Supporting Information

Development of nordihydroguaiaretic acid derivatives as potential Multidrug-Resistant selective agents for cancer treatment

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General Information

All reactions were carried out in oven-dried glassware (120 °C) under an atmosphere of nitrogen, unless as indicated otherwise. Acetone, dichloromethane, 1,4-dioxane, ethyl acetate, hexane, and tetrahydrofuran were purchased Mallinckrodt Chemical Co. Acetone was dried with 4Å molecular sieves and distilled. Dichloromethane, ethyl acetate, and hexane were dried and distilled from CaH_2. 1,4-Dioxane and tetrahydrofuran were dried by distillation from sodium and benzophenone under an atmosphere of nitrogen. Nordihydroguaiaretic acid was purchased from Fluka Chemical Co. 4-(2-Chloroethyl)morpholine hydrochloride, 1-(2-chloroethyl)piperidine monohydrochloride, 1-(2-chloroethyl)pyrrolidine hydrochloride, *N,N'*-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), and potassium carbonate were purchased from Aldrich Chemical Co.

Melting point was obtained with a Buchi 535 melting point apparatus. Analytical thin layer chromatography (TLC) was performed on precoated plates (silica gel 60 F–254), purchased from Merck Inc. Gas chromatographic analyses were performed on a Hewlett–Paclard 5890 Series II instrument equipped with a 25-m crosslinked methyl silicone gum capillary column (0.32 mm i.d.). Nitrogen gas was used as a carrier gas and the flow rate was kept constant at 14.0 mL/min. The retention time t_R was measured under the following conditions: injector temperature 260 °C, isothermal column temperature 280 °C. Gas chromatography and low resolution mass spectral analyses were performed on an Agilent Technology 6890N Network GC System equipped with an Agilent 5973 Network Mass Selective Detector and capillary HP–1 column. Purification by gravity column chromatography was carried out by use of Merck Reagents Silica Gel 60 (particle size 0.063–0.200 mm, 70–230 mesh ASTM). Purity of all compounds was >99.5%, as checked by HPLC or GC.

Ultraviolet (UV) spectra were measured on Hitachi U3300 UV/VIS spectrophotometer. Infrared (IR) spectra were measured on Jasco FT–IR-5300 Fourier transform infrared spectrometer. The wave numbers reported are referenced to the polystyrene 1601 cm^{-1} absorption. Absorption intensities are recorded by the following abbreviations: s, strong; m, medium; w, weak. The fluorescent intensity was measured on Hitach F-4500 Florescence Spectrophotometer. Proton NMR spectra were obtained on a Varian Mercury-400 (400 MHz) spectrometer by use of chloroform-d as the solvent and sodium 3-(trimethylsilyl)propionate as internal standard. Carbon-13 NMR spectra were obtain on a Varian Mercury-400 (100 MHz) spectrometer by use of chloroform-d or D_2O as the solvent. Carbon-13 chemical shifts are referenced to the center of the $CDCl_3$ triplet (δ 77.0 ppm). Multiplicities are recorded by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; *J*, coupling constant (hertz). Hight-resolution mass spectra were obtain by means of a JEOL JMS-HX110 mass spectrometer. Electrospray ionization mass spectrometry (ESI–MS) analyses were performed on a quadrupole ion trap mass analyzer fitted with an electrospray ionization source of Finnigan LCQ, Finnigan MAT.

General Procedure of NDGA Derivatives Synthesis.

In brief, to a solution containing NDGA (**1**, 0.5g, 1.0 eq.) in acetonitrile (40 mL) was added K_2CO_3 (10 equiv) and the suitable benzoyl or sulfonyl chloride (6.0 equiv). After the solution was stirred at 80 °C for overnight, it was cooled down to room temperature. Inorganic solids were filtered off and the filtrate was concentrated under reduced pressure to afford the residue. The crude residue was extracted with ethyl acetate, and washed with brine. Each Product was purified by column chromatography or MPLC to afford the yield of 67–78%.

Standard Procedure for the Syntheses of NDGA Derivatives of Hydrochloride Salt.

To a solution containing NDGA (**1**, 1.0 equiv) and potassium carbonate (6.0–10.0 equiv) in acetone was added a nitrogen-containing organic hydrochloride (5.0 equiv). After the solution was heated at reflux for 24 h, it was quenched with water (20 mL). The solution was extracted with ether (3 × 50 mL) and the combined organic layers were washed with saturated brine, dried over $MgSO_{4(s)}$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (10% methanol in dichloromethane as eluant) and the desired fraction was concentrated. The resultant was dissolved in acetone (250 mL) and then bubbled with excess $HCl_{(g)}$. The precipitates were dissolved in water and re-precipitated twice by use of acetone at room temperature to give the desired NDGA derivative with purity >99.5%, as checked by HPLC.

