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Novel dihydroartemisinin derivatives exhibiting anticancer and antimalarial activities

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Abstract. A two-step procedure was used for the synthesis of six novel derivatives of artemisinin, a main active sesquiterpene lactone, which could be easily isolated on a large scale from the traditional medicinal plant *Artemisia annua* L in Vietnam. Structures of the prepared derivatives were characterized by full-length data of spectra, including ^{1}H-, ^{13}C-NMR and HRMS. Screening for their *in vitro* cytotoxic activity was performed together with dihydroartemisinin (DHA) against three human cancer cell lines: HepG2, A549, and HeLa. Additionally, these derivatives were evaluated for antimalarial activity against *P. plasmodium* using the chloroquine-resistant strain (K1) and the chloroquine-sensitive strain (T96). The results showed that the prepared derivatives exhibited the cytotoxic activity against HepG2, LU-1, and HeLa cells with IC_{50} values ranging from 1.32-18.38 µg/mL, which were stronger than that of DHA. Moreover, the anti-malarial results were promising for the development of new anti-malarial drugs.

Keywords: artemisinin, dihydroartemisinin, antimalarial, cytotoxicity.

Classification numbers: 1.1.2,1.2.4.

1. INTRODUCTION

Dihydroartemisinin **1** (DHA) is a potent antimalarial drug derived from artemisinin **2**, a sesquiterpene lactone abundantly found in whole plant *Artemisia annua* L. DHA and its parent compound artemisinin possesses an endoperoxide pharmacophore that is responsible for expressing both antimalarial and anticancer activities [1]. DHA and several of its derivatives are approved as drugs for the treatment of malarial disease such as arteether **3**, and artesunate **4**

(Figure 1) [2]. These drugs play an important role in the global campaigns against malaria, one of the most popular infectious diseases affecting millions of people each year. DHA is known for its rapid and effective action against the malaria parasite, *Plasmodium*. However, the effectiveness of the above drugs is gradually reduced due to drug resistance, which encourages seeking new drugs to overcome this challenge. In medicinal chemistry, DHA and its analogs are less toxic and safe in use, and therefore DHA has become an attractive template in the design of new derivatives for drug development.

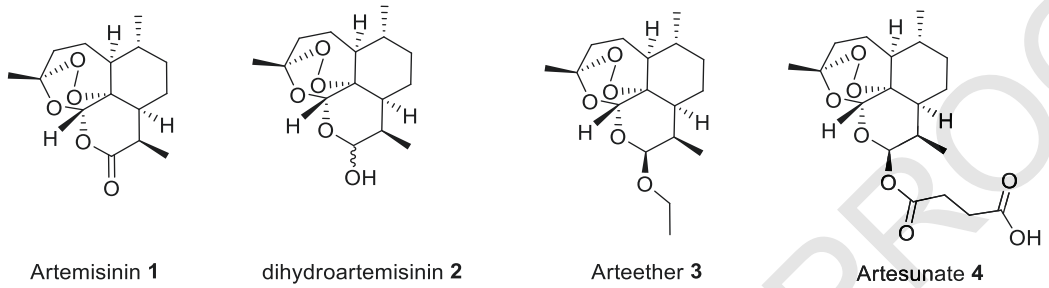

Figure 1. The chemical structures of Artemisinin **1**, Dihydroartemisinin **2**, Arteether **3** and Artesunate **4**.

In addition to its antimalarial properties, DHA has demonstrated potent anticancer activity against various human cancer cell lines, for example, HepG2, A549, HT-29, H460, HL-60, LLC… [3-5]. This anticancer potential arises from the generation of free alkyl radicals which are a result of breaking the endoperoxide. The free alkyl radicals attack and destroy cancer cells, as described by Wang and colleagues [3]. Therefore, the design of new DHA derivatives has focused on retaining the endoperoxide bridge in the end products. Until the current time, numerous DHA derivatives with potent antimalarial and anticancer activities have been successfully synthesized and documented [6-9].

In medicinal chemistry, nucleobases were used for the design of antiviral and anticancer drugs [10, 11], 6-methoxy-7-(3-morpholinopropoxy)quinazolin-4(*3H*)-one and acridone were important precursors for the synthesis of gefitinib [12] and acridone acetic [13], respectively. Our previous study introduced a motif of DHA derivatives containing both thioether and ether linkages with potent anti-inflammatory and anticancer activities [14]. As a continuous result of this strategy, in this contribution, the ethers of DHA with various amines have been designed, synthesized, and screened for both antimalarial and anticancer activities.

