CHAPTER III

EXPERIMENTS

1. Source of geldanamycin

Geldanamycin (**1**) and its natural derivatives, 17-*O*-demethylgeldanamycin (**2**), and a new derivative, 17-*O*-demethylgeldanamycin hydroquinone (**5**) were isolated from fermentation broth of *Streptomyces* sp. TRA 9875-2 as described in Tadtong, 2000.

2. Chromatographic techniques

2.1 Analytical thin-layer chromatography (TLC)

Technique	: one dimension ascending
Adsorbent	: silica gel 60 GF_{254} coated on aluminium sheet (Merck).
Layer thickness	: 250 μm
Distance	: 5 cm
Temperature	: laboratory temperature (25-30°C)
Detection	: 1. Visual detection under daylight 2. Visual detection under ultraviolet light at wavelengths of 254 and 365 nm. 3. Visual detection under daylight after spraying with anisaldehyde reagent (0.5 mL anisaldehyde, 9 mL EtOH, 0.5 mL 97% sulfuric acid, and 0.1 mL glacial acetic acid) and heated until color developed

2.2 Preparative thin-layer chromatography (PLC)

Technique	: one dimension ascending
Adsorbent	: silica gel 60 GF_{254} precoated on glass-plates (Merck).
Layer thickness	: 1 mm

Distance : 10 cm

Temperature : laboratory temperature (25-30°C)

Detection : Visual detection under daylight

2.3 Column chromatography

2.3.1 Normal phase flash column chromatography

Adsorbent : silica gel 60 (No. 7734), particle size 0.063-0.200 nm (70-230 mesh ASTM) (E. Merck)

Packing method : wet packing

Sample loading : The sample was dissolved in a small volume of the eluant and loaded on top of the column.

Detection : Fractions were examined by TLC technique observing under UV light (254 nm).

2.3.2 Reversed phase flash column chromatography

Adsorbent : Cosmosil 75 C-18 OPN (Nacalai tesque)

Packing method : dry packing

Sample loading : The sample was dissolved in a small volume of DMSO and loaded on top of the column.

Detection : Fractions were examined by TLC technique observing under UV light (254 nm).

2.3.3 Gel filtration chromatography

Gel filter : Sephadex LH-20 (Pharmacia Biotech AB)

Packing method : Sephadex gel was suspended in the eluant and left overnight prior to use. It was then poured into the column and allowed to settle.

Sample loading : The sample was dissolved in a small volume of the eluant and loaded on top of the column.

Detection : Fractions were examined by TLC technique observing under UV light (254 nm).

3. Spectroscopy

3.1 Ultraviolet (UV) absorption spectroscopy

UV (in MeOH) spectra were obtained from a Milton Roy Spectronic 3000 Array Spectrometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

3.2 Mass spectroscopy (MS)

The ESI-Q-TOFMS spectra were recorded on a Q-TOF Micromass mass spectrometer equipped with a Z-spray type ESI ion source. All experiments were performed in the positive ion mode. Data were acquired and processed using MassLynx version 3.4 (Laboratory of Organic Chemistry, Graduate School of Bioagricultural Sciences, Nagoya University).

Solvent: Acetonitrile (for LC-MS, Kanto reagent company. Merck)
H_2O (Milli-Q, Millipore)
HCOOH (98~100%, nacalai tesque, code; 163-68 500g)

3.3 Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C-NMR) spectroscopy

^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were obtained from a Bruker AVANCE DPX-300 FT-NMR spectrometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

^{1}H (400 MHz) NMR spectrum was recorded on a Bruker AV400 NMR spectrometer (Laboratory of Organic Chemistry, Graduate School of Bioagricultural Sciences, Nagoya University).

^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectra were recorded on a Bruker AMX-600 NMR spectrometer (Laboratory of Organic Chemistry, Graduate School of Bioagricultural Sciences, Nagoya University).

Deuterated solvents; chloroform-*d* ($CDCl_3$), and dimethylsulfoxide-d_6 (CD_3SOCD_3), were used in NMR experiments. Reference signals were the signals of residual undeuterated solvents at δ 7.24 ppm (^{1}H) and 77.0 ppm (^{13}C) for $CDCl_3$, 2.49 ppm (^{1}H) and 39.7 ppm (^{13}C) for CD_3SOCD_3.

4. Chemical reagents for synthesis

Reagent	Manufacturer
Allylamine	TCI
70% Ammonia	Nacalai tesque
Benzyl alcohol	Aldrich
Benzylamine	TCI
Dichloromethane	Wako
Dimethylformamide	Wako
Dimethylsulfoxide	Wako
Ethyl alcohol	Wako
Ethylamine	TCI
Glutathione	TCI
Methyl alcohol	Wako
Methylamine	TCI
Propanolamine	TCI
n-Propyl alcohol	Aldrich
Propylamine	Nacalai tesque
Sodium hydride 55%	Kanto Chemical
Sodium metal	Nacalai tesque

5. Solvents

Throughout this work, all commercial grade organic solvents were redistilled prior to use.

6. Biological activity

6.1 Cell culture

6.1.1 P19 cells

P19 cells were grown in the P19GM (α-MEM supplemented with 7.5% NCS and 2.5% FBS) in a 5% CO_2 humidified atmosphere, at 37 °C. Cells in monolayer cultures were maintained in exponential growth by subculturing every 2 days (Jones-Villeneuve *et al.*, 1982).

6.1.2 P19 Neuron-like cells

For differentiation, exponentially grown cultures were trypsinized and dissociated into single cells. P19 cells (2×10^6 cells/mL) were then suspended in 10 mL P19IM (α-MEM supplemented with 5% FBS and 0.5 μM RA) and seeded onto a 100-mm bacteriological culture dish. The cells formed large aggregates in suspension. After 4 days of RA treatment, aggregates were dissociated by 5-mL glass measuring pipette, re-plated on poly-L-lysine-pre-coated multi-well plates (multi-well plates were coated with 50 μg/mL poly-L-lysine dissolved in PBS for overnight and steriled under UV light for 30 min) at 7×10^4 cells/mL (150 μL/well in 96-well plate and 1.5 mL/well in 6-well plate), in the P19SM (α-MEM supplemented with 10% FBS) and incubated for 24 h. Ara-C (10 μM) was added at day one after plating and the medium was changed every 2-3 days. The differentiated neuronal cells, P19 neuron-like cells (P19 NLCs), were used after day 14 of the differentiation process (Jones-Villeneuve *et al.*, 1982; MacPherson and McBurney, 1995).

6.2 Cytotoxicity assay

Cytotoxicity against P19 cells was determined with the XTT reduction assay. All assays were performed in triplicate. P19 cells were grown in the P19GM. Cell suspension (5×10^4 cells/mL) was seeded into each well of a 96-well flat-bottom microtiter plate (200 μL/well) and incubated at 37 °C for 24 h. After incubation, DMSO solutions of geldanamycin (**1**), diluted in the P19GM, were added to give the concentrations of 0.001, 0.01, 0.1, 1, and 10 μM. The concentration of DMSO was added to the cultures at 0.5%. The P19GM was added into control wells. The cells were incubated for 18 h in a 5% CO_2 humidified atmosphere, at 37 °C. Then 200 μL of the medium was removed, and 100 μL of XTT solution (1 mg/mL in the presence of 25 μM PMS) in α-MEM was added. The OD value was determined on a microplate reader at 450 nm. The data were expressed as the mean ± SEM (n = 3), with the medium as a control representing 100% cell viability.

The cytotoxicity of geldanamycin analogues was tested at the fixed concentration of 0.1 μM and evaluated as described above then compared to that of geldanamycin (**1**).

6.3 Neurotoxic activity assay

The assays were carried out with P19 NLCs. After 14 days of differentiation process, the P19SM was removed and DMSO solutions of geldanamycin (**1**) and taxol, diluted with the P19SM in the presence of 10 μM Ara-C were added to give the concentrations of 0.0001, 0.001, 0.01, 0.1, 1, and 10 μM. The concentration of DMSO was added to the cultures at 0.5%. The P19SM was added into control wells. The cells were incubated for 18 h at 37 °C. Then 150 μL of the medium was removed, and 100 μL of XTT solution was added. The OD value was determined on a microplate reader at 450 nm. The data were expressed as the mean ± SEM (n = 3), with the medium as a control representing 100% cell viability.

The neurotoxicity on P19 NLCs of geldanamycin analogues were tested at the fixed concentration of 0.001 μM and evaluated as described above then compared to that of geldanamycin (**1**).

6.4 Neuroprotective activity assay

The assays were carried out with P19 NLCs cultured in a 96-well plate. All assays were performed in triplicate. After 14 days of differentiation process, the P19SM was removed and DMSO solutions of geldanamycin (**1**) or its analogues in the presence of taxol, diluted with the P19SM supplemented with 10 μM Ara-C, were added to give the final concentrations of geldanamycin (**1**) or its analogues at 0.001 μM and taxol at 0.65 μM. The concentration of DMSO was added to the cultures at 0.5%. The P19SM was added into control wells. The cells were incubated for 18 h at 37 °C. Then 150 μL of the medium was removed, and 100 μL of XTT solution was added. The OD value was determined on a microplate reader at 450 nm. The data were expressed as the mean ± SEM (n = 3), with the medium as a control representing 100% cell viability.

The P19 NLCs cultured in a 6-well plate treated with 0.001 μM geldanamycins in the presence or absence of taxol were observed for their morphology under a phase-contrast microscope. The appearance of P19 NLCs was compared to the control.