((2*R*,3*S*)-2,3-Dimethylbutane-1,4-diyl)bis(benzene-4,1,2-triyl) tetrabenzoate (5a). The Standard Procedure was followed by use of NDGA (1.43 g, 4.73 mmol, 1.0 equiv), potassium carbonate (6.53 g, 47.3 mmol, 10.0 equiv), acetone (150 mL) and benzoyl chloride (3.33 g, 23.7 mmol, 5.0 equiv) to give pure **5a** (2.55 g, 3.54 mmol) as yellow gum in 75% yield: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.04 (d, J = 6.0 Hz, 8 H, 8 × ArH), 7.50 (dd, J = 7.0, 7.0 Hz 4 H, 4 × ArH),

7.36 (dd, *J* = 7.0, 7.0 Hz, 8 H, 8 × ArH), 7.30 (d, J = 2H, 2 × ArH), 7.19 (s, 2 H, ArH), 7.13 (d, *J* = 8.0 Hz, 2 H, 2 × ArH), 2.72–2.75 (m, 2 H, 2 × ArCH), 2.20 (dd, *J* = 13.6, 9.6 Hz, 2 H, 2 × ArCH), 1.81–1.83 (m, 2 H, 2 × CH), 0.936 (d, *J* = 7.0 Hz, 6 H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.37, 164.29, 142.16, 140.66, 140.40, 133.49, 130.13, 130.12, 128.96, 128.95, 128.41, 127.20, 123.95, 123.10, 39.44, 38.95, 16.19; HRMS (ESI) calcd for $C_{46}H_{69}O_8$: 718.2638, found 718.2565.

((2*R*,3*S*)-2,3-Dimethylbutane-1,4-diyl)bis(benzene-4,1,2-triyl) tetrakis (4-methoxy benzoate) (5b). The Standard Procedure was followed by use of NDGA (1.43 g, 4.73 mmol, 1.0 equiv), potassium carbonate (6.53 g, 47.3 mmol, 10.0 equiv), acetone (150 mL) and 4-methoxybenzoyl chloride (4.04 g, 23.7 mmol, 5.0 equiv) to give pure **5b** (2.96 g, 3.54 mmol) as yellow gum in 72% yield: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.98 (dd, *J* = 6.5, 2.5 Hz, 8 H, 8 × ArH), 7.27 (d, *J* = 7.0 Hz, 2 H, 2 × ArH), 7.15 (s, 2 H, 2 × ArH), 7.08 (d, *J* = 2 Hz, 2 H, 2 × ArH), 6.81 (dd, *J* = 7.5, 1.5 Hz, 8 H, 8 × ArH), 3.83 (s, 12 H, 4 × OCH_3), 2.81 (dd, *J* = 13.5, 4 Hz, 2 H, 2 × ArCH), 2.42 (dd, *J* = 10, 3.0 Hz, 2 H, 2 × ArCH), 1.88–1.89 (m, 2 H, 2 × CH), 0.93 (d, *J* = 5.2 Hz, 6 H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.11, 164.03, 163.75, 163.74, 142.31, 140.56, 140.43, 132.28, 132.26, 127.00, 124.00, 123.12, 121.35, 121.33, 113.68, 55.42, 55.40, 39.48, 38.96, 16.18; HRMS (ESI) calcd for $C_{50}H_{47}O_{12}$: 839.3062, found 839.2990.

((2*R*,3*S*)-2,3-Dimethylbutane-1,4-diyl)bis(benzene-4,1,2-triyl) tetrakis (4-chloro benzoate) (5c). The Standard Procedure was followed by use of NDGA (1.43 g, 4.73 mmol, 1.0 equiv), potassium carbonate (6.53 g, 47.3 mmol, 10.0 equiv), acetone (150 mL), and 4-chlorobenzoyl chloride (4.15 g, 23.7 mmol, 5.0 equiv) to give pure **5c** (3.03 g, 3.54 mmol) as yellow gum in 74% yield: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.97 (dd, *J* = 6.0, 2.0 Hz, 8 H, 8 × ArH), 7.48 (dd, *J* = 7.0, 3.0 Hz, 8 H, 8 × ArH), 7.28 (d, *J* = 7.5 Hz, 2 H, 2 × ArH), 7.12 (dd, *J* = 7.5, 1.5 Hz, 2 H, 2 × ArH), 2.70 (dd, *J* = 8.0, 2.0 Hz, 2 H, 2 × ArCH), 2.18 (d, *J* = 8.5 Hz, 2 H, 2 × ArCH), 1.80–1.82 (m, 2 H, 2 × CH), 0.90 (d, *J* = 6.5 Hz, 6 H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.26, 164.11, 163.85, 163.46, 144.99,

