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Short communication

Synthesis and cytotoxicity of novel artemisinin derivatives containing sulfur atoms

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ABSTRACT

Ten novel artemisinin derivatives containing sulfur atoms were designed and synthesized and their structures were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS technologies in this study. All compounds were reported for the first time. The *in vitro* cytotoxicity against PC-3, SGC-7901, A549 and MDA-MB-435s cancer cell lines was evaluated by MTT assay. Compounds **4a** and **4f** displayed potent antitumor activity against PC-3, SGC-7901 and A549 cells with IC_{50} ranging from 1.6 to 30.5 μM, which values are compared to that of 5-FU (IC_{50} from 6.8 to 42.5 μM). Compounds **4a** and **4f** showed high specificity towards human lung cancer A549 cells compared to normal human hepatic L-02 cells with selectivity index of 16.1 and 50.1 respectively. Our promising findings indicated that the compounds **4a** and **4f** could stand as potential lead compounds for further investigation.

1. Introduction

Artemisinin is extracted from the Chinese herb qinghaosu (*Artemisia annua* or annual wormwood) containing a 1, 2, 4-tiroxane ring system [1], which is a powerful antimalarial drug and has also been proved to possess antiviral [2–6], immunosuppressive [7–9] and anticancer [10–14] activities. Its anticancer activity with strong selectivity [15,16], reversing multidrug resistance [17] and sensitization radiation and chemotherapy [16,18] attracts extensive attention. Many artemisinin derivatives have been synthesized as anticancer agents [2,10,14,19–27]. However, current modification strategies have only focused on the hemiacetal structure of artemisinin due to the difficulty to introduce functionalities on the ring systems by conventional chemical methods. Biotransformation technology successfully solved the above problem. 9α-OH-dihydroartemisinin (9α-OH DHA) with double hydroxyl modification sites was obtained in our previous work [28], which make chemical modification on the ring system come true.

According to the analysis of the elemental composition of U.S. FDA approved drug architectures [29], the sulfur is the fifth most used element beyond C, H, O and N. The appearance of sulfur atom even enhance the cellular uptake percentage and the level of reactive oxygen species (ROS) [30], which is a crucial factor for the antitumor activity of artemisinins [31,32]. Herein, ten novel artemisinin ester derivatives containing sulfur atoms with alkyl or aromatic side chains were reported and *in vitro* cytotoxicity against four cancer cell lines (PC-3, SGC-7901, A549 and MDA-MB-435s) was evaluated.

2. Results and disscussion

2.1. Chemistry

9α-OH DHA was obtained according to our previous work [28,33]. The synthesis of compounds **3a**–**3e** began with *m*-bromobenzoic acid (**1**) and different thiols (**2a**–**2e**) in the presence of $Pd_2(dba)_3$ and Xantphos in 1,4-dioxane, with heating reflux for 6 h under nitrogen. The reaction mixture was acidified with acetic acid to reach pH 3–4 then filtered and concentrated. The crude product was purified by silica gel chromatography to give target compounds (**3a**–**3e**) (Scheme 1). The synthesis of compounds **4a**–**4j** was the esterification of 9α-OH-DHA and compounds **3a**–**3e** respectively using 1-Ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride (EDCI) as coupling agent and 4-dimethylaminopyridine (DMAP) as catalyst in dichloromethane (Scheme 2).

