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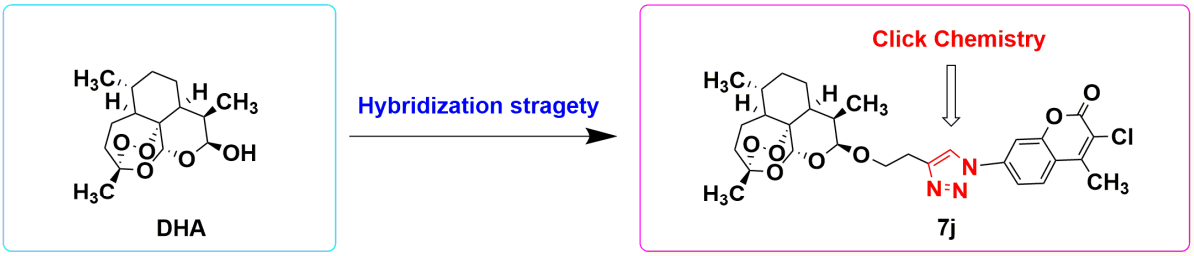

HT-29
Normoxia: IC_{50} >100 uM
Hypoxia: IC_{50} = 18.52 uM

HT-29
Normoxia: IC_{50} = 1.5 uM
Hypoxia: IC_{50} = 0.01 uM

Design, synthesis, cytotoxicity and mechanism of novel dihydroartemisinin-coumarin hybrids as potential anti-cancer agents

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Abbreviations

DHA, Dihydroartemisinin; Carbonic anhydrases, CA; Acetazolamide, AAZ; doxorubicin, DOX.

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Figures: 10

Scheme: 2

Author contributions

Haonan Yu performed experiments, analyzed data and drafted the manuscript. Zhuang Hou performed experiments, analyzed data and revised the manuscript. Ye tian performed experiments. Yanhua Mou and Chun Guo conceived the work, gave critical comments and revised the manuscript.

Abstract

To develop novel agents with anticancer activities, thirty-four new dihydroartemisinin-coumarin hybrids were designed and synthesized in this study. Those compounds were identified that had great anticancer activity against two cancer cell lines (MDA-MB-231 and HT-29). The structure-activity relationships of the derivatives were also discussed, and the results of docking analysis had shown that carbonic anhydrases IX (CA IX) was very likely to be one of the drug targets of the hybrids. Meanwhile, to clarify the mechanism of the anticancer activity of the hybrids molecule, we did further exploration in the bioactivity of the hybrids. The results had shown that these derivatives obviously inhibited proliferation of HT-29 cell lines, arrested G_0/G_1 phase of HT-29 cells, suppressed the migration of tumor cells, and induced a great decrease in mitochondrial membrane potential leading to apoptosis of cancer cells. Interestingly, the hybrids also induced the other cell death pathway-ferroptosis.

Keywords: anticancer; artemisnin; coumarin; hybrid; apoptosis; ferroptosis.

1. Introduction:

Artemisinin, a well-known anti-malarial agent, has been reported to possess a potent and broad antitumor spectrum against human cancer cell lines. Numbers of the artemisinin derivatives had been designed and synthesized and some of them have shown excellent anticancer activities and pharmacokinetic properties [1-5]. Some novel artemisinin derivatives could even overcome multidrug resistance in cancer therapy [6]. Meanwhile, the coumarin derivatives have already been extensively studied as anticoagulant, anti-inflammatory [7, 8], antibacterial [9] and anticancer [10, 11] agents. As the anticancer agents, it selectively target CA IX, by which it inhibited tumor cells [12-15]. Owing to the rapid proliferation, the tumor cells had always been in hypoxia condition along with low pH values. The main function of CA IX is to homeostasis an acidification of extracellular environment that its pH of tumor tissues was about 6.0-6.5 while that of normal tissue was about 7.4. CA IX catalyzed the reaction of carbon dioxide hydration to yield HCO_3^- and H^+. HCO_3^- was transferred into the tumor cells to neutralize H^+ and maintained appropriate micro-environment of tumor tissue through the anion transporter and Na^+/HCO_3^- co-transporter [16-18].

Previously, four series of compounds were obtained referring to the principle of hybridization (**Fig. 1**). Each of them contained coumarin moiety, dihydroartemisinin (DHA) moiety and a triazole ring linker. The cytotoxic activities of those compounds under anoxic condition were 1 to 10 times than that under the normoxic condition. Some compounds had selectivity in inhibiting HT-29 cells (overexpressed CA IX) and compound **A** (**Fig. 1**) was considered a better cytotoxic activity than others *via* experiment [19]. In this article, we designed and synthesized of new molecular hybrids of DHA and coumarin tethered directly or by the alkane chain other than triazole ring. The synthetic approaches have been designed as follows (**Fig. 2**):

(i) no linker

(ii) ethyl and propyl as the linker

(ii) linkers containing triazole ring

Herein, a variety of novel dihydroartemisinin-coumarin molecules containing different linkers (**1b-1e, 2a-2e, 3a-3e, 4a-4d, 4f, 5a-5d, 5f, 6g-6k and 7g-7k**) were synthesized and were also evaluated for their cytotoxic and anti-tumor activities. The structure-activity relationships of dihydroartemisinin-coumarin hydrids were discussed. In addition, a series of pharmacological activity

of compound **A** was tested to reveal the mechanism of the dihydroartemisinin-coumarin.

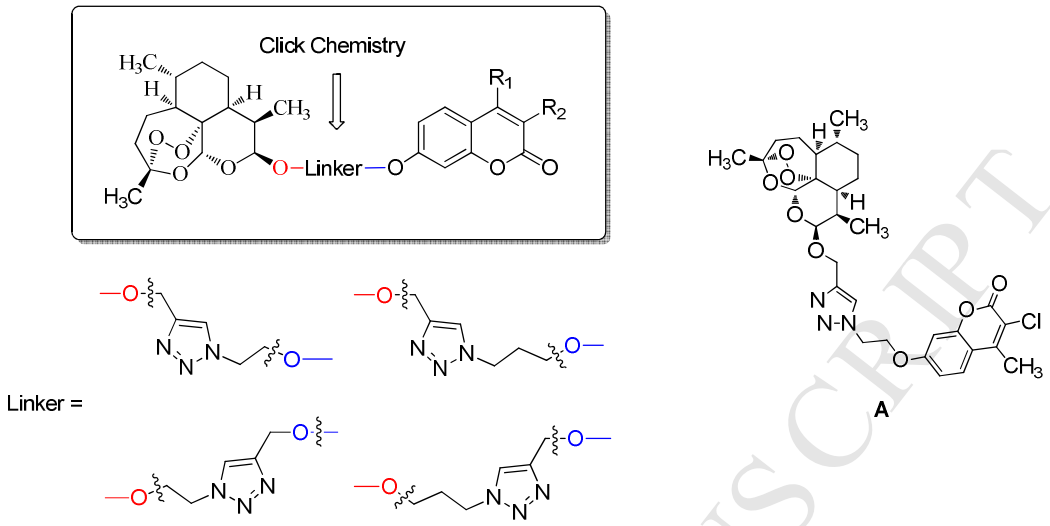


Fig. 1. Structures of previous dihydroartemisinin-coumarin molecules

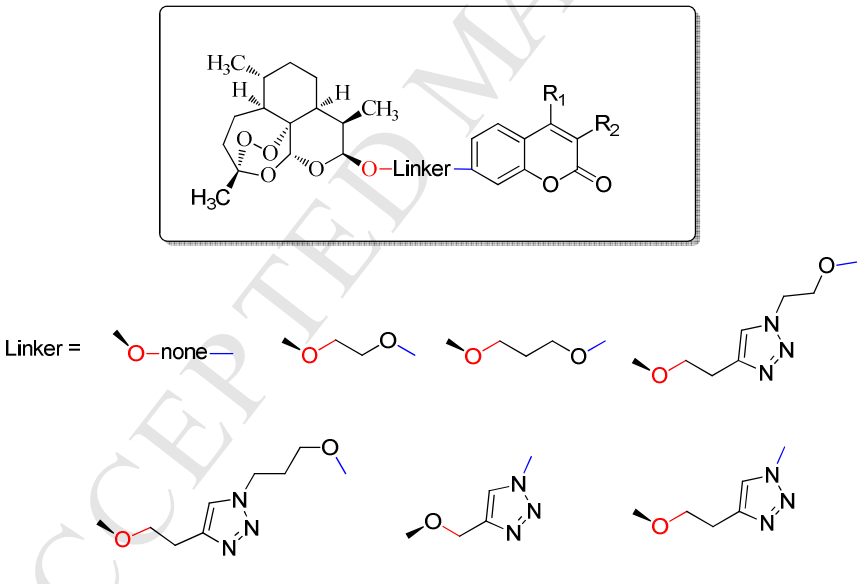
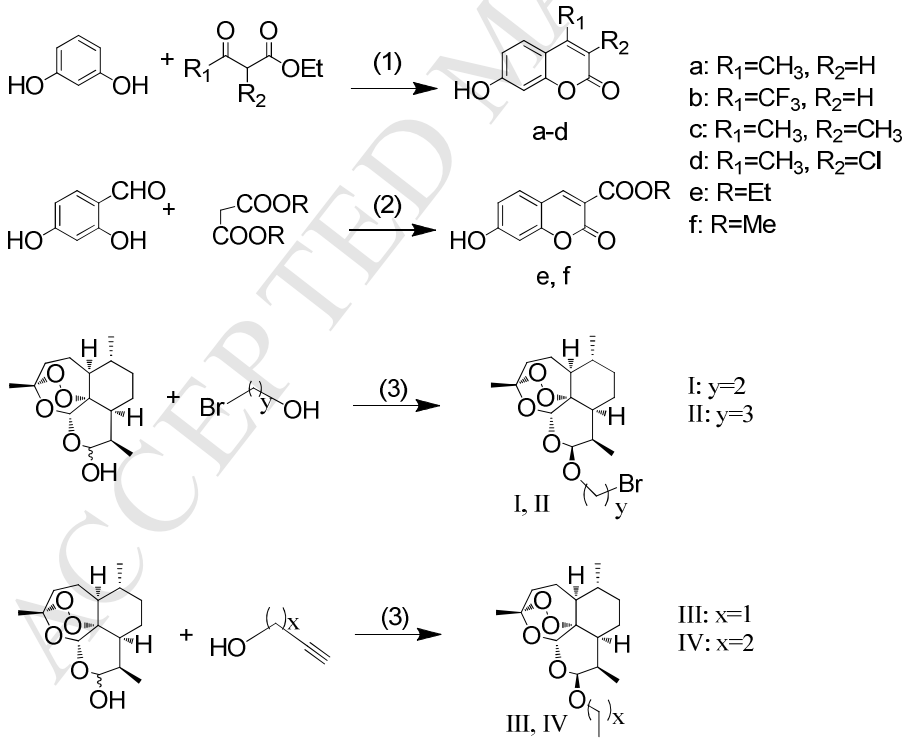


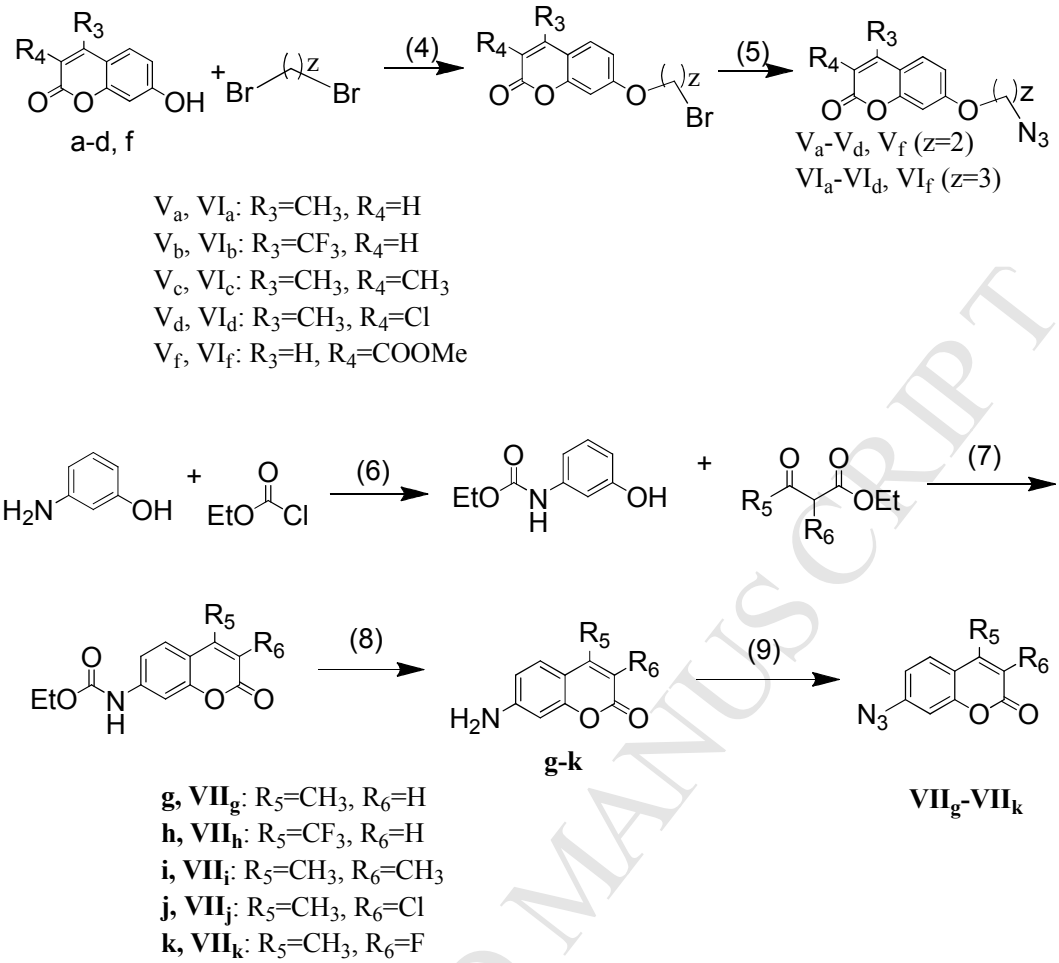
Fig. 2. Structures of new designed dihydroartemisinin-coumarin molecules

2. Results and discussion

2.1. Chemistry

The synthesis of the intermediates **a-f**, **I-VII** was presented at **Scheme 1**. 7-hydroxylcoumarin derivatives **a-d** were obtained by substitution of ethyl acetoacetate in 1, 3-dihydroxybenzene, while **e** and **f** were synthesized by the cyclization between 2, 4-dihydroxybenzaldehyde and diethyl malonate/dimethyl malonate. Azidation reaction was conducted to obtain intermediates $\mathbf{V_a}$-$\mathbf{V_d}$, $\mathbf{V_f}$, $\mathbf{VI_a}$-$\mathbf{VI_d}$ and $\mathbf{VI_f}$ after each coumarin derivative's (**a-d** and **f**) reaction with 1, 2-dibromoethane or 1,3-dibromopropane. The synthesis of 7-aminocoumarin derivatives **g-k** was achieved by the process starting from *m*-aminophenol and its corresponding substituted ethyl acetoacetate. Intermediates $\mathbf{VII_g}$-$\mathbf{VII_k}$ were synthesized by **g-k** *via* diazotization reaction and azidation reaction. Intermediates **I**, **II**, **III**, **IV** were respectively obtained by 2-bromoethanol, 3-bromo-1-propanol, propargyl alcohol and 3-Butyn-1-ol in the substrate of dihydroartemisinin (DHA).

