Chapter 5: Synthesis and biological evaluation of dihydroartemisinyl-chalcone esters - Article 3

Chapter 5 contains the manuscript of an article to be submitted to ChemMedChem. The article contains the background, aim, results, calculated physicochemical properties, DSC, TGA, in vitro biological results and experimental details of synthesised compounds of this study. This article is prepared according to the author's guidelines available in the Author information pack at the journal homepage: http://onlinelibrary.wiley.com/journal/10.1002/%28ISSN%291860-7187/homepage/2452_authors.html

Synthesis and biological evaluation of dihydroartemisinyl-chalcone esters

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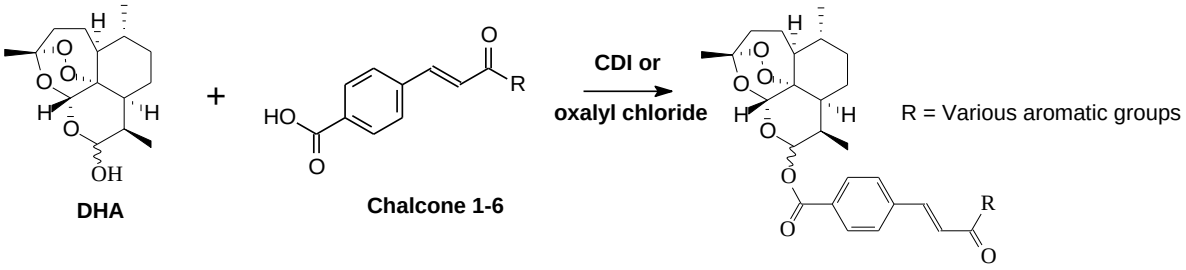
A series of artemisinyl-chalcone esters were synthesized and their in vitro antimalarial activity against 3D7 and W2 strains of *Plasmodium falciparum* determined. The in vitro cytotoxicity were determined against normal Human Fetal Lung Fibroblast (WI-38 cell line), while anticancer activities were determined against a 3-cell line panel consisting of TK-10 (renal), UACC-62 (melanoma) and MCF-7 (breast) cancer cells.



Antimalarial activity

IC_{50} **3D7**: 2 - 11 nM

IC_{50} **W2**: 1.5 - 11 nM

Anticancer activity

IC_{50} **TK-10**: 3 - 24 µM

IC_{50} **UACC-62**: 3 - 29 µM

IC_{50} **MCF-7**: 3 - 15 µM

Abstract

A series of dihydroartemisinyl-chalcone esters were synthesized through esterification of chalcones with DHA. The hybrids were screened against the CQ sensitive (3D7) and CQ resistant (W2) strains of *Plasmodium falciparum*, and were all found to be active, with IC_{50} values ranging between 1.5 - 11 nM against both strains. The dihydroartemisinyl-chalcone esters, featuring oxygenated aryl rings (**7**, **10** and **11**), were found to be equipotent to dihydroartemisinin but were three to four times more active than artesunate against the 3D7 and W2 strains. 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. Thermogravimetric analysis 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 drug-candidate for further investigation as a potential drug in search for new, safe and effective antimalarial drugs.

Keywords: Malaria; Plasmodium falciparum; antiprotozoal agents; antitumor agents; thermogravimetric analysis.

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 genus *Plasmodium falciparum* species 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) currently is the preferred treatment for uncomplicated malaria. Contrary, 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.

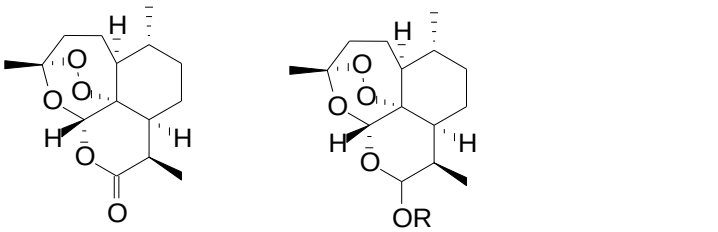
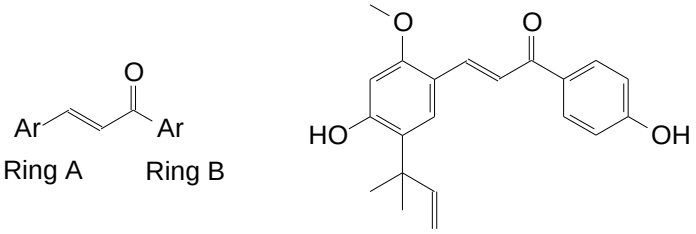


Figure 1: Clinically used artemisinin and its derivatives.

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 are 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] 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 malarial cysteine protease is the hydrolysis of haemoglobin in the food vacuole.[17] This enzyme is 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 DHA had been combined as a hybrid through an amide bond, for use as antitumour agents. These compounds showed superior activity against various cancer cell lines compared to DHA.[20]



General structure of a chalcone

Licochalcone A

Figure 2: General structure of a chalcone and licochalcone A.

While artemisinin-based compounds show high efficacy when administered via systemic routes, they are fairly less effective when given orally.[21] 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.[21] reported that ester derivatives of DHA showed better oral activity than artemether and artesunate, which may be beneficial to the administration of these drugs in rural, malaria endemic areas. It was demonstrated by Cloete et al.[22] that 10-alkyl/aryl ester derivatives of DHA had shown superior activity, compared to 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.[23] 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 anti-cancer drug[24] and for this reason the antitumor properties of the synthesized compounds in this study will also be investigated.

As part of our program focusing 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 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. 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 Physiochemical 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 does 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**.

Table 1: Calculated physicochemical properties of compounds **1** - **12**, CQ, DHA and AS

Compound	log *P*[a]	ADMET Solubility level[b]	ADMET Absorption level[c]
1	2.9	3	0
2	3.7	3	0
3	3.6	3	0
4	3.9	3	0
5	3.5	3	0
6	5.2	2	0
7	6.5	1	2
8	7.2	1	2
9	7.2	1	2
10	7.5	1	2
11	7.0	1	2
12	8.7	0	3
CQ	3.1	2	0
DHA	2.7	2	0
AS	3.2	2	0

[a] Calculated using ACD/Chemsketch v 4.5; [b] ADMET aqueous solubility level (log S_w) at 25°C and pH 7, values = 0, 1, 2 and 3 indicate extremely low, very low, low and good solubility, respectively; [c] ADMET human intestinal absorption, values = 0, 1, 2 and 3 indicate good, moderate, poor and very poor absorption, respectively.

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 poor absorption levels.

2.3 In vitro antimalarial activity and cytotoxicity

The synthesized chalcones **1** - **6** and the artemisinyl-chalcone esters **7** - **12** were screened in vitro, alongside CQ, DHA and AS against the 3D7 and W2 strains of *P. falciparum.* The IC_{50} values are presented in **Table 2**. Compounds **1** - **6** were found to be relatively less active, with IC_{50} values in the 10.8 – 41.0 µM and 10.8 – 62.2 µM ranges against 3D7 and W2, respectively. The targeted hybrid compounds were all found active, with IC_{50} values ranging between 1.9 – 10.7 nM and 1.6 – 10.6 nM against 3D7 and W2, respectively.

Chalcones **1**, **4** and **5** were the most active of all the chalcones tested, all of which were oxygenated, whereas chalcone **6** was found the least active. Generally, the chalcones having IC_{50} values lower than 20 µM (**1**, **4** and **5**) resulted in esters (**7**, **10** and **11**), which demonstrated equipotent antimalarial activities compared to that of DHA against the 3D7 strain. Compound **7** was found to be twice as active as AS and four times more active than CQ, while **10** and **11** were equipotent to AS and about three times more active than CQ. The chalcones with IC_{50} higher than 20 µM (**2**, **3** and **6**) resulted in esters (**8**, **9** and **12**), which showed overall lower activities, compared to DHA and AS, but activities equipotent to CQ against the 3D7 strain.

The same observations were made with respect to antimalarial activities against the W2 strain, except for **8**. Chalcones **1**, **4** and **5** were again the most active of the chalcone intermediates, while esters **7**, **10** and **11** were the most active hybrid compounds, with IC_{50} values of around 1.5 nM. They were equipotent to DHA, four times more potent than AS and more than 40 times more potent than CQ. Incidentally, the three esters that were formed from the most active chalcones **1**, **4** and **5**, were also the three most active hybrid compounds. The majority of the synthesized compounds had resistance index (RI) values of 1.0, or lower.

Compared to the reference drug emetine, none of the compounds were cytotoxic. However, taking into account the selectivity indices (SI), all the intermediate chalcones showed little selectivity, while all the targeted hybrid compounds showed exceptional selectivity towards the parasitic cells in the presence of mammalian cells.

Table 2: Antimalarial activity and cytotoxicity of screened compounds

	Antimalarial activity, IC_{50} (nM) ± S.D[a]		Resistance Index	Cytotoxicity, IC_{50} (µM)[c]	Selectivity Index
Compound	**3D7**	**W2**	**RI[b]**	**WI-38 HFLF[d]**	**SI[e]**
1	18.32* ± 1.14	17.34* ± 1.92	0.9	32.0	1.7
2	29.55* ± 3.60	30.82* ± 2.55	1.0	>100	>3.4
3	40.66* ± 9.90	31.23* ± 3.60	0.8	29.0	0.7
4	10.80* ± 1.10	10.80* ± 1.10	1.0	67.4	6.2
5	14.83* ± 0.91	14.83* ± 0.91	1.0	48.6	3.3
6	40.96* ± 8.27	62.18* ± 23.6	1.5	20.4	0.5
7	1.98 ± 0.25	1.47 ± 0.41	0.7	72.2	36464
8	9.73 ± 1.54	10.59 ± 1.79	1.1	>100	>10277
9	5.87 ± 1.27	5.66 ± 0.87	1.0	>100	>17035
10	3.36 ± 0.59	1.46 ± 0.25	0.4	>100	>29761
11	3.02 ± 0.30	2.04 ± 0.47	0.7	>100	>33112
12	10.67 ± 1.19	5.84 ± 0.54	0.5	61.9	5801
CQ	8.60 ± 0.70	70.10 ± 0.04	8.2	3.63	422
DHA	1.45 ± 0.14	1.31 ± 0.51	0.9	nd	nd
AS	4.00 ± 0.63	5.71 ± 1.15	1.4	nd	nd
Emetine	nd	nd	nd	0.05	nd

* Expressed in µM;

nd = not determined,

[a] Minimum concentration of compound inducing 50% parasitic cells inhibition.

