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ROYAL SOCIETY OF CHEMISTRY

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View Article Online
DOI: 10.1039/C6RA17323C

Journal Name

ARTICLE

# Novel Dihydroisoxazoline-Alkyl Carbon Chain Hybrid Artemisinin Analogues (Artemalogs): Synthesis and Antitumor Activities

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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Two new series of dihydroisoxazoline-alkyl carbon chain hybrid artemisinin analogues (artemalogs) were designed and synthesized though a 1,3-dipolar cycloaddition. Subsequent pharmacological screeneing led to several compounds having dramatically improved antiproliferative effects against several human tumor cell lines compared to artemisinin and dihydroartemisinine. Mechanistic studies on the most potent artemalogs were investigated.

## Introduction

The unique molecular frameworks of natural products have offered medicinal chemists ever-lasting inspiration for the design and development of biologically useful chemical probes and therapeutical drugs.[1-6] Though a few are used directly as disease treatment without any chemical modifications, the majority of natural products needs major cut-offs or adds-on in order to transfer to clinically useful drugs due to the strict molecule druggability and safety criteria.[1-2] Artemisinin, the active component of the sweet wormwood plant (Artemisia *annua L.*) is a natural sesquiterpene lactone containing a 1,2,4-trioxane.[7-8] The unique structure of artemisinin and its significant activity against multidrug resistant malaria has built up a new paradigm of antimalarial drug discovery, and several of these drugs (dihydroartemisinin, artemether, and artesunate) have been used for decades as the standard treatment of *P. falciparum.*[9-10] Meanwhile, artemisinin is also profiled extensively in many other disease fields, especially cancer.[11-17] Although the excise anticancer mechanism of artemisinin has been unknown, it is believed that a similar mechanism as to the antimalarial activity may exist involving cytotoxic carbon-centered free radicals generated by interaction of the endoperoxide moiety of artemisinin with heme iron.[18-21] Among the various artemisinin analogues (artemalogs) that have been reported to show antitumor activities,[22-37] compound **1** bearing a lipophilic carbon chain is one of the most potent artemalogs showing an $IC_{50}$ value of 0.46 μM against the proliferation of HepG2 cell lines, whereas the natural artemisinin only showed a much weak cytotoxicity ($IC_{50}$ = 97 μM).[34] Recently, our group prepared a series of new artemalogs bearing an isoxazoline/isoxazolidine motif and tested them for the antiproliferative effects against quamous carcinoma KB cells, vincristine-resistant KB/VCR cells, and human lung cancer A549 cells. Among these artemalogs, spirobicyclic artemalogue **2** was found to be the most potent with $IC_{50}$s of 1.47, 3.16 and 5.01 μM, respectively against the three tumor cells, which were more than four-fold more potent than that of the parent artemisinin.[37]

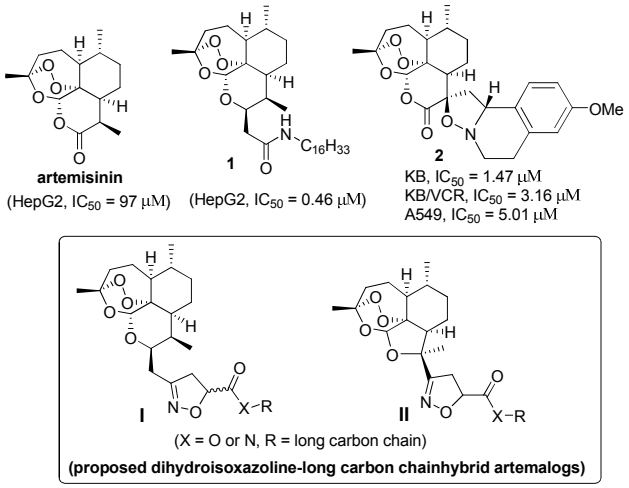


**Fig. 1** Artemisinin and its analogues.

As an extension of our interest in the development of antitumor agents based on the unique endoperoxide motif of artemisinin, we recently proposed to take advantage of both structural features of compounds **1** and **2** to generate new hrbrid artemalogs **I** and **II**, which contains both the lipophilic

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

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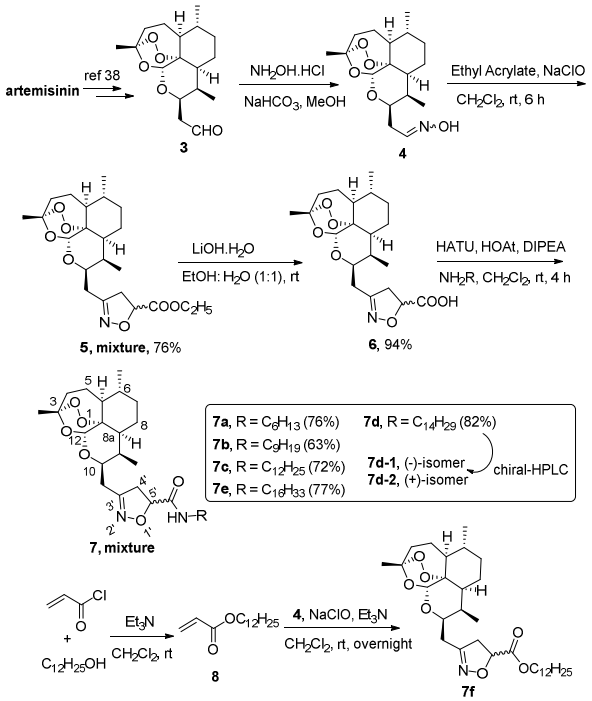
carbon chain as that in **1** as the tail terminus and the isoxazoline heterocycle as the linker as that in **2** (Fig. 1). Herein, we report the synthesis of these two new series of artemalogs and their cytotoxicity against several tumor cells.

## Results and discussion

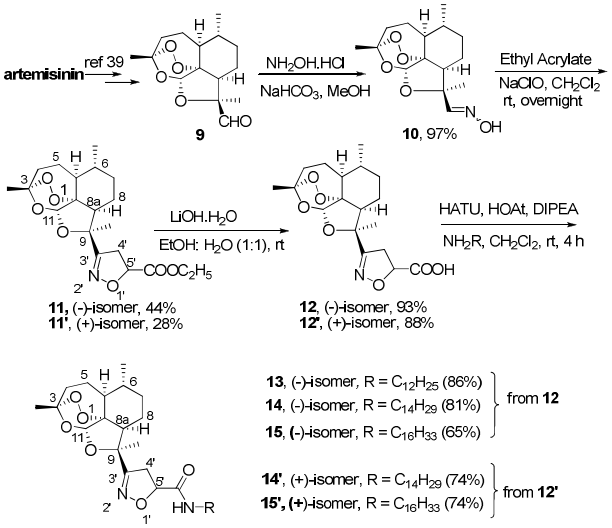
### Chemistry

As shown in Scheme 1, the aldehyde derivative **3** was obtained over 4 steps starting from artemisinin by following a literature procedure.[38] Treatment of aldehyde **3** with hydroxylamine hydrochloride gave the oxime **4**, which was further oxidized into the nitrile oxide intermediate by NaClO to undergo the 1,3-dipolar cycloaddition[39] with ethyl acrylate, affording the dihydroisoxazoline ester product **5** as a mixture of two diastereomers in 76% yield. Without further isolation, hydrolysis of **5** followed by amide condensation with alkyl amines of various length provided the dihydroisoxazoline amides **7a-7e** in 63-82% yields. The dihydroisoxazoline ester **7f** could be achieved in 46% yield when dodecyl acrylate was used as the dipolarophile in the corresponding 1,3-dipolar cycloaddition. It should be noted that neither dihydroisoxazoline esters **5** and **7f** nor dihydroisoxazoline amide **7a-7e** could be separated by TLC or silica gel column, but the NMR spectra indicated that all of them were diastereomeric mixtures. The molar ratio of the isomers of amides **7a**-**7e** was determined by the HPLC data (see SI). To obtain optically pure derivatives, **7d** was separated by preparation chiral-HPLC to provide the (-)-diastereomer **7d-1** and the (+)-diastereomer **7d-2**, respectively (Scheme 1). Nevertheless, their absolute stereochemistry at C-5' could not be assigned by 2D NMR, and crystallization of these isomers were also unsuccessful.




