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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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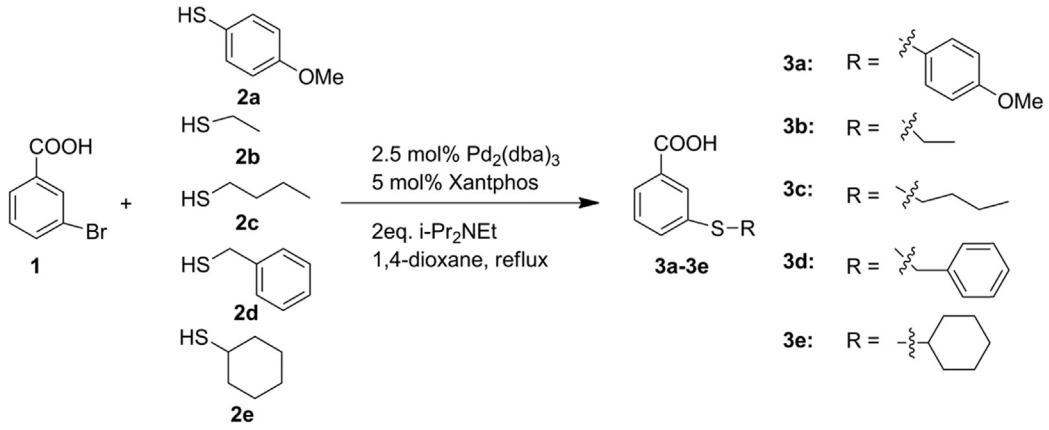
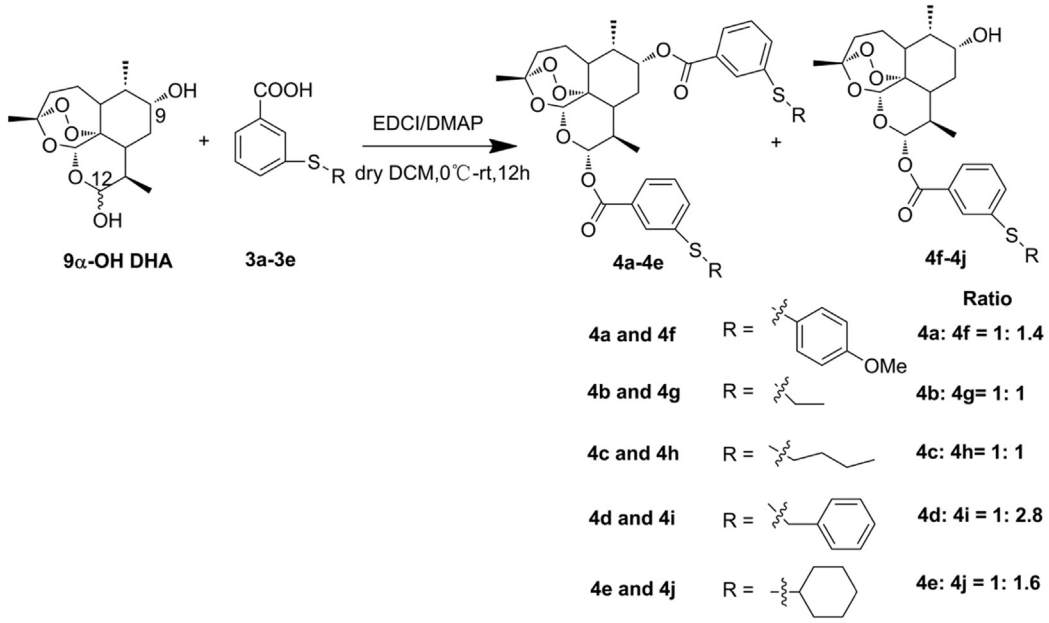


Scheme 1. The synthesis route of compounds **3a**–**3e**. Reagents and conditions: 2.5 mol% $Pd_2(dba)_3$, 5 mol% Xantphos, 2 eq. i-Pr_2NEt, 1,4-dioxane, reflux, 6 h.



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.