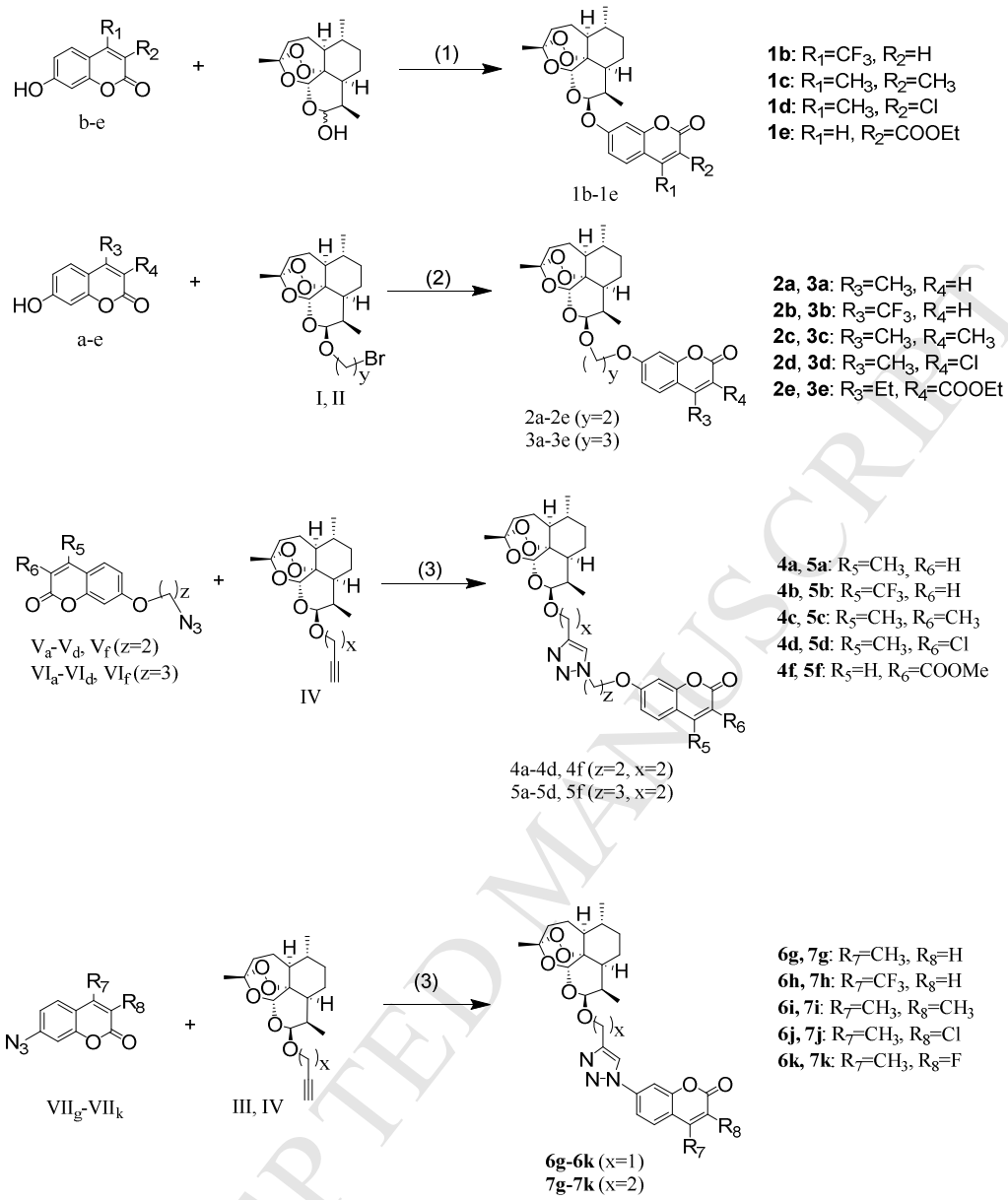




Reagents and conditions: (1) H_2SO_4, r.t.; (2) piperidine (cat.), EtOH, reflux; (3) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0□; (4) K_2CO_3, DMF, 60□; (5) NaN_3, DMF, 60□; (6) Et_3N, CH_2Cl_2; (7) H_2SO_4, r.t.; (8) AcOH, H_2SO_4; (9) ①$NaNO_2$, HCl; ②NaN_3;

Scheme 1. Synthetic routes of intermediates.

The synthesis of the desired new compounds was presented at **Scheme 2**, target compounds **1b-1e** were prepared by the reaction between **b-e** and DHA. Target compounds **2a-2e and 3a-3e** were obtained by the reaction between each coumarin derivatives (**a-e**) with **I** and **II**. Target compounds **4a-4d**, **4f**, **5a-5d** and **5f** were synthesized by intermediates **V** and **VI** with intermediate **IV** *via* click chemistry. Target compounds **6g-6k** and **7g-7k** were obtained by reaction of **VII_g-VII_k** with **III** and **IV**, respectively *via* click chemistry.



Reagents and conditions: (1) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2; (2) K_2CO_3, DMF, 60□; (3) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, DMF, r.t..

Scheme 2. Synthetic routes of target compounds **1b-1e**, **2a-2e**, **3a-3e**, **4a-4d**, **4f**, **5a-5d**, **5f**, **6g-6k** and **7g-7k**.

2.2. Biological assay

2.2.1. In vitro cytotoxicity studies

The cytotoxicity of new compounds toward sensitive HT-29 (CA IX high express) and MDA-MB-231 (CA IX normal express) cells were evaluated by the MTT assay. As the highly-relative

expression of CA IX towards the growth and metastasis of tumor which could be more significant in the anoxia environment, the influence of O_2 was also considered in the experiment. Acetazolamide (AAZ, standard carbonic anhydrase inhibitor), doxorubicin (DOX) and DHA were selected to be positive reference drugs.

The inhibitory concentration 50% (IC_{50}) of the target compounds were shown in **Table 1**. The cytotoxicity of the most compounds on HT-29 cells were higher than that of MDA-MB-231 cells. Meanwhile, there showed a general promotion under hypoxia conditions, indicating the cytotoxicity effects of the coumarin moiety of the target compounds.

The structure-activity relationship of the target compounds was also concluded. **i**): in the same series of compounds, the 3-chloro, 4-methyl substituent in coumarin moiety generally showed a better exhibition than that of the others. **ii**): on the contrary, the 3-ethoxycarbonyl group or 3-methoxycarbonyl group in the 3-position of coumarin moiety showed poorer activity than others in the same series of compouds. **iii**): in the series of compounds **1**, **2** and **3**， compounds **2** and **3** generally had better cytotoxicity exhibition than that of the series of compounds **1** which indicated increasing the distance between DHA and coumarin moiety could improve the activity. i**v**): although the distance between DHA moiety and coumarin moiety were different, the cytotoxicity activity of the series of compounds **4**, **5**, **6** and **7** which having 1, 2, 3- triazole linker showed no difference. **v**): the compounds having 1, 2, 3- triazole linker generally had better cytotoxicity exhibition than the others.

Table 1. IC_{50} of tested samples in two cell lines.

Compd.	IC_{50}(μM)			
	HT-29		MDA-MB-231	
	Hypoxia	Normoxia	Hypoxia	Normoxia
AAZ	>100	>100	>100	>100
DOX	0.25	0.24	5.98	1.07
DHA	18.52	>100	5.64	47.11
A	0.17	1.88	7.51	18.37
1b	22.3	11.09	3.25	56.84
1c	30.28	11.79	6.44	78.81

1d	0.41	1.28	1.4	50.54
1e	7.17	3.45	2.85	21.93
2a	3.69	2.35	0.1	4.29
2b	5.59	54.82	6.14	15.2
2c	57.22	12.82	12.85	57.21
2d	7.02	23.92	5.36	18.03
2e	69.96	5.52	6.02	17.02
3a	8.71	13.82	22.94	29.96
3b	1.19	4.57	8.86	13.57
3c	3.54	8.71	7.92	17.13
3d	8.05	2.55	2.38	0.6
3e	8.69	13.82	22.96	42.89
4a	10.69	5.72	18.25	17
4b	4.01	3.58	14.25	17.88
4c	14.56	3.47	52.28	56.71
4d	2.37	5.28	7.24	7.53
4f	75.68	67.19	58.44	60.97
5a	0.11	6.87	14.4	72.25
5b	4.29	5.88	6.58	32.5
5c	0.11	5	33.19	>100
5d	1.68	1.19	6.07	30.98
5f*	-	-	-	-
6g	5.09	10.84	49.82	21.49
6h	5.33	5.22	49.6	>100
6i	0.02	2.67	4.85	23.44
6j	1.15	4.27	24.68	50.53
6k	7.39	3.35	2.6	3.47
7g	10.93	8.95	9.96	3.78
7h	10.27	6.07	95.74	1.85

7i	0.08	4.71	3.58	3.75
7j	0.01	1.5	1.03	5.19
7k	5.66	9.89	3.36	3.84

*__5f__ was insoluble

2.2.2. Anti-tumor mechanisms of compound ***A****.*

Dihydroartemisinin-coumarin hybrids had exhibited a great value in anticancer activity, but the specific mechanisms were still unclear. Compound **A** as one of the dihydroartemisinin-coumarin hybrids was chosen to be further studied.

2.2.2.1 Docking analysis

Maresca et al. revealed that coumarin moiety were hydrolyzed within the CA active site[20]. Therefore, as shown in **(d)** of **Fig. 3**, the hydrolyzed compound **A** was used as the ligand of this docking analysis. The molecular docking results showed the coumarin segment of compound **A** could integrated closely with the CA IX active site cavity (**Fig. 3**) with minimum binding energy of (ΔG) −11.4 kcal/mol. At the active site of CA IX, compound **A** formed six H-bonds with His-96, His-119, Thr-199, Thr-200, Asn-62 and Gln-67. The triazole substation was involved in the interactions, which might explain why the compounds having 1, 2, 3- triazole linker generally had better cytotoxicity exhibition than the others. Combined with the results of the cytotoxicity studies, CA IX was very likely to be one of the drug targets of the dihydroartemisinin-coumarin hybrids.

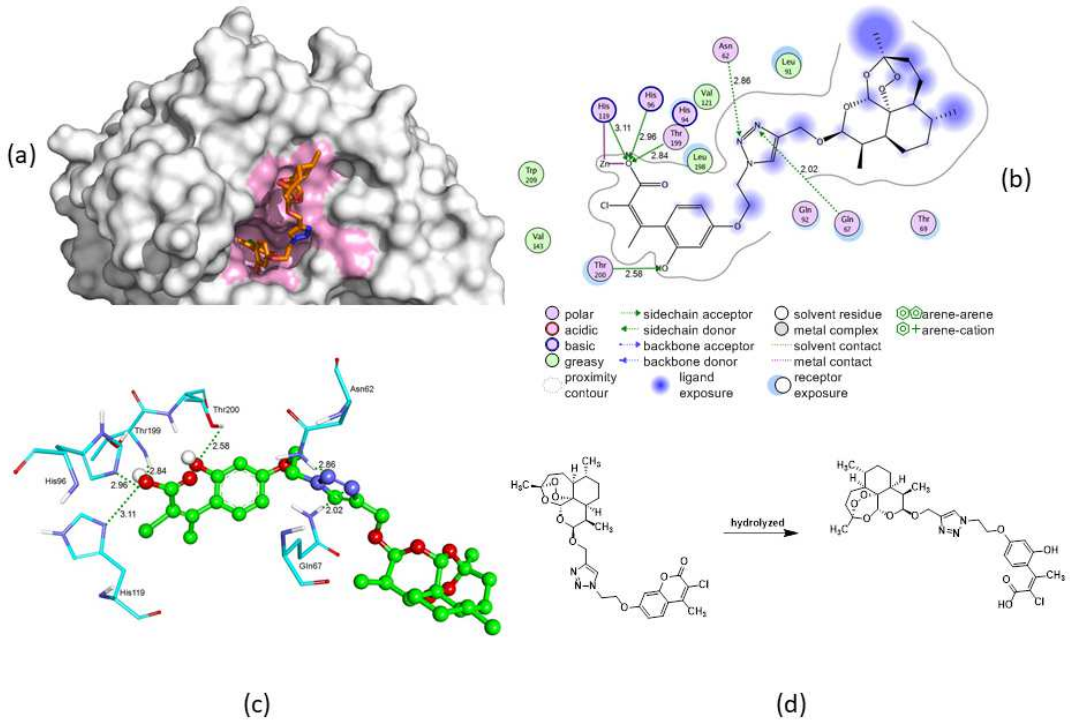


Fig. 3. Interaction diagrams of the selected docked conformations for hydrolyzed compound **A** inside the active site of CA IX enzyme. (a) The surface representation of binding pocket has been shown at the top of the figure. (b) 2D ligand interactions diagram. (c) 3D ligand interactions diagram. (d) The ligand of this docking analysis.

2.2.2.2. Cell morphology

Different concentration of compound **A** were administered to HT-29 and then cultured for 96 h, which showed a proliferation inhibition in a dose dependent manner (**Fig. 4**). As shown in F1 and F2 of **Fig. 4**, the cell volume significantly enlarged, the cell amount, meanwhile, was less than others, indicating the proliferate inhibition effect of compound **A**.