2. MATERIAL AND METHODS

2.1. Materials

Dihydroartemisinin **2** is a commercially available material in Vietnam, other chemicals: acridone **5a**, uracil **5d**, thymine **5e**, and 5-fluorouracil **5f** were purchased from Merck (Germany). 4-(phenylimino)thiazolidine-4-one **5b** was synthesized from aniline. 6-Methoxy-7-(3-morpholinopropoxy)quinazolin-4(*3H*)-one **5c** was synthesized from isovaniline. 2-(10*β*-Dihydroartemisinoxy)-ethyl bromide **6** was prepared from DHA and 2-bromoethanol. ^{1}H-NMR and ^{13}C-NMR spectra were recorded at ambient temperature on a Bruker Avance 500 MHz spectrometer (Bruker Biospin, Germany) in DMSO-d_6. Chemical shifts δ are quoted in parts per million (ppm) referenced to the residual solvent peak, (DMSO at 2.50, 3.32 ppm, and 39.5 ppm) relative to TMS. Mass spectra were recorded by using an Agilent LC/MSD Trap SL. Thin-layer chromatography was performed on the precoated Silica Gel 60 F_{254} aluminum sheets (Merck,

Darmstadt, Germany), and products were visualized under a UV lamp at 254 nm. Column chromatography was carried out on Silica Gel (40-230 mesh).

2.2. Methods

2.2.1. General procedure for the synthesis of 7a-f

In a round bottom flask (100 mL) containing DMF solvent (5 mL) placed on a magnetic stirrer, each amine including acridone **5a,** (phenylimino)thiazolidine-4-one **5b,** quinazolinon-4-one **5c**, uracil **5d**, thymine **5e,** or 5-fluorouracil **5f** (1.0 mmol, 1.0 equiv.) was added, followed by the addition of potassium carbonate K_2CO_3 (138 mg, 1.0 mmol, 1.0 equiv.) in one portion. Subsequently, intermediate **6** (391 mg, 1mmol, 1 equiv.), and DIPEA (0.1 mmol, 0.1 equiv.) were added to the mixture, respectively. The resulting mixture was heated to 70-80 °C and stirred overnight. The reaction progress was controlled by TLC (*n*-hexane: acetone 2.5:1, v/v) until the absence of starting compound **5a-f**. The reaction mass was poured into ice water (20 mL) and acidified to pH 6. The precipitate was filtered on a Buchner funnel and dried in a vacuum desiccator. Crudes **7a-f** were purified by column chromatography using *n*-hexane: acetone 2.5:1 (v/v) as an eluent to give pure **7a-f.**

10-(2-(((3R,5aS,6R,8aS,9R,10S,12R,12aR)-3,6,9-trimethyldecahydro-12H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)oxy)ethyl)acridin-9(10H)-one (7a). Yield 45.8 %, white powder, m.p. 208-210 °C; R_f = 0.40 (*n*-hexane: acetone 2.5:1). ^{1}H-NMR (500 MHz, DMSO-d_6, δ (ppm)): 8.34 (dd, J_1 = 1.5 Hz, J_2 = 8.0 Hz, 2H, H-1', H-8'), 7.94 (d, J = 8.5 Hz, 2H, H-4', H-5'), 7.78 (td, J_1 = 1.5 Hz, J_2 = 8.0 Hz, 2H, H-2', H-7', 7.31 (t, J = 7.5 Hz, 2H, H-3', H-6'), 4.91 (m, 1H, 10-*O*-CH_{2a}-), 4.83 (m, 1H, 10-*O*-CH_{2b}-), 4.77 (s, 1H, H-12), 4.57 (d, J = 3.5 Hz, 1H, H-10), 4.26 (m, 1H, 10-*N*-CH_{2a}-), 3.74 (m, 1H, 10-*N*-CH_{2b}-), 2.19 (m, 1H, H-9), 2.05 (td, J_1 = 4.0 Hz, J_2 = 14.0 Hz ,1H, H-4a), 1.87 (m, 1H, H-4b), 1.63 (m, 1H, H-5a), 1.19 (s, 3H, H-15), 1.13 (m, 1H, H-8a), 1.07 (m, 2H, H-8b), 1.07 (m, 1H, H-7a), 0.93 (m, 3H, H-5b, H-8A, H-6), 0.75 (d, J = 6.0 Hz, 3H, H-13), 0.67 (m, 1H, H-5A), 0.57 (m, 1H, H-7b), 0.45 (d, J = 7.5 Hz, 3H, H-13). ^{13}C-NMR (125 MHz, DMSO-d_6, δ (ppm)): 176.5 (C-9'), 142.0 (C-11', C-14'), 133.7 (C-3', C-6'), 126.5 (C-1', C-8'), 121.6 (C-12', C-13'), 121.2 (C-2', C-7'), 116.7 (C-4', C-5'), 103.2 (C-3), 100.7 (C-10), 86.7 (C-12), 80.2 (C-12A), 64.5 (10-*O*-CH_2-), 51.7 (C-5A), 43.9 (C-8A), 43.4 (10-*N*-CH_2-), 36.4 (C-6), 35.8 (C-4), 33.9 (C-7), 30.8 (C-9), 25.5 (C-15), 24.1 (C-5), 23.5 (C-8), 19.9 (C-14), 12.2 (C-13). ESI-HRMS calculated for $C_{30}H_{36}NO_6$: $[M+H]^+$ *m/z*: 506.25422, found: 506.25372

