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Research Article

Novel Thioethers of Dihydroartemisinin Exhibiting Their Biological Activities

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Eleven conjugates between dihydroartemisinin (DHA) with thiols containing both ether and thioether bonds were designed, synthesized by a two-step procedure including etherification and *S*-alkylation. Analysis of the NMR spectral data indicated that the dimer of DHA with thiols 6-mercaptopurine and 2-mercaptoimidazole was produced with yields of 31% and 62%, respectively. Furthermore, the tautomerization of thiol 5-methoxy-2-mercaptobenzimidazole led to the formation of a mixture of two isomers in which they might be interchangeable through a dynamic tautomeric equilibrium in the solution. Screening *in vitro* biological activities revealed that most of the synthesized conjugates showed good cytotoxic and anti-inflammatory activity, while three of them displayed α-glucosidase inhibitory activity. Notably, two conjugates **5d** and **5e** of DHA with thiols 2-mercaptopyrimidine and 2-mercaptobenzothiazole had an effect in all tested activities in which conjugate **5e** is the most potent.

1. Introduction

Artemisinin, a sesquiterpene lactone, has been isolated on a large-scale from *Artemisia annua L.* in Vietnam [1]. Artemisinin has excellent antimalarial activity due to an endoperoxide bridge that is an active center for biological activity [2]. Discovery of artemisinin was considered a revolution in the treatment of severe malaria in the 90s of the last century [3]. In a short time, many artemisinin derivatives were introduced and screened for antimalarial activity to find new drugs for the treatment of this disease with the aim of improving efficacy and resistance [4]. Among these derivatives, dihydroartemisinin **1** (DHA), artemether **1a**, and artesunate **1b** (Figure 1) are three candidates that have the earliest been approved for use in the treatment of malarial disease [5, 6]. Although the antimalarial activity is improved compared with artemisinin, over time resistance to these drugs has also been discovered, and their effectiveness is also significantly reduced [7, 8]. Thus, various artemisinin derivatives were prepared and probed for antimalarial activity, for example, DHA α-alkylbenzylic ethers [9], derivatives containing a sugar moiety [10], water-soluble derivatives [11, 12]. Apparently, artemisinin scaffold is still the best selection for new antimalarial drug development.

In addition to antimalarial activity, artemisinin and its derivatives have also been discovered as potential candidates for anti-inflammatory and antitumor activities [13–19]. Reports show that artesunate is a potent inhibitor of many cancer cell lines and effectively prevents metastasis of tumor cells [20–23]. Interestingly, artesunate **1b** is less toxic to healthy cells, and suitable for clinical trial studies. Based on the artesunate **1b** structure, a dimethylene bridge in the ether C-O-C linkage at C10 was used as a template for the synthesis of dihydroartemisinin derivatives. Several structures like **1c** and **1d** were also reported to have potential *in vitro*

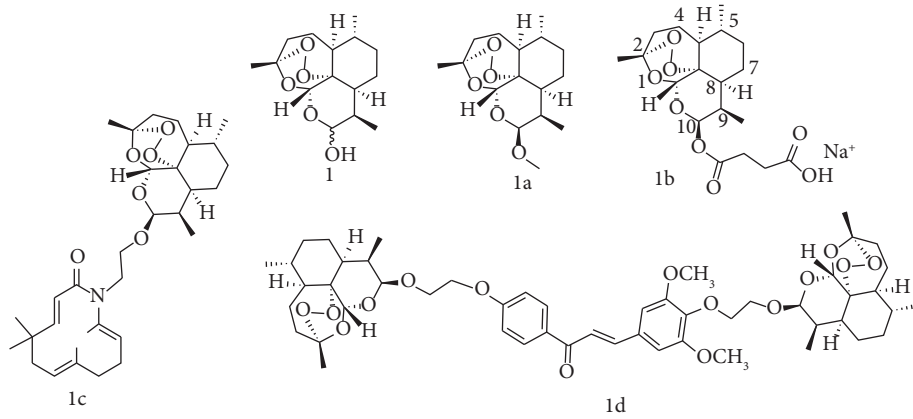


Figure 1: Typical artemisinin derivatives in clinical and pharmacological studies.

anticancer activity [24, 25]. Therefore, artemisinin **1** scaffold is an ideal target for searching and developing a new drug that can be used for both antimalarial and antitumor purposes.

In pharmaceutical chemistry research, thiols are considered as substances with a broad spectrum of activities, including anti-inflammatory and anticancer activities [26–29]. They are widely used in drug design, and several thiols are important components of drugs such as omeprazole and lansomeprazole [30, 31]. In our previous report [32], the conjugates of thiol with zerumbone, another naturally occurring sesquiterpene, exhibited activity against HepG2, A549, and HeLa cell lines with the IC_{50} ranging from 0.93 to 22.40 μM, that were approximately 4-fold to 20-fold stronger cytotoxic activity than that of zerumbone. Therefore, the strategy of using thiols in combination with the artemisinin skeleton can be effective. Currently, there are few studies on the conjugates of thiols with DHA [33–35]. Notably, three thioethers between DHA and mercaptothiadiazoles, mercaptotetrazole *via* a dimethylene bridge as well as their antibacterial activity were reported [35]. In line with our continuing study into the chemical modification at C-10 of DHA, in the present work, new derivatives of DHA with a series of heterocyclic thiols *via* thioether and ether linkages were designed, synthesized, and evaluated for their cytotoxic, anti-inflammatory, and α-glucosidase inhibitory activities.

2. Materials and Methods

2.1. Chemistry. Dihydroartemisinin is commercially available in Vietnam. Other chemicals were purchased from Sigma-Aldrich and used without further purification. ^{1}H-NMR and ^{13}C-NMR spectra were recorded at ambient temperature on a Bruker Avance 600 MHz spectrometer in DMSO-d_6. Chemical shifts δ are quoted in parts per million (ppm) referenced to the residual solvent peak, (DMSO-d_6 at 2.49 ppm and 39.5 ppm) relative to TMS. High-resolution mass spectra were recorded by using a X500R QTOF LC-MS system, SCIEX, US. Thin-layer chromatography (TLC) was performed on a precoated aluminum sheet of Silica Gel 60 F254 (Merck), and products were visualized by using a UV lamp at 254 nm. Column chromatography was carried out on silica gel (40–230 mesh).

Synthesis of 2-(10-β-dihydroarteminoxy) ethyl bromide **3**: 2-(10-β-dihydroarteminoxy) ethyl bromide **3** was obtained in 46% yield by the etherification of DHA with 2-bromoethanol **2** catalyzed by BF_3. Et_2O in dichloromethane according to a known procedure [36].