[b] Resistance Index (RI) = IC_{50} W2/IC_{50} 3D7.

[c] Minimum concentration of compound inducing 50% of WI-38 HFLF cells inhibition.

[d] WI-38 cell line of normal human fetal lung fibroblast.

[e] Selectivity index (SI) = IC_{50} WI-38-HFLF/IC_{50} 3D7.

In 2009, Bhattacharya et al.[7] studied the in vitro pharmacodynamics of chalcone derivatives in combination with artemisinin against *P. falciparum* and found that the combinations had shown synergistic or additive interactions. For this reason, during this study the chalcone intermediates of the three most active hybrid compounds were screened against the CQS strain as a 1:1 molar ratio with DHA, in order to determine the effect of chemically binding these chalcones to DHA. Chalcones **1**, **4** and **5** were therefore combined in a 1:1 molar ratio with DHA, with final concentrations equal to the IC_{50} values of **7**, **10** and **11**, respectively.

The results are represented in **Figure 3**. The concentration, at which each combination was screened, was calculated by M/1000 x IC_{50}, where M was the molar mass of the compound. The calculations resulted in 0.9, 1.8 and 1.7 $ng.ml^{-1}$ for **C1** (**1**:**DHA**), **C2** (**4**:**DHA**) and **C3** (**5**:**DHA**), respectively. Chalcones **1**, **4** and **5** and DHA were also included as references at these specific concentrations. The hybrid compounds were all at 50% proliferation and are not shown in the histogram (**Fig. 3**).

Figure 3 illustrates that the three chalcones were inactive, as had already been established. At the specific concentrations, DHA was highly active and was well above the IC_{50} value against the 3D7 strain. For combination **C1**, the percentage cells proliferation (% proliferation), compared to DHA was very similar (20% versus 13%), while **C2** showed a much higher % proliferation compared to DHA (57% versus 2.4%), with a twenty-three times decrease in activity. For combination **C3**, a 20% proliferation was obtained, compared to 2.4% for DHA, which was a ten-fold decrease in activity.

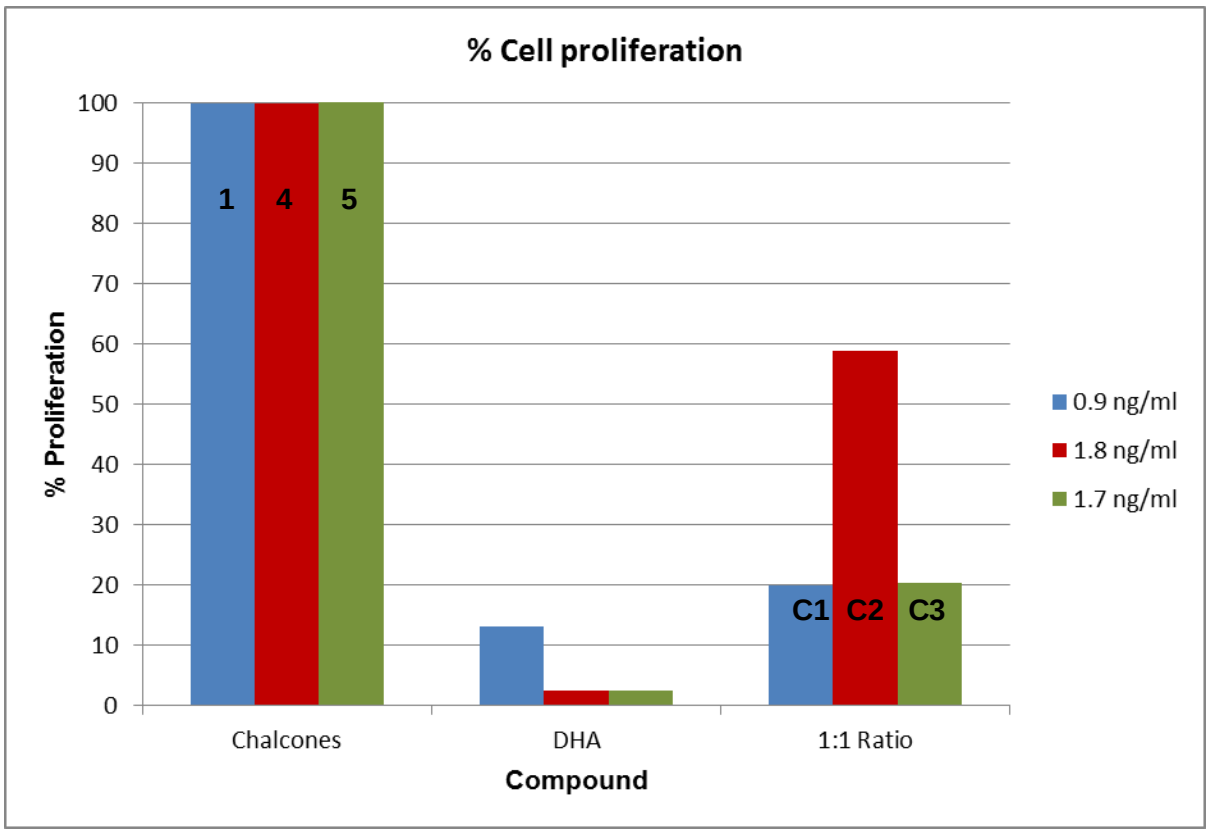


Figure 3: The percentage cell proliferation with the addition of chalcones **1**, **4** and **5**, DHA and the molar combinations thereof, respectively.

2.4 In vitro antitumor activity

The synthesized chalcones **1** - **6** and the artemisinyl-chalcone esters **7** - **12** were screened in vitro, alongside parthenolide against a panel of three cancer cell lines consisting of TK-10, UACC-62 and MCF-7. The IC_{50} values are presented in **Table 3** while **Table 4** illustrates the selective antiplasmodial activity of the different compounds using the W2 strain compared to the individual cell line of the human carcinoma line panel.

All the synthesized compounds, except **7**, were all relatively inactive against the TK-10 cancer cell line, with compound **7** (IC_{50} = 3.6 µM) having analogous activity compared to parthenolide (IC_{50} = 3.7 µM). On contrary, only compounds **1**, **6** and **12** were inactive against UACC-62 cancer cells, with compounds **7** having a threefold increased activity, with an IC_{50} of 3.29 µM. Compounds **8**, **10** and **11** showed a twofold increased activity, while compounds **2** - **5** and **9** were equipotent compared to the reference drug. Against the MCF-7 cancer cells, compounds **7** - **10** were equipotent, while compound **11** were twice as active as parthenolide (IC_{50} = 2.4 µM), with an IC_{50} of 1.02 µM. All other compounds displayed little to no antitumor activity against the MCF-7 cancer cells.

Table 3: Antitumor activity of screened compounds

	Anticancer activity, IC_{50} (μM)[a]			Selectivity Index		
Compound	**TK-10[b]**	**UACC-62[c]**	**MCF-7[d]**	**SI_1[e]**	**SI_2[f]**	**SI_3[g]**
1	90.03	58.02	19.73	0.4	0.6	1.6
2	29.77	13.87	11.91	>3	>7	>8
3	21.13	13.43	9.52	1.4	2.2	3.0
4	18.87	7.87	8.88	3.6	8.6	7.6
5	59.63	6.75	28.06	0.8	7.2	1.7
6	23.64	19.71	8.73	0.9	1.0	2.3
7	3.60	3.29	3.42	20.0	21.9	21.1
8	8.32	5.35	3.59	>12	>19	>28
9	17.04	7.58	2.90	>6	>13	>34
10	5.99	5.19	3.401	>17	>19	>29
11	22.03	4.33	1.02	>5	>23	>100
12	53.7	28.5	14.9	1.2	2.2	4.2
PTD	3.71	9.71	2.40	nd	nd	nd

Parthenolide (PTD)

nd = not determined,

[a] Minimum concentration of compound inducing 50% cells growth inhibition.

[b]TK-10 – renal cancer cells

[c]UACC-62 – melanoma cancer cells

[d] MCF-7 – breast cancer cells

[e] Selectivity index (SI_1) = IC_{50}WI-38-HFLF/IC_{50}TK-10

[f] Selectivity index (SI_2) = IC_{50} WI-38-HFLF/IC_{50} UACC-62

[g] Selectivity index (SI_3) = IC_{50} WI-38-HFLF/IC_{50} MCF-7

Table 4 demonstrate the antitumor SI of the screened compounds against the *P*. *falciparum* W2 strain and indicates that all the chalcone intermediates, except compound **2**, have relatively low selectively towards parasitic cells compared to tumour cells. The targeted hybrid compounds **7** - **12**, on the other hand showed superior selectivity towards the parasitic cells compared to tumour cells.