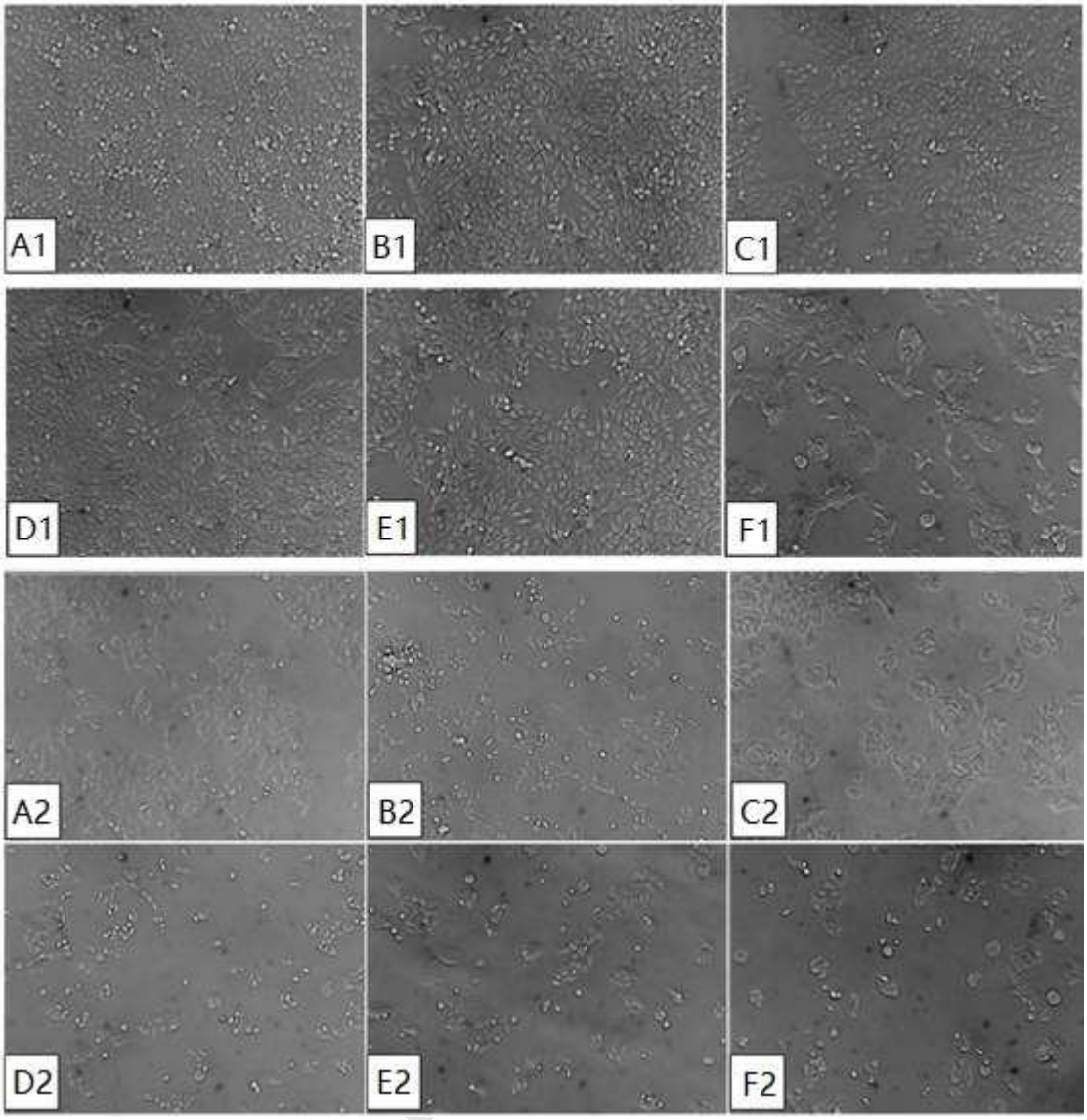


Fig. 4. Inhibition of compound **A** on cell viability under normoxic condition (A1-F1); Inhibition of compound **A** on cell viability under hypoxic condition (A2-F2)**.** Cell were treated with (A1, A2) 0 μM, (B1, B2) 1.25 μM, (C1, C2) 2.5 μM, (D1, D2) 5.0 μM, (E1, E2) 10.0 μM, (F1, F2) 20.0 μM compound **A** for 96 h. Representative images were shown. 100×

2.2.2.3. Analysis of cell cycle progression by flow cytometry

The effect of compound **A** on cell cycle progression and apoptosis was examined by flow cytometry. The distribution of HT-29 in cell cycle was tested after treated with different concentrations of compound **A** (**A1**-**A5** of **Fig. 5**). These results explained the possible correlation between growth inhibition of compound **A** on HT-29 and G_0/G_1 phase arrest. We also took the time factor into the consideration. HT-29 cells were treated with 10 μM compound **A**. As shown in **B** of **Fig. 5**, Sub-G_1

phase was not obviously evident before 48 h. The ratio increased during 48 h to 72 h in a time dependent manner. The maximum ratio of Sub-G_1 reached up to 60%. All these results revealed that compound **A** could arrest the growth of HT-29 within a short time-frame (0 h-48 h). But when prolonged treated period, there induced obviously apoptosis of HT-29 cells, indicating that the duration of the treatment was at least 48 h enough to induce apoptosis.

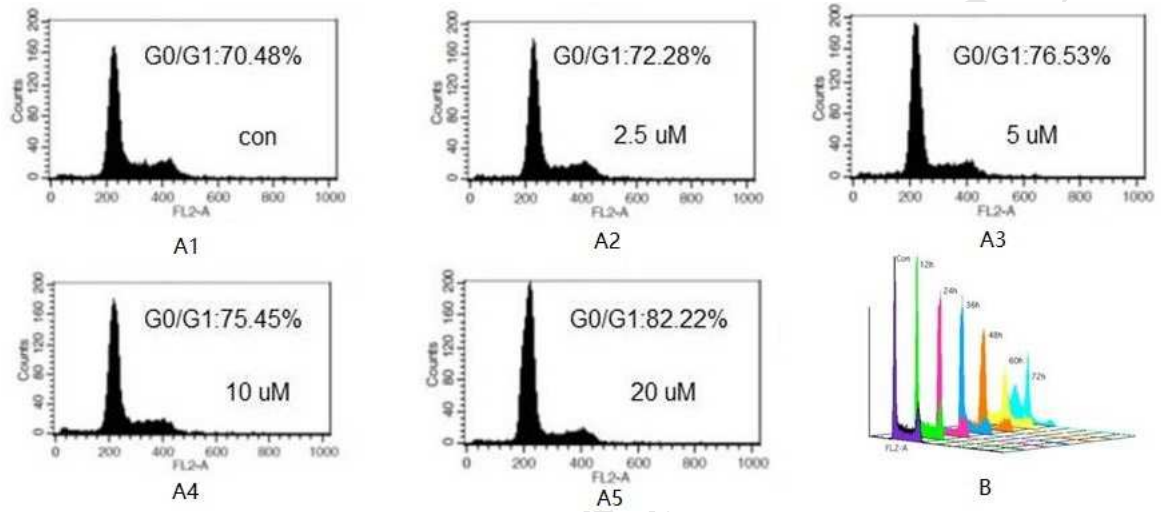


Fig. 5. G_0/G_1-phase cell cycle arrest in HT-29 cell line induced by compound **A** at 48 h (A1-A5); Cell cycle arrest in HT-29 cell line induced by 10 μM compound **A** at different time (B).

2.2.2.4. Evaluation of apoptosis by Annexin V-FITC assay

For further confirming the evidence of apoptosis, early apoptosis was measured by Annexin V-FITC assay. As illustrated in **Fig. 6**, cells in the early apoptotic phase were positive after treatment of HT-29 cells with different compound A concentrations.

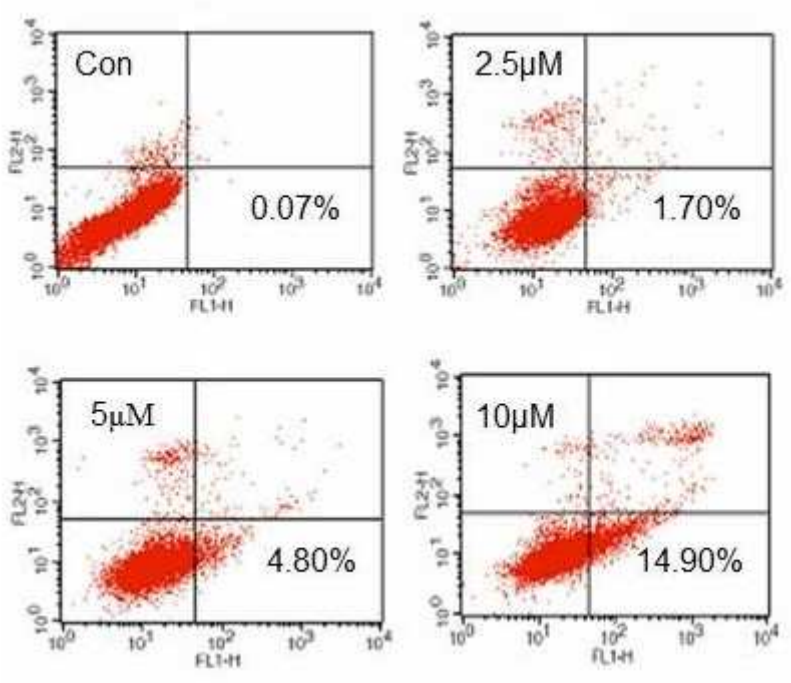


Fig. 6. Compound **A** induced apoptosis in HT-29 at 48 h. Cells were treated with different concentrations of compound **A**, and then treated with 0.25 % trypsin. Cells were collected and washed with PBS, incubated with ANNEX-FITC and PI in binding buffer, and at final detected by flow cytometry.

2.2.2.5. Mitochondrial membrane potential (ΔΨm) analysis

The effect of compound **A** on mitochondrial membrane potential (MMP) of HT-29 was investigated by flow cytometry after treated with rhodamine-123 (Rh-123). As shown in **Fig. 7**, compound **A** induced dramatically decrease of MMP at 3 h, but it was gradually recovered until 24 h. Interestingly, after 24 h, we observed that the MMP of HT-29 gradually inclined again in time dependent manner. Compound **A** was considered to induce cell cycle arrest within 24 h and showed apoptosis effect after 24 h according to the previous experiment. The dramatically decrease at 3 h was possibly related to extra function of Rh-123 on cells. Other than MMP, Rh-123 could also evaluate the ATP level of cells. We tend to think the possibility that compound **A** induced ATP metabolic disorder of HT-29.

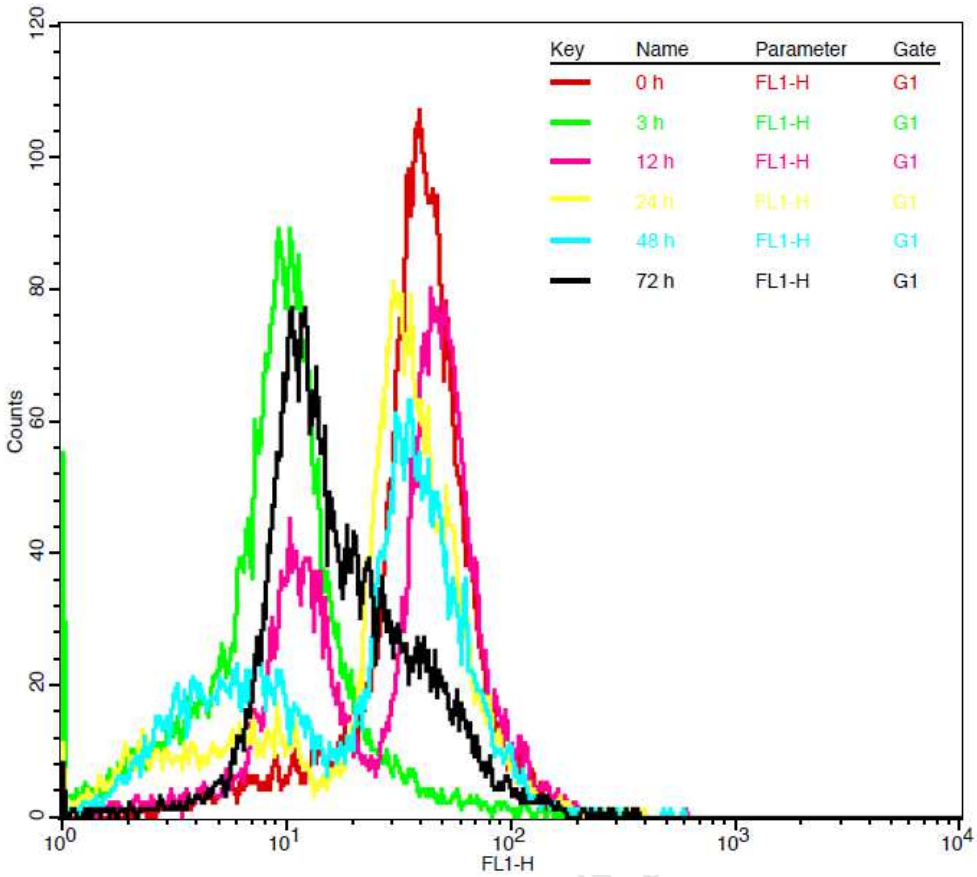


Fig. 7. A induced transmitochondria membrain potential decrease in HT-29 cell line. Cells were treated with 20 μM **A** for different times. Stained with Rh123 for 40 min and then treated with 0.25% trypsin. Cells were collected and washed with PBS for 3 times and detected by flow cytometry.

2.2.2.6. Intracellular pH analysis

Using BCECF-AM as the fluorescent pH indicator, we investigated the effect of compound A on the intracellular pH. As shown in **Fig. 8**, after 3 h and 12 h administration (color green and pink), the fluorescence intensity was obviously lower than 0 h (color red). After 24 h administration (color blue), the fluorescence intensity recovered but still lower than 0 h. These results indicated the acidification occurred in the cells, which showed the possible suppression of CA IX by compound **A** resulted in the H^+accumulated.

