Supporting Information for

Novel hybrids of natural oridonin bearing nitrogen mustards as potential anticancer drug candidates

Shengtao Xu[†], Lingling Pei[†], Chengqian Wang[†], Yun-Kai Zhang[‡], Dahong Li[†,§], Hequan Yao[†], Xiaoming Wu[†], Zhe-Sheng Chen[‡,*], Yijun Sun[Δ] and Jinyi Xu[†,*]

[†]State Key Laboratory of Natural Medicines and Department of Medicinal Chemistry, China Pharmaceutical University, 24 Tongjia Xiang, Nanjing 210009, China

[‡]College of Pharmacy and Health Sciences, St. John's University, 8000 Utopia Parkway, Queens, New York, NY 11439, USA

[§]Key Laboratory of Structure-Based Drug Design & Discovery of Ministry of Education and School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, 103 Wen Hua Road, Shenyang 110016, China

[Δ]Drug Screening Center, Nanjing Key Gen Biotech. Co. Ltd., Nanjing 210012, China

Table of Contents:

Abbreviations Used:

EDCI: 1-Ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride

DMAP: 4-dimethylamiopryidine

TsOH: 4-methylbenzenesulfonic acid

Ac_2O: Acetic anhydride

TEA: Triethylamine

DMP: 2,2-Dimethoxypropane

DCM: Dichloromethane.

THF: Tetrahydrofuran

$(Boc)_2O$: Di-tert-butyl pyrocarbonate

$SOCl_2$: Thionyl chloride

Experimental:

1. Chemistry

All commercially available solvents and reagents were used without further purification. Flash column chromatography was carried out on 200-300 mesh silica gel. ^1H NMR and ^{13}C NMR spectra were recorded with a Bruker AV-300 spectrometer in the indicated solvents (TMS as internal standard): the values of the chemical shifts are expressed in δ values (ppm) and the coupling constants (J) in Hz. Mass spectra were obtained using FTMS-2000. The synthetic method and physicochemical data of compounds **2** and **5** were disclosed in our previous report[1]. The synthetic routes to the benzoic acid mustard, compound **7** and compound **8** were outlined in literature[2-5]. Melphalan and chlorambucil were purchased directly from Sigma-Aldrich.

2. Characterization Data for Target Compounds

ent-1α,6β,7β-trihydroxy-{14β-O-[N-(S)-2-(4-(bis(2-chloroethyl)amino)

phenylpropionic acid methylester)aminoacyl-propionyloxy]}-15-oxo-7,20-epoxy-16-kaurene (**16a**).

Compound **1** (72 mg, 0.2 mmol) was mixed with melphalan acid **10** (83 mg, 0.2 mmol), EDCI and DMAP in 15 mL of dichloromethane and stirred at room temperature for 12 h. The mixture was poured into 15 mL of 10% HCl, and extracted with dichloromethane (10 mL × 3). The organic layer was combined, washed with water and saturated NaCl solution sequentially, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by column chromatography (MeOH/CH_2Cl_2 1:150 v/v) to give the 107mg (83%) **16a** as a white solid. ^{1}H NMR($CDCl_3$, 300 M Hz), δ (ppm): 6.97 (d, J = 7.5 Hz, 2H, Ar-H), 6.63 (d, J = 7.5 Hz, 2H, Ar-H), 6.17 (m, 3H), 5.92 (s, 1H), 5.52 (s, 1H), 4.80 (d, J = 6.0 Hz, 1H), 4.30, 4.11 (dd, $J_A = J_B$ = 9.0 Hz, each 1H, 20-CH_2), 3.78 (s, 3H, -OCH_3), 3.72 (m, 4H), 3.64 (m, 4H), 3.48 (m, 1H), 3.20 (m, 1H), 3.03 (m, 2H) , 2.65 (m, 6H), 2.39 (m, 2H), 1.99 (m, 4H), 1.62 (m, 6H), 1.46 (s, 3H, -CH_3), 1.21 (s, 3H, -CH_3); ^{13}C NMR ($CDCl_3$, 75 M Hz): δ (ppm) 206.9, 172.4, 171.5, 170.9, 150.2, 145.4, 130.8, 125.0, 120.5, 114.8, 112.4, 96.3, 76.2, 74.6, 73.7, 63.6, 62.0, 59.8, 54.9, 53.8, 53.6, 52.6, 41.6, 40.7, 38.9, 35.2, 33.1, 32.8, 30.8, 30.3, 30.0, 21.9, 20.1; ESI-MS *m/z* 765.3 $[M+H]^+$; HR-MS (ESI, M+H) *m/z*: calcd for $C_{38}H_{51}Cl_2N_2O_{10}$: 765.2915, found 765.2933.

ent-1α,6β,7β-trihydroxy-{14β-O-[4-(p-bis(2-chloroethyl)aminophenyl)formyloxy]} -15-oxo-7,20-epoxy-16-kaurene (**16b**).

According to the synthetic procedure from 0.2 mmol **1** to **16a** described above, **16b** (91 mg, 72%) was obtained as a white solid. ^{1}H NMR ($CDCl_3$, 300 M Hz): δ (ppm) 7.78 (d, J = 9.0 Hz, 2H, Ar-H), 6.62 (d, J = 9.0 Hz, 2H, Ar-H), 6.17 (m, 2H), 6.03 (s, 1H), 5.47 (s, 1H), 4.30 (s, 1H), 4.08, 4.33 (dd, $J_A = J_B$ = 10.2 Hz, each 1H), 3.78 (m, 4H), 3.65 (m, 4H), 3.57 (m, 1H), 3.28 (d, J = 9.6 Hz, 1H), 2.67 (m, 1H), 2.39

(m, 1H), 2.04 (m, 1H), 1.78 (m, 2H), 1.68 (m, 3H), 1.49 (m, 2H), 1.36 (m, 2H), 1.13 (s, 3H), 1.12 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 M Hz): δ (ppm) 206.4, 164.3, 149.8, 149.6, 131.4, 119.6, 117.0, 110.6, 95.8, 76.2, 73.5, 72.6, 63.0, 61.5, 59.4, 54.1, 52.7, 41.0, 40.8, 39.6, 38.2, 33.2, 32.1, 30.0, 29.4, 21.1, 19.3; ESI-MS *m/z* 608.2 $[M+H]^+$; HR-MS (ESI, M+H) *m/z*: calcd for $C_{31}H_{40}Cl_2NO_7$: 608.2176, found 608.2179; Anal. Calcd. For $C_{31}H_{39}Cl_2NO_7$; C: 61.18; H: 6.46; N: 2.30, Found: C: 61.21; H: 6.43; N: 2.28.

ent-1α,6β,7β-trihydroxy-{14β-O-[4-(p-bis(2-chloroethyl)aminophenyl)butanoyloxy]} -15-oxo-7,20-epoxy-16-kaurene (**16c**).

Compound **1** (72 mg, 0.2 mmol) was mixed with chlorambucil (60 mg, 0.2 mmol), EDCI and DMAP in 15 mL of dichloromethane and stirred at room temperature for 2 h. The mixture was poured into 15 mL of 10% HCl, and extracted with dichloromethane (10 mL × 3). The organic layer was combined, washed with water and saturated NaCl solution sequentially, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by column chromatography ($MeOH/CH_2Cl_2$ 1:120 v/v) to give 89 mg (71%) **16c** as a white solid. ^{1}H NMR ($CDCl_3$, 300 M Hz), δ(ppm): 6.92 (d, J = 8.4 Hz, 2H, Ar-H), 6.53 (d, J = 8.4 Hz, 2H, Ar-H), 6.07 (s, 1H), 6.06 (d, J = 6.0 Hz, 1H), 5.75 (s, 1H), 5.41 (s, 1H), 4.25 (s, 1H), 4.23, 4.01 (dd, $J_A = J_B$ = 10.2 Hz, each 1H, 20-CH_2), 3.69 (m, 1H), 3.63 (m, 4H), 3.55 (m, 4H), 3.43 (m, 1H), 3.12 (d, J = 9.6 Hz, 1H), 2.65 (m, 1H), 2.43 (t, J = 7.2 Hz, 2H), 2.20 (t, J = 7.2 Hz, 2H), 1.91 (m, 1H), 1.81 (m, 2H), 1.73 (m, 2H), 1.70 (m, 2H), 1.65 (m, 3H), 1.37 (m, 2H), 1.18 (s, 3H, -CH_3), 1.05 (s, 3H, -CH_3); ^{13}C NMR ($CDCl_3$, 75 M Hz): δ (ppm) 206.1, 174.4, 149.4, 143.9, 129.6, 129.1, 119.6, 111.8, 95.7, 76.3, 73.4, 72.8, 63.0, 61.3, 59.3, 54.0, 53.1, 40.8, 40.7, 40.1, 38.2, 33.3, 33.2, 32.3, 30.0, 29.5, 29.2, 25.8, 21.1, 19.2; ESI-MS *m/z* 650.3 $[M+H]^+$; HR-MS (ESI, M+H) *m/z*:

calcd for $C_{34}H_{46}Cl_2NO_7$: 650.2646, found 650.2652.

ent-1α,6β,7β-trihydroxy-{14β-O-[N-(S)-2-(4-(bis(2-chloroethyl)amino) phenylpropionic acid methylester)aminoacyl-butyryloxy]}-15-oxo-7,20-epoxy-16-kaurene (**16d**).