The structures of ten novel artemisinin derivatives were

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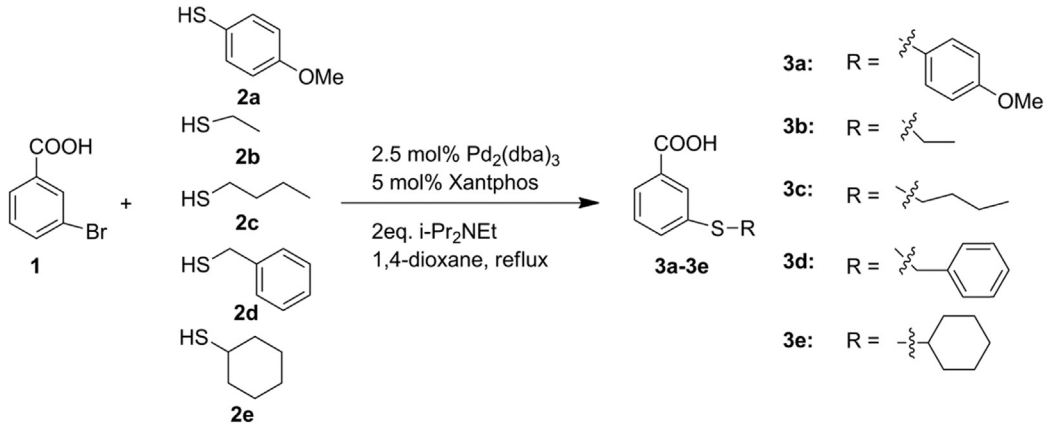
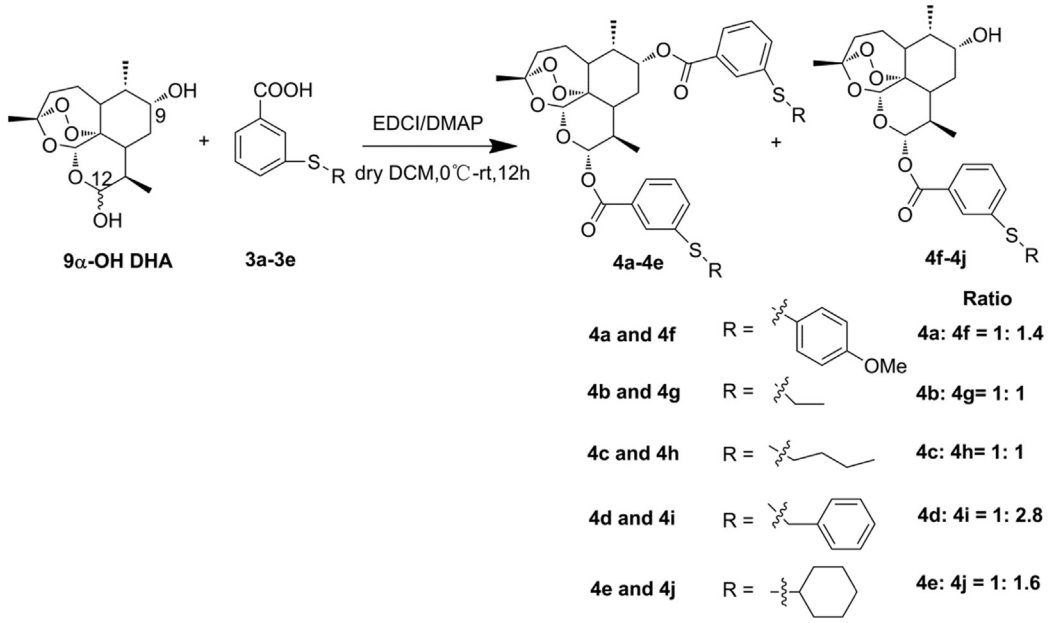


Scheme 1. The synthesis route of compounds **3a**–**3e**. Reagents and conditions: 2.5 mol% $Pd_2(dba)_3$, 5 mol% Xantphos, 2 eq. i-Pr_2NEt, 1,4-dioxane, reflux, 6 h.



Scheme 2. The synthesis route of compounds **4a**–**4j**. Reagents and conditions: EDCI/DMAP, dry DCM, 0 °C-rt, 12 h.

confirmed by ^{1}H NMR, ^{13}C NMR and HRESIMS. For compounds **4a**–**4j**, the 12α-isomer was exclusively attained, which was evident from the large coupling constants ($J > 9$ Hz) of H-11 and H-12 in ^{1}H NMR (the β-isomers, $J \approx 3$–4 Hz) [34]. Additionally, the ^{13}C NMR spectrum chemical shift of C-4 at circa 104 ppm in all compounds suggested the presence of the intact peroxide [35]. The di-esterification or mono-esterification products were determinated by the numbers of carbonyl signals in ^{13}C NMR spectrum. The HMBC correlations of H-12 or H-9 with carbonyl group indicated that acetoxy group were attached to C-12 or C-9.

2.2. Biological evaluation

The biological activities of all compounds were evaluated against selected four cancer cell lines (human prostate cancer PC-3 cells, human gastric cancer SGC-7901 cells, non-small-cell-lung cancer A549 cells and human breast cancer MDA-MB-435s cells) using the MTT assay. DHA and 5-fluorouracil (5-FU) were used as the positive controls. Compounds **3a**–**3e** showed no activity to any cancer cell lines (the data is not shown). The IC_{50} values of compounds **4a**–**4j** were showed in Table 1. The compounds **4a** and

Table 1
Cytotoxicity of compounds **4a**–**4j** against PC-3, SGC-7901, A549 and MDA-MB-435s cell lines.

