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PII: S0960-894X(16)31161-1
DOI: http://dx.doi.org/10.1016/j.bmcl.2016.11.021
Reference: BMCL 24416

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 14 September 2016
Revised Date: 2 November 2016
Accepted Date: 9 November 2016

Please cite this article as: Fang, Y., Wang, R., He, M., Huang, H., Wang, Q., Yang, Z., Li, Y., Yang, S., Jin, Y., Nitric oxide-donating derivatives of hederacolchiside A_1: synthesis and biological evaluation *in vitro* and *in vivo* as potential anticancer agents, *Bioorganic & Medicinal Chemistry Letters* (2016), doi: http://dx.doi.org/10.1016/j.bmcl.2016.11.021

Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

Nitric oxide-donating derivatives of hederacolchiside A_1: synthesis and biological evaluation *in vitro* and *in vivo* as potential anticancer agents

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ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
Nitric oxide;
Hederacolchiside A_1;
Furoxan;
Triterpenoid saponin;
Anticancer activity

ABSTRACT

A series of nitric oxide (NO) donating derivatives of hederacolchiside A_1 bearing triterpenoid saponin motif were designed, synthesized and evaluated for their anticancer activity. All of the tested furoxan-based NO releasing compounds showed significant proliferation inhibitory activities. Especially compound **6a** exhibited strong cytotoxicity (IC_{50} = 1.6 - 6.5 μM) against four human tumor cell lines (SMMC-7721, NCI-H460, U251, HCT-116) *in vitro* and the highest level of NO releasing. Furthermore, compound **6a** was revealed low acute toxicity to mice and weak haemolytic activity with potent tumor growth inhibition against mice H22 hepatocellular cells *in vivo* (51.5%).

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Cancer is one of the terrible disease causes of human death worldwide.[1] The World Health Organization estimated that a number of cancer death would reach to 13.1 million by 2030.[2] In this area, natural products play a highly significant role in the drug discovery and development process.[3]

Triterpenoid saponins are an important structural type amongst bioactive natural products and impart favorable physicochemical and pharmacological attributes, which were reported to possess diverse biological activities such as antitumor, antiviral, antibacterial and so on.[4-14] In our efforts to discover anticancer drugs, a series of triterpenoid saponins were isolated from *pulsatilla chinensis* (Bunge) Regel, a traditional Chinese herb for "blood-cooling" and detoxification.[15-17] Among those compounds, hederacolchiside A_1 (HA_1, **Figure 1**), a known structure which contains a trisaccharide scaffold,[12-14] manifests strongest and broad-spectrum proliferation inhibitory activities (IC_{50} = 2.2 - 8.4 μM) against human cancer cell lines (SMMC-7721, NCI-H460, U251, HCT-116) *in vitro* and best tumor inhibition rate *in vivo* (47.5%). Nevertheless, it's unpractical to develop it directly as a clinic drug because of the haemolytic toxicity. It's reported that the 28-COOH of triterpenoid saponins was the functional group inducing erythrocytes haemolysis.[15, 18] Currently little work about the modification of HA_1 has been reported since the natural product is too "difficult" to obtain it.

In recent decades, NO has attracted a tremendous interest in a broad field of basic and applied research as one of the most significant physiological signaling molecule in the body.[19] High concentrations of NO produce reactive nitrogen species, which along with reactive oxygen species result in DNA base deamination, impaired cellular function and cell apoptosis.[20-21] Several targets have been revealed that the combination between NO and cancer therapy including synergistic effect increased the influx of the anticancer therapy by NO into intracellular compartments and the efficiency of cytostatic therapy.[22-25] Furoxan, an important class of NO releasing moiety, could produce high concentration of NO and exhibit good anticancer activity.[26] We were interested in NO donor hybrids severed as anticancer drug.