According to the synthetic procedure from 0.2 mmol **1** to **16a** described above, **16d** (102 mg, 78%) was obtained as a white solid. ^{1}H NMR($CDCl_3$, 300 M Hz), δ(ppm): 6.98 (d, J = 7.8 Hz, 2H, Ar-H), 6.65 (d, J = 7.8 Hz, 2H, Ar-H), 6.5 (d, J = 6.6 Hz, 1H), 6.35 (d, J = 5.4 Hz, 1H), 6.20 (s, 1H), 5.90 (s, 1H), 5.52 (s, 1H), 4.80 (m, 1H), 4.30, 4.10 (dd, $J_A = J_B$ = 9.0 Hz, each 1H, 20-CH_2), 3.77 (s, 3H, -OCH_3), 3.72 (m, 4H), 3.64 (m, 4H), 3.50 (m, 1H), 3.20 (d, J = 6.0 Hz, 1H), 3.0 (m, 2H), 2.65 (m, 1H), 2.49 (m, 4H), 2.26 (m, 4H), 1.83 (m, 4H), 1.66 (m, 3H), 1.32 (m, 2H), 1.13 (s, 3H, -CH_3), 1.12 (s, 3H, -CH_3); ^{13}C NMR ($CDCl_3$, 75 M Hz): δ (ppm) 207.2, 177.9, 172.7, 172.4, 172.1, 150.3, 145.3, 130.8, 125.1, 120.6, 112.5, 96.3, 75.8, 74.4, 73.7, 63.6, 62.3, 59.6, 54.9, 53.8, 53.5, 52.6, 41.6, 40.7, 38.9, 37.0, 35.3, 33.8, 33.8, 33.1, 32.8, 31.1, 30.5, 22.0, 20.8, 20.1, 20.0; ESI-MS *m/z* 779.3 $[M+H]^+$; HR-MS (ESI, M+H) *m/z*: calcd for $C_{39}H_{53}Cl_2N_2O_{10}$: 779.3072, found 779.3088.

ent-1α,6β,7β-trihydroxy-{14β-O-[3-(4-(bis(2-chloroethyl)aminophenyl))-(S)-2-((tert-butoxycarbonyl)amino) propionyloxy]}-15-oxo-7,20-epoxy-16-kaurene (**16e**).

According to the synthetic procedure from 0.2 mmol **1** to **16a** described above, **16e** (112 mg, 75%) was obtained as a white solid. ^{1}H NMR ($CDCl_3$, 300 M Hz), δ (ppm): 6.99 (d, J = 8.4 Hz, 2H, Ar-H), 6.57 (d, J = 8.4 Hz, 2H, Ar-H), 6.11 (m, 1H), 6.06 (d, J = 10.5 Hz, 1H), 5.88 (s, 1H), 5.45 (s, 1H), 4.93 (d, J = 7.2 Hz, 1H), 4.38 (m, 1H), 4.33, 4.07 (dd, $J_A = J_B$ = 10.2 Hz, each 1H, 20-CH_2), 3.98 (br, 1H), 3.70 (m, 4H), 3.62 (m, 4H), 3.48 (m, 1H), 3.10 (m, 1H), 2.92 (m, 2H), 2.61 (m, 1H), 2.25 (m, 1H),

1.99 (m, 1H), 1.62 (m, 7H), 1.42 (m, 2H), 1.38 (s, 9H), 1.11 (s, 6H, -CH_3); ^{13}C NMR ($CDCl_3$, 75 M Hz): δ(ppm) 205.6, 170.4, 168.0, 149.3, 144.6, 130.2, 130.0, 124.2, 119.7, 111.7, 95.4, 74.0, 73.0, 62.8, 61.5, 59.0, 54.6, 54.3, 53.0, 40.8, 40.0, 38.2, 36.1, 33.2, 32.1, 30.1, 29.5, 27.7, 21.3, 19.4; ESI-MS *m/z* 751.3 $[M+H]^+$; HR-MS (ESI, M+H) *m/z*: calcd for $C_{38}H_{53}Cl_2N_2O_9$: 751.3123, found 751.3134.

ent-1α,6β,7β-trihydroxy-{14β-O-[3-(4-(bis(2-chloroethyl)aminophenyl))-(S)-2-formamido- propionyloxy]}-15-oxo-7,20-epoxy-16-kaurene (**16f**).

According to the synthetic procedure from 0.2 mmol **1** to **16a** described above, **16f** (78 mg, 60%) was obtained as a white solid. ^{1}H NMR ($CDCl_3$, 300 M Hz), δ (ppm): 7.40 (s, 1H), 6.99 (d, J = 8.4 Hz, 2H, Ar-H), 6.55 (d, J = 8.4 Hz, 2H, Ar-H), 6.14 (m, 2H), 5.83 (s, 1H), 5.51 (s, 1H), 4.37 (m, 2H), 4.28, 4.09 (dd, $J_A = J_B$ = 10.2 Hz, each 1H, 20-CH_2), 3.80 (m, 1H), 3.69 (m, 4H), 3.63 (m, 4H), 3.50 (m, 1H), 3.20 (m, 1H), 2.63 (m, 2H), 2.26 (m, 1H), 2.12 (m, 2H), 2.06 (m, 3H), 1.65 (m, 3H), 1.28 (s, 3H), 1.12 (s, 6H, -CH_3); ^{13}C NMR (DMSO-d_6, 75 M Hz): δ (ppm) 209.1, 173.1, 161.6, 152.3, 145.4, 130.6, 130.5, 125.6, 119.9, 112.1, 97.3, 73.6, 72.9, 72.1, 63.2, 59.9, 53.5, 52.7, 52.6, 43.2, 41.6, 36.2, 33.8, 33.1, 30.4, 29.7, 22.1, 19.7; ESI-MS *m/z* 679.3 $[M+H]^+$; HR-MS (ESI, M+H) *m/z*: calcd for $C_{34}H_{45}Cl_2N_2O_8$: 679.2553, found 679.2546.

ent-1α-O-Acetyl-6β,7β-dihydroxy-{14β-O-[N-(S)-2-(4-(bis(2-chloroethyl)amino) phenylpropionic acid methylester)aminoacyl-propionyloxy]}-15-oxo-7,20-epoxy-16-kaurene (**17a**).

According to the synthetic procedure from **1** to **16a** described above, **17a** (132 mg, 82%) was obtained as a white solid from 0.2 mmol **5**. ^{1}H NMR ($CDCl_3$, 300 M Hz): δ (ppm) 6.96 (d, J = 8.4 Hz, 2H, Ar-H), 6.69 (d, J = 8.4 Hz, 2H, Ar-H), 6.13 (m, 2H),

6.00 (d, J = 7.8 Hz, 1H), 5.87 (s, 1H), 5.52 (s, 1H), 4.79 (m, 1H), 4.62 (m, 1H), 4.27, 4.17 (dd, $J_A = J_B$ = 9.9 Hz, each 1H, 20-CH_2), 3.82 (m, 1H), 3.78 (s, 3H, -OCH_3), 3.72 (m, 4H), 3.65 (m, 4H), 3.15 (d, J = 10.2 Hz, 1H), 3.02 (m, 2H) , 2.50 (m, 5H), 1.99 (m, 5H), 1.72 (m, 2H), 1.51 (m, 4H), 1.34 (m, 2H), 1.13 (s, 6H, -CH_3); ^{13}C NMR ($CDCl_3$, 75 M Hz): δ (ppm) 205.8, 171.6, 170.8, 170.0, 169.4, 149.0, 144.7, 130.0, 124.1, 120.0, 111.6, 95.4, 75.3, 74.9, 73.7, 62.9, 61.1, 59.6, 53.2, 53.0, 52.8, 51.8, 40.6, 40.0, 39.2, 37.7, 36.1, 33.1, 31.9, 30.1, 29.7, 29.4, 24.7, 21.1, 21.0, 17.6; ESI-MS m/z 807.3 $[M+H]^+$; HR-MS (ESI, M+H) *m/z*: calcd for $C_{40}H_{53}Cl_2N_2O_{11}$: 807.3021, found 807.3034.

ent-1α-O-Acetyl-6β,7β-dihydroxy-{14β-O-[4-(p-bis(2-chloroethyl)aminophenyl) formyloxy]}-15-oxo-7,20-epoxy-16-kaurene (**17b**).

According to the synthetic procedure from **1** to **16b** described above, **17b** (73 mg, 56%) was obtained as a white solid from 0.2 mmol **5**. ^{1}H NMR ($CDCl_3$, 300 M Hz): δ (ppm) 7.78 (d, J = 9.0 Hz, 2H, Ar-H), 6.62 (d, J = 9.0 Hz, 2H, Ar-H), 6.17 (m, 2H), 6.00 (s, 1H), 5.47 (s, 1H), 4.65 (m, 1H), 4.32 (s, 1H), 4.18, 4.33 (dd, $J_A = J_B$ = 10.5 Hz, each 1H), 3.79 (m, 4H), 3.65 (m, 4H), 3.29 (d, J = 10.2 Hz, 1H), 2.61 (m, 1H), 2.09 (m, 1H), 2.05 (s, 3H), 1.78 (m, 1H), 1.58 (m, 3H), 1.49 (m, 2H), 1.36 (m, 3H), 1.14 (s, 3H), 1.12 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 M Hz): δ (ppm) 205.9, 169.4, 164.2, 149.8, 149.2, 131.4, 119.8, 117.0, 110.6, 95.7, 75.9, 74.9, 73.3, 63.0, 61.1, 59.7, 53.2, 52.7, 40.7, 39.5, 39.3, 37.7, 33.1, 31.9, 29.7, 24.7, 21.0, 17.5; ESI-MS *m/z* 650.2 $[M+H]^+$; HR-MS (ESI, M+H) *m/z*: calcd for $C_{33}H_{42}Cl_2NO_8$: 650.2282, found 650.2298.

ent-1α-O-Acetyl-6β,7β-dihydroxy-{14β-O-[4-(p-bis(2-chloroethyl)aminophenyl) butanoyloxy]}-15-oxo-7,20-epoxy-16-kaurene (**17c**).

According to the synthetic procedure from **1** to **16c** described above, **17c** (87 mg,

62 %) was obtained as a white solid from 0.2 mmol **5**. ^{1}H NMR ($CDCl_3$, 300 M Hz), δ (ppm): 7.01 (d, J = 8.4 Hz, 2H, Ar-H), 6.61 (d, J = 8.4 Hz, 2H, Ar-H), 6.14 (s, 1H), 6.10 (d, J = 10.5 Hz, 1H), 5.80 (s, 1H), 5.49 (s, 1H), 4.62 (m, 1H), 4.26 (m, 1H), 4.26, 4.18 (dd, $J_A = J_B$ = 10.5 Hz, each 1H, 20-CH_2), 3.81 (m, 1H), 3.78 (m, 4H), 3.61 (m, 4H), 3.18 (d, J = 9.9 Hz, 1H), 2.60 (m, 1H), 2.50 (t, J = 7.2 Hz, 2H), 2.28 (t, J = 7.2 Hz, 2H), 2.03 (m, 2H), 1.97 (s, 3H), 1.91 (m, 4H), 1.55 (m, 5H), 1.12 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 M Hz): δ (ppm) 205.7, 171.4, 169.4, 148.9, 143.9, 129.6, 129.2, 119.9, 111.8, 95.6, 76.0, 74.9, 73.2, 63.0, 60.9, 59.8, 53.1, 40.5, 40.0, 39.2, 37.6, 33.3, 33.2, 33.1, 31.9, 29.7, 29.2, 25.8, 24.7, 21.0, 17.4; ESI-MS *m/z* 692.3 $[M+H]^+$; HR-MS (ESI, M+H) *m/z*: calcd for $C_{36}H_{48}Cl_2NO_8$: 692.2751, found 692.2761.

ent-6β,7β-dihydroxy-{14β-O-[N-(S)-2-(4-(bis(2-chloroethyl)amino)phenylpropionic acid methylester)aminoacyl-propionyloxy]}-1,15-dioxo-7,20-epoxy-16-kaurene (**18a**).

