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Electronic Supplementary Information

For

5-(Dimethylamino)-*N*-(4-ethynylphenyl)-1-naphthalenesulfonamide as a Novel Bifunctional Anti-Tumor Agent and Two-Photon Induced Bio-Imaging Probe

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Experimental Section

General: All reactions were performed under nitrogen atmosphere. Solvents were carefully dried and distilled from appropriate drying agents prior to use. Commercially available reagents were used without further purification unless otherwise stated. All reactions were monitored by thin-layer chromatography (TLC) with Merck pre-coated glass plates. Flash column chromatography and preparative TLC were carried out using silica gel from Merck (230–400 mesh). Fast atom bombardment (FAB) mass spectra were recorded on a Finnigan MAT SSQ710 system. Proton and ^{13}C-{^1H} NMR spectra were measured in $CDCl_3$ on a Varian Inova 400 MHz or JEOL GX270 FT-NMR spectrometer; chemical shifts were quoted relative to tetramethylsilane. Model compounds **3**[S1] and **4**[S2] were prepared according to slight modifications of the published methods.

Spectroscopic and photophysical measurements: UV-Visible absorption spectra in the spectral range 200 to 1100 nm were recorded by a HP UV-8453 spectrophotometer. Single-photon luminescence spectra were recorded using a Edinburgh Instrument FLS920 Combined Fluorescence Lifetime and Steady state spectrophotometer that was equipped with a red sensitive single-photon counting photomultiplier in Peltier Cooled Housing. The spectra were corrected for detector response and stray background light phosphorescence. The quantum yields of the compounds were measured by Demountable 142 mm (inner) diameter barium sulphide coated integrating sphere supplied with two access ports. For two-photon experiments, the 820 nm pump source was from the fundamental of a femtosecond mode-locked Ti:Sapphire laser system (output beam ~ 150 fs duration and 1 kHz repetition rate). The 700 nm pump wavelengths were generated from a commercial optical parametric amplifier (Coherent) pumped by the SHG of the 800 nm femtosecond pulses. The lasers were focused to spot size ~ 50 μm via an f = 10 cm lens onto the sample. The emitting light was collected with a backscattering configuration into a 0.5 m spectrograph and detected by a liquid nitrogen-cooled CCD detector. A power meter was used to monitor the uniform excitation.

Synthesis of 4-(trimethylsilylethynyl)aniline: 4-Iodoaniline (1.00 g, 4.57 mmol) was first dissolved in a solution mixture of freshly distilled NEt_3 (50 mL) and THF (50 mL) under nitrogen. Catalytic amounts of CuI (30 mg), $Pd(OAc)_2$ (30 mg) and PPh_3 (90 mg) were added into the mixture. After the solution was stirred for 15 min, $Me_3SiC{\equiv}CH$ (0.65 mL, 4.57 mmol) was then added and the suspension was stirred for 1 h at room temperature. Afterwards, the mixture was heated to 65 ^{o}C for 15 h. The solution was then allowed to cool to room temperature and the solvent mixture was evaporated *in vacuo*. The resulting residue was then redissolved in CH_2Cl_2 and was purified by column chromatography on silica gel using *n*-hexane/CH_2Cl_2 (1:1, v/v) as eluent. The product was collected as a white solid (0.65 g, 75%). IR (KBr): 2144 cm^{-1} (C≡C), 3371 cm^{-1} (NH_2); ^1H NMR ($CDCl_3$): δ = 7.27–7.24 (m, 2H, Ar), 6.56–6.54 (d, *J* = 8.0 Hz, 2H, Ar), 3.77 (s, 2H, NH_2), 0.22 ppm (s, 9H, $Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ = 146.99, 133.60, 114.76, 112.72 (Ar), 106.21, 91.60 (C≡C), 0.36 ppm ($Si(CH_3)_3$); FAB-MS: *m/z* = 189 (M^+); elemental analysis calcd (%) for $C_{11}H_{15}NSi$: C 69.78, H 7.99, N 7.40; found: C 69.89, H 8.10, N 7.62.

Synthesis of 1-TMS: 4-(Trimethylsilylethynyl)aniline (0.35 g, 1.85 mmol) and dansyl chloride (0.50 g, 1.85 mmol) were first dissolved in dry pyridine (20 mL)

under nitrogen. A catalytic amount of 4-(dimethylamino)pyridine (30 mg) was added into the solution mixture. The resulting mixture was refluxed at 95 °C for 15 h. The solution was then allowed to cool to room temperature and the solvent mixture was evaporated *in vacuo*. The resulting residue was then redissolved in CH_2Cl_2 and purified on preparative TLC plates eluting with pure CH_2Cl_2. The product **1-TMS** was collected as a pale yellow solid (0.49 g, 62%). IR (KBr): 1144 cm^{-1} (O=S=O), 2151 cm^{-1} (C≡C), 3244 cm^{-1} (N–H); ^1H NMR ($CDCl_3$): δ = 8.49–8.47 (d, J = 8.0 Hz, 1H, Ar), 8.38–8.36 (d, J = 8.0 Hz, 1H, Ar), 8.21–8.19 (m, 1H, Ar), 7.60 (s, 1H, Ar), 7.53 (t, J = 8.0 Hz, 1H, Ar), 7.42 (t, J = 8.0 Hz, 1H, Ar), 7.20–7.13 (m, 3H, Ar), 6.92–6.89 (d, J = 12.0 Hz, 2H, Ar), 2.85 (s, 6H, $N(CH_3)_2$), 0.18 ppm (s, 9H, $Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ = 152.32, 136.97, 133.90, 133.11, 131.35, 130.73, 130.01, 129.71, 129.05, 123.27, 120.34, 119.56, 118.49, 115.55 (Ar), 104.51, 94.48 (C≡C), 45.61 ($N(CH_3)_2$), 0.14 ppm ($Si(CH_3)_3$); FAB-MS: m/z = 422 (M^+); elemental analysis calcd (%) for $C_{23}H_{26}N_2O_2SSi$: C 65.38, H 6.21, N 6.63; found: C 65.43, H 6.32, N 6.78.