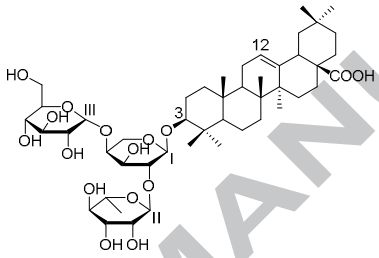
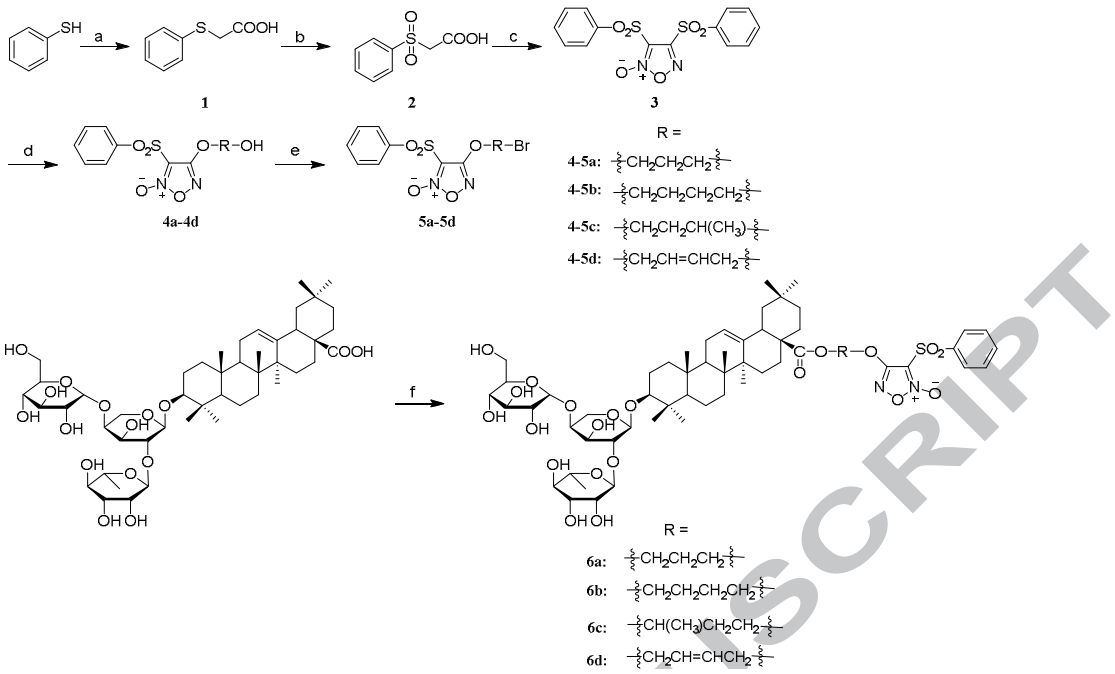


Figure 1: The structure of HA_1

Therefore, due to the potent and broad spectrum antiproliferative activity of HA_1, which was represented as a lead compound and planned to introduce furoxan fragment on 28-COOH as a NO donor. Based on the available structure-activity relationship study (SARs) of furoxan-NO releasing group,[26-29] 4 kinds of furoxans shown significant anticancer activities were explored in our research. It was predicted that these HA_1 derivatives containing NO donor could cause significant pharmacological change and decrease haemolytic toxicity. Herein, we report a series of novel HA_1 derivatives bearing NO donating fragment-furoxans were designed and synthesized. Biological evaluation of these compounds were also carried out including antiproliferative activities *in vitro* and *in vivo*, NO releasing abilities and acute toxicity.

