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In vitro antimalarial activity and molecular modeling studies of novel artemisinin derivartives

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ABSTRACT

Cerebral malaria is a serious and sometimes fatal disease caused by a *Plasmodium falciparum* that infects a certain female anopheles mosquito which feeds on humans. The parasites responsible for the mosquito-borne infectious disease are increasingly resistant to current drug approaches, and almost half of the world is at risk of contracting an illness. A series of twenty five new ether and ester derivatives of dihydroartemisinin (DHA) have been prepared based on *in silico* studies and *in vitro* antimalarial activity and later assessed against the chloroquine sensitive NF-54 strain of *Plasmodium falciparum*. In general the incorporation of nitro functionality in ester derivatives enhances the activity relative to artemisinin. Most of the ether derivatives were found to be as active as DHA, while 11-OH ether derivatives were not as active as DHA. The most potent analogue in the series was compound **21** which was several fold more active than artemisinin against *P. falciparum* used in the study. Molecular docking and ADMET studies were performed to explore the possible mode of interaction of active compounds in to the binding site pocket of malaria parasite target enzyme plasmepsin-II and evaluated compliance with oral bioavailability and pharmacokinetics parameters. The ester derivatives **19** and **20** were found to be twice active than DHA, having nitro functionality showing IC_{50} 10.58nM and 8.54nM respectively.

Keywords: Dihydroartemisinin, *P. falciparum, in vitro,* antimalarial, docking, plasmepsin-II

Abbreviations: MIC, minimum inhibitory concentrations; DEPT, distortionless enhancement by polarization transfer; ESI-MS, electrospray ionization mass spectrometry, COSY, correlation spectroscopy, HSQC,heteronuclear single quantum correlation, HMBC, heteronuclear multiple-bond correlation spectroscopy; MIC, Minimum Inhibitory Concentration

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Introduction

Malaria is one of the world's most deadly diseases and is becoming an increasingly serious problem as malaria parasites have developed resistance to drugs such as chloroquine and mefloquine. Malaria is endemic in many parts of the world. Globally, an estimated 1.2 billion people are at high risk (>1 in 1000 chance of getting malaria in a year). According to the latest estimates, 198 million cases of malaria occurred globally in 2013 and the disease led to 584 000 deaths.[1].Malaria is a major parasitic disease of the tropical and subtropical countries including India[1]. Hence the malaria situation is getting worse with rapid spread of multidrug resistant *Plasmodium falciparum*. Isolation of artemisinin **1** from *Artemisia annua*, as the active principle of the Chinese traditional drug against malaria, is a major breakthrough in malaria chemotherapy[2-12]. Artemisinin owes its antimalarial activity due to the presence of 1,2,4-trioxane system. It is active against both chloroquine-sensitive and chloroquine resistant malaria. The semisynthetic derivatives of artemisinin such as dihydroartemisinin **2**, artemether **3**, arteether **4**, and artesunic acid **5** are more active than artemisinin and are currently used as drugs of choice for the treatment of malaria caused by multidrug- resistant *P. falciparum*[13-14]. Therefore, there is considerable urgency to develop new classes of antimalarials. Artemisinin (**1**) (qinghaosu) is an unusual 1,2,4-trioxane which has been used clinically in China for the treatment of multidrug resistant *P. falciparum* malaria. However the clinical application of artemisinin has been limited by the drug's pharmacokinetic properties. This has provided the impetus for the investigation of derivatives of this compound, some of which include esters and ethers of the corresponding lactol, dihydroartemisinin (DHA) (**2**). Analogues of this type are currently being developed as potent and rapidly acting antimalarials[2]. Chloroquine-resistant *P. falciparum* is present in most of the countries of Asia, Africa, and South America. Resistance to the sulphonamide-

pyrimethamine combination is widespread in southeast Asia and South America. Field trials with mefloquine have met with rapid emergence of malarial parasites resistant to the drug.

Resistance to quinine is not common, but the duration of the treatment with the drug is long and requires hospitalization. Currently, artemisinin **1** and its derivatives dihydroartemisinin **2**, artemether **3**, arteether **4**, and artesunic acid **5** (Figure 1) are the only class of drugs that are effective against multidrug resistant malaria[2-11]. Artesunic acid, the hemisuccinate ester of dihydroartemisinin **2**, is one of the clinically useful derivative of artemisinin[15-19]. Through strong worldwide programs malaria has been eradicated from many countries, resistance to current malarial drugs continues and about 600,000 people a year die from the disease, mostly children. As the disease and carrier mosquitoes build up resistances to the current drugs, researchers are looking for the individual molecules that will kill the malaria parasite to combat resistance. In continuation of our work[12] to meet these objectives, we have prepared a series of novel ether and ester derivatives of dihydroartemisinin **6-18**, **19-25** and **28-32**.

MATERIALS AND METHODS:

General Experimental Procedures

Melting point was determined on a Toshniwal melting point apparatus. IR spectra were recorded on a Perkin Elmer 1719 FT-IR spectrophotometer. NMR spectra were obtained in acetone-d_6, $CDCl_3$ on a Bruker Avance, 300 MHz and 400 MHz instrument using TMS as internal standard. The chemical shift values are reported in ppm and coupling constants in Hz. ESI-MS spectra were recorded on a Perkin Elmer Turbo Mass/Shimadzu LC-MS. TLC analyses were carried out on precoated silica gel 60 F_{254}plates (Merck Mumbai, India) were used using solvent system, hexane:ethylacetate (7:3). All the required solvents and reagents were purchased from Merck (Mumbai, India) and Sigma Aldrich., India. The compounds were visualized by either exposure of TLC plates to I_2 vapors or by spraying with vanillin-

sulfuricacid reagent, followed by heating at 110°C for 15 minutes. Si-gel, 60-120 mesh (spectrochem) was used in the column chromatography for the purification of metabolites. The compounds were identified by their spectral IR, ID (^{1}H, ^{13}C, DEPT) NMR and ESIMS analysis.

Test compound preparation. Isolation of secondary metabolite artemisinin from the plant *Artemisia annua*, grown at the Central Institute of Medicinal and Aromatic Plants (CIMAP) experimental farms, Lucknow, India, was carried out essentially according to the patented procedure[19b]. A schematic outline for the extraction protocol is provided in Fig. 2 summarizes the outline for the preparation of a series of new and known derivatives of artemisinin. Briefly, artemisinin (**1**) was reduced with sodium borohydride at 0 to -5°C to prepare dihydroartemisinin (**2**) in 85% (wt/wt) yield in 2 h.

Synthesis of ether derivatives of artemisinin: After crystallization and drying, Dihydroartemisinin (1.0 g, 3mmol) was dissolved in dichloromethane (20 ml), stirred for 12 min to 4 h at 0-10°C in the presence of appropriate alkylating reagents (3mmol), *viz.*, o-nitrobenzylalcohol, m-nitrobenzylalcohol (for **6** and **7**), 2,3-dimethoxybenzoylalcohol, 2,5-dimethoxybenzoylalcohol, 3,5-dimethoxybenzoylalcohol (for **8**, **9** and **10**), o-bromo, p-cyano-benzoylalcohol, o-chloro,p-flourobenzoylalcohol (for **11** and **12**), 3-phenoxybenzylalcohol (for **13**), 3-methyl-2-butene-1-ol (for **14**), and octadecane-1-ol (for **15**) and the catalyst p-TSA (Fig. 3). After completion of the reaction (as monitored by thin-layer chromatography [TLC], the reaction mixture was quenched with cooled 1% aqueous $NaHCO_3$ (50 ml) and extracted with ethyl acetate (three times with 50 ml each time). The combined extract was washed with water till neutral, dried (Na_2SO_4), and concentrated to get the crude mixture of β/α products (1.2 to 1.8 g). The mixture was subjected to silica gel column chromatography to obtain the pure β-isomer.

12β-Arte-3'-nitrobenzylether (6): Elution of the column with *n*-hexane-ethyl acetate (95:5) yielded an oily compound in 93% (w/w) yield). IR λ_{max} (neat): 1254, 1171, 1103 (ether), 1,527, 1343 (Ar NO_2), 2875, 2925, 1029, 875 cm^{-1}; ^1H, COSY- NMR (300MHz, $CDCl_3$) δ0.87 (6H, d, *J* =6.0 Hz, H_3-13, H_3-14), 1.37 (3H, s, H_3-15), 2.61 (1H, m, C-11), 4.77 (1H, d, *J*=14.4 Hz, Ha-1'), 4.87 (1H, brs, αH-12), 5.18 (1H, d, *J*=14.7 Hz, Hb-1'), 5.37 (1H, s, H-5), 7.37 (1H, d, *J* = 7.8 Hz, H-7'), 7.56 (2H, m, H-5', H-6'), and 7.95 (1H, d, *J*=8.1 Hz, H-4'); ^{13}C, DEPT- NMR(75 MHz, $CDCl_3$) : δ13.3 (C-13), 20.7 (C-14), 24.8 (C-8), 25.0 (C-2), 26.5 (C-15), 31.3 (C-11), 34.9 (C-9), 36.8 (C-3), 37.7 (C-10), 44.6 (C-7), 52.9 (C-1), 67.80 (C-1'), 81.4 (C-6, q), 88.4 (C-5), 102.5 (C-12), 104.5 (C-4,q), 125.1 (C-4'), 128.5 (C-7'), 129.5 (C-5'), 133.7 (C-6'), 134.7 (C-2', q), and 148.1 (C-3', q); ESI-MS(positive): m/z 420 $[M+H]^+$ calcd. for $C_{22}H_{30}O_7N$ found 420 and (negative): 418 $[M-H]^-$ calcd. for $C_{22}H_{28}O_7N$ and found 418.

12β-Arte-4'-nitrobenzylether (7): Elution of the column with *n*-hexane-ethyl acetate (95:5) yielded a colorless oil in 90% (w/w) yield. IR λ_{max} (neat): 1195, 1102 (ether), 1531, 1349 (Ar NO_2), 2925, 2874, 1034, 875 cm^{-1}; ^1H, COSY- NMR (300 MHz, $CDCl_3$) δ0.94 (3H, d, *J* =5.7 Hz, H_3-13), 0.98 (3H, d, *J* =4.2 Hz, H_3-14), 1.44 (3H, s, H_3-15), 2.69 (1H, m, H-11), 4.62 (1H, d, *J*=17.6 Hz, Ha-1'), 4.91 (1H, brs, αH-12), 4.97 (1H, d, *J*=17.6 Hz, Hb-1'), 5.44 (1H, s, H-5), 7.48 (1H, dd, *J* = 7.5, 3.3 Hz, H-6')7.61 (1H, d, *J* = 6.9 Hz, H-7'), 8.11 (1H, d, *J* = 7.2 Hz, H-5'), 8.23 (1H, s, H-3'); ^{13}C, DEPT- NMR (75 MHz, $CDCl_3$): δ 13.4 (C-13), 20.7 (C-14), 24.9 (C-8), 25.0 (C-2), 26.5 (C-15), 31.2 (C-11), 34.9 (C-9), 36.7 (C-3), 37.8 (C-10), 44.6 (C-7), 52.91 (C-1), 68.9 (C-1'), 81.4 (C-6, q), 88.4 (C-5), 102.1 (C-12), 104.6 (C-4, q), 122.3 (C-3'), 122.8 (C-5'), 129.6 (C-6'), 133.3 (C-7'), 141.0 (C-2', q), 148.6 (C-4', q); ESI-MS(positive): m/z 420 $[M+H]^+$ calcd. for $C_{22}H_{30}O_7N$ found 420 and (negative): 418 $[M-H]^-$ calcd. for $C_{22}H_{28}O_7N$ and found 418 and 442 $[M+Na]^+$.

12β-Arte-3', 4'-dimethoxybenzylether (8): Elution of the column with *n*-hexane-ethyl acetate (95:5) yielded a colorless oil in 90% (w/w) yield. IR λ_{max} (neat): 1278, 1098 (ether), 2939, 2875, 1481, 1378, 1012, 787 cm^{-1}; ^{1}H, COSY- NMR (300 MHz, Acetone-d_6): δ0.92 (6H, d, *J* =6.9 Hz, H_3-13, H_3-14), 1.32 (3H, s, H_3-15), 2.56 (1H, m, H-11), 3.75, 3.79 (3H each, s, 2 x O$\underline{C}H_3$), 4.47 (1H, d, *J*=12.3 Hz, Ha-1'), 4.86 (1H, d, *J*=12.3 Hz, Hb-1'), 4.83 (1H, d, *J*=4.5 Hz, αH-12), 5.46 (1H, s, H-5), 6.97 (1H, m, H-5', H-6', H-7'); ^{13}C, DEPT-NMR (75 MHz, Acetone-d_6): δ12.9 (C-13), 20.1 (C-14), 24.7 (C-8), 25.0 (C-2), 25.7 (C-15), 31.3 (C-11), 34.9 (C-9), 36.6 (C-3), 37.6 (C-10), 44.9 (C-7), 53.0 (C-1), 55.6, 60.3 (2 x O$\underline{C}H_3$), 64.8 (C-1'), 81.0 (C-6, q), 88.0 (C-5), 101.5 (C-12), 103.9 (C-4, q), 112.5 (C-5'), 121.1 (C-7'), 124.1 (C-6'), 132.4 (C-2', q), 147.4 (C-4', q), and 153.0 (C-3', q); ESI-MS(positive): m/z 457 $[M + Na]^+$, molecular formula $C_{24}H_{34}O_7$.