7. Physical and chemical properties of the natural occurring geldanamycins

7.1 Geldanamycin (1)

ESI-Q-TOFMS : $[M+Na]^+$ m/z 583.2858 (calcd for $C_{29}H_{40}N_2O_9Na$, 583.2632); Figure A1

UV : λ_{max} nm (log ε), in methanol; 247 (3.982), 322 (4.097), 527 (2.778); Figure A2

^{1}H-NMR : δ_H (ppm), 300 MHz, in $CDCl_3$; 8.67 (br s; 1-NH); 7.22 (s; H-19); 6.90 (d, 12; H-3); 6.53 (t, 12, 11; H-4); 5.83 (t, 11, 10; H-5); 5.77

(d, 10; H-9); 5.14 (br s; H-7); 4.80 (NH_2, br s; 7-OCONH_2); 4.29 (d, 10; H-6); 4.08 (3H, s; 17-OCH_3); 3.48 (m; H-11); 3.35 (m; H-12); 3.31 (3H, s; 12-OCH_3); 3.25 (3H, s; 6-OCH_3); 2.73 (m; H-10); 2.41 (2H, m; H-15); 1.98, (3H, s; 2-CH_3); 1.75 (3H, s; 8-CH_3); 1.73 (2H, m; H-13); 1.63 (br s; H-14); 0.93 (3H, d, 7; 10-CH_3); 0.92 (3H, d, 6; 14-CH_3); (Tadtong, 2000).

^{13}C-NMR : δ_C (ppm), 75 MHz, in $CDCl_3$; 184.70 (s; C-21); 183.88 (s; C-18); 167.99 (s; C-1); 156.75 (s; C-17); 155.77 (s; 7-O$\underline{C}$ONH_2); 137.86 (s; C-20); 136.25 (d; C-5); 134.66 (s; C-2); 133.11 (s; C-8); 132.97 (d; C-9); 127.42 (s; C-16); 127.07 (d; C-3); 126.18 (d; C-4); 111.63 (d; C-19); 81.64 (d; C-7); 81.23 (d; C-6); 80.93 (d; C-12); 72.62 (d; C-11); 61.71 (q; 17-OCH_3); 57.31 (q; 6-OCH_3); 56.77 (q; 12-OCH_3); 34.76 (t; C-13); 32.85 (t; C-15); 32.28 (d; C-10); 27.99 (d; C-14); 23.06 (q; 14-CH_3); 13.01 (q; 8-CH_3); 12.66 (q; 10-CH_3); 12.50 (q; 2-CH_3); (Tadtong, 2000).

7.2 17-*O*-demethylgeldanamycin (2)

HRFABMS : $[M+Na]^+$ *m/z* 569.2479 (calcd for $C_{28}H_{38}N_2O_9Na$, 569.2475)

UV : λ_{max} nm (log ε), in methanol; 246 (3.866), 322 (4.127), 530 (2.875); Figure B1

^{1}H-NMR : δ_H (ppm), 300 MHz, in $CDCl_3$+DMSO-d_6; 8.90 (br s; 1-NH); 7.17 (s; H-19); 6.91 (d, 12; H-3); 6.46 (t, 12, 11; H-4); 5.80 (t, 11, 9; H-5); 5.69 (d, 9; H-9); 5.43 (NH_2, br s; 7-OCONH_2); 5.01 (br s; H-7); 4.23 (d, 9; H-6); 3.49 (m; H-11); 3.33 (m; H-12); 3.24 (3H, s; 12-OCH_3); 3.20 (3H, s; 6-OCH_3); 2.69 (m; H-10); 2.31 (2H, m; H-15); 1.92, (3H, s; 2-CH_3); 1.77 (br s; H-14); 1.67 (3H, s; 8-CH_3); 1.60 (2H, m; H-13); 0.91 (3H, d, 6; 14-CH_3); 0.81 (3H, d, 7; 10-CH_3); (Tadtong, 2000).

^{13}C-NMR : δ_C (ppm), 75 MHz, in $CDCl_3$+DMSO-d_6; 183.16 (s; C-18; C-21); 167.82 (s; C-1); 155.77 (s; 7-O$\underline{C}$ONH_2, C-17); 139.50 (s; C-20); 136.78 (d; C-5); 133.62 (s; C-2); 132.74 (s; C-8); 132.28 (d; C-9); 127.28 (d; C-3); 125.48 (d; C-4); 117.26 (s; C-16); 108.18

(d; C-19); 81.48 (d; C-6); 80.97 (d; C-7); 80.53 (d; C-12); 71.99 (d; C-11); 56.85 (q; 6-OCH_3); 56.29 (q; 12-OCH_3); 33.09 (t; C-13); 32.06 (t; C-15); 31.87 (d; C-10); 27.31 (d; C-14); 23.22 (q; 14-CH_3); 12.60 (q; 8-CH_3); 12.19 (q; 10-CH_3; 2-CH_3); (Tadtong, 2000).

7.3 17-*O*-demethylgeldanamycin hydroquinone (5)

ESI-TOFMS : $[M+Na]^+$ *m/z* 571.2649 (calcd for $C_{28}H_{40}N_2O_9Na$, 571.2632)

UV : λ_{max} nm (log ε), in methanol; 327 (4.175), 527 (2.740); Figure C1

^{1}H-NMR : δ_H (ppm), 300 MHz, in $CDCl_3$+DMSO-d_6; 9.58 (br s; 1-NH); 6.89 (s; H-19); 6.98 (d, 11; H-3); 6.51 (t, 11, 11; H-4); 6.09 (NH_2, br s; 7-OCONH_2); 5.77 (t, 11, 10; H-5); 5.73 (d, 9; H-9); 4.93 (br s; H-7); 4.25 (d, 10; H-6); 3.41 (m; H-11); 3.23 (m; H-12); 3.22 (3H, s; 12-OCH_3); 3.14 (3H, s; 6-OCH_3); 2.59 (m; H-10); 2.24 (2H, m; H-15); 1.92, (3H, s; 2-CH_3); 1.70 (br s; H-14); 1.66 (3H, s; 8-CH_3); 0.99 (2H, br s; H-13); 0.83 (3H, d, 7; 14-CH_3); 0.81 (3H, d, 7; 10-CH_3); (Tadtong, 2000).

^{13}C-NMR : δ_C (ppm), 75 MHz, in $CDCl_3$+DMSO-d_6; 167.81 (s; C-1); 156.18 (s; 7-O$\underline{C}$ONH_2; C-18); 143.82 (s; C-17; C-21); 136.05 (d; C-5); 134.00 (s; C-2); 132.84 (s; C-8; C-20); 132.47 (d; C-9); 126.82 (d; C-3); 125.98 (d; C-4); 113.57 (s; C-16); 106.36 (d; C-19); 81.38 (d; C-6, C-12); 80.51 (d; C-7); 71.76 (d; C-11); 56.39 (q; 6-OCH_3); 56.21 (q; 12-OCH_3); 33.22 (t; C-15); 31.91 (d; C-10); 29.30 (t; C-13); 27.73 (d; C-14); 23.68 (q; 14-CH_3); 12.84 (q; 8-CH_3); 12.43 (q; 10-CH_3; 2-CH_3); (Tadtong, 2000).

8. Derivatization of geldanamycin

8.1 Modification of 17-OCH_3 into other alkoxy derivatives:

8.1.1 Synthesis and purification of 17-*O*-ethyl-17-*O*-demethyl-geldanamycin (6)

Geldanamycin (**1**) (10 mg, 0.0178 mmol) was dissolved in 200 μL of 1:1 EtOH: DMSO and stirred under N_2 atmosphere for 30 min at room temperature. Then 46 μL of 1.1 M EtONa was added into the reaction mixture and continued stirring for 2 h under N_2 atmosphere at room temperature. The reaction mixture was quenched by partitioned with EtOAc (15 mL × 3). The organic layer was further washed with concentrated $NaHCO_3$ and brine, then concentrated under reduced pressure and purified by Si Gel column (gradient MeOH in CH_2Cl_2) to produce a yellow amorphous solid of 17-*O*-ethyl-17-*O*-demethylgeldanamycin (**6**) (7.4 mg, 72.20%).

ESI-Q-TOFMS : $[M+Na]^+$ *m/z* 597.2791 (calcd for $C_{30}H_{42}N_2O_9Na$, 597.2783); Figure D1

UV : λ_{max} nm (log ε), in MeOH; 247 (4.055), 322 (4.033); Figure D2

^{1}H-NMR : δ_H (ppm), 300 MHz, in $CDCl_3$; 8.69 (br s; 1-NH); 7.24 (s; H-19); 6.92 (d, 11; H-3); 6.54 (t, 11, 11; H-4); 5.85 (t, 11, 9; H-5); 5.80 (d, 10; H-9); 5.17 (br s; H-7); 4.77 (NH_2, br s; 7-OCONH_2); 4.44 (2H, m; 17-OC$H_2$$CH_3$); 4.30 (d, 9; H-6); 3.51 (m; H-11); 3.34 (3H, s; 12-OCH_3); 3.27 (3H, s; 6-OCH_3); 3.07 (m; H-12); 2.76 (m; H-10); 2.46 (2H, m; H-15); 2.00, (3H, s; 2-CH_3); 1.77 (3H, s; 8-CH_3; 2H, m; H-13); 1.59 (m; H-14); 1.34 (3H, t, 7, 7; 17-$OCH_2$$CH_3$); 0.84 (3H, d, 7; 10-$CH_3$); 0.83 (3H, d, 7; 14-$CH_3$); Figure D3

^{13}C-NMR : δ_C ppm, 75 MHz, in $CDCl_3$; 184.73 (s; C-21); 184.00 (s; C-18); 167.99 (s; C-1); 156.56 (s; 7-O$C$$ONH_2$); 155.76 (s; C-17); 137.89 (s; C-20); 136.21 (d; C-5); 134.70 (s; C-2); 133.09 (s; C-8; d; C-9); 127.42 (s; C-16); 127.04 (d; C-3); 126.20 (d; C-4); 111.62 (d; C-19); 81.68 (d; C-7); 81.44 (d; C-6); 81.24 (d; C-12); 72.66 (d;

C-11); 70.24 (t; 17-O<u>C</u>H_2CH_3); 57.32 (q; 6-OCH_3); 56.78 (q; 12-OCH_3); 34.91 (t; C-13); 32.97 (t; C-15); 32.32 (d; C-10); 28.14 (d; C-14); 23.17 (q; 14-CH_3); 16.17 (q; 17-OCH_2<u>C</u>H_3); 13.02 (q; 8-CH_3); 12.67 (q; 10-CH_3); 12.53 (q; 2-CH_3); Figure D4

The EtONa solution was prepared form 55% NaH (24 mg). The NaH was washed several times by hexane to remove the coated mineral oil from NaH. Then 500 μL of EtOH was added and stirred until NaH was completely dissolved. The 500 μL of DMSO was added into the EtONa solution to produce 1.1 M EtONa solution in DMSO.