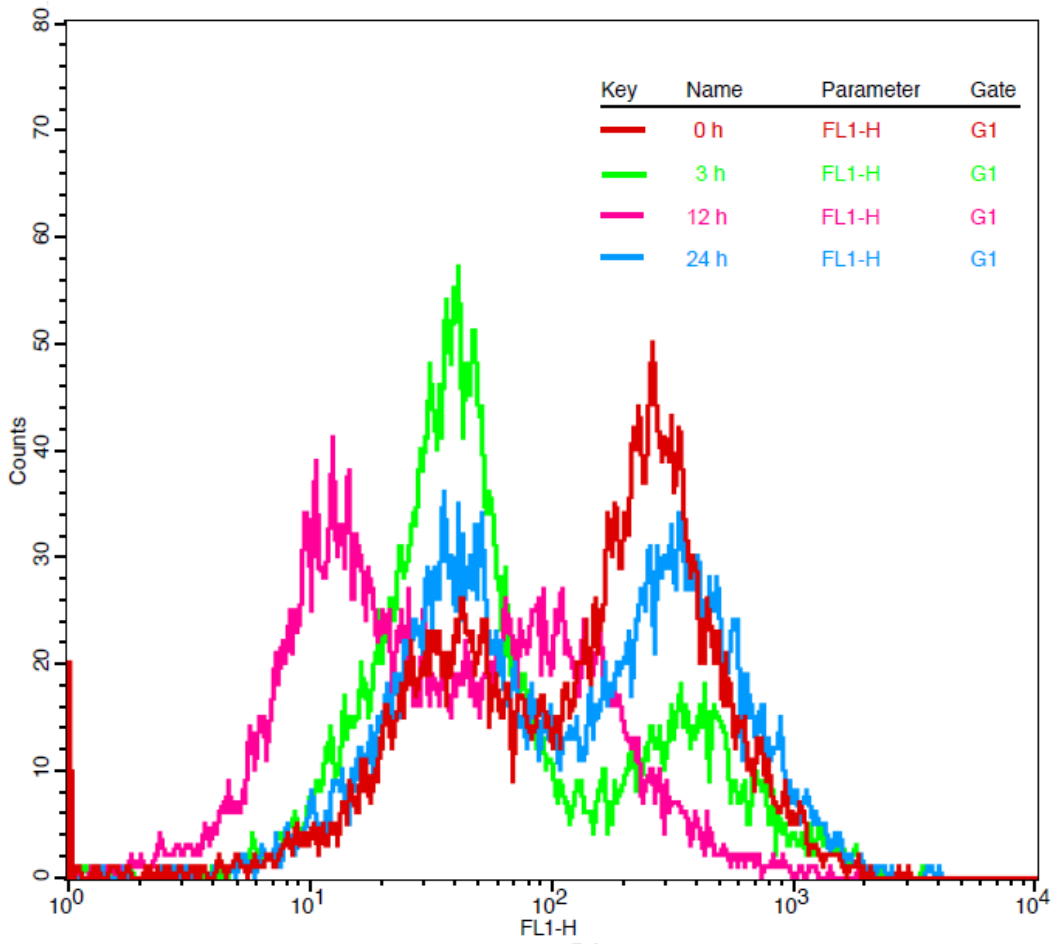


Fig. 8. Compound **A** induced cellular pH decrese in HT-29 cells. Cells were treated with 10 μM **A** for different times. Stained with BCECF-AM, a PH sensetive fluorodye, for 40 min and then treated with 0.25% trypsin. Cells were collected and washed with PBS and detected by flow cytometry.

2.2.2.7. Ferroptosis

Ferroptosis was a newly found cell death pathway. It was demonstrated that overwhelming, iron-dependent accumulation of lethal lipid reactive oxygen species (ROS) could trigger cell death in the form of ferroptosis [21]. Artesunate had already been identified as an activator of ferroptosis[22]. Our target compounds had a similar artesunate DHA group. Therefore, we supposed our target compounds had the same effect as well. As shown in **A1**-**A3** of **Fig. 9**, HT-29 cells were treated with liproxstatin-1 which is ferroptosis inhibitor, which resulted in strong death suppression at 72 h after A/Liproxstatin-1 treatment. As shown in **B** of **Fig. 9**, the death prevention of liproxstatin-1 showed a dose dependent manner at 48 h after HT-29 cells were treated with compound **A**. These results demonstrated that compound **A** induced ferroptosis in HT-29 Cells.

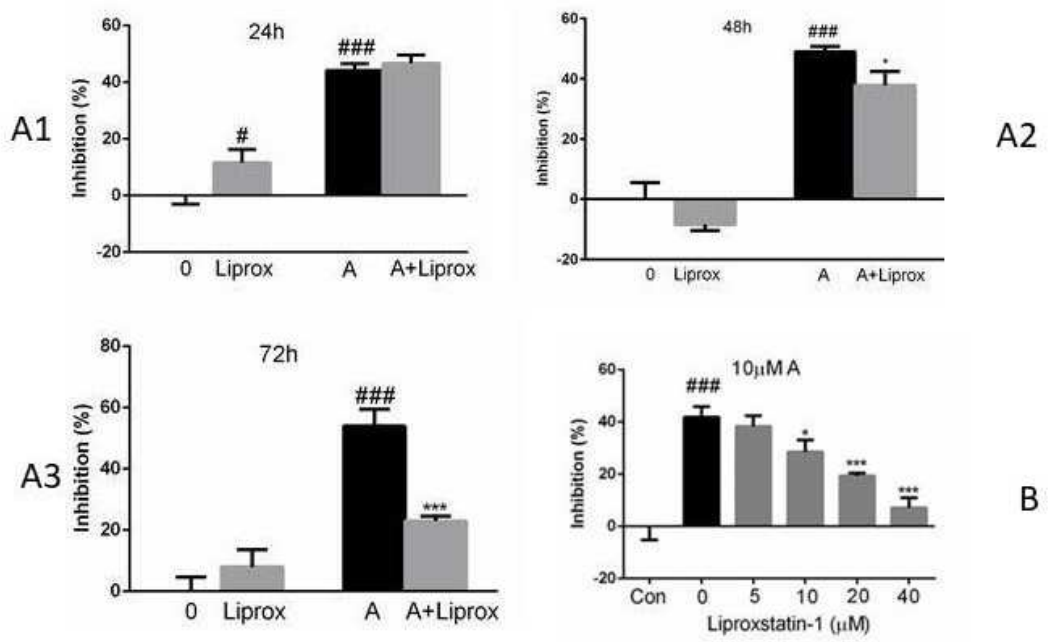


Fig. 9. Compound **A**-induced ferroptosis in HT-29 cells (A1-A3). Cells were pretreated with 10 μM; Liproxstatin-1 reversed compound A-induced growth inhibition in HT-29 cells (B). Cells were pretreated with different concentrations of Liproxstatin-1 for 1 h, and then 10 μM **A** was added and cultured for 48 h. At the end points MTT assay was measured.

2.2.2.8. Wound-Healing Assay

HT-29 cells were plated in 24-well plates for 48 h and then the wound was created. The tumor cells were treated with 0 μM, 0.31 μM, 0.63 μM, 1.25 μM, 2.5 μM and 5 μM compound **A** respectively, then cultured in normoxia condition. 0 h and 72 h were observed by inverted microscope. As shown in **Fig. 10**, there showed the migrate inhibition of compound **A** in a concentration dependent manner.

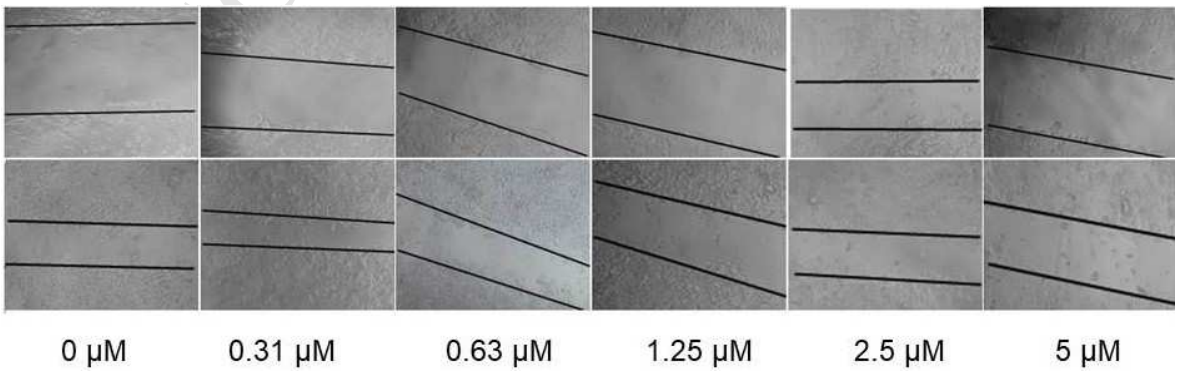


Fig. 10. Inhibition of compound **A** on cell migration in HT-29 cells by wounded healing test. (0 h

above, 72 h below)

2.2.2.9. Transwell assay

The migrate inhibition of compound **A** was tested by Transwell assay. As shown in **Fig. 11**, 1.25 μM and 2.5 μM compound **A** could obviously inhibit HT-29 cell migration.

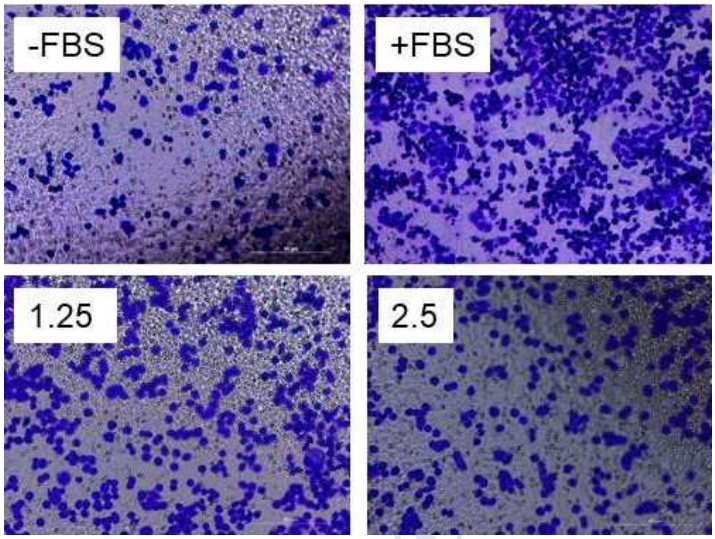


Fig. 11. Inhibition of compound **A** on cell migration in HT-29 cells by transwell test.

3. Conclusion

We designed and synthesized new series of dihydroartemisinin-coumarin hybrids. These compounds exhibited moderate cytotoxic activities against HT-29 and MDA-MB-231 cell lines especially under hypoxia condition. Then we did further study in the compound **A** which was reported before. We found that compound **A** could inhibited proliferation of HT-29 cell lines, arrested the cell cycle progression of HT-29 cells, and induce both apoptosis and ferroptosis of HT-29 cells. It also exhibited the inhibition of the migration of HT-29 cells. Therefore we preliminarily clarified the mechanism of dihydroartemisinin-coumarin hybrids' anticancer activity.

4. Experimental section

4.1 Chemistry

Melting points were recorded on an X-4 microscope melting point apparatus (Beijing Tech

Instrument Co., Ltd., Beijing, China) without calibration. The ^{1}H-NMR and ^{13}C-NMR spectra were measured by a Bruker AV-400 spectrometer (Bruker Bioscience, Billerica, MA, USA), with tetramethylsilane as an internal standard. High-resolution mass spectra (HRMS) were measured with an Agilent Accurate-Mass Q-TOF 6530 (Agilent, Santa Clara, CA, USA) in ESI mode. Reaction progress was monitored by TLC on silica gel precoated GF254 plates (Qingdao Haiyang Chemical Co. Ltd., Qingdao, Shandong, China). Preparative flash column chromatography was performed on the 200–300 mesh silica gel (Qingdao Haiyang Chemical Co. Ltd., Qingdao, Shandong, China). Unless otherwise noted, all solvents and reagents were commercially available and used without further purification.

*4.1.1. General Procedure for the Synthesis of Compounds **1b-1e***

DHA (0.01 mol) and triethylamine (0.02 mol) were added in 40 mL dichloromethane and stirred for 10 min at -5~0□. Trifluoroacetic anhydride was added dropwise while stirring. After the completion of addition, the mixture was stirred at 0~5□. After 10 h, coumarin (**b**-**e**) (0.02 mol) was added and the reaction mixture kept stirring for 8 h. The mixture was washed with saturation sodium bicarbonate (100 mL×5), dried over anhydrous sodium sulfate and evaporated to dryness. The product was purified through column chromatography.

*4.1.1.1. 4-(trifluoromethyl)-7-((10S)-Dihydroartemisin-10-oxy)-2H-1-chromen-2-one (**1b**)*

White solid; 12.1% yield; m.p. 186~187□; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.65-7.59 (m, 1H), 7.18 (d, *J* = 2.4 Hz, 1H), 7.08 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.62 (s, 1H), 5.60 (d, *J* = 3.5 Hz, 1H), 5.41 (s, 1H), 2.91-2.81 (m, 1H), 2.41-2.32 (m, 1H), 2.05-1.99 (m, 1H), 1.98-1.82 (m, 3H), 1.69-1.73 (m, 1H), 1.66-1.57 (m, 1H), 1.45-1.52 (m, 1H), 1.43 (s, 3H), 1.27-1.40 (m, 3H), 1.03 (d, *J* = 7.3 Hz, 3H), 0.96 (d, *J* = 5.8 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) *δ* (ppm): 160.06, 159.28, 158.36, 155.01, 125.37, 121.45, 119.63, 113.44, 111.77, 106.86, 103.75, 99.41, 87.36, 79.73, 76.20, 75.99, 75.78, 51.37, 43.11, 36.43, 35.21, 33.49, 29.71, 24.94, 23.58, 23.37, 19.25, 11.75; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{25}H_{27}F_3O_7Na$: 519.1601. Found: 519.1585.

*4.1.1.2. 3, 4-dimethyl-7-((10S)-Dihydroartemisin-10-oxy)-2H-1-chromen-2-one (**1c**)*

White solid; 17.0% yield; m.p. 162~164□; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.48 (d, *J* = 8.8 Hz, 1H), 7.07 (d, *J* = 2.5 Hz, 1H), 7.01 (dd, *J* = 8.7, 2.4 Hz, 1H), 5.54 (d, *J* = 3.4 Hz, 1H), 5.42 (s, 1H), 2.87-2.77 (m, 1H), 2.40-2.37 (m, 1H), 2.35 (s, 3H), 2.17 (s, 3H), 2.03 (s, 1H), 1.93-1.84 (m, 3H), 1.70

(dd, *J* = 13.2, 3.2 Hz, 1H), 1.61-1.55 (m, 2H), 1.42 (s, 3H), 1.33-1.22 (m, 3H), 1.01 (d, *J* = 7.3 Hz, 3H), 0.95 (d, *J* = 5.9 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 161.34, 158.13, 152.26, 145.00, 124.20, 118.45, 114.15, 112.21, 103.31, 103.23, 99.44, 87.25, 79.83, 76.22, 76.01, 75.80, 51.43, 43.23, 36.40, 35.26, 33.55, 29.84, 25.00, 23.60, 23.39, 19.27, 14.06, 12.20, 11.84; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{26}H_{32}O_7Na$: 479.2040. Found: 479.2045.