12β-Arte-3', 6'-dimethoxybenzylether (9): Elution of the column with *n*-hexane– ethyl acetate (95:5) yielded a colorless oil in 90% (w/w) yield. IR λ_{max} (neat): 1276, 1082 (ether), 2934, 2872, 1476, 1381, 1019 cm^{-1}; ^{1}H, COSY- NMR (300 MHz, $CDCl_3$): δ0.92 (3H, d, *J* =6.6 Hz, H_3-13), 0.96 (3H, d, *J* =7.5 Hz, H_3-14), 1.31 (3H, s, H_3-15), 2.58 (1H, m, H-11), 3.73, 3.78 (3H each, s, 2xO$\underline{C}H_3$), 4.41 (1H, d, *J*=13.2 Hz, Ha-1'), 4.84 (1H, d, *J*=2.7 Hz, αH-12), 4.86 (1H, d, *J*=13.2 Hz, Hb-1'), 5.46 (1H, s, H-5), 6.78 (1H, dd, *J* = 8.7, 3.0 Hz, H-5'), 6.88 (1H, d, *J* = 8.7 Hz, H-4'), 6.97 (1H, d, *J*=3.0 Hz, H-7'); ^{13}C, DEPT- NMR (75 MHz, Acetone-d_6): δ12.9 (C-13), 20.1 (C-14), 24.7 (C-8), 25.0 (C-2), 25.7 (C-15), 31.4 (C-11), 35.0 (C-9), 36.6 (C-3), 37.6 (C-10), 44.9(C-7), 53.0 (C-1), 55.3, 55.6 (2 x O$\underline{C}H_3$), 65.0 (C-1'), 81.0 (C-6, q), 87.9 (C-5), 101.7 (C-12), 103.8 (C-4, q), 111.5 (C-5'), 112.6 (C-4'), 114.4 (C-7'), 128.4 (C-2', q), 151.3 (C-3', q), 154.0 (C-6', q); ESI-MS(positive): m/z 435 $[M+H]^+$ calcd. for $C_{24}H_{35}O_7$ found 435.

12β-Arte-4', 6'-dimethoxybenzylether (10): Elution of the column with *n*-hexane-ethyl acetate (95:5) yielded a colorless oil in 90% (w/w) yield. IR λ_{max} (neat): 1203, 1155 (ether), 2927, 2873, 1600, 1476, 1381, 1029,875 cm^{-1}; ^{1}H, COSY- NMR (300 MHz, Acetone-d_6): δ0.92 (3H, d, *J* =6.6 Hz, H_3-13), 0.96 (3H, d, *J* =7.5 Hz, H_3-14), 1.31 (3H, s, H_3-15), 2.55 (1H, m, H-11), 3.74, 3.76 (3H each, s, 2 x O$C\underline{H}_3$), 4.44 (1H, d, *J*=12.6 Hz, Ha-1'), 4.76 (1H, d, *J*=12.6 Hz, Hb-1'), 4.79 (1H, d, *J*=3.6 Hz, αH-12), 5.42 (1H, s, H-5), 6.38 (1H, s, H-5'), 6.53 (2H, brs, H-3', H-7'); ^{13}C, DEPT- NMR (75 MHz, Acetone-d_6): δ 13.0 (C-13), 20.1 (C-14), 22.1 (C-8), 24.8 (C-2), 25.0 (C-15), 31.3 (C-11), 34.9 (C-9), 36.6 (C-3), 37.6 (C-10), 44.8 (C-7), 53.0 (C-1), 55.0 (2 x O$\underline{C}H_3$), 69.5 (C-1'), 81.0 (C-6, q), 88.0(C-5), 99.5 (C-5'), 101.3 (C-12), 103.9 (C-4, q), 105.2 (C-3', C-7'), 141.5 (C-2', q), 161.4 (C-4', C-6', q); ESI-MS(positive): m/z 435 $[M+H]^+$ calcd. for $C_{24}H_{35}O_7$ found 435 and 457 $[M+Na]^+$.

12α-Arte-2'-bromo-4'-cyanophenylether (11): Elution of the column with *n*-hexane– ethyl acetate (95:5) yielded white crystals in 70% w/w yield. IR λ_{max} (KBr): 1378, 1138 (ether), 2926, 2873, 1450, 875 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ0.90 (6H, d, *J* =6.3 Hz, H_3-13, H_3-14), 1.43 (3H, brs, H_3-15), 2.83 (1H, m, H-11), 5.44 (1H, d, *J*=7.2 Hz, βH-12), 5.54 (1H, s, H-5), 6.90 (1H, dd, *J*=8.1, 8.1 Hz, H-6'), 7.08 (1H, d, *J*=6.9, H-5'), 7.26 (1H, d, *J*=1.8 Hz, H-3'); ESI-MS(positive): m/z 464 $[M+H]^+$ calcd. for $C_{22}H_{27}O_5NBr$ found 464.

12β-Arte-2'-chloro, 4'-flourophenylether (12): Elution of the column with *n*-hexane-ethyl acetate (95:5) yielded a colorless oil, in 80% (w/t) yield. IR λ_{max} (neat): 1371, 1132 (ether), 2870, 1456, 1592, 1102 and 753 (Cl groups) cm^{-1}; ^{1}H, COSY- NMR (300 MHz, $CDCl_3$): δ 0.86 (3H, d, *J* =6.0 Hz, H_3-13), 0.96 (3H, d, *J* =5.1 Hz, H_3-14), 1.42 (3H, s, H_3-15), 2.81 (1H, m, H-11), 5.45 (1H, brs, αH-12), 5.54 (1H, s, H-5), 6.92 (1H, dd, H-5'), 7.08 (1H, d, *J* = 8.4 Hz, H-5'), 7.30 (1H, brs, H-3'); ^{13}C, DEPT- NMR (75 MHz, $CDCl_3$): δ 13.4 (C-13), 20.7 (C-14), 24.6 (C-8), 25.0 (C-2), 26.4 (C-15), 31.5 (C-11), 35.0 (C-9), 36.7 (C-3), 37.8 (C-10),

44.6 (C-7), 52.9 (C-1), 81.3 (C-6, q), 88.9 (C-5), 102.4 (C-12), 104.7 (C-4, q), 114.6 (C-5'), 117.4 (C-6'), 124.7 (C-2', q), 150.3 (C-3'), 155.9 (C-1', q), 159.2 (C-4', q); ESI-MS(positive): m/z 413 $[M+H]^+$ calcd. for $C_{21}H_{27}O_5ClF$ found 413 and 452 $[M+H+K]^+$.

12β-Arte-4'-phenoxybenzylether (13): Elution of the column with *n*-hexane-ethyl acetate (95:5) yielded a colorless oil, 95% (w/w) in yield. IR λ_{max} (neat): 3067, 3040, 1585, 1487, 1253, 1214, 1030, 875, 789, 693 (aromatics), 2873, 1448, 1377, 1140 (ether) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.83 (3H, d, *J* =7.2 Hz, H_3-13), 0.88 (3H, d, *J* =5.4 Hz, H_3-14), 1.38 (3H, s, H_3-15), 2.60 (1H, m, H-11), 4.44 (1H, d, *J*=12.6 Hz, Ha-1'), 4.81 (1H, d, *J*=12.3 Hz, Hb-1'), 4.83 (1H, d, *J*=3.0, αH-12), 5.36 (1H, s, H-5), 6.91 (5H, m, H-3', H-5', H-7', H-9', H-13'), 7.04 (1H, t, *J*=7.5 Hz, H-11'), 7.27 (3H, m, H-6', H-10', H-12'); ^{13}C, DEPT- NMR (75 MHz, $CDCl_3$): δ 13.4 (C-13), 20.7 (C-14), 24.8 (C-8), 25.0 (C-2), 26.5 (C-15), 31.3 (C-11), 35.0 (C-9), 36.8 (C-3), 37.7 (C-10), 44.8 (C-7), 52.9 (C-1), 69.6 (C-1'), 81.4 (C-6, q), 88.4 (C-5), 101.8 (C-12), 104.5 (C-4, q), 117.4 (C-3'), 118.0 (C-5'), 119.5 (C-9', C-13'), 122.0 (C-10', C-12'), 123.7 (C-11'), 129.9 (C-7'), 130.1 (C-6') 140.9 (C-2', q) 157.4 (C-4', q), 157.9 (C-8', q); ESI-MS(positive): m/z 467 $[M+H]^+$ calcd. for $C_{28}H_{35}O_6$ found 467 and (negative): 465 $[M-H]^-$ calcd. for $C_{28}H_{33}O_6$ found 465.

2β-Arte-3'-methyl-2'-butenylether (14): Elution of the column with *n*-hexane-ethyl acetate (95:5) yielded a colorless oil, 90% (w/w) in yield. IR λ_{max} (neat): 1655 (double bond), 1377, 1007 (ether), 2922, 2874,1450, cm^{-1}; ^1H, COSY- NMR (400 MHz, Acetone-d_6): δ 0.89 (3H, d, *J* =7.4 Hz, H_3-13), 0.94 (3H, d, *J* =8.0 Hz, H_3-14), 1.32 (3H, s, H_3-15), 1.69, 1.74 (3H each, s, H_3-4', H_3-5'), 3.63 (1H, m, Ha-1'), 4.20 (1H, m, Hb-1'), 4.71 (1H, d, *J*=3.4 Hz, αH-12), 5.31 (1H, t, *J* =4.5 Hz, H-2'), 5.37 (1H, s, H-5); ^{13}C, DEPT- NMR (100 MHz, Acetone-d_6): δ 12.5 (C-13), 17.3, 24.3 (C-4', C-5'), 19.8 (C-14), 24.6 (C-2), 24.9 (C-8), 25.3 (C-15), 30.8 (C-11), 34.6 (C-9), 36.3 (C-3), 37.2 (C-10), 44.5 (C-7), 52.6 (C-1), 63.9 (C-1'), 80.6

(C-6, q), 87.5 (C-5), 100.5 (C-12), 103.4 (C-4, q), 121.3 (C-2'), 135.8 (C-3', q); ESI-MS(positive): m/z 353 $[M+H]^+$ calcd. for $C_{20}H_{30}O_5$ found 353; 375 $[M+Na]^+$.

12β-Arteoctadecanylether (15): Elution of the column with *n*-hexane-ethyl acetate (95:5) yielded a colorless oil, 90% (w/w) yield. IR ν^{max} (neat): IR λ_{max} (neat): 1377, 1007 (ether) 714, 725 (long chain), 2922 , 2874,1450 cm^{-1}; ^1H NMR (300 MHz, Acetone-d_6): δ 0.91 (3H, d, *J* =7.2 Hz, H_3-13), 0.93 (3H, t, *J* =6.9 Hz, H_3-17'), 0.97 (3H, d, *J* =5.7 Hz, H_3-14), 1.27 (28H, brs, H_2-2'-H_2-16'), (3H, s, H_3-15), 3.37 (1H, m, Ha-1'), 4.79 (1H, m, Hb-1'), 4.80 (1H, d, *J*=2.4 Hz, αH-12), 5.35 (1H, s, H-5); ^{13}C, DEPT- NMR (300 MHz, Acetone-d_6): δ 13.4, 14.5 (C-13, C-18'), 20.7 (C-14), 23.0 (C-8), 25.1 (C-2), 26.6 (C-15), 24.4, 29.7, 30.11, 32.3 (C-2' to C-17'), 31.3 (C-11), 35.1 (C-9), 36.8 (C-3), 37.8 (C-10), 44.9 (C-7), 53.0 (C-1), 68.8 (C-1'), 81.5 (C-6, q), 88.3 (C-5), 102.3 (C-12), 104.4 (C-4, q); ESI-MS(positive): m/z 537 $[M+H]^+$ calcd. for $C_{33}H_{62}O_5$ found 537.