8.1.2 Synthesis and purification of 17,19-di-*O*-ethyl-17-*O*-demethyl-geldanamycin (7)

Geldanamycin (**1**) (17 mg, 0.0304 mmol) was dissolved in 200 μL DMSO and stirred for 30 min at room temperature. Then 2 equivalences of 1 M EtONa were added into the reaction mixture and continued stirring for 16 h at room temperature. The reaction mixture was quenched by partitioned with EtOAc (15 mL × 3). The organic layer was further washed with concentrated $NaHCO_3$ and brine, then concentrated under reduced pressure and purified by Si Gel column (gradient MeOH in CH_2Cl_2) and a PLC (10% MeOH in CH_2Cl_2) to give an amorphous solid of 17,19-di-*O*-ethyl-17-*O*-demethylgeldanamycin (**7**) (2.8 mg, 14.93%).

ESI-Q-TOFMS : $[M+Na+2H]^+$ *m/z* 643.3115 (calcd for $C_{32}H_{48}N_2O_{10}Na$, 643.3201); Figure E1

UV : λ_{max} nm (log ε), in MeOH; 266 (3.829), 331 (2.929), 523 (2.176); Figure E2

^{1}H-NMR : δ_H (ppm), 300 MHz, in $CDCl_3$; 7.45 (d, 12; H-3); 6.54 (t, 12, 12; H-4); 5.57 (t, 12, 10; H-5); 5.29 (d, 10; H-9); 5.02 (br s; H-7); 4.72 (NH_2, br s; 7-OCON<u>H</u>$_2$); 4.40 (2H, q, 14, 7, 7; 17-OC<u>H</u>$_2$$CH_3$); 4.31 (d, 9; H-6); 4.21 (2H, q, 14, 7, 7; 19-OC<u>H</u>$_2$$CH_3$); 3.59 (m; H-11); 3.30 (3H, s; 12-OCH_3); 3.27 (3H, s; 6-OCH_3); 3.12 (m; H-12); 2.38 (m; H-10); 2.29 (2H, m; H-15); 1.94, (3H, s; 2-CH_3);

1.72 (m; H-14); 1.64 (3H, s; 8-CH_3); 1.57 (2H, m; H-13); 1.33 (3H, t, 7, 7; 17-$OCH_2\underline{CH_3}$); 1.29 (3H, t, 7, 7; 19-$OCH_2\underline{CH_3}$); 1.00 (3H, d, 7; 14-CH_3); 0.79 (3H, d, 7; 10-CH_3); Figure E3

The sodium alkoxide solution was prepared form Na metal (23 mg) reacted with alcohol. The Na was cut into small pieces, 1 mL of alcohol was added and stirred until Na was completely dissolved to yield 1 M sodium alkoxide solution.

8.1.3 Synthesis and purification of 17-*O*-n-propyl-17-*O*-demethyl-geldanamycin (8)

Geldanamycin (**1**) (10 mg, 0.0178 mmol) was dissolved in 200 μL of 1:1 n-PrOH: DMSO and stirred under N_2 atmosphere for 30 min at room temperature. Then 46 μL of 1.1 M n-PrONa was added into the reaction mixture and continued stirring for 2 h under N_2 atmosphere at room temperature. The reaction mixture was quenched by partitioned with EtOAc (15 mL × 3). The organic layer was further washed with concentrated $NaHCO_3$ and brine, then concentrated under reduced pressure and purified by Si Gel column (gradient MeOH in CH_2Cl_2) to produce a yellow amorphous solid of 17-*O*- n-propyl-17-*O*-demethylgeldanamycin (**8**) (7.7 mg, 73.33%).

ESI-Q-TOFMS : $[M+Na]^+$ *m/z* 611.2955 (calcd for $C_{31}H_{44}N_2O_9Na$, 611.2939); Figure F1

UV : λ_{max} nm (log ε), in MeOH; 246 (4.176), 316 (4.099); Figure F2

^{1}H-NMR : δ_H ppm, 300 MHz, in $CDCl_3$; 8.70 (br s; 1-NH); 7.24 (s; H-19); 6.92 (d, 12; H-3); 6.55 (t, 12, 12; H-4); 5.85 (t, 12, 9; H-5); 5.80 (d, 10; H-9); 5.17 (br s; H-7); 4.77 (NH_2, br s; 7-OCON$\underline{H_2}$); 4.36 (2H, m; 17-$OC\underline{H_2}CH_2CH_3$); 4.30 (d, 9; H-6); 3.52 (m; H-11); 3.27 (3H, s; 12-OCH_3); 3.15 (m; H-12); 3.14 (3H, s; 6-OCH_3); 2.75 (m; H-10); 2.46 (2H, m; H-15); 2.00, (3H, s; 2-CH_3); 1.77 (3H, s; 8-CH_3, 2H, m; H-13); 1.59 (m; H-14); 1.27 (2H, m; 17-

$OCH_2C\underline{H}_2CH_3$); 1.00 (3H, t, 7, 7; 17-$OCH_2CH_2C\underline{H}_3$); 0.97 (3H, d, 7; 10-$CH_3$; 3H, d, 7; 14-$CH_3$); Figure F3

^{13}C-NMR : δ_C ppm, 75 MHz, in $CDCl_3$; 184.70 (s; C-21); 184.02 (s; C-18); 167.99 (s; C-1); 156.74 (s; 7-$O\underline{C}ONH_2$); 155.74 (s; C-17); 137.91 (s; C-20); 136.24 (d; C-5); 134.72 (s; C-2); 133.10 (s; C-8; d; C-9); 127.27 (s; C-16); 127.07 (d; C-3); 126.18 (d; C-4); 111.63 (d; C-19); 81.70 (d; C-7); 81.26 (d; C-6); 81.05 (d; C-12); 75.90 (d; C-11); 72.69 (t; 17-$O\underline{C}H_2CH_2CH_3$); 57.31 (q; 6-$OCH_3$); 56.77 (q; 12-$OCH_3$); 34.97 (t; C-13); 32.95 (t; C-15); 32.35 (d; C-10); 28.18 (d; C-14); 23.95 (t; 17-$OCH_2\underline{C}H_2CH_3$); 23.23 (q; 14-$CH_3$); 13.02 (q; 8-$CH_3$); 12.66 (q; 10-$CH_3$); 12.55 (q; 2-$CH_3$); 10.45 (q; 17-$OCH_2CH_2\underline{C}H_3$); Figure F4

The n-PrONa solution was prepared form 55% NaH (24 mg). The NaH was washed several times by hexane to remove the coated petroleum oil from NaH. Then 500 μL of n-PrOH was added and stirred until NaH was completely dissolved. The 500 μL of DMSO was added into the n-PrONa solution to produce 1.1 M n-PrONa solution in DMSO.

8.1.4 Synthesis and purification of 17-*O*-benzyl-17-*O*-demethyl-geldanamycin (9)

Geldanamycin (**1**) (10 mg, 0.0178 mmol) was dissolved in 200 μL of 1:1 BnOH: DMSO and stirred under N_2 atmosphere for 30 min at room temperature. Then 46 μL of 1.1 M BnONa was added into the reaction mixture and continued stirring for 2 h under N_2 atmosphere at room temperature. The reaction mixture was quenched by partitioned with EtOAc (15 mL × 3). The organic layer was further washed with concentrated $NaHCO_3$ and brine, then concentrated under reduced pressure and purified by Si Gel column (gradient MeOH in CH_2Cl_2) to produce a yellow amorphous solid of 17-*O*-benzyl-17-*O*-demethylgeldanamycin (**9**) (8.4 mg, 73.95%).

ESI-Q-TOFMS : $[M+Na]^+$ *m/z* 659.2971 (calcd for $C_{35}H_{44}N_2O_9Na$, 659.2939); Figure G1

UV : λ_{max} nm (log ε), in MeOH; 247 (3.860), 329 (3.067); Figure G2

^{1}H-NMR : δ_H ppm, 300 MHz, in $CDCl_3$; 8.60 (br s; 1-NH); 7.20 (5H, m; 17-$OCH_2C_6\underline{H}_5$; 7.08 (s; H-19); 6.82 (d, 11; H-3); 6.44 (t, 11, 11; H-4); 5.74 (t, 11, 10; H-5); 5.68 (d, 9; H-9); 5.35 (2H, dd, 24, 12, 11; 17-$OC\underline{H}_2C_6H_5$); 5.05 (br s; H-7); 4.78 (NH_2, br s; 7-$OCONH\underline{H}_2$); 4.20 (d, 10; H-6); 3.39 (m; H-11); 3.22 (3H, s; 12-OCH_3); 3.20 (m; H-12); 3.16 (3H, s; 6-OCH_3); 2.89 (m; H-10); 2.67 (2H, m; H-15); 1.91 (3H, s; 2-CH_3); 1.68 (3H, s; 8-CH_3, 2H, m; H-13); 1.58 (m; H-14); 0.86 (3H, d, 7; 10-CH_3); 0.82 (3H, d, 7; 14-CH_3); Figure G3

^{13}C-NMR : δ_C ppm, 75 MHz, in $CDCl_3$; 184.51 (s; C-21); 184.05 (s; C-18); 168.02 (s; C-1); 155.90 (s; 7-O$\underline{C}ONH_2$; C-17); 140.63 (s; 17-$OCH_2\underline{C}_6H_5$); 137.94 (s; C-20); 136.29 (d; C-5); 134.59 (s; C-2); 133.07 (s; C-8); 132.81 (d; C-9); 129.13 (d; 17-$OCH_2\underline{C}_6H_5$); 128.93 (s; C-16); 128.77 (d; C-3); 128.69 (d; C-4); 128.34 (d; 17-$OCH_2\underline{C}_6H_5$); 127.65 (d; 17-$OCH_2\underline{C}_6H_5$); 111.64 (d; C-19); 81.56 (d; C-7); 81.26 (d; C-6); 80.92 (d; C-12); 75.47 (d; C-11); 72.67 (t; 17-O$\underline{C}H_2C_6H_5$); 57.25 (q; 6-OCH_3); 56.68 (q; 12-OCH_3); 34.59 (t; C-13); 32.96 (t; C-15); 32.26 (d; C-10); 28.03 (d; C-14); 23.20 (q; 14-CH_3); 12.94 (q; 8-CH_3); 12.60 (q; 10-CH_3); 12.51 (q; 2-CH_3); Figure G4

The BnONa solution was prepared form 55% NaH (24 mg). The NaH was washed several times by hexane to remove the coated petroleum oil from NaH. Then 500 μL of BnOH was added and stirred until NaH was completely dissolved. The 500 μL of DMSO was added into the BnONa solution to produce 1.1 M BnONa solution in DMSO.