(Z)-2-(phenylimino)-3-(2-(((3R,5aS,6R,8aS,9R,10S,12R,12aR)-3,6,9-trimethyldecahydro-12H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)oxy)ethyl)thiazolidin-4-one (7b). Yield 45.5 %, pale yellow powder, m.p. 93-94 °C; R_f = 0.44 (*n*-hexane: acetone 2.5:1). ^{1}H-NMR (500 MHz, DMSO-d_6, δ (ppm)): 7.52 (m, 5H, H-1", H-2", H-6", H-3", H-5"), 5.30 (s, 1H, H-12), 4.67 (d, J = 3.5 Hz, 1H, H-10), 4.24 (m, 2H, 10-*O*-CH_2-), 3.92 (d, J = 12.5 Hz, 1H, H-5'a), 3.84 (d, J = 12.5 Hz, 1H, H-5'b), 3.73 (m, 1H, N3' -CH_{2a}-), 3.64 (m, 1H, 1H, N3' -CH_{2b}-), 2.39 (m, 1H, H-9), 2.17 (m, 1H, H-4a), 1.98 (m, 1H, H-4b), 1.79 (m, 1H, H-5a), 1.55 (m, 1H, H-8a), 1.46 (m, 2H, H-8b, H-7a), 1.34 (m, 2H, H-5b, H-8A), 1.28 (s, 3H, H-15), 1.22 (m, 1H, H-5A), 1.11 (m, 1H, H-6), 0.87 (d, J = 6.0 Hz, 3H, H-14), 0.86 d, J = 7.0 Hz, 3H, H-13). ^{13}C-NMR (125 MHz, DMSO-d_6, δ (ppm)): 186.7 (C-4'), 183.6 (C-2'), 140.1 (C-1"), 129.8 (C-3", C-5"), 129.4 (C-4"), 128.0 (C-2", C-6"), 103.3 (C-3), 100.8 (C-10), 87.0 (C-12), 80.4 (C-12A), 64.2 (10-*O*-CH_2-), 51.9 (C-5A), 43.6 (C-8A), 40.4 (N3'-CH_2-), 36.5 (C-6), 36.0 (C-4), 34.1 (C-7), 30.3 (C-9), 25.6 (C-15), 24.2 (C-5), 23.7 (C-8), 20.0 (C-14), 12.6 (C-13). ESI-HRMS calculated for $C_{27}H_{36}NO_6S$: $[M+H]^+$ *m/z*: 503.22160, found: 503.21875.

6-methoxy-7-(3-morpholinopropoxy)-3-(2-(((3R,5aS,6R,8aS,9R,10S,12R,12aR)-3,6,9-trimethyldecahydro-12H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)oxy)ethyl)quinazolin-4(3H)-one (7c). Yield 51.2 %, white powder, m.p. 102-104 °C; R_f = 0.45 (*n*-hexane: acetone 2.5:1). ^{1}H-NMR (500 MHz, DMSO-d_6, δ (ppm)): 8.19 (s, 1H, H-2'), 7.46 (s, 1H, H-5'), 7.13 (s, 1H, H-8'), 4.83 (s, 1H, H-12), 4.67 (d, *J* = 3.0 Hz, 1H, H-10), 4.27 (m, 1H, 7'-*O*-CH_{2a}-), 4.13 (m, 4H, 7'-*O*-CH_{2b}-, 10-*O*-CH_2-, N3'-CH_{2a}-), 3.90 (s, 3H, 6'-OCH_3), 3.57 (t, *J* = 4.5 Hz, 4H, H-3", H-5"), 3.55 (m, 1H, N3'-CH_{2b}-), 2.44 (t, *J* = 7.0 Hz, 2H, $N_{1"}$-CH_2-) 2.37 (m, 4H, H-2", H-6"), 2.30 (m, 1H, H-9), 2.09 (m, 1H, H-4a), 1.92 (m, 1H, H-4b), 1.65 (m, 1H, H-5a), 1.42 (m, 1H, H-8a), 1.26 (s, 3H, H-15), 1.20 (m, 2H, H-8b, H-7a), 1.11 (m, 1H, H-8A), 1.05 (m, 1H, H-5b), 0.95 (m, 1H, H-5A), , 0.72 (d, *J* = 7.5 Hz, 3H, H-14), 0.64 (m, 1H, H-6), 0.63 d, *J* = 6.5 Hz, 3H, H-13). ^{13}C-NMR (125 MHz, DMSO-d_6, δ (ppm)): 159.5 (C-4'), 154.7 (C-7'), 148.0 (C-6'), 146.8 (C-2'), 144.1 (C-9'), 114.5 (C-10'), 107.9 (C-8'), 105.9 (C-5'), 103.2 (C-3), 100.0 (C-10), 86.6 (C-12), 80.2 (C-12A), 66.8 (7'-*O*-CH_2-), 66.2 (C-3", C-5"), 63.2 (10-CH_2-), 56.0 (6'-OCH_3), 54.7 ($N_{1"}$-CH_2-), 53.3 (C-2", C-6"), 51.7 ($N_{3'}$-CH_2-), 43.6 (C-8A), 36.3 (C-6), 35.9 (C-4), 33.9 (C-7), 30.2 (C-9), 25.6 (C-15), 25.5 (7'-CH_2-CH_2-), 24.1 (C-5), 23.7 (C-8), 19.8 (C-14), 12.5 (C-13). ESI-HRMS calculated for $C_{33}H_{48}N_3O_9$: $[M+H]^+$ *m/z*: 630.33907, found: 630.33909.