General procedure for the synthesis of artemisinin derivatives **5a–k**: Each mercapto compound 2-mercaptopyridine **4a**, 4-mercaptopyridine **4b**, 2-mercaptopyrimidine **4c**, 2-mercaptobenzoxazole **4d**, 2-mercaptobenzothiazole **4e**, methyl 2-mercaptobenzoate **4f**, 2-mercaptoimidazole **4g**, 6-mercaptopurine **4h**, 2-mercaptobenzimidazole **4i**, or 5-methoxy-2-mercaptobenzimidazole **4k** (1 mmol, 1.0 equiv.) was dissolved in dry DMF (5 mL), then potassium carbonate (1.5 equiv.) and compound **2** (1.0 equiv.) were added. Reaction mixtures were stirred at room temperature overnight and monitored by TLC. At the end of the reaction, cool water (15 mL) was added, and then the mixture was extracted with ethyl acetate (3×15 mL). The combined organic layer was dried over anhydrous Na_2SO_4, then filtered and evaporated under reduced pressure. Crude products **5a–5k** were purified by column chromatography on silica gel eluting with *n*-hexane/acetone to obtain the desired products.

4-((2-(((3R,5aS,6R,8aS,9R,10S,12R,12aR)-3,6,9-Trimethyldecahydro-12H-3,12-epoxy[1,2]dio-xepino[4,3-i]isochromen-10-yl)oxy)ethyl)thio)pyridine, **5a**: Yield 69%, white solid, m.p. 95–97°C; ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)): 8.42 (m, 1H, H-6′), 7.60 (m, 1H, H-5′), 7.30 (m, 1H, H-3′), 7.11 (m, 1H, H-4′), 5.36 (s, 1H, H-12), 4.73 (d, $J = 3.0$ Hz, 1H, H-10), 3.88 (m, 1H, 10-O-CH_{2a}-), 3.62 (m, 1H, 10-*O*-CH_{2b}-), 3.40 (m, 2H, *S*-CH_2-), 2.39 (m, 1H, H-9), 2.17 (m, 1H, H-4a), 1.99 (m, 1H, H-4b), 1.79 (m, 1H, H-5a), 1.71 (m, 1H, H-8a), 1.55 (m, 1H, H-8b), 1.49 (m, 1H, H-7a), 1.35 (m, 2H, H-8A, H-5b), 1.27 (s, 3H, H-13), 1.24 (m, 1H, H-6), 1.13 (m, 1H, H-5A), 0.89 (d, $J = 6.6$ Hz, 3H, H-14), 0.85 (m, 1H, H-7b), 0.83 (d, $J = 7.2$ Hz, 3H, H-15); ^{13}C-NMR (150 MHz, DMSO-d_6, δ (ppm)): 157.8 (C-2′), 149.3 (C-6’), 136.5 (C-4′), 121.8 (C-3’), 119.7 (C-5′), 103.2 (C-3), 100.6 (C-10), 87.0 (C-12), 80.4 (C-12A), 66.3 (10-O-CH_2-), 52.0 (C-5A), 43.8 (C-8A), 36.6 (C-6), 36.0 (C-4), 34.1 (C-7), 30.4

(C-9), 29.0 (*S*-CH_2-), 25.6 (C-13), 24.2 (C-5), 23.8 (C-8), 20.1 (C-14), 12.6 (C-15); ESI-HRMS calculated for $C_{22}H_{32}NO_5S$: $[M+H]^+$ 422.2001, found 422.1984.

2-((2-(((3R,5aS,6R,8aS,9R,10S,12R,12aR)-3,6,9-Trimethyldecahydro-12H-3,12-epoxy[1,2]dio-xepino[4,3-i]isochromen-10-yl)oxy)ethyl)thio)pyridine, **5b**: Yield 70%, white solid, m.p. 91–93°C; ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)): 8.36 (dd, $J_1 = 1.5$ Hz, $J_2 = 4.5$ Hz, 2H, H-2′, H-6′), 7.30 (dd, $J_1 = 1.5$ Hz, $J_2 = 4.5$ Hz, 2H, H-3′, H-5′), 5.35 (s, 1H, H-12), 4.74 (d, $J = 3.0$ Hz, 1H, H-10), 3.91 (m, 1H, 10-*O*–CH_{2a}–), 3.63 (m, 1H, 10-*O*–CH_{2b}–), 3.31 (m, 2H, *S*–CH_2–), 2.38 (m, 1H, H-9), 2.17 (m, 1H, H-4a), 1.99 (m, 1H, H-4b), 1.79 (m, 1H, H-5a), 1.68 (m, 1H, H-8a), 1.53 (m, 1H, H-8b), 1.48 (m, 1H, H-7a), 1.34 (m, 2H, H-8A, H-5b), 1.28 (s, 3H, H-13), 1.24 (m, 1H, H-6), 1.13 (m, 1H, H-5A), 0.89 (d, $J = 6.6$ Hz, 3H, H-14), 0.84 (m, 1H, H-7b), 0.81 (d, $J = 6.6$ Hz, 3H, H-15); ^{13}C-NMR (150 MHz, DMSO-d_6, δ (ppm)): 149.1 (C-3′, C-5′), 148.2 (C-1′), 120.6 (C-2′, C-6′), 100.3 (C-3), 100.8 (C-10), 87.0 (C-12), 80.4 (C-12A), 52.0 (C-5A), 65.8 (10-*O*-CH_2-), 43.7 (C-8A), 36.6 (C-6), 36.0 (C-4), 34.1 (C-7), 30.4 (C-9), 29.9 (*S*-CH_2-), 25.6 (C-13), 24.2 (C-5), 23.8 (C-8), 20.1 (C-14), 12.6 (C-15); ESI-HRMS calculated for $C_{22}H_{32}NO_5S$: $[M+H]^+$ 422.2001, found 422.1975.