HA_1 derivatives were prepared from natural product HA_1 isolated in our group, following by the procedures and conditions as shown in **Scheme 1**. Intermediates **1-3** were generated according to the previously reported procedures.[30] Monophenylsulfonylfuroxans **4a-4d** was synthesized from diphenylsulfonylfuroxan **3** treated with diols and aqueous NaOH solution at room temperature.[31-32] Appel reaction of **4a-4d** with CBr_4 and PPh_3 afforded bromide of furoxans **5a-5d**,[33] following by reaction with HA_1 and K_2CO_3 in DMF to give desired products **6a-6d**.[34-37]



Scheme 1. Reagents and conditions: (a) $ClCH_2COOH$, NaOH, reflux, 2 h; (b) 30% H_2O_2, CH_3COOH, R.T., 2.5 h; (c) fuming HNO_3, 90℃, 1.5 h; (d) diol, 25% NaOH aq., THF, R.T., 3 h; (e) CBr_4, PPh_3, CH_2Cl_2, 0℃-R.T., overnight; (f) **5a-5d**, K_2CO_3, DMF, R.T., overnight.

Derivatives **6a-6d** were evaluated for cytotoxicity against SMMC-7721, NCI-H460, U251 and HCT-116 human cancer cell lines using MTT assay.[38] HA_1 was selected as positive control and the IC_{50} values for the inhibition of proliferation were illustrated in **Table 1**. All of compounds shown the promising anticancer activities against the tested 4 human cancer cell lines, especially against SMMC-7221 (IC_{50} = 1.9 - 4.5 μM) and U251 (IC_{50} = 1.6 - 3.1 μM). Derivative **6d** exhibited the weaker antiproliferative activity than other derivatives, which was revealed that the linker of furoxans with alkene group was disadvantage for the cytotoxicity. Comparing with the natural product HA_1, compound **6a** showed the stronger anticancer activity, but compound **6b** showed the similar activity and compound **6c** showed the weaker activity. This results indicated that the shorter linker of furoxans was favorable to bioactivity. Among these derivatives, compound **6a** was observed the potent cytotoxicity against the 4 human cancer cell lines (SMMC-7721: IC_{50} = 1.9 μM; NCI-H460: IC_{50} = 3.1 μM; U251: IC_{50} = 1.6 μM; HCT-116: IC_{50} = 6.5 μM).

Table 1
Cytotoxicity of HA_1 derivatives against 4 human cancer cell lines after 48 h incubation

Compound	IC_{50}[a] (μM)			
	SMMC-7721[b]	NCI-H460[c]	U251[d]	HCT-116[e]
6a	1.9 ± 0.2	3.1 ± 0.2	1.6 ± 0.1	6.5 ± 0.6
6b	2.6 ± 0.2	3.4 ± 0.2	2.1 ± 0.1	7.3 ± 0.5
6c	3.7 ± 0.3	6.2 ± 0.4	2.3 ± 0.2	11.2 ± 1.2
6d	4.5 ± 0.3	6.8 ± 0.5	3.1 ± 0.2	10.1 ± 1.1
HA_1	2.8 ± 0.1	3.7 ± 0.2	2.2 ± 0.2	8.4 ± 0.5

[a] Concentration inhibiting fifty percent of cell growth for 48 h exposure period of tested samples. Assay was done in triplicate.
[b] human hepatocellular cell line.
[c] human large cell lung cancer cell line.
[d] human glioma cell line.
[e] human colon cancer cell line.

The level of NO release abilities from HA_1-furoxan derivatives was determined by the Griess test using nitroglycerin as the positive control.[39] As shown in **Figure 2**, **6c** and **6d** showed weaker NO produce abilities than nitroglycerin, but **6a** and **6b** exhibited better NO release abilities. Of all the derivatives, **6a** released the highest amount of NO at every time point, which was chose to take further research.