2.4.3. General Procedure II: Preparation of 12α-artethioethers 16-18

DHA (1.0 g, 3mmol) was dissolved in dichloromethane (20 ml), stirred for 12 min to 4 h at 0-10°C in the presence of appropriate alkylating reagents, *viz.*, admantanethiol (3mmol for **16**) and butanedithiol (5mmol for **16** and **18**) and the catalyst boron trifluoride etherate. After completion of the reaction, monitored by TLC, the reaction mixture was quenched with cooled 1% aqueous $NaHCO_3$ (50 ml) and extracted with ethyl acetate (3 x 50 ml). The combined extract was washed with water till neutral pH, dried (Na_2SO_4) and concentrated to get the crude mixture of β/α products (1.3 to 1.7g). The mixture was subjected to silica gel column chromatography to obtain the pure β-isomer.

12α-Arteadmantanethioether (16): Elution of the column with *n*-hexane– ethyl acetate (95:5) yielded a white solid, 71% (w/w) yield. IR λ_{max} (KBr): 3178, 2923, 1410 cm^{-1}; ^1H, COSY-NMR (300 MHz, $CDCl_3$): δ 0.90 (3H, m, H_3-14), 1.13 (3H, d, *J*=5.1 Hz, H_3-13), 1.38 (3H, s, H_3-15), 1.66 (4H, brs, H_2-9', H_2-10'), 1.92 (1H, m, H-8'), 1.99 (8H, brs, H_2-2', H_2-4',

H_2-6', H_2-7'), 2.27 (1H, t, *J*=13.65 Hz, H-3', H-5'), 5.42 (1H, s, H-5), 5.44 (1H d, *J*=11.1 Hz, βH-12); ^{13}C, DEPT-NMR (75 MHz, $CDCl_3$): δ 20.2 (C-14), 21.7 (C-13), 25.2 (C-2, C-8), 26.0 (C-15), 30.2 (C-11, C-3', C-5'), 32.0 (C-9'), 34.5 (C-9), 36.7 (C-4', C-10'), 36.9 (C-3), 37.7 (C-10), 41.4 (C-8'), 44.4 (C-2', C-6'), 44.5 (C-7'), 46.5 (C-1', q 47.9 (C-7), 51.7 (C-1), 78.8 (C-5), 82.5 (C-6, q), 91.4 (C-12), 102.6 (C-4, q); ESI-MS(positive): m/z 435 $[M+H]^+$ calcd. for $C_{25}H_{39}O_4S$ found 435 m/z; 457 $[M + Na]^+$, 501 $[M+K]^+$.

12α-Artebutane-4'-thiol, 1'-thioether (17): Elution of the column with *n*-hexane-ethyl acetate (90:10) yielded a viscous compound, 51% (w/w) yield. IR λ_{max} (neat): 689 (C-S-linkage), 2927, 2872, 1453, 1377, 1129,1038, 788 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.93 (3H, d, *J*=5.4 Hz, H_3-13), 0.97 (3H, d, *J*= 4.5 Hz, H_3-14), 1.33 (3H, s, H_3-15), 1.73 (4H, m, H_2-2', H_2-3'), 2.70 (2H, m, H_2-4'), 2.93 (1H, m, H_2-1'), 4.67 (1H, d, *J*=7.86 Hz, βH-12a), 5.26 (1H, d, *J*=3.87 Hz, SH), 5.56 (1H, s, H-5); ^{13}C, DEPT-NMR (100 MHz, $CDCl_3$): δ 15.1 (C-13), 20.6 (C-14), 25.1 (C-8), 25.4 (C-2), 26.2 (C-15), 32.9 (C-11), 35.1 (C-9), 37.1 (C-3), 37.8 (C-10), 46.0 (C-7), 53.6 (C-1), 81.1 (C-6, q), 88.5 (C-5), 92.8 (C-12), 104.5 (C-4, q); ESI-MS(positive): m/z 389 $[M+H]^+$ calcd. for $C_{19}H_{33}O_4S_2$ found 389; m/z 411$[M + Na]^+$.

12α-Diartebutane-1', 4'-dithioether (18, dimer): Elution of the column with *n*-hexane–ethyl acetate (92:8) yielded a viscous compound, 36% (w/w) yield. IR λ_{max} (KBr): 690 (C-S-linkage), 2926, 2871, 1451, 1377, 1129,1037 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.92 (6H, d, *J*=7.2 Hz,, H_3-13a, H_3-13b), 0.97 (6H, m, H_3-14a, H_3-14b,), 1.32 (6H, s, H_3-15a, H_3-15b,), 1.73 (4H, m, H_2-2', H_2-3'), 2.62 (4H, m, H_2-1', H_2-4'), 4.60 (1H, d, *J*=8.02 Hz, βH-12a), 4.75 (1H, d, *J*=8.22 Hz, βH-12b), 5.37 (1H, s, H-5a), 5.98 (1H, s, H-5b); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.8, 15.3 (C-13a, 13b), 20.5, 20.7 (C-14a, 14b), 22.3 (C-8a, 8b), 24.4 (C-2a, 2b), 25.5, 26.1 (C-15a, 15b), 28.1 (C-2'), 31.2 (C-11a, 11b), 32.8 (C-3'), 33.7, 33.8 (C-1', C-4'), 34.9, 35.3 (C-9a, 9b), 36.1 (C-3a, C-3b), 36.9, 37.8 (C-10a, 10b), 47.0, 49.1 (C-

7a, 7b), 52.7, 56.0 (C-1a, 1b), 80.6, 81.1 (C-6a, 6b, q), 83.5 (C-5a, 5b), 92.7, 93.3 (C-12a, 12b), 104.4 (C-4a, 4b, q); ESI-MS(positive): m/z 655 $[M+H]^+$ calcd. for $C_{34}H_{55}O_8S_2$ found 655; 677 $[M + Na]^+$, 693 $[M+K]^+$.

2.4.5. General Procedure IV: Preparation of 12α-arteesters 19-25

Dihydroartemisinin (1.0 g, 3mmol) was dissolved in dichloromethane (20 ml), stirred for 12 min to 4 h at 0-10°C in the presence of appropriate acylating reagents (3mmol), *viz.*, *m*-nitrobenzoylchloride, *p*-nitrobenzoylchloride, (for **19** and **20**), piperonyloylchloride, 4-phenylazobenzoylchloride, lauroyl chloride and palmitoyl chloride (for **21**, **22, 23** and **24**) and furan-3-carboxylic acid (for **25**), catalyst triethyamine for **19-24** and DCC/DMAP for **25**(Fig. 4). After completion of the reaction, monitored by TLC, the reaction mixture was worked up as usual to get crude mixture of β/α products (1.1 to 1.8 g). The mixture was subjected to silica gel column chromatography to obtain the pure α-isomer.

12α-Dihydroartemisinyl-4'-nitrobenzoate (19): Elution of the column with *n*-hexane-ethyl acetate (95:5) yielded a colorless oil, 90% (w/w) yield. IR λ_{max} (neat): 3087, 1439, 1261, 1040, 858, 719 (aromatics), 1732 (ester CO), 1538, 1556 (Ar NO_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.93 (3H, d, *J* =7.2 Hz, H_3-13), 0.97 (3H, d, *J* =5.7 Hz, H_3-14), 1.41 (3H, s, H_3-15), 2.80 (1H, m, H-11), 5.52 (1H, s, H-5), 6.01 (1H, d, *J*=9.9 Hz βH-12), 7.66 (1H, m, H-6'), 8.42 (2H, d, *J* = 8.4 Hz, H-5', H-7'), 8.91 (1H, s, H-3'); ^{13}C, DEPT- NMR (70 MHz, $CDCl_3$): δ 12.2 (C-13), 20.1 (C-14), 22.0 (C-8), 24.5 (C-2), 25.8 (C-15), 31.8 (C-11), 34.0 (C-9), 36.1 (C-3), 37.2 (C-10), 45.2 (C-7), 51.5 (C-1), 80.0 (C-6, q), 91.6 (C-5), 93.3 (C-12), 104.5 (C-4, q), 124.9 (C-3'), 127.7 (C-5'), 129.6 (C-6'), 131.3 (C-2', q), 135.6 (C-7'), 148.2 (C-2', q), 163.2 (C-1', q); EI-MS(positive): m/z 434 $[M+H]^+$ calcd. for $C_{22}H_{28}O_8N$ found 434; 456 $[M+Na]^+$, 472 $[M+K]^+$.

12α-Dihydroartemisinyl-5'-nitrobenzoate (20): Elution of the column with n-hexane– ethyl acetate (95:5) yielded an oily compound in 95% (w/w) yield. IR λ_{max} (neat): 3081, 1437,

1256, 1034, 851, 711 (aromatics), 1734 (ester CO), 1,533, 1558 (Ar NO_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.92 (6H, m, H_3-13, H_3-14), 1.41 (3H, s, H_3-15), 2.75 (1H, m, H-11), 5.52 (1H, s, H-5), 6.01 (1H, d, *J*=9.0 Hz, βH-12), 8.27 (4H, brs, H-3', H-4', H-6', H-7'); ^{13}C, DEPT- NMR(70 MHz, $CDCl_3$): δ 12.62 (C-13), 20.60 (C-14), 22.42, (C-8), 24.96 (C-2), 26.2 (C-15), 32.3 (C-11), 34.4 (C-9), 36.6 (C-3), 37.6 (C-10), 45.6 (C-7), 51.9 (C-1), 81.5 (C-6, q), 92.0 (C-5), 93.7 (C-12), 104.9 (C-4, q), 123.9 (C-4', C-6'), 131.6 (C-3', C-7'), 135.4 (C-2', q), 151.1 (C-5', q), 163.9 (C-1', q); EI-MS(positive): m/z 434 $[M+H]^+$ calcd. for $C_{22}H_{28}O_8N$ found 434; 456 $[M+Na]^+$.

12α-Artepiperonyloylester (21) Elution of the column with *n*-hexane-ethyl acetate (95:5) yielded an oily compound, 90% (w/w) yield. IR λ_{max} (neat): 1626, 1508, 1444, 1260, 1017, 878, 861 (aromatics), 1734 (ester CO), 921 (-O-CH_2-O- group) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.89 (3H, d, *J* =7.2 Hz, H_3-13), 0.96 (3H, d, *J* =5.7 Hz, H_3-14), 1.41 (3H, s, H_3-15), 2.70 (1H, m, H-11), 5.50 (1H, s, H-5), 5.94 (1H, d, *J*=9.9 Hz, βH-12), 6.03 (2H, s, H-8'), 6.83 (1H, d, *J* = 7.8 Hz, H-6'), 7.53 (1H, s, H-3'), 7.73 (1H, d, *J* = 7.8 Hz, H-7'); ^{13}C, DEPT-NMR (70 MHz, $CDCl_3$) = 12.1 (C-13), 20.1 (C-14), 21.9 (C-8), 24.5 (C-2), 25.8 (C-15), 31.9 (C-11), 34.0 (C-9), 36.2 (C-3), 37.2 (C-10), 45.2 (C-7), 51.5(C-1), 80.1 (C-6, q), 91.5 (C-5), 92.4 (C-12), 101.8 (C-8'), 104.3 (C-4, q), 107.8 (C-6'), 109.8 (C-7'), 123.4 (C-2', q), 126.0 (C-3'), 147.6 (C-4', q), 151.9 (C-5', q), 164.5 (C-1', q); ESI-MS(positive): m/z 455 $[M+Na]^+$ calcd. for $C_{23}H_{28}O_8Na$ found 455m/z.

12α-Dihydroartemisinyl-5'-phenylazobenzoate (22): Elution of the column with *n*-hexane–ethyl acetate (95:5) yielded orange crystals of compound **24,** 85% (w/w) yield, mp. 138-140°C. IR λ_{max} (KBr): 2344 (-N=N- group), 1654, 1508, 1458, 1271, 1028, 882, 864, 783 (aromatics), 1732 (ester CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.92 (3H, d, *J* =8.1 Hz, H_3-13), 0.98 (3H, d, *J* =6.0 Hz, H_3-14), 1.49 (3H, s, H_3-15), 2.79 (1H, m, H-11), 5.54 (1H, brs, H-5), 6.02 (1H, d, *J*=9.9 Hz, βH-12), 7.53 (3H, m, H-10', H-11', H-12'), 7.95 (4H, d, *J* =

8.7 Hz, H-4', H-6', H-9', H-13'), 8.27 (2H, d, *J*=8.7 Hz, H-3', H-7'); ^{13}C, DEPT- NMR (75 MHz, $CDCl_3$): δ 12.6 (C-13), 20.6 (C-14), 22.4 (C-8), 25.0 (C-2), 26.3 (C-15), 32.4 (C-11), 34.5 (C-9), 36.6 (C-3), 37.6 (C-10), 45.7 (C-7), 52.0 (C-1), 80.6 (C-6), 92.0 (C-5), 93.2 (C-12), 104.8 (C-4, q), 122.9 (C-4', C-6'), 123.5 (C-9', C-13'), 129.5 (C-10', C-12'), 131.5 (C-3', C-7'), 132.1 (C-11'), 152.9 (C-8', q), 155.7 (C-5', q), 165.1 (C-1', q) ; ESI-MS(positive): m/z 493 $[M+H]^+$ calcd. for $C_{28}H_{33}O_6N_2$ found 493.