Compounds	Cytotoxicity, IC_{50} (μM)			
	PC-3	SGC-7901	A549	MDA-MB-435s
4a	**30.5 ± 4.7**	**6.0 ± 1.7**	**4.3 ± 1.7**	107.6 ± 24.8
4b	30.0 ± 13.6	74.2 ± 13.4	44.4 ± 2.6	111.5 ± 25.4
4c	114.9 ± 12.6	85.0 ± 13.4	106.6 ± 42.3	NA
4d	NA	NA	223.0 ± 24.0	288.9 ± 46.6
4e	NA	NA	NA	NA
4f	**13.0 ± 5.0**	**7.1 ± 1.5**	**1.6 ± 0.6**	123.7 ± 42.8
4g	NA	NA	1435.0 ± 485.3	119.9 ± 14.3
4h	464.4 ± 27.9	172.8 ± 18.7	96.1 ± 14.6	NA
4i	146.2 ± 20.2	NA	95.0 ± 11.1	223.0 ± 37.1
4j	204.4 ± 38.1	553.3 ± 62.2	33.3 ± 11.6	29.60 ± 5.4
DHA	NA	NA	80.4 ± 5.8	21.9 ± 1.1
5-FU	42.5 ± 3.7	11.9 ± 1.3	6.8 ± 0.8	15.2 ± 0.9

NA: no activity.
The bold values idicate a relatively good antitumor activity.

4f displayed potent cytotoxicity against PC-3, SGC-7901 and A549 cell lines with IC_{50} ranging from 1.6 to 30.5 μM. Other derivatives showed poor or no cytotoxic activities against the selected four cancer cell lines. To further investigate the specificity between the cancer cell and the normal cell lines, the cytotoxicity of compounds **4a** and **4f** against human normal hepatic L-02 cells were examined. In A549 cells, compounds **4a** and **4f** were 4.2 fold and 13.2 fold less cytotoxic than that of 5-FU respectively. In SGC-7901 cells, compounds **4a** and **4f** were 5.2 fold and 5.1 fold less cytotoxic than that of 5-FU respectively (Table 2).

The structure activity relationship analysis revealed no clear trend for the effects of the side chains on the cytotoxicity of compounds **4a**–**4j**. Compounds **4a** and **4f** with the *p*-methoxythiophenyl substituent group on benzoic acid showed potent cytotoxicity implied that the different substituents of benzoic acid have a significant impact on cytotoxicity. The cytotoxicity of compound **4b** with ethylthio substituent group was better than that of compound **4c** with butylthio substituent group, which manifested the length of alkyl carbon chain might have a negative effect on the antitumor activity.

3. Conclusions

In conclusion, ten novel artemisinin derivatives containing sulfur atoms were synthesized and evaluated for their cytotoxicity against selected four human cancer cell lines. Compounds **4a** and **4f** with the *p*-methoxythiophenyl substituent group on benzoic acid were potent cytotoxicity and high specificity. The structure-activity relationship study revealed that the substitutes on benzoic acid and the length of alkyl carbon chain had an impact on their cytotoxicity. Our results indicated compounds **4a** and **4f** could be further investigated as antitumor drug candidates.

4. Experimental section

4.1. Materials and measurements

Melting points (mp) were determined in duplicate on an X-4 digital display microscope melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra was obtained in $CDCl_3$ solution on a Brucker Avance-400 or Brucker 300 spectrophotometer using TMS as the internal standard. High resolution ESI mass spectra were measured on Aglient LC-Q-TOF-MS 6520. Column chromatography was done using silica gel (Qingdao Marine Chemical Co., Ltd., China), or Sephadex LH-20 (GE healthcare, Sweden).

All commercially available reagents were used without further purification unless otherwise stated. The progress of the reactions was monitored by analytical thin layer chromatography (TLC) performed on homemade HSGF254 precoated silica gel plates. Visualization was performed by UV or development using vanillin solution in sulfuric acid and ethanol (4/1 v/v). Yields were reported after chromatographic purification.

4.2. Preparation of the compounds

4.2.1. General procedure of synthesis of compounds *3a*–*3e*

The compounds **3a**–**3e** were obtained by palladium-catalyzed coupling according to reference [36]. To a three-necked flask (100 mL) were added 3-bromobenzoic acid (2 mmol), i-Pr_2NEt (4 mmol), dry 1,4-dioxane (8.0 mL). The mixture was evacuated and backfilled with nitrogen (3 cycles). Catalyst $Pd_2(dba)_3$ (0.05 mmol), Xantphos (0.1 mmol) and corresponding thiols (2 mmol) were added and then the mixture was degassed twice more. The mixture was heated to reflux for 6 h. Then the reaction mixture was allowed to reach ambient temperature and acidified with acetic acid to reach pH 3–4, filtered and concentrated. The crude product was purified by column chromatography on silica gel to afford the target compounds.

