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Synthesis and *in vitro* biological evaluation of dihydroartemisinyl-chalcone esters

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ABSTRACT

A series of dihydroartemisinyl-chalcone esters were synthesized through esterification of chalcones with dihydroartemisinin (DHA). The hybrids were screened against chloroquine (CQ) sensitive (3D7) and CQ resistant (W2) strains of intraerythrocytic *Plasmodium falciparum* parasites, and were all found to be active, with IC_{50} values ranging between 1.5 and 11 nM against both strains, with SI values over 5800. The esters featuring oxygenated aryl rings (**7**, **10** and **11**), were found to be equipotent to DHA, but were 2–3 times more active than artesunate against the 3D7 and W2 strains of the malaria parasites. They were also screened *in vitro* against a panel of three cancer cell lines consisting of TK-10, UACC-62 and MCF-7. Compound **7**, bearing a furan ring, displayed the most potent overall antitumor activity against all three cancer cell lines. TGA revealed that the targeted hybrids were all thermally more stable than DHA, which may be beneficial to the high temperature storage conditions that prevail in malaria endemic countries. During this study, ester **7** was identified as the best candidate for further investigation as a potential drug in search for new, safe and effective antimalarial drugs.

1. Introduction

Together with AIDS and TB, malaria is one of the three major communicable diseases, worldwide [1]. Malaria is caused by a protozoan parasite of the genus *Plasmodium*. There are an estimated two billion people at risk of contracting this disease [2]. Malaria is commonly found in tropical and subtropical regions, such as Africa and Asia [3] and is endemic in 97 countries, worldwide. In 2012, it was reported that 627,000 people succumbed of malaria, for which *Plasmodium falciparum* was responsible for the majority of deaths. Of these reported deaths, 90% were in the African region and 86% were children under the age of five [2].

Despite the fact that artemisinins (Fig. 1) suffer from severe drawbacks, such as very short pharmacological half-lives, paired with low water or oil solubility [4], artemisinin based combined therapy (ACT) is currently the preferred treatment for uncomplicated malaria. By contrast, artesunate or quinine-artemether is the drug/drug combination of choice for severe *P. falciparum* malaria [5]. Although the global burden of malaria has reduced by 25% in the past decade, progress might be hindered by the emergence of clinical tolerance towards artemisinin in South Asia and along the Cambodia–Thailand border [2,6]. Widespread resistance towards artemisinins could have dire consequences to the struggle against malaria, since this class of drugs currently comprises the last line of chemotherapeutic defence against this disease.

In an attempt to avoid widespread resistance developing against artemisinins by malaria parasites, as had happened with chloroquine (CQ), the World Health Organization (WHO) recommended that artemisinins be withdrawn from the market as mono-therapy and be replaced by ACT's instead. It is hoped that this may slow the spread of tolerance to some extent. With the on-going use of artemisinins in mono-therapy, especially in Asia, the development of resistant strains towards this class of compounds is unfortunately inevitable [7]. An alternative strategy that could help in overcoming the progress of resistance is by making use of hybridization of the parent molecule with other pharmacophores [8]. Hybrid molecules combine two drugs in a single molecule with the aim of creating a chemical entity with two or more structural domains, having different biological functions and dual activity that are medically/therapeutically more effective than its individual components [9].

The *in vitro* discovery of antimalarial activity of by Licochalcone A (Fig. 2), a natural product isolated from Chinese liquorice roots, led several authors to investigate the activity of chalcone and chalcone-based compounds, but with varying success [6,10–15].

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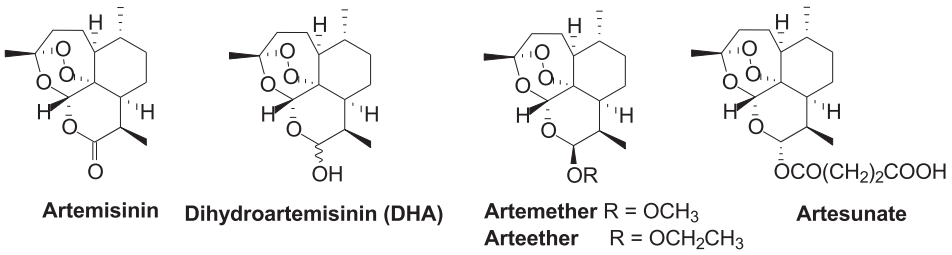

Fig. 1. Artemisinin and its clinically used derivatives.