*4.1.1.3. 3-chloro-4-methyl-7-((10S)-Dihydroartemisin-10-oxy)-2H-1-chromen-2-one (**1d**)*

White solid; 17.5% yield; m.p. 97~98□; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.51 (d, *J* = 8.8 Hz, 1H), 7.11 (d, *J* = 2.4 Hz, 1H), 7.07 (dd, *J* = 8.8, 2.4 Hz, 1H), 5.56 (d, *J* = 3.4 Hz, 1H), 5.41 (s, 1H), 2.86-2.82 (m, 1H), 2.53 (s, 3H), 2.40-2.32 (m, 1H), 2.06-1.98 (m, 1H), 1.96-1.81 (m, 3H), 1.75-1.67 (m, 1H), 1.62-1.57 (m, 1H), 1.50-1.44 (m, 1H), 1.42 (s, 3H), 1.36-1.21 (m, 3H), 1.02 (d, *J* = 7.3 Hz, 3H), 0.95 (d, *J* = 5.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* (ppm): 160.155, 157.308, 152.814, 147.791, 125.878, 118.207, 114.130, 104.389, 104.268, 100.451, 88.324, 80.786, 77.412, 77.094, 76.776, 52.404, 44.181, 37.411, 36.247, 34.533, 30.789, 25.966, 24.609, 24.381, 20.281, 16.180, 14.203, 12.823; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{25}H_{29}ClO_7Na$: 499.1494. Found: 499.1501.

*4.1.1.4. Ethyl 7-((10S)-Dihydroartemisin-10-oxy)-2H-1-chromen-2-one-3-carboxylate (**1e**)*

White solid; 16.7% yield; m.p. 82~83□; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.49 (s, 1H), 7.51 (d, *J* = 8.6 Hz, 1H), 7.13 (d, *J* = 2.2 Hz, 1H), 7.05 (dd, *J* = 8.6, 2.3 Hz, 1H), 5.60 (d, *J* = 3.4 Hz, 1H), 5.40 (s, 1H), 4.39 (q, *J* = 7.1 Hz, 2H), 2.88-2.84 (m, 1H), 2.43-2.32 (m, 1H), 2.06-2.00 (m, 1H), 1.98-1.78 (m, 3H), 1.76-1.68 (m, 1H), 1.64-1.58 (m, 1H), 1.43 (s, 3H), 1.40 (t, *J* = 7.1 Hz, 4H), 1.32-1.21 (m, 3H), 1.03 (d, *J* = 7.3 Hz, 3H), 0.96 (d, *J* = 5.7 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 163.43, 162.65, 157.18, 157.08, 148.83, 130.74, 114.71, 114.53, 112.46, 104.46, 103.86, 100.48, 88.37, 80.72, 77.24, 77.03, 76.81, 61.77, 52.39, 44.13, 37.44, 36.22, 34.51, 30.74, 25.97, 24.60, 24.36, 20.27, 14.28, 12.78; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{27}H_{32}O_9Na$: 523.1939. Found: 523.1939.

*4.1.2. General Procedure for the Synthesis of Compounds **2a-2e** and **3a-3e***

Substituted coumarin (**a-e**) (0.001 mol), substituted DHA (**I**, **II**) (0.001 mol) and anhydrous potassium carbonate (0.0011 mol) were added in 15 mL DMF and stirred for 5 h. After the completion of reaction, the mixture was poured in the water and extracted with ethyl acetate (50 mL×3). The

organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The product was purified through column chromatography.

*4.1.2.1. 4-methyl-7-(2-((10S)-Dihydroartemisin-10-oxy)-ethyoxyl)-2H-1-chromen-2-one (**2a**)*

White solid; 61.7% yield; m.p. 64~65□; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.48 (d, *J* = 8.7 Hz, 1H), 6.85 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.82 (d, *J* = 2.3 Hz, 1H), 6.12 (d, *J* = 1.6 Hz, 1H), 5.44 (s, 1H), 4.88 (d, *J* = 3.4 Hz, 1H), 4.25-4.13 (m, 3H), 3.85-3.76 (m, 1H), 2.68-2.59 (m, 1H), 2.39 (d, *J* = 1.4 Hz, 3H), 2.34 (dd, *J* = 13.9, 3.8 Hz, 1H), 2.06-2.00 (m, 1H), 1.91-1.82 (m, 1H), 1.78-1.52 (m, 3H), 1.51-1.45 (m, 2H), 1.43 (s, 3H), 1.41-1.14 (m, 3H), 0.91 (d, *J* = 6.0 Hz, 3H), 0.88 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 161.90, 161.21, 155.21, 152.51, 125.58, 113.68, 112.56, 112.00, 104.10, 102.24, 101.59, 87.89, 81.04, 67.86, 66.29, 52.51, 44.34, 37.47, 36.38, 34.57, 30.83, 26.17, 24.70, 24.40, 20.34, 18.69, 12.93; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{27}H_{34}O_8Na$: 509.2146. Found: 509.2151.

*4.1.2.2. 4-(trifluoromethyl)-7-(2-((10S)-Dihydroartemisin-10-oxy)-ethyoxyl)-2H-1-chromen-2-one (**2b**)*

White solid; 46.3% yield; m.p. 60~61□; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.61 (dd, *J* = 9.0, 1.9 Hz, 1H), 6.91 (dd, *J* = 8.9, 2.6 Hz, 1H), 6.88 (d, *J* = 2.5 Hz, 1H), 6.61 (s, 1H), 5.42 (s, 1H), 4.88 (d, *J* = 3.4 Hz, 1H), 4.27-4.16 (m, 3H), 3.85-3.78 (m, 1H), 2.68-2.59 (m, 1H), 2.39-2.31 (m, 1H), 2.06-1.99 (m, 1H), 1.89-1.84 (m, 1H), 1.72-1.69 (m 1H), 1.68-1.64 (m, 1H), 1.52 (dd, *J* = 13.3, 3.2 Hz, 1H), 1.45 (d, *J* = 5.7 Hz, 1H), 1.43 (s, 3H), 1.42-1.40 (m, 1H), 1.30-1.16 (m, 3H), 0.91 (d, *J* = 5.5 Hz, 3H), 0.88 (d, *J* = 7.4 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 162.80, 159.31, 156.29, 126.37, 126.36, 113.66, 112.32, 112.29, 107.11, 104.15, 102.31, 102.10, 87.90, 81.00, 77.28, 77.07, 76.85, 68.09, 66.22, 52.49, 44.31, 37.53, 36.37, 34.55, 30.80, 26.15, 24.69, 24.43, 20.31, 12.91; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{27}H_{31}F_3O_8Na$: 563.1863. Found: 563.1862.

*4.1.2.3. 3,4-dimethyl-7-(2-((10S)-Dihydroartemisin-10-oxy)-ethyoxyl)-2H-1-chromen-2-one (**2c**)*

White solid; 58.0% yield; m.p. 146~148□; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.48 (d, *J* = 8.8 Hz, 1H), 6.83 (dd, *J* = 8.8, 2.6 Hz, 1H), 6.79 (d, *J* = 2.5 Hz, 1H), 5.44 (s, 1H), 5.28 (s, 1H), 4.87 (d, *J* = 3.4 Hz, 1H), 4.20-4.13 (m, 3H), 3.84-3.75 (m, 1H), 2.66-2.58 (m, 1H), 2.35 (s, 3H), 2.35-2.30 (m, 1H), 2.16 (s, 3H), 2.06-1.98 (m, 1H), 1.89-1.82 (m, 1H), 1.75-1.64 (m, 2H), 1.53-1.46 (m, 2H), 1.43 (s, 3H), 1.40 (d, *J* = 4.2 Hz, 1H), 1.25-1.16 (m, 2H), 0.90 (d, *J* = 5.3 Hz, 3H), 0.88 (d, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* (ppm): 162.377, 160.807, 153.503, 146.194, 125.278, 118.999, 114.291,

112.375, 104.100, 102.237, 101.318, 87.906, 81.052, 77.398, 77.080, 76.763, 67.780, 66.354, 52.527, 44.367, 37.458, 36.399, 34.583, 30.841, 26.165, 24.698, 24.402, 20.327, 15.071, 13.148, 12.925; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{28}H_{36}O_8Na$: 523.2302. Found: 523.2300.

*4.1.2.4. 3-chloro-4-methyl-7-(2-((10S)-Dihydroartemisin-10-oxy)-ethyoxyl)-2H-1-chromen-2-one (**2d**)*

White solid; 38.4% yield; m.p. 124~126□; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.52 (d, *J* = 8.9 Hz, 1H), 6.90 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.83 (d, *J* = 2.5 Hz, 1H), 5.44 (s, 1H), 4.88 (d, *J* = 3.4 Hz, 1H), 4.23-4.15 (m, 3H), 3.85-3.78 (m, 1H), 2.68-2.58 (m, 1H), 2.54 (s, 3H), 2.40-2.32 (m, 1H), 2.06-2.01 (m, 1H), 1.92-1.82 (m, 1H), 1.79-1.64 (m, 3H), 1.52 (dd, *J* = 13.2, 3.2 Hz, 3H), 1.43 (s, 3H), 1.27-1.19 (m, 2H), 0.91 (d, *J* = 5.4 Hz, 3H), 0.88 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* (ppm): 161.836, 157.371, 153.071, 147.926, 125.926, 117.847, 113.393, 113.278, 104.142, 102.291, 101.507, 87.914, 81.030, 77.389, 77.071, 76.754, 67.991, 66.281, 52.508, 44.337, 37.510, 36.384, 34.578, 30.823, 26.163, 24.709, 24.424, 20.341, 16.184, 12.924; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{27}H_{33}ClO_8Na$: 543.1756. Found: 543.1763.

*4.1.2.5. Ethyl 7-(2-((10S)-Dihydroartemisin-10-oxy)-ethyoxyl)-2H-1-chromen-2-one-3-carboxylate (**2e**)*

White solid; 62.5% yield; m.p. 73~76□; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.48 (s, 1H), 7.49 (d, *J* = 8.7 Hz, 1H), 6.86 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.80 (d, *J* = 2.3 Hz, 1H), 5.41 (s, 1H), 4.86 (d, *J* = 3.5 Hz, 1H), 4.36 (q, *J* = 7.1 Hz, 2H), 4.23-4.14 (m, 3H), 3.84-3.76 (m, 1H), 2.66-2.56 (m, 1H), 2.39-2.28 (m, 1H), 2.04-1.98 (m, 1H), 1.89-1.80 (m, 1H), 1.73-1.62 (m, 2H), 1.54-1.42 (m, 3H), 1.40 (d, *J* = 9.3 Hz, 4H), 1.36 (d, *J* = 7.1 Hz, 3H), 1.78-1.22 (m, 2H), 0.89 (d, *J* = 5.6 Hz, 3H), 0.86 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* (ppm): 164.382, 163.406, 157.483, 157.069, 148.884, 130.768, 114.197, 113.854, 111.727, 104.146, 102.317, 101.070, 87.905, 81.004, 77.400, 77.082, 76.764, 68.167, 66.180, 61.713, 52.492, 44.311, 37.523, 36.367, 34.553, 30.803, 26.148, 24.699, 24.435, 20.333, 14.280, 12.906; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{29}H_{36}O_{10}Na$: 567.2201. Found: 567.2202.

*4.1.2.6. 4-methyl-7-(2-((10S)-Dihydroartemisin-10-oxy)-propoxy)-2H-1-chromen-2-one (**3a**)*

White solid; 65.9% yield; m.p. 66~68□; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.48 (d, *J* = 8.7 Hz, 1H), 6.86 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.80 (d, *J* = 2.2 Hz, 1H), 6.12 (d, *J* = 1.2 Hz, 1H), 5.25 (s, 1H), 4.79 (d, *J* = 3.4 Hz, 1H), 4.10 (t, *J* = 6.1 Hz, 3H), 3.53-3.47 (m, 1H), 2.62-2.57 (m, 1H), 2.38 (s, 3H), 2.33-2.27 (m, 1H), 2.11-2.05 (m, 2H), 2.01-1.96 (m, 1H), 1.81-1.42 (m, 5H), 1.42 (s, 3H), 1.39-1.21 (m, 2H),

1.16-1.09 (m, 1H), 0.86 (d, *J* = 7.2 Hz, 3H), 0.82-0.78 (m, 1H), 0.74 (d, *J* = 5.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 162.07, 161.21, 155.29, 152.52, 125.52, 113.53, 112.70, 111.90, 104.03, 101.87, 101.19, 87.73, 80.94, 77.40, 77.08, 76.76, 65.18, 63.89, 52.38, 44.28, 37.19, 36.37, 34.45, 30.85, 28.87, 26.18, 24.55, 24.50, 20.18, 18.68, 13.00; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{28}H_{36}O_8Na$: 523.2302. Found: 523.2300.

*4.1.2.7. 4-(trifluoromethyl)-7-(2-((10S)-Dihydroartemisin-10-oxy)-propoxy)-2H-1-chromen-2-one (**3b**)*

White solid; 50.6% yield; m.p. 102~104□; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.62 (dd, *J* = 9.0, 2.0 Hz, 1H), 6.93 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.87 (d, *J* = 2.5 Hz, 1H), 6.61 (s, 1H), 5.24 (s, 1H), 4.80 (d, *J* = 3.5 Hz, 1H), 4.15-4.10 (m, 3H), 3.54-3.49 (m, 1H), 2.66-2.57 (m, 1H), 2.36-2.31 (m, 1H), 2.13-2.07 (m, 2H), 2.02-1.96 (m, 1H), 1.82-1.44 (m, 5H), 1.43 (s, 3H), 1.42-1.09 (m, 4H), 0.87 (d, *J* = 7.4 Hz, 3H), 0.76 (d, *J* = 5.1 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 162.96, 159.26, 156.36, 126.32, 126.31, 113.81, 112.18, 112.14, 106.95, 104.04, 101.86, 101.67, 87.70, 80.87, 77.31, 77.10, 76.89, 65.47, 63.73, 53.47, 52.35, 44.24, 37.26, 36.32, 34.44, 30.81, 28.74, 26.13, 24.51, 20.17, 12.98; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{28}H_{33}F_3O_8Na$: 577.2020. Found: 577.2029.

*4.1.2.8. 3, 4-dimethyl-7-(2-((10S)-Dihydroartemisin-10-oxy)-propoxy)-2H-1-chromen-2-one (**3c**)*

White solid; 60.3% yield; m.p. 68~70□; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.48 (d, *J* = 8.9 Hz, 1H), 6.85 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.78 (t, *J* = 2.5 Hz, 1H), 5.26 (s, 1H), 4.79 (d, *J* = 3.4 Hz, 1H), 4.14-4.07 (m, 3H), 3.53-3.47 (m, 1H), 2.61-2.58 (m, 1H), 2.35 (s, 3H), 2.32-2.27 (m, 1H), 2.17 (d, *J* = 2.0 Hz, 3H), 2.07 (t, *J* = 6.2 Hz, 2H), 2.01-1.96 (m, 1H), 1.81-1.57 (m, 3H), 1.46-1.43 (m, 1H), 1.42 (s, 3H), 1.38-1.08 (m, 3H), 0.86 (d, *J* = 7.3 Hz, 3H), 0.85-0.76 (m, 2H), 0.74 (d, *J* = 5.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 162.36, 160.96, 153.56, 146.21, 125.24, 118.87, 114.13, 112.48, 104.02, 101.87, 100.89, 87.73, 80.95, 77.40, 77.08, 76.76, 65.08, 63.95, 52.40, 44.30, 37.16, 36.37, 34.46, 30.85, 28.92, 26.18, 24.55, 24.49, 20.18, 15.07, 13.14, 13.00; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{29}H_{38}O_8Na$: 537.2459. Found: 537.2463.

