Discovery of Artemisinin-Glycolipid Hybrids as Anti-oral Cancer Agents

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Novel artemisinin-glycolipid hybrids were directly synthesized from 12β (C–C)-type deoxoartemisinin and glycolipid and exhibited exceptional *in vitro* anticancer activity, particularly against the oral carcinoma cancer cell lines, respectively. The artemisinin-glycolipid hybrids, with effective concentrations under 20 μM, demonstrated better anticancer activity than either artemisinin or glycolipid alone and showed five times more anti-oral cancer activity than either cisplatin or paclitaxel.

Key words artemisinin; glycolipid; hybrid; anti-oral cancer activity

Artemisinin (**1**) (Fig. 1), also known as *qinghaosu*, is a natural sesquiterpene trioxane isolated from *Artemisia annua* L.[1] Artemisinin and its derivatives have been used clinically to treat drug-resistant malaria.[2—6] The pharmacology and pharmacokinetics of artemisinin and its derivatives are well studied.[7—12] Artemisinin derivatives have also been reported to have antiangiogenic activity.[13—20] We recently reported that 12β(C–C)-type artemisinin show strong anti-angiogenic activity. In fact, they are comparable as potent as fumagillin and thalidomide, known antiangiogenic agents.[17] As we have described previously,[17,18,21,22] a variety of researchers have reported on the potential antitumor properties of artemisinin and its derivatives.[23—28] We have previously demonstrated that 12β(C–C)-type amide derivatives of deoxoartemisinin exhibit higher *in vitro* anticancer activity[18] along with 20 times more acid stability than 12β(C–O)-type derivatives of artemisinin.[29]

The glycolipid daumone (**2**) (Fig. 1), a pheromone isolated from *Caenorhabditis elegans*, was identified by our laboratory and its total synthesis was previously presented.[30] Daumone signals *C. elegans* to enter the dauer stage, an enduring and non-aging stage of the nematode life cycle with distinctive adaptive features and extended life.[30] Although some studies have suggested the possibility of using glycolipids as anticancer chemotherapeutic agents, the relatively weak activity of this class of compounds has generally discouraged further investigation.[31—35] Recently, glycolipids fractionated from spinach have been shown to inhibit the activities of replicative DNA polymerase (pols), mitochondrial pol,[36] and glycosphingolipids found in invertebrates not having sialic acid suppressed cell proliferation, an effect associated with suppression of the activation of focal adhesion kinase (FAK), extracellular signal-regulated kinase (Erk), and AKT in melanoma B16F10 cells.[37] We previously reported a versatile synthesis of novel glycolipid derivatives through stereospecific α-glycosylation to afford di- and tri-rhamnoside daumone derivatives, most of which possessed potent anticancer activity against human cancer cell lines with effective concentrations in the nanomolar range, which is comparable to that of doxorubicin.[38]

In order to improve anticancer activity, treatment using hybrids drugs, an approach that incorporates two drugs into a single molecule, has been developed.[39] The use of hybrid drugs can impact multiple targets simultaneously. Although gefitinib originally developed as anti-lung cancer drug has been later reported to be cytotoxic against oral cell line (HSC-2),[40] no anti-oral cancer agent has been reported in the literature to date.

In this study we report on the practical synthesis and the potent *in vitro* anti-oral cancer activity of derivatives of 12β(C–C)-type deoxoartemisinin, glycolipids, and novel artemisinin-glycolipid hybrids.

Results and Discussion

As outlined in Chart 1, compound **6**[22] was obtained through 5 consecutive reactions from artemisinc acid (**3**)[41] *via* 12-allylic deoxoartemisinin (**4**) and 12-(3-hydroxypropyl)deoxoartemisinin (**5**).[41,42] New amide derivatives **7** and **8** were prepared by reacting compound **6** with 3-aminoquinoline or piperazine in the presence of 1-hydroxybenzotriozole (HOBt) or 4-dimethylaminopyridine (DMAP) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) as coupling agents with the previously described method[18] to give 89% and 52% yields, respectively (Chart 1).

During the stereospecific total synthesis[30] of daumone (**2**) from L-rhamnose in ten steps, the versatile intermediate **9** was briefly used for the preparation of compounds **10**,[38] **12**, and daumone **2** by the known procedure[30] as shown in Chart 2.