2-((2-(((3R,5aS,6R,8aS,9R,10S,12R,12aR)-3,6,9-Trimethyldecahydro-12H-3,12-epoxy[1,2]dio-xepino[4,3-i]isochromen-10-yl)oxy)ethyl)thio)pyrimidine, **5c**: Yield 66%, white solid, m.p. 144–146°C; ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)): 8.64 (dd, $J_1 = 1.2$ Hz, $J_2 = 4.8$ Hz, 2H, H-4′, H-6′), 7.21 (t, $J_1 = 4.8$ Hz, 1H, H-5′), 5.35 (s, 1H, H-12), 4.74 (d, $J = 3.0$ Hz, 1H, H-10), 3.91 (m, 1H, 10-*O*–CH_{2a}–), 3.67 (m, 1H, 10-*O*–CH_{2b}–), 3.39 (t, $J = 5.7$ Hz, 2H, *S*–CH_2–), 2.31 (m, 1H, H-9), 2.17 (m, 1H, H-4a), 1.99 (m, 1H, H-4b), 1.80 (m, 1H, H-5a), 1.69 (m, 1H, H-8a), 1.55 (m, 1H, H-8b), 1.49 (m, 1H, H-7a), 1.34 (m, 2H, H-8A, H-5b), 1.28 (s, 3H, H-13), 1.24 (m, 1H, H-6), 1.13 (m, 1H, H-5A), 0.89 (d, $J = 6.6$ Hz, 3H, H-14), 0.85 (m, 1H, H-7b), 0.83 (d, $J = 7.2$ Hz, 3H, H-15); ^{13}C-NMR (150 MHz, DMSO-d_6, δ (ppm)): 170.7 (C-2′), 157,7 (C-4′, C-6’), 117.2 (C-5′), 103.3 (C-3), 100,6 (C-10), 87.0 (C-12), 80.4 (C-12A), 66.0 (10-*O*-CH_2-), 52.0 (C-5A), 43.8 (C-8A), 36.6 (C-6), 36.0 (C-4), 34.1 (C-7), 30.4 (C-9), 30.1 (*S*-CH_2-), 25.6 (C-13), 24.2 (C-5), 23.8 (C-8), 20.1 (C-14), 12.6 (C-15); ESI-HRMS calculated for $C_{21}H_{31}N_2O_5S$: $[M+H]^+$ 423.1953, found 423.1933.

2-((2-(((3R,5aS,6R,8aS,9R,10S,12R,12aR)-3,6,9-Trimethyldecahydro-12H-3,12-epoxy[1,2]di-oxepino[4,3-i]isochromen-10-yl)oxy)ethyl)thio)benzo[d]oxazole, **5d**: Yield 63%, colorless oil; ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)): 7.63 (dd, $J_1 = 1.8$ Hz, $J_2 = 7.2$ Hz, 2H, 2H, H-5′, H-6′), 7.32 (m, 2H, H-4′, H-7′), 5.34 (s, 1H, H-12), 4.76 (d, $J = 3.6$ Hz, 1H, H-10), 4.04 (m, 1H, 10-*O*-CH_{2a}-), 3.75 (m, 1H, 10-*O*-CH_{2b}-), 3.61 (t, $J = 5.7$ Hz, 2H, *S*-CH_2-), 2.39 (m, 1H, H-9), 2.16 (m, 1H, H-4a), 1.97 (m, 1H, H-4b), 1.76 (m, 1H, H-5a), 1.57 (m, 1H, H-8a), 1.44 (m, 1H, H-8b), 1.29 (m, 3H, H-7a, H-8A, H-5b), 1.27 (s, 3H, H-13), 1.16 (m, 1H, H-6), 1.09 (m, 1H, H-5A), 0.83 (d, $J = 6.6$ Hz, 3H, H-14), 0.80 (d, $J = 7.8$ Hz, 3H, H-15), 0.74 (m, 1H, H-7b); ^{13}C-NMR (150 MHz, DMSO-d_6, δ (ppm)): 164.2 (C-2′), 151.2 (C-3′a), 141.3 (C-7′a), 124.5 (C-6′), 124.1 (C-5), 118.1 (C-7′), 110.0 (C-4′), 103.2 (C-3), 100.7 (C-10), 87.0 (C-12), 80.3 (C-12A), 65.9 (10-*O*-CH_2-), 51.9 (C-5A), 43.6 (C-8A), 36.6 (C-6), 35.9 (C-4), 33.9 (C-7), 32.1 (*S*-CH_2-), 30.4 (C-9), 25.5 (C-13), 24.1 (C-5), 23.7 (C-8), 20.0 (C-14), 12.5 (C-15); ESI-HRMS calculated for $C_{24}H_{32}NO_6S$: $[M+H]^+$ 462.1950, found 462.1928.

2-((2-(((3R,5aS,6R,8aS,9R,10S,12R,12aR)-3,6,9-Trimethyldecahydro-12H-3,12-epoxy[1,2]di-oxepino[4,3-i]isochromen-10-yl)oxy)ethyl)thio)benzo[d]oxazole, **5e**: Yield 68%, colorless oil; ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)): 8.00 (dd, $J_1 = 1.2$ Hz, $J_2 = 7.8$ Hz, 1H, H-7′), 7.84 (d, $J = 7.8$ Hz, 1H, H-4′), 7.46 (dt, $J_1 = 1.2$ Hz, $J_2 = 7.8$ Hz, 1H, H-5′), 7.36 (dt, $J_1 = 1.2$ Hz, $J_2 = 7.8$ Hz, 1H, H-6′), 5.34 (s, 1H, H-12), 4.75 (d, $J = 3.0$ Hz, 1H, H-10), 4.02 (m, 1H, 10-*O*-CH_{2a}-), 3.74 (m, 1H, 10-*O*-CH_{2b}-), 3.64 (m, 2H, *S*-CH_2-), 2.38 (m, 1H, H-9), 2.16 (m, 1H, H-4a), 1.97 (m, 1H, H-4b), 1.76 (m, 1H, H-5a), 1.59 (m, 1H, H-8a), 1.45 (m, 1H, H-8b), 1.31 (m, 2H, H-7a, H-8A), 1.27 (s, 3H, H-13), 1.25 (m, 1H, H-5b), 1.15 (m, 1H, H-6), 1.08 (m, 1H, H-5A), 0.82 (m, 6H, H-14, H-15), 0.73 (m, 1H, H-7b); ^{13}C-NMR (150 MHz, DMSO-d_6, δ (ppm)): 166.6 (C-2′), 152.6 (C-7′a), 134.5 (C-3′a), 126.3 (C-5′), 124.4 (C-6′), 121.7 (C-7′), 121.0 (C-4′), 103.3 (C-3), 100.7 (C-10), 87.0 (C-12), 80.4 (C-12A), 66.0 (10-*O*-CH_2-), 52.0 (C-5A), 43.7 (C-8A), 36.6 (C-6), 35.9 (C-4), 34.0 (C-7), 33.0 (*S*-CH_2-), 30.4 (C-9), 25.5 (C-13), 24.2 (C-5), 23.8 (C-8), 20.0 (C-14), 12.6 (C-15); ESI-HRMS calculated for $C_{24}H_{32}NO_5S_2$: $[M+H]^+$ 478.1722, found 478.1701.