*4.1.2.9. 3-chloro-4-methyl-7-(2-((10S)-Dihydroartemisin-10-oxy)-propoxy)-2H-1-chromen-2-one (**3d**)*

White solid; 64.2% yield; m.p. 85~87□; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.52 (d, *J* = 8.9 Hz, 1H), 6.91 (d, *J* = 8.9 Hz, 1H), 6.81 (s, 1H), 5.27 (d, *J* = 1.9 Hz, 1H), 4.82-4.77 (m, 1H), 4.12-4.09 (m, 3H), 3.55-3.47 (m, 1H), 2.62-2.59 (m, 1H), 2.55-2.52 (m, 3H), 2.36-2.28 (m, 1H), 2.12-2.04 (m, 2H),

2.03-1.94 (m, 1H), 1.86-1.56 (m, 4H), 1.49-1.44 (m, 1H), 1.42 (d, *J* = 2.0 Hz, 3H), 1.39-1.32 (m, 1H), 1.31-1.22 (m, 1H), 1.20-1.09 (m, 1H), 0.94 (dd, *J* = 6.0, 1.8 Hz, 1H), 0.86 (dd, *J* = 7.5, 1.8 Hz, 3H), 0.77 (dd, *J* = 6.1, 2.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* (ppm): 162.013, 157.374, 153.152, 147.956, 125.898, 117.742, 113.405, 113.252, 104.077, 101.950, 101.134, 87.774, 80.952, 77.405, 77.088, 76.770, 65.400, 64.011, 52.406, 44.295, 37.283, 36.376, 34.489, 30.858, 28.925, 26.185, 24.599, 24.524, 20.233, 16.182, 13.019; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{28}H_{35}ClO_8Na$: 557.1913. Found: 557.1917.

*4.1.2.10. Ethyl 7-(2-((10S)-Dihydroartemisin-10-oxy)-propoxy)-2H-1-chromen-2-one-3-carboxylate (**3e**)*

White solid; 66.2% yield; m.p. 82~84□; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.50 (s, 1H), 7.50 (d, *J* = 8.7 Hz, 1H), 6.89 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.80 (d, *J* = 2.3 Hz, 1H), 5.28 (d, *J* = 6.8 Hz, 1H), 4.80 (d, *J* = 3.5 Hz, 1H), 4.39 (q, *J* = 7.1 Hz, 2H), 4.17-4.06 (m, 3H), 3.55-3.50 (m, 1H), 2.66-2.57 (m, 1H), 2.38-2.27 (m, 1H), 2.13-2.07 (m, 2H), 2.02-1.97 (m, 1H), 1.86-1.57 (m, 4H), 1.51-1.46 (m, 1H), 1.42 (s, 3H), 1.39 (t, *J* = 7.1 Hz, 4H), 1.36-1.26 (m, 1H), 1.19-1.12 (m, 1H), 0.99-0.90 (m, 1H), 0.87 (d, *J* = 7.4 Hz, 3H), 0.79 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 164.54, 163.45, 157.60, 157.09, 148.93, 130.69, 114.11, 114.08, 111.61, 104.10, 101.97, 100.65, 87.78, 80.94, 77.34, 77.02, 76.71, 65.64, 63.95, 61.74, 52.40, 44.28, 37.36, 36.36, 34.48, 30.85, 28.87, 26.18, 24.62, 24.55, 20.30, 14.29, 13.01; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{30}H_{38}O_{10}Na$: 581.2357. Found: 581.2366.

*4.1.3. General Procedure for the Synthesis of Compounds **4a-4d**, **4f**, **5a-5d** and **5f***

Substituted coumarin (**V_a-V_d**, **V_f**, **VI_a-VI_d**, **VI_f**) (10 mmol) and compound **IV** (10 mmol) were added in 20 mL CH_2Cl_2. A mixture of $CuSO_4 \cdot 5H_2O$ (12.5 mg) and sodium ascorbate (30 mg) in water (10 mL) was added and the reaction mixture was stirred at room temperature for 8 h. The reaction mixture was filtered, washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The product was purified through column chromatography.

4.1.3.1.

*4-methyl-7-((2-(4-(2-((10S)-Dihydroartemisin-10-oxy)-ethyl)-1H-1,2,3-triazol-1-yl))-ethoxy)-2H-1-chromen-2-one (**4a**)*

White solid; 37.4% yield; m.p. 73~75□; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.53 (s, 1H), 7.50 (d, *J*

= 8.7 Hz, 1H), 6.82 (dd, *J* = 8.7, 2.5 Hz, 1H), 6.79 (d, *J* = 2.5 Hz, 1H), 6.15 (t, *J* = 1.4 Hz, 1H), 5.27 (s, 1H), 4.85-4.71 (m, 3H), 4.41 (t, *J* = 5.0 Hz, 2H), 4.16-4.07 (m, 1H), 3.70-3.64 (m, 1H), 3.07-2.95 (m, 2H), 2.60-2.56 (m, 1H), 2.39 (s, 3H), 2.32 (dd, *J* = 14.1, 4.0 Hz, 1H), 2.03-1.97 (m, 1H), 1.87-1.82 (m, 1H), 1.73-1.63 (m, 2H), 1.60-1.54 (m, 1H), 1.47-1.38 (m, 5H), 1.31-1.13 (m, 3H), 0.92 (d, *J* = 6.0 Hz, 3H), 0.80 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 160.92, 160.64, 155.11, 152.27, 145.60, 125.83, 122.38, 114.40, 112.59, 112.07, 104.07, 101.87, 101.81, 87.87, 81.02, 77.36, 77.04, 76.72, 67.22, 66.89, 52.49, 49.33, 44.30, 37.36, 36.39, 34.60, 30.80, 26.59, 26.17, 24.67, 24.34, 20.37, 18.68, 12.98; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{31}H_{39}N_3O_8Na$: 604.2629. Found: 604.2647.

4.1.3.2.

*4-(trifluoromethyl)-7-((2-(4-(2-((10S)-Dihydroartemisin-10-oxy)-ethyl)-1H-1,2,3-triazol-1-yl))-ethoxy)-2H-1-chromen-2-one (**4b**)*

White solid; 39.1% yield; m.p. 71~73□; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.64 (dd, *J* = 9.0, 1.8 Hz, 1H), 7.52 (s, 1H), 6.89 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.86 (d, *J* = 2.5 Hz, 1H), 6.65 (s, 1H), 5.28 (s, 1H), 4.82-4.76 (m, 3H), 4.45 (t, *J* = 5.1 Hz, 2H), 4.12-4.08 (m, 1H), 3.71-3.67 (m, 1H), 3.10-2.95 (m, 2H), 2.61-2.58 (m, 1H), 2.37-2.32 (m, 1H), 2.05-1.97 (m, 1H), 1.88-1.83 (m, 1H), 1.75-1.62 (m, 2H), 1.59-1.55 (m, 1H), 1.48-1.35 (m, 5H), 1.30-1.17 (m, 2H), 0.93 (d, *J* = 6.2 Hz, 3H), 0.91-0.84 (m, 1H), 0.82 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 161.51, 159.00, 156.13, 145.63, 126.66, 126.65, 122.38, 113.20, 112.99, 107.80, 104.07, 102.24, 101.86, 87.87, 81.00, 77.27, 77.06, 76.85, 67.20, 67.07, 53.46, 52.47, 49.20, 44.28, 37.37, 36.38, 34.59, 30.78, 26.56, 26.16, 24.66, 24.34, 20.36, 12.99; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{31}H_{36}F_3N_3O_8Na$: 658.2347. Found: 658.2374.

4.1.3.3.

*3,4-dimethyl-7-((2-(4-(2-((10S)-Dihydroartemisin-10-oxy)-ethyl)-1H-1,2,3-triazol-1-yl))-ethoxy)-2H-1-chromen-2-one (**4c**)*

White solid; 33.2% yield; m.p. 77~79□; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.53 (s, 1H), 7.49 (d, *J* = 8.9 Hz, 1H), 6.80 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.76 (d, *J* = 2.6 Hz, 1H), 5.27 (s, 1H), 4.79-4.72 (m, 3H), 4.39 (t, *J* = 5.0 Hz, 2H), 4.13-4.07 (m, 1H), 3.70-3.64 (m, 1H), 3.09-2.94 (m, 2H), 2.61-2.51 (m, 1H), 2.36 (s, 3H), 2.34-2.29 (m, 1H), 2.17 (s, 3H), 2.02-1.97 (m, 1H), 1.88-1.82 (m, 1H), 1.75-1.62 (m, 2H), 1.59-1.53 (m, 1H), 1.49-1.42 (m, 1H), 1.40 (s, 3H), 1.39-1.36 (m, 1H), 1.30-1.12 (m, 3H), 0.92 (d, *J* = 6.0 Hz, 3H), 0.79 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* (ppm): 162.124, 159.562,

153.412, 145.941, 145.576, 125.538, 122.411, 119.675, 115.022, 111.900, 104.063, 101.880, 101.524, 87.874, 81.024, 77.365, 77.047, 76.729, 67.228, 66.834, 52.503, 49.386, 44.310, 37.359, 36.401, 34.609, 30.799, 26.584, 26.155, 24.663, 24.332, 20.357, 15.085, 13.202, 12.965; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{32}H_{41}N_3O_8Na$: 618.2786. Found: 618.2801.

*4.1.3.4. 3-chloro-4-methyl-7-((2-(4-(2-((10S)-Dihydroartemisin-10-oxy)-ethyl)-1H-1,2,3-triazol-1-yl))-ethoxy)-2H-1-chromen-2-one (**4d**)*

White solid; 35.8%; m.p. 85~88□; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.53 (d, *J* = 5.2 Hz, 1H), 7.52 (s, 1H), 6.86 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.79 (d, *J* = 2.5 Hz, 1H), 5.27 (s, 1H), 4.80-4.75 (m, 3H), 4.42 (t, *J* = 5.1 Hz, 2H), 4.11-4.08 (m, 1H), 3.69-3.65 (m, 1H), 3.06-2.96 (m, 2H), 2.54 (s, 3H), 2.36-2.30 (m, 1H), 2.04-1.97 (m, 1H), 1.88-1.82 (m, 1H), 1.76-1.63 (m, 3H), 1.58-1.55 (m, 1H), 1.48-1.42 (m, 1H), 1.40 (s, 3H), 1.40-1.35 (m, 1H), 1.26-1.15 (m, 3H), 0.92 (d, *J* = 6.2 Hz, 3H), 0.80 (d, *J* = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) *δ* (ppm): 160.481, 157.035, 152.840, 147.629, 145.513, 126.092, 122.321, 118.351, 113.991, 112.693, 103.989, 101.786, 101.631, 87.787, 80.929, 77.184, 76.972, 76.759, 67.131, 66.899, 52.395, 49.197, 44.204, 37.276, 36.302, 34.517, 30.703, 26.490, 26.074, 24.578, 24.251, 20.282, 16.117, 12.897; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{31}H_{38}ClN_3O_8Na$: 638.2240. Found: 638.2260.

*4.1.3.5. Methyl 7-((2-(4-(2-((10S)-Dihydroartemisin-10-oxy)-ethyl)-1H-1,2,3-triazol-1-yl))-ethoxy)-2H-1-chromen-2-one-3-carboxylate (**4f**)*

White solid; 39.3% yield; m.p. 81~84□; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.51 (s, 1H), 7.52 (d, *J* = 2.1 Hz, 1H), 7.49 (d, *J* = 2.0 Hz, 1H), 6.85 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.78 (d, *J* = 2.3 Hz, 1H), 5.26 (s, 1H), 4.78 (t, *J* = 4.9 Hz, 3H), 4.45 (t, *J* = 5.0 Hz, 2H), 4.11-4.06 (m, 1H), 3.91 (s, 3H), 3.69-3.63 (m, 1H), 3.07-2.92 (m, 2H), 2.60-2.54 (m, 1H), 2.37-2.28 (m, 1H), 2.03-1.95 (m, 1H), 1.89-1.61 (m, 4H), 1.59-1.52 (m, 1H), 1.49-1.41 (m, 1H), 1.40 (s, 3H), 1.25-1.16 (m, 3H), 0.91 (d, *J* = 6.1 Hz, 3H), 0.79 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 163.84, 163.14, 157.30, 156.78, 149.10, 145.59, 131.04, 122.37, 114.49, 113.46, 112.28, 104.05, 101.84, 101.24, 87.85, 80.99, 77.39, 77.07, 76.76, 67.18, 67.15, 52.77, 52.47, 49.17, 44.28, 37.35, 36.38, 34.59, 30.78, 26.55, 26.14, 24.65, 24.33, 20.35, 12.97; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{32}H_{39}N_3O_{10}Na$: 648.2528. Found: 648.2555.

4.1.3.6.

*4-methyl-7-((2-(4-(2-((10S)-Dihydroartemisin-10-oxy)-ethyl)-1H-1,2,3-triazol-1-yl))-propoxy)-2H-1-chromen-2-one (**5a**)*

White solid; 33.2% yield; m.p. 65~68□; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.47 (d, *J* = 8.8 Hz, 1H), 7.35 (s, 1H), 6.81 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.74 (d, *J* = 2.5 Hz, 1H), 6.10 (d, *J* = 1.4 Hz, 1H), 5.23 (s, 1H), 4.75 (d, *J* = 3.4 Hz, 1H), 4.53 (t, *J* = 6.8 Hz, 2H), 4.08-4.04 (m, 1H), 4.01 (t, *J* = 5.8 Hz, 2H), 3.66-3.62 (m, 1H), 3.02-2.92 (m, 2H), 2.59-2.52 (m, 1H), 2.42-2.38 (m, 2H), 2.37 (d, *J* = 1.2 Hz, 3H), 2.34-2.28 (m, 1H), 2.02-1.95 (m, 1H), 1.85-1.80 (m, 1H), 1.71-1.59 (m, 2H), 1.55-1.51 (m, 1H), 1.45-1.34 (m, 5H), 1.28-1.11 (m, 3H), 0.90 (d, *J* = 6.1 Hz, 3H), 0.79 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) *δ* (ppm): 161.277, 160.991, 155.058, 152.395, 145.299, 125.650, 121.662, 113.847, 112.078, 112.045, 103.935, 101.757, 101.535, 87.734, 80.900, 77.254, 77.042, 76.829, 67.175, 64.623, 52.369, 46.633, 44.187, 37.236, 36.280, 34.493, 30.695, 29.668, 26.469, 26.051, 24.554, 24.222, 20.251, 18.576, 12.879; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{32}H_{41}N_3O_8Na$: 618.2786. Found: 618.2805.