The controlled ozonolysis of compound **9** using ozone in methylene chloride and subsequent reduction with $NaBH_4$ provided compound **11** in one step with 66% yield.

Artemisinin-glycolipid hybrids **14**, **15** and **16** were prepared by following the procedures (Chart 3).

Oxidation of the double bond of the intermediate **4** gave carboxymethyldeoxoartemisinin **13a** by the known procedure.[41] The coupling between compound **13a** and compound **10** using EDC and DMAP gave hybrids **14** and **15** with 31%

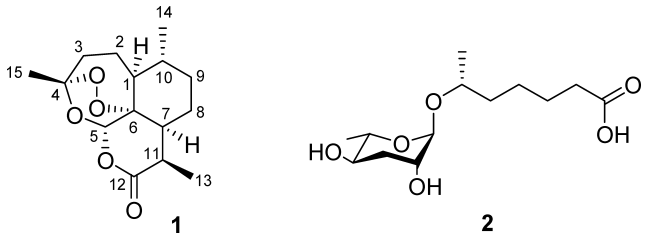

Fig. 1. Structure of Artemisinin (**1**) and Daumone (**2**)

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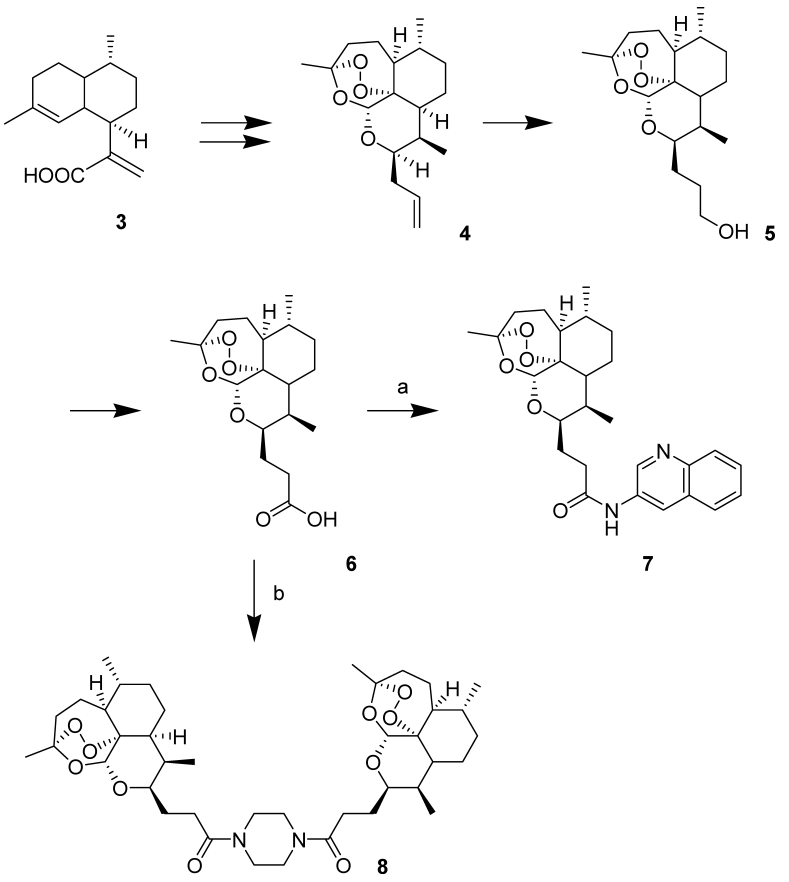
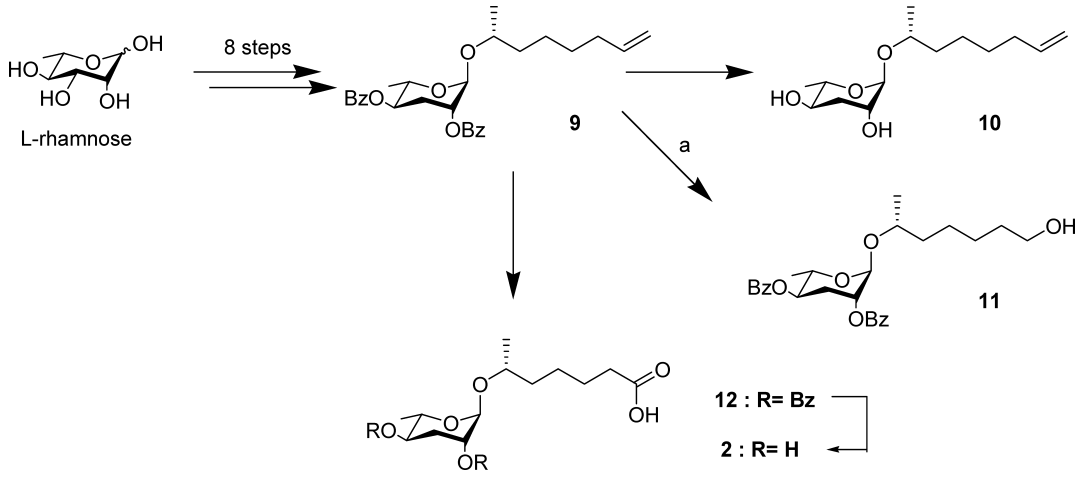