Synthesis of 1: 1-TMS (0.50 g, 1.18 mmol) was first dissolved in THF (30 mL) and MeOH (20 mL) under nitrogen and K_2CO_3 (0.25 g, 1.77 mmol) was added as a base. The reaction mixture was allowed to stir at room temperature under nitrogen for 15 h. All volatile components were removed under reduced pressure to leave a pale brown residue. This residue was dissolved in the minimum amount of CH_2Cl_2 and purified on preparative TLC plates eluting with *n*-hexane/ethyl acetate (2:1, v/v) as eluent. The product **1** was obtained as a pale yellow solid (0.31 g, 75%). IR (KBr): 1144 cm^{-1} (O=S=O), 2101 cm^{-1} (C≡C), 3266 cm^{-1} (N–H), 3283 cm^{-1} (≡CH); ^1H NMR ($CDCl_3$): δ = 8.51–8.49 (d, J = 8.0 Hz, 1H, Ar), 8.34–8.32 (d, J = 8.0 Hz, 1H, Ar), 8.22–8.20 (d, J = 8.0 Hz, 1H, Ar), 7.56 (t, J = 8.0 Hz, 1H, Ar), 7.44 (t, J = 8.0 Hz, 1H, Ar), 7.30 (s, 1H, Ar), 7.25–7.22 (m, 2H, Ar), 7.17–7.15 (d, J = 8.0 Hz, 1H, Ar), 6.92–6.90 (m, 2H, Ar), 2.98 (s, 1H, C≡CH), 2.86 ppm (s, 6H, $N(CH_3)_2$); ^{13}C NMR ($CDCl_3$): δ = 152.39, 137.24, 134.00, 133.30, 131.38, 130.67, 130.05, 129.72, 129.03, 123.31, 120.50, 118.64, 118.43, 115.54 (Ar), 83.09, 77.51 (C≡C), 45.61 ppm ($N(CH_3)_2$); FAB-MS: m/z = 350 (M^+); elemental analysis calcd (%) for $C_{20}H_{18}N_2O_2S$: C 68.55, H 5.18, N 8.00; found: C 68.34, H 5.29, N 8.16.

Synthesis of 1-Ph: To an ice-cooled mixture of 4-iodoaniline (219 mg, 1.00 mmol) in freshly distilled triethylamine (20 mL) and CH_2Cl_2 (20 mL) solution under nitrogen was added $Pd(OAc)_2$ (20 mg), PPh_3 (60 mg) and CuI (20 mg). After the solution was stirred for 30 min, phenylacetylene (150 mg, 1.50 mmol) was then added and the suspension was stirred for another 30 min in the ice-bath before being warmed to room temperature. After reacting for 30 min at room temperature, the mixture was heated to 50 °C for 48 h. The solution was then allowed to cool to room temperature and the solvents were removed on a rotary evaporator *in vacuo*. The crude product was purified by column chromatography on silica gel eluting with CH_2Cl_2 to provide 4-(2-phenylethynyl)aniline (200 mg) as a brown solid. The crude product was used in the next step without further purification.

In an ice-cooled round-bottom flask, 4-(2-phenylethynyl)aniline (58 mg, 0.30 mmol) was dissolved in pyridine (5 mL) and dansyl chloride (81 mg, 0.30 mmol) was added. The mixture was warmed to room temperature and stirred overnight. The solvents were removed on a rotary evaporator *in vacuo*. The crude product was purified by column chromatography on silica gel eluting with CH_2Cl_2 to provide **1-Ph** (74 mg, 58%) as a brown yellow solid. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.51-8.49 (d, 1H, Ar), 8.40-8.37 (d, 1H, Ar), 8.24-8.22 (m, 1H, Ar), 7.57-7.42 (m, 5H, Ar), 7.30-

7.24 (m, 4H, Ar), 7.16-7.14 (d, 1H, Ar), 6.97-6.94 (d, 2H, Ar), 2.85 ppm (s, 6H, $N(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 152.13, 136.56, 133.86, 132.51, 131.51, 131.14, 130.47, 129.84, 129.55, 128.84, 128.34, 123.10, 120.44, 119.63, 118.34, 115.36 (Ar), 89.50, 88.65 (C≡C), 45.40 ppm ($N(CH_3)_2$); FAB-MS: m/z = 426 (M^+); elemental analysis calcd (%) for $C_{26}H_{22}N_2O_2S$: C 73.21, H 5.20, N 6.57; found: C 73.34, H 5.31, N 6.65.

Synthesis of 1-Me: A mixture of 4-iodoaniline (1.10 g, 5.0 mmol), iodomethane (0.35 mL, 5.0 mmol) and K_2CO_3 (0.76 g, 5.5 mmol) in EtOH (30 mL) was refluxed for 24 h under N_2.[S3] After the mixture was cooled to room temperature, it was poured into H_2O (50 mL), and extracted with CH_2Cl_2 (25 mL) three times. The organic layer was dried over anhydrous Na_2SO_4. After removal of the solvent, the crude product was purified by column chromatography on silica gel eluting with CH_2Cl_2/hexane (1:2, v/v) to provide 4-iodo-*N*-methylaniline (495 mg, 2.12 mmol, 43%) as a brown oil, together with 4-iodo-*N*,*N*-dimethylaniline (150 mg, 0.60 mmol, 12%) and 4-iodoaniline (220 mg, 1.00 mmol, 20%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43-7.41 (d, 2H, Ar), 6.40-6.38 (d, 2H, Ar), 3.74 (m, 1H, N*H*), 2.81-2.80 ppm (d, 3H, NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 148.84, 137.72, 114.62, 77.71 (Ar), 30.56 ppm (NCH_3). FAB-MS: m/z = 233 (M^+).