**Scheme 1** Synthesis of compounds **7a-7f**.

To diversify artemisinin scaffold, we shifted our synthetic effort to the ring-contracted artemisinin derivatives. The aldehyde derivative **9**, readily accessible starting from artemisinin according to the literature procedure,[40] was treated with hydroxylamine hydrochloride to give the oxime **10** in 97% yield, which was then used to generate the nitrile oxide intermediate in the presence of excess NaClO. Further 1,3-dipolar cycloaddition afforded **11** and **11'** in 44% and 28% yields, respectively. In this case, diastereoisomers **11** and **11'** could be separated by silica gel column. The absolute configuration of the newly formed C-5' center could not be deduced by NOE and efforts to crystallize these isomers were unsuccessful. As shown in experiment procedure section, $[\alpha]_D^{20}$ value of compound **11** is -50.0 (*c* 0.051, MeOH), while the $[\alpha]_D^{20}$ value of **11'** is +130.0 (*c* 0.058, MeOH). After hydrolysis of the dihydroisoxazoline esters **11** and **11'**, acid **12** was condensed with dodecan-1-amine, tetradecan-1-amine and hexadecan-1-amine, respectively, to provide dihydroisoxazoline amides **13-15** in 65-86% yields, whereas condensation of **12'** with tetradecan-1-amine and hexadecan-1-amine yielded **14'**and **15'** in 74% yields (Scheme 2).




**Scheme 2** Synthesis of compounds **13-15** and **14'-15'**.

To improve aqueous solubility, substituted piperazines bearing carbon chains of various length were employed to couple with the dihydroisoxazoline carbolic acid **12** leading to the dihydroisoxazoline amides **18a-18c** in 43-64% yields (Scheme 3a). The dihydroisoxazoline amide **22** bearing an oxa-alkyl carbon chain could be achieved when tetraoxatetradecan-1-amine was used (Scheme 3b). To further diversify artemisinin scaffold as well as investigate the role of the endoperoxide moiety on antitumor activity, **14** was subjected to $FeCl_2{\cdot}4H_2O/CH_3CN$ reduction leading to the Ring-contracted furan acetate product **23** in 82% yield (Scheme 3c).[41]

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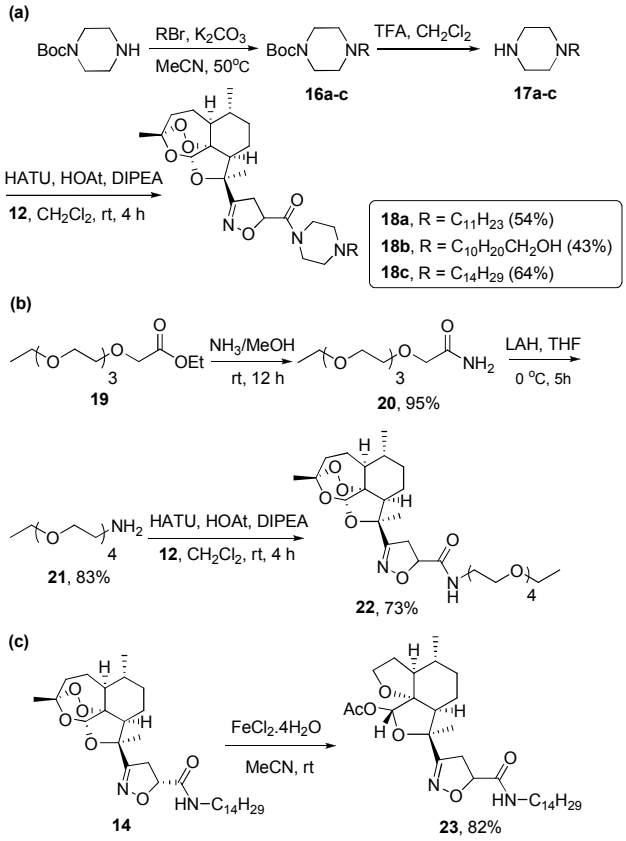
**Biological activity**

***In vitro* cytotoxic activity**. The growth inhibitory effects of all the synthesized dihydroisoxazoline artemalogs bearing long alkyl chains were evaluated against three human cancer cell lines, including squamous carcinoma KB cells, vincristine-resistant KB/VCR cells, and human lung cancer A549 cells using SRB assays as described in the in vitro screening protocol (Experimental Section).[42] The result was summarized in Table 1 and Table 2. Both artemisinin and dihydroartemisinine were chosen as positive controls. As shown in Table 1, most of the newly synthesized artemalogs (**7a–7f**) not only exhibited significantly improved antiproliferative activity against KB and A549 cells, but also displayed marked growth inhibitory effects against vincristine-resistant KB/VCR cells, for which artemisinin had only weak activity with an average $IC_{50}$ value greater than 20 μM. Among them, amide analogue **7d** with a tetradecyl chain displayed the most potent antiproliferative activity against all tested cancer cell lines with $IC_{50}$ values of 0.68, 0.47 and 0.49 μM, respectively. Both the (-)-diastereomer **7d-1** and the (+)-diastereomer **7d-2** exhibited comparable antiproliferative potency to that of **7d**, indicating the stereochemistry at C-5' position generally has no significant impact on the cellular activity. Thus, diastereomeric mixture **7d** could be directly used without separation in the following pharmacological characterization. Artemalogs with longer or shorter alkyl chains exhibited less potent activity. Replacement of the amide bond with an ester bond also led to the decreased activity against all tested cell lines, especially for A549 cell.



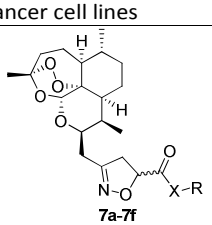

**Scheme 3** Synthesis of compounds **18a**-**18c**, **22** and **23**.

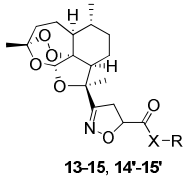
In the case of ring-contracted artemalogs **13-15** and **14'**,**15'**, most of them possessed similar potency with submicromolar $IC_{50}$ values relative to the aforementioned artemalogs. New artemalogs bearing a tetradecyl chain also showed the most potent activity. Generally, the stereochemistry at C-5' position also has no significant impact on the cellular effect. The (-)-diastereomer **14** and the (+)-diastereomer **14'** possessed similar potency against KB (0.21 *vs* 0.24 uM) and KB/VCR (0.58 *vs* 0.72 μM) cells, but **14** is about one-fold more potent than **14'** in terms of A549 cell (0.64 *vs* 1.21 μM). Similarly, for artemalogs **15** and **15'**, there is no significant difference on their potency against KB (0.55 *vs* 0.49 μM) and A549 (0.87 *vs* 1.11 μM) cells, but **15'** is one-fold more potent than **15** against KB/VCR cells (0.86 *vs* 1.61 μM).

As shown in Table 2, slightly lower activity was observed when piperazine was inserted into the lipophilic alkyl carbon chain to give artemalog **18c** with $IC_{50}$ values of 0.88 and 2.42 μM against KB and KB/VCR cells, respectively. Shortening the length of the alkyl carbon chain led to **18a** with dramatically decreased potency against the two tested cancer cell lines. Installation of the aqueous hydroxyl group at the end of the alkyl carbon chain resulted in **18b** totally losing the activity. Artemalog **22** with the tetraoxatetradecanyl chain is completely inactive, indicating the crucial role of an all carbon alkyl chain. The ring-contracted furan acetate artemalog **23** lacking the endoperoxide bridge moiety only displayed a marginal activity (around 15 μM), suggesting the endoperoxide moiety is another key pharmacophoric function.

**Compounds 7d and 14 elicited broad-spectrum *in vitro* antitumor effects.** To characterize the anticancer activity of the most potent compounds **7d** and **14**, they were further tested against a panel of 7 human cancer cell lines, including leukemia, breast cancer, ovarian cancer, gastric cancer, colon cancer, hepatoma, and lung cancer cells. As shown in Table 3, compounds **7d** and **14** exhibited a similar *in vitro* anticancer spectrum with $IC_{50}$ values ranging from 0.15 to 1.01 μM against almost all of the above human cancer cell lines, except the leukemia K562 cells in which compounds **7d** and **14** were much less effective with $IC_{50}$ values of ~10 μM. The results indicate that the new artemalogs possess a broad anticancer spectrum.