Reagents and conditions: (a) 3-aminoquinoline, DMAP, EDC, DMF, rt, 3 h, 89%; (b) piperazine, HOBt, EDC, CH_2Cl_2, rt, 12 h, 52%.
Chart 1. Synthesis of Deoxoartemisinin Derivatives, **7** and **8**



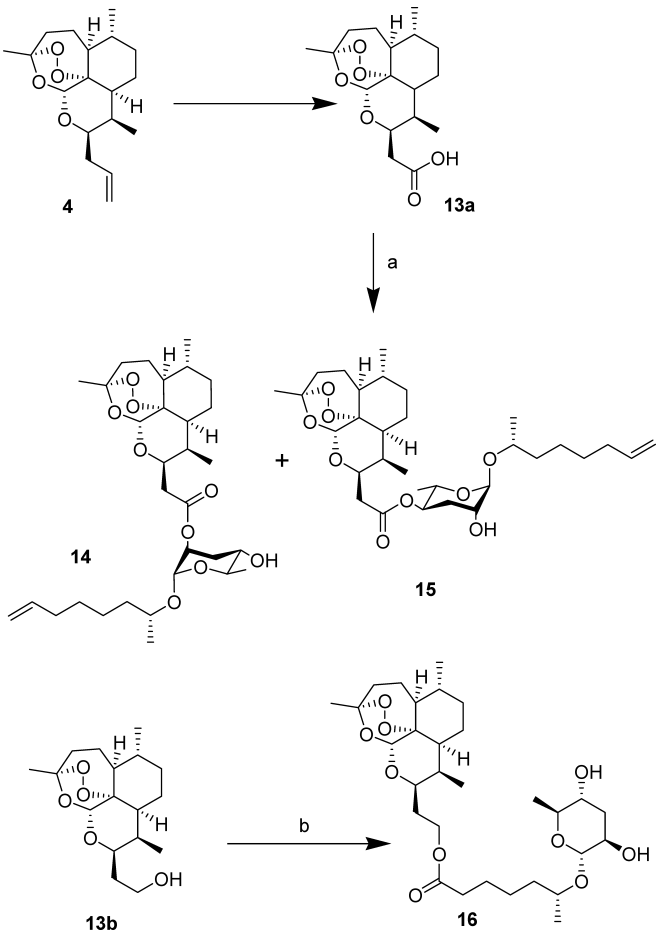
Reagents and conditions: (a) O_3, CH_2Cl_2, −76 °C, 5 min, $NaBH_4$, EtOH, 0 °C, 16 h, 66%.
Chart 2. Synthesis of Daumone Derivatives, **11** and **12**

and 32% yields, respectively. The hybrid **16** was prepared in a fashion similar to the above coupling step from 12-hydroxyethyldeoxoartemisinin, **13b**[43] obtained from compound **4**.

In order to determine the *in vitro* anticancer activities of artemisinin (**1**), daumone (**2**), compounds **7**, **8**, **11**, **12**, and hybrids **14**, **15**, and **16**, we tested the cell proliferation inhibitory activity of these derivatives against the cancer cell lines, MDA-MB-231, A549, HSC-2 and Ca.9-22 using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric method.