12α-Dihydroartemisinyllauroyloate (23): Elution of the column with *n*-hexane-ethyl acetate (95:5) yielded a colorless oil, 90% (w/w) in yield. IR λ_{max} (neat): 1736 (ester CO), 714, 725(long chain), 2922, 2874, 1450, 1377, 1007 cm^{-1}; ^{1}H, COSY- NMR (300 MHz, $CDCl_3$): δ 0.78 (3H, t, *J*= 7.2, H_3-12'), 0.79 (3H, d, *J* =7.2 Hz, H_3-13), 0.89 (3H, d, *J* =5.4 Hz, H_3-14), 1.18 (18H, m, H_2-3' - H_2-11'), 1.36 (3H, s, H_3-15), 2.31 (2H, t, *J*=7.2, H_2-2'), 2.48 (1H, m, H-11), 5.37 (1H, s, H-5), 5.72 (1H, d, *J*=9.6 Hz, βH-12); ^{13}C, DEPT- NMR (75 MHz, $CDCl_3$): δ 12.4 (C-12'), 14.4 (C-13), 20.6 (C-14), 22.4 (C-8), 23.0 (C-2), 24.9, 25.0, 29.4, 29.6, 29.7, 29.8, 29.9, 30.0, 32.2, 34.7 (C-2' to C-11',C-8), 26.3 (C-15), 32.2 (C-11), 34.5 (C-9), 36.6 (C-3), 37.6 (C-10), 45.6 (C-7), 52.0 (C-1), 80.5 (C-6, q), 91.8 (C-5), 92.0 (C-12), 104.8 (C-4, q), 172.9 (C-1', q); ESI-MS(positive): m/z 467 $[M+H]^+$ calcd. for $C_{27}H_{47}O_6$ found 467; 489 $[M+Na]^+$(negative): 465 $[M-H]^-$.

12α-Dihydroartemisinylpalmitoyloate (24): Elution of the column with *n*-hexane-ethyl acetate (95:5) yielded a colorless oil, 90% (w/w) in yield. IR λ_{max} (neat): 1751 (ester CO), 722 (long chain), 2924, 2853, 1459, 1376, 1037 cm^{-1}; ^{1}H, COSY- NMR (300 MHz, $CDCl_3$): δ 0.93 (6H, m, H_3-13, H_3-16'), 0.97 (3H, d, *J* =4.2 Hz, H3-14), 1.23 (26H, m, H_2-3' - H_2-15'), 1.41 (3H, s, H3-15), 2.34 (2H, t, *J*=8.4, H_2-2'), 2.54 (1H, m, H-11), 5.42 (1H, s, H-5), 5.77 (1H, d, *J*=9.9 Hz, βH-12); ^{13}C, DEPT- NMR (75 MHz, $CDCl_3$); δ 12.5 (C-16'), 14.5 (C-13), 20.6 (C-14), 22.4 (C-8), 23.0 (C-2), 24.9, 25.0, 29.4, 29.6, 29.7, 29.8, 29.9 (12 x $\underline{C}H_2$), 26.3 (C-15), 32.2 (C-11), 30.0, 34.7 (2 x $\underline{C}H_2$), 34.7 (C-9), (C-2'), 36.6 (C-3), 37.6 (C-10),

44.6 (C-7), 52.0 (C-1), 80.5 (C-6, q), 91.8 (C-5), 92.0 (C-12), 104.8 (C-4, q), 172.9 (C-1', q); ESI-MS(positive): m/z 561 $[M + K]^+$ calcd. for $C_{31}H_{54}O_6K$ found 561.

12α-Dihydroartemisinylfuranoate (25): Elution of the column with *n*-hexane– ethyl acetate (95:5) yielded creamish crystals of compound **27,** 85% (w/w) yield, mp. 140°C IR λ_{max} (KBr): 3145, 1509, 876 (furan moiety), 1739 (ester CO), 2926, 1306, 1164, 1036 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.86 (3H, d, *J* =6.9 Hz, H_3-13), 0.95 (3H, d, *J* =5.4 Hz, H_3-14), 1.31 (3H, s, H_3-15), 2.65 (1H, s, H-11), 5.47 (1H, s, H-5), 5.91 (1H, d, *J*=9.6 Hz, βH-12), 6.77 (1H, s, H-5'), 7.41 (1H, s, H-4'), 8.08 (1H, s, H-3'); ^{13}C, DEPT- NMR (75 MHz, $CDCl_3$): δ 12.5 (C-13), 20.6 (C-14), 22.4 (C-8), 24.9 (C-2), 26.3 (C-15), 32.3 (C-11), 34.4 (C-9), 36.6 (C-3), 37.6 (C-10), 45.6 (C-7), 52.0 (C-1), 80.5 (C-6, q), 91.9 (C-5), 92.4 (C-12), 104.8 (C-4, q), 110.3 (C-5'), 119.2 (C-2', q), 144.1 (C-4'), 148.9 (C-3'), 162.1 (C-1', q); ESI-MS(positive): m/z 417 $[M + K]^+$ calcd. for $C_{20}H_{26}O_7K$ found 417; (negative): m/z 377 [M-H].

2.4.4.1. Preparation ofanhydrodihydroartemisinin (26): To a solution of DHA (10g) in diethylether 500 ml added dropwise $BF_3.OEt_2$ solution and stirred the reaction mixture overnight at RT. After completion of reaction it was washed with 5% $NaHCO_3$ solution (3x100 ml), dried (Na_2SO_4) and concentrated[19c]. The residue, obtained was then purified by column chromatography (10% EtOAc in hexane) followed by crystallisation (EtOAc-hexane) to afford shiny white crystals of 9, 10-anhydrodihydroartemisinin (**26**), (7.1 gm), mp 98-100 °C ;IR λ_{max}(KBr): 1684 (double bond), 2928, 2850, 1452, 1109 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 0.92 (3H, d, *J*=6.0 Hz, H_3-14), 1.42 (3H, s, H_3-15), 1.56 (3H, d, *J* =1.2, H_3-13), 1.66 (2H, m), 1.87 (3H, m), 2.04 (2H, m), 1.92 (1H, m, H-1), 2.39 (1H, dt, *J* =13.9, 4.8 Hz, H-7), 5.52 (1H, s, H-5), 6.16 (1H, s, H-12); ^{13}C, DEPT-NMR (75 MHz, $CDCl_3$) *δ* 16.5 (C-13), 20.6 (C-14), 24.8 (C-2), 26.2 (C-15), 30.3 (C-8), 34.5 (C-9), 36.6 (C-3), 37.8 (C-10),

44.8 (C-7), 51.8 (C-1), 79.3 (C-6, q), 90.0 (C-5), 104.9 (C-4, q), 108.4 (C-11, q), 135.3 (C-12)[27a] ; ESI-MS(positive): m/z 267 $[M+H]^+$ calcd. for $C_{15}H_{23}O_4$ found 417.

2.4.4.2. Preparation of11β, 12β-arteepoxide (27): Anhydrodihydroartemisinin (**26)** (6g) was dissolved in CH_2Cl_2 (150 ml) and *m*-chloroperbenzoic acid (5.7g in 0.5 N $NaHCO_3$) was added[19c]. The reaction mixture was stirred at room temperature for 2h[19d]. After completion of the reaction, monitored by TLC the reaction mixture was diluted to 300 ml with CH_2Cl_2 and was washed with 5% Na_2SO_3 (3 x 200 ml), 5% $NaHCO_3$ (200 ml x 3) and finally with water (200 ml x 3). After drying over anhydrous Na_2SO_4 and evaporation of solvent followed by column chromatography of the crude residue afforded 11β, 12β-arteepoxide, 4.2g (**27),** mp 112 °C ; IR λ_{max}(KBr): 2928, 2857, 1680, 1449, 1109, 876 cm^{-1}; ^{1}H, COSY- NMR (300 MHz, $CDCl_3$) δ 0.91 (3H, d, *J*= 4.8 Hz, H_3-14), 1.09 (1H, m, H-9α), 1.24 (1H, m, H-10), 1.28 (3H, s, H_3-13), 1.38-1.56 (2H, m, H-8β, H-2β), 1.71 (2H, m, H-1, H-9β), 1.76-1.96 (3H, m, H-8α, H-7, H-2α), 2.05 (1H, m, H-3β), 2.32 (1H, ddd, *J*=4.2, 12.8, 14.2 Hz, H-3α), 4.91 (1H, s, H-12), 5.24 (1H,s, H-5); ^{13}C, DEPT- NMR (75 MHz, $CDCl_3$) :*δ* 20.0 (C-14), 22.0 (C-13), 24.1 (C-8), 24.2 (C-2), 25.9 (C-15), 33.3 (C-9), 36.1 (C-3), 36.9(C-10), 45.0 (C-7), 51.6 (C-1), 56.2 (C-11, q), 78.1 (C-6, q), 82.5 (C-12), 90.4 (C-5), 104.5 (C-4, q) ESI-MS(positive): m/z 283 $[M+H]^+$ calcd. for $C_{15}H_{23}O_5$ found 283.

2.4.4.3. General Procedure III: Preparation of 11β-hydoxy12β-arteethers 28-33:

Further, the epoxide **27** was alkylated, using appropriate alcohols, 1-admantaneethanol, cinnamyl alcohol, cyclopropylmethanol, 2,5-dimethoxybenzyl alcohol, allyl alcoholpiperonylyl alcohol, catalyst $BF_3.OEt_2$ in CH_2Cl_2 to yield 11-hydoxy12β-arteethers **28-33** (Fig. 5).

11β-Hydroxy-12β-arteallylether (28): Elution of the column with *n*-hexane– ethyl acetate (90:10) yielded a white solid in 85% (w/w) yield. IR λ_{max} (KBr): 3423 (OH), 1103, 1029

(ether linkage), 1655 (double bond), 2923, 2853, 1463, 1376, 877 cm^{-1}. ^{1}H, COSY- NMR (400 MHz, Acetone-d_6): δ 0.96 (3H, d, J =4.76 Hz, H_3-14), 1.34 (3H, s, H_3-15), 1.47 (3H, s, H_3-13), 4.08 (1H, dd, *J* =12.62, 4.94 Hz, Ha-1'), 4.32, d, *J*= 10.12 Hz, Hb-1'), 4.60 (1H, brs, αH-12), 5.18 (1H, d, *J*=10.44 Hz, Ha-3'), 5.33 (1H, d, *J*=17.16 Hz, Hb-3'), 5.42 (1H, s, H-5), 5.99 (1H, m, H-2'); ^{13}C, DEPT- NMR (100 MHz, Acetone-d_6): δ 19.7 (C-14), 24.3 (C-2, C-8), 25.0 (C-15), 28.3 (C-13), 34.2 (C-9), 36.2 (C-3), 37.2 (C-10), 50.4 (C-7), 52.7 (C-1), 68.9 (C-1'), 70.0 (C-11, q), 82.6 (C-6, q), 87.4 (C-5), 103.0 (C-12), 104.3 (C-4, q), 116.9 (C-3'), 135.5 (C-2'); ESI-MS(positive): m/z 341 $[M+H]^+$ calcd. for $C_{18}H_{29}O_6$ found 341; (negative): 339 $[M-H]^-$.

11β-Hydroxy-12β-artecyclopropylmethylether (29): Elution with *n*-hexane– ethyl acetate (90:10) yielded a white solid, 78% (w/w) yield. IR λ_{max} (KBr):3363 (OH), 1272, 1103, (ether linkage), 2921, 2863, 1469, 1378 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.25/0.53 (2H each, m H_2-3'/H_2-4'), 0.97 (3H, brs, H_3-14), 1.43 (3H, s, H_3-15), 1.56 (3H, s, H_3-13), 2.36 (1H, m, H-2'), 3.43 (1H, m, Ha-1'), 3.60 (1H, m, Hb-1'), 4.67 (1H, d, *J*=2.1 Hz, αH-12), 5.42 (1H, s, H-5); ^{13}C, DEPT-NMR (75 MHz, $CDCl_3$): δ 3.1/3.6 (C-3' , C-4'), 10.9 (C-2'), 20.6 (C-14), 24.7 (C-2, C-8), 26.2 (C-15), 28.3 (C-13), 34.5 (C-9), 36.8 (C-3), 37.9 (C-10), 50.6 (C-7), 53.0 (C-1), 69.9 (C-1'), 74.0 (C-11, q), 82.3 (C-6, Q), 88.2 (C-5), 103.6 (C-12), 104.3(C-4, q); ESI-MS(positive): m/z 355 $[M+H]^+$ calcd. for $C_{19}H_{31}O_6$ found 355; (negative): 353 $[M-H]^-$.