According to the synthetic procedure from **1** to **16a** described above, **18a** (119 mg, 78%) was obtained as a white solid from 0.2 mmol **2**. ^{1}H NMR ($CDCl_3$, 300 M Hz): δ (ppm) 6.95 (d, J = 8.7 Hz, 2H, Ar-H), 6.63 (d, J = 8.7 Hz, 2H, Ar-H), 6.24 (s, 1H), 6.04 (d, J = 7.8 Hz, 1H), 5.92 (s, 1H), 5.62 (s, 1H), 5.40 (br, 1H), 4.79 (m, 1H), 4.29, 4.01 (dd, $J_A = J_B$ = 9.0 Hz, each 1H, 20-CH_2), 3.74 (s, 3H, -OCH_3), 3.70 (m, 4H), 3.66 (m, 4H), 3.10 (m, 1H), 3.03 (m, 1H) , 2.50 (m, 6H), 2.39 (m, 2H), 1.99 (m, 4H), 1.62 (m, 5H), 1.19 (s, 3H, -CH_3), 0.99 (s, 3H, -CH_3); ^{13}C NMR ($CDCl_3$, 75 M Hz): δ (ppm) 211.2, 204.4, 173.1, 171.2, 148.8, 144.4, 130.1, 121.5, 111.6, 96.5, 73.9, 72.9, 64.3, 59.3, 53.0, 52.8, 51.8, 50.2, 48.1, 40.9, 40.0, 37.9, 36.1, 35.3, 32.4, 30.2, 30.0, 29.5, 29.2, 22.7, 18.7; ESI-MS *m/z* 763.3 $[M+H]^+$; HR-MS (ESI, M+H) *m/z*: calcd for $C_{38}H_{49}Cl_2N_2O_{10}$: 763.2759, found 763.2754.

ent-6β,7β-dihydroxy-{14β-O-[4-(p-bis(2-chloroethyl)aminophenyl)formyloxy]}-1,15-

dioxo-7,20-epoxy-16-kaurene (**18b**).

According to the synthetic procedure from **1** to **16a** described above, **18b** (89 mg, 74%) was obtained as a white solid from 0.2 mmol **2**. ^{1}H NMR ($CDCl_3$, 300 M Hz): δ (ppm) 7.79 (d, J = 9.0 Hz, 2H, Ar-H), 6.63 (d, J = 9.0 Hz, 2H, Ar-H), 6.27 (s, 1H), 6.00 (s, 1H), 5.59 (s, 1H), 5.45 (d, J = 11.4 Hz, 1H), 4.35 (m, 1H), 4.03, 4.33 (dd, J_A = J_B = 10.2 Hz, each 1H), 3.78 (m, 5H), 3.63 (m, 4H), 3.27 (d, J = 9.6 Hz, 1H), 2.67 (m, 1H), 2.41 (m, 3H), 2.04 (m, 3H), 1.80 (m, 1H), 1.71 (m, 1H), 1.36 (m, 1H), 1.21 (s, 3H), 1.01 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 M Hz): δ (ppm) 211.3, 204.5, 165.9, 149.7, 149.1, 131.5, 121.5, 116.4, 110.6, 96.6, 76.1, 74.8, 72.6, 64.4, 60.8, 59.8, 52.8, 50.2, 48.1, 41.0, 39.5, 38.1, 35.3, 32.4, 30.0, 29.5, 22.8, 18.6; ESI-MS *m/z* 606.2 $[M+H]^+$ HR-MS (ESI, M+H) *m/z*: calcd for $C_{31}H_{38}Cl_2NO_7$: 606.2022, found 606.2021.

ent-6β,7β-dihydroxy-{14β-O-[4-(p-bis(2-chloroethyl)aminophenyl)butanoyloxy]}
-1,15-dioxo-7,20-epoxy-16-kaurene (**18c**).

According to the synthetic procedure from **1** to **16a** described above, **18c** (92 mg, 72%) was obtained as a white solid from 0.2 mmol **2**. ^{1}H NMR($CDCl_3$, 300 M Hz), δ (ppm): 7.01 (d, J = 8.7 Hz, 2H, Ar-H), 6.60 (d, J = 8.7 Hz, 2H, Ar-H), 6.25 (s, 1H), 5.85 (s, 1H), 5.60 (s, 1H), 5.40 (d, J = 11.7 Hz, 1H), 4.22 (s, 1H), 4.29, 4.03 (dd, J_A = J_B = 10.2 Hz, each 1H, 20-CH_2), 3.72 (m, 1H), 3.71 (m, 4H), 3.60 (m, 4H), 3.13 (d, J = 9.3 Hz, 1H), 2.60 (m, 1H), 2.50 (t, J = 7.2 Hz, 2H), 2.35 (m, 2H), 2.26 (t, J = 7.2 Hz, 2H), 1.96 (m, 2H), 1.84 (m, 2H), 1.73 (m, 2H), 1.68 (m, 1H), 1.25 (m, 2H), 1.19 (s, 3H, -CH_3), 1.00 (s, 3H, -CH_3); ^{13}C NMR ($CDCl_3$, 75 M Hz): δ (ppm) 211.1, 204.4, 171.4, 148.9, 143.9, 129.8, 129.3, 129.2, 121.5, 111.8, 96.5, 74.5, 72.6, 64.4, 60.7, 59.6, 53.1, 50.2, 48.0, 40.9, 40.0, 38.0, 35.3, 33.3, 32.4, 30.0, 29.5, 25.8, 22.7, 18.6; ESI-MS *m/z* 648.3 $[M+H]^+$; HR-MS (ESI, M+H) *m/z*: calcd for $C_{34}H_{44}Cl_2NO_7$: 648.2489, found 648.2496.

3. MTT assay *in vitro*

The MTT assay was performed in 96-well plates. K562 cells (CaEs-17, Bel-7402 and MGC-803 cells, L-O2, SW620, SW620/AD300, NCI-H460, NCI-H460/MX20) at the log phase of their growth cycle (5×10^4 cells/mL) were added to each well (100 μL/well), then treated in the presence or absence of test compounds, and incubated for 24 h at 37 ℃ in a humidified atmosphere of 5 % CO_2. After 72 h, 20 μL of MTT solution (5 mg/mL) per well was added to each cultured medium, which was incubated for another 4 h. Then, DMSO was added to each well (150 μL/well). After 10 min at room temperature, the OD of each well was measured on a Microplate Reader (BIO-RAD Instruments Inc NO.550) at the wavelength of 490 nm. In these experiments, the negative reference agent was 0.1 % DMSO, and Taxol was used as the positive reference with the concentration of 10 μg/mL.

4. Cell cycle study

Progression through the cell cycle was assessed by flow cytometry DNA determination with propidum iodide (PI). Bel-7402 cells were planted in 6-well plates (5.0×10^3 cells/well) and incubated at 37℃ for 24 h. Cells were incubated with tested compound at certain concentrations. Cells treated with the solvent (DMSO) were included. After 48 h treatment, cells were fixed with 70% ethanol, treated with RNase, and the stained with PI. Cellular DNA content for the cell cycle distribution analysis, was measured using a flow cytometer (FACS Calibur Becton-Dickinson).

5. Analysis of cellular apoptosis

This assay was applied to determine the capacity of inducing cell death of the

compounds under investigation. The Bel-7402 cells were incubated with the compounds as described above. Cells were then exposed to tested compound for 48 h and apoptosis was analyzed using Annexin V and propidium iodide double staining by flow cytometry according to the manufacturer's instructions in order to detect apoptotic cells. Three cell populations, including viable (annexin V-FITC, negative; PI, negative), early apoptotic (annexin V-FITC, positive; PI, negative), and late apoptotic cells or dead cells (annexin V-FITC, positive; PI, positive), were utilized.