**Table 1** Antiproliferative effects of new artemalogs against human cancer cell lines




| Compound | X | R | ($IC_{50}$, μM) KB | KB/VCR | A549 |
|---|---|---|---|---|---|

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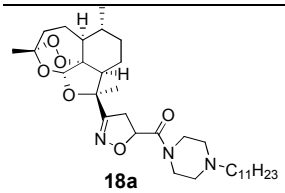
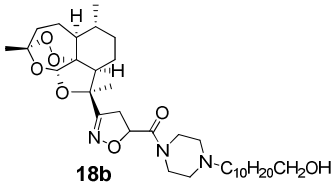
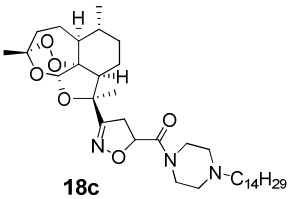
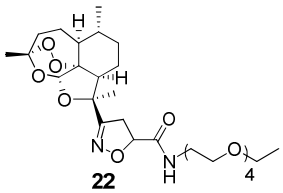
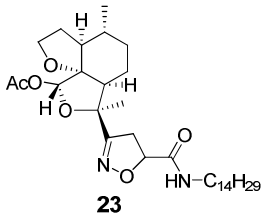
| | | | | | |
|---|---|---|---|---|---|
| **Artemisinin** | | | >20 | >20 | >20 |
| **Dihydroartemisinin** | | | 4.79 | 3.09 | 10.66 |
| **7a** | NH | $C_6H_{13}$ | 10.3 | 12.6 | ND |
| **7b** | NH | $C_9H_{19}$ | 5.36 | 5.49 | ND |
| **7c** | NH | $C_{12}H_{25}$ | 3.65 | 3.82 | ND |
| **7d** | NH | $C_{14}H_{29}$ | 0.68 | 0.47 | 0.49 |
| **7d-1** | NH | $C_{14}H_{29}$ | 0.41 | 0.88 | 1.52 |
| **7d-2** | NH | $C_{14}H_{29}$ | 0.63 | 0.84 | 0.67 |
| **7e** | NH | $C_{16}H_{33}$ | 1.55 | 3.13 | 3.28 |
| **7f** | O | $C_{12}H_{25}$ | 3.13 | 6.21 | > 20 |
| **13** | NH | $C_{12}H_{25}$ | 1.30 | 5.27 | ND |
| **14** | NH | $C_{14}H_{29}$ | 0.21 | 0.58 | 0.64 |
| **14'** | NH | $C_{14}H_{29}$ | 0.24 | 0.72 | 1.21 |
| **15** | NH | $C_{16}H_{33}$ | 0.55 | 1.61 | 0.87 |
| **15'** | NH | $C_{16}H_{33}$ | 0.49 | 0.86 | 1.11 |

ND - not determined.

**Table 2** Cytotoxicities of **18a-c** and **22, 23** against human cancer cell lines

| Compound | ($IC_{50}$, μM) | | |
|---|---|---|---|
| | KB | KB/VCR | A549 |
|  **18a** | 15.66 | 8.07 | ND |
|  **18b** | > 20 | 15.83 | ND |
|  **18c** | 0.88 | 2.42 | ND |
|  **22** | >20 | >20 | ND |
|  **23** | 15.77 | 14.94 | ND |

ND - not determined.

**Compounds 7d and 14 produced direct cytotoxic effects on multidrug resistant cancer cell lines.** Multidrug resistance (MDR), especially to drugs of natural origin, is an important impediment to the effective chemotherapy of cancer.[43] Therefore, new agents with capacity to circumvent MDR are of critical importance. To examine the activity of artemalogs **7d** and **14** against MDR, we used three classical MDR cell lines (K562/A02, KB/VCR, and MCF-7/ADR) that were significantly resistant to the corresponding drugs.[44]

**Table 3** Inhibitory effects of **7d** and **14** against cell proliferation

| Cell lines | ($IC_{50}$, μM) | |
|---|---|---|
| | **7d** | **14** |
| K562 | 12.48 | 9.25 |
| MCF-7 | 0.52 | 0.88 |
| SK-OV-3 | 0.73 | 0.80 |
| KB | 0.68 | 0.21 |
| SGC-7901 | 0.23 | 0.28 |
| SW-620 | 0.75 | 1.01 |
| BEL-7402 | 0.35 | 0.38 |
| SPC-A4 | 0.15 | 0.19 |

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As shown in Table 4, the $IC_{50}$ values of compound **7d** against these MDR cell lines were 10.23, 1.71, and 0.47 μM, while to their corresponding sensitive cell lines (K562, MCF-7, and KB), its $IC_{50}$ values were 12.48, 0.52, and 0.68 μM, respectively. The results indicated that compound **7d** showed approximately equipotent cytotoxicity against each MDR cell lines as compared with their parental cell lines. The corresponding resistance factors are 0.82, 3.29, and 0.69, respectively (Table 4). Compared to **7d**, Compound **14** displayed similar antiproliferative effects against MDR cell lines, with slightly less sensitivity against KB/VCR with higher RF values.

**Table 4** Inhibitory effects of **7d** and **14** on proliferation of drug-resistant tumor cells

| Cell lines | 7d | | 14 | |
|---|---|---|---|---|
| | $IC_{50}$ (μM) | RF | $IC_{50}$ (μM) | RF |
| K562/A02 | 10.23 | 0.82 | 9.81 | 1.06 |
| MCF-7/ADR | 1.71 | 3.29 | 2.13 | 2.42 |
| KB/VCR | 0.47 | 0.69 | 0.58 | 2.76 |

NOTE: Resistance factor (RF) was calculated as the ratio of the $IC_{50}$ value of the drug-resistant cells to that of the corresponding parent cells in Table 3.

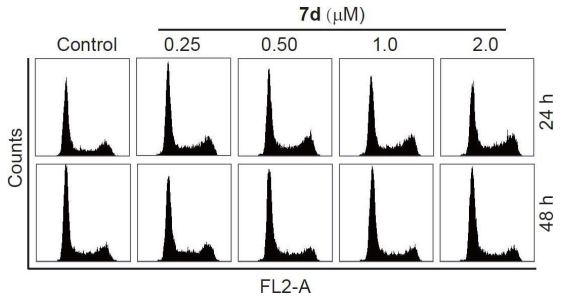


**Fig. 2** **7d** did not induce typical cell cycle arrest in SGC-7901 cells.

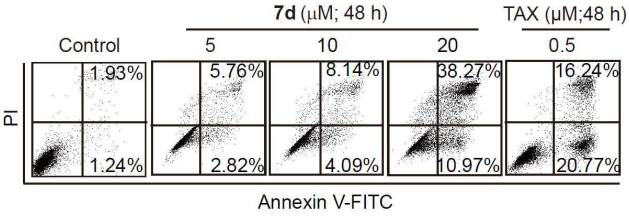


**Fig. 3** **7d** induced apoptosis in SW-620 cells. TAX, taxol.

**Cell-cycle analysis.** On the basis of the promising antiproliferative effect, compound **7d** was selected for further mechanistic study to determine whether the growth inhibitory effect was induced by a cell-cycle arrest. We treated SGC-7901 cells with compound **7d** for 24 or 48 h at 0.25 μM, 0.5 μM, 1.0 μM and 2.0 μM, respectively, and the DNA content of cell nuclei was detected by flow cytometry. The result showed that **7d** did not induce typical cell cycle arrest in SGC-7901 cells at any concentrations (Fig. 2).

**Compound 7d induced apoptosis of SW-620 cells.** To determine whether the growth inhibition induced by **7d** was attributed to apoptosis. SW-620 cells were treated with vehicle and Taxol (TAX) as controls. Meanwhile, similar treatments of SW-620 cells with **7d** were conducted at different concentrations (5, 10, or 20 μM) for 48 h and then stained with FITC-Annexin V and propidium iodide (PI). The percentages of apoptotic SW-620 cells were determined by flow cytometry. As shown in Fig. 3, compound **7d** displayed moderate effects to induce apoptosis of SW-620 cells in a dose-dependent manner, and resulted in 8.6%, 12.2%, and 49.2% of apoptotic cells (early and late apoptosis) with 5, 10, and 20 μM, respectively, as compared to 3.1% in an untreated vehicle control.

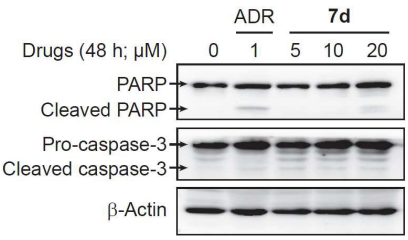


**Fig. 4** **7d** induced the cleavage of PARP and Caspase-3 in SW-620 cells. ADR, adriamycin.

**Effects of 7d on cell apoptosis proteins PARP and caspase-3.** To elucidate the potential mechanism of cell apoptosis induced by the new artemalog **7d**, several proteins related to apoptosis were determined by Western blotting. As shown in Fig. 4, treatment of SW-620 cells with compound **7d** at moderate concentrations (5–20 μM) slightly triggered PARP and caspase-3 cleavages from their full-length form to the cleaved form as indicated by the weak appearance of PARP fragments and activated caspase-3 in a concentration-dependent manner.

## Conclusions

Two new series of dihydroisoxazoline-lipophilic carbon chain hybrid artemalogs were designed and synthesized though 1,3-dipolar cycloaddition. Most of these compounds displayed significantly improved antiproliferative effects against three human tumor cell lines, compared to artemisinin and dihydroartemisinine. Among these new artemalogs, dihydroisoxazoline amide artemalogs **7d** and **14** with tetradecyl chain demonstrated the most potent activity with submicromolar $IC_{50}$ values. Further investigations indicated that artemalogs **7d** and **14** not only elicited broad-spectrum in vitro antitumor effects, but also produced direct cytotoxic

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effects on multidrug resistant cancer cell lines. Mechanism studies showed that **7d** did not induce cell cycle arrest significantly, but at least partially mediated apoptosis to exert its antiproliferative activity. Further in vivo studies and antitumor mechanism investigation of the new potent artemalogs are undergoing.