8.2 Modification of 17-OCH_3 into alkylamino derivatives:

8.2.1 Synthesis and purification of 17-amino-17-demethoxy-geldanamycin (10)

Geldanamycin (**1**) (10 mg, 0.0178 mmol) was dissolved in 500 μL DMF and stirred for 30 min at room temperature. Then 10 equivalences of 70% NH_3 were added into the reaction mixture and continued stirring for 16 h at room temperature. The reaction mixture was concentrated under reduced pressure and purified by reversed phase open column (gradient MeOH in water) to give a purple needle crystal of 17-amino-17-demethoxygeldanamycin (**10**) (6.8 mg, 68.48%).

ESI-Q-TOFMS : $[M+Na]^+$ *m/z* 568.3027 (calcd for $C_{28}H_{39}N_3O_8Na$, 568.2635); Figure H1

UV : λ_{max} nm (log ε), in MeOH; 246 (4.035), 329 (4.300); Figure H2

^{1}H-NMR : δ_H ppm, 600 MHz, in $CDCl_3$; 9.11 (br s; 1-NH); 7.28 (s; H-19); 6.95 (d, 12; H-3); 6.57 (t, 12, 11; H-4); 5.85 (t, 11, 11; H-5); 5.90 (d, 9; H-9); 5.57 (NH_2, br s; 7-OCONH_2); 5.32 (br s; H-7); 4.89 (NH_2, br s; 17-NH_2); 4.30 (d, 11; H-6); 3.68 (t, 8, 8; H-11); 3.45 (m; H-12); 3.37 (3H, s; 6-OCH_3); 3.27 (3H, s; 12-OCH_3); 2.79 (t, 7, 7; H-10); 2.73 (1H, m; H-15); 2.03 (3H, s; 2-CH_3); 1.99 (1H, m; H-15); 1.89 (2H, br s; H-13); 1.82 (3H, s; 8-CH_3; 1H, m; H-14); 1.02 (3H, d, 7; 10-CH_3); 1.00 (3H, d, 6; 14-CH_3); Figure H3

^{13}C-NMR : δ_C ppm, 150 MHz, in $CDCl_3$; 183.06 (s; C-18); 180.40 (s; C-21); 167.86 (s; C-1); 156.03 (s; 7-O$\underline{C}ONH_2$); 145.93 (s; C-17); 140.37 (s; C-20); 135.76 (d; C-5); 134.93 (s; C-2); 133.86 (s; C-8); 132.92 (d; C-9); 126.87 (d; C-3); 126.50 (d; C-4); 110.25 (s; C-16); 108.51 (d; C-19); 81.75 (d; C-11); 81.11 (d; C-6; C-12); 72.17 (d; C-7); 57.01 (q; 6-OCH_3); 56.68 (q; 12-OCH_3); 34.99 (t; C-13); 34.64 (t; C-15); 32.17 (d; C-10); 28.64 (d; C-14); 23.72 (q; 14-CH_3); 12.73 (q; 2-CH_3); 12.42 (q; 8-CH_3); 12.18 (q; 10-CH_3); Figure H4

8.2.2 Synthesis and purification of 17,19-di-methylamino-17-demethoxy-geldanamycin (11)

Geldanamycin (**1**) (10 mg, 0.0178 mmol) was dissolved in 500 μL DMF and stirred for 30 min at room temperature. Then 1 equivalence of CH_3NH_2 was added into the reaction mixture and continued stirring for 14 h at room temperature. The reaction mixture was concentrated under reduced pressure and purified by reversed phase open column (gradient MeOH in water) to give a purple needle crystal of 17,19-di-methylamino-17-demethoxygeldanamycin (**11**) (8.6 mg, 81.90%).

ESI-Q-TOFMS : $[M+Na+2H]^+$ *m/z* 613.3977 (calcd for $C_{30}H_{46}N_4O_8Na$, 613.3208); Figure I1

UV : λ_{max} nm (log ε), in MeOH; 254 (4.270), 343 (4.316); Figure I2

^{1}H-NMR : δ_H ppm, 400 MHz, in $CDCl_3$; 7.33 (d, 12; H-3); 6.84 (NH, m; 17-N<u>H</u>CH$_3$); 6.68 (NH, m; 19-N<u>H</u>CH$_3$); 6.51 (t, 12, 12; H-4); 5.55 (t, 12, 10; H-5); 5.26 (d, 10; H-9); 5.05 (d, 6; H-7); 4.92 (NH_2, br s; 7-OCON<u>H</u>$_2$); 4.33 (dd, 10, 6, 6; H-6); 3.60 (d, 8; H-11); 3.29 (3H, s; 12-OCH$_3$); 3.26 (3H, s; 6-OCH$_3$); 3.15 (3H, d, 6; 17-NHC<u>H</u>$_3$); 3.13 (m; H-12); 2.84 (3H, d, 5; 19-NHC<u>H</u>$_3$); 2.39 (m; H-10); 2.65 (1H, m; H-15); 2.47 (1H, m; H-15); 1.96 (3H, s; 2-CH$_3$); 1.71 (2H, m; H-13); 1.64 (3H, s; 8-CH$_3$); 1.55 (m; H-14); 1.03 (3H, d, 7; 10-CH$_3$); 0.76 (3H, d, 6; 14-CH$_3$); Figure I3

^{13}C-NMR : δ_C ppm, 150 MHz, in $CDCl_3$; 183.76 (s; C-18); 180.91 (s; C-21); 168.29 (s; C-1); 155.97 (s; 7-O<u>C</u>ONH$_2$); 144.62 (s; C-19); 141.17 (s; C-17); 135.78 (s; C-20); 134.89 (d; C-5); 134.72 (d; C-9); 133.66 (s; C-2); 132.71 (s; C-8); 126.86 (d; C-3); 126.45 (d; C-4); 108.74 (s; C-16); 81.58 (d; C-7); 81.38 (d; C-12); 81.14 (d; C-6); 72.57 (d; C-11); 57.04 (q; 12-OCH$_3$); 56.63 (q; 6-OCH$_3$); 47.72 (q; 17-NH<u>C</u>H$_3$; q; 19-NH<u>C</u>H$_3$); 34.97 (t; C-13); 34.27 (t; C-15); 32.24 (d; C-10); 28.40 (d; C-14); 22.81 (q; 14-CH$_3$); 12.69 (q; 8-CH$_3$); 12.51 (q; 10-CH$_3$); 12.29 (q; 2-CH$_3$); Figure I4

8.2.3 Synthesis and purification of 17-ethylamino-17-demethoxy-geldanamycin (12)

Geldanamycin (**1**) (10 mg, 0.0178 mmol) was dissolved in 500 μL ACN and stirred for 30 min at room temperature. Then 1 equivalence of 70% $CH_3CH_2NH_2$ was added into the reaction mixture and continued stirring for 2 h at room temperature. The reaction mixture was concentrated under reduced pressure and purified by reversed phase open column (gradient MeOH in water) to give a purple needle crystal of 17-ethylamino-17-demethoxygeldanamycin (**12**) (4.4 mg, 43.01%).

ESI-Q-TOFMS : $[M+Na]^+$ *m/z* 596.3008 (calcd for $C_{30}H_{43}N_3O_8Na$, 596.2948); Figure J1

UV : λ_{max} nm (log ε), in MeOH; 245 (4.163), 332 (4.340), 527 (2.602); Figure J2

^{1}H-NMR : δ_H ppm, 600 MHz, in $CDCl_3$; 9.17 (br s; 1-NH); 6.94 (d, 12; H-3); 6.56 (t, 12, 11; H-4); 6.20 (NH, t, 5, 5; 17-N<u>H</u>CH_2CH_3); 5.87 (t, 11, 11; H-5); 5.82 (d, 11; H-9); 5.16 (br s; H-7); 4.80 (NH_2, br s; 7-OCON<u>H</u>$_2$); 4.29 (d, 11; H-6); 3.51 (m; H-11); 3.62 (1H, m; 17-NHC<u>H</u>$_2$$CH_3$); 3.45 (1H, m; 17-NHC<u>H</u>$_2$$CH_3$); 3.41 (m; H-12); 3.34 (3H, s; 12-OCH_3); 3.24 (3H, s; 6-OCH_3); 2.74 (m; H-10); 2.68 (1H, m; H-15); 2.40 (1H, m; H-15); 2.00 (3H, s; 2-CH_3); 1.70 (2H, br s; H-13); 1.84 (3H, s; 8-CH_3); 1.63 (br s; H-14); 1.31 (3H, t, 7, 7; 17-NHCH$_2$C<u>H</u>$_3$); 0.97 (3H, d, 7; 10-CH_3); 0.94 (3H, d, 7; 14-CH_3); Figure J3