Methyl-2-((2-(((3R,5aS,6R,8aS,9R,10S,12R,12aR)-3,6,9-trimethyldecahydro-12H-3,12-epoxy-[1,2]di-oxepino[4,3-i] isochromen-10-yl)oxy)ethyl)thio)benzoate, **5f**: Yield 60%, colorless oil; ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)): 7.85 (dd, $J_1 = 1.2$ Hz, $J_2 = 7.8$ Hz, 1H, H-6′), 7.52 (m, 2H, H-3′, H-4′), 7.24 (dt, $J_1 = 1.2$ Hz, $J_2 = 7.8$ Hz, 1H, H-5′), 5.36 (s, 1H, H-12), 4.73 (d, $J = 3.0$ Hz, 1H, H-10), 3.89 (m, 1H, 10-*O*-CH_{2a}-), 3.82 (s, 3H, OCH_3), 3.64 (m, 1H, 10-*O*-CH_{2b}-), 3.21 (m, 2H, *S*-CH_2-), 2.39 (m, 1H, H-9), 2.17 (m, 1H, H-4a), 1.98 (m, 1H, H-4b), 1.78 (m, 2H, H-5a, H-8a), 1.55 (m, 1H, H-8b), 1.49 (m, 1H, H-7a), 1.34 (m, 4H, H-5b, H-8A, H-6), 1.27 (s, 3H, H-13), 1.12 (m, 1H, H-5A), 0.89 (d, $J = 6.6$ Hz, 3H, H-14), 0.85 (m, 1H, H-7b), 0.83 (d, $J = 7.2$ Hz, 3H, H-15); ^{13}C-NMR (150 MHz, DMSO-d_6, δ (ppm)): 166.1 (C-7′), 140.2 (C-2′), 132.5 (C-4′), 130.6 (C-6’), 127.7 (C-1′), 126.1 (C-3′), 124.1 (C-5′), 103.2 (C-3), 100.6 (C-10), 87.0 (C-12), 80.4 (C-12A), 65.3 (10-*O*-CH_2-), 52.0 (C-5A), 51.9 (C-8′), 43.8 (C-8A), 36.4 (C-6), 36.0 (C-4), 34.1 (C-7), 31.3 (*S*-CH_2-), 30.4 (C-9), 25.6 (C-13), 24.2 (C-5), 23.8 (C-8), 20.1 (C-14), 12.6 (C-15). ESI-HRMS calculated for $C_{25}H_{34}O_7SNa$: $[M+Na]^+$ 501.1922, found 501.1900.

2-((2-(((3R,5aS,6R,8aS,9R,10S,12R,12aR)-3,6,9-Trimethyldecahydro-12H-3,12-epoxy[1,2]di-oxepino[4,3-i]isochromen-10-yl)oxy)ethyl)thio)imidazole, **5g1**: Yield 46%, colorless oil; ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)): 7.27 (d, $J = 1.2$ Hz, 1H, H-5′), 6.95 (d, $J = 1.2$ Hz, 1H, H-4′), 5.34 (s, 1H, H-12), 4.97 (s, 1H, H-12″), 4.73 (d, $J = 3.6$ Hz, 1H, H-10), 4.64 (d, $J = 3.6$ Hz, 1H, H-10″), 4.19 (m, 1H, 10″-*O*-CH_{2a}-), 4.10 (m, 1H, 10″-*O*-CH_{2b}-), 4.00 (m, 1H, 10-*O*-CH_{2a}-), 3.84 (m, 1H, 10-*O*-CH_{2b}-), 3.58 (*N*-CH_2-), 3.18 (*S*-CH_2-), 2.40 (m, 1H, H-9), 2.34 (m, 1H, H-9″), 2.17 (m, 2H, H-4a, H-4″a), 1.99 (m, 2H, H-4b, H-4″b), 1.78 (m, 3H, H-5a, H-5″a, H-8a), 1.64 (m, H-8b), 1,54 (m, 2H, H-8″a, H-8″b), 1.49 (m,

2H, H-7a, H-7″a), 1.34 (m, 6H, H-8A, H-8″A, H-5b, H-5″b, H-6, H-6″), 1,28 (s, 3H, H-13), 1.27 (s, 3H, H-13″), 1.16 (m, 2H, H-5A, H-5″A), 1.08 (m, 1H, H-7b), 0.90 (d, J = 6.6 Hz, 3H, H-14), 0.87 (d, J = 6.6 Hz, 3H, H-14″), 0.85 (d, J = 7.8 Hz, 1H, H-5), 0,80 (m, 1H, H-7″b), 0.75 (d, J = 7.2 Hz, 3H, H-15″); ^{13}C-NMR (150 MHz, DMSO-d_6, δ (ppm)): 140.2 (C-2′), 128.6 (C-4′), 121.7 (C-5'), 103.3 (C-3), 103.2 (C-3″), 100.7 (C-10), 100.4 (C-10″), 87.0 (C-12), 86.8 (C-12″), 80.4 (C-12A), 80.3 (C-12″A), 66.3 (10-*O*-CH_2-), 65.8 (10″-*O*-CH_2-), 52.0 (C-5A), 51.9 (C-5″A), 45.6 (*N*-CH_2-), 43.8 (C-8A), 43.6 (C-8″A), 36.7 (C-6), 36.4 (C-6″), 36.0 (C-4), 35.9 (C-4″), 34.1 (C-7), 34.0 (C-7″), 30.6 (C-9), 30.4 (C-9″), 30.2 (*S*-CH_2-), 25.6 (C-13), 25.5 (C-13″), 24.3 (C-5), 24.2 (C-5″), 23.9 (C-8), 23.8 (C-8″), 12.7 (C-15), 12.5 (C-15″); ESI-HRMS calculated for $C_{37}H_{57}N_2O_{10}S$: $[M+H]^+$ 721.3734, found 721.3657.

1-(2-(((3R,5aS,6R,8aS,9R,10S,12R,12aR)-3,6,9-Trimethyldecahydro-12H-3,12-epoxy[1,2]dio-xepino[4,3-i]isochromen-10-yl)oxy)ethyl)-2-((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)thio)-1H-imidazole, **5g2**: Yield 31%, colorless oil; ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)): 12.17 (s, 1H, N-H), 7.02 (s, 2H, H-4′, H-5'), 5.36 (s, 1H, H-12), 4.72 (d, J = 3.6 Hz, 1H, H-10), 3.85 (m, 1H, 10-O−CH_{2a}−), 3.58 (m, 1H, 10-O−CH_{2b}−), 3.22 (m, 2H, S−CH_2−),2.39 (m, 1H, H-9), 2.18 (m, 1H, H-4a), 1.99 (m, 1H, H-4b), 1.80 (m, 1H, H-5a), 1.73 (m, 1H, H-8a), 1.61 (m, 1H, H-8b), 1.56 (m, 1H, H-7a), 1.36 (m, 4H, H-5b, H-8A, H-6), 1.28 (s, 3H, H-13), 1.14 (m, 1H, H-5A), 0.90 (d, J = 6.6 Hz, 3H, H-14), 0.87 (m, 1H, H-7b), 0.85 (d, J = 7.2 Hz, 3H, H-15); ^{13}C-NMR (150 MHz, DMSO-d_6, δ (ppm)): 138.7 (C-2′), 103.3 (C-3), 100.7 (C-10), 87.0 (C-12), 80.4 (C-12A), 66.6 (10-O-CH2-), 52.1 (C-5A), 43.8 (C-8A), 36.5 (C-6), 36.0 (C-4), 34.1 (C-7), 32.9 (*S*-CH2-), 30.4 (C-9), 25.6 (C-13), 24.2 (C-5), 23.8 (C-8), 20.1 (C-14), 12.7 (C-15); ESI-HRMS calculated for $C_{20}H_{31}N_2O_5S$: $[M+H]^+$ 411.1953, found 411.1932.