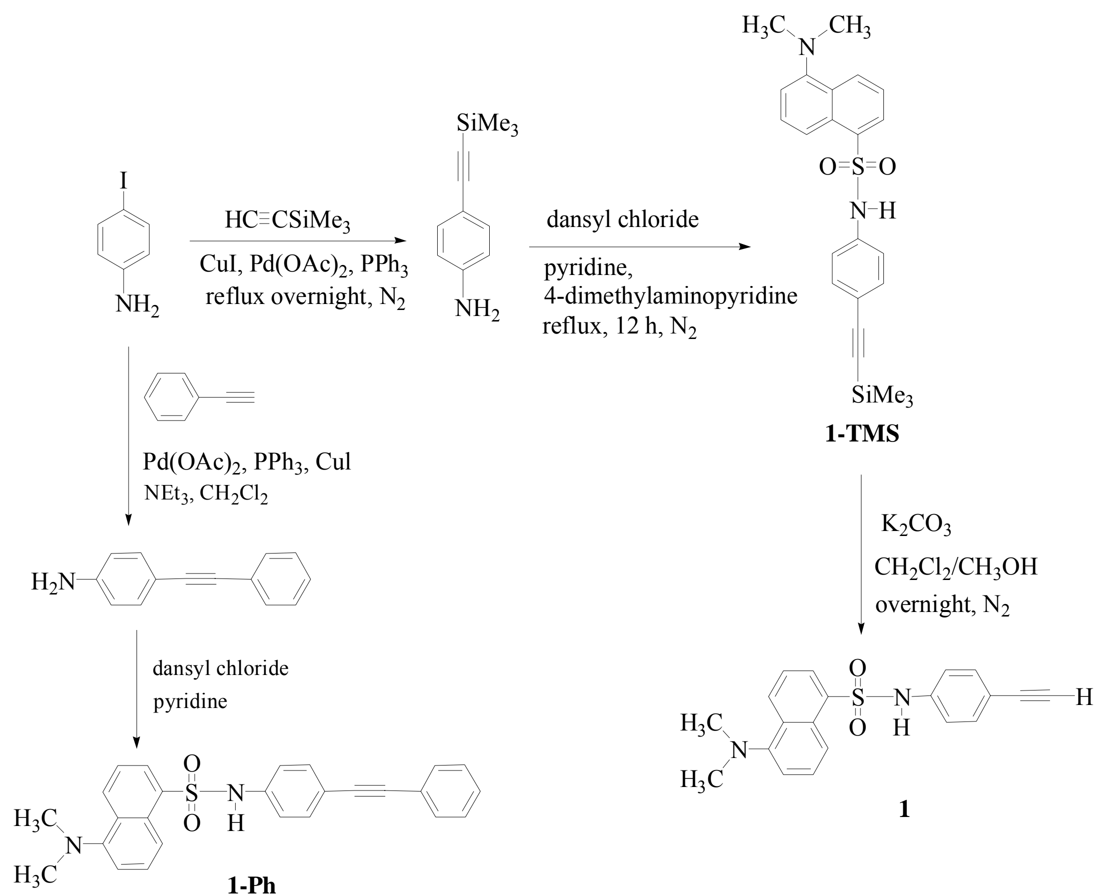
To an ice-cooled mixture of 4-iodo-*N*-methylaniline (495 mg, 2.12 mmol) in freshly distilled triethylamine (15 mL) and CH_2Cl_2 (15 mL) solution under nitrogen was added $Pd(OAc)_2$ (30 mg), PPh_3 (90 mg) and CuI (30 mg). After the solution was stirred for 30 min, trimethylsilylacetylene (0.6 mL, 4.24 mmol) was then added and the suspension was stirred for another 30 min in the ice-bath before being warmed to room temperature. After stirring for 4 h at room temperature, the solvents were removed on a rotary evaporator *in vacuo*. The crude product was purified by column chromatography on silica gel using CH_2Cl_2/hexane (2:1, v/v) as eluent to provide *N*-methyl-4-[(trimethylsilyl)ethynyl]aniline (306 mg, 71%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.31-7.29 (d, 2H, Ar), 6.50-6.48 (d, 2H, Ar), 3.87 (m, 1H, N*H*), 2.84-2.83 (d, 3H, NCH_3), 0.22 ppm (s, 9H, TMS). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 149.32, 133.24, 111.71, 110.76 (Ar), 106.46, 90.98 (C≡C), 30.26 (NCH_3), 0.15 ppm (TMS). FAB-MS: m/z = 203 (M^+).

In an ice-cooled 25 mL round-bottom flask, *N*-methyl-4-[(trimethylsilyl)ethynyl]aniline (220 mg, 1.08 mmol) was dissolved in the mixture of CH_2Cl_2 (30 mL) and pyridine (10 mL) under a N_2 atmosphere, and dansyl chloride (321 mg, 1.19 mmol) was added. The mixture was warmed to room temperature and stirred for 3 days. The solvents were removed on a rotary evaporator *in vacuo*. The crude product was purified by column chromatography on silica gel eluting with CH_2Cl_2/hexane(1:2, v/v) to give **1-Me-TMS** (370 mg, 79%) as a yellow-green oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.53-8.51(d, 1H, Ar), 8.08-8.06 (dd, 1H, Ar), 8.03-8.00 (d, 1H, Ar), 7.47-7.43 (m, 1H, Ar), 7.38-7.31 (m, 3H, Ar), 7.14-7.12 (dd, 1H, Ar), 7.07-7.05 (m, 2H, Ar), 3.23 (s, 3H, NCH_3), 2.87 (s, 6H, $N(CH_3)_2$), 0.24 ppm (s, 9H, TMS). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 151.47, 141.55, 133.14, 132.38, 130.87, 130.60, 130.24, 129.84, 127.80, 126.35, 122.94, 121.64, 119.74, 115.14 (Ar), 104.21, 95.02 (C≡C), 45.33, 37.98 ppm (CH_3). FAB-MS: m/z = 436 (M^+).