Chalcones have been proven to be cysteine protease inhibitors [16], which mediates protein hydrolysis via nucleophilic attack on a carbonyl of a susceptible peptide bond. The main function of malaria parasite cysteine protease is the hydrolysis of haemoglobin in the food vacuole [17]. These enzymes are also presumed to be involved in the rupture of the erythrocyte membrane [18]. Besides its antimalarial activity, chalcones display an immense range of biological functions [6,19]. It was not until 2009 that chalcones and dihydroartemisinin (DHA) had been combined by two independent research groups, as a hybrid through an amide, ester or ether bond respectively, for use as antitumor agents. These compounds showed superior activity against various cancer cell lines compared to DHA [20,21].

While artemisinin-based compounds show high efficacy when administered via systemic routes, they are fairly less effective when given orally [22]. Due to an increasing emphasis on fixed-dose combinations in antimalarial therapy, the combination of DHA and various chalcones into hybrid molecules may offer several advantages over combination therapy. Furthermore, Singh et al. [22] reported that ester derivatives of DHA showed better oral activity than artemether, which may be beneficial to the administration of these drugs in rural, malaria endemic areas. It was demonstrated by Cloete et al. [23] that 10-alkyl/aryl ester derivatives of DHA had shown superior activity, compared to and DHA and 10-aminoethylethers of artemisinin.

Furthermore, more than 60% of world's total new annual cases of cancer also occur in Africa, Asia and Central and South America. In 2012, 8.2 million cancer related deaths were reported with 70% of the deaths occurring within these regions [24]. It is thus clear that besides the major burden malaria places on Africa and Asia; cancerous diseases also contribute tremendously to both the socio-economic distress and total deaths within these regions. Interestingly, it has also been found that DHA is an effective antitumor agents [25] and for this reason the antitumor properties of the synthesized compounds in this study will also be investigated.

As part of our program focussing on the discovery of novel antimalarial compounds and in light of the above considerations, ester compounds, formed by linking various chalcones to DHA, were investigated. In each instance ring B of the chalcone would be varied with different aromatic moieties ranging from un-

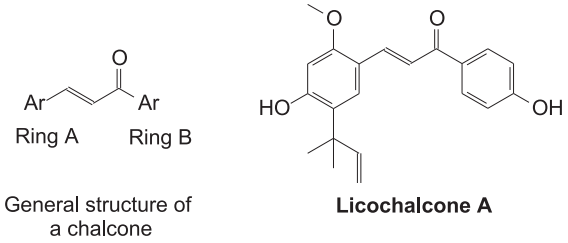

Fig. 2. General structure of a chalcone and licochalcone A.

substituted phenyl rings to substituted phenyl rings with both electron donating- and -withdrawing groups. In this paper, the synthesis and *in vitro* antimalarial, cytotoxicity and antitumor activities of these novel dihydroartemisinyl-chalcone esters are reported.

2. Results

2.1. Chemistry

The chalcone intermediates **1**–**6** were easily attained in high yields (70–96%) through Claisen–Schmidt condensation of 4-formylbenzoic acid and the appropriate aryl ketone in basic methanolic water. This reaction resulted in the exclusive formation of the *E*-isomer, as corroborated by the large coupling constant (*J*) of ~15 Hz [26]. The targeted compounds, **7**–**11**, were synthesized in low to moderate yields (8–61%) through esterification of the carboxylic acid-functionalized chalcone and the hydroxyl group of DHA, using 1,1′-carbonyldiimidazole (CDI) as coupling agent. The reaction of compound **6** with DHA, using oxalyl chloride as coupling reagent, resulted in the desired compound **12** in low yields (9%). All compounds were characterized by routinely used methods, such as NMR, IR and HRMS. Most of the hybrid compounds proved to be unstable towards the harsh conditions of MS and therefore the nominal mass was not observed. Elemental analysis (EA) was thus performed on all the hybrid compounds.

2.2. Physicochemical properties

Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were employed to determine the physical state, different phase transitions and thermal stability of the targeted hybrid compounds. Both DSC and TGA thermograms are supplied as Supporting Information.

The ADMET properties, such as log*P*, solubility and absorption levels were determined using ACD/Chemsketch and Discovery Studio 3.1 software. It should be noted that these calculated values are theoretical values and do not necessarily portray the true ADMET properties. Preliminary ADMET calculations were performed to determine the drug-like properties of the synthesized compounds **1**–**12**, together with DHA, artesunate (AS) and CQ as references and are summarized in Table 1.

Chalcones **1**–**5** demonstrated favourable drug-like properties, such as log*P* values lower than 4, coupled with good absorption levels, similar to CQ, DHA and AS. These chalcones also displayed increased solubility levels, compared to CQ, DHA and AS. Contrary, chalcone **6** had a higher log*P* value, with solubility and absorption levels similar to CQ, DHA and AS. The esters **7**–**11** showed poor drug-like properties, such as log*P* values above 6.5, very low solubility and deprived absorption levels, compared to those of CQ, DHA and AS. In terms of drug-like properties, compound **12** had very poor drug-like properties, with extremely low solubility and very