Table 4: Selective antiplasmodial activity versus anticancer activity of synthesized compounds

	Selectivity Index		
Compound	**SI_1**[a]	**SI_2**[b]	**SI_3**[c]
1	5.2	3.3	1.1
2	>1.0	>0.5	>0.4
3	0.7	0.4	0.3
4	1.7	0.7	0.8
5	4.0	0.5	1.9
6	0.4	0.3	0.1
7	2451.7	2240.1	2325.9
8	>786	>505	>339
9	>3010	>1340	>512
10	>4105	>3556	>2329
11	>10799	>2123	>500
12	9198.6	4873.3	2549.7

[a] Selectivity index (SI_1) = IC_{50}TK-10/IC_{50} W2

[b] Selectivity index (SI_2) = IC_{50} UACC-62/IC_{50} W2

[c] Selectivity index (SI_3) = IC_{50}MCF-7/IC_{50} W2

3. Discussion

3.1 Chemistry

The synthesis of chalcones **1** - **6** through Claisen-Schmidt condensation in basic methanolic water led to the exclusive formation of the *E*-stereoisomer.[25] This was established by the large *J* value of 15.7 Hz, typically associated with vinylic protons having a large dihedral angle that is in a staggered conformation. As a result of the resonance of the unsaturated aryl system near the vinylic carbons, C-6' and C-7' showed a larger chemical shift, due to the existence of a formal positive charge on C-6'.

A broad, highly deshielded peak at ~13 ppm was found on the ^{1}H NMR of all intermediate chalcones, which was evident of the H-α of the carboxylic acid. The carbon of the carboxylic acid C-1' could be assigned to the peak at ~166 ppm. Additionally, the IR spectra showed a

broad stretching frequency at 2800 – 3000 cm^{-1}, which further supported the presence of the carboxylic acid. A strong stretching band was found at ~1650 cm^{-1} for all of the intermediate chalcones, which was generally attributed to an unsaturated ketone. The ^{13}C NMR showed a shielded carbon at approximately 175 ppm for **1**, while the other chalcones had a shielded peak at around 190 ppm, which could be accredited to the conjugated carbonyl C-8'. The shielding of the carbonyl was a result of the conjugation of the chalcone entity, which donated electron density.

For chalcone **1**, evidence, such as six conjugated tertiary carbons (as determined with DEPT90) and six doublets (^1H NMR) further confirmed its structure. The integration of the two doublets at 7.9 ppm accounted for four protons, which were assigned to H-3' and H-4', indicating that the *ortho*- and *meta*-position were non-equivalent. Due to the electronic effect of both the ketone and heterocyclic oxygen in the furan ring, quaternary carbon C-2' was deshielded and was assigned to the peak at 158 ppm. The presence of the methyl group was indisputably assigned to the only aliphatic signal at 13 ppm on ^{13}C NMR. Chalcone **2**, however, had seven conjugated tertiary carbons (as determined with DEPT90) and five doublets (^1H NMR), as well as two triplets (C-11' and C-12').

For the tri-substituted chalcone **3**, six doublets and one singlet peak were observed on the ^1H NMR, together with seven conjugated tertiary carbons (as determined with DEPT90). Interestingly, the isolated protons (protons with no adjacent protons: H-10' for **3** and **4**, and H-11' for **5**) of the phenyl ring B were observed as doublets, which weakly coupled to the protons in the *meta*-position (H-14' for **3** and **4**, and H-13' for **5**), resulting in these protons to be displayed as doublets of doublets. These doublets of doublets were the result of higher order coupling (^4J), which was attributed to the fact that these protons were not magnetically equivalent. This is also known as *meta*-coupling and is it a common phenomenon among aromatic systems, due to the delocalization of electrons.[26]

Besides *meta*-coupling of compound **4**, H-3' and H-4' were displayed as a singlet instead of two doublets. This could be explained by the fact that as long as the chemical shift difference in hertz ($\Delta\nu$) is larger than the coupling constant (J), a simple splitting pattern appears, but within some compounds $\Delta\nu/J$ is very small, which consequently results in multiplets to be displayed as singlets.[26] Chalcone **6** displayed six doublets on ^1H NMR, together with six tertiary carbons (as determined with DEPT90). The spitting patterns of **6** were comparable to that of the expected pattern. The three chloride atoms were clearly identified by means of HRMS bearing an $[M+2]^+$ ion peak being in a 1:1 height ratio to the $[M+H]^+$ peak.

The esterification of chalcones **1** - **6** with DHA resulted in the formation of the desired compounds. For compound **7**, the α-isomer was exclusively attained, which was evident from the large *J* value of 9.8 Hz of H-9 and H-10, consistent with an *anti*-periplanar (*trans*-axial) arrangement of these protons in a chair pyranose ring.[27] Chalcones **8** - **11**, however, resulted in a mixture of α- and β-forms, with the α-isomer as the major compound, as was apparent from the large *J* value of 9.8 Hz of H-9 and H-10. The fractions of the isomers were quantified by the ratio of the integration of H-10 of the α- and β-forms. In contrast, compound **12** exclusively presented as the β-isomer, which was unswerving from the small *J* of 1.6 Hz of H-9 and H-10, and conclusive of the *cis*-equatorial-axial arrangement of these protons in a chair pyranose ring.[4]

The elemental analysis of all compounds showed a slight increase in carbon content, with a hydrogen content which is relatively consistent with the calculated values. The increase in carbon content can be explained by the presence of solvent trapped within or adsorbed onto the surface of each compound's surface, which is evident from both the NMR as well as TGA.

3.2 Physicochemical properties

All of the compounds prepared and investigated during this study (**6** - **12**) displayed small endothermic phase transitions, consistent with glass transitions, with onset temperatures ranging between 65 - 75 °C. In the case of **10**, the onset temperature was at 126 °C, making this compound the extremity in terms of the glass transition profile of these compounds. It was possible that some of the less pronounced endothermic events of **8**, **9** and **11** may have also represented glass transitions, but may have been obscured, because of differences in thermal history among the compounds.[28] These thermally obscured glass transitions could be readily elucidated by means of annealing.[29] However, a thorough physicochemical analysis of the solid-state properties of the synthesized compounds, including strength and fragility parameters,[30] was not the main objective of the study. For the purpose of this investigation, it was sufficient to conclude that the DSC thermograms suggested that all of the compounds had been amorphous.

With regards to the TGA analyses, weight loss occurred right from the start of the heating runs. The onset temperatures of the weight loss did, however, not coincide with the onset temperatures of the small endothermic events, as observed in the DSC thermograms. This may have been consistent with a loss of solvent from the surface of the solid, possibly due to adsorption. Since the absence of a clear baseline before and after the thermal events made

it difficult to elucidate the exact cause of the weight loss, the presence of solvent trapped within crystalline regions of the solid, could not be ruled out.

Of particular interest though, were the large exothermic events taking place just before what appeared to be melting endotherms.[29] At first glance, these exothermic peaks seemed to have corresponded with the recrystallization peaks commonly found after glass transitions. However, TGAs revealed dramatic onset of weight loss coinciding with these exothermic events. Rather than assigning them to recrystallization, the data suggested that these peaks were indicative of exothermic decomposition of the compounds tested, the exact mechanism of which would still need to be investigated. Compared to DHA, all esters were found thermally more stable, which may have been indicative of the positive effect that the chalcone moiety had conveyed onto the DHA.

It is important to determine drug-like properties already during the early stages of the development of any drug, due to drug development that may be largely hampered by poor pharmacokinetics and toxicity.[31] The aqueous solubility of any compound largely influences its ability to be absorbed when taken orally. Lipophilic compounds show poor aqueous solubility and tend to be taken up in fatty globules in the intestine. If they reach the blood stream, they may be absorbed into tissue. Their slow release may exacerbate toxicity, such as neurotoxicity. Contrary, hydrophilic drugs may be excreted directly by the kidneys, or should they be able to penetrate a cell membrane, become entrapped in intracellular, aqueous media. These two properties are thus of high importance when designing new drugs, as they may influence bioavailability to a significant extent.

For drug uptake to occur by means of passive diffusion through biological membranes, the drug must possess favourable drug-like properties and be neither too lipophilic, nor too hydrophilic. An ideal drug must therefore possess balanced lipophilic/hydrophilic properties to both permeate biological membranes and be taken up by the systemic circulation. The *n*-octanol/water partition coefficient (log *P*) offers a reliable measure of this balance, with values between 1 - 5 being targeted, and values between 1 - 3 being ideal.[32],[33]

All of the chalcones' log *P* values fell comfortably within the targeted range of 1 - 5. Chalcone **6** had a log *P* of 5.2, which could still have been considered as being within the targeted range. Due to the favourable solubility and absorption levels, similar or superior to CQ, DHA and AS, it was expected that these chalcones would display satisfactory antiplasmodial activity. In contrast, the targeted final esters **7** - **12** displayed log *P* values much higher than the ideal range. This was indicative of the high lipophilicity of these

compounds, as a result of the blocking of the polar carboxylic group of the chalcone with the notoriously, lipophilic artemisinin moiety, and was this supported by the low solubility and poor absorption levels displayed by these compounds. These compounds were thus expected to show inferior activity, compared to DHA.

3.3 In vitro antimalarial and cytotoxicity

The *P. falciparum* 3D7 strain, a line cloned from NF54, is of African origin and is CQ susceptible. It has often been used to evaluate in vitro antigametocytes activity of potential antimalarial compounds.[34] In contrast, the Indochina/Laos clone W2 is a multi-drug resistant strain. It is known to be the most resistant of all *P. falciparum* strains and has shown resistance against all traditional antimalarial drugs, including chloroquine, cycloguanil, pyrimethamine and sulfadoxine, [35] except to artemisinins. Both strains were used during this study to determine the in vitro antiplasmodial activity of the synthesized target compounds.