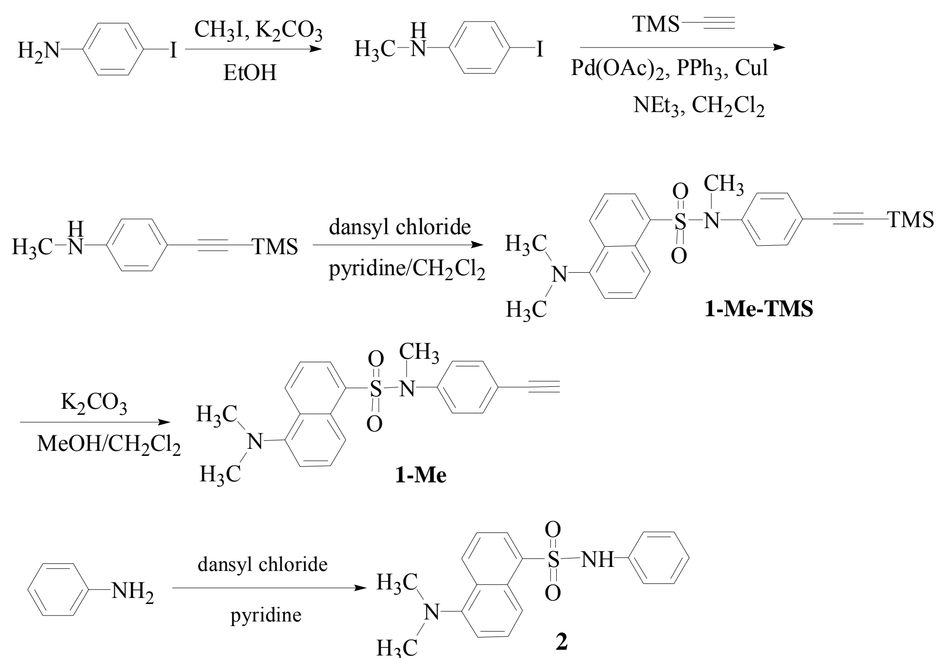
A mixture of **1-Me-TMS** (300 mg, 0.68 mmol) and K_2CO_3 (116 mg, 0.86 mmol) in methanol (10 mL) and CH_2Cl_2 (20 mL) solution mixture, under a nitrogen atmosphere, was stirred at room temperature for 4 h. The mixture was added to CH_2Cl_2 (50 mL) and washed with water (3 × 20 mL), and dried over anhydrous Na_2SO_4. The solvents were removed on a rotary evaporator *in vacuo*. The crude

product was purified by column chromatography on silica gel eluting with CH_2Cl_2/hexane (1:1, v/v) to produce **1-Me** (202 mg, 75%) as a yellow green oil. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.54-8.52 (d, 1H, Ar), 8.10-8.08 (dd, 1H, Ar), 7.48-7.46 (m, 1H, Ar), 7.37-7.32 (m, 3H, Ar), 7.14-7.08 (m, 3H, Ar), 3.23 (s, 3H, NCH_3), 3.08 (s, 1H, C≡C*H*), 2.87 ppm (s, 6H, $N(CH_3)_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 151.54, 141.95, 133.10, 132.63, 130.91, 130.72, 130.31, 129.90, 127.84, 126.55, 123.02, 120.69, 119.77, 115.21 (Ar), 82.89, 77.98 (C≡C), 45.40, 38.05 ppm (CH_3). FAB-MS: *m/z* = 364 (M^+).

Synthesis of 2: Similar to the dansylation protocol for the preparation of **1-Ph**, a pale yellow solid was obtained for **2**. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.50-8.48 (d, 1H, Ar), 8.33-8.31 (d, 1H, Ar), 8.26-8.14 (dd, 1H, Ar), 7.61-7.57 (m, 1H, Ar), 7.44-7.40 (m, 1H, Ar), 7.20-7.18 (d, 1H, Ar), 7.15-7.11 (m, 2H, Ar), 7.04-7.01 (m, 1H, Ar), 6.92-6.90 (d, 2H, Ar), 6.67 (m, 1H, Ar), 2.87 ppm (s, 6H, $N(CH_3)_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 152.03, 136.55, 134.18, 130.85, 130.32, 129.80, 129.65, 129.30, 128.67, 125.04, 123.10, 121.25, 118.66, 118.57, 115.26 (Ar), 45.10 ppm (CH_3). FAB-MS: *m/z* = 326 (M^+).



Scheme 1. Synthesis of dansyl-derived ligands **1-TMS**, **1** and **1-Ph**.



Scheme 2. Synthesis of **1-Me** and **2**.

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X-Ray crystallography: X-Ray diffraction data of **1** were collected at 293 K using graphite-monochromated Mo–Kα radiation (λ = 0.71073 Å) on a Bruker Axs SMART 1000 CCD diffractometer. The collected frames were processed with the software SAINT+[S4] and an absorption correction (SADABS)[S5] was applied to the collected reflections. The structure was solved by the Direct methods (SHELXTL)[S6] in conjunction with standard difference Fourier techniques and subsequently refined by full-matrix least-squares analyses on F^2. Hydrogen atoms were generated in their idealized positions and all non-hydrogen atoms were refined anisotropically. The crystallographic data for all the complexes (excluding structure factors) have been deposited in the Cambridge Crystallographic Data Centre with the deposition numbers CCDC 746813. These data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44)1223-336-033; E-mail: deposit@ccdc.cam.ac.uk). Crystal data for **1**: $C_{20}H_{18}N_2O_2S$, M_w = 350.42, monoclinic, space group *Cc*, a = 8.5113(5), b = 23.013(2), c = 9.8663(6) Å, β = 115.160(1)°, V = 1749.2(2) Å^3, Z = 4, ρ_{calcd} = 1.331 Mg m^{-3}, $\mu(Mo_{K\alpha})$ = 0.201 mm^{-1}, $F(000)$ = 736. 5250 reflections measured, of which 2851 were unique (R_{int} = 0.0136). Final R_1 = 0.0272 and wR_2 = 0.0751 for 2828 observed reflections with $I > 2\sigma(I)$.

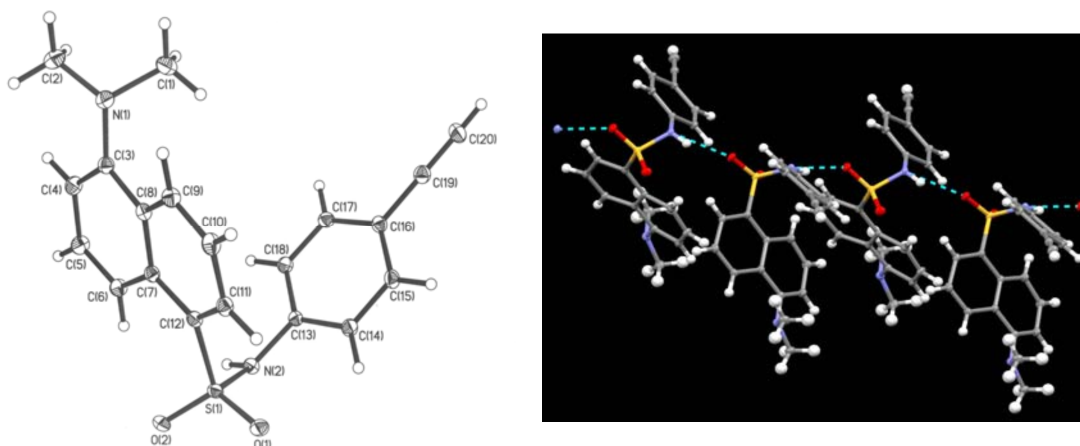


Fig. S1 Left) An ORTEP view of the molecular structure of **1**; Right) X-ray structure of **1**, showing polymeric N-H···O=S hydrogen bonds (O···H, 2.098 Å; O···NH, 2.908 Å).