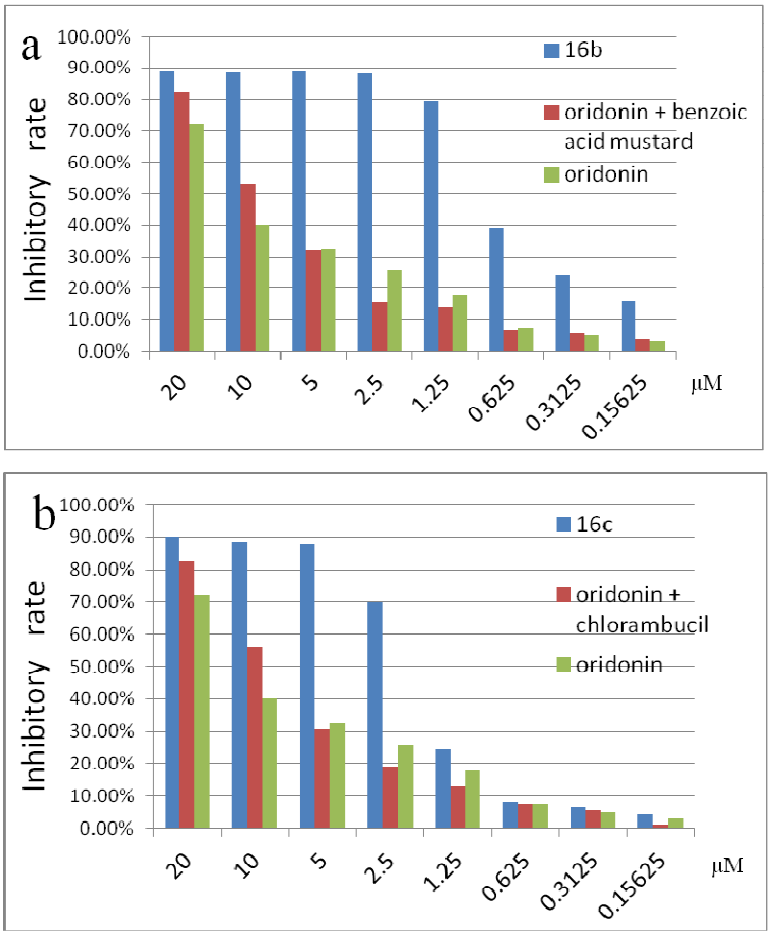
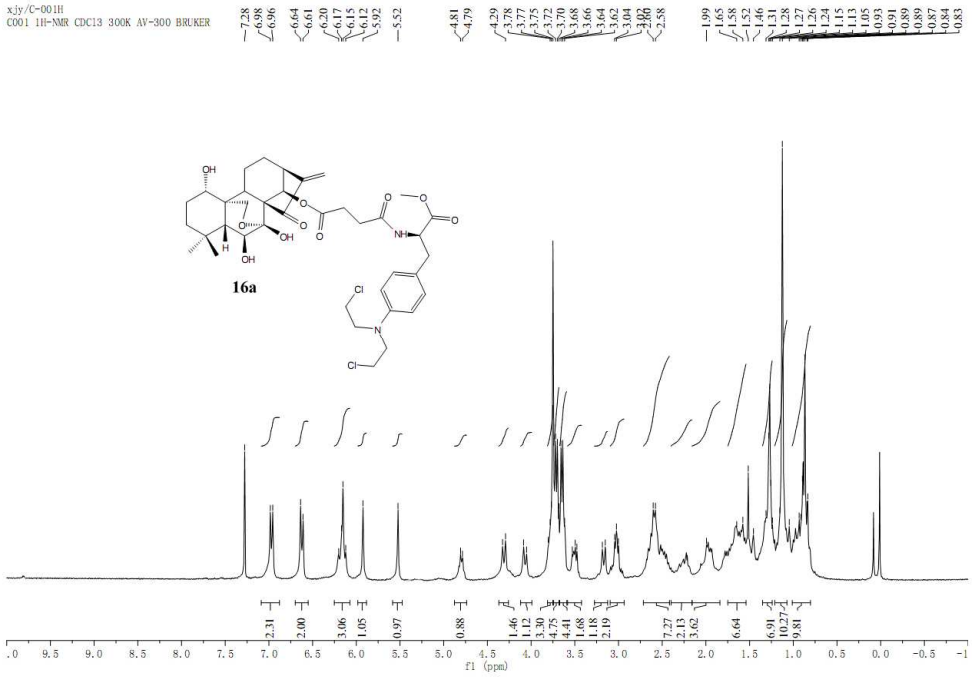


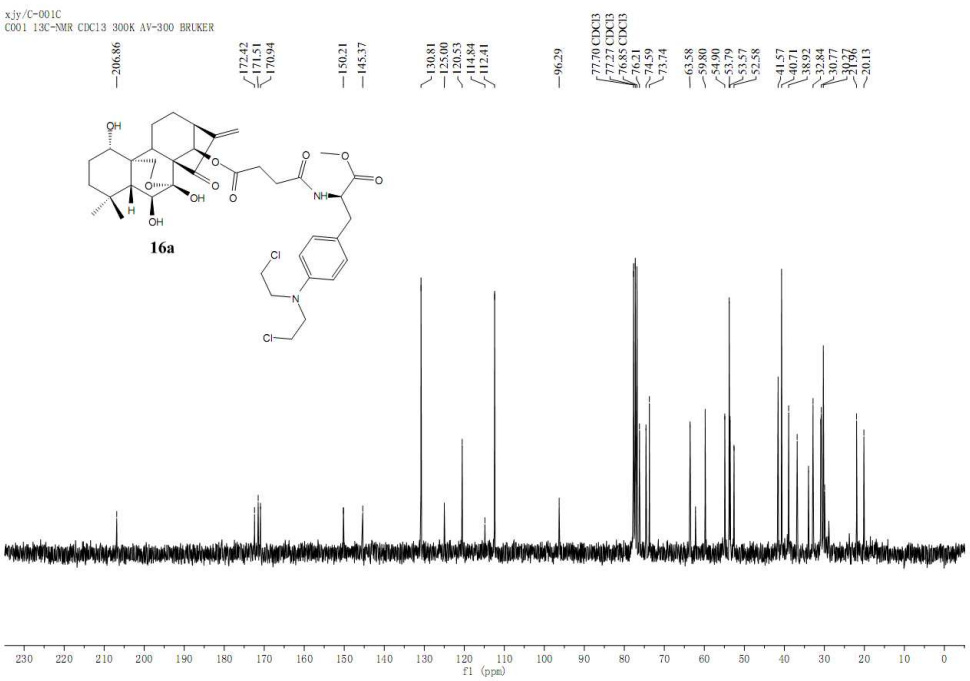
Figure 1. (a) Inhibitory rate of the conjugate (**16b**), oridonin and oridonin + benzoic acid mustard against the MCF-7 cells; (b) Inhibitory rate of the conjugate (**16c**), oridonin and oridonin + chlorambucil against the MCF-7 cells.

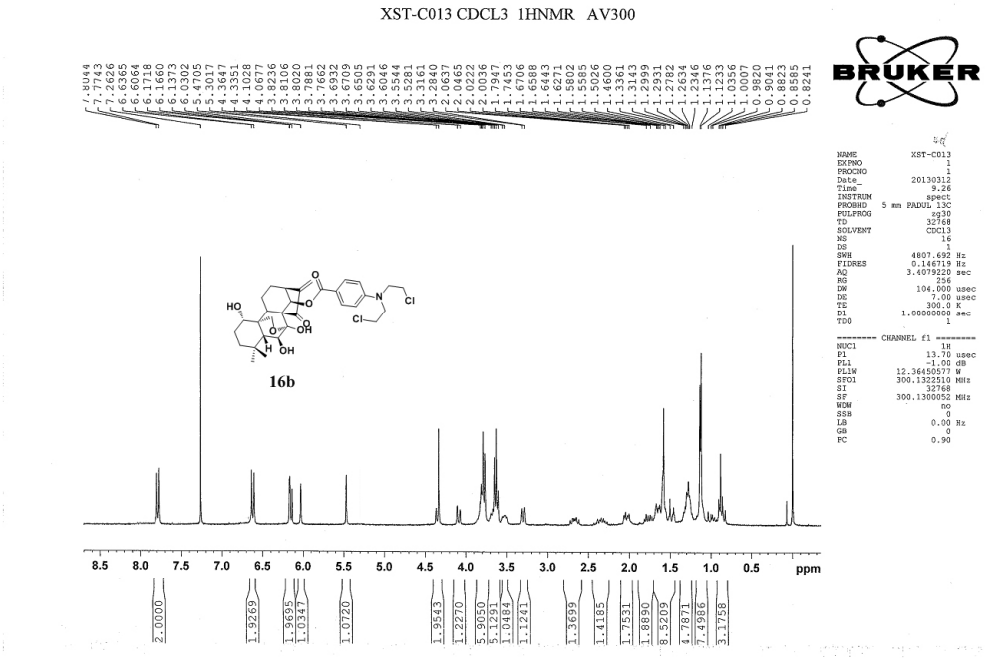
Table 1. The influence of cell cycle progression in Bel-7402 cells by compound **16b** at different concentrations

Group		G_1(%)	S(%)	G_2(%)
Negative control		39.66	40.82	19.52
Compound **16b**	0.125μM	45.65	41.33	13.02
	0.25μM	45.25	44.77	9.98
	0.5μM	51.98	43.56	4.46

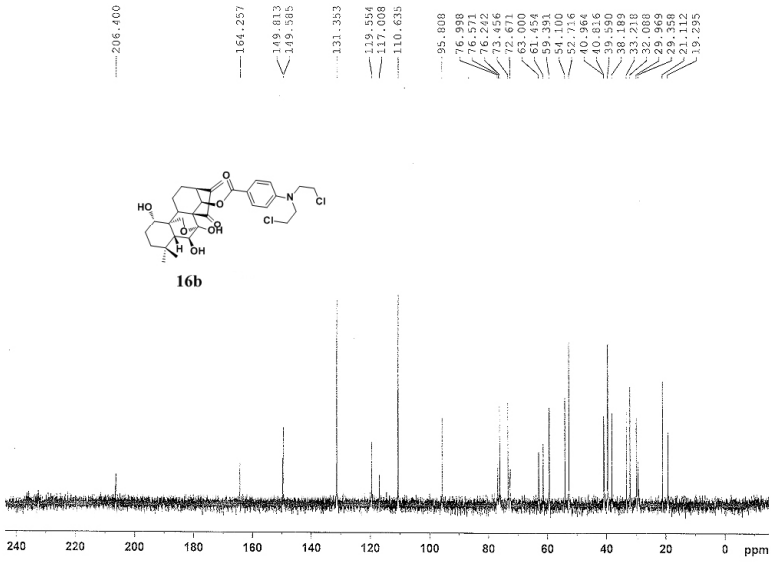
^{1}H NMR and ^{13}C NMR Spectrum of Target Compounds.




xjy/C-001C
C001 13C-NMR CDCl3 300K AV-300 BRUKER
206.86
172.42
171.51
170.94
150.21
145.37
130.81
125.00
120.53
114.84
112.41
96.29
77.70 CDCl3
77.27 CDCl3
76.85 CDCl3
76.21
74.59
73.74
63.58
59.80
54.00
53.79
53.57
52.58
41.57
40.71
38.92
32.84
30.77
30.27
20.13
16a
f1 (ppm)