141.84, 140.98, 140.68, 131.90, 131.69, 131.48, 131.40, 128.99, 128.92, 127.80, 127.42, 127.32, 127.24, 39.57, 39.38, 16.97, 16.26; HRMS (ESI) calcd for $C_{46}H_{35}O_8Cl_4$: 854.1085, found 854.1012.

((2*R*,3*S*)-2,3-Dimethylbutane-1,4-diyl)bis(benzene-4,1,2-triyl) tetramethanesulfonate (5d). The Standard Procedure was followed by use of NDGA (**1**, 1.43 g, 4.73 mmol, 1.0 equiv), potassium carbonate (6.53 g, 47.3 mmol, 10.0 equiv), acetone (150 mL), and methanesulfonyl chloride (2.71 g, 23.7 mmol, 5.0 equiv) to give pure **5d** (2.17 g, 3.53 mmol) as yellow gum in 74% yield: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.28 (d, J = 8 Hz, 2 H, 2 × ArH), 7.21 (d, J = 2 Hz, 2 H, 2 × ArH), 7.14 (dd, J = 6.5, 2.0 Hz, 2 H, 2 × ArH), 3.82 (s, 12 H, 4 × $SOCH_3$), 2.79 (dd, J = 9.0, 5.0 Hz, 2 H, 2 × ArCH), 2.34 (dd, J = 9.5, 5.0 Hz, 2 H, 2 × ArCH), 1.73 (m, 2 H, 2 × CH), 0.79 (d, J = 6.5 Hz, 6 H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 142.70, 140.73, 138.85, 128.86, 124.70, 124.00, 39.10, 38.70, 38.50, 38.38, 16.42; HRMS (ESI) calcd for $C_{22}H_{31}O_{12}S_4$: 614.0691, found 614.0681.

((2*R*,3*S*)-2,3-Dimethylbutane-1,4-diyl)bis(benzene-4,1,2-triyl) tetrabenzenesulfonate (5e). The Standard Procedure was followed by use of NDGA (**1**, 1.43 g, 4.73 mmol, 1.0 equiv), potassium carbonate (6.53 g, 47.3 mmol, 10.0 equiv), acetone (150 mL) and benzenesulfonyl chloride (4.19 g, 23.7 mmol, 5.0 equiv) to give pure **5e** (3.05 g, 3.53 mmol) as yellow gum in 72% yield: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.65 (dd, J = 9.0, 5.0 Hz, 4 H, 4 × ArH), 7.44 (dd, J = 9.0, 4.5 Hz, 8 H, 8 × ArH), 7.13 (d, J = 8.5 Hz, 2 H, 2 × ArH), 7.27 (d, J = 7.0 Hz, 2H, 2 × ArH), 6.96 (dd, J = 6.5, 1.5 Hz, 2 H, 2 × ArH), 2.68 (dd, J = 8.5, 4.5 Hz, 2 H, 2 × ArCH), 2.28 (dd, J = 9.5, 4.0 Hz, 2 H, 2 × ArCH), 1.66 (m, 2 H, 2 × CH), 0.78 (d, J = 6.5 Hz, 6 H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.50, 163.59, 142.89, 141.88, 140.11, 131.93, 131.91, 131.61, 131.49, 129.02, 129.00, 127.71, 127.42, 123.83, 123.05, 39.18, 38.84, 16.24; HRMS (ESI) calcd for $C_{42}H_{39}O_{12}S_4$: 862.1319, found 862.1246.