1,3-bis(2-(((3R,5aS,6R,8aS,9R,10S,12R,12aR)-3,6,9-trimethyldecahydro-12H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)oxy)ethyl)pyrimidine-2,4(1H,3H)-dione (7d). Yield 48.1 %, white powder, m.p. 104-106 °C; R_f = 0.42 (*n*-hexane: acetone 2.5:1). ^{1}H-NMR (500 MHz, DMSO-d_6, δ (ppm)): 7.65 (d, *J* = 7.75 Hz, 1H, H-6'), 5.58 (d, *J* = 7.75 Hz, 1H, H-5'), 5.20 and 515 (s, 2H, H-12 and H12"), 4.67 and 4.54 (d, *J* = 3.0 Hz,, 2H, H-10 and H-10"), 4.08 (m, 1H, 10"-CH_{2a}-), 3.98 (m, 3H, 10"-CH_{2b}-, 10-CH_{2a}-, 10-CH_{2b}-), 3.86 (m, 2H, N3'-CH_2-), 3.52 (m, 2H, N1'-CH_2-), 2.36 (m, 2H, H-9, H-9"), 2.16 (m, 2H, H-4a, H-4"a), 1.99 (m, 2H, H-4b, H-4"b), 1.79 (m, 2H, H-5a, H-5'a), 1.58 (m, 4H, H-8, H-8"), 1.51(m, 2H, H-7a, H-7"a), 1.35 (m, 2H, H-8a, H-8"a), 1.27 (s, 6H, H-15, H-15"), 1.14 (m, 2H, H-5A, H-5"A), 0.86 (m, 6H, H-14, H-14"), 0.84 (m, 2H, H-7b, H-7"b), 0.78 (m, 6H, H-13, H-13"). ^{13}C-NMR (125 MHz, DMSO-d_6, δ (ppm)): 162.3 (C-4'), 151.0 (C-2'), 144.6 (C-6'), 103.30 and 103.25 (C-12 and C-12"), 100.7 and 100.6 (C-10 and C-10"), 99.6 (C-5'), 86.9 and 86.8 (C-12 and C-12"), 80.4 and 80.3 (C-12A and C-12"A), 64.3 and 63.6 (10-*O*-CH_2- and 10"-*O*-CH_2-), 52.0 and 51.9 (N3'-CH_2- and N1'-CH_2-), 43.7 and 43.6 (C-8A and C-8"A), 36.7 (C-6, C-6"), 36.0 and 35.9 (C-4 and C-4"), 34.1 and 34.0 (C-7 and C-7"), 30.31 and 30.28 (C-9 and C-9"), 25.6 (C-15 and C-15"), 24.2 (C-5 and C-5"), 23.9 and 23.7 (C-8 and C-8"), 20.1 and 20.0 (C-14 and C-14"), 12.6 and 12.5 (C-13 and C-13"). ESI-HRMS calculated for $C_{38}H_{57}N_2O_{12}$: $[M+H]^+$ *m/z*: 733.39060, found: 733.39119.