XST-C013 CDCL3 1HNMR AV300
BRUKER
NAME XST-C013
EXPNO 1
PROCNO 1
Date_ 20130312
Time 9.26
INSTRUM spect
PROBHD 5 mm PADUL 13C
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 16
DS 2
SWH 4807.692 Hz
FIDRES 0.146719 Hz
AQ 3.4079220 sec
RG 256
DW 104.000 usec
DE 7.00 usec
TE 300.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 13.70 usec
PL1 -1.00 dB
PLW 12.36450577 W
SFO1 300.1322510 MHz
SI 32768
SF 300.1300052 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 0.90
16b
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
2.0000
1.9269
1.9695
1.0347
1.0720
1.9543
1.2270
5.9050
5.1291
1.0484
1.1241
1.3699
1.4185
1.7531
1.8990
8.5209
4.7871
7.4986
3.1758

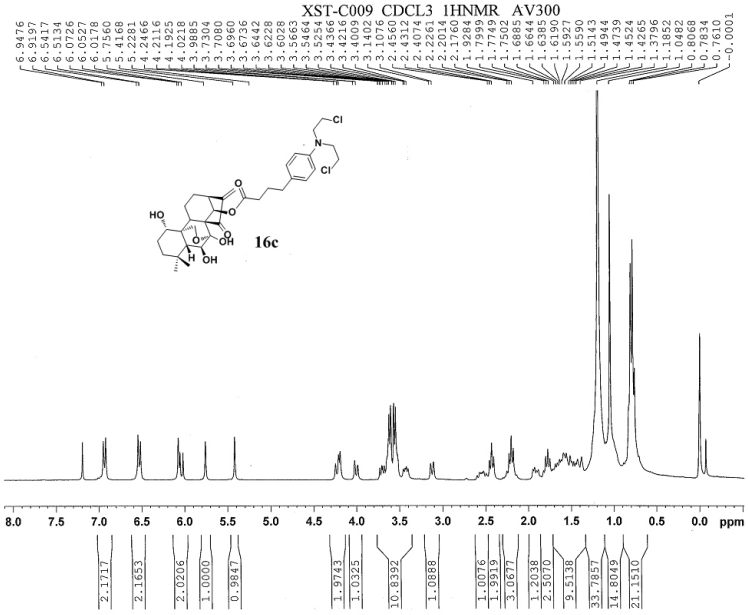
XST-C013 C13-NMR CDCl3 303K AV-300



16b

```
NAME                 CD13
EXPNO                   1
PROCNO                  1
Date_           20130830
Time                10.40
INSTRUM             spect
PROBHD   5 mm PADUL 13C
PULPROG             zgdc
TD                 65536
SOLVENT            CDCl3
NS                    64
DS                     4
SWH             19531.250 Hz
FIDRES          0.298023 Hz
AQ              1.6777716 sec
RG                  50.8
DW                 25.600 usec
DE                  7.00 usec
TE                300.0 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  13C
P1                  11.80 usec
PL1                  1.00 dB
PL1W          26.73651505 W
SFO1          75.4764278 MHz

======== CHANNEL f2 ========
CPDPRG2           waltz16
NUC2                  1H
PCPD2              80.00 usec
PL2                -1.00 dB
PL12               14.30 dB
PL2W          12.36450577 W
PL12W          0.36490241 W
SFO2          300.1312005 MHz
SI                 32768
SF            75.4677867 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

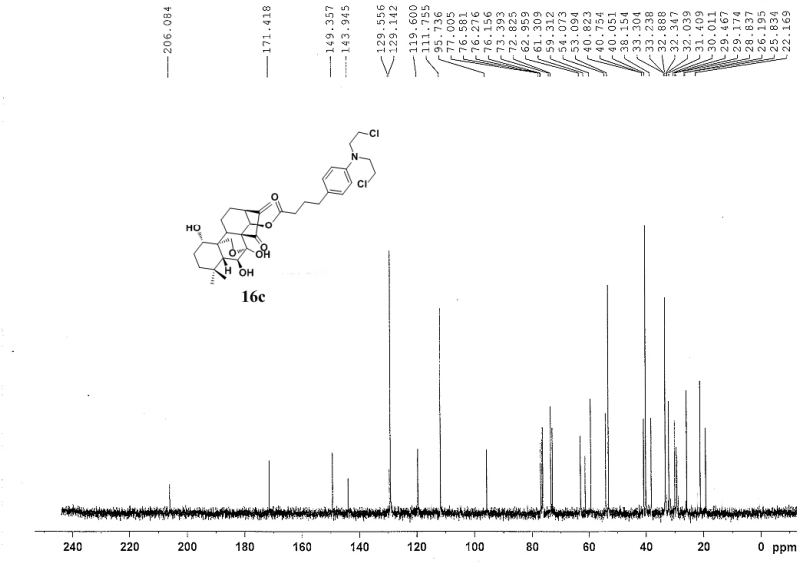
XST-C009 CDCL3 1HNMR AV300



16c