9-(2-(((3R,5aS,6R,8aS,9R,10S,12R,12aR)-3,6,9-Trimethyldecahydro-12H-3,12-epoxy[1,2]dio-xepino[4,3-i]isochromen-10-yl)oxy)ethyl)-6-((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)thio)-9H-purine, **5h**: Yield 62%, colorless oil; ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)): 8.70 (s, 1H, H-8′), 8.50 (s, 1H, H-82'), 5.34 (s, 1H, H-12), 4,75 (d, J = 3.3 Hz, 1H, H-10), 4.64 (, J = 3.3 Hz, 1H, H-10″), 4.55 (m, 1H, 10″-*O*-CH_{2a}-), 4.48 (s, 1H, H-12″), 4.40 (m, 1H, 10″-*O*-CH_{2b}-), 4.22 (m, 1H, 10-*O*-CH_{2a}-), 3.97 (m, 1H, 10-*O*-CH_{2b}-), 3.71 (m, 3H, *S*-CH_2-, *N*-CH_{2a}-), 3.57 (m, 1H, *N*-CH_{2b}-), 2.39 (m, 1H, H-9), 2.26 (m, 1H, H-9″), 2.15 (m, 1H, H-4a), 2.08 (m, 1H, H-4″a), 1.94 (m, 2H, H-4b, H-4″b), 1.78 (m, 1H, H-5a), 1.69 (m, 1H, H-5″a), 1.63 (m, 1H, H-8a), 1.50 (m, 1H, H-8b), 1.31 (m, 4H, H-7a, H-7″a, H-5b, H-5″b), 1,27 (s, 3H, H-13), 1.24 (s, 3H, H-13″), 1.17 (m, 2H, H-8A, H-8″A), 1.11 (m, 2H, H-6, H-6″), 0.95 (m, 1H, H-5A), 0.87 (m, 1H, H-5″A), 0.84 (d, J = 7.2 Hz, 3H, H-14), 0.82 (d, J = 7.2 Hz, 3H, H-14″), 0.80 (d, J = 6.0 Hz, 3H, H-15), 0.65 (d, J = 7.2 Hz, 3H, H-15″), 0.63 (m, 1H, H-7b), 0,54 (m, 1H, H-7″b); ^{13}C-NMR (150 MHz, DMSO-d_6, δ (ppm)): 159.1 (C-6′), 151.1 (C-2′), 148.6 (C-4'), 144.6 (C-8′), 130.6 (C-5'), 103.24 (C-3), 103.16 (C-3″), 100.6 (C-10), 100.1 (C-10″), 87.0 (C-12), 86.6 (C-12″), 80.4 (C-12A), 80.0 (C-12″A), 66.2 (10-*O*-CH_2-), 64.6 (10″-*O*-CH_2-), 52.0 (C-5A), 51.7 (C-5″A), 43.7 (*N*-CH_2-), 43.3 (C-8A), 43.2 (C-8″A), 36.5 (C-6), 36.4 (C-6″), 36.0 (C-4), 35.9 (C-4″), 34.0 (C-7), 33.7 (C-7″), 30.4 (C-9), 30.1 (C-9″), 25.6 (C-13), 25.5 (C-13″), 24.2 (C-5), 24.1 (C-5″), 23.7 (C-8), 23.4 (C-8″), 20.03 (C-14), 20.00 (C-14″), 12.6 (C-15), 12.4 (C-15″); ESI-HRMS calculated for $C_{39}H_{57}N_4O_{10}S$: $[M+H]^+$ 773.3795, found 773.3800.

2-((2-(((3R,5aS,6R,8aS,9R,10S,12R,12aR)-3,6,9-Trimeth yldecahydro-12H-3,12-epoxy[1,2]di -oxepino[4,3-i]isochromen-10-yl)oxy)ethyl)thio)-1H-benzo[d]imidazole, **5i**: Yield 65%, colorless oil; ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)): 12.50 (s, 1H, N-H), 7.49 (s, 1H, H-4′, H-7'), 7.34 (s, 1H, H-7′), 7.10 (dt, J_1 = 3.6 Hz, J_2 = 7.2 Hz 2H, H-5′, H-6′), 5.37 (s, 1H, H-12), 4.75 (d, J = 3.6 Hz, 1H, H-10), 3.98 (m, 1H, 10-O−CH_{2a}−), 3.72 (m, 1H, 10-O−CH_{2b}−), 3.55 (m, 2H, S−CH_2−), 2.39 (m, 1H, H-9), 2.17 (m, 1H, H-4a), 1.97 (m, 1H, H-4b), 1.78 (m, 1H, H-5a), 1.65 (m, 1H, H-8a), 1.50 (m, 1H, H-8b), 1.34 (m, 3H, H-7a, H-5b, H-8A), 1.27 (s, 3H, H-13), 1.22 (m, 1H, H-6), 1.10 (m, 1H, H-5A), 0.85 (d, J = 6.0 Hz, 3H, H-14), 0.83 (d, J = 7.2 Hz, 3H, H-15), 0.77 (m, 1H, H-7b); ^{13}C-NMR (150 MHz, DMSO-d_6, δ (ppm)): 150.0 (C-2′), 143.6 (C-3′a), 135.5 (C-7′a), 121.4 (C-4′), 121.0 (C-7'), 117.2 (C-6′), 110.2 (C-5'), 103.3 (C-3), 100.7 (C-10), 87.0 (C-12), 80.4 (C-12A), 66.5 (10-CH2-), 52.0 (C-5A), 43.8 (C-8A), 36.6 (C-6), 36.0 (C-4), 34.0 (C-7), 31.3 (S-CH2-), 30.4 (C-9), 25.6 (C-13), 24.2 (C-5), 23.8 (C-8), 20.1 (C-14), 12.6 (C-15); ESI-HRMS calculated for $C_{24}H_{33}N_2O_5S$: $[M+H]^+$ 461.2110, found 461.2083.