## Experimental Section

### General experimental information

All reactions were performed in glassware containing a Tefloncoated stir bar. Solvents and chemical reagents were obtained from commercial sources and used without further purifications. Optical rotations were measured on Autopol VI, serial number 90079, manufactured by Rudolph Research Analytical, Hackettstown, NJ. $^{1}$H and $^{13}$C spectra were recorded on Varian Mercury 300 MHz, 400MHz and 500 MHz and the data were recorded using $CDCl_3$ as the solvent. Chemical shifts (δ) are reported in ppm downfield from an internal TMS standard. Low and high-resolution mass spectra were obtained in the ESI and EI mode. Flash column chromatography on silica gel (200-300 mesh) was used for the routine purification of reaction products. The column output was monitored by TLC on silica gel (100-200 mesh) precoated on glass plates (15 x 50 mm). HPLC analysis was conducted for all bioassayed compounds on an Agilent Technologies 1260 series LC system (Agilent ChemStation Rev.A.10.02; SB-C18, 4.6 mm × 150 mm, 2 μM, MeOH/$H_2O$, rt) with the ultraviolet wavelengths of 214 nm. All the assayed compounds displayed chemical purity greater than 95%.

### Preparation of intermediate 4

The aldehyde **3** was prepared according to the literature procedure.[39]

To a mixture of compound **3** (310 mg, 1 mmol) and $NaHCO_3$ (420 mg, 5 mmol) in MeOH (5 mL) was added hydroxylamine hydrochloride (345 mg, 5 mmol). The resulting mixture was stirred at rt for 1 h, and concentrated *in vacuo*. The residue was diluted with water (20 mL) and washed with $CH_2Cl_2$ (3×10 mL). The combined organic layers were washed with water and brine, dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure to give compound **4** (286 mg, 88%) as a colorless oil. The crude product was used to the next step without further purification.

### Preparation of 10-(5'-(methoxycarbonyl)-4',5'-dihydroisoxazol-3'-yl)methyldeoxoartemisinin (5)

To a solution of compound **4** (130 mg, 0.4 mmol) and ethyl acrylate (44 mg, 0.44 mmol) in dry $CH_2Cl_2$ (10 mL) was added a solution of sodium hypochlorite (2.24 mL, 13 % active chlorine), then triethylamine (61 mg, 0.6 mmol) was added dropwise at rt. The mixture was stirred at rt for 6 h and then washed with water (2 x 10 mL). The organic layer was washed with brine, dried over anhydrous $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel chromatography using petroleum ether/ethyl acetate (5/1) to afford a mixture of diastereoisomers **5** (128 mg, 76%) as a colorless oil. $^{1}$H NMR (300 MHz, Chloroform-*d*) δ 5.33 – 5.32 (m, 1H), 5.03 – 4.94 (m, 1H), 4.59 – 4.51 (m, 1H), 4.28 – 4.19 (m, 1H), 3.52 – 3.32 (m, 2H), 2.78 – 2.49 (m, 3H), 2.36 – 2.26 (m, 1H), 2.06 – 1.92 (m, 2H), 1.83 – 1.65 (m, 3H), 1.50 – 1.21 (m, 9H), 0.98 – 0.84 (m, 8H).

### Preparation of the acid intermediate 6

To a solution of compound **5** (85 mg, 0.2 mmol) in EtOH (2 mL) and $H_2O$ (2 mL) was added lithium hydroxide monohydrate (10 mg, 0.24 mmol), and the reaction was stirred at rt for 4 h. The aqueous layer was adjusted to pH 3 by adding hydrochloride acid (0.5 M) and extracted with $CH_2Cl_2$ (3 × 10 mL). The combined organic layers were washed with water and brine, dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure to give compound **6** (74 mg, 94%). The crude product was used to the next step without further purification.

### Preparation of 7a-7e

To a solution of compound **6** (0.1 mmol) in $CH_2Cl_2$ (2 mL), HATU (0.2 mmol) and HOAT (0.1 mmol) was added at 0 °C. After stirring for 10 min at 0 °C, an appropriate aliphatic amine (0.1 mmol) and *N*, *N*-diisopropylethylamine (0.3 mmol) were added to this solution. The resulting mixture was stirred at rt for 4 h and then diluted with $CH_2Cl_2$ (15 mL), washed with saturated $NH_4Cl$ solution, water, and brine. The organic layer was dried over anhydrous $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified *via* silica gel chromatography with petroleum ether/ethyl acetate (2/1) as the eluent to afford a mixture of diastereoisomers **7a-7e**.

**10-(5'-(Hexylcarbamoyl)-4',5'-dihydroisoxazol-3'-yl)methyldeoxoartemisinin (7a)**. Colorless oil (36 mg, 76%); $^{1}$H NMR (400 MHz, Chloroform-*d*) δ 6.76 – 6.71 (m, 1H), 5.32 and 5.29 (s, s, 1H, H-12), 4.97 – 4.91 (m, 1H), 4.59 – 4.55 (m, 1H), 3.53 – 3.15 (m, 4H), 2.77 – 2.61 (m, 2H), 2.47 – 2.42 (m, 1H), 2.34 – 2.22 (m, 1H), 2.05 – 1.92 (m, 2H), 1.81 – 1.66 (m, 4H), 1.50 – 1.25 (m, 16H), 0.97 – 0.88 (m, 8H); $^{13}$C NMR (126 MHz, Chloroform-*d*) δ 170.53 and 170.37 (1C), 158.40 and 158.37 (1C), 102.34 and 102.31 (1C), 89.36 and 89.24 (1C), 80.49, 77.73 and 77.56 (1C), 69.96 and 69.37 (1C), 51.38, 43.18, 41.39, 40.67, 38.66, 37.03 and 36.99 (1C), 36.11, 33.80, 30.97 and 30.91 (1C), 29.74 and 29.69 (1C), 28.86, 28.45 and 28.34 (1C), 26.02 and 25.99 (1C), 25.43 and 25.38 (1C), 24.32 and 24.29 (1C), 22.03, 19.53, 13.53 and 13.50 (1C), 11.94 and 11.91 (1C); MS (ESI) 501.2 $[M + Na]^+$. HRMS (ESI) calcd for $C_{26}H_{43}N_2O_6$, 479.3116; found, 479.3118. $[\alpha]_D^{20}$ = +54 (c 0.049, MeOH).

**10-(5'-(Nonylcarbamoyl)-4',5'-dihydroisoxazol-3'-yl)methyldeoxoartemisinin (7b)**. Colorless oil (33 mg, 63%); $^{1}$H NMR (400 MHz, Chloroform-*d*) δ 6.79 – 6.73 (m, 1H), 5.32 and 5.30 (s, s, 1H, H-12), 4.98 – 4.91 (m, 1H), 4.60 – 4.54 (m, 1H), 3.46 – 3.17 (m, 4H), 2.78 – 2.60 (m, 2H), 2.48 – 2.42 (m, 1H), 2.35 – 2.27 (m, 1H), 2.06 – 1.92 (m, 2H), 1.82 – 1.65 (m, 3H), 1.52 – 1.21 (m, 23H), 0.98 – 0.86 (m, 8H); $^{13}$C NMR (126 MHz, Chloroform-*d*) δ 170.52 and 170.36 (1C), 158.36 and 158.34 (1C), 102.32 and 102.28 (1C), 89.34 and 89.23 (1C), 80.46,



 

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77.71 and 77.55 (1C), 69.90 and 69.36 (1C), 51.36, 43.16, 41.36, 40.66, 38.65, 37.01 and 36.97 (1C), 36.10 and 36.08 (1C), 33.79, 31.36 and 31.33 (1C), 29.72 and 29.68 (1C), 29.18 – 28.33 (5C), 26.35 and 26.32 (1C), 25.42 and 25.37 (1C), 24.30 and 24.27 (1C), 22.15, 19.51, 13.60, 11.91 and 11.89 (1C); MS (ESI) 543.3 $[M + Na]^+$. HRMS (ESI) calcd for $C_{29}H_{49}N_2O_6$, 521.3585; found, 521.3597. $[\alpha]_D^{20}$ = +72 (c 0.050, MeOH).