4.1.3.7.

*4-(trifluoromethyl)-7-((2-(4-(2-((10S)-Dihydroartemisin-10-oxy)-ethyl)-1H-1,2,3-triazol-1-yl))-propoxy)-2H-1-chromen-2-one (**5b**)*

White solid; 37.8% yield; m.p. 70~73□; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.64 (d, *J* = 8.4 Hz, 1H), 7.35 (s, 1H), 6.90 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.85 (s, 1H), 6.63 (s, 1H), 5.26 (s, 1H), 4.79 (d, *J* = 3.5 Hz, 1H), 4.55 (d, *J* = 6.8 Hz, 2H), 4.12-4.02 (m, 4H), 2.63-2.57 (m, 1H), 2.44 (t, *J* = 6.2 Hz, 2H), 2.37-2.32 (m, 1H), 2.04-1.99 (m, 1H), 1.89-1.83 (m, 1H), 1.74-1.62 (m, 3H), 1.56 (dd, *J* = 13.2, 3.3 Hz, 1H), 1.52-1.44 (m, 1H), 1.41 (s, 3H), 1.41 (s, 2H), 1.30-1.17 (m, 3H), 0.93 (d, *J* = 6.1 Hz, 3H), 0.83 (d, *J* = 7.4 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 162.23, 159.20, 156.23, 145.50, 126.54, 121.67, 113.29, 112.61, 107.42, 104.08, 102.11, 101.88, 100.11, 91.10, 87.86, 81.01, 77.25, 77.04, 76.83, 67.27, 64.93, 52.47, 46.62, 44.29, 37.37, 36.38, 34.60, 30.79, 29.68, 26.58, 26.17, 24.68, 24.35, 20.36, 13.00; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{32}H_{38}F_3N_3O_8Na$: 672.2503. Found: 672.2523.

4.1.3.8.

*3,4-dimethyl-7-((2-(4-(2-((10S)-Dihydroartemisin-10-oxy)-ethyl)-1H-1,2,3-triazol-1-yl))-propoxy)-2H-1-chromen-2-one (**5c**)*

White solid; 35.4% yield; m.p. 60~62□; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.46 (d, *J* = 8.8 Hz, 1H), 7.41 (s, 1H), 6.79 (dd, *J* = 8.8, 2.7 Hz, 1H), 6.71 (d, *J* = 2.5 Hz, 1H), 4.55-4.51 (m, 3H), 3.99 (t, *J* = 5.7 Hz, 2H), 3.83-3.77 (m, 1H), 3.68-3.59 (m, 2H), 3.45-3.36 (m, 1H), 2.92 (t, *J* = 6.6 Hz, 2H), 2.51-2.43 (m, 1H), 2.38 (t, *J* = 6.2 Hz, 3H), 2.32 (s, 3H), 2.31-2.22 (m, 1H), 2.13 (s, 3H), 2.06 (s, 3H), 1.92-1.86 (m, 2H), 1.83-1.77 (m, 1H), 1.73-1.67 (m, 2H), 1.50-1.37 (m, 3H), 1.01 (d, *J* = 5.8 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 162.31, 160.23, 153.45, 146.15, 130.92, 128.83, 125.44, 122.02, 119.26, 111.99, 111.04, 109.10, 101.30, 97.36, 91.13, 77.26, 77.04, 76.83, 64.60, 61.58, 52.40, 46.92, 45.34, 39.29, 36.76, 35.82, 34.41, 30.56, 29.93, 29.72, 29.46, 28.60, 19.18, 15.11, 13.74, 13.19; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{33}H_{43}N_3O_8Na$: 632.2942. Found: 632.3321.

4.1.3.9.

*3-chloro-4-methyl-7-((2-(4-(2-((10S)-Dihydroartemisin-10-oxy)-ethyl)-1H-1,2,3-triazol-1-yl))-propoxy)-2H-1-chromen-2-one (**5d**)*

White solid; 38.1% yield; m.p. 81~83□; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.52 (d, *J* = 8.9 Hz, 1H), 7.35 (s, 1H), 6.87 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.78 (d, *J* = 2.5 Hz, 1H), 5.25 (s, 1H), 4.77 (d, *J* = 3.5 Hz, 1H), 4.55 (t, *J* = 6.8 Hz, 2H), 4.10-4.06 (m, 1H), 4.03 (t, *J* = 5.8 Hz, 2H), 3.68-3.64 (m, 1H), 3.05-2.94 (m, 2H), 2.61-2.56 (m, 1H), 2.54 (s, 3H), 2.44-2.39 (m, 2H), 2.39-2.29 (m, 1H), 2.01-1.98 (m, 1H), 1.86-1.82 (m, 1H), 1.79-1.62 (m, 3H), 1.57-1.53 (m, 1H), 1.47-1.41 (m, 1H), 1.40 (s, 3H), 1.39-1.37 (m, 1H), 1.27-1.17 (m, 2H), 0.92 (d, *J* = 6.1 Hz, 3H), 0.81 (d, *J* = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) *δ* (ppm): 161.191, 157.175, 152.922, 147.762, 145.371, 125.988, 121.609, 117.988, 113.582, 112.742, 103.980, 101.798, 101.489, 87.770, 80.922, 77.186, 76.975, 76.763, 67.207, 64.755, 52.390, 46.611, 44.207, 37.275, 36.298, 34.521, 30.710, 29.668, 26.498, 26.078, 24.581, 24.253, 20.271, 16.106, 12.904; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{32}H_{40}ClN_3O_8Na$: 652.2396. Found: 652.2415.

4.1.3.10. Methyl

*7-((2-(4-(2-((10S)-Dihydroartemisin-10-oxy)-ethyl)-1H-1,2,3-triazol-1-yl))-propoxy)-2H-1-chromen-2-one-3-carboxylate (**5f**)*

White solid; 39.2% yield; m.p. 145~148□; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.47 (s, 1H), 7.47 (d, *J* = 8.8 Hz, 1H), 7.35 (s, 1H), 6.82 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.72 (d, *J* = 2.3 Hz, 1H), 5.20 (s, 1H), 4.73 (d, *J* = 3.4 Hz, 1H), 4.52 (t, *J* = 6.8 Hz, 2H), 4.08-3.99 (m, 3H), 3.87 (s, 3H), 3.64-3.58 (m, 1H), 3.00-2.88 (m, 2H), 2.55-2.49 (m, 1H), 2.42-2.36 (m, 2H), 2.33-2.23 (m, 1H), 1.98-1.92 (m, 1H),

1.81-1.77 (m, 1H), 1.65-1.56 (m, 2H), 1.53-1.48 (m, 1H), 1.41-1.33 (m, 5H), 1.24-1.11 (m, 3H), 0.87 (d, *J* = 6.0 Hz, 3H), 0.77 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 164.01, 163.88, 157.46, 156.93, 149.27, 145.49, 130.97, 121.63, 114.16, 113.50, 111.98, 104.06, 101.89, 101.17, 87.86, 81.00, 77.35, 77.03, 76.72, 67.29, 65.12, 52.75, 52.49, 46.63, 44.30, 37.37, 36.40, 34.62, 30.80, 29.68, 26.59, 26.17, 24.68, 24.35, 20.35, 12.98; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{33}H_{41}N_3O_{10}Na$: 662.2684. Found: 662.2697.

*4.1.4. General Procedure for the Synthesis of Compounds **6g-6k** and **7g-7k***

Substituted coumarin (**VII_g-VII_k**) (10mmol) and Substituted DHA (**III**, **IV**) (10mmol) were added in 20mL CH_2Cl_2. A mixture of $CuSO_4 \cdot 5H_2O$ (12.5mg) and sodium ascorbate (30mg) in water (10mL) was added and the reaction mixture was stirred at room temperature for 8h. The reaction mixture was filtered, washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The product was purified through column chromatography.

4.1.4.1.

*4-methyl-7-((4-((10S)-Dihydroartemisin-10-oxy)-methyl)-1H-1,2,3-triazol-1-yl)-2H-1-chromen-2-one (**6g**)*

White solid; 48.6% yield; m.p. 161~163□; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.04 (s, 1H), 7.82 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.76 (d, *J* = 8.6 Hz, 1H), 7.69 (d, *J* = 2.1 Hz, 1H), 6.35 (d, *J* = 1.5 Hz, 1H), 5.44 (s, 1H), 5.02 (d, *J* = 12.8 Hz, 1H), 4.98 (d, *J* = 3.5 Hz, 1H), 4.78 (d, *J* = 12.8 Hz, 1H), 2.71-2.64 (m, 1H), 2.49 (d, *J* = 1.2 Hz, 3H), 2.41-2.33 (m, 1H), 2.09-2.00 (m, 1H), 1.92-1.83 (m, 1H), 1.79-1.73 (m, 2H), 1.61 (dd, *J* = 13.2, 3.3 Hz, 1H), 1.54-1.42 (m, 5H), 1.39-1.19 (m, 3H), 0.94 (s, 3H), 0.92 (d, *J* = 1.6 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 159.86, 154.08, 151.51, 146.31, 138.91, 126.19, 120.63, 115.85, 115.52, 108.23, 104.14, 101.96, 87.92, 81.01, 77.18, 76.97, 76.76, 61.56, 52.40, 50.68, 44.25, 37.28, 36.29, 34.44, 30.77, 26.06, 24.57, 24.38, 20.22, 18.62, 12.91; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{28}H_{33}N_3O_7Na$: 546.2211. Found: 546.2228.

4.1.4.2.

*4-(trifluoromethyl)-7-((4-((10S)-Dihydroartemisin-10-oxy)-methyl)-1H-1,2,3-triazol-1-yl)-2H-1-chromen-2-one (**6h**)*

White solid; 46.1% yield; m.p. 168~170□; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.07 (s, 1H),

7.93-7.88 (m, 1H), 7.88-7.85 (m, 1H), 7.84 (d, *J* = 2.2 Hz, 1H), 6.87 (s, 1H), 5.43 (s, 1H), 5.02 (d, *J* = 12.8 Hz, 1H), 4.99 (d, *J* = 3.6 Hz, 1H), 4.81 (d, *J* = 12.9 Hz, 1H), 4.37-4.32 (m, 1H), 2.71-2.68 (m, 1H), 2.41-2.35 (m, 1H), 2.07-2.03 (m, 1H), 1.93-1.85 (m, 1H), 1.79-1.74 (m, 2H), 1.62 (dd, *J* = 13.3, 3.4 Hz, 1H), 1.51-1.49 (m, 1H), 1.47 (s, 3H), 1.39-1.21 (m, 3H), 0.94 (s, 3H), 0.93 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 157.98, 155.08, 146.76, 139.88, 127.14, 127.12, 120.58, 120.34, 116.51, 116.47, 113.30, 108.75, 104.27, 102.20, 88.03, 81.09, 77.23, 77.02, 76.80, 61.71, 52.48, 44.31, 37.40, 36.38, 34.52, 30.87, 26.18, 24.68, 24.49, 20.33, 13.03; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{28}H_{30}F_2N_3O_7Na$: 600.1928. Found: 600.1954.

4.1.4.3.

*3,4-dimethyl-7-((4-((10S)-Dihydroartemisin-10-oxy)-methyl)-1H-1,2,3-triazol-1-yl)-2H-1-chromen-2-one (**6i**)*

White solid; 39.6% yield; m.p. 158~160□; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.02 (s, 1H), 7.78 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.75 (d, *J* = 8.7 Hz, 1H), 7.66 (d, *J* = 2.1 Hz, 1H), 5.44 (s, 1H), 5.02 (d, *J* = 12.7 Hz, 1H), 5.00-4.96 (m, 1H), 4.78 (d, *J* = 12.8 Hz, 1H), 4.30 (t, *J* = 6.7 Hz, 1H), 2.70-2.66 (m, 1H), 2.45 (d, *J* = 1.1 Hz, 3H), 2.40-2.34 (m, 1H), 2.25 (d, *J* = 1.0 Hz, 3H), 2.06-2.02 (m, 1H), 1.89-1.85 (m, 1H), 1.80-1.73 (m, 2H), 1.64-1.57 (m, 1H), 1.52-1.48 (m, 1H), 1.46 (s, 3H), 1.45-1.40 (m, 1H), 1.38-1.29 (m, 1H), 1.27-1.21 (m, 1H), 0.94 (d, *J* = 1.9 Hz, 3H), 0.92 (d, *J* = 3.0 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 161.28, 152.55, 146.31, 145.14, 137.89, 125.93, 123.19, 120.61, 120.55, 115.84, 108.05, 104.22, 102.03, 88.01, 81.11, 77.25, 77.03, 76.82, 61.69, 52.50, 44.34, 37.38, 36.39, 34.53, 30.86, 26.18, 24.67, 24.48, 20.33, 15.21, 13.61, 13.03; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{29}H_{35}N_3O_7Na$: 560.2367. Found: 560.2381.

4.1.4.4.

*3-chloro-4-methyl-7-((4-((10S)-Dihydroartemisin-10-oxy)-methyl)-1H-1,2,3-triazol-1-yl)-2H-1-chromen-2-one (**6j**)*

White solid; 42.5% yield; m.p. 175~177□; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.05 (s, 1H), 7.86 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.79 (d, *J* = 8.7 Hz, 1H), 7.73 (d, *J* = 2.2 Hz, 1H), 5.44 (s, 1H), 5.02 (d, *J* = 12.8 Hz, 1H), 4.98 (d, *J* = 3.4 Hz, 1H), 4.82-4.77 (m, 1H), 2.71-2.66 (m, 1H), 2.64 (s, 3H), 2.40-2.34 (m, 1H), 2.08-2.02 (m, 1H), 1.91-1.84 (m, 1H), 1.78-1.74 (m, 2H), 1.62-1.59 (m, 1H), 1.52-1.47 (m, 1H), 1.46 (s, 3H), 1.46-1.43 (m, 1H), 1.35-1.29 (m, 1H), 1.26-1.20 (m, 1H), 0.94 (d, *J* = 1.5 Hz, 3H), 0.92 (d,

J = 2.6 Hz, 3H), 0.92-0.87 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 156.28, 152.02, 146.87, 146.55, 138.83, 126.55, 121.77, 120.60, 119.59, 116.48, 108.23, 104.25, 102.11, 88.03, 81.10, 77.23, 77.02, 76.80, 61.68, 52.49, 44.33, 37.40, 36.39, 34.53, 30.87, 26.19, 24.68, 24.49, 20.33, 16.34, 13.03; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{28}H_{32}ClN_3O_7Na$: 580.1821. Found: 580.1835.

4.1.4.5.