The results of this analysis are summarized in Table 1. The standard drugs used for comparison were cisplatin, paclitaxel and gefitinib as positive controls. We found that the inhibitory effects of all compounds (at micromolar concentrations) differed according to the type of cancer cell line.

In our conditions, artemisinin and daumone, as well as cisplatin and paclitaxel, failed to show notable activities at concentrations under 100 μM. For the breast cancer (MCF-7) cell line, all tested molecules had IC_{50} values at 100 μM. In lung cancer (A549) cell lines, only the artemisinin-glycolipid hy-



Reagents and conditions: (a) compound **10**, EDC, DMAP, CH_2Cl_2, rt, 12 h, 31% for **14** and 32% for **15**; (b) compound **2**, EDC, DMAP, CH_2Cl_2, rt, 12 h, 60%.

Chart 3. Synthesis of Artemisinin-Glycolipid Hybrids, **14**, **15** and **16**

brid **15** showed three times more potent anticancer activity than those of the references[44,45] at a concentration of 29.7 μM. For breast cancer (MDA-MB-231) cell lines, compounds **8**, **11**, **12**, **14** and **15** showed one to two times more potent anticancer activity compared with cisplatin[46] or paclitaxel.[47] For the oral squamous carcinoma cells lines (HSC-2 and Ca.9-22), artemisinin derivatives **7** and **8** and glycolipid derivatives **11** and **12** generally possessed more potent anticancer activities at up to three times that of the reference drugs. However, artemisinin derivatives showed more potent activity than glycolipid derivatives. Particularly, the hybrids **14** and **15**, with IC_{50} values under 20 μM, showed five to six times more potent anti-oral cancer activity than either cisplatin or paclitaxel and comparable activity to that of gefitinib. In comparison, the 12β(C–C)-type dimer artemisinin derivative (**8**) generally showed better anticancer activity than the 12β(C–C)-type monomer (**7**). Moreover, 12β(C–O)-type derivatives exhibited, as shown in artemisinin, weaker activities than 12β(C–C)-type derivatives. These results are in accordance with previous observations.[17] In the case of glycolipid derivatives, the benzoyl protected glycolipids **11** and **12** showed better activities than the non-protected glycolipid daumone (**2**). This trend was also observed with the artemisinin-glycolipid hybrids. Interestingly, hybrid **16** showed lower anticancer activity, suggesting the importance of regiospecificity. The artemisinin derivative should be connected with one of the hydroxy groups of the tetrahydropyran ring of glycolipid. As expected, the anticancer activities of hybrids **14** and **15** were much more potent than single monomers of artemisinin or glycolipid, respectively. In a mode of proposed action, selective approach of the hybrids with proper combination of lipophilicity and hydrophilicity to the cancer cell membrane may enhance anticancer activity arisen by homolytic cleavage of internal peroxide of artemisinin.

Table 1. Cytotoxic Activities of Artemisinin-Glycolipid Derivatives (**1**, **2**, **7**, **8**, **11**, **12**, **14**, **15**, **16**) against Four Human Cancer Cell Lines

Compounds	IC_{50} values (μM)			
	MDA-MB-231	A549	HSC-2	Ca.9-22
Artemisinin (**1**)	>100	>100	>100	>100
7	>100	>100	36.9	49.7
8	30.0	>100	25.7	33.2
Daumone (**2**)	>100	>100	>100	>100
11	33.2	>100	69.7	83.8
12	75.6	>100	80.3	69.5
14	37.7	>100	19.1	19.4
15	43.6	29.7	15.7	15.1
16	>100	>100	>100	>100
Cisplatin	>100	>100	>100	>100
Paclitaxel	>100	>100	>100	>100
Gefitinib	—	18.7	23.6	—

MDA-MB-231: metastatic breast cancer cells (estrogen receptor-negative); A549: lung cancer cells; HSC-2: oral squamous carcinoma cells (mouth origin); Ca.9-22: oral squamous carcinoma cells (gingiva origin).