Compared to the IC_{50} of 6.5 µM of Licochalcone A against 3D7 clone,[15] all chalcones were found inactive against both the 3D7 and W2 strains, despite their predicted favourable drug-like properties. Antiplasmodial activity of a series of chalcones, synthesized by Yadav et al.,[36] had indicated that substitution of alkoxylated groups on the 2nd and 4th positions of ring A had higher activity than with other substituents. Liu et al.[37] had also come to a similar conclusion regarding ring B, and had additionally found chalcones, with electron deficient groups on ring A, to display strong, antiparasitic activity. Despite low activity, the same trends were observed for compounds **1** - **6**. The chalcone intermediates **1**, **4** and **5**, with oxygenated groups, displayed overall higher potencies than compounds with both rings bearing electron withdrawing groups, such as **3** and **6**. Chalcone **2**, featuring only a benzene ring, displayed activity similar to those of chalcones with electron withdrawing groups. The position of substitution appeared to have had a limited effect on antimalarial activity, compared to the substituent type. Even though, the chalcones did not display potent antimalarial activity, an overall retention of activity against the CQR strain was observed. In view of the fact that the chalcone moiety does not have the same mode of action as CQ, drug resistance was not associated with this pharmacophore, which might have explained this retention.

The esterification of chalcones **1** - **6** with DHA resulted in hybrid compounds **7** - **12**, which were structurally similar to AS, with all bearing an ester linkage to DHA. Since these compounds were tested in vitro and not metabolized by metabolic enzymes, they would act as new entities and not as prodrugs. The observed IC_{50} values were therefore most likely those of hybrids', rather than those of any active metabolite.

None of the targeted hybrid compounds proved to be superior to DHA, which were in accordance with their high log *P* values, low solubility and poor absorption levels. The observed activities were consequently most likely due to the DHA pharmacophore, rather than the chalcone. This suggested that the chalcone moiety antagonized the antimalarial action of DHA pharmacophore in all cases, as supported by **Figure 3**. These preliminary results were indicative that chemical binding of the chalcone to DHA resulted in an antagonistic effect. **Figure 3** also illustrates that the molar ratios of **C1** and **C3** had a two-fold increase in activity, compared to the hybrid compounds. For **C2**, no effect could be observed, signifying that there was no difference between chemically binding the chalcone with DHA, or combining it in a 1:1 molar ratio, which was indicative of an additive effect. Additionally, the findings in this study contradicted those of Cloete et al.,[22] which may also have been attributed to the poor drug-like properties displayed by these compounds.

Regardless of lower/equipotent activities of the targeted hybrids, compared to DHA, the observation that chalcones with higher IC_{50} values (**1**, **4** and **5**) resulted in hybrid compounds with increased/equipotent activity and increased thermal stability, may be encouraging. The incorporation of a chalcone with increased activity could therefore result in a hybrid with improved activity.

Although compared to the reference drug emetine, none of the compounds were cytotoxic against human fetal lung fibroblast WI-38 cells, analysis of the selectivity index (SI), which is indicative of the effectiveness of a drug to clear only parasitic cells and not healthy cells, enabled the reaching of the following conclusions: All chalcone intermediates, except **2**, had very low (lower than 10) selectivity indices, indicating high cytotoxicity. Thus, the observed antiplasmodial activity is most likely due to the cytotoxicity rather than intrinsic activity. Compound **2** had a cytotoxic activity of >100 µM, which is indicative of no to little toxicity and thus the observed antiplasmodial activity is likely intrinsic. In contrast to the chalcones, all the artemisinyl-chalcone esters were highly selective toward the parasitic cells in the presence of mammalian cells, with a SI of over 5800, which is without a doubt an indication of intrinsic activity.

3.4 In vitro antitumor activity

The intermediate chalcones displayed overall poor anticancer activity, even though compounds **4** and **5**, did display equipotent activity against the UACC-62 cancer cell line. In terms of structure activity relationship, no clear trend could be observed between the different compounds or between the different cell lines. This also holds true for the targeted

hybrid compounds. Although compounds **7**, **10** and **11**, the most potent antiplasmodial compounds with oxygenated aryl rings, were overall the most active against all three cell lines.

Against all three cancer cell lines, compounds **2** and **8** - **11**, all showed intrinsic activity, hence not exerted by cytotoxicity, but rather through other mechanisms, since these compounds showed no or little cytotoxicity. The selectivity indices of compounds **1**, **3** - **6** and **12** all indicate that the resulting IC_{50} values of these compounds are most likely due to cytotoxicity, rather than intrinsic activity. Compound **7**, on the other hand, displayed moderate cytotoxicity, with a moderate SI (~20). The observed antitumor activity is therefore likely due to a combination of cytotoxicity and other mechanisms. Overall, compound **7** displayed the best antitumor activity, with an IC_{50} of around 3 µM against all three cancer cell lines.

3.5 Selective antiplasmodial activity of hybrids

Because both DHA and chalcone pharmacophores have been described as potential anticancer agents, it was interesting to assess the antimalarial selectivity of the synthesized compounds. Comparing the SI values (**Table 4**), it is clear that all the chalcone intermediates had no selective antiplasmodial activity in the presence all three cancer cell-types. This, in conjunction with the cytotoxicity data, suggests that these compounds would indiscriminately stop the growth of healthy, parasitic and cancerous cells as result of their systemic cytotoxicity once in use. Conversely, the targeted hybrid compounds **7** -**12**, showed superior selectivity towards the parasitic cells compared to tumour cells as result of their intrinsic activity.

4. Conclusion

In this study, a series of novel, dihydroartemisinyl-chalcone esters were prepared in a two-step process, involving the synthesis of carboxylic acid-functionalized chalcones and esterification thereof with DHA, using either 1,1'-carbonyldiimidazole as coupling reagent, or oxalyl chloride as activation reagent. Routinely used techniques, such NMR, HRMS, IR and EA served to confirm their structures. DSC and TGA were used to reveal their amorphous structures and thermal stability, respectively, while ADMET served to predict their drug-like properties. In screens alongside CQ, DHA and AS, all of the esters proved to be active with IC_{50} values ranging between 1.9 – 10.7 nM and 1.6 – 10.6 nM against 3D7 and W2 strains of *P. falciparum*, respectively, despite possessing predicted unfavourable drug-like properties. The carboxylic acid-functionalized chalcones **1** - **6**, however, were found less active, with IC_{50} values ranging between 10.8 – 41.0 µM and 10.8 – 62.2 µM against 3D7

and W2, respectively, irrespective of their favourable drug-like properties. The dihydroartemisinyl-chalcone esters, **7**, **10** and **11**, featuring oxygenated aryls as ring B in the chalcone, were found to be equipotent to DHA, three to four times more potent than artesunate against both the 3D7 and W2 strains, and more than forty-fold higher activity than chloroquine against the W2 strain. The esters displayed an overall gain of activity against the CQR strain, as compared to the CQS, which resulted in resistance index values under the unit. In this study, the antimalarial activity was found to increase with the presence of oxygenated aryl groups (electron donating ring B), independent of the position of substitution. Compounds with electron withdrawing groups seemed less potent. Several of the targeted hybrid compounds displayed potent antitumor activity, with compounds **7**, being overall the most active. During this study, hybrid **7** performed admirable compared to DHA and AS against both the CQS and CQR strains of *P*. *falciparum*, with a good RI value (0.7), excellent SI value (36464), overall potent antitumor activities (~3 µM) against all three cancer cells lines, as well as increased thermal stability. Ester **7** was thus identified as the best drug-candidate for further investigation as a potential drug in search for new, safe and effective antimalarial drugs. All compounds displayed higher thermal stability compared to DHA.

5. Materials and Methods

5.1 Materials

4-formylbenzoic acid, 2-acetyl-5-methylfuran, acetophenone, 3'-methoxy-4'-nitroacetophenone, 3',4'-dimethoxyacetophenone, 2',4'-dimethoxyacetophenone, 2',3',4'-trichloroacetophenone, hydrochloric acid, sodium hydroxide, 1,1'-carbonyldiimidazole (CDI), oxalyl chloride and magnesium sulphate were purchased from Sigma-Aldrich (South-Africa). All solvents used were purchased from Associated Chemical Enterprises (ACE, South Africa). All chemicals and reagents were of analytical grade and were used without further purification, except for dichloromethane (DCM) that was distilled with calcium hydride and kept on molecular sieves (4Å) prior to use in reactions.

5.2 General procedures

The ^{1}H and ^{13}C NMR spectra were recorded on a BrukerAvance™ III 600 spectrometer at a frequency of 600 and 150 MHz, respectively, in deuterated dimethyl sulfoxide (DMSO-d_6), deuterated methanol (MeOD), or deuterated chloroform ($CDCl_3$). Chemical shifts are reported in parts per million δ (ppm), with the residual protons of the solvent as reference.

The splitting pattern abbreviations are as follows: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), p (pentet), and m (multiplet).

High resolution mass spectrometry (HRMS) was recorded on a Bruker MicroTOF Q II mass spectrometer that had an APCI or an ESI source set at 300°C or 180°C respectively, using Bruker Compass DataAnalysis 4.0 software. A full scan between 50 - 1500 m/*z* was generated, at a capillary voltage of 4500 V, an end plate offset voltage of -500 V and a collision cell RF voltage of 100 Vpp.

Infrared (IR) spectra were recorder on a Bruker Alpha-P FTIR instrument. Melting points (mp) were determined on a BÜCHI melting point B-545 instrument and were uncorrected.

The CHN analysis was performed on a CE-440 Elemental Analyzer, supplied by Exeter Analytical, Inc made in the USA. The samples were incinerated at 1040 ºC and the temperature of reduction tube was set at 700 ºC. The C, H and N content was determined for each sample with the %R is calculated as rest of mass, and is normally O when no other elements are present.

Thin layer chromatography (TLC) was performed using silica gel plates ($60F_{254}$), acquired from Merck (South Africa).