**10-(5'-(Dodecylcarbamoyl)-4',5'-dihydroisoxazol-3'-yl)methyldeoxoartemisinin (7c)**. Colorless oil (40 mg, 72%); $^1$H NMR (400 MHz, Chloroform-*d*) δ 6.75 – 6.72 (m, 1H), 5.32 and 5.29 (s, s, 1H, H-12), 4.97 – 4.90 (m, 1H), 4.59 – 4.54 (m, 1H), 3.50 – 3.14 (m, 4H), 2.77 – 2.59 (m, 2H), 2.48 – 2.42 (m, 1H), 2.34 – 2.26 (m, 1H), 2.04 – 1.92 (m, 2H), 1.81 – 1.65 (m, 3H), 1.50 – 1.21 (m, 29H), 0.97 – 0.88 (m, 8H); $^{13}$C NMR (126 MHz, Chloroform-*d*) δ 170.52 and 170.35 (1C), 158.37 and 158.35 (1C), 102.32 and 102.29 (1C), 89.36 and 89.25 (1C), 80.48, 77.72 and 77.56 (1C), 69.91 and 69.35 (1C), 51.37, 43.17, 41.38, 40.67, 38.66, 37.03 and 36.98 (1C), 36.11 and 36.09 (1C), 33.80, 31.41, 29.74 and 29.69 (1C), 29.17 – 28.35 (8C), 26.37 and 26.34 (1C), 25.43 and 25.38 (1C), 24.31 and 24.28 (1C), 22.18, 19.52, 13.62, 11.93 and 11.90 (1C); MS (ESI) 585.3 $[M + Na]^+$. HRMS (ESI) calcd for $C_{32}H_{55}N_2O_6$, 563.4055; found, 563.4046. $[\alpha]_D^{20}$ = +64 (c 0.057, MeOH).

**10-(5'-(Tetradecylcarbamoyl)-4',5'-dihydroisoxazol-3'-yl)methyldeoxoartemisinin (7d)**. Colorless oil (48 mg, 82%); $^1$H NMR (300 MHz, Chloroform-*d*) δ 6.77 – 6.71 (m, 1H), 5.32 and 5.29 (s, s, 1H, H-12), 4.97 – 4.90 (m, 1H), 4.61 – 4.53 (m, 1H), 3.54 – 3.14 (m, 4H), 2.78 – 2.57 (m, 2H), 2.48 – 2.41 (m, 1H), 2.36 – 2.25 (m, 1H), 2.07 – 1.92 (m, 2H), 1.82 – 1.64 (m, 3H), 1.52 – 1.13 (m, 33H), 1.00 – 0.83 (m, 8H); $^{13}$C NMR (126 MHz, Chloroform-*d*) δ 171.04 and 170.88 (1C), 158.90 and 158.55 (1C), 102.86 and 102.82 (1C), 89.87 and 89.75 (1C), 81.00, 78.23 and 78.07 (1C), 70.45 and 69.89 (1C), 51.89, 43.69, 41.89, 41.18, 39.18, 37.54 and 37.50 (1C), 36.60, 34.30, 31.93, 30.24 and 30.20 (1C), 29.70 – 28.85 (10C), 26.88 and 26.85 (1C), 25.94 and 25.89 (1C), 24.79, 22.70, 20.03, 14.13, 12.44 and 12.42 (1C); MS (ESI) 613.4 $[M + Na]^+$. HRMS (ESI) calcd for $C_{34}H_{59}N_2O_6$, 591.4368; found, 591.4354. $[\alpha]_D^{20}$ = +75 (c 0.044, MeOH).

**(-)-10-(5'-(Tetradecylcarbamoyl)-4',5'-dihydroisoxazol-3'-yl)methyldeoxoartemisinin (7d-1)**. Colorless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 6.75 – 6.74 (m, 1H), 5.31 (s, 1H), 4.95 (dd, *J* = 11.6, 6.0 Hz, 1H), 4.60 – 4.56 (m, 1H), 3.49 (dd, *J* = 18.0, 11.6 Hz, 1H), 3.40 – 3.18 (m, 3H), 2.73 – 2.61 (m, 2H), 2.48 (dd, *J* = 11.2, 3.2 Hz, 1H), 2.36 – 2.28 (m, 1H), 2.05 – 1.94 (m, 2H), 1.83 – 1.67 (m, 2H), 1.51 – 1.27 (m, 33H), 0.99 – 0.86 (m, 8H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 171.72, 159.75, 103.66, 90.60, 81.83, 79.08, 71.29, 52.72, 44.52, 42.75, 40.02, 38.38, 37.46, 35.15, 32.77, 31.09, 30.69 – 29.83 (9C), 29.70, 27.72, 26.79, 25.66, 23.53, 20.87, 14.96, 13.25. $[\alpha]_D^{20}$ = -8 (c 0.049, MeOH).

**(+)-10-(5'-(Tetradecylcarbamoyl)-4',5'-dihydroisoxazol-3'-yl)methyldeoxoartemisinin (7d-2)**. Colorless oil. $^1$H NMR (500 MHz, $CDCl_3$) δ 6.76 – 6.74 (m, 1H), 5.32 (s, 1H), 4.95 (dd, *J* = 11.5, 6.0 Hz, 1H), 4.57 (ddd, *J* = 11.5, 6.5, 3.0 Hz, 1H), 3.49 (dd, *J* = 17.5, 12.0 Hz, 1H), 3.36 – 3.26 (m, 2H), 3.15 (td, *J* = 13.0, 7.0 Hz, 1H), 2.73 (dd, *J* = 15.0, 11.5 Hz, 1H), 2.66 – 2.57 (m, 1H), 2.44 (dd, *J* = 15.0, 3.0 Hz, 1H), 2.30 (tt, *J* = 14.5, 7.5 Hz, 1H), 2.05 – 1.92 (m, 2H), 1.81 – 1.65 (m, 3H), 1.52 – 1.25 (m, 33H), 1.03 – 0.83 (m, 8H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 171.87, 159.70, 103.66, 90.71, 81.82, 78.91, 70.68, 52.72, 44.52, 42.00, 39.99, 38.32, 37.43, 35.13, 32.75, 31.03, 30.70 – 29.97 (9C), 29.80, 27.68, 26.71, 25.62, 23.52, 20.85, 14.95, 13.25. $[\alpha]_D^{20}$ = +100 (c 0.051, MeOH).

**10-(5'-(Hexadecylcarbamoyl)-4',5'-dihydroisoxazol-3'-yl)methyldeoxoartemisinin (7e)**. colorless oil (48 mg, 77%); $^1$H NMR (400 MHz, Chloroform-*d*) δ 6.76 – 6.70 (m, 1H), 5.32 and 5.29 (s, s, 1H, H-12), 4.97 – 4.91 (m, 1H), 4.60 – 4.55 (m, 1H), 3.49 – 3.16 (m, 4H), 2.73 – 2.62 (m, 2H), 2.48 – 2.42 (m, 1H), 2.34 – 2.22 (m, 1H), 2.05 – 1.92 (m, 2H), 1.81 – 1.65 (m, 3H), 1.49 – 1.25 (m, 37H), 0.97 – 0.84 (m, 8H); $^{13}$C NMR (126 MHz, Chloroform-*d*) δ 170.54 and 170.38 (1C), 158.40 and 158.37 (1C), 102.34 and 102.31 (1C), 89.37 and 89.25 (1C), 80.50, 77.73 and 77.57 (1C), 69.93 and 69.37 (1C), 51.39, 43.19, 41.39, 40.67, 38.67, 37.03 and 36.99 (1C), 36.10, 33.80, 31.43, 29.70, 29.20 – 28.35 (12C), 26.37 and 26.35 (1C), 25.44 and 25.39 (1C), 24.29, 22.20, 19.53, 13.63, 11.94; MS (ESI) 641.4 $[M + Na]^+$. HRMS (ESI) calcd for $C_{36}H_{63}N_2O_6$, 619.4681; found, 619.4695. $[\alpha]_D^{20}$ = +67 (c 0.046, MeOH).

**Preparation of 7f**

To a solution of compound **4** (65 mg, 0.2 mmol) and dodecyl acrylate (53 mg, 0.22 mmol) in dry $CH_2Cl_2$ (5 mL) was added a solution of sodium hypochlorite (1.12 mL, 13 % active chlorine), then triethylamine (31 mg, 0.3 mmol) was added dropwise. The mixture was stirred at rt overnight and then washed with water (2 x 10 mL). The organic layer was washed with brine, dried over anhydrous $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica column chromatography using petroleum ether/ethyl acetate (5/1) to afford a mixture of diastereoisomers **7** (52 mg, 46%) as a colorless oil.

**10-(5'-Dodecyloxycarbonyl)-4',5'-dihydroisoxazol-3'-yl)methyldeoxoartemisinin (7f).** $^1$H NMR (400 MHz, Chloroform-*d*) δ 5.32 (s, 1H), 5.01 – 4.93 (m, 1H), 4.57 – 4.52 (m, 1H), 4.18 – 4.13 (m, 2H), 3.50 – 3.30 (m, 2H), 2.76 – 2.49 (m, 3H), 2.35 – 2.27 (m, 1H), 2.04 – 1.92 (m, 2H), 1.82 – 1.58 (m, 5H), 1.38 – 1.25 (m, 27H), 0.97 – 0.86 (m, 8H); $^{13}$C NMR (126 MHz, Chloroform-*d*) δ 170.66, 157.56 and 156.30 (1C), 102.87 and 102.81 (1C), 89.82 and 89.79 (1C), 81.04 and 81.01 (1C), 77.31 and 77.20, (1C), 71.06 and 70.38 (1C), 65.87, 51.95 and 51.91 (1C), 43.77 and 43.71 (1C), 41.18, 40.49, 37.54 and 37.49 (1C), 36.60, 34.31, 31.92, 30.30 and 30.19 (1C), 29.66 – 28.48 (8C), 25.89, 25.78, 24.80, 22.69, 20.05 and 19.96 (1C), 14.13, 12.49 and 12.40 (1C); MS (ESI) 586.4 $[M + Na]^+$. HRMS (ESI) calcd for $C_{32}H_{54}NO_7$, 564.3895; found, 564.3886. $[\alpha]_D^{20}$ = +59 (c 0.051, MeOH).