*4.2.1.1. 3-(4′-Methoxyphenyl)sulfanylbenzoic acid (**3a**).* Yield: 60%. White solid. mp. 120–121 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.89–7.86 (m, 1H), 7.84 (ddd, *J* = 7.9, 5.6, 3.7 Hz, 1H), 7.49–7.43 (m, 2H), 7.37–7.29 (m, 2H), 6.99–6.88 (m, 2H), 3.85 (s, 3H). The ^{1}H NMR data were consistent with the reported data [36]. HR-ESI-MS *m*/*z* 259.0437 $[M - H]^-$, (calcd for $C_{14}H_{11}O_3S$, 259.0434).

*4.2.1.2. 3-(Ethylthio)benzoic acid (**3b**).* Yield: 66%. White solid. mp. 107–110 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.26 (t, *J* = 1.7 Hz, 1H), 7.93–7.88 (m, 1H), 7.55 (ddd, *J* = 7.8, 1.9, 1.1 Hz, 1H), 7.40 (t, *J* = 9.4 Hz, 1H), 3.01 (q, *J* = 7.4 Hz, 2H), 1.35 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 171.68, 137.98, 133.87, 131.21, 129.90, 128.81, 128.91, 27.38, 14.18. HR-ESI-MS *m*/*z* 181.0321 $[M - H]^-$, (calcd for $C_9H_9O_2S$, 181.0329).

*4.2.1.3. 3-(Butylthio)benzoic acid (**3c**).* Yield: 50%. White solid. mp. 135–138 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.04 (t, *J* = 1.7 Hz, 1H), 7.92–7.88 (m, 1H), 7.54 (ddd, *J* = 7.8, 1.8, 1.1 Hz, 1H), 7.38 (t, *J* = 7.8 Hz, 1H), 2.99 (t, *J* = 7.3 Hz, 2H), 1.66 (ddd, *J* = 12.6, 8.4, 6.4 Hz, 2H), 1.48 (dq, *J* = 14.4, 7.3 Hz, 2H), 0.94 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 171.99, 138.38, 133.69, 129.94, 129.63, 128.87, 127.23, 32.96, 31.01, 21.95, 13.62. HR-ESI-MS *m*/*z* 209.0660 $[M - H]^-$, (calcd for $C_{11}H_{13}O_2S$, 209.0642).

*4.2.1.4. 3-(benzylthio)benzoic acid (**3d**).* Yield: 52%. White solid. mp. 128–130 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.07 (s, 1H), 7.91 (d, *J* = 7.7 Hz, 1H), 7.50 (d, *J* = 7.9 Hz, 1H), 7.39–7.27 (m, 6H), 4.18 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 171.17, 137.47, 136.81, 134.67, 130.81, 129.89, 128.90, 128.86 (×2), 128.59 (×2), 127.93, 127.39, 38.70. HR-ESI-MS *m*/*z* 243.0495 $[M - H]^-$, (calcd for $C_{14}H_{11}O_2S$, 243.0485).

*4.2.1.5. 3-(cyclohexylthio)benzoic acid (**3e**).* Yield: 79%. White solid. mp. 84–86 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.13 (t, *J* = 1.6 Hz, 1H), 7.97–7.92 (m, 1H), 7.62 (ddd, *J* = 7.8, 1.9, 1.2 Hz, 1H), 7.40 (q, *J* = 7.7 Hz, 1H), 3.24–3.15 (m, 1H), 2.05–1.96 (m, 2H), 1.83–1.72 (m, 2H), 1.63 (dd, *J* = 10.5, 4.3 Hz, 1H), 1.47–1.20 (m, 5H). ^{13}C NMR

Table 2
Cytotoxicity of compounds **4a** and **4f** on human normal hepatic L-02 cells.

Compounds	IC_{50} (μM)			SI ($IC_{50}^{L-02}/IC_{50}^{A549}$)	SI ($IC_{50}^{L-02}/IC_{50}^{SGC-7901}$)
	A549	SGC-7901	L-02		
4a	4.3 ± 1.7	6.0 ± 1.7	69.2 ± 19.6	**16.1**	11.5
4f	1.6 ± 0.6	7.1 ± 1.5	80.2 ± 5.9	**50.1**	11.3
DHA	80.4 ± 5.8	NA	145.9 ± 3.6	1.8	
5-FU	6.8 ± 0.8	11.9 ± 1.3	25.9 ± 2.3	3.8	2.2

NA: no activity.
The bold values idicate a relatively good antitumor activity.

(100 MHz, $CDCl_3$) δ = 171.51, 136.69, 136.48, 132.80, 129.87, 128.86, 128.18, 46.52, 33.25 (×2), 25.97 (×2), 25.72. HR-ESI-MS *m/z* 235.0814 $[M - H]^-$, (calcd for $C_{13}H_{15}O_2S$, 235.0798).

