ATP and Mg^{2+}, although the affinity for ethanolamine is much lower.[5] However, it differs with regard to several aspects; thus the genome of this parasite contains a single gene encoding *Pf*CK, it lacks isoforms and is active as a monomer.[5,11]

The Kennedy pathway is the most common route for the synthesis of PC and PE, consequently the enzymes involved, CK, CTP: phosphocholine cytidylyltransferase (CCT) and choline/ethanolamine-phosphate transferase (CEPT), have been validated in *Plasmodium* as targets for new antimalarial therapies.[5–7] Examples of inhibitors of the pathway are **PG12** (which inhibits *Pf*CCT) and **amodiaquine** which inhibit *P. falciparum* phosphoethanolamine methyl-transferase (*Pf*PMT).[12] This enzyme belongs to an alternative route that involves serine decarboxylase-phosphoethanolamine methyltransferase (SDPM)[13] and has received special attention for the development of selective antimalarials since it is not present in mammals.

Numerous studies support the interest of biscationic compounds as attractive candidates for new drugs against malaria.[2,8,13–16] **HC-3**, **MN58b** and **RSM-932A** (Fig. 1) represent the first three generations of compounds with antiproliferative and antiplasmodial properties that exhibit CK inhibition. In addition **HC-3** has also been postulated as an inhibitor of choline uptake.[14–16]

Regarding the mechanism of action and antiparasitic activity of these compounds, it has recently been published that both **HC-3** and

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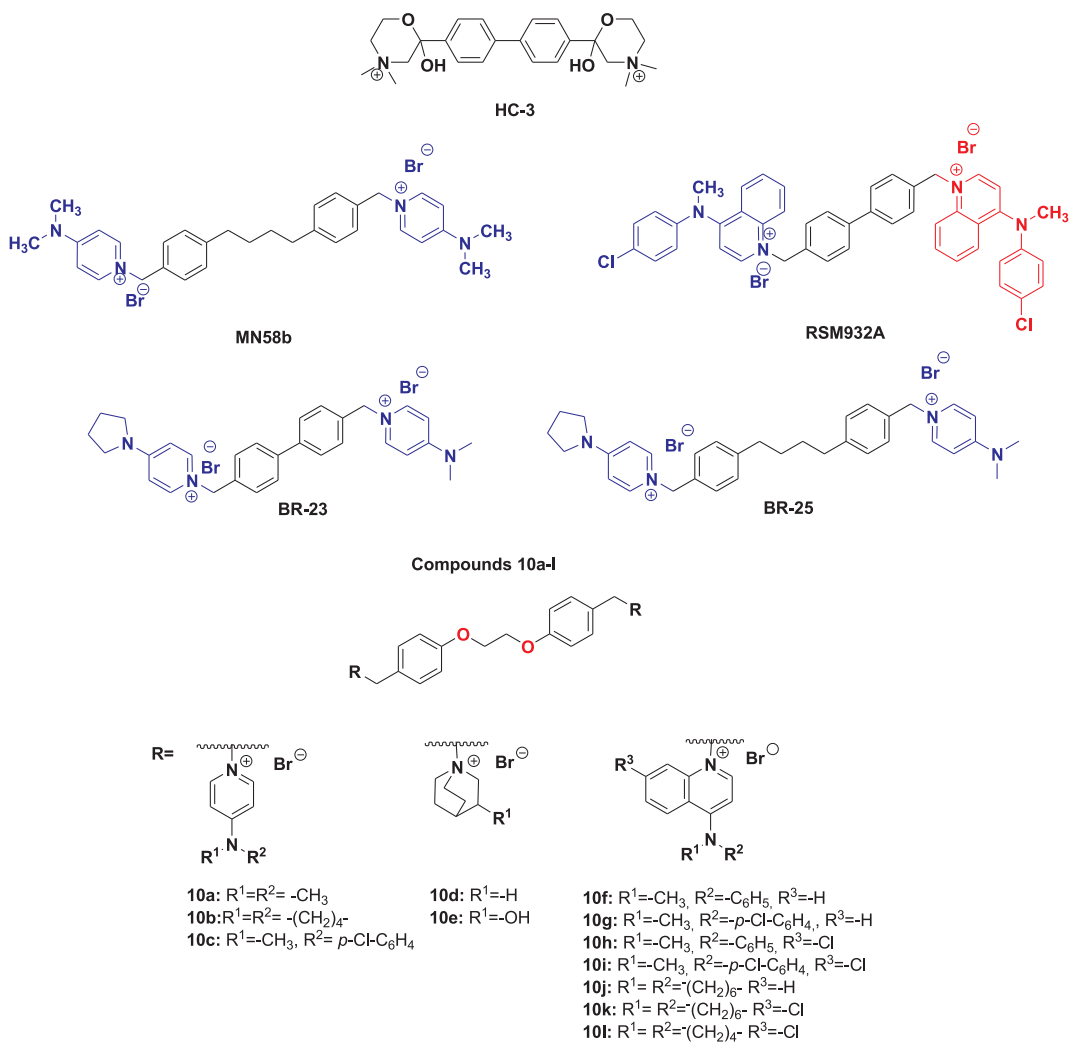