Conclusion

In summary, two new 12β(C–C)-type artemisinin amide derivatives were synthesized and they showed potent anti-oral cancer activity. Hybrids of 12β(C–C)-types of artemisinin and glycolipid were synthesized in one-step reactions and these hybrids showed exceptional anti-oral cancer activity. It is also noteworthy that the hybrid compounds generally showed drastic increases in anticancer activity in comparison with the non-coupled molecules artemisinin (**1**) or daumone (**2**) alone. The artemisinin-glycolipid hybrids deserve further evaluation as possible anti-oral cancer drug candidates.

Experimental

Chemistry Commercial reagents were obtained from commercial suppliers and used as received. All solvents were purchased in septum sealed bottles stored under an inert atmosphere. All reactions were sealed with septa through which an argon atmosphere was introduced unless otherwise noted. Liquid reagents and solvents were transferred under a positive pressure of argon *via* syringe. Reactions were conducted in round-bottomed flasks containing Teflon-coated magnetic stir bars. Reactions were monitored by thin layer chromatography (TLC) on precoated TLC glass plates (silica gel 60 F254, 250 μm thickness). Visualization of the developed TLC chromatogram was performed by fluorescence quenching. Flash chromatography was performed on an automated purification system using prepacked silica gel columns. ^{1}H-NMR were recorded on NMR spectra (250 MHz) were obtained on Bruker AC250 spectrometer using Me_4Si as an internal standard and ^{13}C-NMR spectra (100 MHz) were measured on the same instrument; chemical shifts (δ) are reported relative to residual protio solvent signals. Data for NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity (s=singlet, brs=broad singlet, d=doublet, t=triplet, q=quartet, dd=doublet of doublets, m=multiplet), coupling constant (Hz), integration.

(3*R*,5a*S*,6*R*,8a*S*,9*R*,10*R*,12*R*,12a*R*)-[2′-(3-Amidequinoline)]-decahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin-10-

propanamide (7) A stirred solution of **6** (20 mg, 0.06 mmol), EDC (35 mg, 0.18 mmol) and DMAP (7 mg, 0.06 mmol) in DMF (1 ml) was combined with 3-aminoquinoline (8 mg, 0.06 mmol) at room temperature during 3 h. The reaction mixture was quenched with slow addition of saturated citric acid (2 ml), extracted with ethyl acetate (3×5 ml) and washed with $NaHCO_3$ (5 ml) and brine (5 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The mixture was purified by flash column chromatography on silica gel with hexane/EtOAc (2 : 1, v/v) as eluant to give **7** (24 mg, 89%). *Rf*=0.11 (*n*-hexane/EtOAc=2 : 1 v/v); $[\alpha]_D^{20}$ +18.0° (*c*=0.05, $CHCl_3$); ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 8.89 (br s, 1H), 8.83—8.86 (m, 1H), 8.82 (s, 1H), 8.00 (d, *J*=7.90 Hz, 1H), 7.77 (d, *J*=8.21 Hz, 1H), 7.55—7.62 (m, 1H), 7.49 (t, *J*=7.42 Hz, 1H), 5.36 (s, 1H), 4.14—4.25 (m, 1H), 1.33 (s, 3H), 0.93 (d, *J*=5.69 Hz, 3H), 0.89 (d, *J*=7.58 Hz, 3H); ^{13}C-NMR (63 MHz, $CDCl_3$) δ: 172.5, 145.1, 144.5, 132.2, 129.0, 128.4, 128.1, 127.9, 127.1, 123.8, 103.6, 89.1, 81.3, 76.3, 52.4, 44.4, 37.5, 36.6, 36.2, 34.50, 30.4, 26.2, 25.2, 25.0, 24.7, 20.3, 13.1; IR (KBr, cm^{-1}) ν_{max} 2965, 2855, 1684, 1559, 1490, 1375, 1188, 1125, 1093, 1052, 1012, 787, 756; MALDI-TOF MS: Found 467.1881 $[M+H]^+$. HR-MS (FAB) Calcd for $C_{27}H_{34}N_2O_5$ $[M+H]^+$ *m/z* 467.2546, Found 467.2542. *Anal.* Calcd for $C_{27}H_{34}N_2O_5$: C, 69.52; H, 7.29; N, 6.00. Found: C, 69.48; H, 7.31; N, 6.10.