5-Methoxy-2-((2-(((3R,5aS,6R,8aS,9R,10S,12R,12aR)-3,6,9-trimethyldecahydro-12H-3,12-ep oxy[1,2]dioxepino[4,3-i]isochromen-10-yl)oxy)ethyl)thio)-1H-benzo[d]imidazole, **5k1**: Yield 41%, white solid, m.p. 94–96°C; ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)):12.34 (s, 1H, NH), 7.37 (d, J = 7.8 Hz, 1H, H-7′), 6.85 (s, 1H, H-4'), 6.73 (d, J = 7.8 Hz, 1H, H-6′), 5.36 (s, 1H, H-12), 4.74 (d, J = 3.0 Hz, 1H, H-10), 3.96 (m, 1H, 10-*O*-CH_{2a}-), 3.75 (s, 3H, -OCH_3), 3.70 (m, 1H, 10-*O*-CH_{2b}-), 3.49 (m, 2H, *S*-CH_2-), 3.38 (m, 1H, H-9), 2.16 (m, 1H, H-4a), 1.97 (m, 1H, H-4b), 1.78 (m, 1H, H-5a), 1.65 (m, 1H, H-8a)1.51 (m, 1H, H-8b), 1.35 (m, 3H, H-8A, H-7a, H-5b), 1.27 (s, 3H, H-13), 1.23 (m, 1H, H-6), 1.11 (m, 1H, H-5A), 0.86 (d, J = 7.2 Hz, 3H, H-14), 0.83 (d, J = 7.2 Hz, 3H, H-15), 0.78 (m, 1H, H-7b); ^{13}C-NMR (150 MHz, DMSO-d_6, δ (ppm)): 103.3 (C-3), 100.7 (C-10), 87.0 (C-12), 80.4 (C-12A), 65.5 (10-*O*-CH_2-), 55.8 (OCH_3), 52.0 (C-5A), 43.8 (C-8A), 36.6 (C-6), 36.0 (C-4), 34.0 (C-7), 30.6 (*S*-CH_2-), 30.4 (C-9), 25.6 (C-13), 24.2 (C-5), 23.8 (C-8), 20.1 (C-14), 12.6 (C-15); ESI-HRMS calculated for $C_{25}H_{35}N_2O_6S$: $[M+H]^+$ 491.2216, found 491.2186.

6-Methoxy-2-((2-(((3R,5aS,6R,8aS,9R,10S,12R,12aR)-3,6,9-trimethyldecahydro-12H-3,12-ep oxy[1,2]dioxepino[4,3-i]isochromen-10-yl)oxy)ethyl)thio)-1H-benzo[d]imidazole, **5k2**: Yield 33%, white solid, m.p. 95–97°C; ^{1}H-NMR (600 MHz, DMSO-d_6, δ (ppm)):12.34 (s, 1H, NH), 7.21 (d, J = 7.8 Hz, 1H, H-4′), 7.06 (s, 1H, H-7'), 6.73 (d, J = 7.8 Hz, 1H, H-5′), 5.36 (s, 1H, H-12), 4.74 (d, J = 3.0 Hz, 1H, H-10), 3.96 (m, 1H, 10-*O*-CH_{2a}-), 3.75 (s, 3H, -OCH_3), 3.70 (m, 1H, 10-*O*-CH_{2b}-), 3.49 (m, 2H, *S*-CH_2-), 3.38 (m, 1H, H-9), 2.16

(m, 1H, H-4a), 1.97 (m, 1H, H-4b), 1.78 (m, 1H, H-5a), 1.65 (m, 1H, H-8a), 1.51 (m, 1H, H-8b), 1.35 (m, 3H, H-8A, H-7a, H-5b), 1.27 (s, 3H, H-13), 1.23 (m, 1H, H-6), 1.11 (m, 1H, H-5A), 0.86 (d, $J = 7.2$ Hz, 3H, H-14), 0.83 (d, $J = 7.2$ Hz, 3H, H-15), 0.78 (m, 1H, H-7b); ^{13}C-NMR (150 MHz, DMSO-d_6, δ (ppm)): 103.3 (C-3), 100.7 (C-10), 87.0 (C-12), 80.4 (C-12A), 65.5 (10-*O*-CH_2-), 55.8 (OCH_3), 52.0 (C-5A), 43.8 (C-8A), 36.6 (C-6), 36.0 (C-4), 30.6 (*S*-CH_2-), 34.0 (C-7), 30.4 (C-9), 25.6 (C-13), 24.2 (C-5), 23.8 (C-8), 20.1 (C-14), 12.6 (C-15); ESI-HRMS calculated for $C_{25}H_{35}N_2O_6S$: $[M+H]^+$ 491.2216, found 491.2186.

2.2. Biology. The *in vitro* anti-inflammatory activity of tested compounds was determined using a nitrite assay in murine macrophages following published methods with minor modifications [37, 38]. Prior to nitrite assay, cell viability of RAW 264.7 macrophages in exposure to test compounds was evaluated by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric method [39].

The alpha-glucosidase inhibitory activity of test compounds was determined based on the conversion of substrate *p*-nitrophenyl-*α*-*D*-glucopyranoside (*p*NPG) to yellow-colored product *p*-nitrophenol (pNP) following the described method of Ting and coworkers [40] with modifications.

The *in vitro* cytotoxic evaluation against Hep-G2 and LU-1 cancer cell lines was carried out according to the described protocols [41, 42].

3. Results and Discussion

3.1. Chemistry. The objective of this research was to synthesize the thioethers of dihydroartemisinin that were obtained by the connection of thiols **4a–4k** with the DHA *via* ether and thioether linkages as outlined in Scheme 1. The ether bridge of intermediate **3** was created by an etherification between DHA with 2-bromoethanol according to a known procedure [36], followed by the *S*-alkylation of thiols **4a–4k** with **3** at room temperature catalyzed by a weak base K_2CO_3 in DMF to produce thioethers **5a–k** in 31–70% yields.

Most of the conjugates between DHA **1** and thiols were successfully obtained as designed. However, some thiols are tautomerized leading to the formation of products that are not as originally designed. Besides, dimer products of both *S*- and *N*-alkylation were also observed and isolated. The first is the case of 2-mercaptoimidazole **4g**. TLC showed that two products were formed including **5g2** and **5g1** that were easily separated by column chromatography/silica gel eluting with *n*-hexane : acetone = 2 : 1. Their ^{1}H and ^{13}C-NMR spectra indicated that **5g1** is a product of *S*-alkylation, and **5g2** is another product of both the *S*-alkylation and *N*-alkylation reactions that contains two DHA units (Scheme 1). The assignment of signals was accomplished based on the current NMR data of **5g1** and references [43]. Accordingly, the assigned data of the DHA moiety are also in good agreement with the 1D- and 2D-NMR spectra of **5f**.

In the case of 6-mercaptopurine **4h**, this compound can exist in several structural forms due to the tautomerization [42]. Theoretically, the alkylation reaction can create products with different structures. However, the observation of the TLC of the reaction under a UV lamp at 254 nm revealed that only one product **5h** was formed and easily purified by column chromatography/silica gel eluted with *n*-hexane: acetone = 2 : 1. The NMR spectra of the product show that alkylation occurred at both -SH and -NH groups, evidenced by the observation that all signals of the protons and carbons in the two DHA moieties appear in pairs at the same positions, respectively. For example, 2 doublets of H-10 and H-10″ ($J = 3.3$ Hz) can be found in the ^{1}H-NMR spectrum at 4.75 and 4.64 ppm, respectively. The signals of C-10 and C-10″ atoms were also found to be 100.7 and 100.4 ppm, respectively.