5-methyl-1,3-bis(2-(((3R,5aS,6R,8aS,9R,10S,12R,12aR)-3,6,9-trimethyldecahydro-12H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)oxy)ethyl)pyrimidine-2,4(1H,3H)-dione (7e). Yield 47.2 %, colorless oil, R_f = 0.42 (*n*-hexane: acetone 2.5:1). ^{1}H-NMR (500 MHz, DMSO-d_6, δ (ppm)): 7.56 (s. 1H, H-6), 5.17 and 5.11 (s, 2H, H-12 and H-12"), 4.67 and 4.64 (d and d , *J* = 3.5 Hz and 3.0 Hz, 2H, H-10 and H-10"), 4.13 and 3.97 (10-*O*-CH_2- and 10"-*O*-CH_2-), 3.85 (m, 2H, N1'-CH_{2a}-, N3'-CH_{2a}-), 3.53 (m, 2H, N1'-CH_{2b}-, N3'-CH_{2b}-), 2.35 (m, 2H, H-9, H-9"), 2.15 (m, 2H, H-4a, H-4"a), 1.98 (m, 2H, H-4b, H-4"b), 1.79 (H-5a, H-5"a), 1.77 (s, 3H, 5-CH_3), 1.55 (m, 4H, H-8, H-8"), 1.48 (m, 2H, H-7a, H-7"a), 1.32 (m, 4H, H-5a, H-5"a, H-8A, H8"A), 1.27 (s, 6H, H-15, H-15"), 1.20 (m, 2H, H-6, H-6"), 1.12 (m, 2H, H-5A, H-5"A), 0.88 (m, 6H, H-14, H-14"), 0.84 (m, 2H, H-7b, H-7"b), 0.77 (m, 6H, H-13, H-13"). ^{13}C-NMR (125 MHz, DMSO-d_6, δ (ppm)): 163.0 (C-4'), 150.8 (C-2'), 140.7 (C-6'), 107.1 (C-5'), 103.29 and 103.24 (C-12 and C-12"), 100.7 and 100.6 (C-10 and C-10"), 86.9 and 86.8 (C-12 and C-12"), 80.34 and 80.29 (C-12A and C-12"A), 64.4 and 63.6 (10-*O*-CH_2- and 10"-*O*-CH_2-), 51.9 and 51.8 (N3'-CH_2- and

N1'-CH_2-), 43.64 and 43.58 (C-8A and C-8"A), 36.7 (C-6, C-6"), 36.0 and 35.9 (C-4 and C-4"), 34.13 and 34.07 (C-7 and C-7"), 30.3 (C-9, C-9"), 25.55 and 25.54 (C-15 and C-15"), 24.26 and 24.22 (C-5 and C-5"), 23.9 and 23.6 (C-8 and C-8"), 20.0 (C-14, C-14"), 12.6 and 12.51 and 12.49 (C-13 and C-13"), 12.4 (5'-CH_3). ESI-HRMS calculated for $C_{39}H_{59}N_2O_{12}$: $[M+H]^+$ *m/z*: 747.40625, found: 747.40688.

5-fluoro-1,3-bis(2-(((3R,5aS,6R,8aS,9R,10S,12R,12aR)-3,6,9-trimethyldecahydro-12H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)oxy)ethyl)pyrimidine-2,4(1H,3H)-dione (7f). Yield 47.7 %, white powder, m.p. 175-177 °C; R_f = 0.43 (*n*-hexane: acetone 2.5:1). ^{1}H-NMR (500 MHz, DMSO-d_6, δ (ppm)): 8.15 (d, *J* = 6.5 Hz, 1H, H-6'), 5.19 and 5.15 (s, 2H, H-12 and H-12''), 4.68 and 4.65 (d and d, 2H, *J* = 3.0 Hz and 3.5 Hz, H-10 and H-10''), 4.12 and 3.97 (m, 4H, 10-*O*-CH_2- and 10''-*O*-CH_2-), 3.87 (m, , 2H, N1'-CH_{2a}-, N3'-CH_{2a}-), 3.55 (m, 2H, m, 2H, N1'-CH_{2b}-, N3'-CH_{2b}-), 2.36 (m, 2H, H-9, H-9''), 2.17 (m, 2H, H-4a, H-4''a), 1.99 (m, 2H, H-4b, H-4''b), 1.79 (H-5a, H-5''a), 1.58 (m, 4H, H-8, H-8''), 1.50 (m, 2H, H-7a, H-7''a), 1.32 (m, 4H, H-6, H-6'', H-8A, H8''A), 1.29 (m, 2H, H-5b, H-5''b), 1.27 (s, 6H, H-15, H-15''), 1.13 (m, 2H, H-5A, H-5''A), 0.89 (d, *J* = 4.5 Hz, 6H, H-14, H-14''), 0.86 (m, 2H, H-7b, H-7''b), 0.77 (m, 6H, H-13, H-13''). ^{13}C-NMR (125 MHz, DMSO-d_6, δ (ppm)): 156.6 (d, *J* = 25.0 Hz, C-4'), 149.4 (C-2'), 138.7 (d, *J* = 226.25 Hz, C-5'), 129.5 (d, *J* = 33.75 Hz, C-6'), 103.32 and 103.28 (C-12 and C-12''), 100.8 and 100.7 (C-10 and C-10''), 86.88 and 86.85 (C-12 and C-12''), 80.32 and 80.28 (C-12A and C-12''A), 64.3 and 63.5 (10-*O*-CH_2- and 10''-*O*-CH_2-), 51.9 and 51.8 (N3'-CH_2- and N1'-CH_2-), 43.6 and 43.5 (C-8A and C-8''A), 36.71 and 36.68 (C-6, and C-6''), 36.0 and 35.9 (C-4 and C-4''), 34.1 and 34.0 (C-7 and C-7''), 30.6 and 30.3 (C-9 and C-9''), 25.5 (C-15 and C-15''), 24.2 (C-5 and C-5''), 23.9 and 23.7 (C-8 and C-8''), 20.1 and 20.0 (C-14 and C-14''), 12.52 and 12.47 (C-13 and C-13''). ESI-HRMS calculated for $C_{38}H_{58}N_2O_{12}F$: $[M+H]^+$ *m/z*: 751.38118, found: 751.38178.