```
NAME              XST-C009
EXPNO                    1
PROCNO                   1
Date_            20130226
Time                13.55
INSTRUM             spect
PROBHD   5 mm PADUL 13C
PULPROG             zg30
TD                 32768
SOLVENT            CDCl3
NS                    16
DS                     1
SWH              4807.692 Hz
FIDRES          0.146719 Hz
AQ              3.4078220 sec
RG                   256
DW                104.000 usec
DE                  7.00 usec
TE                300.0 K
D1              1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                   1H
P1                  13.70 usec
PL1                -1.00 dB
PL1W          12.36450577 W
SFO1          300.1322510 MHz
SI                 32768
SF            300.1300261 MHz
WDW                   no
SSB                    0
LB                  0.00 Hz
GB                     0
PC                  0.90
```

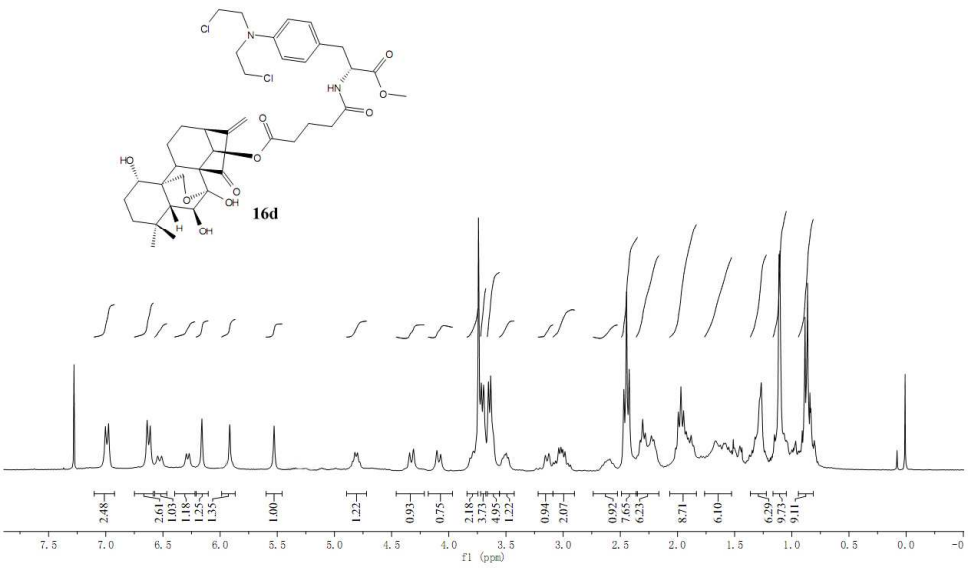
XST-C009 C13-NMR DMSO 303K AV-300



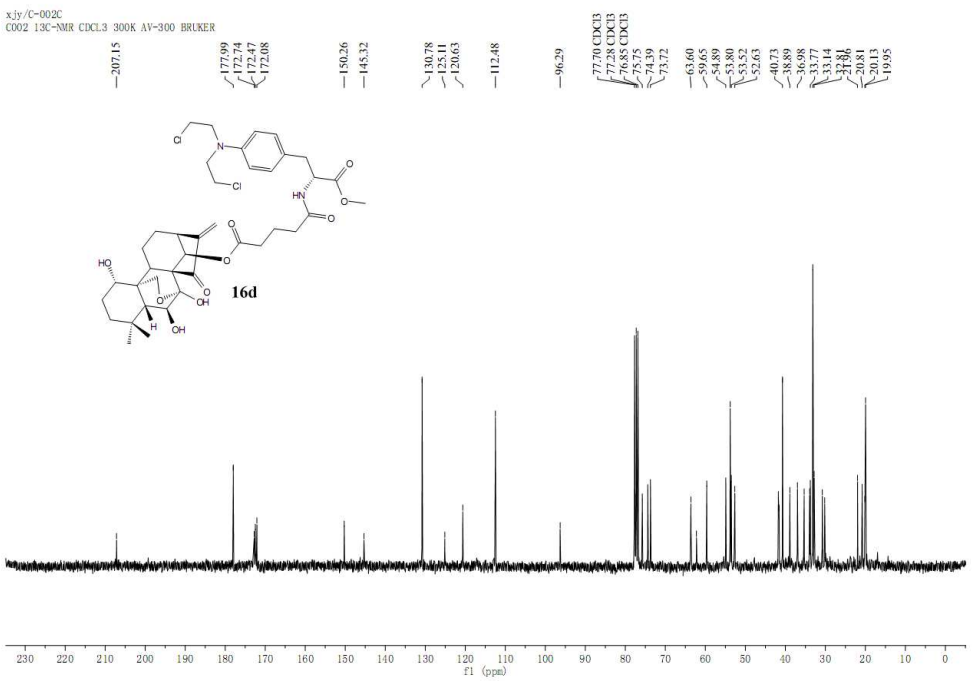
16c

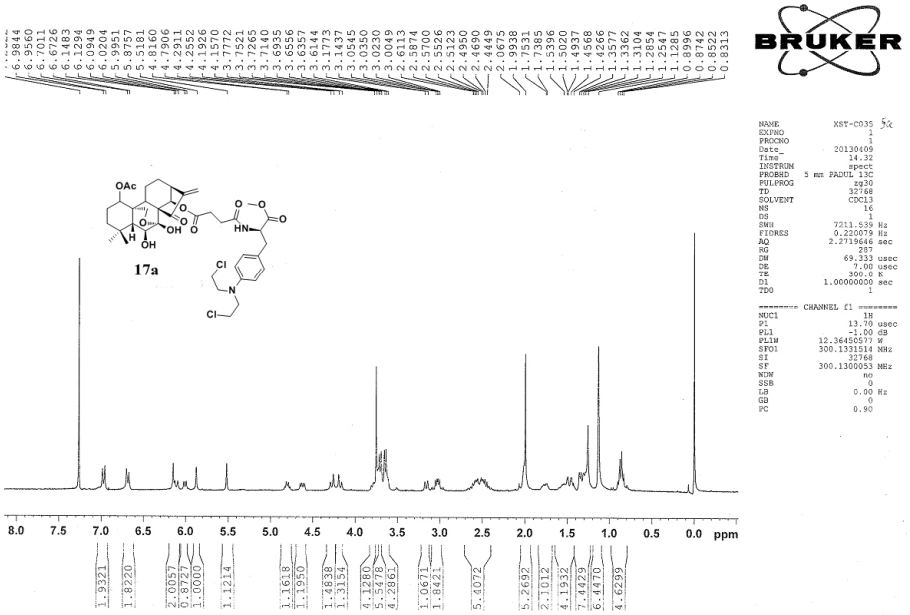
NAME	XST-C009
EXPNO	1
PROCNO	1
Date_	20130712
Time	9.36
INSTRUM	spect
PROBHD	5 mm PADUL 13C
PULPROG	zgdc
TD	65536
SOLVENT	CDCl3
NS	140
DS	4
SWH	19531.250 Hz
FIDRES	0.298023 Hz
AQ	1.6777716 sec
RG	50.6
DW	25.600 usec
DE	7.00 usec
TE	300.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	11.80 usec
PL1	1.00 dB
PL1W	26.73651505 W
SFO1	75.4764278 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-1.00 dB
PL12	14.30 dB
PL2W	12.36450577 W
PL12W	0.36490241 W
SFO2	300.1312005 MHz
SI	32768
SF	75.4677867 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

xjy/C-002H
C002 1H-NMR CDCL3 300K AV-300 BRUKER

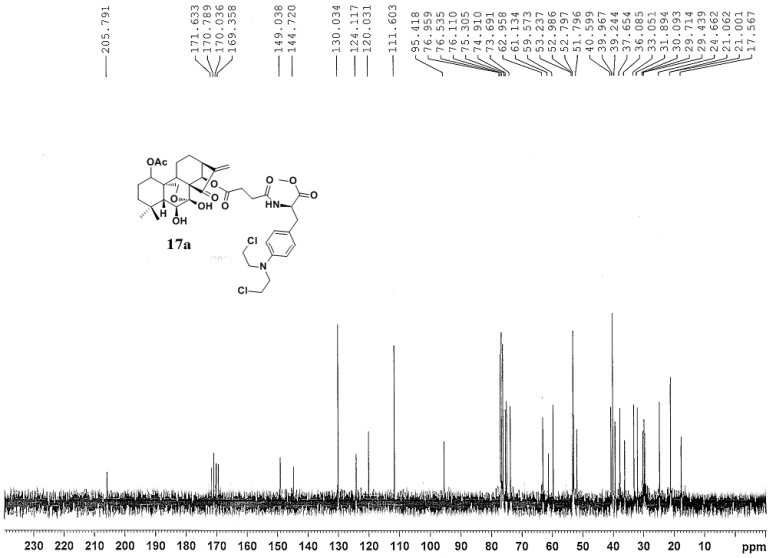


16d

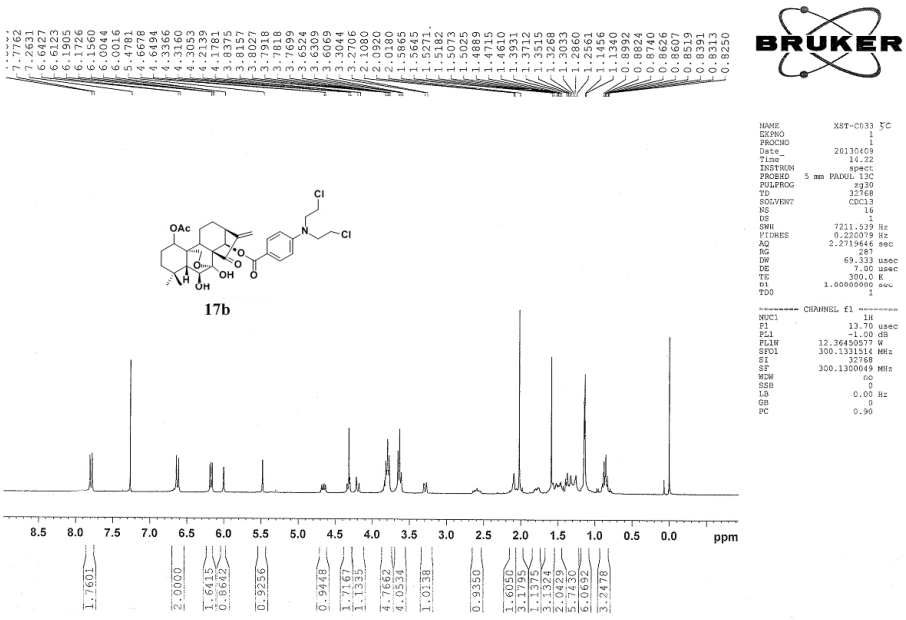

xjy/C-002C
C002 13C-NMR CDCL3 300K AV-300 BRUKER
16d
f1 (ppm)


BRUKER
17a
ppm

XST-C035A C13-NMR DMSO 303K AV-300

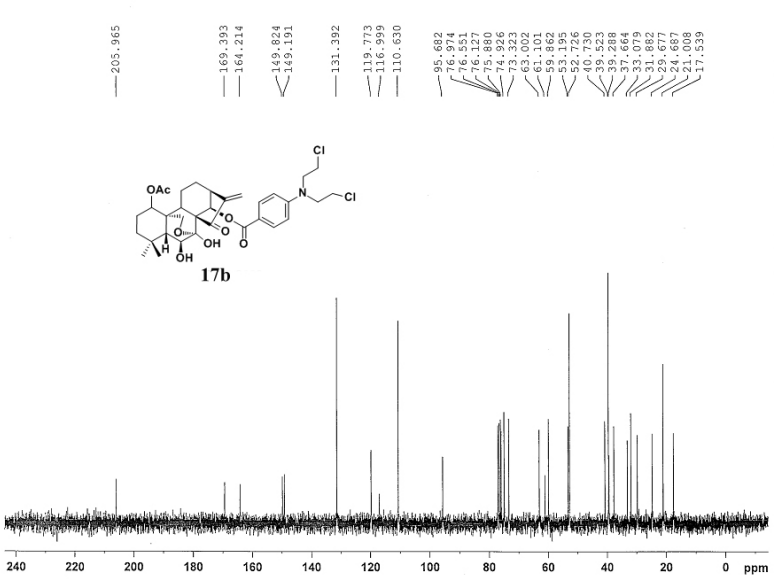



```
NAME                XST-C035A
EXPNO                       1
PROCNO                      1
Date_                20130909
Time                    11.45
INSTRUM                 spect
PROBHD  5 mm PADUL 13C
PULPROG                 zgdc
TD                      65536
SOLVENT                 CDCl3
NS                        160
DS                          4
SWH                 19531.250 Hz
FIDRES              0.298023 Hz
AQ                  1.6777716 sec
RG                       50.8
DW                     25.600 usec
DE                      7.00 usec
TE                     300.0 K
D1                  2.00000000 sec
D11                 0.03000000 sec
TD0                         1

======== CHANNEL f1 ========
NUC1                       13C
P1                     11.80 usec
PL1                     1.00 dB
PL1W               26.73651505 W
SFO1                75.4764278 MHz

======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                        1H
PCPD2                  80.00 usec
PL2                    -1.00 dB
PL12                   14.30 dB
PL2W               12.36450577 W
PL12W               0.36490241 W
SFO2               300.1312005 MHz
SI                       32768
SF                  75.4677867 MHz
WDW                         EM
SSB                          0
LB                      1.00 Hz
GB                           0
PC                        1.40
```



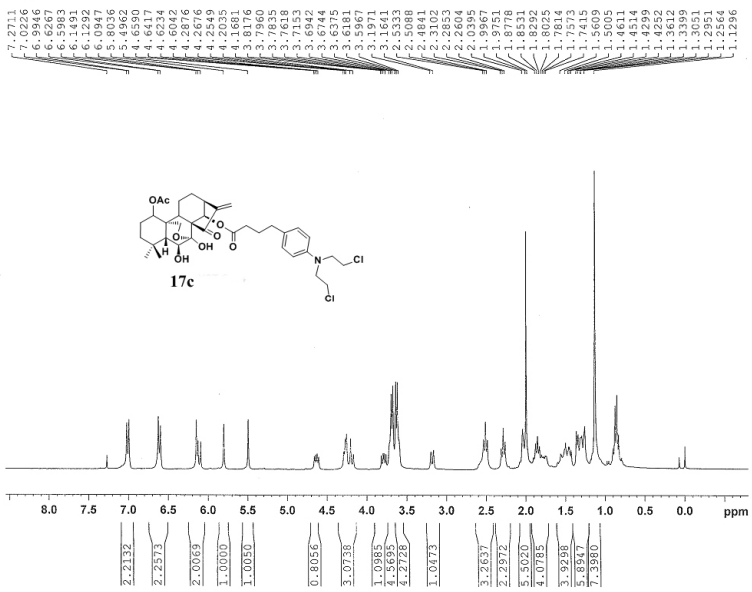

```
NAME                XST-C033
EXPNO                       1
PROCNO                      1
Date_                20130409
Time                    14.22
INSTRUM                 spect
PROBHD  5 mm PADUL 13C
PULPROG                 zg30
TD                      32768
SOLVENT                 CDCl3
NS                         16
DS                          1
SWH                 7211.539 Hz
FIDRES              0.220079 Hz
AQ                  2.2719646 sec
RG                         287
DW                     69.333 usec
DE                      7.00 usec
TE                     300.0 K
D1                  1.00000000 sec
TD0                         1