^{13}C-NMR : δ_C ppm, 150 MHz, in $CDCl_3$; 183.87 (s; C-18); 181.67 (s; C-21); 168.38 (s; C-1); 156.04 (s; 7-O<u>C</u>ONH_2); 144.81 (s; C-17); 141.43 (s; C-20); 135.79 (d; C-5); 134.99 (s; C-2); 133.80 (s; C-8); 132.74 (d; C-9); 126.90 (d; C-3); 126.53 (d; C-4); 108.64 (d; C-19); 108.35 (s; C-16); 81.66 (d; C-7); 81.51 (d; C-6); 81.22 (d; C-12); 72.65 (d; C-11); 57.09 (q; 6-OCH_3); 56.69 (q; 12-OCH_3); 40.66 (t; 17-NH<u>C</u>H$_2$$CH_3$); 35.09 (t; C-13); 34.37 (t; C-15); 32.32 (d; C-10); 28.50 (d; C-14); 22.83 (q; 14-CH_3); 15.12 (q; 17-

$NHCH_2\underline{C}H_3$); 12.74 (q; 8-CH_3); 12.58 (q; 10-CH_3); 12.35 (q; 2-CH_3); Figure J4

8.2.4 Synthesis and purification of 17-n-propylamino-17-demethoxy-geldanamycin (13)

Geldanamycin (**1**) (10 mg, 0.0178 mmol) was dissolved in 200 μL CH_2Cl_2 and stirred for 30 min at room temperature. Then 1 equivalence of $CH_3CH_2CH_2NH_2$ was added into the reaction mixture and continued stirring for 10 min at room temperature. The reaction mixture was concentrated under reduced pressure and purified by reversed phase open column (gradient MeOH in water) to give a purple needle crystal of 17-n-propylamino-17-demethoxygeldanammycin (**13**) (7.6 mg, 72.52%).

ESI-Q-TOFMS : $[M+Na]^+$ *m/z* 610.3177 (calcd for $C_{31}H_{45}N_3O_8Na$, 610.3104); Figure K1

UV : λ_{max} nm (log ε), in MeOH; 247 (4.169), 332 (4.359), 528 (2.602); Figure K2

^{1}H-NMR : δ_H ppm, 600 MHz, in $CDCl_3$; 9.17 (br s; 1-NH); 6.94 (d, 12; H-3); 6.56 (t, 12, 11; H-4); 6.29 (NH, t, 5, 5; 17-$N\underline{H}CH_2CH_2CH_3$); 5.87 (t, 11, 11; H-5); 5.83 (d, 11; H-9); 5.17 (br s; H-7); 4.77 (NH_2, br s; 7-OCON$\underline{H}_2$); 4.30 (d, 11; H-6); 3.54 (m; H-11); 3.50 (2H, m; 17-$NHC\underline{H}_2CH_2CH_3$); 3.43 (m; H-12); 3.35 (3H, s; 6-OCH_3); 3.25 (3H, s; 12-OCH_3); 2.73 (m; H-10); 2.65 (1H, m; H-15); 2.41 (1H, m; H-15); 2.00 (3H, s; 2-CH_3); 1.78 (3H, s; 8-CH_3); 1.68 (2H, m; 17-$NHCH_2C\underline{H}_2CH_3$); 1.67 (2H, br s; H-13); 1.59 (m; H-14); 1.02 (3H, d, 7; 10-CH_3); 0.97 (3H, d, 7; 14-CH_3); 0.93 (3H, t, 8, 8; 17-$NHCH_2CH_2C\underline{H}_3$); Figure K3

^{13}C-NMR : δ_C ppm, 150 MHz, in $CDCl_3$; 183.89 (s; C-18); 180.59 (s; C-21); 168.37 (s; C-1); 155.98 (s; 7-O$\underline{C}ONH_2$); 144.94 (s; C-17); 141.46 (s; C-20); 135.78 (d; C-5); 134.99 (s; C-2); 133.81 (s; C-8); 132.71 (d; C-9); 126.53 (d; C-3); 126.53 (d; C-4); 108.64 (d; C-19); 108.33 (s; C-16); 81.68 (d; C-7); 81.51 (d; C-6); 81.20

(d; C-12); 72.64 (d; C-11); 57.10 (q; 6-OCH_3); 56.68 (q; 12-OCH_3); 47.56 (t; 17-NH$\underline{C}H_2CH_2CH_3$); 35.09 (t; C-13); 34.38 (t; C-15); 32.32 (d; C-10); 28.52 (d; C-14); 23.13 (q; 14-CH_3); 22.85 (t; 17-NHCH$_2\underline{C}H_2CH_3$); 12.73 (q; 8-CH_3); 12.57 (q; 10-CH_3); 12.34 (q; 2-CH_3); 11.25 (q; 17-NHCH$_2$CH$_2\underline{C}H_3$); Figure K4

8.2.5 Synthesis and purification of 17,19-di-n-propylamino-17-demethoxygeldanamycin (14)

Geldanamycin (**1**) (10 mg, 0.0178 mmol) was dissolved in 500 µL CH_2Cl_2 and stirred for 30 min at room temperature. Then 1 equivalence of $CH_3CH_2CH_2NH_2$ was added into the reaction mixture and continued stirring for 2 h at room temperature. The reaction mixture was concentrated under reduced pressure and purified by reversed phase open column (gradient MeOH in water) to give a purple needle crystal of 17,19-di-n-propylamino-17-demethoxygeldanamycin (**14**) (9.4 mg, 81.84%).

ESI-Q-TOFMS : $[M+Na+2H]^+$ *m/z* 669.3936 (calcd for $C_{34}H_{54}N_4O_8Na$, 669.3834); Figure L1

UV : λ_{max} nm (log ε), in MeOH; 254 (4.415), 346 (4.462); Figure L2

^{1}H-NMR : δ_H ppm, 600 MHz, in $CDCl_3$; 7.34 (d, 12; H-3); 6.51 (t, 12, 12; H-4); 6.77 (NH, t, 6, 6; 17-N$\underline{H}$CH$_2$CH$_2$CH$_3$); 6.60 (NH, t, 6, 6; 19-N$\underline{H}$CH$_2$CH$_2$CH$_3$); 5.56 (t, 12, 10; H-5); 5.26 (d, 10; H-9); 5.10 (d, 6; H-7); 4.85 (NH_2, br s; 7-OCON$\underline{H}_2$); 4.32 (dd, 9, 6, 6; H-6); 3.60 (m; H-11); 3.45 (2H, m; 17-NHC$\underline{H}_2$CH$_2$CH$_3$); 3.29 (3H, s; 6-OCH_3); 3.27 (3H, s; 12-OCH_3); 3.13 (m; H-12); 3.07 (2H, m; 19-NHC$\underline{H}_2$CH$_2$CH$_3$); 2.41 (1H, m; H-10; 2H, m; H-15); 1.96 (3H, s; 2-CH_3); 1.69 (2H, m; H-13); 1.65 (3H, s; 8-CH_3; 2H, m; 17-NHCH$_2\underline{C}H_2$CH$_3$; 2H, m; 19-NHCH$_2\underline{C}H_2$CH$_3$); 1.55 (m; H-14); 1.04 (3H, d, 7; 10-CH_3); 0.77 (3H, d, 7; 14-CH_3); 0.95 (3H, t, 8, 8; 17-NHCH$_2$CH$_2\underline{C}H_3$; 3H, t, 8, 8; 19-NHCH$_2$CH$_2\underline{C}H_3$); Figure L3

^{13}C-NMR : δ_C ppm, 150 MHz, in $CDCl_3$; 179.29 (s; C-18); 179.01 (s; C-21); 170.83 (s; C-1); 156.00 (s; 7-O$\underline{C}$ONH$_2$); 150.74 (s; C-19); 147.50 (s; C-17); 131.54 (d; C-5); 131.29 (s; C-2; s; C-8); 130.93 (d; C-9); 128.99 (d; C-3); 127.80 (d; C-4); 105.93 (s; C-16); 91.91 (s; C-20; d; C-7); 80.31 (d; C-6); 79.80 (d; C-12); 74.14 (d; C-11); 56.89 (q; 6-OCH$_3$); 56.83 (q; 12-OCH$_3$); 46.32 (t; 17-NH$\underline{C}$H$_2$CH$_2$CH$_3$); 44.23 (t; 19-NH$\underline{C}$H$_2$CH$_2$CH$_3$); 34.78 (t; C-13); 34.47 (t; C-15); 31.36 (d; C-10); 30.43 (d; C-14); 23.62 (t; 17-NHCH$_2\underline{C}$H$_2$CH$_3$); 21.60 (t; 19-NHCH$_2\underline{C}$H$_2$CH$_3$); 18.71 (q; 14-CH$_3$); 17.45 (q; 8-CH$_3$); 14.33 (q; 10-CH$_3$); 12.84 (q; 2-CH$_3$); 11.46 (q; 19-NHCH$_2$CH$_2\underline{C}$H$_3$); 11.19 (q; 17-NHCH$_2$CH$_2\underline{C}$H$_3$); Figure L4

8.2.6 Synthesis and purification of 17-allylamino-17-demethoxy-geldanamycin (15)

Geldanamycin (**1**) (10 mg, 0.0178 mmol) was dissolved in 500 μL CH_2Cl_2 and stirred for 30 min at room temperature. Then 40 equivalences of allylamine were added into the reaction mixture and continued stirring for 20 h at room temperature. The reaction mixture was concentrated under reduced pressure and purified by reversed phase open column (gradient MeOH in water) to give a purple needle crystal of 17-allylamino-17-demethoxygeldanamycin (**15**) (10.3 mg, 98.57%).