11β -Hydroxy-12β-arte-2'-admantanethylether (30): Elution of the column with *n*-hexane–ethyl acetate (90:10) yielded a white solid, 80% (w/w) yield. IR λ_{max} (KBr): 3448 (OH), 1301, 1141, 1091, 1010 (ether linkage), 2857, 3178, 2923, 1410 cm^{-1}; ^{1}H, COSY-NMR (300 MHz, $CDCl_3$): δ 0.95 (3H, d, *J*=5.1 Hz, H_3-14), 1.45 (3H, s, H_3-15), 1.51 (7H, brs, H_3-13, H_2-11', H_2-12'), 1.63 (10H, m, H_2-4', H_2-6', H-7', H_2-8', H_2-9', H-10') 1.87 (2H, t, *J*=18.9 Hz, H_2-2'), 2.35 (1H, t, *J*=13.95 Hz, H-5'), 3.51 (1H, m, Ha-1'), 3.96 (1H, m, Hb-1'), 4.65

(1H, brs, αH-12), 5.40 (1H, s, H-5); ^{13}C, DEPT-NMR (75 MHz, $CDCl_3$): δ 20.6 (C-14), 24.6 (C-8), 24.8 (C-2), 26.2 (C-15), 28.3 (C-13), 29.0(C-5', C-7'), 32.3(C-3', q), 34.5 (C-9), 36.8 (C-3), 37.4 (C-6', C-11', C-12'), 37.8 (C-10), 43.1 (C-2', C-4', C-8', C-9'), 44.0 (C-10'), 50.5 (C-7), 53.0 (C-1), 65.6 (C-1'), 70.0 (C-11, q), 82.3 (C-6, q), 88.2 (C-5), 104.1 (C-12), 104.4 (C-4, q); ESI-MS(positive): m/z 485 $[M+Na]^+$ calcd. for $C_{27}H_{42}O_6Na$ found 485.

11β-Hydroxy-12β-artecinnamylether (31): Elution of the column with *n*-hexane-ethyl acetate (90:10) yielded a white solid, 88% (w/w) yield. IR λ_{max} (KBr): 3405 (OH), 1276, 1119, (ether linkage), 1652 (double bond), 2855, 1603, 1508, 1444, 1260, 1017, 878, 861 (aromatics) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.96 (3H, d, *J* =6.0 Hz, H_3-14), 1.40 (3H, s, H_3-15), 1.58 (3H, s, H_3-13), 4.28 (1H, t, J= 6.0 Hz Ha-1'), 4.53 (1H, m, Hb-1'), 4.82 (1H, brs, αH-12), 5.47 (1H, brs, H-5), 6.30 (1H, m, H-2'), 6.64 (1H, m, H-3'), 7.40 (5 H, m, H-5', H-6', H-7', H-8', H-9'); ^{13}C, DEPT- NMR (75 MHz, $CDCl_3$); δ 20.6 (C-14), 24.7 (C-2, C-8), 26.2 (C-15), 28.4 (C-13), 34.5 (C-9), 36.8 (C-3), 37.8 (C-10), 50.5 (C-7), 53.0 (C-1), 69.6 (C-1'), 70.0 (C-11, q), 82.3 (C-6, q), 88.3 (C-5), 103.2 (C-12), 104.4 (C-4, q), 125.2 (C-2'), 127.0 (C-6', C-8'), 127.8 (C-7'), 128.3 (C-5', C-9'), 133.7 (C-3'), 137.08(C-4', q); ESI-MS(positive): m/z 417 $[M+H]^+$ calcd. for $C_{24}H_{33}O_6$ found 417.

11β-Hydroxy-12β-artepiperonylylether (32): Elution of the column with *n*-hexane– ethyl acetate (90:10) yielded an oily compound, 90% (w/w) yield. IR λ_{max} (neat): 3398 (OH), 1121, 1031 (ether linkage), 1606, 1514 1432, 1256, 1011, 871 (aromatics), 914 (-O-CH_2-O- group) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.93 (3H, d, *J* =5.4 Hz, H_3-14), 1.44 (3H, s, H_3-15), 1.55 (3H, s, H_3-13), 4.45 (1H, d, *J*=11.4 Hz, Ha-1'), 4.75 (1H, d, *J*=11.4 Hz, Hb-1'), 4.78 (1H, brs, αH-12), 5.45 (1H, brs, H-5), 5.95 (2H, s, H-8'), 6.78 (3H, m, H-3', H-4', H-5'); ^{13}C, DEPT- NMR (70 MHz, $CDCl_3$): δ 20.6 (C-14), 24.7 (C-2, C-8), 26.2 (C-15), 28.4 (C-13), 34.4 (C-9), 36.8 (C-3), 37.8 (C-10), 50.5 (C-7), 52.9 (C-1), 70.0 (C-11, q), 70.8 (C-1'), 82.3 (C-6, q), 88.3 (C-5), 101.5 (C-8'), 103.0 (C-12), 104.4 (C-4, q), 108.6 (C-5'), 109.0 (C-3'),

122.1 (C-4'), 131.2 (C-2', q), 148.2 (C-7'), 148.2 (C-6'); ESI-MS(positive): m/z 435 $[M+H]^+$ calcd. for $C_{23}H_{31}O_8$ found 435.

11β-Hydroxy-12β-arte-3', 6'-dimethoxybenzylether (33): Elution of the column with *n*-hexane-ethyl acetate (90:10) yielded a colourless oil in 90% (w/w) yield. IR λ_{max} (neat): 3408 (OH), 1102, 1038 (ether linkage), 1606, 1501, 1432, 1262, 1011, 874, 864 (aromatics) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.94 (3H, m, H_3-14), 1.47 (3H, s, H_3-15), 1.56 (3H, s, H_3-13), 3.77, 3.79 (3H each, s, 2xOC$\underline{H}_3$), 4.53 (1H, d, *J*=11.4 Hz, Ha-1'), 4.80 (1H, brs, αH-12), 4.92 (1H, d, *J*=11.4 Hz, Hb-1'), 5.55 (1H, s, H-5), 6.69 (2H, dd, *J* = 6.9, 3.0 Hz, H-4', H-5'), 6.86 (1H, s, H-7'); ^{13}C, DEPT- NMR (75 MHz, Acetone-d_6): δ 20.1 (C-14), 24.6 (C-8), 24.8 (C-2), 26.2 (C-15), 28.3 (C-13), 34.5 (C-9), 36.8 (C-3), 37.8 (C-10), 50.5 (C-7), 53.0 (C-1), 56.1 (2 x O$\underline{C}H_3$), 67.6 (C-1'), 69.9 (C-11, q), 82.4 (C-6, q), 88.3 (C-5), 104.1 (C-12), 104.4 (C-4, q), 111.5 (C-5'), 112.6 (C-4'), 114.4 (C-7'), 128.12 (C-2', q), 152.2 (C-3, q'), 153.7 (C-6', q); ESI-MS(positive): m/z 451 $[M+H]^+$ calcd. for $C_{24}H_{35}O_8$ found 451; 473 $[M+Na]^+$.

***In vitro* anti-malarial activity**

Anti-malarial assay: The *in vitro* inhibitory assays were performed using *P. falciparam* (NF54 strain) in human blood. *P. falciparum* cultures were carried out according to the method described[20]. Compounds were dissolved in DMSO and then diluted. Synchronous cultures with parasitaemia of 1-1.5% and 2% final haematocrit were incubated in 96 well microtitre plates with multiple concentrations of compounds for 48 h at 37°C. Parasite growth was determined spectrophotometrically by measuring the activity of the pLDH, in control and drug-treated cultures. At the end of the incubation, the cultures were carefully resuspended, and aliquots of 20 µL were removed and added to 0.1mL of Malstat reagent in a 96 well microtitre plates. The spectrophotometric assessment of pLDH activity is facilitated by adding 25 µL of a solution of 1.9 µM NBT and 0.24 µM PES. As APADH is formed, the NBT is reduced and forms a blue formazan product that can be measured at 650nm. The anti-

malarial activity of the test compound was expressed as the 50% inhibitory concentration (IC_{50}) determined from the dose-response curve (Table 1, Table 2, Table 3).

In silico molecular docking study

Molecular docking and visualization studies of artemisinin and its derivatives was performed through AutoDock Vina (www.vina.scripps.edu/), an open-source program for doing molecular docking with 78% binding mode prediction accuracy on test set (Molecular Graphics Lab at The Scripps Research Institute, USA).[21] PyRx v0.8 (http://pyrx.sourceforge.net), an open-source virtual screening software of computational drug discovery used to screen libraries of compounds against potential drug targets. The 3D crystallographic structure of anti-malarial protein target was retrieved through RCSB Protein Data Bank (PDB) (www.rcsb.org/). To find the possible bioactive conformations of artemisinin derivatives, these programs were used to dock the artemisinin derivatives into the binding site of *P. falciparum* target hydrolase enzyme Plasmepsin-II (PDB: 2IGY).[22] During docking procedure all parameters were assigned to their default values.

Screening through *in silico* pharmacokinetic parameters

Most of drugs fail during discovery process to cross human clinical trials because of poor pharmacokinetic (PK).[23] The important parameters of PK are absorption, distribution, metabolism, excretion, and toxicity (ADMET). These PK parameters are important descriptors for human therapeutic use of any compounds. These PK parameters were calculated and later evaluated through ADMET modules in Discovery Studio v3.5 software (Accelrys, USA).From this module, mathematical predictive ADMET QSAR models for different pharmacokinetics parameters namely, aqueous solubility, blood–brain barrier penetration, cytochrome P450 2D6 inhibition, hepatotoxicity, human intestinal absorption and plasma protein binding were used to quantitatively predict properties of a set of rules that

specify ADMET characteristics of the chemical structure of the artemisinin derivatives. These ADMET descriptors allow us to eliminate compounds with unfavourable ADMET characteristics early, to avoid expensive reformulation, preferably before synthesis and also help to evaluate proposed structural refinements that are designed to improve ADMET properties. The studied compounds were also evaluated against Lipinski's rule of five for oral bioavailability, since 90% orally active existing drugs/compounds follows Lipinski's rule.[24] To predict a variety of toxicities that are often used in drug development, the recommended toxicity screening models for carcinogenicity, developmental toxicity, mutagenicity and skin irritancy or sensitization were used for calculations of artemisinin derivatives through DS_TOPKAT module of Discovery Studio 3.5 software (Accelrys, USA).[25] These predictions will help in optimizing therapeutic ratios of lead compounds for further development and assessing their potential safety concerns. These predictions will also help in evaluating intermediates, metabolites and pollutants, along with setting dose range for animal assays.

RESULT AND DISCUSSION

Dihydroartemisinin (**2)** was prepared by $NaBH_4$ reduction of artemisinin using the known procedure[19]. $BF_3.OEt_2$-catalyzed reaction of **2** with appropriate alcohols (Figure 2) in CH_2-Cl_2 at subzero temperature (-10°C to -5°C) furnished the corresponding ether derivatives **6-18** and **28-32** in 65-99% yields as diastereomeric mixtures of β and α isomers, with β-isomers isolated as the major products. In the case of ester derivatives, α isomers appeared as major spot on TLC and was separated by column chromatography, and the pure isomers were evaluated for antimalarial activity. β-isomer of ether derivatives and α-isomers of ester derivatives were used for bioevaluation. The acid chlorides were reacted with dihydroartemisinin in the presence of triethylamine in dry dichloromethane at 0°C for 2h to furnish corresponding ester derivatives **19-24** in 40-90% yield. Ester derivative **25** was

synthesised by reacting furan-2-carboxylic acid and dihydroartemisinin (**2**) in the presence of DCC and DMAP in dry dichloromethane at RT for 2h.

Derivatives **28-32** were synthesized by dissolving anhydrodihydroartemisinin (200mg) in CH_2Cl_2 (15 ml) and m-chloroperbenzoic acid (190 mg in 0.5 N $NaHCO_3$) was added. The reaction mixture was vigorously stirred at room temp. for 75 min. Further epoxide **27** was hydrolysed by using $BF_3.OEt_2$ in CH_2Cl_2 in the presence of appropriate alcohols which resulted in the products **28-32**[26]. The configuration at C-12 stereocenter is assigned based on the vicinal coupling constant *J*H-9:H-10. A large coupling constant (7-10 Hz) is generally found for the 10α-isomer, indicating the relative trans-configuration. The 10β-isomer, indicating cis-configuration at C-10, on the other hand, has a smaller coupling constant (2-5 Hz).[26]

Artemisinin derivatives such as artemether **3**, arteether **4**, and artesunic acid **5** have excellent antimalarial activity when given by systemic routes. They are fast acting drugs and are increasingly being used for the treatment of complicated cases of malaria caused by multidrug-resistant *P. falciparum*. These drugs, however, have serious limitation such as short half-life and poor bioavailability when given by oral route[27]. Both the short half-life and poor oral bioavailability are believed to be due to the poor stability of C10-O linkage which is prone to acid hydrolysis and P450-catalyzed oxidation[28]. Since C-10 acetal derivatives are unstable, several workers have in recent past prepared C10-C linked derivatives which are more stable and have shown improved antimalarial activity by oral route[29-33]. In a parallel program on synthetic antimalarial 1,2,4-trioxanes, we had observed that incorporation of particular functionality enhances the activity relative to artemisinin[34]. Also there are several reports in the literature, ether and ester derivatives show promising biological activities[35] On the basis of these considerations, we have prepared ether derivatives **6-18** and ester

derivatives **19-25** and 11-hydroxy ether derivatives of artemisinin **28-32** and compared their biological activity against *P. falciparum*.