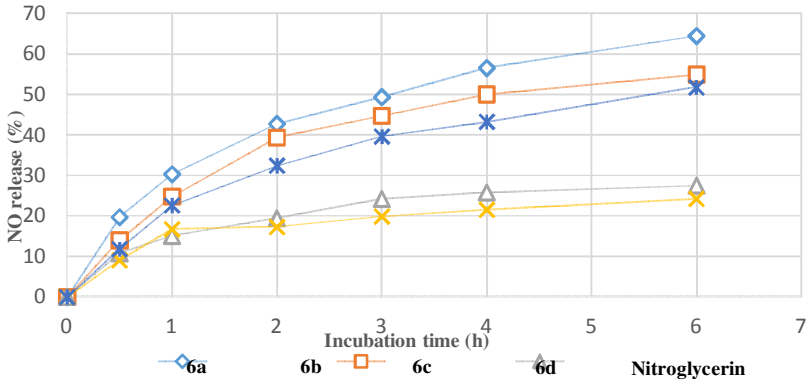


Figure 2: Griess test results of HA_1-furoxan derivatives

Because derivative **6a** exhibited potent anticancer activities to 4 human cancer cell lines (IC_{50} = 1.6 - 6.5 μM) and the highest level of NO releasing ability, which was chosen to identify the mice acute toxicities with natural product HA_1 as a positive control. Due to the poor solubility, these 2 compounds were made in submicron emulsion formation at 0.8 mg/mL concentration of HA_1, 2.4 mg/ml concentration of compound **6a**. The mice were injected the solutions via caudal vein at the dose of 0.1 mL/10 g/d for 7 days and continued to observe for another 7 days without injection. As shown in **Table 2**, the tolerance dose of natural product HA_1 was less than 8 mg/kg and the 12 mice were all dead in 1 h after injection. The tolerance dose of compound **6a** was more than 168 mg/kg and no mouse was dead in 14 days. The results indicated that compound **6a** declined the acute toxicity greatly and supplied an evidence to design the suitable dose for tumor inhibition rate trial. The haemolytic activity of compound **6a** against rabbit erythrocytes was assayed using the reported method.[40] Compared with HA_1, **6a** showed very weak haemolytic activity (HD_{50} = 279.5 μM), which revealed that the reduced toxicity should be caused by the weakening of haemolysis.

Table 2
Haemolytic activity and mice acute toxicity of compound **6a**

Compound	Number of mice	Tolerance dose (mg/kg)	Death number	Death time	HD_{50}[a] (μM)
6a	12	>168	0	>14 days	279.5 ± 14.2
HA_1	12	<8	12	<1 h	6.7 ± 0.3

[a] Concentration inducing fifty percent of rabbit erythrocytes haemolysis.

In vitro studies suggested that compound **6a** exhibited significant and consistent cytotoxicity in 4 human cancer cell lines and the highest level of NO releasing ability. The activity of compound **6a** on tumor growth inhibition was evaluated using H22 hepatocellular carcinoma model in male nude mice with HA_1 as a positive control.[41] Compounds **6a** and HA_1 were made in submicron emulsion formation at 2.0 mg/ml concentration of compounds **6a**, 0.6 mg/mL concentration of HA_1. And compound **6a** was injected via caudal vein at dose of 10 mg/kg/d and 20 mg/kg/d for 14 days respectively, HA_1 at dose of 6 mg/kg/3 d for 5 times. The results were shown in **Table 3**. Treatment with **6a** at 10 mg/kg significantly inhibited the growth of H22 tumor (tumor inhibition rates 38.3%), but treatment with 20 mg/kg of **6a** enhanced the inhibitory effect on the growth of H_{22} tumor (51.5%). And its inhibitory effect was slightly stronger than the treatment of HA_1 (47.5%). More importantly, no mice were dead after treatment with **6a** in 14 days, which demonstrated that derivatives **6a** could reduce the toxicity greatly.

Table 3
In vivo H22 xenograft studies of compound **6a**

Compound	Number of mice	Dose(mg/kg)	Death number	Tumor weights(g)	Tumor inhibition rates (%)
Model group	15	-	0	2.27±1.76	
6a	15	10	0	1.40±1.35*	38.3
6a	15	20	0	1.10±1.27*	51.5
HA_1	15	6	5	1.19±1.22**	47.5