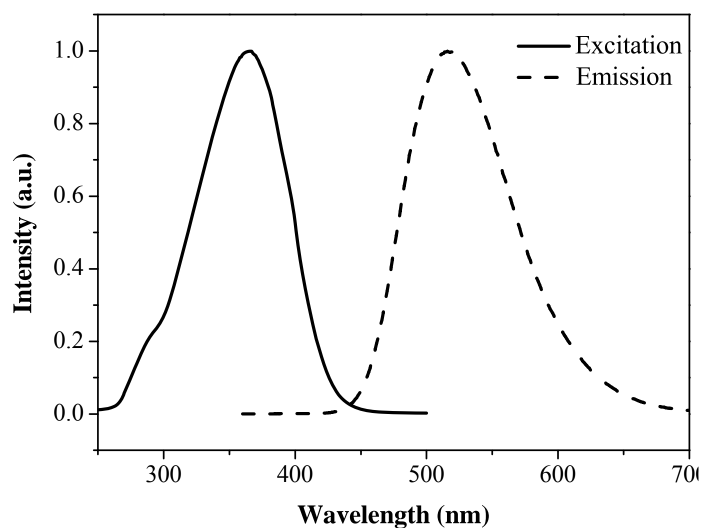


Fig. S2 Excitation (solid line) and emission (dashed line) spectra of **1** in CH_2Cl_2 solution at 293 K.

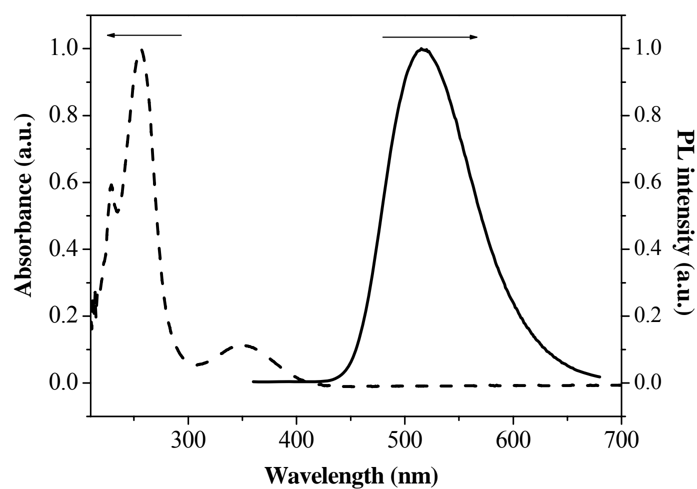


Fig. S3 Absorption (dashed line) and PL spectra (solid line) of **1** in CH_2Cl_2 solution at 293 K.

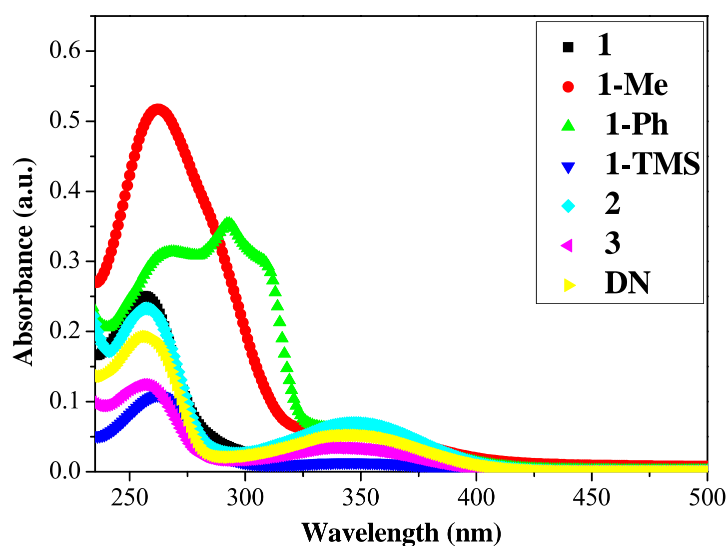


Fig. S4 Absorption spectra of dansylated compounds in DMSO.

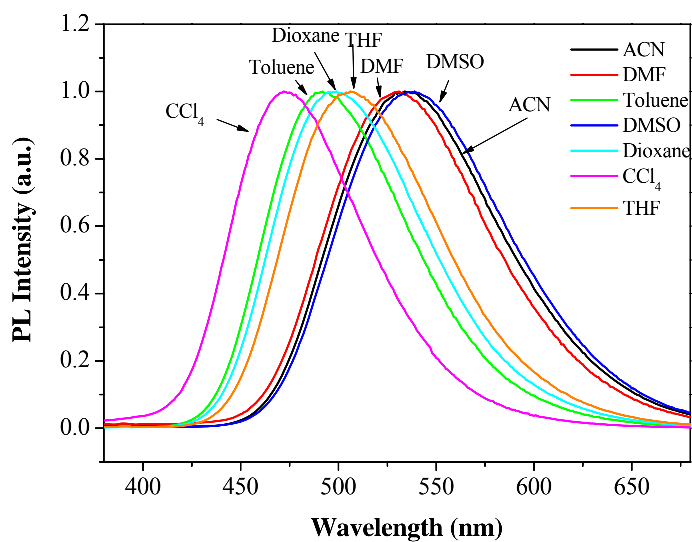


Fig. S5 Solvatochromic effects of the PL spectra of **1**.