*4.2.2. General procedure of synthesis of compounds **4a**–**4j***

To a three-necked flask (100 mL) that contained a solution of 9α-OH DHA (1 equiv), 4-dimethyaminopyridine (DMAP, 2.6 equiv) and 1-Ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride (EDCI, 3 equiv) in dry CH_2Cl_2 (20 mL) was added the corresponding **3a**–**3e** (2.6 equiv) at ice bath. The resulting mixture was stirred at 0 °C-rt for 12 h, the solvent was moved. Then the residue was taken up with EtOAc, washed with water (20 mL) and saturated NaCl solution (20 mL), dried over anhydrous $MgSO_4$, and purified using column chromatography to obtain the target compounds (Scheme 2).

*4.2.2.1. Dihydroartemisinin 9α,12α-di-[3-(p-methoxyphenyl)thio]-benzoate (**4a**).* Yield: 31.0%. White powder. mp. 75 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.89–7.82 (m, 3H), 7.80–7.74 (m, 1H), 7.47–7.41 (m, 4H), 7.30 (td, *J* = 5.3, 1.9 Hz, 4H), 6.95–6.89 (m, 4H), 5.92 (d, *J* = 9.8 Hz, 1H), 5.61 (s, 1H), 4.68 (td, *J* = 10.8, 4.4 Hz, 1H), 3.83 (s, 3H), 3.82 (s, 3H), 2.79–2.66 (m, 1H), 2.41 (dd, *J* = 18.5, 8.8 Hz, 1H), 2.18 (ddd, *J* = 15.0, 9.7, 5.3 Hz, 1H), 2.14–2.06 (m, 1H), 2.00–1.88 (m, 2H), 1.78–1.51 (m, 4H), 1.44 (s, 3H), 0.99 (d, *J* = 6.2 Hz, 3H), 0.86 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.64, 164.71, 160.26, 160.23, 140.08, 139.89, 135.92 (×2), 135.90 (×2), 132.46, 132.24, 130.72, 130.19, 129.00, 128.87, 128.74, 128.71, 127.25, 126.68, 123.18, 123.10, 115.40 (×4), 104.58, 92.34, 91.22, 79.08, 76.26, 55.40 (×2), 49.09, 42.77, 41.33, 36.08, 31.63, 27.48, 25.88, 24.54, 15.44, 12.05. HR-ESI-MS *m/z* 807.2262 $[M+Na]^+$, (calcd for $C_{43}H_{44}NaO_{10}S_2$, 807.2268).

*4.2.2.2. Dihydroartemisinin 9α,12α-di-(3-ethylthio)-benzoate (**4b**).* Yield: 40.3%. White powder. mp. 69–72 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.21 (dt, *J* = 26.8, 1.7 Hz, 2H), 8.06–7.96 (m, 2H), 7.76–7.68 (m, 2H), 7.40–7.30 (m, 2H), 6.01 (d, *J* = 9.7 Hz, 1H), 5.65 (s, 1H), 4.76 (td, *J* = 10.8, 4.3 Hz, 1H), 3.01 (q, *J* = 7.4 Hz, 2H), 2.99 (q, *J* = 7.5 Hz, 2H), 2.85–2.74 (m, 1H), 2.43 (dt, *J* = 14.4, 3.8 Hz, 1H), 2.26–2.17 (m, 1H), 2.12 (t, *J* = 12.4 Hz, 1H), 2.00–1.91 (m, 2H), 1.83–1.51 (m, 4H), 1.46 (s, 3H), 1.34 (t, *J* = 11.4 Hz, 3H), 1.32 (t, *J* = 11.4 Hz, 3H), 1.03 (d, *J* = 6.3 Hz, 3H), 0.93 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.73, 164.81, 136.68, 136.38, 136.16, 133.18, 132.60, 130.07 (×2), 128.73, 128.18, 127.41, 122.60, 122.46, 104.65, 92.37, 91.27, 79.02, 76.53, 49.07, 42.79, 41.33, 36.08, 31.68, 29.69, 27.55 (×2), 25.86, 24.55, 15.50, 14.20 (×2), 12.11. HR-ESI-MS *m/z* 651.2061 $[M+Na]^+$, (calcd for $C_{33}H_{40}NaO_8S_2$, 651.2057).