*Control with model group, *P<0.05, **P<0.01

In summary, 4 NO-donor HA_1 derivatives were designed and synthesized. All of compounds were evaluated the antiproliferative activities against 4 human cancer cell lines (SMMC-7721, NCI-H460, U251 and HCT-116). As a result, all derivatives exhibited the significant anticancer activities. The NO releasing abilities of 4 derivatives was measured by Griess assay. Compound **6a** was observed the highest NO-releasing ability. It is exciting that compound **6a** also showed the most potent cytotoxicity against tested 4 cancer cell lines (IC_{50} = 1.6 - 6.5 μM), that indicated the high level of NO concentration could increase the anticancer activity. More importantly, compound **6a** revealed strong tumor growth inhibition *in vivo* at dose of 20mg/kg (51.5%) and low acute toxicity comparing with

natural product HA_1. These results encourage us to discover the novel anticancer agents from natural product. The follow-up studies on mechanism of action and pharmacokinetics of candidate derivatives **6a** are in progress and the results will be reported in due time.

Acknowledgements

This work was supported by the Education Department of Jiangxi Province on Science and Technology Project Foundation (GJJ150842) and Doctoral Scientific Research Foundation of Jiangxi University of Traditional Chinese Medicine (2015BS012).

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32. Typical synthetic procedure of **4**: To a solution of compound **3** (0.3 g, 0.82 mmol) in THF (3 mL) was added diol (3 mmol) and 25% NaOH aq. (0.15 mL, 0.9 mmol) respectively. The reaction was stirred at room temperature for 3 h. Then the mixture was extracted by ethyl acetate (25 mL × 2). The organic phase was combined, washed with saturated brine (15 mL × 2), dried over anhydrous $MgSO_4$ and filtered. The filtrate was evaporated to give crude product, which was purified by column chromatography (petroleum ether: ethyl acetate = 2: 1) to give compounds **4a-4d**. Compound **4a** as white solid, 92% yield. ^{1}H-NMR (600 MHz, $CDCl_3$) δ (ppm): 8.07-8.05 (m, 2H), 7.78-7.75 (m, 1H), 7.67-7.61 (m, 2H), 4.60 (t, *J*=6.0, 2H), 3.89 (s, 2H), 2.16-2.12 (m, 2H), 1.91 (s, 1H); MS-ESI: $[M+Na]^+$: 323.2.
33. Typical synthetic procedure of **5**: To a solution of compound **4** (0.83 mmol) in CH_2Cl_2 (3 mL) was added CBr_4 (0.3 g, 0.92 mmol) at 0 ℃. The mixture of PPh_3 (0.24 g, 0.92 mmol) in CH_2Cl_2 (1.5 mL) was added dropwise under N_2 at 0 ℃. The reaction was stirred at room temperature for overnight. Then the solution was diluted by CH_2Cl_2. The organic phase was washed with saturated brine (15 mL × 2), dried over anhydrous $MgSO_4$ and filtered. The filtrate was evaporated to give crude product, which was purified by column chromatography (petroleum ether: ethyl acetate = 4: 1) to give compounds **5a-5d**. Compound **5a** as white solid, 78 yield. ^{1}H-NMR (600 MHz, $CDCl_3$) δ (ppm): 8.09-8.07 (m, 2H), 7.80 (m, 1H), 7.67-7.66 (m, 2H), 4.61 (m, 2H), 3.62 (m, 2H), 2.43 (m, 2H); MS-ESI: $[M-H]^-$: 361.8.