Among ether derivatives **6**, **8**, **13**, **14** and **16** displayed better activity than arteether, and **16** was most active with IC_{50} 9.21nM. Except **7** and **15** having nitro group functionality at meta position and long chain ether group respectively all the derivatives were better than artemisinin. Thus, against NF54 strain, the esters were found still more active than the ethers. All esters were distinctively the most active of all artemsinin derivatives synthesized during this study. They were significantly more potent than arteether and artemisinin. As the lipophilicity of the compound increased, there was an increase in activity[36,37]. This increase in activity is in accordance with earlier observations[16] that lipophilic derivatives are more active than their more polar counterparts; i.e. esters and ethers are more active than the corresponding alcohols. Decrease in lipophilicity decreases activity. In the 11-OH-12-ether series, none of the derivatives were as active as artemisinin.

Molecular docking revealed binding affinity against Plasmepsin II

The aim of docking studies was to explore the binding conformation of active artemisinin derivatives against antimalarial target plasmepsin II. The results of docking studies suggest that active compounds inhibit the activity of plasmepsin II due to high binding affinity as indicated by docking score. Predicted results were also complimentary to our prior studies.[38] In the studied work, we explored the orientations and binding affinity of dihydroartemisinin derivatives towards antimalarial target plasmepsin II (PDB: 2IGY). Plasmepsins are a class of at least 10 enzymes produced by the plasmodium parasite. Through their haemoglobin-degrading activity, they are an important cause of symptoms in malaria sufferers. Consequently this family of enzymes is a potential target for antimalarial drugs. The aspartic protease of plasmodium species are known as plasmepsins. Plasmepsins are aspartic acid protease, which means their active site contains two aspartic acid residues.

The binding affinity obtained in the docking study allowed the activity of the artemisinin derivatives to be compared to that of the standard antimalarial compounds artemisinin and DHA. The docking study suggest that studied artemisinin and their derivatives inhibit the plasmepsin II enzymatic activity by indicating acceptable binding affinity score. All the derivatives showed significant binding affinity against plasmepsin II.

When we compared how the binding site pocket residues of plasmepsins interacted with the artemisinin derivatives, we found that compounds **19**, **20** and **21** showed interaction with specific amino acid residues thus lead to more stability and potency in these cases and also showed the hydrogen (H-) bond formation between the ligand and receptor (Table 4). During docking, compounds **19**, **20** and **21** showed quiet lower binding energy (i.e., higher binding affinity) in compare to reference drugs DHA (binding energy -6.9 Kcal/mol) (Fig. 6b) and artemisinin (binding energy -6.8 Kcal/mol) (Fig. 6c). The docking results for the active artemisinin derivatives namely, compounds **19**, **20** and **21**, docked on plasmepsin enzyme of *P. falciparum* with high docking score (i.e., low docking binding energy) of-8.0 Kcal/mol (Fig. 6d), -7.6 Kcal/mol (Fig. 6e) and -8.2 Kcal/mol (Fig. 6f), respectively. For compound **19**, docking result showed formation of H-bond with binding site polar basic residue HIS-164 having bond length of 2.19 Å. Likewise compound **20**, also showed H-bond formation with same polar basic residue histidine (HIS-164) having bond length of 1.99 Å. Similarly, compound **21**, showed H-bond formation with chemically similar family i.e., polar basic amino acid residue arginine (ARG-307) having bond length of 2.37Å. Compound 21 seems best docked artemisinin derivative even potent then DHA and artemisinin. During docking against plasmepsin II, results showed similar binding site residues for most favourable conformations of predicted active artemisinin derivatives as well as for DHA and artemisinin (Table-4).

Compliance with *in silico* pharmacokinetics properties

Since the docking studies were found to be promising, the chemical descriptors for the pharmacokinetic properties were also calculated, so as to check the compliance of studied compounds with standard range. Calculating these ADMET properties was intended as the first step toward analysing the novel chemical entities in order to check the failure of lead candidates, which may cause toxicity or be metabolized by the body into an inactive form or one unable to cross the intestinal membranes. Results revealed that artemisinin and its derivatives *viz*., compound **19**, **20** and **21** follows Lipinski's rule of five for oral bioavailability (Table-5). The bi-plot showed two confidence ellipses of 95% and 99% for the blood–brain barrier penetration and human intestinal absorption models (Fig.7). The polar surface area (PSA) was shown to have an inverse relationship with percent human intestinal absorption and membrane permeability. Compounds **19**, **20** showed comparatively higher PSA and logP, therefore reflect moderate intestinal absorption and very low or no blood-brain barrier penetration. Compound **21** showed comparatively low PSA and logP, therefore reflect good intestinal absorption and medium blood-brain barrier penetration, similar to DHA & artemisinin. However, compounds **19**, **20** and **21** showed no or very low aqueous solubility, while DHA and artemisinin comparatively showed higher but low aqueous solubility (calculated for water at 25°C). All derivatives showed poorly bound property while evaluating plasma-protein binding prediction, similar to reference drugs DHA and artemisinin. Besides, artemisinin derivatives were found to be non-inhibitors of cytochrome P450 2D6, similar to reference drugs DHA and artemisinin, thus may not metabolized frequently. The CYP2D6 enzyme is one of the important enzyme involved in drug metabolism (Table-6).

Toxicity risk assessment screening

Toxicity risk assessment screening was performed for artemisinin derivatives. Predicted results showed that compounds **19**, **20** have rodent (mouse & rat both male & female)

carcinogenicity similar to DHA and artemisinin, while compound **21** is non-carcinogenic. Similarly, compounds **19**, **20**, **21** showed non-mutagenicity (Ames) similar to DHA & artemisinin. Compounds **19**, **20**, **21** showed mild skin irritancy similar to DHA and artemisinin. Except compound **21**, other two compounds **19**, **20** showed developmental/reproductive toxicity, similar to DHA and artemisinin. Likewise, compounds **19**, **20** showed non-degradable properties (aerobic), while compound **21** showed degradable property similar to DHA & artemisinin (Table-7).

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Figure Captions

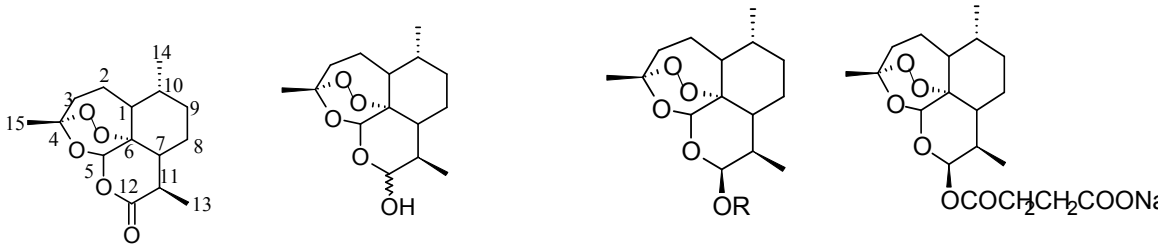
Figure 1. Artemisinin and its derivatives.

Figure 2. Extraction of artemisinin: Outline of extraction protocol (see reference 19for details).

Fig 3. Synthesis of ether derivatives

Fig 4. Synthesis of ester derivatives

Fig 5. Synthesis of derivatives **26-33**



Artemisinin (**1**) Dihydroartemisinin (**2**) R=Me Artemether (**3**) Artesunate (**5**)

R=Et Arteether (**4**)

Figure 1. Artemisinin and its derivatives.

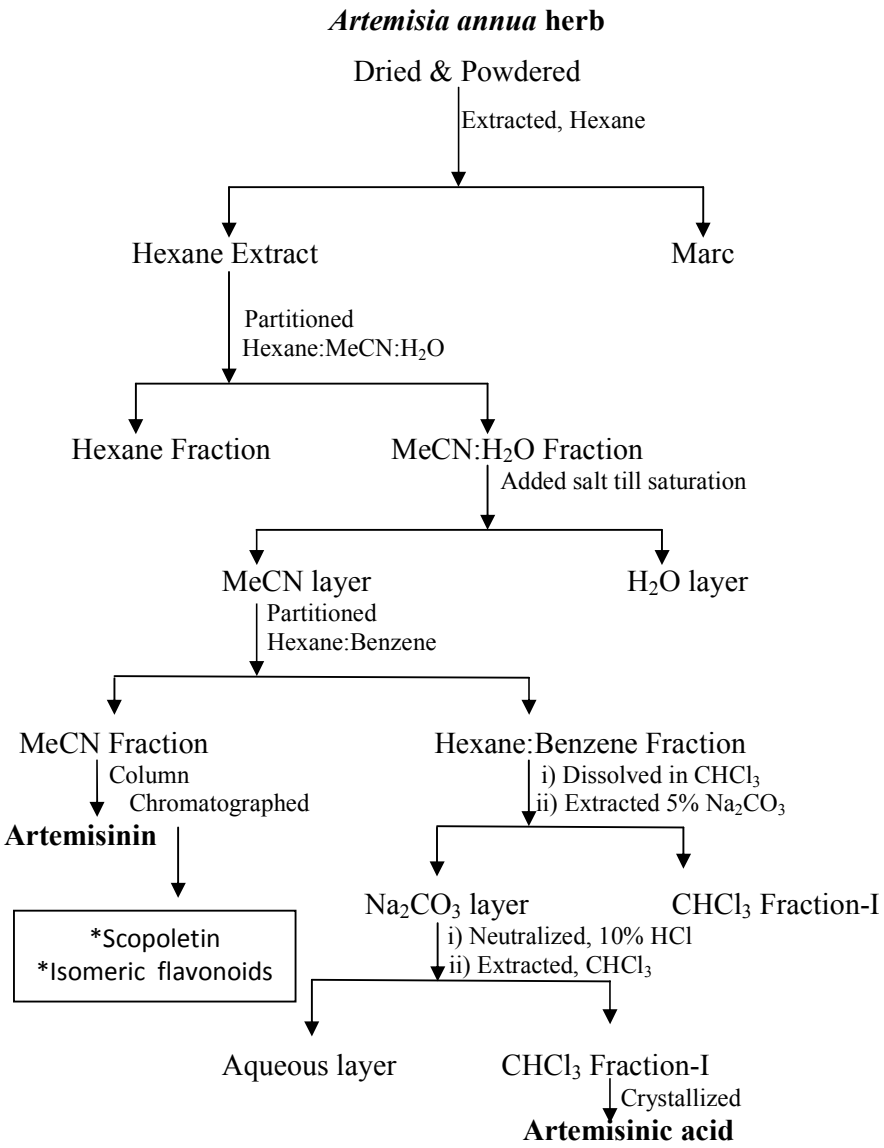


Fig. 2. Extraction of artemisinin: Outline of extraction protocol (see reference 19for details).