**Preparation of intermediate 10**

The ring-contracted artemisinin aldehyde **9** was prepared according to the literature procedure.[40]

To a mixture of compound **9** (282 mg, 1 mmol) and $NaHCO_3$ (420 mg, 5 mmol) in MeOH (5 mL) was added hydroxylamine hydrochloride (345 mg, 5 mmol). The resulting mixture was

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stirred at rt for 1 h, concentrated *in vacuo*. The residue was diluted with water (20 mL) and washed with $CH_2Cl_2$ (3 × 10 mL). The combined organic layer were washed with water and brine, dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure to give compound **10** (286 mg, 97%) as colorless oil. The crude product was used to the next step without further purification.

**Preparation of 11 and 11'**

To a solution of compound **10** (118 mg, 0.4 mmol) and ethyl acrylate (44 mg, 0.44 mmol ) in dry $CH_2Cl_2$ (10 mL) was added a solution of sodium hypochlorite (2.24 mL, 13 % active chlorine) and then triethylamine (61 mg, 0.6 mmol) was added dropwise at rt. The mixture was stirred at rt overnight and then washed with water (2 x 10 mL). The organic layer was washed with brine, dried over anhydrous $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel chromatography using petroleum ether/ethyl acetate (5/1) to afford **11** (70 mg, 44%) and **11'** (44 mg, 28%) as a colorless oil.

**(-)-9-(5'-(Methoxycarbonyl)-4',5'-dihydroisoxazol-3'-yl) ring-contracted artemalog (11)**. $^1$H NMR (300 MHz, Chloroform-*d*) δ 5.71 (s, 1H), 4.97 (dd, *J* = 11.6, 5.6 Hz, 1H), 4.23 (q, *J* = 7.1 Hz, 2H), 3.58 (dd, *J* = 18.0, 11.6 Hz, 1H), 3.29 (dd, *J* = 18.0, 5.6 Hz, 1H), 2.37 – 2.29 (m, 2H), 2.11 – 1.95 (m, 3H), 1.85 (s, 3H), 1.57 – 1.47 (m, 5H), 1.41 – 1.22 (m, 5H), 1.03 – 0.98 (m, 5H). $[\alpha]_D^{20}$ = -50 (c 0.051, MeOH).

**(+)-9-(5'-(Methoxycarbonyl)-4',5'-dihydroisoxazol-3'-yl) ring-contracted artemalog (11')**. H NMR (300 MHz, Chloroform-*d*) δ 5.63 (s, 1H), 4.89 (dd, *J* = 11.6, 6.4 Hz, 1H), 4.16 (q, *J* = 7.2 Hz, 2H), 3.50 (dd, *J* = 17.9, 6.4 Hz, 1H), 3.21 (dd, *J* = 17.9, 11.4 Hz, 1H), 2.30 – 2.21 (m, 2H), 2.04 – 1.89 (m, 3H), 1.76 (s, 3H), 1.54 – 1.40 (m, 5H), 1.36 – 1.17 (m, 5H), 1.00 – 0.86 (m, 5H). $[\alpha]_D^{20}$ = +130 (c 0.058, MeOH).

**Preparation of the acid 12 and 12'**

To a solution of compound **11** or **11'** (79 mg, 0.2 mmol) in EtOH (2 mL) and $H_2O$ (2 mL) was added lithium hydroxide monohydrate (10 mg, 0.24 mmol). The reaction was stirred at rt for 4 h, and then adjusted to pH 3 by adding hydrochloride acid (0.5 M) and extracted with $CH_2Cl_2$ (3 × 10 mL). The combined organic layers were washed with water and brine, dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure. The crude product **12** (68 mg, 93%) or **12'** (64 mg, 88%) was used to the next step without further purification.

**Preparation of 13-15 and 14'-15'**

To a solution of compound **12** or **12'** (0.1 mmol) in $CH_2Cl_2$ (2 mL), HATU (0.2 mmol) and HOAT (0.1 mmol) was added at 0 $^oC$. After stirring for 10 min at 0 $^oC$, an appropriate aliphatic amine (0.1 mmol) and *N,N*-diisopropylethylamine (0.3 mmol) were added to this solution. The resulting mixture was stirred at rt for 4 h, and then diluted with $CH_2Cl_2$ (15 mL), washed with saturated $NH_4Cl$ solution, water, and brine. The organic layer was dried over anhydrous $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified *via* silica gel chromatography with petroleum ether/ethyl acetate (2/1) as the eluent to afford **13-15** and **14'-15'**.

**(-)-9-(5'-(Dodecylcarbamoyl)-4',5'-dihydroisoxazol-3'-yl) ring-contracted artemalog (13)**. Colorless oil (46 mg, 86%); $^1$H NMR (400 MHz, Chloroform-*d*) δ 6.83 – 6.79 (m, 1H), 5.69 (s, 1H), 4.93 (dd, *J* = 11.5, 3.9 Hz, 1H), 3.58 (dd, *J* = 18.3, 11.5 Hz, 1H), 3.36 (dd, *J* = 18.3, 3.9 Hz, 1H), 3.31 – 3.13 (m, 2H), 2.36 – 2.22 (m, 2H), 2.12 – 1.95 (m, 2H), 1.84 (s, 3H), 1.75 – 1.70 (m, 1H), 1.55 – 1.25 (m, 27H), 0.99 – 0.86 (m, 8H); $^{13}$C NMR (126 MHz, Chloroform-*d*) δ 170.56, 165.20, 103.38, 97.13, 85.51, 83.97, 77.43, 53.26, 48.05, 41.62, 38.73, 36.57, 36.06, 31.95, 31.40, 29.20 – 28.78 (7C), 27.97, 26.35, 25.90, 24.75, 23.73, 22.18, 19.24, 13.62; MS (ESI) 557.4 $[M + Na]^+$. HRMS (ESI) calcd for $C_{30}H_{51}N_2O_6$, 535.3742; found, 535.3743. $[\alpha]_D^{20}$ = -33 (c 0.057, MeOH).

**(-)-9-(5'-(Tetradecylcarbamoyl)-4',5'-dihydroisoxazol-3'-yl) ring-contracted artemalog (14)**. Colorless oil (45 mg, 81%); $^1$H NMR (400 MHz, Chloroform-*d*) δ 6.84 – 6.81 (m, 1H), 5.69 (s, 1H), 4.93 (dd, *J* = 11.6, 4.0 Hz, 1H), 3.58 (dd, *J* = 18.4, 11.6 Hz, 1H), 3.36 (dd, *J* = 18.4, 4.0 Hz, 1H), 3.30 – 3.14 (m, 2H), 2.36 – 2.23 (m, 2H), 2.12 – 1.95 (m, 2H), 1.84 (s, 3H), 1.75 – 1.68 (m, 1H), 1.56 – 1.25 (m, 31H), 1.02 – 0.86 (m, 8H); $^{13}$C NMR (126 MHz, Chloroform-*d*) δ 170.57, 165.21, 103.39, 97.13, 85.52, 83.97, 77.43, 53.25, 48.06, 41.62, 38.73, 36.57, 36.07, 31.95, 31.42, 29.18 – 28.78 (9C), 27.97, 26.35, 25.90, 24.75, 23.73, 22.19, 19.24, 13.62; MS (ESI) 585.4 $[M + Na]^+$. HRMS (ESI) calcd for $C_{32}H_{55}N_2O_6$, 563.4055; found, 563.4070. $[\alpha]_D^{20}$ = -33 (c 0.044, MeOH).

**(+)-9-(5'-(Tetradecylcarbamoyl)-4',5'-dihydroisoxazol-3'-yl) ring-contracted artemalog (14')**. Colorless oil (42 mg, 74%); $^1$H NMR (400 MHz, Chloroform-*d*) δ 6.76 – 6.73 (m, 1H), 5.71 (s, 1H), 4.93 (dd, *J* = 11.2, 4.8 Hz, 1H), 3.48 (dd, *J* = 17.6, 4.8 Hz, 1H), 3.34 – 3.14 (m, 3H), 2.36 – 2.20 (m, 2H), 2.11 – 1.96 (m, 2H), 1.78 – 1.72 (m, 4H), 1.60 – 1.25 (m, 31H), 1.10 – 0.86 (m, 8H); $^{13}$C NMR (126 MHz, Chloroform-*d*) δ 171.12, 163.60, 103.35, 96.84, 85.69, 84.16, 77.67, 52.53, 48.08, 41.41, 38.72, 36.58, 36.27, 31.91, 31.43, 29.20 – 28.80 (9C), 27.21, 26.34, 26.19, 24.71, 23.83, 22.20, 19.30, 13.63; MS (ESI) 585.4 $[M + Na]^+$. HRMS (ESI) calcd for $C_{32}H_{55}N_2O_6$, 563.4055; found, 563.4054. $[\alpha]_D^{20}$ = +82 (c 0.053, MeOH).