ESI-Q-TOFMS : $[M+Na]^+$ *m/z* 608.3555 (calcd for $C_{31}H_{43}N_3O_8Na$, 608.2948); Figure M1

UV : λ_{max} nm (log ε), in MeOH; 244 (4.241), 332 (4.394), 522 (2.477); Figure M2

^{1}H-NMR : δ_H ppm, 600 MHz, in $CDCl_3$; 9.14 (br s; 1-NH); 6.94 (d, 12; H-3); 6.56 (t, 12, 12; H-4); 6.37 (NH, t, 6, 6; 17-N$\underline{H}$CH$_2$CH=CH$_2$); 5.92 (1H, m; 17-NHCH$_2\underline{C}$H=CH$_2$); 5.86 (d, 12; H-9); 5.83 (t, 12, 11; H-5); 5.29 (2H, m; 17-NHCH$_2$CH=C$\underline{H}_2$); 5.17 (br s; H-7); 4.79 (NH$_2$, br s; 7-OCON$\underline{H}_2$); 4.29 (d, 11; H-6); 4.15 (2H, m; 17-NHC$\underline{H}_2$CH=CH$_2$); 3.54 (m; H-11); 3.42 (m; H-12); 3.34 (3H,

s; 12-OCH_3); 3.28 (3H, s; 6-OCH_3); 2.73 (m; H-10); 2.62 (1H, m; H-15); 2.35 (1H, m; H-15); 2.00 (3H, s; 2-CH_3); 1.78 (3H, s; 8-CH_3); 1.76 (2H, br s; H-13); 1.70 (m; H-14); 0.98 (3H, d, 7; 10-CH_3); 0.96 (3H, d, 7; 14-CH_3); Figure M3

^{13}C-NMR : δ_C ppm, 150 MHz, in $CDCl_3$; 183.76 (s; C-18); 180.91 (s; C-21); 168.29 (s; C-1); 155.95 (s; 7-O$\underline{C}ONH_2$); 144.61 (s; C-17); 141.17 (s; C-20); 135.78 (d; C-5); 134.89 (s; C-2); 133.67 (s; C-8); 132.71 (d; C-9); 132.47 (d; 17-$NHCH_2\underline{C}H{=}CH_2$); 126.87 (d; C-3); 126.45 (d; C-4); 118.46 (t; 17-$NHCH_2CH{=}\underline{C}H_2$); 108.74 (s; C-16; d; C-19); 81.59 (d; C-7); 81.38 (d; C-6); 81.14 (d; C-12); 72.57 (d; C-11); 57.04 (q; 6-OCH_3); 56.63 (q; 12-OCH_3); 47.72 (t; 17-$NH\underline{C}H_2CH{=}CH_2$); 34.97 (t; C-13); 34.27 (t; C-15); 32.24 (d; C-10); 28.40 (d; C-14); 22.81 (q; 14-CH_3); 12.67 (q; 8-CH_3); 12.51 (q; 10-CH_3); 12.29 (q; 2-CH_3); Figure M4

8.2.7 Synthesis and purification of 17,19-di-hydroxypropylamino-17-demethoxygeldanamycin (16)

Geldanamycin (**1**) (10 mg, 0.0178 mmol) was dissolved in 500 μL ACN and stirred for 30 min at room temperature. Then 1 equivalence of propanolamine was added into the reaction mixture and continued stirring for 1.5 h at room temperature. The reaction mixture was concentrated under reduced pressure and purified by reversed phase open column (gradient MeOH in water) to give a purple needle crystal of 17,19-di-hydroxypropylamino-17-demethoxygeldanamycin (**16**) (7.0 mg, 58.00%).

ESI-Q-TOFMS : $[M+Na+2H]^+$ *m/z* 701.3751 (calcd for $C_{34}H_{54}N_4O_{10}Na$, 701.3732); Figure N1

UV : λ_{max} nm (log ε), in MeOH; 251 (4.502), 343 (4.389); Figure N2

^{1}H-NMR : δ_H ppm, 600 MHz, in DMSO-d_6; 7.14 (d, 12; H-3); 6.52 (t, 12, 11; H-4); 7.67 (NH, t, 6, 6; 19-$N\underline{H}CH_2CH_2CH_2OH$); 7.47 (OH, br s; 19-$NHCH_2CH_2CH_2O\underline{H}$); 7.19 (NH, t, 6, 6; 17-$N\underline{H}CH_2CH_2CH_2OH$); 6.99 (OH, br s; 17-$NHCH_2CH_2CH_2O\underline{H}$); 6.38 ($NH_2$, br s; 7-$OCON\underline{H}_2$);

5.41 (t, 11, 10; H-5); 5.26 (d, 10; H-9); 4.60 (2H, m; 17-NHCH$_2$CH$_2$C<u>H$_2$</u>OH); 4.84 (d, 10; H-7); 4.35 (dd, 10, 5, 5; H-6); 3.31 (m; H-11); 3.47 (2H, m; 17-NHC<u>H$_2$</u>CH$_2$CH$_2$OH); 3.45 (2H, m; 17-NHCH$_2$CH$_2$C<u>H$_2$</u>OH); 3.16 (3H, s; 6-OCH$_3$); 3.15 (2H, m; 19-NHC<u>H$_2$</u>CH$_2$CH$_2$OH); 3.14 (3H, s; 12-OCH$_3$); 2.97 (d, 10; H-12); 2.34 (2H, d, 7; H-15); 2.20 (m; H-10); 1.82 (3H, s; 2-CH$_3$); 1.67 (2H, m; H-13; 2H, m, 19-NHCH$_2$C<u>H$_2$</u>CH$_2$OH); 1.65 (2H, m, 17-NHCH$_2$C<u>H$_2$</u>CH$_2$OH); 1.52 (3H, s; 8-CH$_3$); 1.45 (m; H-14); 0.85 (3H, d, 6; 10-CH$_3$); 0.67 (3H, d, 7; 14-CH$_3$); Figure N3

^{13}C-NMR : δ_C ppm, 150 MHz, in DMSO-d_6; 178.36 (s; C-18); 178.28 (s; C-21); 169.71 (s; C-1); 155.93 (s; 7-O<u>C</u>ONH$_2$); 150.47 (s; C-19); 146.98 (s; C-17); 133.31 (d; C-5); 132.13 (s; C-2); 131.55 (s; C-8); 130.35 (d; C-9); 128.16 (d; C-3); 126.42 (d; C-4); 105.32 (s; C-16); 91.06 (s; C-20); 79.77 (d; C-6); 78.95 (d; C-7); 76.38 (d; C-12); 72.88 (d; C-11); 58.58 (t; 19-NHCH$_2$CH$_2$<u>C</u>H$_2$OH); 58.36 (t; 17-NHCH$_2$CH$_2$<u>C</u>H$_2$OH); 55.86 (q; 6-OCH$_3$); 55.68 (q; 12-OCH$_3$); 41.41 (t; 17-NH<u>C</u>H$_2$CH$_2$CH$_2$OH); 40.03 (t; 19-NH<u>C</u>H$_2$CH$_2$CH$_2$OH); 34.73 (t; C-13); 34.26 (d; C-10); 32.70 (t; 17-NHCH$_2$<u>C</u>H$_2$CH$_2$OH); 31.01 (t; C-15); 30.53 (t; 19-NHCH$_2$<u>C</u>H$_2$CH$_2$OH); 29.54 (d; C-14); 18.80 (q; 14-CH$_3$); 17.13 (q; 8-CH$_3$); 13.90 (q; 10-CH$_3$); 12.60 (q; 2-CH$_3$); Figure N4

8.2.8 Synthesis and purification of 17-benzylamino-17-demethoxy-geldanamycin (17)

Geldanamycin (**1**) (10 mg, 0.0178 mmol) was dissolved in 200 μL CH_2Cl_2 and stirred for 30 min at room temperature. Then 10 equivalences of benzylamine were added into the reaction mixture and continued stirring for 20 h at room temperature. The reaction mixture was concentrated under reduced pressure and purified by reversed phase open column (gradient MeOH in water) to give a purple needle crystal of 17-benzylamino-17-demethoxygeldanamycin (**17**) (8.8 mg, 68.06%).

ESI-Q-TOFMS : $[M+Na]^+$ *m/z* 658.3829 (calcd for $C_{35}H_{45}N_3O_8Na$, 658.3104); Figure O1

UV : λ_{max} nm (log ε), in MeOH; 254 (4.380), 333 (4.422), 530 (2.699); Figure O2

^{1}H-NMR : δ_H ppm, 600 MHz, in $CDCl_3$; 9.15 (br s; 1-NH); 7.40 (5H, m; 17-NHCH$_2$C$_6$H$_5$); 7.29 (s; H-19); 6.95 (d, 12; H-3); 6.56 (t, 12, 12; H-4); 6.45 (NH, t, 5, 5; 17-NHCH$_2$C$_6$H$_5$); 5.87 (t, 12, 10; H-5); 5.84 (d, 10; H-9); 5.17 (br s; H-7); 4.77 (NH_2, br s; 7-OCONH$_2$); 4.75 (1H, dd, 15, 6, 6; 17-NHCH$_2$C$_6$H$_5$); 4.61 (1H, dd, 15, 6, 6; 17-NHCH$_2$C$_6$H$_5$); 4.30 (d, 10; H-6); 3.55 (m; H-11); 3.44 (m; H-12); 3.34 (3H, s; 12-OCH_3); 3.26 (3H, s; 6-OCH_3); 2.72 (m; H-10); 2.65 (1H, m; H-15); 2.46 (1H, m; H-15); 2.01 (3H, s; 2-CH_3); 1.78 (3H, s; 8-CH_3); 1.76 (2H, br s; H-13); 1.65 (m; H-14); 1.01 (3H, d, 6; 10-CH_3); 0.96 (3H, d, 12; 14-CH_3); Figure O3