Experimental details for biological studies

Sulforhodamine B assay for cell viability: Cancer cells seeded in the 96 wells microtitre plates at a concentration of approximately 1×10^5 cells/mL for 24 h were prepared for the screening of synthesized compounds. The compounds were dissolved in molecular biology grade DMSO. Doxorubicin, dissolved in saline buffer, was used as the positive reference compound and added at a starting concentration of 50 μM. All of the compounds showed reasonably good solubility following serial dilution in cell culture medium. Compounds were added at a starting concentration of 50 μM followed by a serial of two-fold dilutions and incubated for a further period of 48 h. The maximum concentration of DMSO employed was 0.1% by volume. We confirmed that cell proliferation was not affected by DMSO at 0.1% by volume. Afterwards, the evaluation of possible anti-proliferative potential of our synthesized compounds was performed by the sulforhodamine B protein staining methods. Briefly, cancer cells were fixed with trichloroacetic acid, washed and stained with sulforhodamine B. Afterwards, cells were washed again with acetic acid and stained cells were dissolved in unbuffered Tris-base. Finally, optical absorptions were measured at 575 nm using a microplate reader (Victor V form Perkin Elmer, Life Sciences). The 50% inhibitory concentrations of compounds and CDDP were calculated from these experimental results.

Fluorescence detection and confocal imaging: Hep3B cells were harvested on coverslips in a 24 well plate, and after 24 h of treatment with **1**, the cells were snapped with Coolsnap HQ camera connected to Zeiss axiovert 200M fluorescence microscope and with camera Princeton Instruments VersArray 512B connected to Zeiss axiovert 200 with CARV II™ confocal imager from BD™.

Two-photon absorption cross-section measurements: Two-photon induced luminescence and two-photon absorption cross section: For two-photon experiment, the 720-800 nm pump source was from the fundamental of a femtosecond mode-locked Ti:Sapphire laser system (output beam ~ 150 fs duration and 1 kHz repetition rate). The lasers were focused to spot size ~ 50 μm via an f = 10 cm lens onto the sample. The light emitted was collected with a backscattering configuration into a 0.5 m spectrograph and detected by a liquid nitrogen-cooled CCD detector. A power meter was used to monitor the uniform excitation.

The theoretical framework and experimental protocol for the two-photon cross-section measurement have been outlined by Webb and Xu.[S7] In this approach, the two-photon excitation (TPE) ratios of the reference and sample systems are given by:

$$\frac{\sigma_2^S \cdot \phi^S}{\sigma_2^R \cdot \phi^R} = \frac{C_R \cdot n_S \cdot F^S(\lambda)}{C_S \cdot n_R \cdot F^R(\lambda)}$$

where ϕ is the quantum yield, C is the concentration, n the refractive index, and $F(\lambda)$ is the integrated photoluminescent spectrum. In our measurements, we have ensured that the excitation flux and the excitation wavelengths are the same for both the sample and the reference. The two-photon absorption cross-section σ_2 of **1** was determined using Rhodamine B as a reference.[S8]

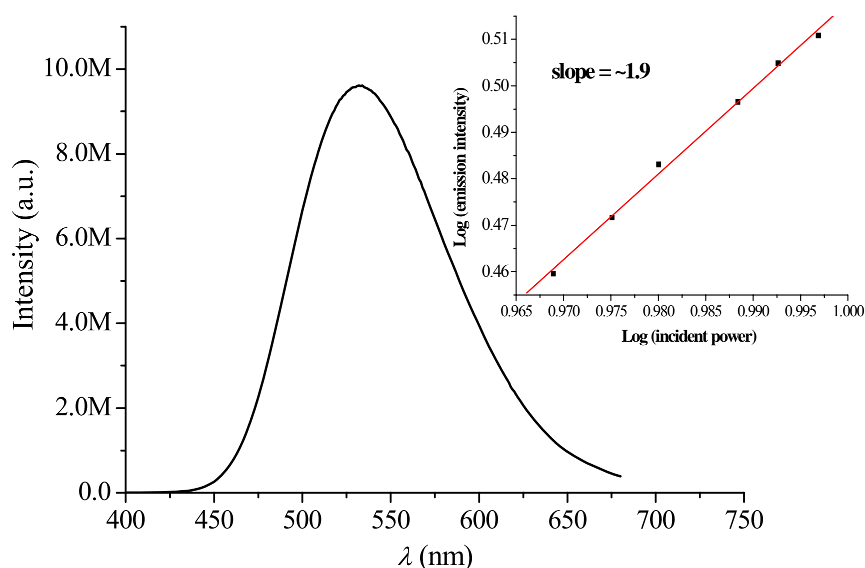


Fig. S6 The two-photon induced emission spectrum of **1** (λ_{ex} = 720 nm) with the power dependence response curve (inset).

Two-photon *in vitro* imaging: Human cervical carcinoma cells (HeLa) (American Type Culture Collection, USA) and Hep 3B hepatic cell were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin streptomycin in 5% CO_2. Thirty hours prior to imaging, 0.5 × 10^6 cells were seeded onto 60 mm culture dishes (MatTek Corporation, MA, USA). The cells were allowed to attach overnight. Culture medium in each dish was changed prior to exposure to **1**. To study the localization behaviour of **1**, experiments were carried out in the commercial multi-photon confocal microscope. For the two-photon *in-vitro* imaging, the cells were imaged in the tissue culture chamber (5% CO_2, 37 ^oC) using a Zeiss 510 LSM (upright configuration) confocal microscope equipped with a femtosecond-pulsed Ti:Sapphire laser (Libra II, Coherent). The excitation beam produced by the femtosecond laser, which was tunable from 720 – 900 nm, (λ_{ex} = 720 nm, ~ 10 mW), passed through an LSM 510 microscope with HFT 650 dichroic (Carl Zeiss, Inc.) and focused on coverslip-adherent cells using a 63x oil immersion objective.

FACS analysis and cell cycle distribution: The effects on the cell cycle distribution were studied on human hepatoma line Hep3B by flow cytometric analysis after staining with propidium iodide. Cells were exposed for 24 h to an increasing concentration (5, 10, 20 and 50 μM) of compound **1**. After 24 h treatment, the cells were washed once in ice-cold PBS 1x and resuspended at 1 × 10^6 per mL in a hypotonic fluorochrome solution containing propidium iodide (Sigma) at 50 μg/mL plus 0.03% (v/v) nonidet P-40 (Sigma) and RNAse (Sigma). After a 30 min incubation, the fluorescence of each sample was analyzed as single-parameter frequency histogram, using a FACScan flow cytometer (Becton–Dickinson, San Jose, CA). Analysis of the proportion of sub-G_0-G_1 (apoptotic peak) cells indicated the effects of the tested compound on apoptosis.