**(-)-9-(5'-(Hexadecylcarbamoyl)-4',5'-dihydroisoxazol-3'-yl) ring-contracted artemalog (15).** Colorless oil (38 mg, 65%); $^1$H NMR (400 MHz, Chloroform-*d*) δ 6.83 – 6.80 (m, 1H), 5.69 (s, 1H), 4.93 (dd, *J* = 11.6, 4.0 Hz, 1H), 3.58 (dd, *J* = 18.4, 11.6 Hz, 1H), 3.36 (dd, *J* = 18.4, 4.0 Hz, 1H), 3.31 – 3.13 (m, 2H), 2.36 – 2.20 (m, 2H), 2.11 – 1.96 (m, 2H), 1.84 (s, 3H), 1.75 – 1.70 (m, 1H), 1.55 – 1.25 (m, 35H), 1.02 – 0.86 (m, 8H); $^{13}$C NMR (126 MHz, Chloroform-*d*) δ 170.56, 165.20, 103.37, 97.13, 85.51, 83.97, 77.43, 53.26, 48.05, 41.62, 38.73, 36.57, 36.06, 31.95, 31.42, 29.19 – 28.79 (11C), 27.96, 26.35, 25.90, 24.75, 23.73, 22.19, 19.24, 13.62; MS (ESI) 591.3 $[M + H]^+$. HRMS (ESI) calcd for $C_{34}H_{59}N_2O_6$, 591.4368; found, 591.4366. $[\alpha]_D^{20}$ = -24 (c 0.043, MeOH).

**(+)-9-(5'-(Hexadecylcarbamoyl)-4',5'-dihydroisoxazol-3'-yl) ring-contracted artemalog (15').** Colorless oil (44 mg, 74%); $^1$H NMR (400 MHz, Chloroform-*d*) δ 6.79 – 6.75 (m, 1H), 5.73 (s, 1H), 4.94 (dd, *J* = 11.6, 4.8 Hz, 1H), 3.48 (dd, *J* = 18.0, 4.8 Hz,

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DOI: 10.1039/C6RA17323C




1H), 3.36 – 3.16 (m, 3H), 2.37 – 2.25 (m, 2H), 2.12 – 1.97 (m, 2H), 1.80 – 1.70 (m, 4H), 1.58 – 1.27 (m, 35H), 1.12 – 0.88 (m, 8H); $^{13}$C NMR (126 MHz, Chloroform-*d*) δ 170.12, 163.67, 103.33, 96.83, 85.68, 84.15, 77.67, 52.52, 48.07, 41.41, 38.71, 36.57, 36.26, 31.91, 31.43, 29.20 – 28.80 (11C), 27.20, 26.34, 26.18, 24.71, 23.83, 22.19, 19.30, 13.63; MS (ESI) 591.3 [M + H]$^+$. HRMS (ESI) calcd for $C_{34}H_{59}N_2O_6$, 591.4368; found, 591.4381. $[\alpha]_D^{20}$ = +74 (c 0.051, MeOH).

**Preparation of intermediates 16a-16c**

To a solution of tert-Butyl 1-piperazinecarboxylate (3 mmol) and $K_2CO_3$ (6 mmol) in MeCN (10 mL) was added the appropriate alkyl bromide (3.15 mmol), the resulting mixture was stirred at rt for 2 h. The residue was diluted with water (20 mL) and washed with $CH_2Cl_2$ (3 × 10 mL). The combined organic layer were washed with water and brine, dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure. The crude product was used to the next step without further purification.

**Preparation of intermediates 17a-17c**

To a solution of **16a**, **16b** or **16c** (1 mmol) in $CH_2Cl_2$ (10 mL) was added trifluoroacetic acid (30 mmol). The reaction was stirred at rt for 4 h and then concentrated *in vacuo*. The residue was used to the next step without purification.

**Preparation of 18a-18c**

To a solution of compound **12** (0.1 mmol) in $CH_2Cl_2$ (2 mL), HATU (0.2 mmol) and HOAT (0.1 mmol) was added at 0 $^o$C. After stirring for 10 min at 0 $^o$C, **17a**, **17b** or **17c** (0.1 mmol) and *N, N*-diisopropylethylamine (0.3 mmol) were added to this solution. The resulting mixture was stirred at rt for 4 h and then diluted with $CH_2Cl_2$ (15 mL), washed with saturated $NH_4Cl$ solution, water, and brine. The organic layer was dried over anhydrous $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified *via* silica gel chromatography with MeOH/$CH_2Cl_2$ (40/1) as the eluent to afford **18a**, **18b** or **18c**.

**(-)-9-((5'-(4-Undecylpiperazine-1-carbonyl)-4',5'-dihydroisoxazol-3'-yl) ring-contracted artemalog (18a)**. Colorless oil (32 mg, 54%); $^1$H NMR (400 MHz, Chloroform-*d*) δ 5.73 (s, 1H), 5.16 (dd, *J* = 11.3, 7.3 Hz, 1H), 3.85 – 3.72 (m, 3H), 3.59 – 3.47 (m, 2H), 3.37 (dd, *J* = 18.0, 11.3 Hz, 1H), 2.58 – 2.45 (m, 3H), 2.40 – 2.26 (m, 5H), 2.10 – 2.05 (m, 1H), 2.01 – 1.94 (m, 1H), 1.87 – 1.81 (m, 4H), 1.62 – 1.36 (m, 5H), 1.29 – 1.09 (m, 19H), 0.99 – 0.85 (m, 7H); $^{13}$C NMR (126 MHz, Chloroform-*d*) δ 165.32, 164.92, 103.31, 97.09, 85.82, 84.24, 76.19, 58.01, 53.29, 52.77, 52.15, 48.21, 45.27, 41.96, 38.77, 36.64, 36.18, 32.00, 31.40, 29.20 – 28.83 (3C), 28.31, 26.98, 26.13, 24.83, 23.78, 22.18, 19.30, 13.62; MS (ESI) 590.4 [M + H]$^+$. HRMS (ESI) calcd for $C_{33}H_{56}N_3O_6$, 590.4164; found, 590.4178. $[\alpha]_D^{20}$ = -108 (c 0.052, MeOH).

**(-)-9-((5'-(4-(11-Hydroxyundecyl)piperazine-1-carbonyl)-4',5'-dihydroisoxazol-3'-yl) ring-contracted artemalog (18b).** Colorless oil (26 mg, 43%); $^1$H NMR (400 MHz, Chloroform-*d*) δ 5.74 (s, 1H), 5.17 (dd, *J* = 11.2, 7.2 Hz, 1H), 3.86 – 3.73 (m, 3H), 3.65 – 3.51 (m, 4H), 3.38 (dd, *J* = 18.0, 11.2 Hz, 1H), 2.52 – 2.49 (m, 3H), 2.41 – 2.27 (m, 5H), 2.13 – 2.06 (m, 1H), 2.01 – 1.97 (m, 1H), 1.88 – 1.84 (m, 4H), 1.63 – 1.40 (m, 9H), 1.37 – 1.11 (m, 17H), 0.99 – 0.86 (m, 4H); $^{13}$C NMR (126 MHz, Chloroform-*d*) δ 165.82, 165.42, 103.82, 97.58, 86.32, 84.73, 76.69, 63.00, 58.53, 53.78, 53.32, 52.70, 48.70, 45.80, 42.50, 39.26, 37.14, 36.68, 32.79, 32.50, 29.69 – 29.40 (3C), 28.80, 27.44, 26.64, 25.73, 25.32, 24.27, 19.80; MS (ESI) 606.4 [M + H]$^+$. HRMS (ESI) calcd for $C_{33}H_{56}N_3O_7$, 606.4113; found, 606.4095. $[\alpha]_D^{20}$ = -82 (c 0.049, MeOH).