*4.2.2.3. Dihydroartemisinin 9α,12α-di-(3-butylthio)-benzoate (**4c**).* Yield: 30.3%. White powder. mp. 115–118 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.00 (dt, *J* = 24.0, 1.6 Hz, 2H), 7.92–7.86 (m, 1H), 7.84–7.80 (m, 1H), 7.54–7.48 (m, 2H), 7.36 (dd, *J* = 17.1, 7.8 Hz, 2H), 6.00 (d, *J* = 9.8 Hz, 1H), 5.65 (s, 1H), 4.74 (td, *J* = 10.9, 4.4 Hz, 1H), 2.97 (q, *J* = 5.6 Hz, 4H), 2.83–2.73 (m, 1H), 2.49–2.38 (m, 1H), 2.22 (dt, *J* = 12.8, 4.4 Hz, 1H), 2.10 (dd, *J* = 15.0, 3.8 Hz, 1H), 2.03–1.91 (m, 2H), 1.77 (td, *J* = 10.8, 6.1 Hz, 1H), 1.72–1.55 (m, 9H), 1.51–1.46 (m, 2H), 1.45 (s, 3H), 1.04 (d, *J* = 6.3 Hz, 3H), 0.92 (d, *J* = 7.2 Hz, 3H), 0.93 (t, *J* = 7.3 Hz, 3H), 0.94 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.74, 164.82, 138.25, 138.05, 133.45, 133.12, 130.67, 130.12, 129.86, 129.32, 128.86, 128.73, 127.27, 126.67, 104.60, 92.36, 91.27, 79.10, 76.22, 49.12, 42.82, 41.38, 36.09, 33.13, 33.09, 31.68, 31.08, 31.05, 27.55, 25.88, 24.56, 21.93 (×2), 15.49, 13.63, 13.61, 12.12. HR-ESI-MS *m/z* 707.2700 $[M+Na]^+$, (calcd for $C_{37}H_{48}NaO_8S_2$, 707.2683).

*4.2.2.4. Dihydroartemisinin 9α,12α-di-(3-benzylthio)-benzoate (**4d**).* Yield: 17.2%. White powder. mp. 72 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.00 (dt, *J* = 33.1, 1.7 Hz, 2H), 7.93–7.89 (m, 1H), 7.86–7.82 (m, 1H), 7.47 (tdd, *J* = 8.8, 1.9, 1.1 Hz, 2H), 7.36–7.21 (m, 12H), 6.00 (d, *J* = 9.8 Hz, 1H), 5.65 (s, 1H), 4.73 (td, *J* = 10.9, 4.4 Hz, 1H), 4.18 (s, 2H), 4.15 (s, 2H), 2.82–2.72 (m, 1H), 2.50–2.40 (m, 1H), 2.20 (dt, *J* = 12.9, 4.4 Hz, 1H), 2.11 (dt, *J* = 7.2, 6.2 Hz, 1H), 2.02–1.92 (m, 2H), 1.79–1.54 (m, 4H), 1.46 (s, 3H), 1.01 (d, *J* = 6.3 Hz, 3H), 0.90 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.58, 164.67, 137.35, 137.16, 136.91, 134.49, 134.16, 130.90, 130.66, 130.36, 130.11, 128.84 (×6), 128.73, 128.57 (×4), 127.97, 127.40, 127.35 (×2), 104.61, 92.34, 91.26, 79.07, 76.18, 49.09, 42.78, 41.36, 38.81 (×2), 36.09, 31.66, 27.52, 25.88, 24.57, 15.51, 12.12. HR-ESI-MS *m/z* 775.2388 $[M+Na]^+$, (calcd for $C_{43}H_{44}NaO_8S_2$, 775.237).

*4.2.2.5. Dihydroartemisinin 9α,12α-di-(3-cyclohexylthio)-benzoate (**4e**).* Yield: 16.7%. White powder. mp. 95–97 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.08 (dt, *J* = 18.4, 1.7 Hz, 2H), 7.91 (dd, *J* = 20.2, 7.7 Hz, 2H), 7.63–7.53 (m, 2H), 7.36 (q, *J* = 7.7 Hz, 2H), 6.01 (d, *J* = 9.8 Hz, 1H), 5.65 (s, 1H), 4.74 (td, *J* = 10.7, 4.3 Hz, 1H), 3.23–3.11 (m, 2H), 2.86–2.72 (m, 1H), 2.52–2.35 (m, 1H), 2.24 (dt, *J* = 12.7, 4.4 Hz, 1H), 2.06–1.93 (m, 8H), 1.78 (d, *J* = 4.6 Hz, 7H), 1.62 (d, *J* = 10.4 Hz, 6H), 1.45 (s, 3H), 1.43–1.22 (m, 6H), 1.04 (d, *J* = 6.2 Hz, 3H), 0.93 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 165.67, 164.75, 136.43, 136.34, 136.26, 136.16, 132.92, 132.46, 130.63, 130.09, 128.79, 128.65, 128.17, 127.60, 104.56, 92.35, 91.25, 79.07, 76.24, 49.12, 46.61, 42.81, 41.36, 36.08, 33.24(×3), 33.20(×3), 31.66, 27.52, 25.91 (×3), 25.85, 25.69 (×2), 24.54, 15.45, 12.09. HR-ESI-MS *m/z* 759.3000 $[M+Na]^+$, (calcd for $C_{41}H_{52}NaO_8S_2$, 759.2996).