Annexin V-FITC analysis: Hep3B cells were seeded in sterilized coverslips, put in a 24 well plate, and treated with compound **1**. After 24 h, they were washed with PBS 1x and stained with AnnexinV-FITC (Clontech, Mountain View, CA). The pictures of the stained cells were taken with Coolsnap HQ camera in Zeiss axiovert 200M fluorescence microscope.

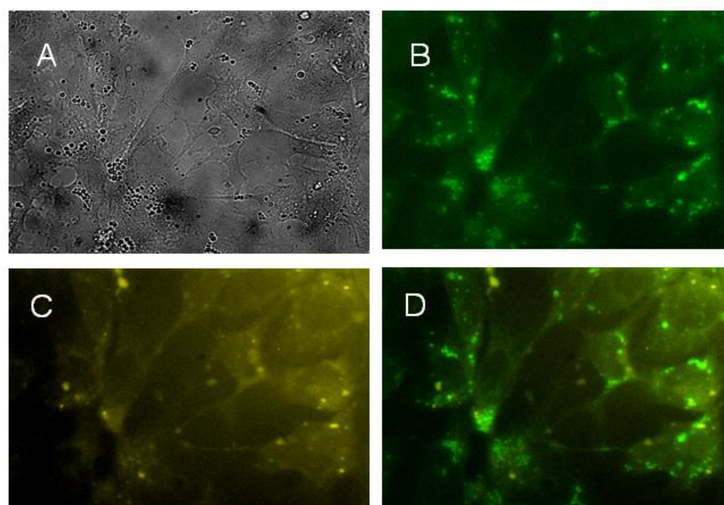


Fig. S7 Annexin labeling of Hep 3B hepatic cell treated with **1** (20 μM) and analyzed with inverted fluorescence microscope after 24 h of incubation. A: phase-contrast analysis; B: fluorescence imaging; C: annexin labeling. D: superimposed image of fluorescence imaging and annexin labelling.

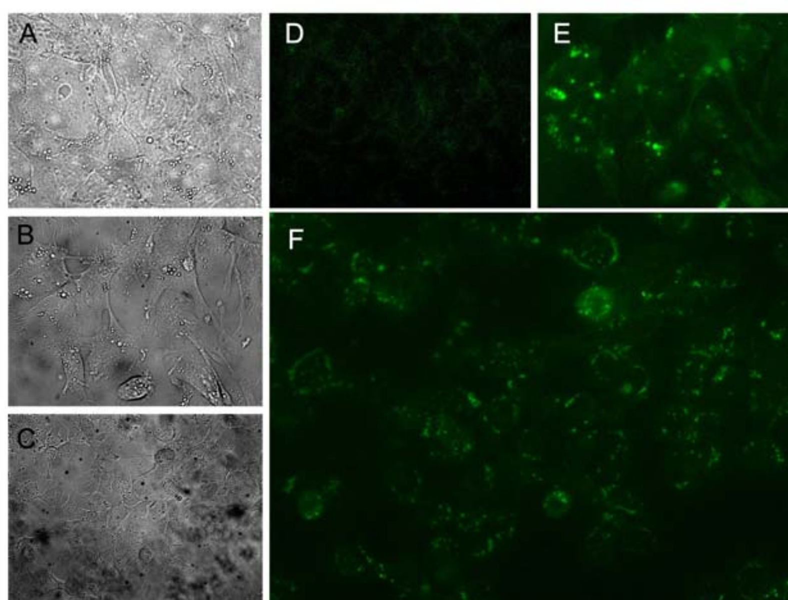
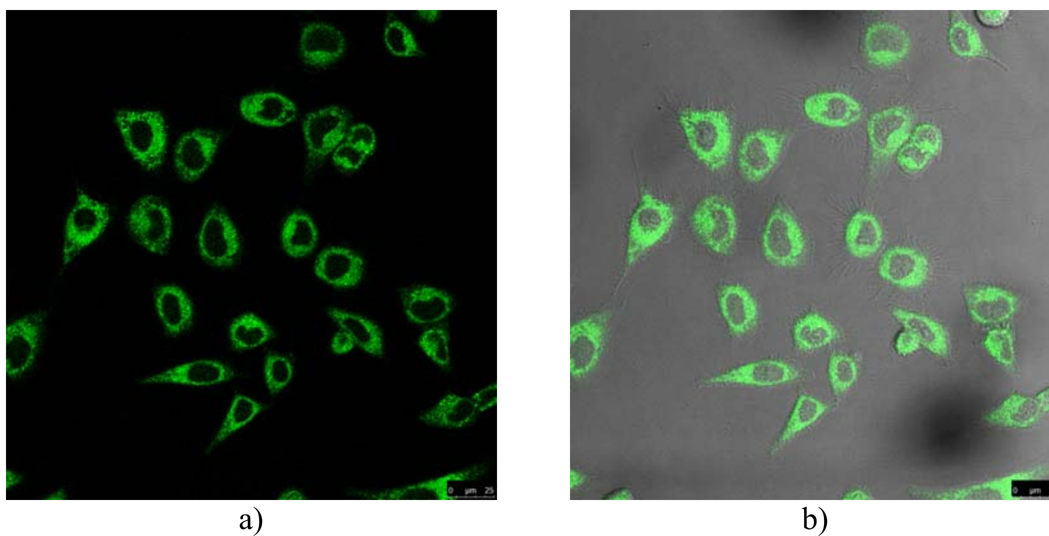


Fig. S8 Hep 3B hepatic cells were treated with compound 1 and analyzed with inverted fluorescence microscope after 24 h of incubation at the stated concentrations. (A,D): Control; (B,E): 20 μM ; (C,F): 50 μM. A-C: phase-contrast analysis; D-F: fluorescence imaging.



a) b)

Fig. S9 a) Two-photon *In-vitro* cytoplasm staining microscopy study in HeLa cells with **1** (λ_{ex} = 720 nm, dosage concentration = 15 μM for 1 hr; b) the corresponding bright field image.

