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Modified quaternary ammonium salts as potential antimalarial agents

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ABSTRACT

A series of new quaternary ammonium salts containing a polyconjugated moiety has been synthesized and characterized; their biological activity as potential antimalarial agents was investigated, as well. All compounds were screened against chloroquine resistant W-2 (CQ-R) and chloroquine sensitive, D-10 (CQ-S) strains of *Plasmodium falciparum* showing IC_{50} in the submicromolar range and low toxicity against human endothelial cells.

1. Introduction

Malaria is the most prevalent tropical disease in the world with 207 million estimated new cases in 2012 and an annual death toll of 627,000 people. At present, there are 104 countries in which malaria is considered endemic.[1]

The increasing drug resistance of *Plasmodium falciparum* (*P. falciparum*) responsible of the most lethal form of malaria and the resistance of mosquitoes to various pesticides stimulates the search for new weapons to fight this disease.

This is particularly urgent now that the resistance to the artemisinin derivatives, essential component of the combination treatments presently available, has been confirmed in SE Asia.[2]

Quaternary ammonium salts are known to play an active role inhibiting the growth of *P. falciparum* parasites and have gained attention as a new, effective, and relatively cheap anti-malarial drugs. Ancielin et al.[3,4] reported that the in vitro lethal effect of quaternary ammonium compounds on *P. falciparum* are predominantly related to the inhibition of phosphatidylcholine and phosphatidylethanolamine biosynthesis as a consequence of a reduced choline transport into infected erythrocytes. Choline cannot be synthesized by the parasites which relies on exogenous supply. The treatment with phospholipids polar head analogs interferes with natural phospholipids biosynthesis by competition or substitution.[5,6] From these observations, a series of bis-thiazolium compounds have been tested and shown to possess potent antimalarial activity.[7,8] The bis-thiazolium series provided the clinical candidate albitiazolium[9] (albitiazolium bromide, SAR97276), that is in clinical phase II trials to treat severe malaria by the parenteral route.[10]

As widely reported in literature,[11,12] polyconjugated systems such as retinoids and carotenoids possess many biochemical and pharmacological properties including antioxidant and antimicrobial activities.

The retinol and some retinoid-like compounds are able to inhibit the in vitro growth of *P. falciparum* suggesting that this parasite is retinol-sensitive and that, in patients with malaria, adjunctive retinol therapy may accelerate parasite dysfunction and death.[13] Moreover, it is also reported the high antiplasmodial activity of a marine carotenoid, fucoxanthin, extracted from marine brown seaweeds, macroalgae, diatoms and microalgae which activity could be related to its antioxidant properties.[14]

In the last decade our interest was turned on the synthesis of polyunsaturated molecules[15,16] whose structure is shown in Figure 1 which share with carotenoids and retinoids some structural features, antioxidant properties, effects on cell proliferation and antibacterial activity.[17]

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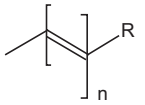

R = CHO, CH_2OH, COOH, Ph

n=2-7

Figure 1. Polyunsaturated compounds.

These compounds are chemically homologous of sorbic acid (2*E*,4*E*-hexadienoic acid), a natural preservative used as antimicrobial agent especially in food, drinks and cosmetics.

From the preliminary biological evaluations[18] (2*E*,4*E*,6*E*)-octatrien-1-ol, the corresponding acid and potassium salt showed interesting pharmacological properties. With the aim to develop a new class of compounds with potential antimalarial activity, in this work we describe the synthesis, the molecular characterization and the preliminary evaluation of the antimalarial activity in vitro of new quaternary ammonium salts derived from (2*E*,4*E*,6*E*)-octatrien-1-ol.

2. Results and discussion

2.1. Chemistry

The compounds **4a**–**e** and **6a**–**e** were obtained following Scheme 1. Firstly, (2*E*,4*E*,6*E*)-octatrien-1-ol of industrial origin was oxidized to (2*E*,4*E*,6*E*)-octatrienal **1** with manganese dioxide and then reacted in anhydrous toluene with primary amines with long alkyl chain to give the corresponding Schiff bases **2a**–**e**. The quantitative reduction of imines with sodium borohydride in refluxed anhydrous ethanol allowed to obtain the secondary amines **3a**–**e** which were directly quaternized in a stainless steel autoclave using an excess of methyl iodide in presence of a stoichiometric amount of an hindered base to give ammonium salts **4a**–**e**. Otherwise for the synthesis of ammonium salts **6a**–**e**, the secondary amines **3a**–**e** were at first reacted with benzyl chloride in the presence of a stoichiometric amount of diisopropylethylamine and finally reacted with methyl iodide.