*4.2.2.6. 9α-Hydroxy-dihydroartemisinin 12α-[3-(p-methoxyphenyl) thio]-benzoate (**4f**).* Yield: 44.8%. White powder. mp. 64 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.90 (d, *J* = 0.9 Hz, 1H), 7.89–7.85 (m, 1H), 7.47–7.40 (m, 2H), 7.33–7.27 (m, 2H), 6.97–6.88 (m, 2H), 5.95 (d, *J* = 9.8 Hz, 1H), 5.57 (s, 1H), 3.84 (s, 3H), 3.25 (td, *J* = 10.6, 4.3 Hz, 1H), 2.77–2.58 (m, 1H), 2.40 (td, *J* = 14.4, 3.9 Hz, 1H), 2.09 (ddd, *J* = 8.9, 4.5, 1.7 Hz, 1H), 1.90 (ddd, *J* = 16.4, 8.6, 5.1 Hz, 1H), 1.83 (dd, *J* = 9.3, 4.9 Hz, 1H), 1.64–1.47 (m, 3H), 1.44 (s, 3H), 1.40–1.29 (m, 2H), 1.08 (d, *J* = 6.1 Hz, 3H), 0.88 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 164.75, 160.21, 139.85, 135.91, 135.88, 132.44, 130.24, 129.18, 128.87, 127.29, 123.18, 115.22 (×2), 104.54, 92.50, 91.31, 79.45, 73.81, 55.40, 49.10, 44.15, 42.99, 36.12, 31.60, 31.07, 25.90, 24.49, 15.43, 12.15. HR-ESI-MS *m/z* 565.1858 $[M+Na]^+$, (calcd for $C_{29}H_{34}NaO_8S$, 565.1867).

*4.2.2.7. 9α-Hydroxy-dihydroartemisinin 12α-(3-ethylthio)-benzoate (**4g**).* Yield: 42.5%. White powder. 75–77 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.26–7.88 (m, 1H), 8.08–8.01 (m, 1H), 7.72–7.48 (m, 1H), 7.42–7.30 (m, 1H), 6.01 (d, *J* = 9.7 Hz, 1H), 5.65 (s, 1H), 4.76 (td, *J* = 10.8, 4.3 Hz, 1H), 3.00 (q, *J* = 7.4 Hz, 2H), 2.84–2.72 (m, 1H), 2.39 (dt, *J* = 14.4, 3.7 Hz, 1H), 2.26–2.16 (m, 1H), 2.10 (d, *J* = 14.4 Hz, 1H), 1.97 (dd, *J* = 21.4, 11.6 Hz, 2H), 1.85–1.53 (m, 4H), 1.45 (s, 3H), 1.32 (t, *J* = 7.8 Hz, 3H), 1.03 (d, *J* = 6.3 Hz, 3H), 0.91 (t, *J* = 8.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 164.83, 136.35, 133.59, 133.02, 129.93, 127.40, 122.45, 104.58, 92.71, 91.37, 79.46, 73.80, 49.11, 44.16, 42.98, 36.12, 31.65, 31.09, 27.55, 25.89, 24.50, 15.43, 14.21, 12.21. HR-ESI-MS *m/z* 487.1712 $[M+Na]^+$, (calcd for $C_{24}H_{32}NaO_7S$, 487.1761).

*4.2.2.8. 9α-Hydroxy-dihydroartemisinin 12α-(3-butylthio)-benzoate (**4h**).* Yield: 33.7%. White powder. mp. 72–75 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.04 (t, *J* = 1.6 Hz, 1H), 7.94–7.87 (m, 1H), 7.51 (ddd, *J* = 7.8, 1.7, 1.1 Hz, 1H), 7.35 (t, *J* = 7.8 Hz, 1H), 6.01 (d, *J* = 9.8 Hz, 1H), 5.57 (s, 1H), 3.25 (td, *J* = 10.7, 4.3 Hz, 1H), 3.02–2.92 (m, 2H), 2.83–2.67 (m, 1H), 2.48–2.31 (m, 1H), 2.07 (dt, *J* = 13.7, 4.2 Hz, 2H), 1.86 (m, 2H), 1.71–1.45 (m, 6H), 1.43 (s, 3H), 1.40–1.24 (m, 2H), 1.10 (d, *J* = 5.9 Hz, 3H), 0.94 (d, *J* = 7.1 Hz, 3H), 0.95 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 164.84, 138.03, 133.42, 130.19, 129.85,