Lastly, in the cases of **5k,** the tautomerization of the benzimidazole ring also resulted in the formation of a mixture of two isomers. The TLC of the reaction (*n*-hexane : acetone = 2 : 1) gives an unique spot visualized at 254 nm under a UV lamp. However, the NMR signal in the aromatic region of the obtained product suggests that this is a mixture of 5-OMe and 6-OMe forms [44] (Figure 2), these isomers could not separate from each other by column chromatography.

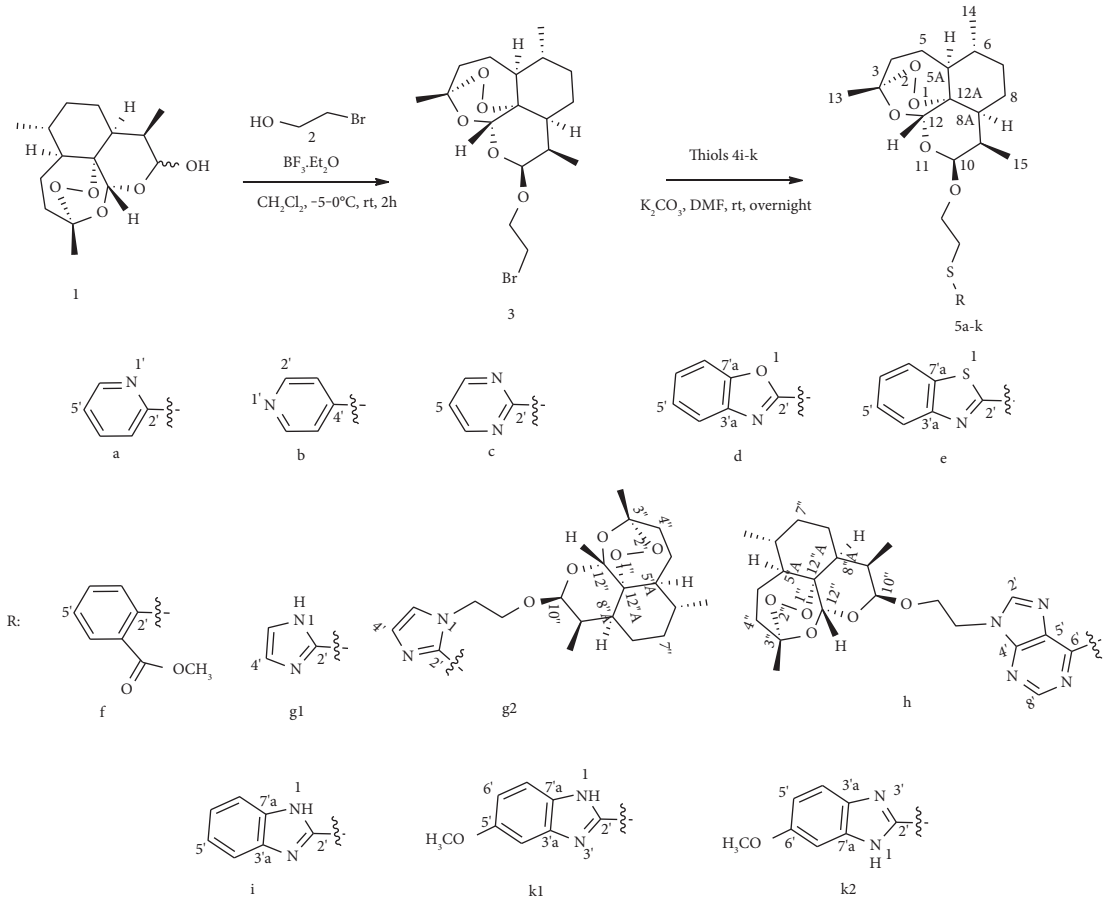
The ^{1}H-NMR and ^{13}C-NMR spectra of **5k** (**5k1** and **5k2**) show all 5 protons of the benzimidazole moiety including 12.5 ppm (s, 1H, N1-H), 7.49 ppm (s, broad, 1H, H-7), 7.34 ppm (s, broad, H-4′), and 7.10 ppm (m, 2H, H-5′, H-6′). However, the tautomerization leads to no splitting of these signals. Moreover, in the ^{13}C-NMR spectrum the signals of the carbon atoms in the benzimidazole ring were found at low intensity.

Spectral analysis of the mixture of **5k1** and **5k2** isomers also revealed that the signals of H-4′, H-6′ of the 5-OMe isomer and H-5′, H-7′ of 6-OMe one were split as pairs, corresponding to a 5 : 4 ratio, which was determined by its ^{1}H-NMR. While the signal region of aromatic carbon atoms of this moiety in the ^{13}C-NMR spectrum was very weak and difficult to attribute.

3.2. Biological Activities

3.2.1. Anti-Inflammatory Activity. (1) Cell Viability. Figure 3 depicts the cytotoxic half-maximal inhibitory concentrations (IC_{50}) of test compounds on RAW 264.7 cells, as well as concentrations eliciting no significant cytotoxicity for further assessment.

The effects of test compounds on RAW 264.7 viability were determined in terms of viable cell percentages measured by the MTT test. Results revealed cell viabilities ranging from 0 to 23.77 ± 2.18% when incubating with test compounds at a concentration of 20 μg/mL (data not



Scheme 1: Synthesis of novel thioethers **5a–k**.

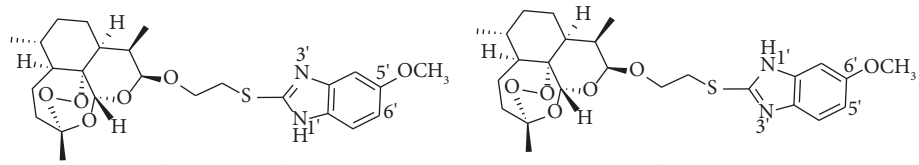


Figure 2: Structures of two isomers **5k1** and **5k2**.

shown), suggesting lower amounts of test compounds for application in nitrite assay.

3.2.2. Reduction of NO Production. The *in vitro* anti-inflammatory activity of test compounds was evaluated by measuring the reduced NO production in cell culture supernatants of LPS-stimulated RAW 264.7 cells. The results showed that nine out of ten compounds inhibited NO production with percentages of inhibition ranging from 59.74 ± 3.04 to $77.93 \pm 0.88\%$ (Table 1). Among the tested compounds, 5i was the sole causing no NO reduction. While the inhibition of NO production to the culture medium in wells treated with LPS was taken as 0%, the percentage by incubation with positive control (dexamethasone) was $62.46 \pm 1.27\%$ (Table 1).