Fig. 1. General structures of the previously reported [18] antiplasmodial biscationic compounds and symmetrical compounds **10a–l** evaluated in this work.

MN58b would interact with the catalytic site, however they do not behave as competitive inhibitors.[16] **RSM-932A** appears to have an altogether novel mechanism of inhibition, in which this inhibitor traps *Pf*CK in a phosphorylated intermediate state blocking phosphate transfer to choline. **HC-3**, **MN58b** and **RSM932A** exhibited IC_{50} values for enzyme inhibition of 250 μM, 106 μM, and 1.75 μM, respectively[16] while the inhibition data for *in vitro* assays against the parasite were higher than 50 μM, 3.5 nM and 26.5 nM, respectively.[16]

On the other hand it has been recently reported that the *Pf*CK is responsible for the production of the vast majority of PE and PC in the parasite. The inhibition of this enzyme depends on the presence of the alternative substrates, ethanolamine or choline, but also on the different binding modes in the choline-binding site of *Pf*CK compared to the human enzyme. The asymmetrical bispyridinic compounds described in Fig. 1 (**BR-23** and **BR-25**) inhibited the formation of PE directly by reducing its biosynthesis via the Kennedy pathway and consequently resulted lethal for the parasite. **BR-23** and **BR-25** were selective inhibitors of *Pf*CK when the substrate was ethanolamine.[17]

We have also previously reported a series of 1,1′-(((ethane-1,2-diylbis(oxy))bis(4,1-phenylene))bis(methylene))-bispyridinium and bisquinolinium bromide derivatives with general structure **10**[18], containing a pair of oxygen atoms in the linker of their structure, **Scheme 1**. These compounds were good inhibitors of *Hs*CKα1 and exhibited nanomolar activity against a panel of eight cancer cell lines. Of the tested compounds, derivatives **10a** and **10l** displayed IC_{50} values of 0.027–0.12 and 0.007–0.8 μM against a panel of eight solid and leukemic tumours and IC_{50} values for the inhibition of *Hs*CKα1 of 1.00 and 0.92 μM, respectively. These values of *Hs*CKα1 inhibition were very similar to those reported for **MN58b** y **RSM932A**.[18] Docking and crystal structure studies have revealed that the 1,2-diphenoxyethane linker allows these molecules to adopt both an antiperiplanar and synclinal conformation, which means that they could bind to the enzyme in a conformation similar to both **BR-23** (with a shorter linker), and **BR-25** (compound with the longer linker). However, as suggested, compounds **10a–b**, **d–e** would adopt a conformation similar to **BR-23** and compounds **10c**, **g–l** would behave preferably as **BR-25**.[17] These observations together with the low toxicity described for some of these compounds have encouraged us to evaluate compounds **10a-l** as antiplasmodials and to study their possible mechanism of action as inhibitors of *Pf*CK.

Here we present a preliminary study of the compounds previously analysed in human tumour and non-tumour cells[18] in order to investigate their behaviour as antiplasmodial agents. We have evaluated **their** *in vitro* activity and selected the most potent to study their activity against *Pf*CK[19,20]

The results are shown in Table 1, where the compounds were grouped in three series depending on the cationic head present in their structure: bispyridinium (**A**), bisquinuclidinium (**B**) and bisquinolinium (subdivided in two, **C-1** and **C-2**). In general, the bispyridinium compounds (**10a–b**) exhibited the highest antiplasmodial activity although there were no significant differences between a bulky group and a dimethylamino substituent.