128.73, 127.26, 104.54, 92.50, 91.36, 79.46, 73.81, 49.11, 44.17, 43.01, 36.14, 33.13, 31.65, 31.12, 31.07, 25.89, 24.50, 21.93, 15.43, 13.61, 12.21. HR-ESI-MS *m/z* 515.2078 $[M+Na]^+$, (calcd for $C_{26}H_{36}NaO_7S$, 515.2074).

*4.2.2.9. 9α-Hydroxy-dihydroartemisinin 12α-(3-benzylthio)-benzoate (**4i**).* Yield: 47.4%. White powder. mp. 83 °C. ^1H NMR (300 MHz, $CDCl_3$) δ = 8.05 (s, 1H), 7.91 (d, *J* = 7.8 Hz, 1H), 7.46 (d, *J* = 7.8 Hz, 1H), 7.31 (dd, *J* = 8.8, 5.6 Hz, 6H), 5.99 (d, *J* = 9.8 Hz, 1H), 5.57 (s, 1H), 4.16 (s, 2H), 3.25 (td, *J* = 10.6, 4.2 Hz, 1H), 2.80–2.66 (m, 1H), 2.40 (dt, *J* = 14.8, 3.7 Hz, 1H), 2.12–1.99 (m, 2H), 1.96–1.77 (m, 2H), 1.61–1.45 (m, 2H), 1.44 (s, 3H), 1.41–1.24 (m, 2H), 1.08 (t, *J* = 7.4 Hz, 3H), 0.91 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 164.66, 137.13, 136.93, 134.45, 130.89, 130.77, 130.19, 128.82, 128.69, 128.55, 127.95, 127.84, 127.31, 104.51, 92.48, 91.33, 79.41, 73.82, 49.11, 44.16, 43.00, 38.82, 36.13, 31.61, 31.10, 25.85, 24.48, 15.39, 12.16. HR-ESI-MS *m/z* 549.1925 $[M+Na]^+$, (calcd for $C_{29}H_{34}NaO_7S$, 549.1917).

*4.2.2.10. 9α-Hydroxy-dihydroartemisinin 12α-(3-cyclohexylthio)-benzoate (**4j**).* Yield: 26.8%. White powder. mp. 90–92 °C. ^1H NMR (300 MHz, $CDCl_3$) δ = 8.11 (dd, *J* = 4.5, 2.9 Hz, 1H), 7.98–7.91 (m, 1H), 7.61–7.55 (m, 1H), 7.37 (q, *J* = 7.4 Hz, 1H), 6.00 (d, *J* = 9.8 Hz, 1H), 5.57 (s, 1H), 3.32–3.10 (m, 2H), 2.83–2.68 (m, 1H), 2.40 (dt, *J* = 14.4, 4.0 Hz, 1H), 2.11–1.95 (m, 5H), 1.92–1.72 (m, 4H), 1.67–1.47 (m, 4H), 1.43 (s, 3H), 1.41–1.24 (m, 5H), 1.10 (d, *J* = 5.9 Hz, 3H), 0.94 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 164.77, 136.40, 136.15, 132.90, 130.16, 128.65, 128.16, 104.50, 92.48, 91.33, 79.42, 73.81, 49.11, 46.60, 44.16, 43.01, 36.13, 33.24, 33.21, 31.62, 31.11, 25.92 (×2), 25.86, 25.70, 24.48, 15.39, 12.17. HR-ESI-MS *m/z* 541.2224 $[M+Na]^+$ (calcd for $C_{28}H_{38}NaO_7S$, 541.223).

4.3. Biological activity assays

The cytotoxicity of all compounds was assessed using the PC-3, SGC-7901, A549 and MDA-MB-435s cancer cell lines and normal hepatic L-02 cell lines via the MTT assay. The cells were grown in DMEM supplemented with 10% fetal bovine serum and cultured at a density of 5000 cells/well in a 96-well microtiter plate. Five different concentrations of each compound in DMSO were subsequently added to the wells. Each concentration was tested in triplicate. After incubation under 5% CO_2 at 37 °C for 72 h, 20 μL of MTT (5 mg/mL) was added to each well, and the cells were incubated for another 4 h. Then, the liquid in each well was removed, and DMSO (150 μL) was added. The absorbance (OD values) at 570 nm with a 650 nm reference was measured on an Epoch Microplate Spectrophotometer.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.08.015.

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