34. Compound **6a** as white solid, 78% yield. ^{1}H-NMR (600 MHz, CD_3OD) δ (ppm): 8.07 (d, *J*=7.7 Hz, 2H, SO_2Ph), 7.87 (t, *J*=7.4 Hz, 1H, SO_2Ph), 7.74 (t, *J*=7.9 Hz, 2H, SO_2Ph), 5.25 (t, *J*=3.2 Hz, 1H, H-12), 5.20 (d, *J*=1.2 Hz, 1H, H-1^{II}), 4.58-4.55 (m, 1H, -$CH_2CH_2CH_2$-), 4.52-4.49 (m, 1H, -$CH_2CH_2CH_2$-), 4.47 (d, *J*=5.4 Hz, 1H, H-1^{III}), 4.45 (d, *J*=7.7 Hz, 1H, H-1^{I}), 4.27-4.20 (m, 2H, -$CH_2CH_2CH_2$-), 2.27-2.24 (m, 2H, -$CH_2CH_2CH_2$-), 1.25 (d, *J*=6.2 Hz, 3H, H-6^{II}), 1.13 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.64 (s, 3H, CH_3), 0.36 (s, 3H, CH_3); ^{13}C-NMR (150 MHz, CD_3OD) δ: 177.6, 159.2, 144.0, 138.1, 136.1, 130.0 (× 2), 128.4 (× 2), 122.4, 110.6, 104.9, 104.0, 100.6, 88.7, 78.3, 76.9, 76.6, 75.7, 74.0, 72.6, 72.4, 70.8, 70.7, 70.1, 68.8, 67.8, 63.3, 61.4, 60.0, 55.6, 46.8, 45.8, 41.4, 41.3, 39.2, 38.9, 38.8, 38.5, 36.5, 33.4, 32.5, 32.4, 32.3, 30.4, 27.6, 27.4, 27.3, 25.9, 25.3, 23.1, 22.9, 22.7, 17.9, 17.1, 16.4, 16.1, 14.6; HRMS-ESI $(M+Na)^+$ m/z calcd. for $C_{58}H_{86}N_2NaO_{21}S$, 1201.5341, found, 1201.5380.
35. Compound **6b** as white solid, 80% yield. ^{1}H-NMR (600 MHz, CD_3OD) δ (ppm): 8.05-8.04 (m, 2H, SO_2Ph), 7.85-7.83 (m, 1H, SO_2Ph), 7.71-7.69 (m, 2H, SO_2Ph), 5.25 (t, *J*=3.4 Hz, 1H, H-12), 5.16 (d, *J*=1.3 Hz, 1H, H-1^{II}), 4.48 (t, *J*=6.4 Hz, 2H,-$CH_2CH_2CH_2CH_2$-), 4.45 (d, *J*=5.4 Hz, 1H, H-1^{III}), 4.42 (d, *J*=7.7 Hz, 1H, H-1^{I}), 4.15-4.06 (m, 2H, -$CH_2CH_2CH_2CH_2$-), 1.98-1.94 (m, 2H, -$CH_2CH_2CH_2CH_2$-), 1.86-1.78 (m, 2H, -$CH_2CH_2CH_2CH_2$-), 1.21 (d, *J*=6.2 Hz, 3H, H-6^{II}), 1.14 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.71 (s, 3H, CH_3); ^{13}C-NMR (150 MHz, CD_3OD) δ: 179.6, 160.6, 145.2, 139.8, 137.1, 131.2 (× 2), 129.7 (× 2), 124.1, 111.9, 106.0, 105.3, 102.2, 90.6, 79.1, 78.2, 78.0, 77.3, 75.5, 74.1, 73.5, 72.4, 72.3, 71.5, 70.3, 65.2, 64.4, 62.8, 57.2, 49.6, 48.3, 47.2, 43.0, 42.9, 40.8, 40.4, 40.1, 38.0, 34.9, 34.1, 33.9, 33.6, 31.8, 30.3, 28.9, 28.8, 27.2, 26.6, 26.5, 26.2, 24.7, 24.2, 24.1, 19.4, 18.1, 18.0, 17.2, 16.1; HRMS-ESI $(M+Na)^+$ m/z calcd. for $C_{59}H_{88}N_2O_{21}NaS$, 1215.5498, found, 1215.5613.
36. Compound **6c** as white solid, 76% yield. ^{1}H-NMR (600 MHz, CD_3OD) δ (ppm): 8.10-8.05 (m, 2H, SO_2Ph), 7.88-7.86 (m, 1H, SO_2Ph), 7.77-7.72 (m, 2H, SO_2Ph), 5.25 (s, 1H, H-12), 5.20 (s, 1H, H-1^{II}), 5.16-5.14 (m, 1H, -$CH(CH_3)CH_2CH_2$-), 4.49 (m, 1H, -$CH(CH_3)CH_2CH_2$-), 4.47 (d, *J*=5.4 Hz, 1H, H-1^{III}), 4.45 (d, *J*=7.7 Hz, 1H, H-1^{I}), 4.40 (m, 1H, -$CH(CH_3)CH_2CH_2$-), 2.26-2.19 (m, 1H, -$CH(CH_3)CH_2CH_2$-), 2.13-2.09 (m, 1H, -$CH(CH_3)CH_2CH_2$-), 1.33 (d, *J*=2.5 Hz, 3H, -$CH(CH_3)CH_2CH_2$-), 1.25 (d, *J*=6.2 Hz, 3H, H-6^{II}), 1.14 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.83 (s, 3H, CH_3), 0.66 (s, 3H, CH_3); ^{13}C-NMR (150 MHz, CD_3OD) δ: 178.8, 160.7, 145.3, 139.6, 137.2, 131.3 (× 2), 129.9 (× 2), 123.9, 112.0, 106.0, 105.3, 102.1, 90.6, 79.1, 78.2, 78.0, 77.2, 75.5, 74.1, 73.6, 72.2, 71.5, 70.3, 68.8, 68.1, 64.4, 62.8, 61.6, 57.1, 49.7, 48.2, 47.3, 42.9,