2.2.2. Evaluation biology

Antimalarial activity

The antimalarial activity of the above conjugates was tested against chloroquine-sensitive (T96) and chloroquine-resistant *P. falciparum* monoclonal (K1) on the 96-well microplates at The Vietnam National Institute of Malariology Parasitology and Entomology. Briefly, The antimalarial assay includes the following steps: dissolving the test samples in DMSO and shaking them until completely miscible; diluting sample solutions by culture medium supplemented with 15 % human type O serum for targeted concentrations; then pipetting 100 µL culture medium (15 % human-type O serum supplemented) containing test samples at serially diluted concentrations into wells of microtiter plates, each replicated twice. Control wells were prepared by pipetting 100 µL culture medium (15 % human type O serum). Next, 10 µL of *P. falciparum*-infected red blood cells (0.25 % in hematocrit 40 %) was pipetted into each well of microtiter plates, mildly shook the plates for equal dispersal of the parasites and transferred the microtiter plates into an anaerobic jar (37 °C, 48 h). After 48 h of incubation, the supernatant of the culture medium for collection of red blood cells was discarded in each well. An aliquot of the harvested red blood cells was used to prepare blood film on a slide, followed by Giemsa staining (3 % Giemsa solution in 45 min). After the enumeration of parasitemia (parasites in 10,000 RBCs), the IC_{50} and MIC values were determined using the WERSDORFE and WWARN softwares.

Cytotoxicity

The *in vitro* cytotoxic evaluation of the six prepared conjugates along with DHA against Hep-G2, HeLa, and LU-1 cancer cell lines was carried out according to the described methods [15, 16].

3. RESULT AND DISCUSSION

3.1. Results synthesis of 7a-f

The preparation of novel DHA conjugates **7a-f** was initiated from heterocyclic amines, such as acridone **5a**, (*Z*)-2-(phenylimino)thiazolidine-4-one **5b**, 6-methoxy-7-(3-morpholinopropoxy)quinazolin-4(*3H*)-one **5c**, and nucleobases comprised uracil **5d**, thymine **5e**, and 5-fluorouracil **5f**. Among these amines, **5b** was obtained from aniline in a 70 % yield using the published procedure of Roszczenko and colleagues [17], while **5c** was synthesized from isovaniline according to a known protocol of Ramanadham and co-workers [12]. Additionally, 2-(10*β*-dihydroartemisinoxy)-ethyl bromide **6** was also prepared by the etherification of DHA with 2-bromoethanol in a 46 % yield using a recipe as described by Li *et al.* [18]. The synthetic pathway of the conjugates **7a-f** was outlined in Scheme 1.

In this step, amines **5a-f** were coupled with the intermediate **6** in DMF in the presence of K_2CO_3 and Hünig's base (DIPEA) catalysts, resulting in the formation of products **7a-f** in 45.5-51.8 % yields. Indeed, DIPEA catalyst was used to improve the rate and efficiency of reactions due to the weak nucleophilic property of heterocyclic amines and nucleobases.

In the initial design, the conjugates of the above amines with one DHA unit were expected results to ensure that their molecular weights were below 500 Da according to Lipinski's rule. However, in the cases of nucleobases, the target products containing two DHA units were major products at any molar ratio of nucleobase: intermediate **6**. After completion of the reaction, the resulting mass was poured into ice water during vigorous stirring. The formed precipitate was filtrated off, washed thoroughly with water, and dried in a desiccator. Crudes **7a-f** were purified by column chromatography/silica gel eluted with *n*-hexane: acetone.

The structures of **7a-f** were elucidated by spectroscopic methods including ^{1}H-NMR, ^{13}C-NMR, HSQC, HMBC, and HRMS. The data of NMR and HRMS spectra were in excellent agreement with the proposed structures. The case of **7d** was a typical example of its structural interpretation. The structure of **7d** consisted of two structurally identical DHA units attached to the N1' and N3' of uracil. However, the basicity of N1 and N3, and the DHA spatiality were not large enough to cause a clear difference in the chemical shifts of identical proton and carbon pairs of these DHA moieties. As a result, the resonance of the same protons and carbons in the two DHA parts of **7d** gave pairwise identical signals in terms of multiplicity, constants *J*, and chemical shifts observed in both ^{1}H-NMR and ^{13}C-NMR spectra. For example, the resonance of the H-10 and -10" arises a pair of the proton signals observed at 4.77 and 4.75 ppm, while the signals of H-3, H-3" appeared as a pair at 5.20 and 5.15 ppm, with a minimal chemical shift difference of only 0.05 ppm. The signal of other proton pairs overlapped in its ^{1}H-NMR spectrum.