((2*R*,3*S*)-2,3-Dimethylbutane-1,4-diyl)bis(benzene-4,1,2-triyl) tetrakis(4-methyl benzenesulfonate) (5f). The Standard Procedure was followed by use of NDGA (**1,** 1.43 g, 4.73 mmol, 1.0 equiv), potassium carbonate (6.53 g, 47.3 mmol, 10.0 equiv), acetone (150 mL) and 4-methylbenzenesulfonyl chloride (4.50 g, 23.7 mmol, 5.0 equiv) to give pure **5f** (3.24 g, 3.53 mmol) as yellow gum in 72% yield: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.44 (dd, J = 9.0, 4.5 Hz, 4 H, 4 × ArH), 7.23 (dd, J = 9.0, 4.5 Hz, 8 H, 8 × ArH), 7.10 (d, J = 8.5 Hz, 2 H, 2 × ArH), 6.98 (d, J = 7 Hz, 2H, 2 × ArH), 6.96 (dd, J = 6.5, 1.5 Hz, 2 H, 2 × ArH), 2.77 (dd, J = 13.5, 5.0 Hz, 2 H, 2 × ArCH), 2.38 (dd, J = 9.5, 4.5 Hz, 2 H, 2 × ArCH), 1.75–1.78 (m, 2 H, 2 × CH), 0.85 (d, J = 6.5 Hz, 6 H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 145.60, 15.97, 21.73, 38.76, 39.32, 124.03, 124.73, 128.22, 128.49, 128.53, 129.63, 132.14, 132.23, 139.13, 140.87, 141.97, 145.56,; HRMS (ESI) calcd for $C_{46}H_{47}O_{12}S_4$: 918.1942, found 918.1869.

((2*R*,3*S*)-2,3-Dimethylbutane-1,4-diyl)bis(benzene-4,1,2-triyl) tetrakis(4-methoxy benzenesulfonate) (5g). The Standard Procedure was followed by use of NDGA (**1,** 1.43 g, 4.73 mmol, 1.0 equiv), potassium carbonate (6.53 g, 47.3 mmol, 10.0 equiv), acetone (150 mL) and 4-methoxybenzenesulfonyl chloride (4.90 g, 23.7 mmol, 5.0 equiv) to give pure **5g** (3.37 g, 3.43 mmol) as yellow gum in 71% yield: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.63 (dd, J = 10, 5.0 Hz, 8 H, 8 × ArH), 7.11 (d, J = 8.5 Hz, 2 H, 2 × ArH), 7.01 (s, 2 H, 2 × ArH), 6.94 (dd, J = 8.5, 2.0 Hz, 2 H, 2 × ArH), 6.87 (dd, J = 8.5, 2.0 Hz, 4 H, 4 × ArH), 3.82 (s, 12× ArOCH), 2.66 (dd, J = 13, 4.5 Hz, 2 H, 2 × ArCH), 2.24 (dd, J = 13.5, 9.5 Hz, 2 H, 2 × ArCH), 1.72–1.74 (m, 2 H, 2 × CH), 0.75–0.77 (d,

J = 7.0 Hz, 6 H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.31, 164.27, 141.95, 140.98, 139.24, 130.84, 130.81, 126.38, 124.77, 124.08, 114.24, 114.22, 39.29, 38.77, 16.03; HRMS (ESI) calcd for $C_{46}H_{47}O_{16}S_4$: 982.1740, found 982.1667.

((2*R*,3*S*)-2,3-dimethylbutane-1,4-diyl)bis(benzene-4,1,2-triyl) tetrakis(4-nitrobenz

enesulfonate) (5h). The Standard Procedure was followed by use of NDGA (**1**, 1.43 g, 4.73 mmol, 1.0 equiv), potassium carbonate (6.53 g, 47.3 mmol, 10.0 equiv), acetone (150 mL) and 4- 4-nitrobenzenesulfonyl chloride (5.24 g, 23.7 mmol, 5.0 equiv) to give pure **5h** (3.54 g, 3.43 mmol) as yellow gum in 70% yield: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.70 (dd, J = 10.5, 2.5 Hz, 8 H, 8 × ArH), 7.43 (dd, J = 11, 2.5 Hz, 2 H, 2 × ArH), 7.13 (d, J = 8 Hz, 2 H, 2 × ArH), 7.03 (dd, J = 8.5, 2.0 Hz, 2 H, 2 × ArH), 7.00 (dd, J = 9.0, 2.0 Hz, 2 H, 2 × ArH), 2.70 (dd, J = 13, 4.5 Hz, 2 H, 2 × ArCH), 2.28 (dd, J = 13.5, 10 Hz, 2 H, 2 × ArCH), 1.67–1.71 (m, 2 H, 2 × CH), 0.78 (d, J = 6.5 Hz, 6 H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.73, 157.93, 151.05, 146.06, 144.03, 140.69, 140.30, 138.65, 129.71, 129.48, 127.04, 126.91, 125.35, 124.65, 122.64, 116.46, 39.46, 38.74, 16.16; HRMS (ESI) calcd for $C_{42}H_{34}N_4O_{20}S_4$: 1042.9816, found 1042.9782.