======== CHANNEL f1 ========
NUC1                        1H
P1                     13.70 usec
PL1                    -1.00 dB
PL1W               12.36450577 W
SFO1               300.1331514 MHz
SI                       32768
SF                 300.1300049 MHz
WDW                         no
SSB                          0
LB                      0.00 Hz
GB                           0
PC                        0.90
```

XST-C033 C13-NMR DMSO 303K AV-300



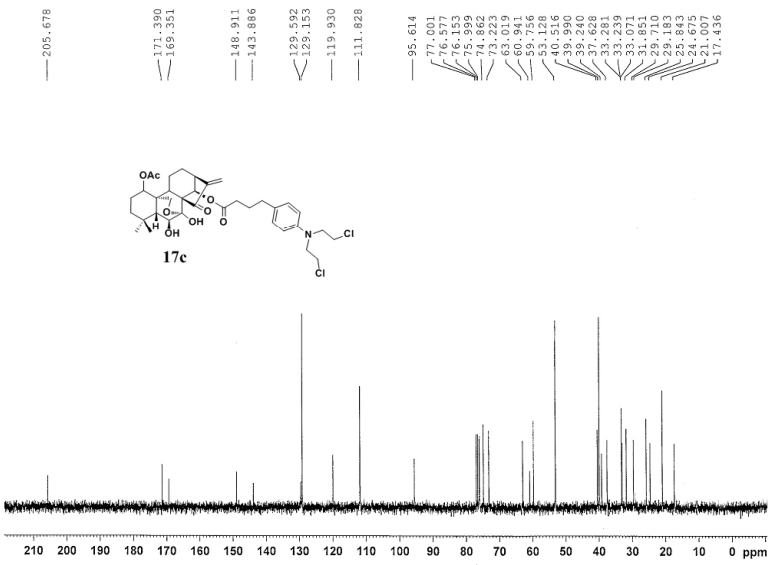

```
NAME                XST-C035 5C
EXPNO                        1
PROCNO                       1
Date_                 20130909
Time                     11.36
INSTRUM                  spect
PROBHD   5 mm PADUL 13C
PULPROG                  zgdc
TD                      65536
SOLVENT                  CDCl3
NS                          85
DS                           4
SWH                 19531.250 Hz
FIDRES              0.298023 Hz
AQ                  1.6777716 sec
RG                        50.8
DW                      25.600 usec
DE                        7.00 usec
TE                       300.0 K
D1                  2.00000000 sec
D11                 0.03000000 sec
TD0                          1

======== CHANNEL f1 ========
NUC1                       13C
P1                       11.80 usec
PL1                       1.00 dB
PL1W                26.73651505 W
SFO1                 75.4764278 MHz

======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                        1H
PCPD2                    80.00 usec
PL2                      -1.00 dB
PL12                     14.30 dB
PL2W                12.36450577 W
PL12W               0.36490241 W
SFO2                300.1312005 MHz
SI                       32768
SF                   75.4677867 MHz
WDW                         EM
SSB                          0
LB                        1.00 Hz
GB                           0
PC                        1.40
```

XST-C034 CDCL3 1HNMR AV300




```
NAME                XST-C034 1H
EXPNO                        1
PROCNO                       1
Date_                 20130702
Time                     9.24
INSTRUM                  spect
PROBHD   5 mm PADUL 13C
PULPROG                  zg30
TD                      32768
SOLVENT                  CDCl3
NS                          16
DS                           1
SWH                  7211.539 Hz
FIDRES               0.220079 Hz
AQ                   2.2719646 sec
RG                          57
DW                      69.333 usec
DE                        7.00 usec
TE                       300.0 K
D1                  1.00000000 sec
TD0                          1

======== CHANNEL f1 ========
NUC1                        1H
P1                       13.70 usec
PL1                      -1.00 dB
PL1W                12.36450577 W
SFO1                300.1331314 MHz
SI                       32768
SF                  300.1300024 MHz
WDW                         no
SSB                          0
LB                        0.00 Hz
GB                           0
PC                        0.90
```

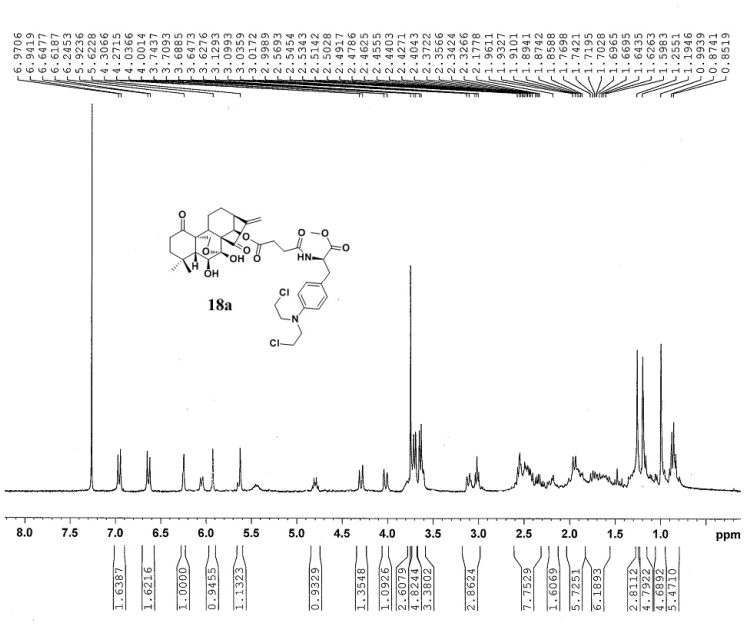
XST-C034 C13-NMR CDCl3 303K AV-300



BRUKER

NAME	XST-C034
EXPNO	1
PROCNO	1
Date_	20130702
Time	9.28
INSTRUM	spect
PROBHD	5 mm PADUL 13C
PULPROG	zgdc
TD	65536
SOLVENT	CDCl3
NS	48
DS	4
SWH	19531.250 Hz
FIDRES	0.298023 Hz
AQ	1.6777716 sec
RG	64
DW	25.600 usec
DE	7.00 usec
TE	300.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	11.80 usec
PL1	1.00 dB
PL1W	26.73651505 W
SFO1	75.4764278 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-1.00 dB
PL12	14.30 dB
PL2W	12.36450577 W
PL12W	0.36490241 W
SFO2	300.1312005 MHz
SI	32768
SF	75.4677867 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

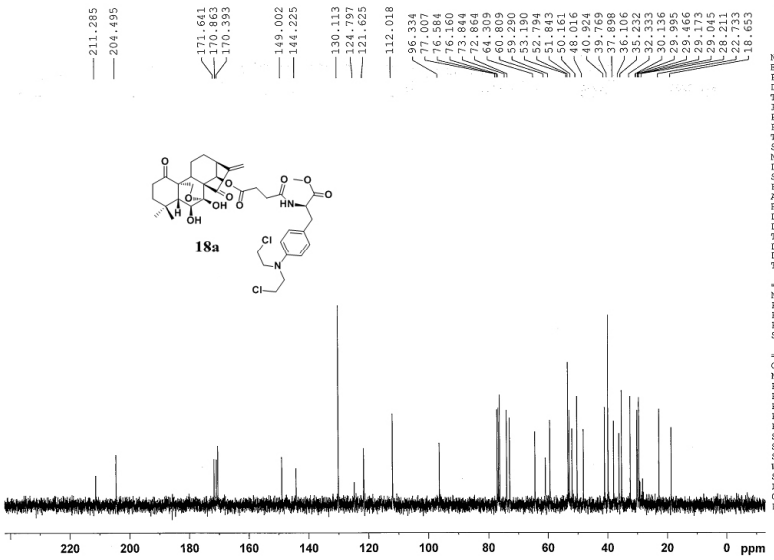
XST-C023 CDCL3 1HNMR AV300



BRUKER

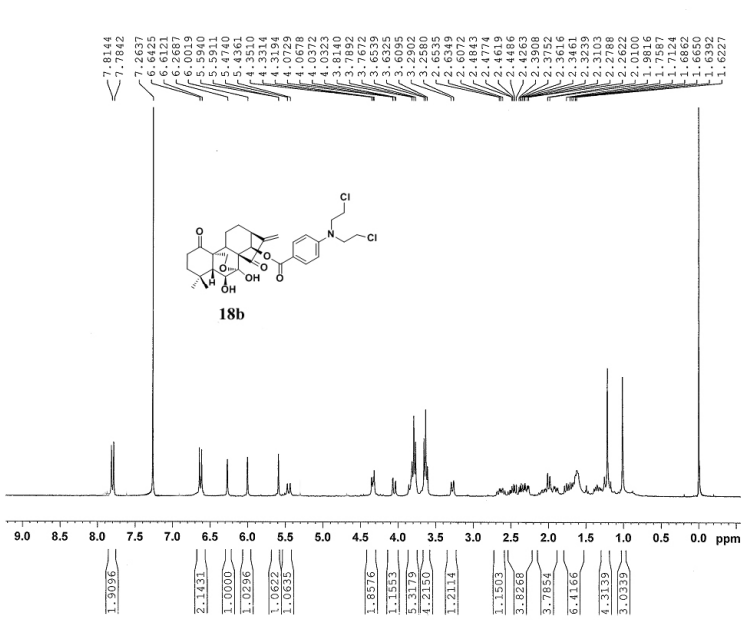
NAME	XST-C023
EXPNO	1
PROCNO	1
Date_	20130329
Time	9.15
INSTRUM	spect
PROBHD	5 mm PADUL 13C
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	16
DS	4
SWH	7211.539 Hz
FIDRES	0.220079 Hz
AQ	2.2719646 sec
RG	203
DW	69.333 usec
DE	7.00 usec
TE	300.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	13.70 usec
PL1	-1.00 dB
PL1W	12.36450577 W
SFO1	300.1331514 MHz
SI	32768
SF	300.1300050 MHz
WDW	no
SSB	0
LB	0.00 Hz
GB	0
PC	0.90

XST-C023A C13-NMR CDCl3 303K AV-300



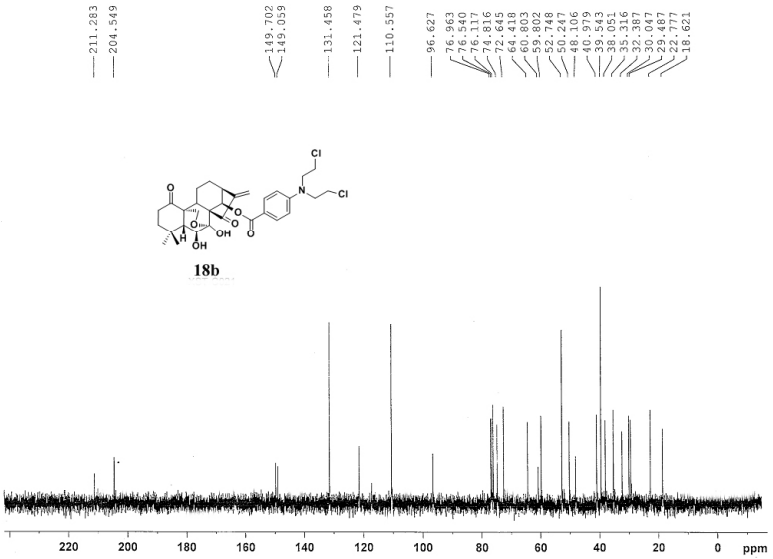