The ^{13}C-NMR of **7d** also showed similar results, the resonance of identical carbon pairs also arose the pairs of signals which were found clearly in its spectrum. The position of the signal pairs was so close together that the difference in chemical shift is only 0.5 ppm, for example, the signal pair at 103.4 and 103.5 ppm belonged to C-3, C-3", two signals at 100.6,

and 100.7 ppm attributed to C-10 and C-10", another pair of signals at 80.35 and 70.27 ppm was assigned to C-12A and C-12"A, the difference between signal of N1-CH_2- and N3-CH_2- was found to be 0.08 ppm. Moreover, the resonance of some pairs only gave a single signal, such as C-6 and C-6" gave only one signal at 36.7 ppm, or the signal of C-15 and C-15" was found at 35.6 ppm.

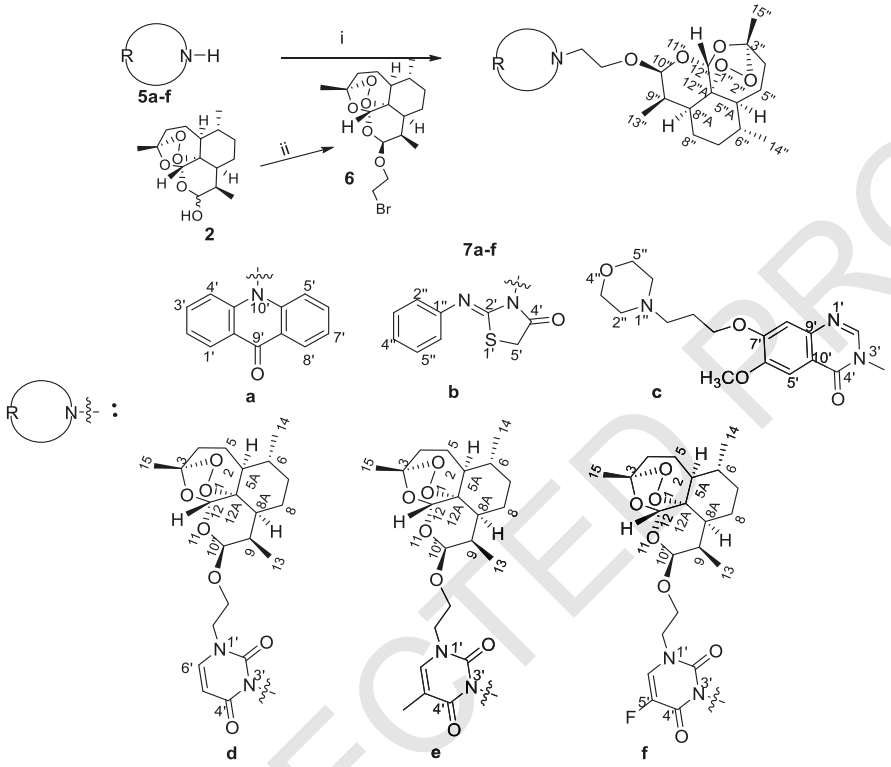

Figure 2. The synthesis of conjugates **7a-f**
Conditions: i) K_2CO_3, DIPEA, DMF, 70-80 ^{0}C, overnight; ii) bromoethanol, BF_3-OEt_2, CH_2Cl_2, -5 ^{0}C, 5 h.

As discussed above, the accurate assignment for every proton or carbon of two DHA moieties was not easy, even with the valuable aid of two-dimensional spectra such as HSQC and HMBC. Therefore, the spectral data of DHA conjugates with uracil, thymine, and 5-fluorouracil were listed in pairs corresponding to the same proton and carbon pairs in the two DHA moieties. Finally, the NMR spectral and HRMS data of **7a-f** perfectly matched their structures and molecular formulas.

3.2. Biology

3.2.1. Antimalarial activity

All six tested conjugates **7a-f** showed the inhibition of the growth of the malarial parasites. For the monoclonal (K1) of *P. falciparum*, the minimum inhibitory concentration (MIC) values of **7a-f** were ranged from < 0.05 to 3 nmol/L, and the concentration at which a substance exerts half of its maximal inhibitory effect (IC_{50}) were intervals 1.46-856.9 nmol/L. The MIC and IC_{50} values for chloroquine-sensitive *P. falciparum* monoclonal (T96) were in the region of 1.71-31.34 nmol/L. Notably, the conjugates **7d**, **7c**, and **7d** expressed efficiency at low

concentrations. Minimum inhibitory concentrations (MICs), IC_{50} vaues, and the parasite clearance concentrations of the six tested compounds against chloroquine-sensitive (T96) and chloroquine-resistant monoclonal (K1) are represented in Table 1.