Cell culture and regents

The human uterine sarcoma cell line (MES-SA), and multidrug resistant uterine sarcoma cell line (MES-SA/Dx5) were obtained from Bioresource Collection and Research Center (Hsinchu, Taiwan). The cells were maintained routinely and passed in the culture medium DMEM supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics under a humidified atmosphere of 5% CO2 and 95% air at 37 ^{o}C. The culture medium was changed every 2–3 days until the cells reached confluence. The synthesized compounds were dissolved in DMSO (SigmaeAldrich). The final concentration of DMSO in all reactions was maintained at 0.1% in all experiments. Morphology of cell was assessed by microscopy and Giemsa staining method.

Cytotoxic effect of the compounds on OVCAR-3 and SKOV3 uterine sarcoma cells

The uterine sarcoma cells were seeded onto 96-well plates at a density of 1 x 10^4 cells/well. After the cells reached 80% confluence, the medium was replaced with fresh medium containing graded concentrations of compounds up to 1000 nM for 48 hr at 37^{o}C. The MTT assay was performed to measure cell proliferation with modifications.[ref 1] This assay is based on the cleavage of the yellow

tetrazolium salt MTT by mitochondrial dehydrogenases of metabolically active cells to purple formazan crystals, which are then solubilised and spectrophotometrically quantified at 590 nm.[ref] At the end of the experiments, MTT (0.5 mg/ml medium) was added to each well and the plates were then incubated at 37°C for 4 h. After adding an equal volume of solubilisation solution (10% SDS in 0.01 M HCl) to each well, the plates were incubated at 37 °C to ensure that all dark purple crystals were dissolved. The plates were then read on a microplate reader (Power Wave 200 microplate reader, Bio-Tek Instruments, Winooski, VT) at 590 nm and 690 nm.

[ref 1]: Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods 1983; 65:55-63.

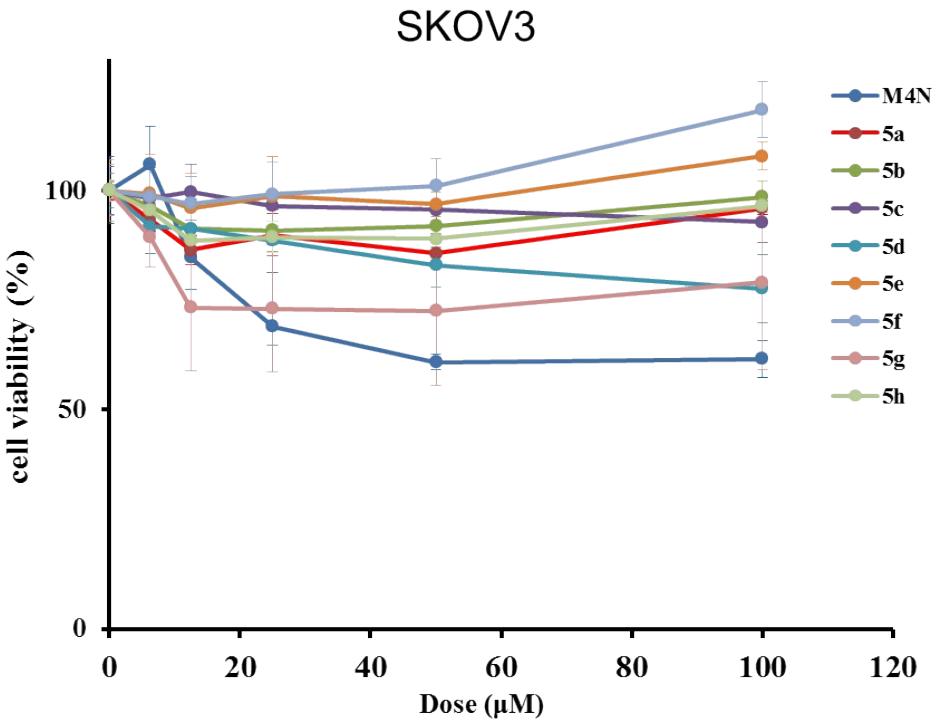

Figure S1. Dose measurement of Terameprocol (M4N) and synthesize derivatives on inhibit human ovarian cancer cell line SKOV3 after 48 incubation.