42.6, 40.0, 37.9, 36.0, 35.1, 34.0, 33.6, 31.7, 29.3, 28.8, 28.6, 27.1, 26.6, 24.6, 24.1, 23.9, 20.5, 17.9, 17.3, 17.2, 16.3, 15.8; HRMS-ESI $(M+Na)^+$ m/z calcd. for $C_{59}H_{88}N_2NaO_{21}S$, 1215.5498, found, 1215.5622.

37. Compound **6d** as white solid, 70% yield. ^{1}H-NMR (600 MHz, CD_3OD) δ (ppm): 8.08 (d, *J*=7.4 Hz, 2H, SO_2Ph), 7.86 (t, *J*=7.6 Hz, 1H, SO_2Ph), 7.72 (t, *J*=8.1 Hz, 2H, SO_2Ph), 5.97-5.90 (m, 2H, -CH_2CH=$CHCH_2$-), 5.27 (t, *J*=3.2 Hz, 1H, H-12), 5.19 (s, 1H, H-1^{II}), 5.11 (d, *J*=5.8 Hz, 2H,-CH_2CH=$CHCH_2$-), 4.76 (dd, *J*=6.6, 13.7 Hz, 1H,-CH_2CH=$CHCH_2$-), 4.68 (dd, *J*=6.1, 13.4 Hz, 1H,-CH_2CH=$CHCH_2$-), 4.48 (d, *J*=5.4 Hz, 1H, H-1^{III}), 4.45 (d, *J*=7.7 Hz, 1H, H-1^{I}), 1.24 (d, *J*=6.2 Hz, 3H, H-6^{II}), 1.17 (s, 3H, CH_3), 1.04 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 0.74 (s, 3H, CH_3); ^{13}C-NMR (150 MHz, CD_3OD) δ: 177.6, 158.7, 143.5, 138.1, 135.6, 129.8, 129.6 (× 2), 128.3 (× 2), 126.1, 122.5, 110.5, 104.5, 103.8, 100.6, 89.1, 77.5, 76.7, 76.5, 75.7, 73.9, 72.5, 71.9, 70.7, 70.0, 68.8, 66.5, 62.8, 61.3, 59.6, 55.7, 48.2, 46.7, 45.7, 41.4, 41.3, 39.3, 38.7, 38.5, 36.5, 33.4, 32.5, 32.2, 32.1, 30.2, 29.4, 27.4, 27.2, 25.6, 25.0, 23.1, 22.7, 22.6, 17.9, 16.6, 16.5, 15.7, 14.6; HRMS-ESI $(M+Na)^+$ m/z calcd. for $C_{59}H_{86}N_2NaO_{21}S$, 1213.5341, found, 1213.5737.
38. Cell culture and cytotoxicity test: The human cancer cell lines were purchased from Shanghai Institutes for Biological Sciences and maintained in a humidified atmosphere at 37 ℃ in 5% CO2. The cells were grown in RPMI-1640 (GIBCO) media containing 10% heat-inactivated fetal bovine serum (FBS). Cytotoxicity was determined by the MTT assay according to the manufacturer's protocol. Briefly, cells were seeded in 96-well microtiter plates at a density of 8 × 103 cells per well. After 24 h incubation, cells were treated with various concentrations of HA1 derivatives, cultured for 48 h. At the end of the treatment period, 20 μL of the MTT (5 mg/mL) reagent was