All synthesized compounds gave satisfactory analytical and spectroscopic data which were in full accordance with their depicted structures.

2.2. Biological activity

Quaternary ammonium compounds with a long alkyl chain on the nitrogen atom are emerging as potentially drug alternatives to conventional antimalarial chemotherapy. The studies on antimalarial activity have suggested that choline transport from plasma to the infected erythrocyte might be the primary target of these compounds[19,20] and this activity seems to be related to shape, electronegativity, and lipophilicity of these compounds. The aim of this study was to synthesize a new class of lipophilic quaternary ammonium salts bearing at the nitrogen atom a lipophilic electron rich eight carbon polyconjugated system and to evaluate their potential antimalarial effects in vitro. The lipophilicity and the electronic density of these compounds was then further modulated by introducing a C_{12}–C_{18} saturated alkyl chain and dimethyl (compounds **4a**–**e**) or methyl-benzyl (compounds **6a**–**e**) substituents. The antimalarial activity of a fully saturated dimethylammonium salt (compound **7**) and of octatrienoic potassium salt was also evaluated as control.

The compounds under study were tested for their antimalarial activity against chloroquine resistant, W-2 (CQ-R) and chloroquine sensitive, D-10 (CQ-S) strains of *P. falciparum*. Their antimalarial activity was quantified as inhibition of parasite growth, measured as the activity of parasite lactate dehydrogenase (pLDH). The results are summarized in Table 1 which shows the concentration of drugs inducing 50% of growth inhibition (IC_{50}). The majority of the tested compounds exhibited moderate activity against both parasite strains, whereas the (2*E*,4*E*,6*E*)-octatrienoic acid potassium salt was inactive. Indeed, seven compounds (**4b**, **4c**, **4d**, **6a**, **6b**, **6c**, **6e**) showed IC_{50} values less than 1 μM against both strains. The remaining three compounds were considered inactive with IC_{50} well above 1 μM. Moreover, compound **7**, analogous of **4a** with a saturated alkyl chain with eight carbon units, exhibited an activity comparable with that of compounds **4c**, **6a** and **6c**, suggesting that the impact of the polyconjugated chain is modest. In both series the effects of chain-length modifications on the antimalarial activity is maximal at 14 carbon chain. However, in the series **4a**–**e**, a linear trend of IC_{50} values is observed even beyond the influence of the carbon chain length; whereas there is not such a close correlation for the compounds **6a**–**e**, suggesting the influence of the steric hindrance of the benzyl system and of the electronic density distribution. Varying a methyl group on the nitrogen atom of compounds **4** with a benzyl residue, the activity patterns is slightly improved with the exclusion of compound **6d**.

What is interesting is the fact that the most potent compounds (**6a** and **6c**) showed comparable activity against both the CQ-S and the CQ-R strains. Indeed, all the compounds of this study were equally active against both the CQ-S and the CQ-R strains. The resistance index (RI), that is the ratio between the IC_{50} of each compound against the two strains of *P. falciparum*, and thus an indication of possible cross-resistance with CQ, was significantly lower than that of CQ (Table 1); this is a characteristic shared with other quaternary ammonium salts.[20]

The cellular cytotoxicity on a human microvascular cell line (HMEC-1) was also assayed. All compounds exhibited low toxicity against this human cell line with a selectivity index (SI), calculated on D10 and W2 strains, ranging between 7 and 102. Interestingly, the most active compounds (**4c**, **6a**, **6c**) were also the less toxic which is indicative of selective action against parasitized red blood cells.

3. Conclusions

In this paper, two new series of quaternary ammonium salts derived from all *trans* 2,4,6-octatrienol have been synthesized and evaluated for their in vitro activity against D-10 (CQ-S) and W-2 (CQ-R) strains of *P. falciparum*. The most active of the tested compounds displayed a significant inhibitory activity with IC_{50} in the submicromolar range with no cross resistance with CQ and very low cytotoxicity against a human endothelial cell line, suggesting a high therapeutic index. Considering that the structural requirements for antiplasmodial activity (polar head and lipophilicity around nitrogen) of these compounds are similar to quaternary ammonium derivatives previously described,[8] we can suppose that they act through inhibition of choline transport. The complete saturated analog **7** was approximately equipotent to the most active compounds.

The introduction of the polar head on the polyconjugated chain allowed to obtain compounds suitable of further optimization to improve in vitro activity against the parasites before considering in vivo experiments and studies for target identification.

4. Experimental

4.1. General

All commercial available solvents and reagents were used after distillation or treatment with drying agents. (2*E*,4*E*,6*E*)-octatrienol, a gift of Giuliani s.p.a. (Milan, Italy), was purified before use by