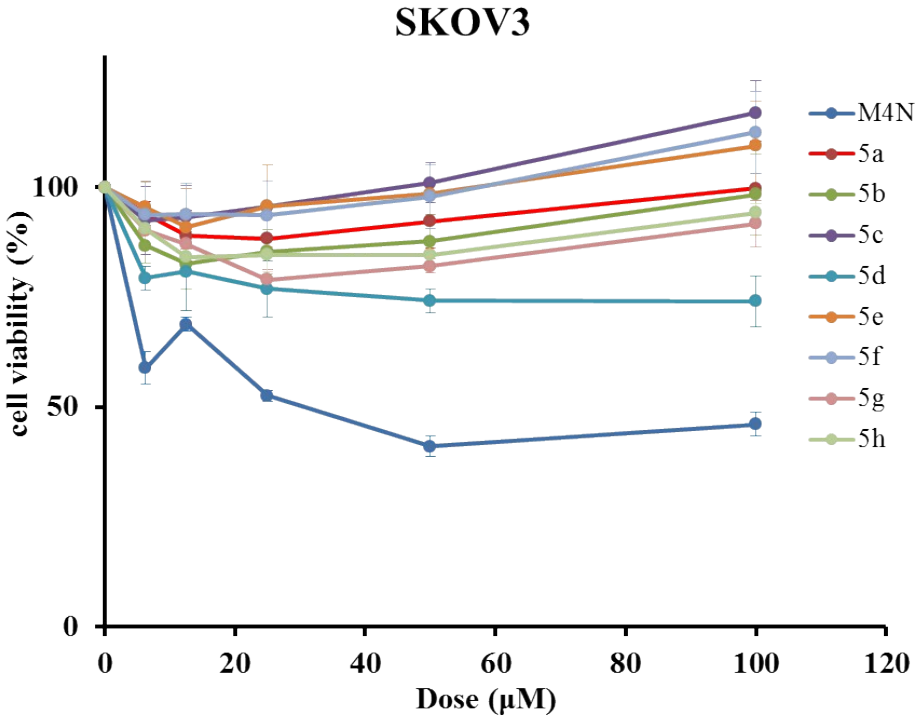

Figure S2. Dose measurement of Terameprocol (M4N) and synthesize derivatives on inhibit human ovarian cancer cell line SKOV3 after 72 incubation.

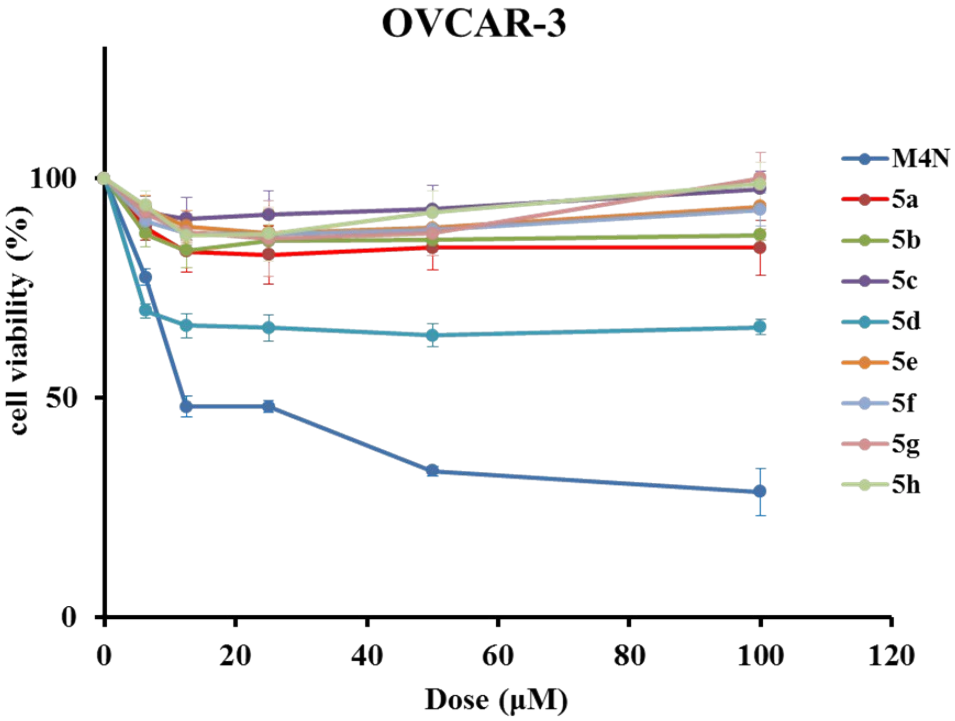

Figure S3. Dose measurement of Terameprocol (M4N) and synthesize derivatives on inhibit human ovarian cancer cell line OVCAR-3 after 48 incubation.