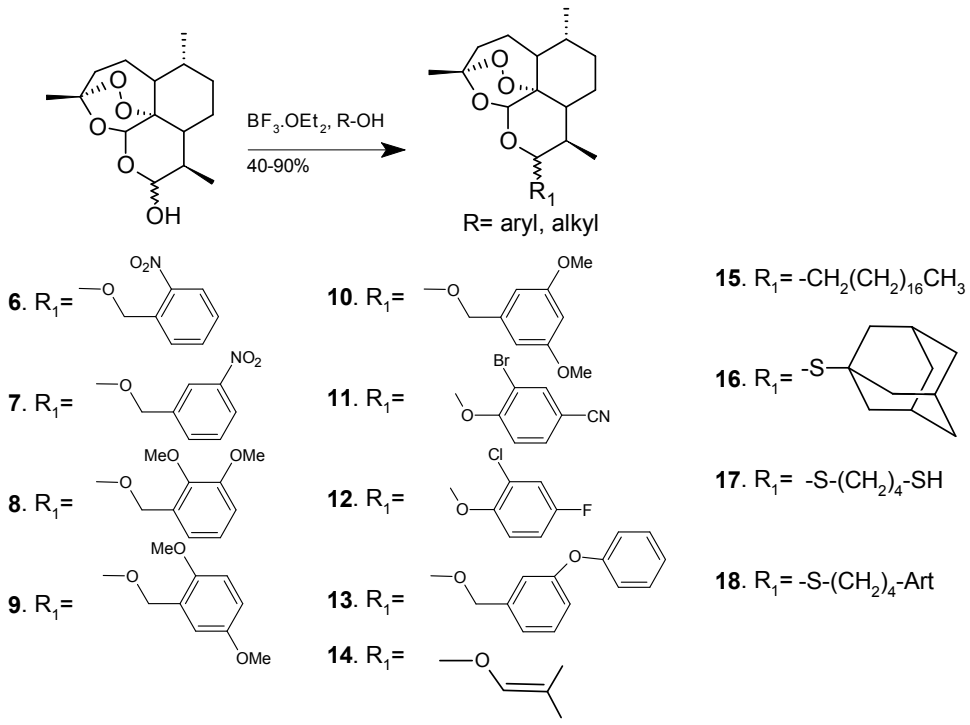


Fig 3. Synthesis of ether derivatives

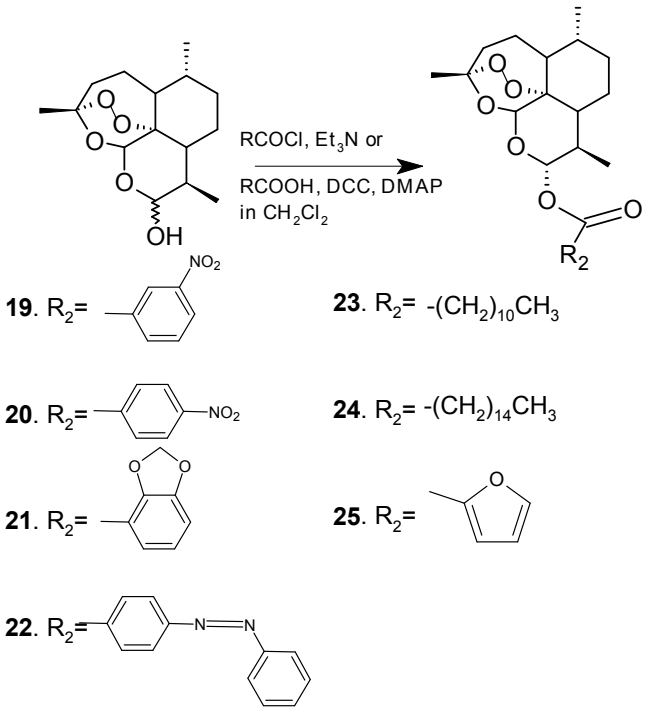


Fig 4. Synthesis of ester derivatives

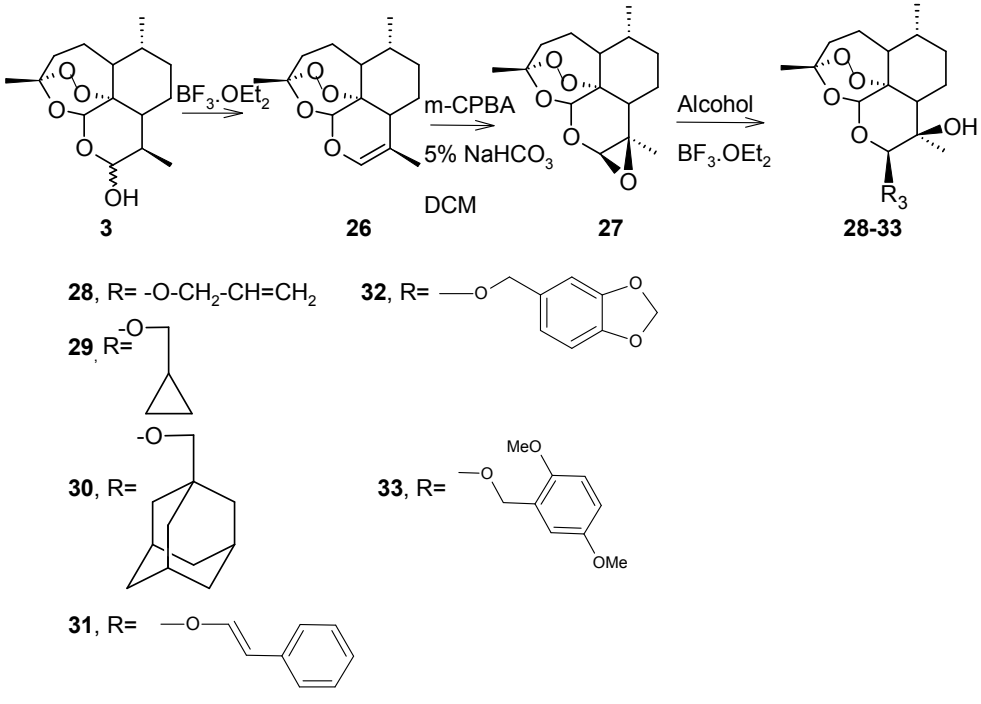
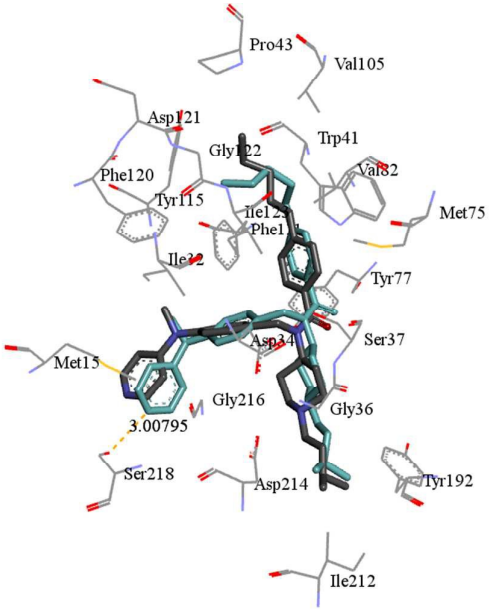
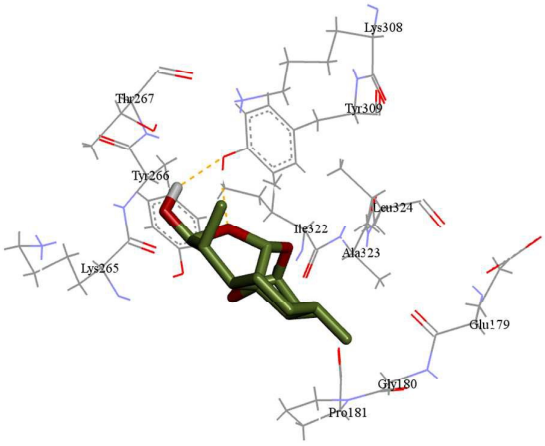


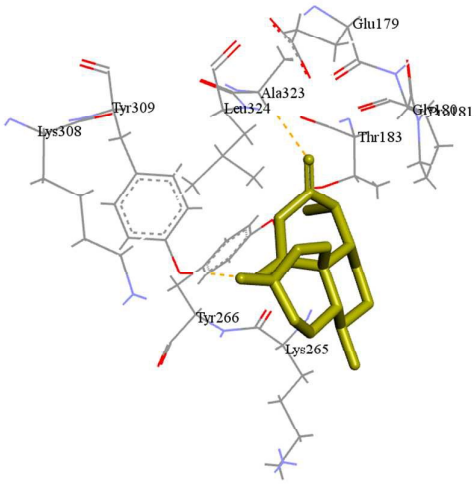
Fig 5. Synthesis of derivatives **26-33**



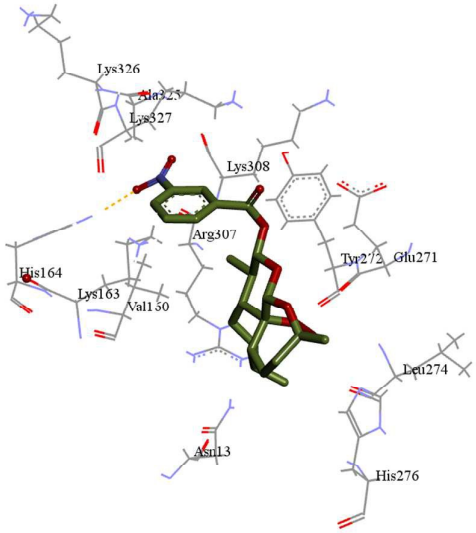
(a)



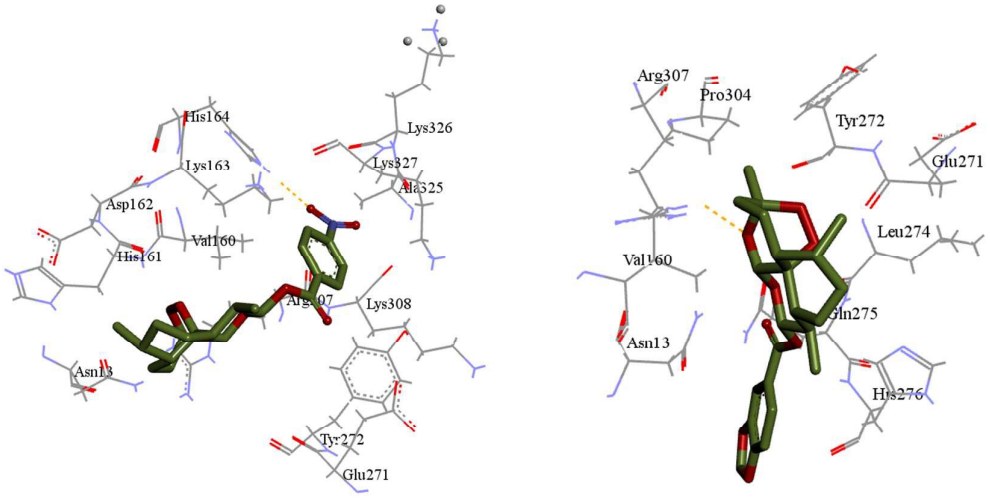
(b)



(c)



(d)



(e) **(f)**

Fig.6. Revealing binding site molecular interactions and involved amino acid residues of malaria parasite *P. falciparum* target hydrolase enzyme plasmepsin-II (PDB ID: 2IGY) through *in silico* docking experiments. (a) Re-docking of co-crystallized inhibitor A2T (N-[1-(3-METHYLBUTYL)PIPERIDIN-4-YL]-N-{4-[METHYL(PYRIDIN-4-YL)AMINO] BENZYL}-4-PENTYLBENZAMIDE) on *P. falciparum* enzyme plasmepsin-II (PDB: 2IGY) with binding energy -8.1 kcal/mol and H-bond of 3.00 Å with polar, uncharged residue serine (SER-218), and revealing similar structural superimposition of docked and co-crystallized plasmepsin-II inhibitor A2T conformations through AutoDock Vina v0.8 in PyRx virtual screening tool. (b) DHA showed a H-bond of 1.79 Å with polar uncharged residue tyrosine (TYR-309), having docking binding energy-6.9 kcal/mol, (c) Artemisinin showed two H-bonds of 2.09 Å and 2.16 Å with polar uncharged residue tyrosine (TYR-309) and non-polar hydrophobic residue leucine (LEU-324) respective, having docking binding energy -6.8 kcal/mol, (d) Compound **19** showed docking binding energy score -8.0 kcal/mol and revealed a H-bond of 2.19Å with polar basic residue histidine (HIS-164), (e) Compound **20** showed docking binding energy score -7.6 kcal/mol and revealed a H-bond of 1.99Å with polar basic residue histidine (HIS-164), similar to compound **19**, and (f) Compound **21** showed docking binding energy score -8.2 kcal/mol and revealed a H-bond of 2.37Å with polar basic residue arginine (ARG-307).