3.2.3. Antidiabetes Activity. Table 2 presented the result of the α-glucosidase inhibitory assay for the obtained conjugates. Accordingly, three products **5a**, **5e**, and **5i** expressed a good effect in suppressing the α-glucosidase, especially compound **5i** with the IC_{50} of 0.21 mM was found to be stronger than the positive control. This is expected to be a promising finding in research of antidiabetes activity of DHA derivatives that has not drawn a worthy attention.

3.2.4. Cytotoxic Activity. The result of *in vitro* cytotoxicity evaluation of the prepared products was summarized in Table 3. The synthesized thioethers were screened for cytotoxic activity against HepG2 and LU-1 cell lines together with DHA. The results showed that eight compounds had stronger cytotoxic activity than DHA against both tested

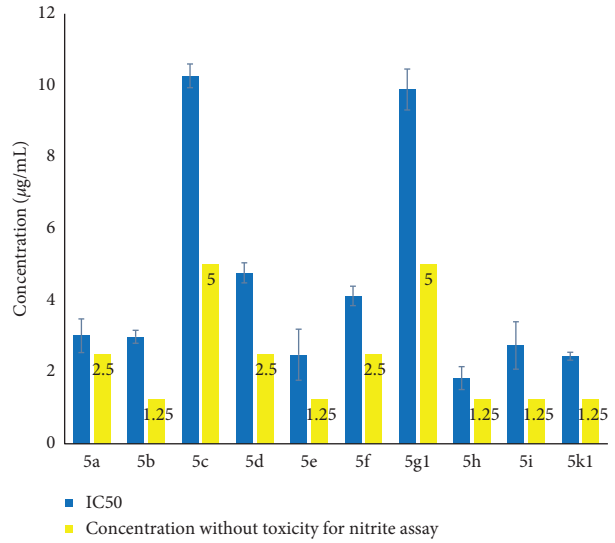


Figure 3: Inhibitory concentrations of test compounds on the viability of RAW264.7 macrophages.

Table 1: Nitric oxide inhibitory activity of test compounds on RAW 264.7 macrophages.

Sample's name**	Test concentration (μg/mL)	NO inhibition* (%)	Cell survival* (%)	NO half-maximal inhibitory concentration (IC_{50}, μM)
(+) control	0.39	62.46 ± 1.27	98.22 ± 1.56	0.87 ± 0.07
5a	2.5	67.24 ± 1.78	96.91 ± 1.71	4.30 ± 0.18
5b	1.25	67.68 ± 0.81	93.01 ± 2.14	2.64 ± 0.09
5c	5	69.77 ± 0.52	96.70 ± 1.05	8.39 ± 0.21
5d	2.5	77.93 ± 0.88	93.48 ± 2.38	3.54 ± 0.11
5e	1.25	59.74 ± 3.04	96.52 ± 0.93	2.56 ± 0.07
5f	2.5	77.22 ± 1.30	94.11 ± 1.89	3.35 ± 0.13
5g1	5	75.43 ± 1.57	97.50 ± 0.47	7.88 ± 0.05
5h	1.25	67.93 ± 2.25	93.57 ± 1.56	1.48 ± 0.10
5i	1.25	0	96.87 ± 1.72	—
5k	1.25	65.84 ± 0.92	97.41 ± 2.61	2.06 ± 0.12

*Data represent the mean ± standard deviation of three independent wells. **Positive control: dexamethasone 1 mM (Merck, Germany).

Table 2: α-Glucosidase inhibitory activity of the synthesized products.

Sample's name	Test concentration (μg/mL)	Inhibition (%)	IC_{50} (mM)
Voglibose*	100	60.33 ± 2.78	0.35 ± 0.06
5a	200	50.05 ± 1.65	0.47 ± 0.01
5b	200	0	—
5c	200	0	—
5d	200	45.50 ± 1.29	—
5e	200	94.92 ± 1.82	0.21 ± 0.02
5f	200	40.48 ± 1.14	—
5g1	200	0	—
5h	200	0	—
5i	200	69.03 ± 1.77	0.41 ± 0.04
5k1	200	43.33 ± 2.06	—

*Voglibose (Merck, Germany) was employed as a positive sample.

cancer cell lines except for **5c** and **5g1** that contained heterocycles 2-mercaptopyrimidine and 2-mercaptoimidazole. Among synthesized thioethers, the conjugate of DHA with 6-mercaptopurine **5h** exhibited the most potent activity against HepG2 and LU-1 cell lines with the IC_{50} of 2.73 and 13.06 μM, that is about 6-fold to 8-fold stronger than that of DHA. Notably, compounds **5a** and **5e** displayed an effect in all three tested assays.

Table 3: In vitro cytotoxic activity of the synthesized conjugates.

No.	Compounds	IC_{50} (μM)	
		Hep-G2	LU-1
1	**5a**	10.76 ± 0.13	41.73 ± 0.59
2	**5b**	11.33 ± 0.65	41.90 ± 0.25
3	**5c**	—	—
4	**5d**	5.75 ± 0.51	21.90 ± 0.14
5	**5e**	5.70 ± 0.33	20.86 ± 0.09
6	**5f**	6.34 ± 0.10	22.36 ± 0,24
7	**5g1**	—	—
8	**5h**	2.73 ± 0.06	13.06 ± 0,15
9	**5i**	11.09 ± 0.32	33.74 ± 0,51
10	**5k1**	10.43 ± 0.30	38.60 ± 0,29
11	DHA	21.00 ± 0.91	79,63 ± 0.68
12	Ellipticine*	1.06 ± 0,05	1.38 ± 0,12

*Ellipticine (Merck, Germany) was employed as a positive control.

4. Conclusions

A convenient procedure was applied to prepare novel derivatives of DHA containing both C-O-C and C-S-C linkages. Anomalies of the signal in NMR spectra were discussed and explained by the tautomerization of thiols bearing imidazole and benzimidazole rings. Screening for the *in vitro* cytotoxicity has indicated that almost all the synthesized compounds had a stronger activity than DHA against HepG2 and LU-1 cancer cell lines. For evaluation of *in vitro* anti-inflammatory activity, apart from **5c**, nine out of ten tested products showed NO production inhibition with the IC_{50} values ranging from 1.48 to 8.39 μM. Additionally, three compounds **5a**, **5e**, and **5i** showed inhibitory activity on α-glucosidase with the IC_{50} values of 0.47, 0.41, and 0.21 mM, respectively. In view of the above obtained findings, it is appropriate to conclude that the presence of 2-mercaptopyridine or 2-mercaptobenzothiazole can be beneficial structures for the biological activities of the conjugates of DHA in this study.

Data Availability

The data used to support the study can be made available upon request to the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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