***In vivo* athymic nude mice experiment:** Eight weeks old athymic nude mice, weighing approximately 15-20 g, were maintained in a sterile facility, in accordance with the institutional guidelines on animal care, with the required consistent temperature and relative humidity. All the procedures were approved by the Animal Research Ethics Committee. Six athymic nude mice were injected subcutaneously with the human hepatocellular carcinoma Hep3B cells. Tumor size was measured by the electronic calliper daily. When tumor size reached a mean volume of about 200 mm^3 where tumor volume was calculated by the formula (length × width × width)/2, they were randomly divided into two groups. Compound at a concentration of 10 mg/kg body weight/day was administrated intraperitoneally for a continuous duration of 10 days starting from day 1 to day 10. Control group received only carrier. Each group consists of 3 mice. On day 11, mice were sacrificed. and whole blood was also collected and plasma liver enzymes including alanine aminotransferase and asparate aminotransferase were measured by the Vet biochemistry assay kits for the IDEXX laboratories machine to determine if there are any liver failure phenomena from both groups of mice by comparing with the normal control ranges.

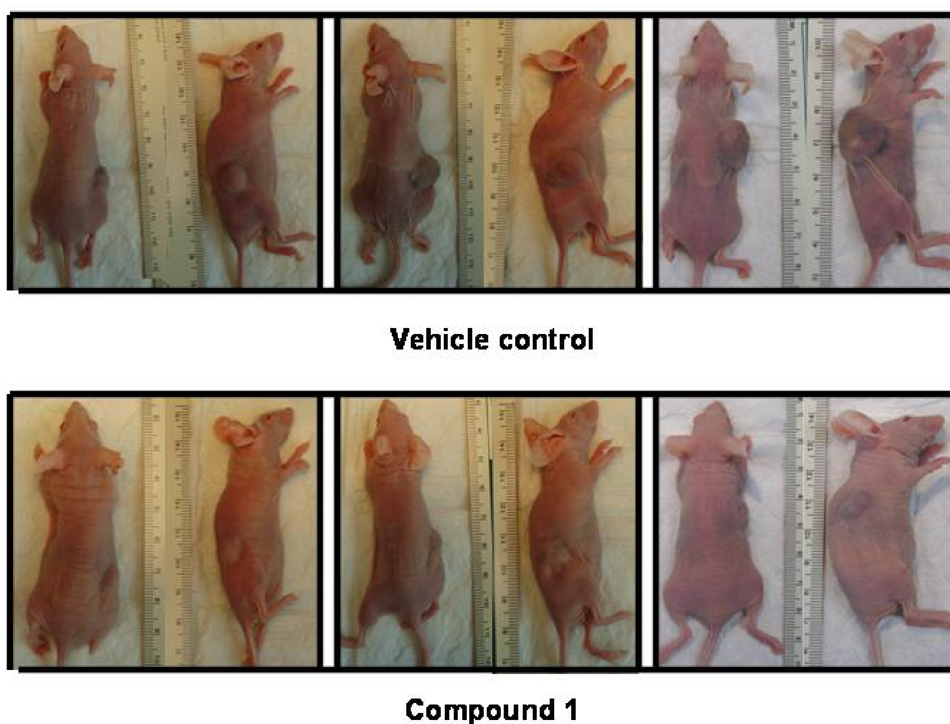


Fig. S10 Hep3B xenografted nude mice treated with vehicle (upper) or **1** (lower) for single dose daily continuously for 10 days from day 1 to day 10. Notice that the tumor size of Hep3B xenograft from control group is much bigger than that of treatment group.

Table S1. Photophysical data for **1** in DMSO at 10^{-5} M.

	λ_{abs} (nm) [a]	τ (ns) [b]	ϕ [c]	σ_2 (GM) [d]	λ_{em} (nm) [e]
1	257 (2.5), 347 (0.33)	13.6	0.31	92	533
1-Me	262 (5.2), 350 (0.73)	15.9	0.43	38	541
1-Ph	264 (3.2), 292 (3.7), 348 (0.6)	16.2	0.30	58	549
1-TMS	263 (1.1), 343 (0.15)	15.8	0.15	75	528
2	259 (2.3), 341 (0.8)	17.1	0.28	21	530
3	260 (1.3), 345 (0.36)	16.2	0.33	10	522
DN [f]	265 (1.9), 348 (0.5)	15.9	0.47	3	530

[a] Molar absorption coefficient (10^5 M^{-1} cm^{-1}) in parentheses; [b] Lifetime (λ_{ex} = 350 nm, λ_{em} = 510 nm); [c] Quantum yield (λ_{ex} = 350 nm); [d] Maximum two-photon absorption cross section against Rhodamine 6G; [e] λ_{ex} = 350 nm and 700 nm; [f] DN = dansylhydrazine.

Table S2. Plasma liver enzyme assays of nude mice for vehicle control with xenograft (column 1), **1** with xenograft (column 2), vehicle control without xenograft (column 3) and **1** without xenograft. N=3 for all the groups. Enzymatic levels were determined by the IDEXX machine using its veterinary biochemistry kits and are expressed as units per litre. Results represent the mean ± SD.

	Control With xenograft	**1** [10 mg/kg/d] With xenograft	Control No xenograft	**1** [30 mg/kg/d] No xenograft	Reference (U/L)
ALT	42.3±4.5	45.0±9.2	31.5±3.7	32.3±4.6	28–132
AST	105.7±40.5	71.3±13.1	50.6±13.5	57.3±4.9	59–247
ALKP	126.3±43.1	81.7±33.6	96.1±2.2	88.7±4.0	62–209

Tolerance test: Eight weeks old athymic nude mice, weighing approximately 15-20g, were maintained in a sterile facility, in accordance with the institutional guidelines on animal care, with the required consistent temperature and relative humidity. All the procedures were approved by the Animal Research Ethics Committee. Compound at a concentration of 30 mg/kg body weight/day was administrated intraperitoneally for a continuous period of 10 days starting from day 1 to day 10. Control group received only carrier. Each group consists of 3 mice. On day 11, mice were sacrificed and whole blood was also collected and plasma liver enzymes including alanine aminotransferase, asparate aminotransferase and alkaline phosphatase were measured by the Vet biochemistry assay kits for the IDEXX laboratories machine to determine if there are any liver failure phenomena from both groups of mice by comparing with the normal control ranges.

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