5.3 Physicochemical properties

A Shimadzu (Kyoto, Japan) DSC-60 instrument was used to record DSC thermograms. Samples (3 - 5 mg) were accurately weighed and sealed in aluminium crimp cells with pierced lids. The samples were heated from 25 - 300 °C at a 10 $C.min^{-1}$ rate and a nitrogen gas purge of 35 ml/min.

A Shimadzu (Kyoto, Japan) TGA-60 instrument was used to determine percentage weight loss (%) of samples during heating. Samples (3 - 5 mg) were accurately weighed into open aluminium crucibles. The samples were heated from 25 - 300 °C at a heating rate of 10 $°C.min^{-1}$ and a nitrogen gas purge of 35 ml/min.

Accelrys Discovery Studio 3.1 was used to calculate the ADMET properties of compounds **1** - **12**, CQ, DHA and AS. ACD/ChemSketch, (2000), version 4.54, was used to calculate log *P* values.

5.4 Biological evaluation

5.4.1 In vitro antimalarial assay

The in vitro antimalarial activity of test samples against the 3D7 or W2 strains of the malaria parasite, *P. falciparum,* was measured by assessing parasite survival after drug exposure, using a parasite SYBR green I fluorescence linearity. Since mature erythrocytes lack RNA and DNA, binding of SYBR green I is specific for malarial DNA in any erythrocytic stage of *P. falciparum* development. SYBR green I also displays preferential binding to double-stranded DNA, versus single-stranded DNA or RNA and are significantly more sensitive, have better defined spectral peaks and are less mutagenic, compared to ethidium bromide.[38]

The parasites were cultivated by the method of Trager and Jensen,[39] with minor modifications made during this study. Cultures were maintained in fresh group A-positive human erythrocytes, suspended at 2% heamatocrit in RPMI 1640, containing 10% human serum, 3 $g.\ell^{-1}$ of glucose, 45 $g.\ell^{-1}$ of hypoxanthine and 50 $g.\ell^{-1}$ of gentamicin. Flasks were incubated at 37 °C under a gas mixture of 5% O_2, 5% CO_2, and 90% N_2. The stock culture was synchronized with 5% sorbitol prior to experiments.[40]

Compound inhibitory activity was determined by preparing test samples in parasite culture medium in transparent 96-well, flat bottom plates in three-fold staggered serial dilutions, to obtain sixteen (16) decreasing concentrations (n = 3 for each data point). Parasitized red blood cells were added to a final concentration of 1% haematocrit, 2% parasitaemia and the plates incubated for 96 hours before proceeding with the SYBR green I fluorescence linearity assay. Percentage parasite survival in each well was calculated relative to control wells that had not been exposed to any drug.

Results are presented as percentage parasite viability at the various compound concentrations, with the 50% inhibitory concentration (IC_{50}) of individual compounds calculated from fitted sigmoidal dose-response curves.

5.4.2 In vitro cytotoxicity assay

The cytotoxic effects of the prepared ester compounds were tested by sulforhodamine B (SRB) assay on the WI-38 cell line. The SRB assay was developed by Skehan et al.[41] to measure drug induced cytotoxicity and cell proliferation. Its principle is based on the ability of the protein dye, sulforhodamine B (Acid Red 52), to bind electrostatically in a pH dependent manner to basic protein amino acid residues of trichloroacetic acid-fixed cells. Under mild acidic conditions it binds to the fixed cellular protein, while under mild basic conditions it can

be extracted from cells and solubilised for measurement.[42] The SRB assay was performed at CSIR in accordance with the protocol of the Drug Evaluation Branch, NCI, and the assay has been adopted for the screening tests compounds of this study.

The WI-38 cell line (normal human fetal lung fibroblast) from ECACC was routinely maintained as a monolayer cell culture at 37 ºC, 5% CO_2, 95% air and 100% relative humidity in EMEM containing 10% fetal bovine serum, 2 mM L-glutamine and 50 µg/ml gentamicin. For this screening experiment, the cells (21 - 50 passages) were inoculated in a 96-well microtiter plates at plating densities of 10 000 cells/well and were incubated for 24 hours. After 24 hours the cells were treated with the experimental drugs, which had previously been dissolved in DMSO and diluted in medium to produce five concentrations. Neat cells served as control. The blank contained complete medium without cells. Parthenolide was used as a standard. The plates were incubated for 48 hours after addition of the compounds. Viable cells were fixed to the bottom of each well with cold 50% trichloroacetic acid, washed, dried and dyed by SRB. Unbound dye was removed and protein bound dye was extracted with 10 mM Tris base for optical density determination at a wavelength of 540 nm, using a multiwell spectrophotometer. Data analysis was performed using GraphPad Prism software. 50% of cell growth inhibition (IC_{50}) was determined by non-linear regression. The results are summarized in **Table 2**.

5.4.3 In vitro anticancer assay

The growth inhibitory effects of the prepared ester compounds were tested by Sulforhodamine B (SRB) assay in a 3-cell line panel consisting of TK-10 (renal), UACC-62 (melanoma) and MCF-7 (breast) cancer cells. The SRB Assay was performed at CSIR in accordance with the protocol of the Drug Evaluation Branch, NCI, and the assay has been adopted for this study.

The human cell lines TK-10, UACC-62 and MCF-7 was obtained from NCI in the framework a collaborative research program between CSIR and NCI. Cell lines was routinely maintained as a monolayer cell culture at 37 ºC, 5% CO_2, 95% air and 100% relative humidity in RPMI containing 5% fetal bovine serum, 2 mM L-glutamine and 50 µg/ml gentamicin.

For this screening experiment, the cells (3 - 19 passages) were inoculated in a 96 - well microtiter plates at plating densities of 10 000 cells/well and were incubated for 24 h. After 24 h the cells were treated with the experimental drugs which were previously dissolved in DMSO and diluted in medium to produce 5 concentrations. Neat cells served as control. The

blank contained complete medium without cells. Parthenolide was used as a standard. The plates were incubated for 48 h after addition of the compounds. Viable cells were fixed to the bottom of each well with cold 50% trichloroacetic acid, washed, dried and dyed by SRB. Unbound dye was removed and protein-bound dye was extracted with 10 mM Tris base for optical density determination at the wavelength 540 nm using a multiwell spectrophotometer. Data analysis was performed using GraphPad Prism software. 50% of cell growth inhibition (IC_{50}) was determined by non-linear regression. The results are summarized in **Table 3**.

5.5 Synthesis

5.5.1 Claisen-Schmidt condensation, 1 - 6

Chalcones **1** - **6** were synthesized, adopting a literature reported method[19] and described as follows: Formylbenzoic acid (10 mmol, 1.5 g, 1 eq.) and the appropriate aryl ketone (10.3 mmol, 1.03 eq.) were successively added to MeOH (60 ml) upon stirring at room temperature. Sodium hydroxide solution, NaOH (1M, 20 ml) was added and stirring was continued for 12 hours [Scheme 1, step (i)]. The progress of the reaction was followed by TLC. After completion, the pH of the solution was adjusted to 2 with HCl solution (1M), upon which an off-white to yellow precipitate formed. The precipitate was subsequently collected by suction filtration and washed with water, then with a 10% MeOH solution and dried to yield the desired pure compound in high yields.

5.5.1.1. 4-[(1E)-3-(5-methylfuran-2-yl)-3-oxoprop-1-en-1-yl]benzoic acid, 1

Off-white to yellow powder; yield 2.45 g (90%); mp 221-228 ºC; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 13.14 (s, 1H, H-α), 7.97 (d, *J* = 8.4 Hz, 2H, H-4'), 7.94 (d, *J* = 8.4 Hz, 2H, H-3'), 7.80 (d, *J* = 3.5 Hz, 1H, H-10'), 7.75 (d, *J* = 15.7 Hz, 1H, H-6'), 7.72 (d, *J* = 15.7 Hz, 1H, H-7'), 6.44 (d, *J* = 3.3 Hz, 1H, H-11'), 2.40 (s, 3H, H-13'); ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm): 175.46 (C-8'), 166.86 (C-1'), 158.97 (C-9'), 151.83 (C-12'), 140.75 (C-6'), 138.65 (C-2'), 131.98 (C-5'), 129.73 (C-3'), 128.74 (C-4'), 124.16 (C-7'), 122.02 (C-10'), 109.64 (C-11'), 13.78 (C-13'); IR (ATR) ν_{max}/cm^{-1} 3104, 3076, 2982, 2924, 2931, 1684, 1654, 1601, 1509, 1288, 1211, 1065, 1027, 845, 772, 754; HRMS (APCI) *m/z* $[M+H]^+$ 257.0890 (Calcd for $C_{15}H_{13}O_4$: 257.0814).

5.5.1.2. 4-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]benzoic acid, 2

Off-white powder; yield: 1.9 g (70%), mp: 229-237 ºC; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 13.15 (s, 1H, H-α), 8.18 – 8.15 (m, 2H, H-10'), 8.05 (d, *J* = 15.7 Hz, 1H, H-7'), 8.02 – 7.94 (m, 4H, H-3' and H4'), 7.78 (d, *J* = 15.7 Hz, 1H, H-6'), 7.68 (t, *J* = 7.4 Hz, 1H, H-12'), 7.57 (t, *J* = 7.7 Hz, 2H, H-11'); ^{13}C NMR (151 MHz, DMSO- d_6) δ (ppm): 189.11 (C-8'),

166.86 (C-1'), 142.56 (C-6'), 138.76 (C-5'), 137.33 (C-9'), 133.37 (C-11'), 132.11 (C-2'), 129.72 (C-3'), 128.94 (C-4'), 128.86 (C-12'), 128.64 (C-10'), 124.20 (C-7');IR (ATR) ν_{max}/cm^{-1} 2839, 1666, 1606, 1289, 754, 681; IR (ATR) ν_{max}/cm^{-1} 2839, 1666, 1606, 1289, 754, 681; HRMS (APCI) *m/z* $[M+H]^+$ 253.0871 (Calcd for $C_{16}H_{13}O_3$: 253.0864).