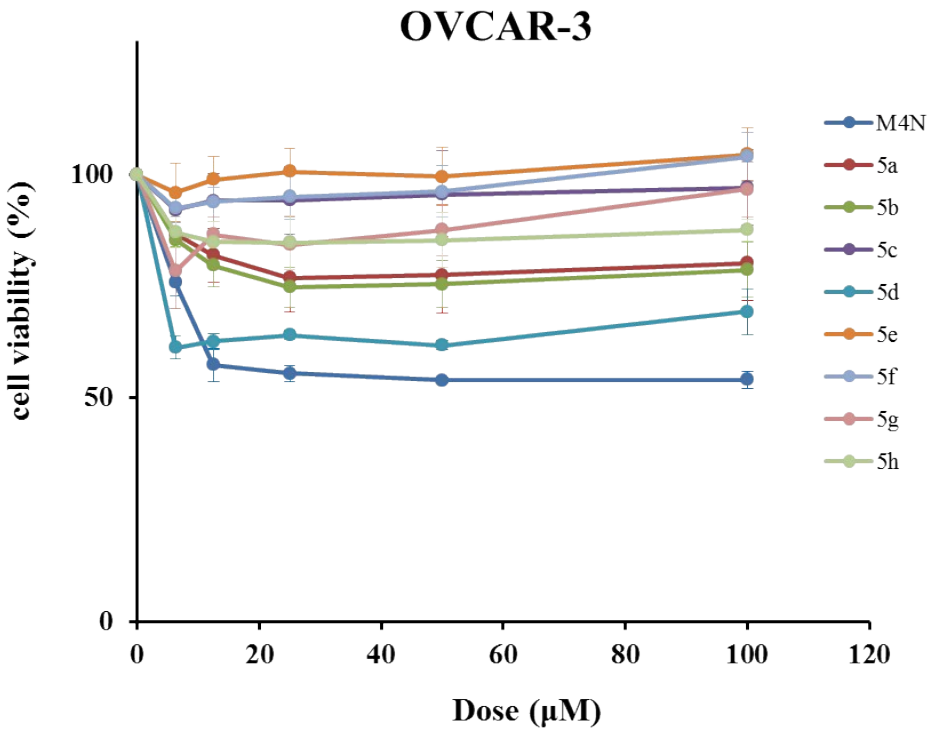

Figure S4. Dose measurement of Terameprocol (M4N) and synthesize derivatives on inhibit human ovarian cancer cell line OVCAR-3 after 72 incubation.

Cytotoxic effect of the compounds on MES-SA and MES-SA/Dx5 uterine sarcoma cells

The uterine sarcoma cells were seeded onto 96-well plates at a density of 1 x 10^4 cells/well. After the cells reached 80% confluence, the medium was replaced with fresh medium containing graded concentrations of compounds up to 1000 nM for 48 hr at 37^{o}C. The MTT assay was performed to measure cell proliferation with modifications.[ref 1] This assay is based on the cleavage of the yellow tetrazolium salt MTT by mitochondrial dehydrogenases of metabolically active cells to purple formazan crystals, which are then solubilised and spectrophotometrically quantified at 590 nm.[ref] At the end of the experiments, MTT (0.5 mg/ml medium) was added to each well and the plates were then incubated at 37^{o}C for 4 h. After adding an equal volume of solubilisation solution (10% SDS in 0.01 M HCl) to each well, the plates were incubated at 37 ^{o}C to ensure that all dark purple crystals were dissolved. The plates were then read on a microplate reader (Power Wave 200 microplate reader, Bio-Tek Instruments, Winooski, VT) at 590 nm and 690 nm.

[ref 1]: Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods 1983; 65:55-63.