added to each well. After 4 h incubation at 37 ℃, the supernatant was aspirated, and formazan crystals were dissolved in 150 μL DMSO for 10 min with gentle agitation. The absorbance per well was measured at 490 nm with a SpectraMax i3 (Molecular Devices Corp.). Assay was done in triplicate. The IC50 values were then determined for each compound from a plot of log (drug concentration) versus percentage of loss of viability.
39. Griess test procedure: The test compound (0.05 mmol) and nitroglycerin (0.017 mmol) were dissolved in DMSO (10 mL) respectively and cysteine (4.34 mmol) was dissolved in 10 mL phosphoric acid buffer solution (1 mol/L). Compounds (10 mL) mixed with cysteine mixture (36 equivalents, 4.15 mL) were incubated at 37℃. The solution (1 mL) was taken out at 0.5h, 1h, 2h, 3h, 4h, 6h respectively, then mixed with Griess regent (1 mL) for 10 min, and followed by measurement at 540 nm by a microplate reader. NaNO2 at different concentrations were prepared to give the nitrite concentration for the establishment of a standard curve.
40. Gong, W.; Jiang, Z. H.; Sun, P.; Li, L.; Jin, Y. S.; Shao, L. C.; Zhang, W.; Liu, B. S.; Zhang, H. W.; Tang, H.; Chen, Y. F.; Yi, Y. H.; Zhang, D. Z. *Chem. Biodivers.* **2011**, *8*, 1833.
41. Tumor inhibition test: Male KM nude mice with weight of 18-22 gram were inoculated subcutaneously with 5×106 H22 cells, which was purchased from Chinese Academy of Medical Sciences. From the second of injection, the tumor-bearing nude mice were randomized for 4 groups and treated via caudal vein injection at 6 mg/kg/3 d of HA1, and 10 mg/kg/d or 20 mg/kg/d for 14 days, respectively. After 24 h of the last feed, the mice were sacrificed, and their tumors were dissected out and weighted.

Graphical Abstract

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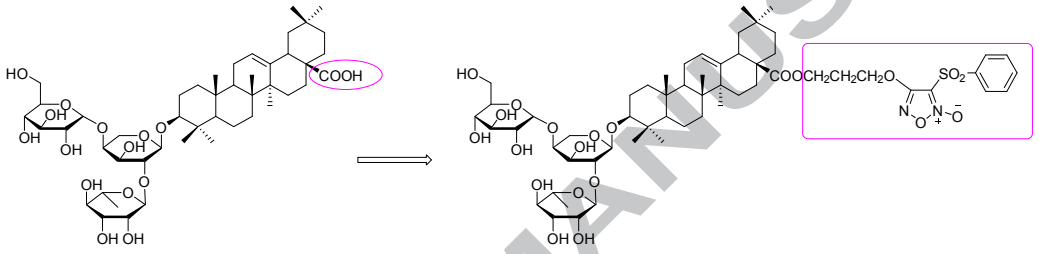

Hederacolchiside A_1

6a: SMMC-7721: IC_{50} = 1.9 uM
NCI-H460: IC_{50} = 3.1 uM
U251: IC_{50} = 1.6 uM
HCT-116: IC_{50} = 6.5 uM
Tumor inhibition rate: 51.5%