5.5.1.3. 4-[(1E)-3-(3-methoxy-4-nitrophenyl)-3-oxoprop-1-en-1-yl]benzoic acid, 3

Light brown powder; yield: 3.4 g (97%), mp 286-288 ºC; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 13.12 (s, 1H, H-α), 8.67 (d, *J* = 2.3 Hz, 1H, H-10'), 8.43 (dd, *J* = 8.8, 2.4 Hz, 1H, H-14'), 8.07 (d, *J* = 15.6 Hz, 1H, H-7'), 7.98 (q, *J* = 8.1 Hz, 4H, H-3' and H-4'), 7.77 (d, *J* = 15.5 Hz, 1H, H-6'), 7.50 (d, *J* = 8.9 Hz, 1H, H-13'), 4.02 (s, 3H, H-15'); ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm): 186.08 (C-8'), 166.87 (C-1'), 155.32 (C-12'), 143.07 (C-6'), 139.38 (C-5'), 138.68 (C-11'), 134.57 (C-14'), 132.22 (C-2'), 129.71 (C-3'), 129.59 (C-9'), 129.10 (C-4'), 125.52 (C-10'), 123.41 (C-7'), 114.51 (C-13'), 57.33 (C015'); IR (ATR) ν_{max}/cm^{-1} 2848, 1670, 1603, 1534, 1282, 1206, 770; HRMS (APCI) *m/z* $[M+H]^+$ 328.0789 (Calcd for $C_{17}H_{14}NO_6$: 328.0821).

5.5.1.4. 4-[(1E)-3-(3,4-dimethoxyphenyl)-3-oxoprop-1-en-1-yl]benzoic acid, 4

Off-white powder; yield: 2.5g (74%), mp 219-221 ºC; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 8.03 (d, *J* = 15.6 Hz, 1H, H-7'), 7.98 (s, 4H, H-3' and H-4'), 7.91 (dd, *J* = 8.5, 2.0 Hz, 1H, H-14'), 7.72 (d, *J* = 15.6 Hz, 1H, H-6'), 7.60 (d, *J* = 2.0 Hz, 1H, H-10'), 7.09 (d, *J* = 8.5 Hz, 1H, H-13'), 3.85 (d, *J* = 7.4 Hz, 6H, H-15' and H-16'); ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm): 187.30 (C-8'), 166.95 (C-1'), 153.47 (C-11'), 148.90 (C-12'), 141.71 (C-6'), 139.00 (C-5'), 131.95 (C-2'), 130.33 (C-9'), 129.76 (C-3'), 128.88 (C-4'), 124.15 (C-7'), 123.69 (C-14'), 110.94 (C-13'), 110.76 (C-10'), 55.85 (C-15'), 55.65 (C-16'); IR (ATR) ν_{max}/cm^{-1} 2837, 1676, 1581, 1515, 1260, 1021, 773; HRMS (APCI) *m/z* $[M+H]^+$ 313.1054 (Calcd for $C_{18}H_{17}O_5$: 313.1076).

5.5.1.5. 4-[(1E)-3-(2,4-dimethoxyphenyl)-3-oxoprop-1-en-1-yl]benzoic acid, 5

Light yellow powder; yield: 3.2 g (96%), mp 199-201 ºC; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 13.09 (s, 1H, H-α) 7.96 (d, *J* = 8.3 Hz, 2H, H-3'), 7.82 (d, *J* = 8.2 Hz, 2H, H-4'), 7.65 (d, *J* = 9.7 Hz, 1H, H-7'), 7.63 (d, *J* = 2.5 Hz, 1H, H-14'), 7.56 (d, *J* = 15.8 Hz, 1H, H-6'), 6.68 (d, *J* = 2.2 Hz, 1H, H-11'), 6.64 (dd, *J* = 8.6, 2.3 Hz, 1H, H-13'), 3.90 (s, 3H, H-15'), 3.84 (s, 3H, H-16'); ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm): 189.07 (C-8'), 166.88 (C-1'), 164.27 (C-12'), 160.49 (C-10'), 139.66 (C-6'), 139.10 (C-5'), 132.24 (C-14'), 131.76 (C-2'), 129.87 (C-3'), 129.18 (C-7'), 128.40 (C-4'), 121.13 (C-9'), 106.17 (C-13'), 98.65 (C-11'), 56.07 (C-15'),

55.67 (C-16'); IR (ATR) ν_{max}/cm^{-1} 2839, 1678, 1604, 1415, 1280, 1253, 1018, 772; HRMS (APCI) *m/z* $[M+H]^+$ 313.1050 (Calcd for $C_{18}H_{17}O_5$: 313.1076).

5.5.1.6. 4-[(1E)-3-oxo-3-(2,3,4-trichlorophenyl)prop-1-en-1-yl]benzoic acid, 6

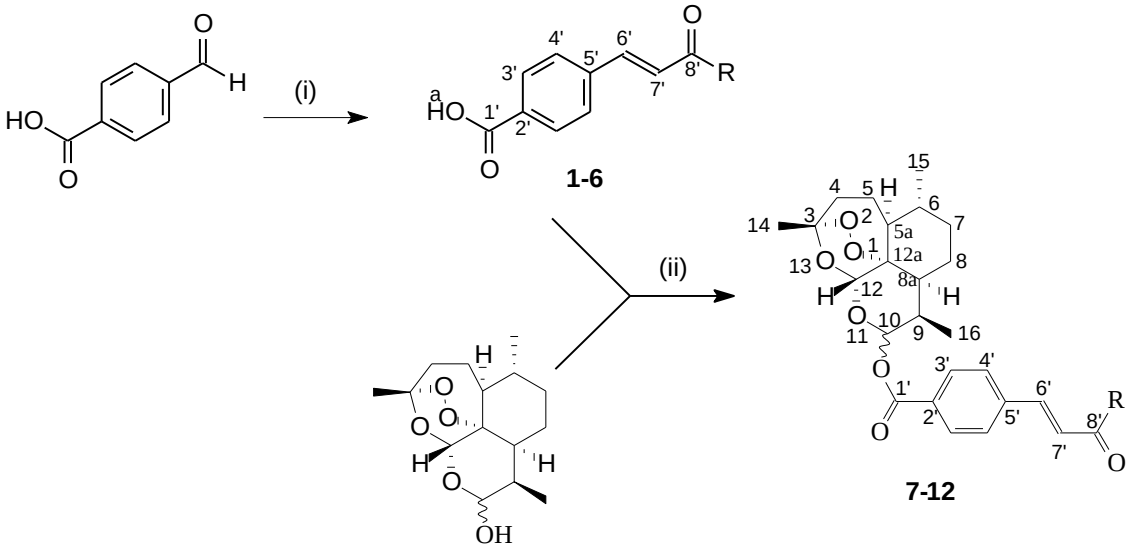
Off-white powder; yield: 3.37 g (89%), mp 297-282 ºC; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 13.12 (s, 1H, H-α), 7.95 (d, *J* = 8.2 Hz, 2H, H-3'), 7.89 (d, *J* = 8.3 Hz, 2H, H-4'), 7.79 (d, *J* = 8.3 Hz, 1H, H-13'), 7.57 (d, *J* = 8.3 Hz, 1H, H-14'), 7.52 (d, *J* = 16.2 Hz, 1H, H-6'), 7.33 (d, *J* = 16.3 Hz, 1H, H-7'); ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm): 192.15 (C-8'), 166.73 (C-1'), 146.27 (C-6'), 139.12 (C-5'), 138.04 (C-12'), 134.56 (C-9'), 132.63 (C-10'), 131.23 (C-11'), 130.00 (C-2'), 129.74 (C-3'), 129.38 (C-13'), 129.10 (C-4'), 127.92 (C-14'), 127.84 (C-7'); IR (ATR) ν_{max}/cm^{-1} 2826, 1669, 1604, 1288, 1173, 773; HRMS (APCI) *m/z* $[M+H]^+$ 354.9677 (Calcd for $C_{16}H_{10}Cl_3O_3$: 354.9695).

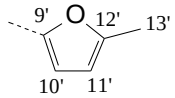
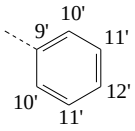
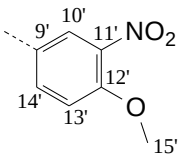
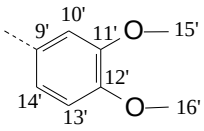
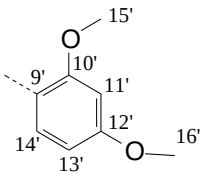
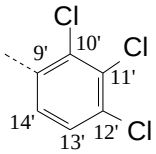
5.5.2 Esterification of chalcone with dihydroartemisinin

Artemisinyl-chalcones **6 - 11**: The chalcone (3.9 mmol, 1 eq.) and CDI (4.9 mmol, 0.8 g, 1.3 eq.) were added to anhydrous DCM (50 ml) and stirred for 3 hours at room temperature. Afterwards, DHA (1.33 mmol, 1.2 eq.) was added and the resulting solution was stirred for an additional 24 hours. The solution was quenched with the addition of distilled water (50 ml). The organic phase was separated and the aqueous phase was extracted three times with DCM (50ml). The combined organic layers were washed water (3 x 50 ml) and then with saturated $NaHCO_3$solution (50 ml), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography, eluting with DCM and then with DCM:EtOAc (95:5, v/v) as eluent afforded the pure compound. NMR, IR and HRMS data is reported below.