Cloning of human MDR1 promoter, plasmid construction and transfection

The 5'-flanking region (2056 bp) upstream of the translation start site of MDR1 gene (GenBank accession no, AY910577) was cloned by PCR amplication using genomic DNA obtained from MES-SA cells as a template in conduction with the following primer set: 5'-GGATCCGGAGCAAAGAAATGGAATACA-3' encoded a *Bam*HI restriction enzyme site, the reverse primer 5'-GAATTCAGTAGCTCCCAGCTTTGC GTG-3' encoded the *Eco*RI restriction enzyme site. PCR was conducted using Taq DNA polymerase (New England BioLab, Beverly, MA, USA) with following conditions: samples were subjected to initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 2 min, and a final extension at 74°C for 10 min. PCR product of MDR1 promoter was digested

with *Bam*HI and *Eco*RI restriction enzymes prior to ligation to the pMetLuc-reporter vector containing the luciferase reporter gene (Clontech, Palo Alto, CA, USA). MES-SA/Dx5 cells were transfected with pMetLuc-MDR1 promoter vector using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Stably expressing clones were isolated by limiting dilution and selection with G418 sulfate (Sigma Chemixal Co., St Louis, MO, USA) at a concentration of 400 μg/mL and cells surviving this treatment were cloned.

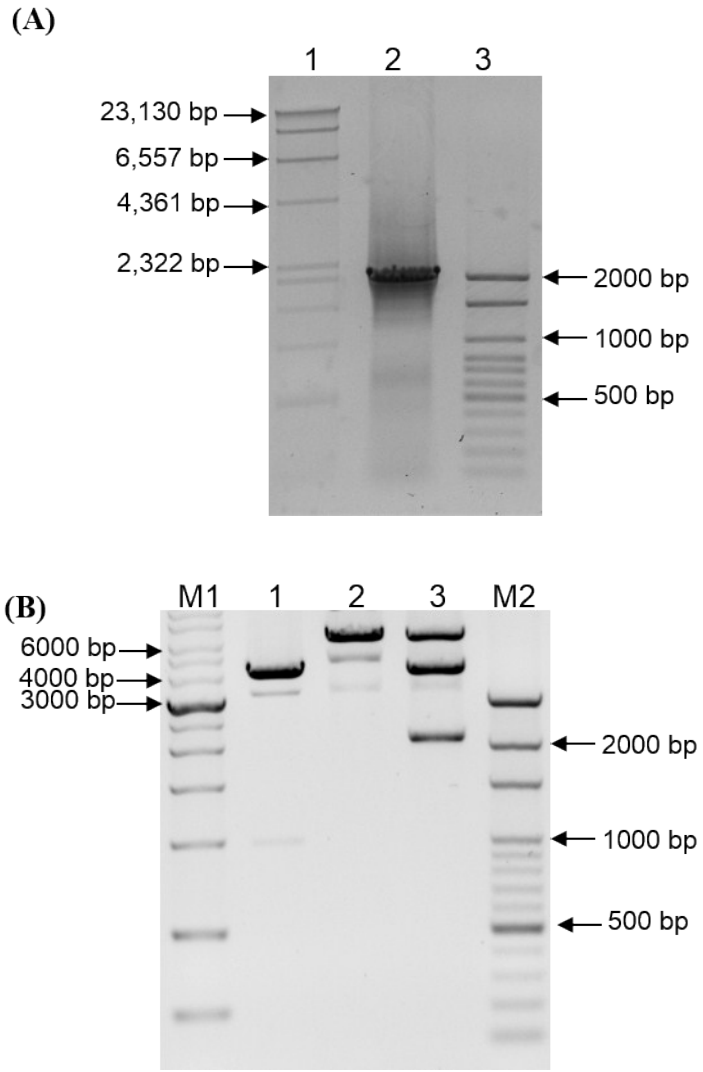

Figure S5. Molecular cloning of human MDR1 promoter and construction of reporter vector. (A) The MDR1 promoter fragment specifically amplified by PCR. Lane 1: λ DNA digested with *Hind*III. Lane 2: PCR product of human MDR1 promoter. Lane 3: 100 bp DNA markers. (B) pMetLuc-MDR1 vector screen with restriction enzyme. Lane M1 and M2 are molecular weight 1000-bp ladder and 100-bp ladder markers with specific molecular weights indicated. Lane

1:pMetLuc digested with *Bam*HI. Lane 2: pMetLuc-MDR1 digested with *Bam*HI. Lane 3: pMetLuc-MDR1 digested with *Bam*HI and *Eco*RI.

Reporter gene assay

Culture supernatants were removed from culture dishes after 48 hours of drug treatment. The luciferase reporter assay was performed using the Ready-To-Glow™ secreted luciferase reporter system according to the manufacturer's instructions. Bioluminescence was detected using a 1420 Multilable Counter Victor 2 (Perkin-Elmer, Wellesley, USA).

Statistical analysis

All experiments were performed in triplicate and statistical significance was determined using the Student's *t* test. A $P < 0.05$ was considered to be statistically significant.