*3-fluoro-4-methyl-7-((4-((10S)-Dihydroartemisin-10-oxy)-methyl)-1H-1,2,3-triazol-1-yl)-2H-1-chromen-2-one (**6k**)*

White solid; 39.2% yield; m.p. 162~164□; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.04 (d, *J* = 2.7 Hz, 1H), 7.90-7.83 (m, 1H), 7.75-7.74 (m, 1H), 7.74 (d, *J* = 3.4 Hz, 1H), 5.43 (d, *J* = 1.9 Hz, 1H), 5.01 (dd, *J* = 12.7, 2.6 Hz, 1H), 4.98 (d, *J* = 3.6 Hz, 1H), 4.80-4.77 (m, 1H), 2.70-2.66 (m, 1H), 2.49-2.43 (m, 3H), 2.43-2.33 (m, 1H), 2.08-2.02 (m, 1H), 1.92-1.83 (m, 1H), 1.79-1.72 (m, 2H), 1.62-1.59 (m, 1H), 1.53-1.47 (m, 2H), 1.47-1.43 (m, 3H), 1.33-1.23 (m, 3H), 0.94 (d, *J* = 2.4 Hz, 3H), 0.92 (d, *J* = 2.1 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 150.89, 146.42, 134.78, 130.92, 128.83, 124.08, 123.59, 121.13, 120.76, 120.48, 120.11, 104.23, 102.02, 88.03, 81.09, 77.23, 77.02, 76.80, 61.61, 52.48, 44.32, 37.40, 36.39, 34.53, 30.85, 29.33, 26.19, 24.67, 24.48, 20.33, 13.03; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{28}H_{32}N_3FO_7Na$: 564.2116. Found: 564.2127.

4.1.4.6.

*4-methyl-7-((4-((10S)-Dihydroartemisin-10-oxy)-ethyl)-1H-1,2,3-triazol-1-yl)-2H-1-chromen-2-one (**7g**)*

White solid; 41.1% yield; m.p. 88~91□; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.90 (s, 1H), 7.80 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.74 (d, *J* = 8.6 Hz, 1H), 7.66 (d, *J* = 2.1 Hz, 1H), 6.34 (d, *J* = 1.3 Hz, 1H), 5.24 (s, 1H), 4.83 (d, *J* = 3.4 Hz, 1H), 4.21-4.17 (m, 1H), 3.78-3.74 (m, 1H), 3.18-3.06 (m, 2H), 2.66-2.58 (m, 1H), 2.48 (d, *J* = 1.3 Hz, 3H), 2.36-2.30 (m, 1H), 2.05-1.98 (m, 1H), 1.84-1.79 (m, 1H), 1.71-1.58 (m, 2H), 1.51-1.48 (m, 1H), 1.43 (s, 3H), 1.42-1.36 (m, 2H), 1.30-1.08 (m, 3H), 0.87 (d, *J* = 7.3 Hz, 3H), 0.85 (d, *J* = 5.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) *δ* (ppm): 159.864, 154.151, 151.484, 146.839, 139.055, 126.155, 119.600, 119.425, 115.596, 115.444, 107.860, 104.037, 101.811, 87.752, 80.874, 77.186, 76.974, 76.763, 66.859, 52.358, 44.152, 37.273, 36.279, 34.447, 30.750, 26.476, 26.060, 24.549, 24.279, 20.195, 18.624, 12.943; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{29}H_{35}N_3O_7Na$: 560.2367. Found: 560.2381.

4.1.4.7.

*4-(trifluoromethyl)-7-((4-((10S)-Dihydroartemisin-10-oxy)-ethyl)-1H-1,2,3-triazol-1-yl)-2H-1-chromen-2-one (**7h**)*

White solid; 42.7% yield; m.p. 93~95□; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.94 (s, 1H), 7.88 (d, *J* = 6.2 Hz, 1H), 7.85 (d, *J* = 2.2 Hz, 1H), 7.82 (d, *J* = 2.1 Hz, 1H), 6.85 (s, 1H), 5.24 (s, 1H), 4.84 (d, *J* = 3.5 Hz, 1H), 4.21-4.17 (m, 1H), 3.80-3.76 (m, 1H), 3.16-3.07 (m, 2H), 2.65-2.59 (m, 1H), 2.37-2.30 (m, 1H), 2.01 (m, 1H), 1.86-1.79 (m, 1H), 1.72-1.60 (m, 2H), 1.50 (dd, *J* = 13.1, 3.1 Hz, 1H), 1.43 (s, 3H), 1.41-1.34 (m, 2H), 1.32-1.12 (m, 3H), 0.87 (d, *J* = 7.5 Hz, 3H), 0.86 (d, *J* = 5.7 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 158.02, 155.11, 147.24, 140.00, 127.07, 127.06, 119.44, 116.24, 113.06, 108.36, 104.15, 101.93, 100.25, 91.14, 87.84, 80.94, 77.28, 77.07, 76.86, 66.92, 52.41, 44.21, 37.37, 36.34, 34.52, 30.82, 26.54, 26.13, 24.64, 24.36, 20.28, 13.02; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{29}H_{32}F_3N_3O_7Na$: 614.2085. Found: 614.2099.

4.1.4.8.

*3,4-dimethyl-7-((4-((10S)-Dihydroartemisin-10-oxy)-ethyl)-1H-1,2,3-triazol-1-yl)-2H-1-chromen-2-one (**7i**)*

White solid; 45.9% yield; m.p. 102~104□; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.88 (s, 1H), 7.75 (d, *J* = 2.1 Hz, 1H), 7.73 (s, 1H), 7.62 (d, *J* = 2.0 Hz, 1H), 5.23 (s, 1H), 4.83 (d, *J* = 3.4 Hz, 1H), 4.20-4.17 (m, 1H), 3.77-3.73 (m, 1H), 3.16-3.06 (m, 2H), 2.62-2.58 (m, 1H), 2.46-2.43 (m, 3H), 2.36-2.29 (m, 1H), 2.24 (s, 3H), 2.04-1.98 (m, 1H), 1.83-1.78 (m, 1H), 1.69-1.59 (m, 2H), 1.50-1.46 (m, 1H), 1.42 (s, 3H), 1.40-1.36 (m, 2H), 1.31-1.12 (m, 3H), 0.87 (d, *J* = 7.3 Hz, 3H), 0.85-0.83 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) *δ* (ppm): 161.201, 152.493, 146.689, 145.099, 137.934, 125.815, 122.924, 120.246, 119.431, 115.455, 107.569, 104.005, 101.776, 87.730, 80.867, 77.212, 77.001, 76.789, 66.855, 52.354, 44.148, 37.244, 36.270, 34.433, 30.744, 26.469, 26.049, 24.532, 24.264, 20.187, 15.101, 13.481, 12.936; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{30}H_{37}N_3O_7Na$: 574.2524. Found: 574.2530.

4.1.4.9.

*3-chloro-4-methyl-7-((4-((10S)-Dihydroartemisin-10-oxy)-ethyl)-1H-1,2,3-triazol-1-yl)-2H-1-chromen-2-one (**7j**)*

White solid; 47.2% yield; m.p. 96~98□; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.85 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.78 (d, *J* = 8.7 Hz, 1H), 7.71 (d, *J* = 2.1 Hz, 1H), 5.24 (s, 1H), 4.84 (d, *J* = 3.5 Hz, 1H),

4.21-4.18 (m, 1H), 3.81-3.75 (m, 1H), 3.18-3.06 (m, 2H), 2.64 (s, 3H), 2.64-2.62 (m, 1H), 2.37-2.31 (m, 1H), 2.05-1.99 (m, 1H), 1.86-1.80 (m, 1H), 1.70-1.65 (m, 1H), 1.62-1.57 (m, 2H), 1.50 (dd, *J* = 13.2, 3.2 Hz, 1H), 1.44 (s, 3H), 1.42-1.39 (m, 1H), 1.31-1.14 (m, 3H), 0.88 (d, *J* = 7.3 Hz, 3H), 0.86 (d, *J* = 5.8 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 156.31, 152.04, 147.04, 146.92, 138.94, 126.53, 121.62, 119.47, 119.39, 116.22, 107.84, 104.16, 101.91, 87.84, 80.95, 77.25, 77.03, 76.82, 66.93, 52.42, 44.21, 37.37, 36.35, 34.52, 30.83, 26.54, 26.15, 24.64, 24.37, 20.30, 16.33, 13.04; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{29}H_{34}ClN_3O_7Na$: 594.1977. Found: 594.1997.

4.1.4.10.

*3-fluoro-4-methyl-7-((4-((10S)-Dihydroartemisin-10-oxy)-ethyl)-1H-1,2,3-triazol-1-yl)-2H-1-chromen-2-one (**6k**)*

White solid; 40.6% yield; m.p. 94~97□; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.91 (s, 1H), 7.85 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.73 (d, *J* = 8.7 Hz, 1H), 7.72 (d, *J* = 2.1 Hz, 1H), 5.24 (s, 1H), 4.83 (d, *J* = 3.5 Hz, 1H), 4.21-4.17 (m, 1H), 3.79-3.75 (m, 1H), 3.17-3.05 (m, 2H), 2.65-2.58 (m, 1H), 2.45 (d, *J* = 2.9 Hz, 3H), 2.38-2.30 (m, 1H), 2.05-1.98 (m, 1H), 1.86-1.79 (m, 1H), 1.72-1.57 (m, 2H), 1.54-1.48 (m, 1H), 1.43 (s, 3H), 1.42-1.35 (m, 2H), 1.33-1.06 (m, 3H), 0.87 (d, *J* = 7.4 Hz, 3H), 0.85 (d, *J* = 5.7 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 154.35, 154.15, 150.75, 146.90, 144.91, 143.23, 138.23, 126.13, 119.40, 116.36, 107.90, 104.04, 101.82, 87.75, 80.86, 77.17, 76.96, 76.75, 66.87, 52.34, 44.14, 37.28, 36.27, 34.44, 30.74, 26.47, 26.06, 24.55, 24.27, 20.20, 12.93, 10.16; ESI-HRMS $[M+Na]^+$: (*m/z*) Calcd. for $C_{29}H_{34}FN_3O_7Na$: 578.2273. Found: 578.2297.

4.2. Biology

4.2.1. In Vitro Cytotoxicity Study (MTT Assay)

The cells suspended in the corresponding culture medium were inoculated in 96-well microtiter plates at a density of 1500-3000 cells per well, and incubated for 24 h at 37□ in a humidified atmosphere with 95% air and 5% CO_2. The two line of cells (HT-29, MDA-MB-231) were treated with varying concentrations (100, 50, 25 and 6.25μM) of DOX, AAZ, DHA or the test compounds, and incubated for 96 h. The cells were incubated with 20 μL of 5 mg/mL MTT solution for 4h. The supernatant was discarded, and the media were then replaced with 100 μL of dimethyl sulfoxide to dissolve the purple colored formazan crystals formed in the wells, and their absorbance were measured

at 492 nm with a microplate reader (Synergy-HT, BioTek Instruments, Winooski, VT, USA); 100 μL DMSO was set as the blank control.

The anoxia condition was achieved by placing cells in a sealed hypoxia incubator chamber (Catalog Number 27310, Stemcell Technologies, Inc., Vancouver, BC, Canada) filled with 5% CO_2 and 95% N_2. For the hypoxia group, the cells were treated with varying concentrations (100, 50, 25 and 6.25μM) of DOX, AAZ, DHA or the test compounds, and incubated for 24h under hypoxia condition, and then moved into normoxic condition and cultured for additional 72h.

4.2.2. cell cycle progression arrest

HT-29 cells were immobilized with 70% ice cold ethanol. After 24 h, 1% ribonuclease and PI (50 μg/ml) was added. After 15min, FL-2 signal was collected to measure the DNA content through flow cytometer. A minimum of 10,000 events/sample was acquired.

4.2.3. Annexin V-FITC assay

The cells were washed with PBS for 3 times and digested using 0.25% trypsin. After centrifugation (1500 rpm), the cells were suspended in 300 μL binding buffer. 5 μL Annexin V-FITC was added to the suspension, which thereafter was incubated for 10 min in darkness at room temperature. Subsequently, the cells were treated with 200 μL pre-diluted binding buffer, 10 μL PI and then analyzed by flow cytometry.

4.2.4. Mitochondrial membrane potential analysis (ΔΨm)

Rh123 is a cell-permeable fluorescent dye by accumulating in active mitochondria, was used to evaluate mitochondrial transmembrane potential (ΔΨm). After specific treatment, the HT-29 cells were incubated with 5 μM Rh123 for 30 min in the dark at 37□, and then washed with PBS for 3 times. Subsequently, 400 μL PBS was added and the mitochondrial ΔΨ was measured through flow cytometer using the FSC, SSC, FL-1 channels (fluorescence 488/525nm).

4.2.5. Intracellular pH analysis

After the treatment, BCECF-AM was added and the suspension incubated for 30 min at 37□. Then,

the cells were washed with PBS and the fluorescence intensity of cells was determined using the flow cytometer system's FSC, SSC, FL-1 channels (fluorescence 488/525 nm).

4.2.6. Wound healing assay

HT-29 cells were grown to confluency. A linear wound was made by 200 μL pipette tips across the confluent cell layer. Cells were washed thrice to remove detached cells and debris. Then size of wounds were observed at the indicated times.

4.2.7. Transwell assay

After specific treatment, cells were plated in transwell inserts and stimulated with 500 μL FBS attractant and incubated for 24 h. Then, cells were washed with PBS and fixed with methyl alcohol. Discarded the solution and the cells were stained with crystal violet staining solution. The cells were detected through inverted microscope.

4.2.8. Molecular docking simulations

The experimental crystallographic structures of CA IX complex was retrieved from the Protein DataBank (PDB ID: 4ZAO). The molecular model of the ligand were built by Discovery Studio 3.5. Both the protein and the ligand were prepared by adding polar hydrogen atoms and partial charges with the assistance of AutoDockTools 1.5.6. A lattice box with the size of 62×56×66 was built to cover the active pocket of the receptor. 200 independent runs of genetic algorithm were performed and the energy evaluations were set as the maximum of 2.5×10^6 in the grid point spacing of 0.375Å. All other parameters were set to default unless stated otherwise.

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Supplementary data

The ^{1}H and ^{13}C NMR spectra of synthesized compounds are provided in supporting information.

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Highlights

1. Thirty-four new dihydroartemisinin-coumarin hybrids were designed and synthesized.
2. The hybrids obviously inhibited proliferation of HT-29 cell lines, arrested G_0/G_1 phase of HT-29 cells, suppressed the migration of tumor cells.
3. The hybrids induced a great decrease in mitochondrial membrane potential leading to apoptosis of cancer cells.
4. The hybrids could also induce the other cell death pathway-ferroptosis.