(3*R*,3′*R*,5a*S*,5′a*S*,6*R*,6′*R*,8a*S*,8′a*S*,9*R*,9′*R*,10*R*,10′*R*,12*R*,12′*R*,12a*R*,12′a*R*)-*N*10,*N*10′-(1,4-Piperazinepropanediyl)-bis[decahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin-10-propanamide (8) A stirred solution of **6** (25 mg, 0.074 mmol), HOBt (30 mg, 0.22 mmol) and EDC (42 mg, 0.22 mmol) in dry CH_2Cl_2 (1 ml) was combined with piperazine (3 mg, 0.037 mmol) at room temperature during 12 h. The reaction mixture was quenched with slow addition of saturated citric acid (2 ml), extracted with CH_2Cl_2 (3×10 ml) and washed with $NaHCO_3$ (5 ml) and brine (5 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The mixture was purified by flash column chromatography on silica gel with CH_2Cl_2/MeOH (15 : 1, v/v) as eluant to give **8** (28 mg, 52%). *Rf*=0.54 (CH_2Cl_2/MeOH=15 : 1 v/v); $[\alpha]_D^{24}$ +31.1° (c=0.05, $CHCl_3$); ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 5.21 (s, 2H), 3.99—3.97 (m, 2H), 3.71—3.69 (m, 1H), 3.65—3.57 (m, 4H), 3.56—3.43 (m, 4H), 2.70—2.68 (m, 4H), 2.30—2.22 (m, 4H), 1.97—1.92 (m, 4H), 1.80—1.73 (m, 7H), 1.60—1.56 (m, 3H), 1.51—1.46 (m, 3H), 1.39—1.35 (m, 4H), 1.30 (s, 6H), 1.25—1.16 (m, 4H), 0.89 (d, *J*=6.00 Hz, 6H), 0.83 (d, *J*=7.63 Hz, 6H); ^{13}C-NMR (63 MHz, $CDCl_3$) δ: 171.8, 171.7 103.2, 88.6, 81.2, 74.2, 52.6, 45.5, 44.6, 41.7, 41.4, 37.4, 37.4, 34.5, 31.1, 31.0, 30.1, 26.2, 24.9, 24.5, 20.3, 13.4; IR (KBr, cm^{-1}) ν_{max} 3403, 2941, 1725, 1712, 1605, 1511, 1451, 1384, 1263, 1174, 1118, 1025, 811, 767; MALDI-TOF MS: found 731.1315 $[M+H]^+$. HR-MS (FAB) Calcd for $C_{40}H_{63}N_2O_{10}$ $[M+H]^+$ *m/z* 731.4483, Found 731.4485. *Anal.* Calcd for $C_{40}H_{63}N_2O_{10}$: C, 65.75; H, 8.63; N, 3.84. Found: C, 65.68; H, 8.62; N, 3.86.

(6*R*)-6-Hydroxyheptyl-2,4-*O*-benzoyl-3,6-dideoxy-L-rhamnoside (11) To the solution of benzoyl-3,6-dideoxy-L-rhamnosepyranosyl)heptene **9** (200 mg, 0.42 mmol) in methylene chloride(20 ml), O_3 was added through bubbling at −78 °C for 3 min. After methylene chloride was removed *in vacuo*, the reaction mixtures were dissolved in ethanol (30 ml) at 0 °C. $NaBH_4$ (195 mg, 5.14 mmol) was in several times added to the solution. After 16 h stirring, the reaction was quenched by addition with distilled water, extracted with EtOAc(150 ml×3). Washing with sat. brine, drying with anh. $MgSO_4$, and concentration *in vacuo* afforded a solid. Purification with flash column chromatography gave compound **11**, a colorless solid (126 mg, 66%).