```
NAME                XST-C023A
EXPNO                       1
PROCNO                      1
Date_              20131128
Time                    10.03
INSTRUM                 spect
PROBHD     5 mm PADUL 13C
PULPROG                 zgdc
TD                     65536
SOLVENT                CDCl3
NS                         86
DS                          4
SWH                19531.250 Hz
FIDRES             0.298023 Hz
AQ                 1.67777116 sec
RG                       50.8
DW                     25.600 usec
DE                      7.00 usec
TE                     300.0 K
D1                 2.00000000 sec
D11                0.03000000 sec
TD0                         1

======== CHANNEL f1 ========
NUC1                      13C
P1                      11.80 usec
PL1                      1.00 dB
PL1W               26.73651505 W
SFO1               75.4764278 MHz

======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                       1H
PCPD2                   80.00 usec
PL2                     -1.00 dB
PL12                    14.30 dB
PL2W               12.36450577 W
PL12W               0.36490241 W
SFO2               300.1312005 MHz
SI                        32768
SF                 75.4677867 MHz
WDW                        EM
SSB                         0
LB                       1.00 Hz
GB                          0
PC                       1.40
```

XST-C021 CDCL3 1HNMR AV300



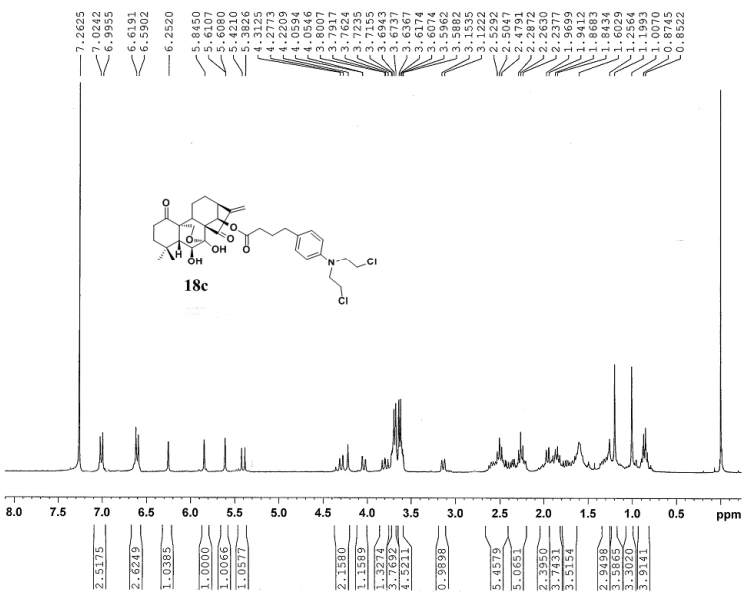

```
NAME                XST-C021
EXPNO                       1
PROCNO                      1
Date_              20130329
Time                    9.04
INSTRUM                 spect
PROBHD     5 mm PADUL 13C
PULPROG                 zg30
TD                     32768
SOLVENT                CDCl3
NS                         16
DS                          1
SWH                7211.539 Hz
FIDRES             0.220079 Hz
AQ                 2.2719646 sec
RG                        287
DW                     69.333 usec
DE                      7.00 usec
TE                     300.0 K
D1                 1.00000000 sec
TD0                         1

======== CHANNEL f1 ========
NUC1                       1H
P1                      13.70 usec
PL1                     -1.00 dB
PL1W               12.36450577 W
SFO1               300.1331514 MHz
SI                        32768
SF                 300.1300048 MHz
WDW                        no
SSB                         0
LB                       0.00 Hz
GB                          0
PC                       0.90
```

XST-C021 C13-NMR DMSO 303K AV-300

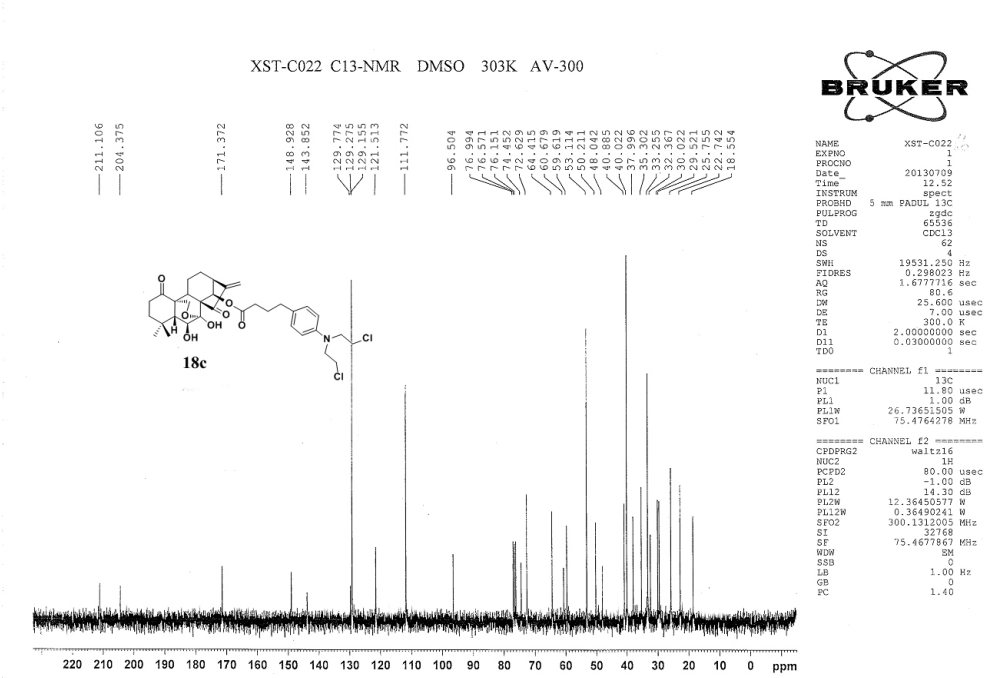



```
NAME                XST-C021
EXPNO                      1
PROCNO                     1
Date_               20130709
Time                   13.01
INSTRUM                spect
PROBHD  5 mm PADUL 13C
PULPROG                zgdc
TD                    65536
SOLVENT                CDCl3
NS                       105
DS                         4
SWH               19531.250 Hz
FIDRES             0.298023 Hz
AQ                 1.6777716 sec
RG                        57
DW                   25.600 usec
DE                    7.00 usec
TE                   300.0 K
D1                2.00000000 sec
D11               0.03000000 sec
TD0                        1

======== CHANNEL f1 ========
NUC1                      13C
P1                      11.80 usec
PL1                      1.00 dB
PL1W               26.73651505 W
SFO1                75.4764278 MHz

======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                       1H
PCPD2                   80.00 usec
PL2                     -1.00 dB
PL12                    14.30 dB
PL2W               12.36450577 W
PL12W               0.36490241 W
SFO2               300.1312005 MHz
SI                       32768
SF                  75.4677867 MHz
WDW                         EM
SSB                          0
LB                        1.00 Hz
GB                           0
PC                        1.40
```

XST-C022 CDCL3 1HNMR AV300




```
NAME                XST-C022
EXPNO                      1
PROCNO                     1
Date_               20130329
Time                    9.09
INSTRUM                spect
PROBHD  5 mm PADUL 13C
PULPROG                zg30
TD                    32768
SOLVENT                CDCl3
NS                        16
DS                         1
SWH                7211.539 Hz
FIDRES             0.220079 Hz
AQ                 2.2719646 sec
RG                       203
DW                  69.333 usec
DE                    7.00 usec
TE                   300.0 K
D1                1.00000000 sec
TD0                        1

======== CHANNEL f1 ========
NUC1                       1H
P1                      13.70 usec
PL1                     -1.00 dB
PL1W               12.36450577 W
SFO1               300.1331514 MHz
SI                       32768
SF                 300.1300052 MHz
WDW                         no
SSB                          0
LB                        0.00 Hz
GB                           0
PC                        0.90
```



References:

1. Wang, L.; Li, D. H.; Xu, S. T.; Yao, H. Q.; Zhang, Y. H.; Jiang, J. Y.; Xu, J. Y. The conversion of oridonin to spirolactone-type or enmein-type diterpenoid: synthesis and biological evaluation of *ent*-6,7-*seco*-oridonin derivatives as novel potential anticancer agents. *Eur. J. Med. Chem*. **2012**, *52*, 242-250.
2. Luo, W.; Zhao, Y. M.; Wang, Y. X.; Xie, S. Q.; Zhao, J.; Wang, C. J. Synthesis and antitumor activity of benzoic nitrogen mustard derivatives. *Acta Pharm. Sinica* **2007**, *42*, 1327-1329. (for benzoic acid mustard)
3. Morris, A. D.; Atassi, G.; Guilbaud, N.; Cordi. A. A. The synthesis of novel melphalan derivatives as potential antineoplastic agents. *Eur. J. Med. Chem*. **1997**, *32*, 343-349.
4. Scutaru, A. M.; Wenzel, M.; Gust, R.; Bivalent bendamustine and melphalan derivatives as anticancer agents. *Eur. J. Med. Chem*. **2011**, *46*, 1604-1615. (for compound **7**)

5. Bergel, F.; Stock, J. A. Cyto-active amino-acids and peptides. Part VIII. N^{α}-acyl, amide, ester and peptide derivatives of melphalan. *J. Chem. Soc.* **1960**, 3658-3669. (for compound **8**)