^{13}C-NMR : δ_C ppm, 150 MHz, in $CDCl_3$; 183.72 (s; C-18); 180.99 (s; C-21); 168.29 (s; C-1); 155.97 (s; 7-OCONH$_2$); 144.65 (s; C-17); 141.16 (s; C-20); 136.57 (s; 17-NHCH$_2$C$_6$H$_5$); 135.80 (d; C-5); 134.89 (s; C-2); 133.66 (s; C-8); 132.73 (d; C-9); 129.31 (d; 17-NHCH$_2$C$_6$H$_5$); 128.34 (d; 17-NHCH$_2$C$_6$H$_5$); 127.65 (d; 17-NHCH$_2$C$_6$H$_5$); 126.88 (d; C-3); 126.45 (d; C-4); 108.93 (s; C-16); 108.78 (d; C-19); 81.58 (d; C-7); 81.37 (d; C-6); 81.15 (d; C-12); 72.58 (d; C-11); 57.04 (q; 6-OCH_3); 56.64 (q; 12-OCH_3); 50.04 (t; 17-NHCH$_2$C$_6$H$_5$); 34.96 (t; C-13); 34.38 (t; C-15); 32.25 (d; C-10); 28.44 (d; C-14); 22.85 (q; 14-CH_3); 12.69 (q; 8-CH_3); 12.52 (q; 2-CH_3); 12.29 (q; 10-CH_3); Figure O4

8.3 Modification at C-19 position:

8.3.1 Synthesis and purification of 19-*O*-methylgeldanamycin (18)

Geldanamycin (**1**) (17 mg, 0.0304 mmol) was dissolved in 200 μL MeOH and stirred for 30 min at room temperature. Then 2 equivalences of 1 M MeONa were added into the reaction mixture and continued stirring for 3 h at room

temperature. The reaction mixture was quenched by partitioned with EtOAc (15 mL × 3). The organic layer was further washed with concentrated $NaHCO_3$ and brine, then concentrated under reduced pressure and purified by Si Gel column (gradient MeOH in CH_2Cl_2) and a PLC (10% MeOH in CH_2Cl_2) to give an amorphous solid of 19-*O*-methylgeldanamycin (**18**) (13.1 mg, 73.14%).

ESI-Q-TOFMS : $[M+Na+2H]^+$ *m/z* 615.2804 (calcd for $C_{30}H_{44}N_2O_{10}Na$, 615.2888); Figure P1

UV : λ_{max} nm (log ε), in MeOH; 266 (4.004), 307 (3.255), 530 (2.602); Figure P2

^{1}H-NMR : δ_H ppm, 300 MHz, in $CDCl_3$; 7.45 (d, 12; H-3); 6.54 (t, 12, 12; H-4); 5.57 (t, 12, 10; H-5); 5.29 (d, 7; H-9); 5.01 (d, 6; H-7); 4.76 (NH_2, br s; 7-OCONH_2); 4.32 (dd, 10, 6, 6; H-6); 4.04 (3H, s; 17-OCH_3); 3.75 (3H, s; 19-OCH_3); 3.59 (m; H-11); 3.29 (3H, s; 12-OCH_3); 3.27 (3H, s; 6-OCH_3); 3.12 (m; H-12); 2.36 (m; H-10); 2.26 (2H, m; H-15); 1.94 (3H, s; 2-CH_3); 1.80 (m; H-14); 1.64 (3H, s; 8-CH_3); 1.54 (2H, m; H-13); 1.00 (3H, d, 7; 10-CH_3); 0.78 (3H, d, 7; 14-CH_3); Figure P3

^{13}C-NMR : δ_C ppm, 75 MHz, in $CDCl_3$; 183.75 (s; C-21); 181.99 (s; C-18); 168.44 (s; C-1; s; C-19); 158.03 (s; C-17); 155.63 (s; 7-O$CONH_2$); 146.94 (s; C-20); 133.67 (d; C-5); 131.66 (d; C-3); 130.97 (d; C-9); 130.90 (s; C-2); 127.88 (d; C-4); 126.35 (s; C-8; s; C-16); 80.44 (d; C-12); 79.81 (d; C-7); 76.58 (d; C-6); 74.12 (d; C-11); 61.54 (q; 17-OCH_3); 56.87 (q; 12-OCH_3); 56.92 (q; 6-OCH_3); 52.10 (q; 19-OCH_3); 34.79 (t; C-13); 34.56 (d; C-10); 31.14 (t; C-15); 29.18 (d; C-14); 19.61 (q; 14-CH_3); 17.62 (q; 10-CH_3); 12.84 (q; 8-CH_3); 12.69 (q; 2-CH_3); Figure P4

The sodium alkoxide solution was prepared form Na metal (23 mg) reacted with MeOH. The Na was cut into small pieces, 1 mL of MeOH was added and stirred until Na was completely dissolved to yield 1 M MeONa solution.

8.3.2 Synthesis and purification of 19-aminogeldanamycin (19)

Geldanamycin (**1**) (10 mg, 0.0178 mmol) was dissolved in 200 μL MeOH and stirred for 30 min at room temperature. Then 2 equivalences of 70% NH_3 were added into the reaction mixture and continued stirring for 16 h at room temperature. The reaction mixture was concentrated under reduced pressure and purified by reversed phase open column (gradient MeOH in water) to give a reddish-purple needle crystal of 19-aminogeldanamycin (**19**) (7.5 mg, 73.03%).

ESI-Q-TOFMS : $[M+Na+2H]^+$ *m/z* 600.3427 (calcd for $C_{29}H_{43}N_3O_9Na$, 600.2892); Figure Q1

UV : λ_{max} nm (log ε), in MeOH; 260 (4.445), 332 (4.497); Figure Q2

^{1}H-NMR : δ_H ppm, 600 MHz, in DMSO-d_6; 7.40 (d, 12; H-3); 6.91 (NH_2, br s; 7-OCON$\underline{H}_2$); 6.60 (t, 12, 11; H-4); 5.59 (t, 11, 10; H-5); 5.25 (NH_2, m; 19-NH_2); 4.86 (d, 7; H-9); 4.41 (d, 5; H-7); 4.21 (t, 10, 8; H-6); 3.68 (3H, s; 17-OCH_3); 3.15 (m; H-11; 3H, s; 12-OCH_3); 3.14 (3H, s; 6-OCH_3); 2.99 (m; H-12); 2.21 (m; H-10); 2.10 (2H, m; H-15); 1.87 (3H, s; 2-CH_3); 1.72 (2H, br s; H-13); 1.52 (3H, s; 8-CH_3); 0.92 (t, 13, 11; H-14); 0.84 (3H, d, 6; 10-CH_3); 0.68 (3H, d, 6; 14-CH_3); Figure Q3

^{13}C-NMR : δ_C ppm, 150 MHz, in DMSO-d_6; 178.87 (s; C-21); 178.25 (s; C-18); 167.88 (s; C-1; s; C-17); 149.34 (s; C-19); 155.99 (s; 7-O$\underline{C}$ONH$_2$); 105.99 (s; C-16; s; C-20); 134.29 (d; C-5); 129.25 (d; C-3); 130.32 (d; C-9); 132.72 (s; C-2); 127.64 (d; C-4); 131.60 (s; C-8); 73.03 (d; C-12); 80.15 (d; C-7); 77.25 (d; C-6); 78.91 (d; C-11); 52.02 (q; 17-OCH_3); 55.91 (q; 12-OCH_3); 56.11 (q; 6-OCH_3); 34.67 (t; C-13); 31.24 (d; C-10); 34.36 (t; C-15); 28.15 (d; C-14); 19.39 (q; 14-CH_3); 17.14 (q; 10-CH_3); 14.20 (q; 8-CH_3); 12.42 (q; 2-CH_3); Figure Q4

8.3.3 Synthesis and purification of 19-glutathionylgeldanamycin (20)

Geldanamycin (**1**) (10 mg, 0.0178 mmol) was dissolved in 200 μL DMSO and stirred under argon atmosphere for 30 min at room temperature. Then 1 equivalence of glutathione (5.49 mg, 0.0178 mmol) was added into the reaction mixture and continued stirring overnight under argon atmosphere at room temperature. The reaction mixture was purified by reversed phase open column (gradient MeOH in water) to yield 19-glutathionylgeldanamycin (**20**) as a red amorphous solid (10.8 mg, 69.90%).

ESI-Q-TOFMS : $[M+H]^+$ *m/z* 866.3986 (calcd for $C_{39}H_{56}N_5O_{15}S$, 866.3494); Figure R1

UV : λ_{max} nm (log ε), in MeOH; 256 (4.172), 329 (3.301); Figure R2

^{1}H-NMR : δ_H ppm, 400 MHz, in DMSO-d_6; 8.64 (NH_2, br s; 19-SCH_2CH-($CONHCH_2COOH$)$NHCOCH_2CH_2CH(N\underline{H_2})COOH$); 8.15 (br s; 1-NH); 6.33 (t, 11, 11; H-4); 6.30 (NH_2, br s; 7-$OCON\underline{H_2}$); 6.23 (d, 11; H-3); 5.23 (t, 11, 10; H-5); 5.14 (d, 10; H-9); 4.85 (d, 9; H-7); 4.66 (m; H-6); 4.25 (m; 19-$SCH_2C\underline{H}$($CONHCH_2COOH$)-$NHCOCH_2CH_2CH(NH_2)COOH$); 3.96 (3H, s; 17-OCH_3); 3.95 (2H, m; 19-$SCH_2CH(CONHC\underline{H_2}COOH)NHCOCH_2CH_2CH$-($NH_2$)COOH); 3.65 (1H, m; 19-$SC\underline{H_2}CH(CONHCH_2COOH)$-$NHCOCH_2CH_2CH(NH_2)COOH$); 3.45 (1H, m; 19-$SCH_2CH$-($CONHCH_2COOH$)$NHCOCH_2CH_2C\underline{H}(NH_2)COOH$); 3.34 (1H, m; 19-$SC\underline{H_2}CH(CONHCH_2COOH)NHCOCH_2CH_2CH(NH_2)$-$COOH$); 3.20 (m; H-11); 3.04 (3H, s; 12-OCH_3); 3.03 (m; H-12); 3.01 (3H, s; 6-OCH_3); 2.08 (m; H-10; 2H, br s; 19-SCH_2-$CH(CONHCH_2COOH)NHCOC\underline{H_2}CH_2CH(NH_2)COOH$); 1.85 (2H, m; H-15); 1.84 (3H, s; 2-CH_3); 1.75 (2H, m; 19-SCH_2CH-($CONHCH_2COOH$)$NHCOCH_2C\underline{H_2}CH(NH_2)COOH$); 1.63 (2H, m; H-13); 1.34 (3H, s; 8-CH_3); 0.92 (m; H-14); 0.85 (3H, d, 6; 10-CH_3); 0.60 (3H, d, 6; 14-CH_3); Figure R3