**(-)-9-((5'-(4-Tetradecylpiperazine-1-carbonyl)-4',5'-dihydroisoxazol-3'-yl) ring-contracted artemalog (18c).** Colorless oil (40 mg, 64%); $^1$H NMR (400 MHz, Chloroform-*d*) δ 5.74 (s, 1H), 5.18 (dd, *J* = 11.2, 7.2 Hz, 1H), 3.87 – 3.73 (m, 3H), 3.58 – 3.51 (m, 2H), 3.39 (dd, *J* = 18.0, 11.2 Hz, 1H), 2.53 – 2.48 (m, 3H), 2.39 – 2.27 (m, 5H), 2.11 – 2.06 (m, 1H), 2.01 – 1.96 (m, 1H), 1.88 – 1.84 (m, 4H), 1.67 – 1.47 (m, 7H), 1.42 – 1.11 (m, 23H), 0.99 – 0.86 (m, 7H); $^{13}$C NMR (126 MHz, Chloroform-*d*) δ 165.37, 165.49, 103.37, 97.14, 85.87, 84.29, 76.24, 58.06, 53.34, 52.82, 52.20, 48.26, 45.27, 41.96, 38.81, 36.69, 36.24, 32.05, 31.48, 29.24 – 28.91 (7C), 28.36, 27.01, 26.19, 24.89, 23.83, 22.25, 19.35, 13.69; MS (ESI) 632.4 [M + H]$^+$. HRMS (ESI) calcd for $C_{36}H_{62}N_3O_6$, 632.4633; found, 632.4647. $[\alpha]_D^{20}$ = -80 (c 0.053, MeOH).

**Preparation of 3,6,9,12-tetraoxatetradecan-1-amide (20)**

A solution of compound **19** (528 mg, 2 mmol) and $NH_3$.MeOH (5 mL) was stirred at rt for 12 h, the reaction was evaporated under reduced pressure to afford the crude product **20** (445 mg, 95%) as a colorless oil.

**Preparation of 3,6,9,12-tetraoxatetradecan-1-amine (21)**

To a stirred solution of compound **20** (235 mg, 1 mmol) in anhydrous THF (5 mL) at 0 $^o$C under nitrogen atmosphere was carefully added $LiAlH_4$ (152 mg, 4 mmol). The resulting mixture was stirred at rt for 5 h. The reaction was then slowly quenched at 0 $^o$C with water and filtered over a pad of celite. The filtrate was washed with $CH_2Cl_2$ (3 × 10 mL). The combined organic layer were washed with water and brine, dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure to give reduced compound **21** (184 mg, 83%) as a colorless oil.

**Preparation of 22**

To a solution of compound **12** (37 mg, 0.1 mmol) in $CH_2Cl_2$ (2 mL), HATU (76 mg, 0.2 mmol) and HOAT (14 mg, 0.1 mmol) was added at 0 $^o$C. After stirring for 10 min at 0 $^o$C, compound **21** (22 mg, 0.1 mmol) and *N, N*-Diisopropylethylamine (0.3 mmol) were added to this solution. The resulting mixture was stirred at rt for 4 h and then diluted with $CH_2Cl_2$ (15 mL), washed with saturated $NH_4Cl$ solution, water, and brine. The organic layer was dried over anhydrous $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified *via* silica gel chromatography with MeOH/$CH_2Cl_2$ (30/1) as the eluent to afford **22** (42 mg, 73%) as a colorless oil.

**(-)-9-((5'-((3,6,9,12-Tetraoxatetradecylcarbamoyl)-4',5'-dihydroisoxazol-3'-yl) ring-contracted artemalog (22).** $^1$H NMR

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(400 MHz, Chloroform-*d*) δ 7.27 – 7.25 (m, 1H), 5.69 (s, 1H), 4.94 (dd, *J* = 11.6, 4.0 Hz, 1H), 3.68 – 3.33 (m, 20H), 2.36 – 2.26 (m, 2H), 2.13 – 1.96 (m, 2H), 1.84 (s, 3H), 1.78 – 1.73 (m, 1H), 1.56 – 1.33 (m, 6H), 1.26 – 1.20 (m, 5H), 0.99 – 0.94 (m, 4H); $^{13}$C NMR (126 MHz, Chloroform-*d*) δ 170.73, 164.87, 103.34, 97.10, 85.53, 83.98, 77.44, 70.11, 70.06, 69.98, 69.83, 69.27, 69.00, 66.10, 53.26, 48.03, 41.51, 38.43, 36.55, 36.05, 32.02, 27.94, 25.76, 24.73, 23.72, 19.24, 14.62; MS (ESI) 571.2 [M + H]$^+$. HRMS (ESI) calcd for $C_{28}H_{46}N_2NaO_{10}$, 593.3045; found, 593.3057. $[\alpha]_D^{20}$ = -62 (c 0.050, MeOH).

#### Preparation of 23

To a solution of compound **14** (56mg, 0.1 mmol) in MeCN (2 mL) was added $FeCl_2{\cdot}4H_2O$ (38 mg, 0.2 mmol) at rt. After 10 min, the reaction mixture was filtered over Celite and the filtrate concentrated under reduced pressure. Purification by silica gel chromatography using petroleum ether/ethyl acetate (3/1) as the eluent gave compound **23** (46 mg, 82%) as a colorless oil.

**Ring-contracted furan acetate artemalog (23).** $^1$H NMR (400 MHz, Chloroform-*d*) δ 6.78 – 6.75 (m, 1H), 6.28 (s, 1H), 4.94 – 4.89 (m, 1H), 4.13 – 4.09 (m, 1H), 3.88 – 3.82 (m, 1H), 3.42 – 3.37 (m, 2H), 3.31 – 3.16 (m, 2H), 2.28 – 2.20 (m, 1H), 2.13 (s, 3H), 2.03 – 1.99 (m, 1H), 1.84 – 1.74 (m, 5H), 1.58 – 1.52 (m, 2H), 1.49 – 1.33 (m, 3H), 1.30 – 1.11 (m, 23H), 1.01 – 0.86 (m, 7H); $^{13}$C NMR (101 MHz, Chloroform-*d*) δ 170.84, 169.82, 163.95, 96.85, 88.90, 85.86, 77.91, 67.71, 52.62, 50.91 , 41.73, 39.20, 34.38, 31.93, 30.32, 29.70, 29.66, 29.61, 29.53, 29.37, 29.29, 27.56, 26.86, 26.59, 26.24, 22.70, 21.50, 20.05, 14.14; MS (ESI) 585.4 [M + Na]$^+$. HRMS (ESI) calcd for $C_{32}H_{54}N_2NaO_6$, 585.3874; found, 585.3881. $[\alpha]_D^{20}$ = -72 (c 0.054, MeOH).

#### Biological assays

#### Cell culture

Human cancer KB, K562, MCF-7, SW-620, A549 and SPC-A4 cell lines were purchased from the the American Type Culture Collection (ATCC; Manassas, VA). The K562/A02 subline was from the Institute of Hematology, Chinese Academy of Medical Sciences (Tianjin, China). Both KB/VCR and MCF-7/ADR sublines were from the Sun Yat-Sen University of Medical Sciences (Guangzhou, China). SK-OV-3 cells were from the Japanese Foundation of Cancer Research (Tokyo, Japan). SGC-7901 and BEL-7402 cell lines were kept in the Shanghai institute of *Materia Medica* of the Chinese Academy of Sciences (Shanghai, China). Cells were periodically authenticated with morphologic inspection and tested for mycoplasma contamination. The cell lines were cultured according to the suppliers' instructions.

#### Proliferation inhibition assays

Cells were seeded into 96-well plates, cultured overnight and treated with gradient concentrations of the tested agents for 72 h. The $IC_{50}$ values of different agents in adherent and suspension cells were measured by the sulforhodamine B (SRB; Sigma, MO) assay and the Cell counting kit-8 (CCK-8) (Dojindo Laboratories, Japan) assay, respectively.

#### Cell cycle assays

SGC-7901 were seeded into 6-well plates, cultured overnight and treated with different concentrations of **7d** for 24 h and 48 h. Cells were then harvested and washed with PBS, fixed with pre-cooled 70% ethanol at 4°C. Staining went along in PBS containing 40 g/ml RNase A and 10 g/ml propidium iodide (PI) in the dark for 30 min. For each sample, at least $1\times10^4$ cells were collected with a FACS Calibur (BD Biosciences, Franklin Lakes, NJ) and analyzed using the CELLQUEST software (BD Biosciences, Franklin Lakes, NJ).

#### Annexin V-FITC apoptosis assays

SW-620 were seeded into 6-well plates, cultured overnight and treated with different agents. Then, cells were harvested, washed and stained by using a AnnexinV-FITC apoptosis detection kit (KeyGEN BioTECH, Nanjing, China). Fluorescence of the cells was determined immediately by flow cytometry (BD Biosciences, Franklin Lakes, NJ).

#### Western blotting

SW-620 cells were treated with **7d** for 48 h. Western blotting was used to detect the cleavage of PARP and caspase-3.

## Acknowledgements

This work was supported by grants from the Pujiang Talent Project from Shanghai Commission of Science and Technology (14PJ1410700), and Chinese NSF (81402790, 81125021, 81373277, 81430080), and National Program on Key Basic Research Project of China (2015CB910603-004).

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DOI: 10.1039/C6RA17323C


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