Artemisinyl-chalcone **12**: An adapted method of Fisher et al.[43] was used for the synthesis of this chalcone and is described as follows:

Chalcone **6** (2.8 mmol, 1 g, 1 eq.) was added to anhydrous DCM (50 ml) in an ice bath whilst stirring. Oxalyl chloride (5.6 mmol, 0.5 ml, 2 eq.) was added slowly over a period of 15 minutes and 2 drops of DMF were added as catalyst. Stirring was continued for 1 hour, after which the ice bath was removed and the solution was allowed warming to room temperature (r.t.) over a period of 1 hour. Subsequently, the solvent was removed *in vacuo* and the residue dissolved in DCM (50 ml), cooled to 0 °C, after which DHA (3.4 mmol, 0.95 g, 1.2 eq.) was added. The solution was allowed to heat to r.t. and stirred for an additional 4 hours. The solvent was removed and the residue subjected to column chromatography with DCM as eluent, to yield the desired compound **12**.



Intermediates	R	Hybrids	R of intermediate
1		**7**	**1**
2		**8**	**2**
3		**9**	**3**
4		**10**	**4**
5		**11**	**5**
6		**12**	**6**

Scheme 1: Multi-step synthesis of artemisinyl-chalcone esters **7 – 12**.

Reagents and conditions: (i) **1** - **6**: aryl ketone, MeOH, NaOH, 12 h, r.t. (ii) **7** - **11**: chalcones**1** - **5**,CDI/DCM, 3 h, r.t. then DHA, 24 h, r.t.; **12**:oxalyl chloride/DCM/DMF, 1 h, 0 °C, then DHA, 4 h, r.t.

5.5.2.1. 10α-dihydroartemisinyl-4-[(1E)-3-(5-methylfuran-2-yl)-3-oxoprop-1-en-1-yl]benzoate, 7

Light yellow powder; yield 0.6 g (61%); R_f = 0.2 (DCM); mp 177-183 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.12 (d, *J* = 8.3 Hz, 2H, H-3'), 7.82 (d, *J* = 15.8 Hz, 1H, H-6'), 7.67 (d, *J* = 8.3 Hz, 2H, H-4'), 7.43 (d, *J* = 15.7 Hz, 1H, H-7'), 7.27 (d, *J* = 3.5 Hz, 1H, H-10'), 6.22 (d, *J* = 3.4 Hz, 1H, H-11'), 5.99 (d, *J* = 9.8 Hz, 1H, H-10), 5.51 (s, 1H, H-12), 2.74 (dqd, *J* = 14.3, 7.2, 4.4 Hz, 1H, H-9), 2.43 (s, 3H, H-13'), 2.40 – 2.32 (m, 1H, H-4α), 2.06 – 1.99 (m, 1H, H-4β), 1.88 (ddt, *J* = 13.7, 6.8, 3.6 Hz, 1H, H-5α), 1.80 (dq, *J* = 13.5, 3.7 Hz, 1H, H-8α), 1.72 (dq, *J* = 13.6, 3.4 Hz, 1H, H-7α), 1.68 – 1.63 (m, 1H, H-8a), 1.53 – 1.42 (m, 2H, H-5β and H-8β), 1.40 (s, 3H, H-14), 1.37 – 1.32 (m, 1H, H-6), 1.29 (td, *J* = 11.3, 6.5 Hz, 1H, H-5a), 1.02 (qd, *J* = 13.2, 3.6 Hz, 1H, H-7β), 0.96 (d, *J* = 6.2 Hz, 3H, H-15), 0.90 (d, *J* = 7.1 Hz, 3H, H-16); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 176.69 (C-8'), 164.64 (C-1'), 158.52 (C-9'), 152.32 (C-12'), 141.62 (C-6'), 139.43 (C-5'), 130.78 (C-2'), 130.59 (C-3'), 128.13 (C-4'), 123.55 (C-7'), 120.04 (C-10'), 109.49 (C-11'), 104.42 (C-3), 92.68 (C-10), 91.59 (C-12), 80.14 (C-12a), 51.58 (C-5a), 45.27 (C-8a), 37.24 (C-6), 36.19 (C-4), 34.06 (C-7), 31.94 (C-9), 25.92 (C-14), 24.54 (C-5), 22.02 (C-8), 20.20 (C-15), 14.21 (C-13'), 12.21 (C-16); IR (ATR) ν_{max}/cm^{-1} 2925, 1717, 1655, 1604, 1509, 1275, 1014, 767; EA found: C 71.43%, H 6.94%, R 21.34% (Calcd: C 68.95%, H 6.56%, O 24.49%).

5.5.2.2. 10-dihydroartemisinyl-4-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]benzoate, 8

Light yellow powder, mixture of α and β (12:1); yield 0.17g (8%); R_f = 0.34 (DCM); mp 167-170 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.15 – 8.12 (m, 2H, H-3'), 8.02 (dd, *J* = 7.4, 1.5 Hz, 2H, H-4'), 7.79 (d, *J* = 15.7 Hz, 1H, H-6'), 7.68 (d, *J* = 8.2 Hz, 2H, H-10'), 7.62 – 7.56 (m, 2H, H-11'), 7.50 (t, *J* = 7.7 Hz, 2H, H-12'), 6.00 (d, *J* = 9.8 Hz, 1H, H-10), 5.51 (s, 1H, H-12), 2.75 (dqd, *J* = 14.2, 7.1, 4.3 Hz, 1H, H-9), 2.41 – 2.34 (m, 1H, H-4α), 2.05 – 1.99 (m, 1H, H-4β), 1.89 (ddt, *J* = 13.9, 7.0, 3.6 Hz, 1H, H-5α), 1.80 (dt, *J* = 13.6, 3.9 Hz, 1H, H-8α), 1.73 (dq, *J* = 13.3, 3.0 Hz, 1H, H-7α), 1.67 (ddt, *J* = 12.6, 7.9, 3.7 Hz, 1H, H-8a), 1.53 – 1.42 (m, 2H, H-5β and H-8β), 1.41 (s, 3H, H-14), 1.38 – 1.24 (m, 2H, H-6 and H-5a), 1.07 – 1.00 (m, 1H, H-7β), 0.96 (d, *J* = 6.0 Hz, 3H, H-15), 0.91 (d, *J* = 7.1 Hz, 3H, H-16); ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.12 (C-8'), 164.63 (C-1'), 143.14 (C-6'), 139.44 (C-5'), 137.82 (C-9'), 134.93 (C-2'), 133.04 (C-11'), 130.65 (C-3'), 128.69 (C-10'), 128.55 (C-4'), 128.14 (C-12'), 124.24 (C-7'), 104.45 (C-3), 92.72 (C-10), 91.61 (C-12), 80.16 (C-12a), 51.60 (C-5a), 45.29 (C-8a), 37.26 (C-6), 36.21 (C-4), 34.07 (C-7), 31.96 (C-9), 25.93 (C-14), 24.55 (C-5), 22.03 (C-8), 20.21

(C-15), 12.23 (C-16); IR (ATR) ν_{max}/cm^{-1} 2923, 1733, 1601, 1264, 1012, 757; HRMS (APCI) *m/z* $[M+H]^+$ 519.2377 (Calcd for $C_{31}H_{35}O_7$: 519.2382); EA found: C 69.9%, H 6.86%, R 22.96% (Calcd: C 71.80%, H 6.61%, O 21.6%).

5.5.2.3. 10-dihydroartemisinyl-4-[(1E)-3-(3-methoxy-4-nitrophenyl)-3-oxoprop-1-en-1-yl]benzoate, 9

Light yellow powder; mixture of α and β (7:1); yield 0.5 g (31%); R_f = 0.35 (DCM); mp 178-183 ºC;^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.53 (d, *J* = 2.3 Hz, 1H, 10'), 8.26 (dd, *J* = 8.8, 2.2 Hz, 1H, H-14'), 8.16 – 8.12 (m, 2H, H-3'), 7.84 (d, *J* = 15.8 Hz, 1H, H-6'), 7.71 – 7.67 (m, 2H, H-4'), 7.56 (d, *J* = 15.5 Hz, 1H, H-7'), 7.20 (d, *J* = 8.8 Hz, 1H, H-13'), 5.99 (d, *J* = 9.8 Hz, 1H, H-10), 5.51 (s, 1H, H-12), 4.04 (s, 3H, H-15'), 2.74 (dqd, *J* = 14.3, 7.2, 4.4 Hz, 1H, H-9), 2.37 (td, *J* = 14.0, 3.9 Hz, 1H, H-4α), 2.06 – 2.00 (m, 1H, H-4β), 1.89 (ddt, *J* = 13.8, 6.8, 3.7 Hz, 1H, H-5α), 1.81 (dq, *J* = 13.7, 3.8 Hz, 1H, H-8α), 1.77 – 1.70 (m, 1H, H-7α), 1.67 (dt, *J* = 14.0, 4.6 Hz, 1H, H-8a), 1.48 (m, 2H, H-5β and H-8β), 1.40 (s, 3H, H-14), 1.36 – 1.26 (m, 2H, H-6 and H-5a), 1.02 (qd, *J* = 13.1, 3.6 Hz, 1H, H-7β), 0.96 (d, *J* = 6.2 Hz, 3H, H-15), 0.91 (d, *J* = 7.2 Hz, 3H, H-16); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 186.38 (C-8'), 164.55 (C-1'), 156.22 (C-12'), 144.10 (C-6'), 139.37 (C-5'), 138.99 (C-11'), 134.42 (C-14'), 131.25 (C-2'), 130.68 (C-3'), 130.15 (C-9'), 128.33 (C-4'), 126.29 (C-10'), 122.60 (C-7'), 113.47 (C-13'), 104.45 (C-3), 92.77 (C-10), 91.61 (C-12), 80.15 (C-12a), 56.94 (C-15'), 51.59 (C-5a), 45.27 (C-8a), 37.25 (C-6), 36.20 (C-4), 34.06 (C-7), 31.95 (C-9), 25.92 (C-14), 24.54(C-5), 22.03 (C-8), 20.21 (C-15), 12.22 (C-16); IR (ATR) ν_{max}/cm^{-1} 2923, 1727, 1609, 1532, 1263, 1006; EA found: C 66.21%, H 6.60%, N 2.34%, R 24.85% (Calcd: C 64.75%, H 5.95%, N 2.36%, O 26.95%).