$C_{27}H_{34}O_7$, *Rf*=0.21 (*n*-hexane/EtOAc, 4 : 1, v/v), $[\alpha]_D^{20}$ −6.1° (*c*=0.5, $CHCl_3$), ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 8.10 (d, *J*=7.1 Hz, 2H, aromatic H), 8.03 (d, *J*=7.1 Hz, 2H, aromatic H), 7.54—7.60 (m, 2H, aromatic H), 7.42—7.49 (m, 4H, aromatic H), 5.13—5.23 (m, 2H), 4.96 (s, 1H, H-1′), 4.07—4.15 (m, 1H, H-4′), 3.82—3.86 (m, 1H, H-6), 3.64 (t, *J*=6.5 Hz, 2H, H-2), 2.38—2.46 (m, 1H, H-3′ eq), 2.16—2.27 (m, 1H, H-3′ ax), 1.89 (s, 1H, alcohol), 1.53—1.67 (m, 3H), 1.42—1.53 (m, 5H), 1.27 (d, *J*=6.2 Hz, 3H, H-6′), 1.21 (s, 1H), 1.15 (d, *J*=7.4 Hz, 3H, H-7); ^{13}C-NMR (63 MHz, $CDCl_3$) δ: 165.9, 165.7, 133.3, 133.2, 130.1, 129.9, 129.7, 128.5, 93.9 (C-1′, a), 72.7 (C-4′), 71.3 (C-2′), 70.7 (C-6), 67.0 (C-5′), 62.9 (C-1), 37.1 (C-5), 32.8 (C-2), 29.8 (C-3), 25.8 (C-3′), 25.6 (C-4), 19.2 (C-7), 17.9 (C-6′) IR ν_{max} cm^{-1} 3519, 3431, 2971, 2933, 2859, 1722, 1601, 1451, 1376, 1316, 1267, 1176, 1108, 1068, 1025, 943, 711. LC mass ESI probe 469.8 Da/e, Scan ES+4.41e. *Anal.* Calcd for $C_{27}H_{34}O_7$: C, 68.94; H, 7.23. Found: C, 68.89; H, 7.25.

2-[(3*R*,5a*S*,6*R*,8a*S*,9*R*,10*R*,12*R*,12a*R*)-Decahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin-10-acetate]-(1*R*)-1-methyl-6-hepten-1-yl 3,6-Dideoxy-α-L-arabino-hexopyranoside (14) A stirred solution of carboxymethyldeoxoartemisinin (15.0 mg, 0.046 mmol), EDC (88.0 mg, 0.46 mmol) and DMAP (56.1 mg, 0.46 mmol) in DMF (2 ml) was combined with olefinic daumone **10** (15.0 mg, 0.046 mmol) at room temperature during 12 h. The reaction mixture was quenched with slow addition of saturated citric acid (2 ml), extracted with ethyl acetate (3×5 ml) and washed with $NaHCO_3$ (5 ml) and brine (5 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The mixture was purified by flash column chromatography on silica gel with petroleum ether/ethyl acetate (1 : 1 v/v) as eluant to give compound **14** (4.1 mg, 0.007 mmol, 15.7% yield) and compound **15** (2.0 mg, 0.004 mmol, 7.7% yield), respectively.

Compound **14**: ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 0.87 (d, *J*=7.58 Hz, 3H), 0,96 (d, *J*=5.37, 3H), 1.07—1.24 (m, 4H), 1.12 (d, *J*=6.32 Hz, 3H), 1.21 (d, *J*=6.00 Hz, 3H), 1.40 (s, 3H), 1.48—2.23 (m, 17H), 2.24—2.56 (m, 2H), 2.60—2.84 (m, 2H), 3.72—3.85 (m, 2H), 3.89 (m, 1H), 4.67—4.82 (m, 1H), 4.71 (s, 1H), 4.83—4.92 (m, 1H), 4.95 (d, *J*=10.23 Hz, 1H), 5.00 (d, *J*=16.94 Hz, 1H), 5.32 (s, 1H), 5.82 (tdd, *J*=16.94, 10.23, 6.79, 6.79 Hz, 1H). IR (KBr, cm^{-1}) ν_{max} 3473, 2927, 2873, 2360, 2342, 1736, 1463, 1373, 1202, 1130, 1103, 1039. HR-MS (FAB) Calcd for $C_{31}H_{50}NaO_9$ $[M+Na]^+$ *m/z* 589.3353, Found 589.3377. *Anal.* Calcd for $C_{31}H_{50}O_9$: C, 65.72; H, 8.83. Found: C, 65.74; H, 8.91.