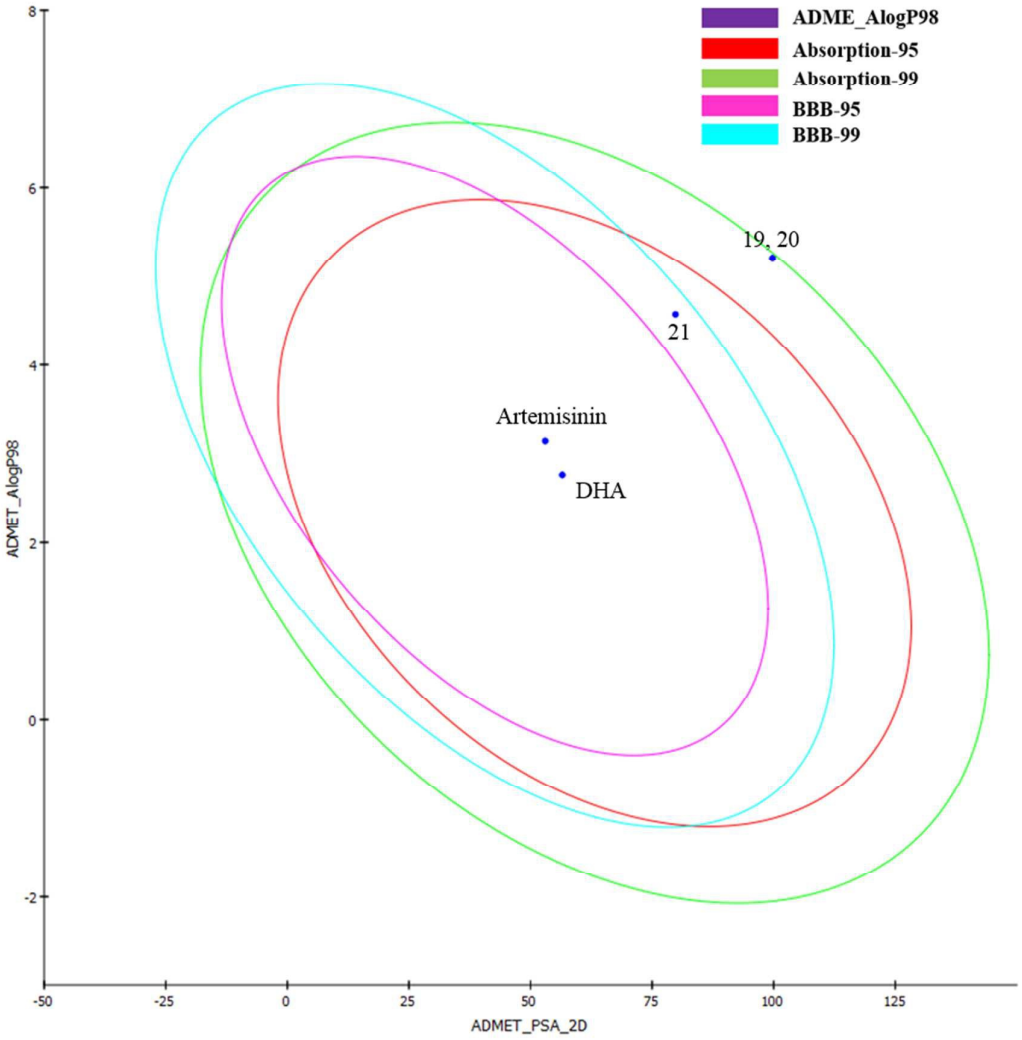
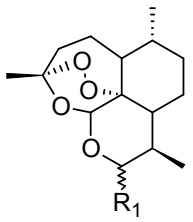
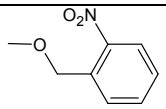
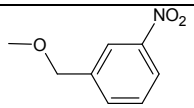
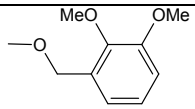
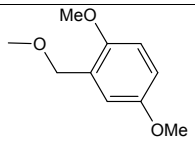
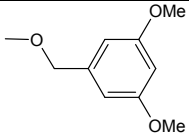
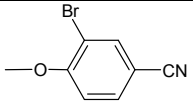
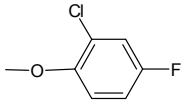
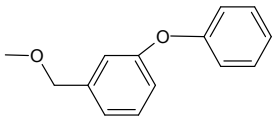


Fig. 7. Plot of PSA versus LogP for DHA, artemisinin & its derivatives (compounds **19**, **20**, **21**) showing the 95% and 99% confidence limit ellipses corresponding to the blood–brain barrier and intestinal absorption models. Abbreviations: ADMET (absorption, distribution, metabolism, excretion, and toxicity), A logP (logarithm of the partition coefficient between n-octanol and water), BBB (blood–brain barrier penetration) and PSA (polar surface area).

Table-1. *In vitro* antimalarial activity of ether derivatives of dihydroartemisinin against *P. falciparum*

Compound	 **R**=	IC_{50} (nM)
6		10.74
7		190.93
8		11.52
9		13.82
10		13.82
11		12.93
12		13.35
13		10.73

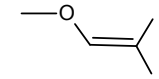
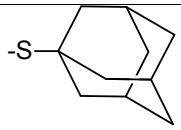
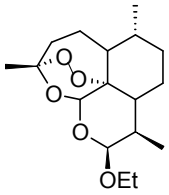
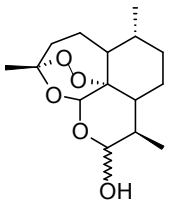
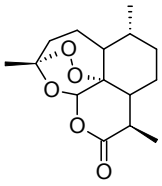
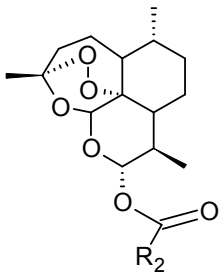
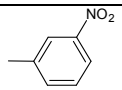
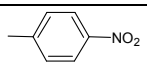
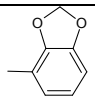
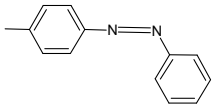
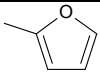
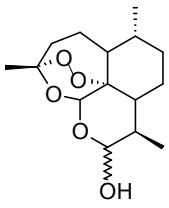
14		9.94
15	$-CH_2(CH_2)_{16}CH_3$	>190
16		9.21
17	$-S-(CH_2)_4-SH$	12.88
18	$-S-(CH_2)_4$-Art	11.46
Artether		12.82
DHA		14.4
Artemisinin		18.43

Table-2.*In vitro* antimalarial activity of ester derivatives of dihydroartemisinin against *P. falciparum*

Compound	 **R**=	IC_{50} (nM)
19		10.58
20		8.54
21		8.08
22		11.19
23	$-(CH_2)_{10}CH_3$	10.58
24	$-(CH_2)_{14}CH_3$	>10.58
25		10.58
DHA		14.4

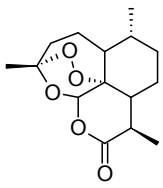
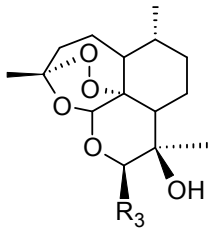
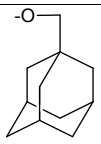
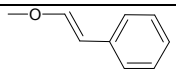
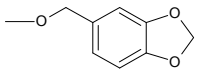
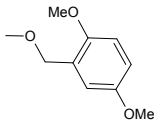
Artemisinin		18.43

Table-3. *In vitro* antimalarial activity of 11-OH-12-artether derivatives of dihydroartemisinin against *P. falciparum*

Compound	 **R_3=**	IC_{50} (nM)
28	-O-CH_2-CH=CH_2	174.84
29		158.19
30		20.56
31		105.76
32		109.50
33		125.0

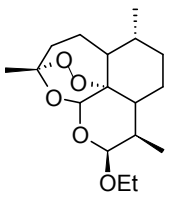
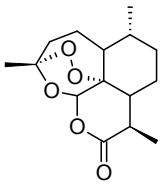
Artether		12.82
Artemisinin		18.43

Table-4. Details of docking results of active artemisinin derivatives **19**, **20**, and **21** against antimalarial target plasmepsin II of *P. falciparum*.

Compound	AutoDock-Vina Binding affinity energy/ docking score (Kcal/mol)	Amino acid residues of binding site pocket (with in 4 Ă region of bound compound)	H-bond forming amino acid residue & its position	Number of H- bond	Length of H-bond (Ă)
19	-8.0	LYS-326, ALA-325, LYS-308, ARG-307, TYR-272, GLU-271, LEU-274, HIS-276, ASN-13, VAL-160, LYS-163, HIS-164, LYS327	HIS-164	1	2.19
20	-7.6	ASN-13, HIS-161, LYS-163, VAL-160, GLU-271, LYS-327, HIS-164, ALA-325, LYS-308, TYR-272, ARG-307, ASP-162	HIS-164	1	1.99
21	-8.2	GLN-275, HIS-276, ASN-13, PRO-304, ARG-307, TYR-272, GLU-271, VAL-160, LEU-274	ARG-307	1	2.37
DHA (Control)	-6.9	LYS-265, LYS-308, THR-183, PRO-181, ALA-323, GLU-179, TYR-266, LEU-324, GLY-180, TYR-309	TYR-309	1	1.79
Artemisinin (Control)	-6.8	LYS-265, LYS-308, THR-183, PRO-181, ALA-323, GLU-179, TYR-266, LEU-324, GLY-180, TYR-309	TYR-309, LEU-324	2	2.09, 2.16

Table-5. Compliance of artemisinin derivatives with computational parameters of drug likeness (oral bioavailability) through Lipinski's rule of five.

Compound	Molecular weight (≤500)	LogP (≤5)	H-bond donors (≤5)	H-bond acceptors (≤10)	Rule of 5 violations (≤1)
19	434.46	3.455	1	8	0
20	434.46	3.455	1	8	0
21	432.46	3.432	0	8	0
DHA	284.348	1.621	1	5	0
Artemisinin	282.332	1.998	0	5	0

Table-6. Compliance of artemisinin derivatives with the standard range of computational pharmacokinetic parameters (ADME).

Compound	Aqueous solubility level	Blood-Brain Barrier (BBB) penetration level	Cytochrome (CYP-2D6) binding	Hepato-toxicity	Intestinal absorption level	Plasma-Protein Binding (PPB) prediction	AlogP98	PSA_2D
19	1 (No, very low, but possible)	4 (No, very low penetrant)	False (Non inhibitor)	False (Non toxic)	1 (Moderate)	False (Poorly bounded)	5.211	99.756
20	1 (No, very low, but possible)	4 (No, very low penetrant)	False (Non inhibitor)	False (Non toxic)	1 (Moderate)	False (Poorly bounded)	5.211	99.756
21	1 (No, very low, but possible)	2 (Medium penetrant)	False (Non inhibitor)	False (Non toxic)	0 (Good)	False (Poorly bounded)	4.573	79.811
DHA	2 (Yes, low)	2 (Medium penetrant)	False (Non inhibitor)	True (Toxic)	0 (Good)	False (Poorly bounded)	2.762	56.535
Artemisinin	2 (Yes, low)	2 (Medium penetrant)	False (Non inhibitor)	False (Non toxic)	0 (Good)	False (Poorly bounded)	3.139	53.021

Footnote: AlogP, the logarithm of the partition coefficient between n-octanol and water; PSA, polar surface area

Table-7. Compliance of the artemisinin derivatives with computational toxicity risk assessment parameters (DS TOPKAT, Accelrys, USA).

Compound	**19**	**20**	**21**	**DHA**	**Artemisinin**
Rat oral LD50 (g/kg body weight)	0.608732	0.372949	0.262126	0.816323	0.83021
Rat inhalational LC50 (mg/m3/h)	1.52426	0.806181	1.96141	13.8258	6.14271
Carcinogenic potency TD50 (mg/kg body weight/day)					
Mouse	5.48795	2.10316	1.61607	1.33707	3.16164
Rat	0.845194	0.33488	1.21525	0.61607	0.538544
Rat maximum tolerated dose (g/kg body weight)	0.0576745	0.0576745	0.0273091	0.0319298	0.0284095
Developmental toxicity potential	Toxic	Toxic	Non-Toxic	Toxic	Toxic
US FDArodent carcinogenicity					
Mouse female	Carcinogen	Carcinogen	Non-Carcinogen	Carcinogen	Carcinogen
Mouse male	Carcinogen	Carcinogen	Non-Carcinogen	Carcinogen	Carcinogen
Rat female	Carcinogen	Carcinogen	Non-Carcinogen	Carcinogen	Carcinogen
Rat male	Carcinogen	Carcinogen	Carcinogen	Carcinogen	Carcinogen
Ames mutagenicity	Non-Mutagen	Non-Mutagen	Non-Mutagen	Non-Mutagen	Non-Mutagen
Daphnia EC50 (mg/L)	1.19787	1.44362	0.53483	16.4186	3.85738
Skin sensitization	Strong	Strong	Strong	Weak	Weak
Rat chronic LOAEL(g/kg body weight)	0.0105119	0.0081405	0.022304	0.0166997	0.019262
Fathead minnow LC50 (g/L)	0.00324	0.00324	0.001444	0.250221	0.019018
Aerobic biodegradability	Non-Degradable	Non-Degradable	Degradable	Degradable	Degradable
Ocular irritancy	Mild	Mild	Mild	Severe	Moderate
Skin irritancy	Mild	Mild	Mild	Mild	Mild

Footnote: EC50, effective concentration 50%; US FDA, United States Food and Drug Administration; LC50, lethal concentration 50%; LD50, lethal dose 50%; LOAEL, lowest observed adverse effect level; TD50, tumorigenic dose 50%.