Table 1. Antimalarial activity of the synthesized derivatives against chloroquine-sensitive *P. falciparum* monoclonal (T96) and chloroquine-resistant monoclonal (K1).

No	Compounds	K1		T96	
		MIC (nmol/L)	IC_{50} (nmol/L)	MIC (nmol/L)	IC_{50} (nmol/L)
1	**7a**	3	51.15	6	31.34
2	**7b**	3	856.90	6	759.00
3	**7c**	1	1.46	0.5	1.82
4	**7d**	< 0.05	2.24	< 0.05	2.32
5	**7e**	1	3.33	4	3.79
6	**7f**	< 0.05	1.63	< 0.05	1.71
7	**Chloroquine**	3	179.66	4	175.07
8	**Piperaquine**	2	48.35	1	55.14
9	**DHA**	1	6.30	2	9.28

In term of MIC values, compounds **7d**, **7f** expressed the most potent activity among the 6 tested derivatives against *P. falciparum* malaria parasite (< 0.05 nmol/L) on both sensitive and resistant monoclonal chloroquine. This is probably explained due to the good biocompatibility of uracil and the 5-fluorouracil derivatives (uracil is one of the four nucleobases as block building of the DNA chain) To determine accurately the MIC values of these two derivatives, it is necessary to test at the concentrations below 1.0 nmol/L. In relation to IC_{50} values, the screening of antimalarial activity of the six conjugates gave a wide range of IC_{50} values, from 1.46-856.9 nmol/L. Among the tested conjugates, conjugate **7c** displayed the best inhibitory effect against chloroquine-resistant monoclonal (K1) with the lowest IC_{50} value of 1.46 nmol/L, while compound **7b** exhibited the weakest activity with the highest IC_{50} value of 856.9 nmol/L.

Comparison of the above-obtained results to currently used antimalarial drugs such as chloroquine, piperaquine, and dihydroartemisinin resulted in the conclusions that five out of six of the tested derivatives **7a**, **7c-f** had IC_{50} values less than those of chloroquine and piperaquine. Finally, four conjugates **7c-f** exhibited antimalarial activity stronger than that of DHA in which the best IC_{50} values of **7c** and **7f** were 1.46 and 1.63 nnmol/L, respectively, which were approximately four times lower than the IC_{50} value of DHA. These results were considered as a basis for further studies to apply these conjugates as drugs in the treatment of malaria.

3.2.2. Cytotoxicity

The result of *in vitro* cytotoxicity of the prepared conjugates and DHA were given in Table 2.

Table 2. In vitro cytotoxic activity of six synthesized conjugates.

No	Compounds	IC_{50} value (μg/ml)		
		HepG2	Hela	LU-1
1	**Ellipticine**	0.25	0.38	0.31
2	**7a**	2.77	3.17	3.54

3	**7b**	4.41	18.38	-
4	**7c**	3.55	8.89	9.91
5	**7d**	4.47	3.43	9.99
6	**7e**	4.01	3.61	4.00
7	**7f**	1.32	2.61	4.29
8	**DHA**	10.19	9.92	-

*Ellipticine was used as a positive control.

All synthesized conjugates expressed cytotoxic effects against three tested cancer cell lines Hep-G2, Hela, and LU-1 except for conjugate **7b**, which exhibited weak activity against the LU-1 cancer cell line. Apparently, the result of this cytotoxic activity greatly depends on the structural moiety attached to the DHA skeleton. Conjugates **7d** and **7e** of DHA with nucleobases including thymine and uracil, respectively, two important nucleobases making DNA chains, exhibited a weaker cytotoxic activity than that of the remaining conjugates. Especially, the conjugate of DHA **7e** with 5-fluorouracil, a generic drug used for the treatment of colon cancer, displayed a promising cytotoxicity with the IC_{50} values from 1.32 to 4.29 μg/ml. This finding can suggest a potential approach for the application of this conjugate as a new candidate in anticancer therapy.

4. CONCLUSIONS

The *N*-alkylation of heterocyclic amines and nucleobases with 2-(10*β*-dihydroartemisinoxy)ethyl bromide gave six conjugates that contained one and two DHA units with full data of NMR and HRMS spectra. Screening for antimalarial and cytotoxic activities showed that four out of six DHA conjugates containing uracil, thymine, 5-fluorouracil, and 6-methoxy-7-(3-morpholinopropoxy)quinazolin-4(*3H*)-one expressed activity against both chloroquine-sensitive *P. falciparum* monoclonal (T96) and chloroquine-resistant monoclonal (K1), and stronger than that of parent compound DHA. Additionally, the four mentioned conjugates also exhibited potent cytotoxic activity against human cancer cell lines HepG2, HeLa, and LU-1. These compounds may be further investigated to develop dual-acting drugs in therapy.

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Declaration of competing interest. The authors declare no competing interests.

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