8.4 Modification of 11-OH into other alkoxy derivatives:

8.4.1 Synthesis and purification of 11-*O*-methylgeldanamycin (21)

Geldanamycin (**1**) (15 mg, 0.0268 mmol) was dissolved in 1 mL DMF and stirred for 30 min at room temperature. Then 4 equivalences of anhydrous potassium carbonate and methyl iodide (2 drops) were added into the reaction mixture and continued stirring under N_2 atmosphere for 16 h at room temperature. The reaction mixture was quenched by partitioned with EtOAc (15 mL × 3). The organic layer was further washed with concentrated $NaHCO_3$ and brine, then concentrated under reduced pressure and purified a PLC (10% MeOH in CH_2Cl_2) to give an yellow amorphous solid of 11-*O*-methylgeldanamycin (**21**) (7 mg, 45.51%).

HRFABMS : $[M+Na+2H]^+$ *m/z* 599.2925 (calcd for $C_{30}H_{44}N_2O_9Na$, 599.2939); (Tadtong, 2000)

UV : λ_{max} nm (log ε), in MeOH; 320 (3.063), 522 (1.544); Figure S1

^{1}H-NMR : δ_H ppm, 300 MHz, in $CDCl_3$; 6.30 (d, 11; H-3); 6.26 (t, 11, 11; H-4); 5.21 (t, 11, 10; H-5); 5.19 (d, 10; H-9); 4.99 (d, 10; H-7); 4.58 (NH_2, br s; 7-OCONH_2); 4.07 (3H, s; 17-OCH_3); 4.00 (t, 10, 10; H-6); 3.60 (m; H-11); 3.34 (3H, s; 11-OCH_3); 3.30 (3H, s; 12-OCH_3); 3.11 (3H, s; 6-OCH_3); 2.85 (m; H-12); 2.59 (1H, m; H-15); 2.51 (1H, m; H-15); 2.35 (br s; H-10); 2.17 (m; H-14); 1.95 (3H, s; 2-CH_3); 1.62 (2H, m; H-13); 1.23 (3H, s; 8-CH_3); 1.03 (3H, d, 6; 10-CH_3); 0.59 (3H, d, 7; 14-CH_3); Figure S2

^{13}C-NMR : δ_C ppm, 75 MHz, in $CDCl_3$; 182.65 (s; C-18); 182.46 (s; C-21); 173.03 (s; C-1); 156.51 (s; C-17); 155.61 (s; 7-O$\underline{C}$ONH_2); 149.94 (s; C-20); 140.85 (s; C-2); 134.56 (d; C-9); 129.92 (d; C-5); 129.50 (s; C-8; s; C-16); 128.73 (d; C-4); 123.95 (d; C-3); 118.61 (d; C-19); 81.83 (d; C-7); 79.84 (d; C-12); 74.66 (d; C-6); 72.43 (d; C-11); 61.26 (q; 17-OCH_3); 56.58 (q; 11-OCH_3; q; 12-OCH_3); 56.27 (q; 6-OCH_3); 34.93 (d; C-10); 30.96 (t; C-13); 30.00 (t; C-15); 28.98 (d; C-14); 18.77 (q; 10-CH_3); 18.52 (q; 14-CH_3); 14.81 (q; 2-CH_3); 12.27 (q; 8-CH_3); Figure S3

8.4.2 Synthesis and purification of 11-*O*-acetylgeldanamycin (22)

Geldanamycin (**1**) (15 mg, 0.0268 mmol) was dissolved in 1 mL anhydrous pyridine and stirred for 30 min at room temperature. Then excess acetic anhydride (1 mL) was added into the reaction mixture and continued stirring under N_2 atmosphere for 16 h at room temperature. The reaction mixture was quenched by partitioned with EtOAc (15 mL × 3). The organic layer was further washed with concentrated $NaHCO_3$ and brine, then concentrated under reduced pressure and purified a PLC (10% MeOH in CH_2Cl_2) to give an yellow amorphous solid of 11-*O*-acetyl-geldanamycin (**22**) (7 mg, 43.40%).

HRFABMS : $[M+Na]^+$ *m/z* 625.2737 (calcd for $C_{31}H_{42}N_2O_{10}Na$, 625.2732); (Tadtong, 2000)

UV : λ_{max} nm (log ε), in MeOH; 251 (3.041), 328 (3.204), 530 (1.699); Figure T1

^{1}H-NMR : δ_H ppm, 300 MHz, in $CDCl_3$+benzene-d_6; 8.60 (br s; 1-NH); 7.39 (d, 11; H-3); 6.25 (t, 11, 11; H-4); 5.71 (t, 11, 10; H-5); 5.12 (br s; H-7); 5.11 (d, 10; H-9); 4.92 (m; H-11); 4.33 (d, 10; H-6; NH_2, br s; 7-OCON$\underline{H}_2$); 3.78 (3H, s; 17-OCH_3); 3.22 (3H, s; 12-OCH_3); 3.21 (m; H-12); 3.11 (3H, s; 6-OCH_3); 3.02 (m; H-10); 2.36 (1H, m; H-15); 2.20 (1H, m; H-15); 1.93 (m; H-14); 1.83 (3H, s; 2-CH_3); 1.78 (3H, s; 8-CH_3); 1.50 (3H, s; 11-OCOC$\underline{H}_3$); 1.26 (2H, br s; H-13); 1.05 (3H, d, 6; 14-CH_3); 0.86 (3H, d, 7; 10-CH_3); Figure T2

^{13}C-NMR : δ_C ppm, 75 MHz, in $CDCl_3$+benzene-d_6; 184.10 (s; C-18); 183.82 (s; C-21); 170.69 (s; 11-O$\underline{C}$OCH$_3$); 169.60 (s; C-1); 156.11 (s; 7-O$\underline{C}$ONH$_2$; s; C-17); 130.64 (s; C-20); 127.86 (s; C-2); 129.41 (d; C-9); 134.35 (d; C-5); 130.52 (s; C-8); 129.67 (s; C-16); 126.98 (d; C-4); 128.33 (d; C-3); 110.30 (d; C-19); 80.16 (d; C-7); 79.82 (d; C-6); 78.93 (d; C-12); 75.24 (d; C-11); 61.32 (q; 17-OCH_3); 56.98 (q; 12-OCH_3); 56.66 (q; 6-OCH_3); 32.28 (d; C-10); 32.00 (t; C-15); 30.10 (t; C-13); 29.00 (d; C-14); 22.87 (q; 14-CH_3); 20.45 (q; 11-OCO$\underline{C}$H$_3$); 13.53 (q; 8-

CH_3; q; 10-CH_3); 12.42 (q; 2-CH_3); Figure T3

8.5 Reduction of double bond at C-2 to C-5 positions:

8.5.1 Synthesis and purification of 2,3,4,5-tetrahydrogeldanamycin (23)

Geldanamycin (**1**) (20 mg, 0.0356 mmol) was dissolved in 400 μL MeOH and stirred for 30 min at room temperature. Then 5 mg of 10% PdC was added into the reaction mixture and continued stirring for 2 h under H_2 atmosphere at room temperature. The reaction mixture was filtured through filter paper and washed several times by MeOH. The filtrate was concentrated under reduced pressure and purified by Si Gel column (gradient MeOH in CH_2Cl_2) and a PLC (10% MeOH in CH_2Cl_2) to give a yellow amorphous solid of 2,3,4,5-tetrahydrogeldanamycin (**23**) (8.8 mg, 43.69%).

ESI-Q-TOFMS : $[M+Na]^+$ *m/z* 587.2928 (calcd for $C_{29}H_{44}N_2O_{10}Na$, 587.2939); Figure U1

UV : λ_{max} nm (log ε), in MeOH; 245 (2.653), 304 (3.720), 527 (2.699); Figure U2

^{1}H-NMR : δ ppm, 300 MHz, in $CDCl_3$; 1:1 mixture of isomers: 8.76 (br s; 1-NH); 8.41 (br s; 1-NH); 7.27 (s; H-19), 7.20 (s; H-19); 5.54 (d, 10; H-9); 5.27 (br s; H-7); 5.10 (d, 4; H-6); 5.00 (d, 7; H-6); 4.07 (3H, s; 17-OCH_3); 4.06 (NH_2, br s; 7-OCONH_2; 3H, s; 17-OCH_3); 3.62 (m; H-11); 3.33 (3H, s; 12-OCH_3); 3.31 (3H, s; 12-OCH_3); 3.29 (3H, s; 6-OCH_3); 3.27 (3H, s; 6-OCH_3; 1H, m; H-12); 2.63 (m; H-10); 2.45 (1H, m; H-15); 2.42 (m; H-2); 2.26 (1H, m; H-15); 1.65 (2H, m; H-3); 1.56 (3H, s; 8-CH_3); 1.55 (3H, s; 8-CH_3); 1.53 (2H, br s; H-13); 1.50 (2H, m; H-5); 1.40 (2H, m; H-4); 1.35 (m; H-14); 1.22 (3H, s; 2-CH_3); 1.18 (3H, s; 2-CH_3); 0.93 (3H, d, 6; 14-CH_3); 0.87 (3H, d, 6; 10-CH_3); Figure U3