4-[(3*R*,5a*S*,6*R*,8a*S*,9*R*,10*R*,12*R*,12a*R*)-Decahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin-10-acetate]-(1*R*)-1-methyl-6-hepten-1-yl 3,6-Dideoxy-α-L-arabino-hexopyranoside (15) Compound **15**: ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 0.87 (d, *J*=7.58 Hz, 3H,) 0.97 (d, *J*=5.69 Hz, 3H), 1.11 (d, *J*=6.00 Hz, 3H), 1.16—1.27 (m, 4H), 1.28 (d, *J*=5.69 Hz, 3H), 1.41 (s, 3H), 1.49—2.23 (m, 17H), 2.25—2.49 (m, 2H), 2.61—2.83 (m, 1H), 3.58—3.85 (m, 3H), 3.63—3.71 (m, 1H), 4.70—4.82 (m, 1H), 4.73 (s, 1H), 4.91 (br s, 1H), 4.94 (d, *J*=10.19 Hz, 1H), 5.00 (d, *J*=16.98 Hz, 1H), 5.34 (s, 1H), 5.70—5.93 (tdd, *J*=16.98, 10.19, 6.63, 6.63 Hz, 1H). IR (KBr, cm^{-1}) ν_{max} 2924, 2851, 2369, 2337, 1734, 1456, 1368. HR-MS (FAB) Calcd for $C_{31}H_{49}O_9$ $[M+H]^+$ *m/z* 567.9899, Found 567.9833. *Anal.* Calcd for $C_{31}H_{49}O_9$: C, 65.84; H, 8.67. Found: C, 65.79; H, 8.66.

(6*R*)-6-[(3,6-Dideoxy-α-L-arabino-hexopyranosyl)oxy]-heptanoic Acid 2-[(3*R*,5a*S*,6*R*,8a*S*,9*R*,10*R*,12*R*,12a*R*)-Decahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin-10-yl]ethyl Ester (16) A stirred solution of hydroxyethyldeoxoartemisinin (22.6 mg, 0.072 mmol), EDC (139.0 mg, 0.72 mmol) and DMAP (88.0 mg, 0.72 mmol) in DMF (2 ml) was combined with daumone **2** (20.0 mg, 0.072 mmol) at room temperature during 12 h. The reaction mixture was quenched with slow addition of satured citric acid (2 ml), extracted with ethyl acetate (3×5 ml) and washed with $NaHCO_3$ (5 ml) and brine (5 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The mixture was purified by flash column chromatography on silica gel with ethyl acetate as eluant to give compound **16** (24.4 mg, 0.043 mmol, 59.1% yield). ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 0.87 (d, *J*=7.27 Hz, 3H), 0.96 (d, *J*=4.74 Hz, 3H), 1.12 (d, *J*=6.00 Hz, 3H), 1.28 (d, *J*=5.69 Hz, 3H), 1.41 (s, 3H), 1.53—2.15 (m, 19H), 2.24—2.42 (m, 3H), 2.58—2.79 (m, 1H), 3.49—3.92 (m, 4H), 4.06—4.38 (m, 3H), 4.70 (s, 1H), 5.30 (s, 1H). ^{13}C-NMR (63 MHz, $CDCl_3$) δ: 12.91, 17.70, 18.90, 20.16, 24.70, 24.77, 24.89, 25.19, 26.05, 29.69, 34.00, 34.26, 34.43, 35.21, 36.53, 36.76, 37.47, 44.26, 52.30, 62.46, 68.11, 69.34, 69.88, 70.97, 71.76, 81.06, 88.96, 95.82, 103.23, 173.78. IR (KBr, cm^{-1}) ν_{max} 3450, 2928, 2878, 2361, 2337, 1734, 1455, 1376, 1130, 1100, 1044. HR-MS (FAB) Calcd for $C_{30}H_{50}O_{10}$ $[M]^+$ *m/z* 570.3302, Found 570.3382. *Anal.* Calcd for $C_{30}H_{50}O_{10}$: C, 63.16; H, 8.77. Found: C, 63.21; H, 8.82.

***In Vitro* Antitumor Assay** The *in vitro* cytotoxicity of artemisinin, daumone and their synthesized hybrids to the human cancer cells was defined by the microculture tetrazolium assay as described by Carmichel *et al.*[48] Paclitaxel and cisplatin were used as the reference substances, exhibiting the activity with an IC_{50} (μM) as shown in Table 1 toward human cell lines.

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