5.5.2.4. 10-dihydroartemisinyl-4-[(1E)-3-(3,4-dimethoxyphenyl)-3-oxoprop-1-en-1-yl]benzoate, 10

Light yellow platelets; mixture of α and β (7:1); yield 0.6 g (32%); R_f = 0.1 (DCM); mp 81-82 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.13 (d, *J* = 8.3 Hz, 2H, H-3'), 7.78 (d, *J* = 15.5 Hz, 1H, H-6'), 7.68 (m, 3H, H-4' and H-14'), 7.64 – 7.58 (m, 2H, H-7' and H-10'), 6.92 (d, *J* = 8.4 Hz, 1H, H-13'), 5.99 (d, *J* = 9.8 Hz, 1H, H-10), 5.51 (s, 1H, H-12), 3.95 (s, 6H, H-15' and H-16'), 2.74 (dqd, *J* = 14.4, 7.2, 4.4 Hz, 1H, H-9), 2.37 (td, *J* = 13.9, 3.9 Hz, 1H, H-4α), 2.12 – 2.02 (m, 1H, H-4β), 1.88 (ddd, *J* = 13.6, 6.8, 3.5 Hz, 1H, H-5α), 1.80 (dq, *J* = 13.9, 3.9 Hz, 1H, H-8α), 1.76 – 1.60 (m, 2H, H-7α and H-8a), 1.53 – 1.25 (m, 7H, H-5β, H-8β, H-14, H-6 and H-5a), 1.06 – 0.98 (m, 1H, H-7β), 0.96 (d, *J* = 6.1 Hz, 3H, H-15), 0.91 (d, *J* = 7.1 Hz, 3H, H-16); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 188.13 (C-8'), 164.66 (C-1'), 153.48 (C-12'), 149.31 (C-11'), 142.30 (C-6'), 139.66 (C-5'), 130.97 (C-2'), 130.76 (C-9'), 130.62 (C-3'),

128.06 (C-4'), 123.89 (C-7'), 123.22 (C-14'), 110.68 (C-10'), 109.96 (C-13'), 104.44 (C-3), 92.70 (C-10), 91.60 (C-12), 80.16 (C-12a), 56.10 (C-15'), 56.05 (C-16'), 51.60 (C-5a), 45.29 (C-8a), 37.25 (C-6), 36.21 (C-4), 34.07 (C-7), 31.96 (C-9), 25.93 (C-14), 24.55 (C-5), 22.03 (C-8), 20.20 (C-15), 12.22 (C-16); IR (ATR) ν_{max}/cm^{-1} 2924, 1726, 1658, 1580, 1260, 1007, 757; HRMS (ESI) *m/z* $[M+H]^+$ 579.2568 (Calcd for $C_{33}H_{39}O_9$: 579.2594); EA found: C 69.48%, H 6.89%, R 23.42% (Calcd: C 68.50%, H 6.62%, O 23.42%).

5.5.2.5. 10-dihydroartemisinyl-4-[(1E)-3-(2,4-dimethoxyphenyl)-3-oxoprop-1-en-1-yl]benzoate, 11

Light yellow powder; mixture of α and β (7:1);yield 0.5g (55%); R_f = 0.26 (DCM:EtOAc, 4:1, v/v); mp 89-94 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.10 (d, *J* = 8.3 Hz, 2H, H-3'), 7.77 (d, *J* = 8.6 Hz, 1H, H-14'), 7.65 (d, *J* = 15.8 Hz, 1H, H-6'), 7.62 (d, *J* = 8.3 Hz, 2H, H-4'), 7.59 (d, *J* = 15.8 Hz, 1H, H-7'), 6.55 (dd, *J* = 8.7, 2.2 Hz, 1H, H-13'), 6.48 (d, *J* = 2.3 Hz, 1H, H-11'), 5.98 (d, *J* = 9.8 Hz, 1H, H-10), 5.50 (s, 1H, H-12), 3.90 (s, 3H, H-15'), 3.85 (s, 3H, H-16'), 2.73 (ddd, *J* = 9.8, 7.2, 4.5 Hz, 1H, H-9), 2.36 (td, *J* = 13.9, 3.9 Hz, 1H, H-4α), 2.03 (dd, *J* = 4.9, 3.0 Hz, 1H, H-4β), 1.88 (ddt, *J* = 13.8, 6.8, 3.5 Hz, 1H, H-5α), 1.79 (dt, *J* = 13.6, 3.9 Hz, 1H, H-8α), 1.70 (dq, *J* = 7.1, 3.2 Hz, 1H, H-7α), 1.66 (dt, *J* = 13.8, 4.5 Hz, 1H, H-8a), 1.53 – 1.42 (m, 2H, H-5β and H-8β), 1.40 (s, 3H, H-14), 1.37 – 1.25 (m, 2H, H-6 and H-5a), 1.03 – 0.98 (m, 1H, H-7β), 0.95 (d, *J* = 6.1 Hz, 3H, H-15), 0.89 (d, *J* = 6.4 Hz, 3H, H-16); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 189.84 (C-8'), 164.74 (C-1'), 164.46 (C-12'), 160.56 (C-10'), 140.21 (C-5'), 140.07 (C-6'), 133.04 (C-14'), 130.53 (C-3'), 130.35 (C-2'), 129.40 (C-7'), 127.95 (C-4'), 121.80 (C-9'), 105.32 (C-13'), 104.41 (C-3), 98.54 (C-11'), 92.63 (C-10), 91.57 (C-12), 80.14 (C-12a), 55.75 (C-15'), 55.55 (C-16'), 51.59 (C-5a), 45.28 (C-8a), 37.24 (C-6), 36.20 (C-4), 34.06 (C-7), 31.94 (C-9), 25.92 (C-14), 24.54 (C-5), 22.02 (C-8), 20.20 (C-15), 12.21 (C-16); IR (ATR) ν_{max}/cm^{-1} 2925, 1726, 1604, 1261, 1006; HRMS (APCI) *m/z* $[M+H]^+$ 579.2560 (Calcd for $C_{33}H_{39}O_9$: 579.2594); EA found: C 71.01%, H 7.21%, R 21.54 % (Calcd: C 68.50%, H 6.62%, O 24.88%).

5.5.2.6. 10β-dihydroartemisinyl-4-[(1E)-3-oxo-3-(2,3,4-trichlorophenyl)prop-1-en-1-yl]benzoate, 12

Light yellow powder; yield 0.12g (9%); R_f = 0.57 (DCM:EtOAc, 4:1, v/v); mp 100-107 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.05 (dd, *J* = 8.5, 2.1 Hz, 2H, H-3'), 7.60 (dd, *J* = 8.5, 2.5 Hz, 2H, H-4'), 7.48 (d, *J* = 8.3 Hz, 1H, H-13'), 7.44 (d, *J* = 15.9 Hz, 1H, H-6'), 7.28 (d, *J* = 8.3 Hz, 1H, H-14'), 7.11 (dd, *J* = 16.0, 1.7 Hz, 1H, H-7'), 6.16 (d, *J* = 1.6 Hz, 1H, H-10), 5.51 (s, 1H, H-12), 2.38 (ddd, *J* = 14.8, 13.3, 4.0 Hz, 1H, H-4α), 2.02 (m, 3H, H-8α, H-9 and H-4β), 1.89 (ddt, *J* = 13.6, 6.6, 3.6 Hz, 1H, H-5α), 1.68 (dd, *J* = 12.6, 4.5 Hz, 1H, H-8a), 1.63 (dq, *J*

= 13.3, 3.2 Hz, 1H, H-7α), 1.56 (d, *J* = 1.4 Hz, 3H, H-16), 1.55 – 1.49 (m, 1H, H-5β), 1.46 – 1.41 (m, 1H, H-5a), 1.40 (s, 3H, H-14), 1.39 – 1.35 (m, 1H, H-6), 1.16 (qd, *J* = 13.4, 3.1 Hz, 1H, H-8β), 1.11 – 1.02 (m, 1H, H-7β), 0.95 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 191.73 (C-8'), 166.23 (C-1'), 145.16 (C-6'), 138.99 (C-5'), 138.16 (C-12'), 136.29 (C-9'), 134.93 (C-10), 132.95 (C-10'), 132.06 (C-11'), 131.37 (C-12'), 130.17 (C-3'), 128.69 (C-13'), 128.44 (C-4'), 127.52 (C-7'), 127.01 (C-14'), 104.49 (C-3), 89.64 (C-12), 78.92 (C-12a), 51.38 (C-5a), 44.39 (C-8a), 37.44 (C-6), 36.19 (C-4), 34.07 (C-7), 29.95 (C-8), 25.84 (C-14), 24.37 (C-5) 20.26 (C-15), 16.17 (C-16); IR (ATR) ν_{max}/cm^{-1} 2923, 1731, 1604, 1267, 1110, 991, 879, 820; EA found: C 62.11%, H 5.95%, R 31.82% (Calcd: C 59.87%, H 5.02%, O 18.01%, Cl 17.10%).

Acknowledgements This work is based upon research supported by the National Research Foundation (NRF). The authors thank the North-West University, Potchefstroom Campus, for the financial support, Dr J Jordaan for MS analysis, Mr A Joubert for NMR analysis and the University of Pretoria for conducting biological screening.

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