Targeted delivery of maytansine to liver cancer cells *via* galactose-modified supramolecular two-dimensional glycomaterial

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S1. Experimental section

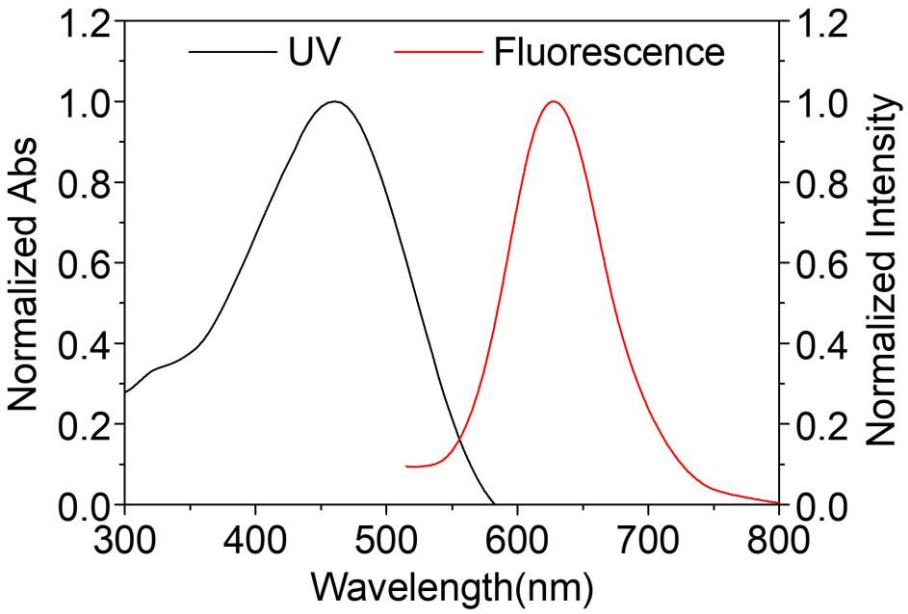


Fig. S1 UV-vis absorption and fluorescence spectra of **Gal-DCM** in Tris-HCl (0.01 M, pH 7.4).

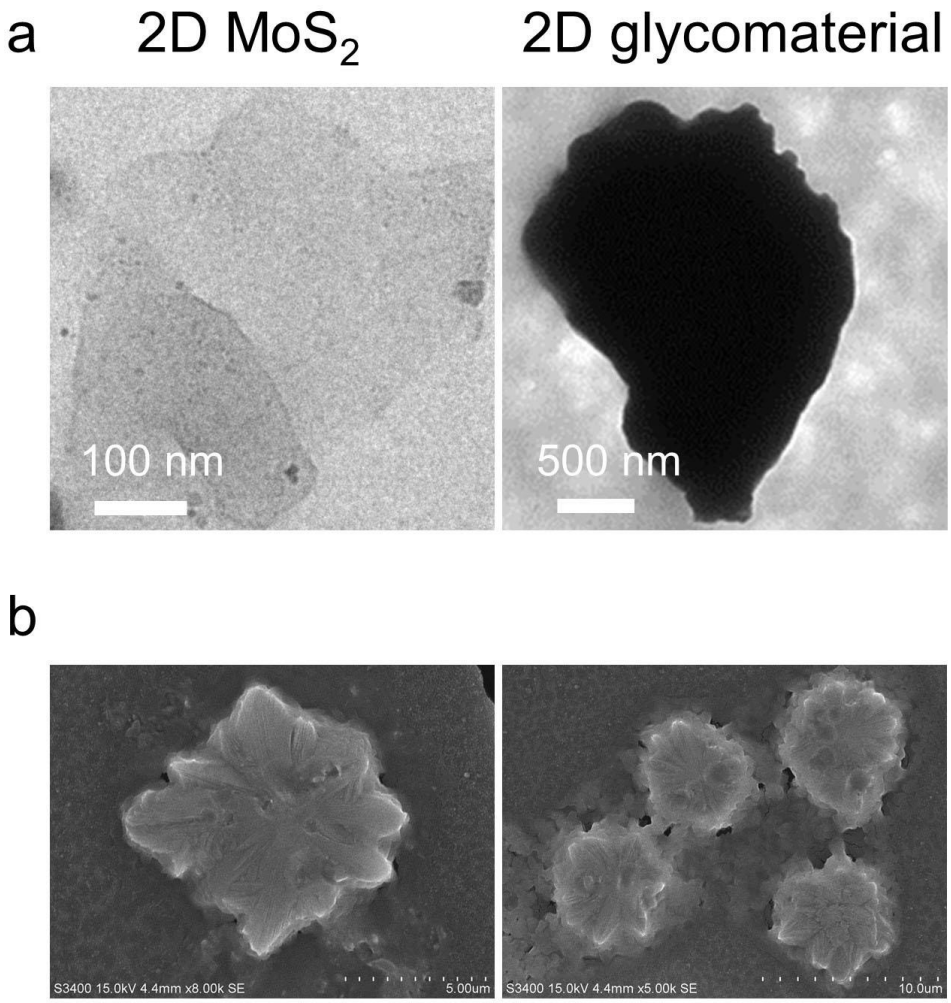


Fig. S2 (a) High-resolution transmission electron microscopy (HRTEM) and (b) scanning electron microscopy (SEM) images of 2D MoS_2 (left, 1 μg mL^{-1}) and 2D glycomaterial (right, **Gal-DCM**/**Maytansine**/HSA/2D MoS_2 = 1 μM/20 nM/1 μM/1 μg mL^{-1}).

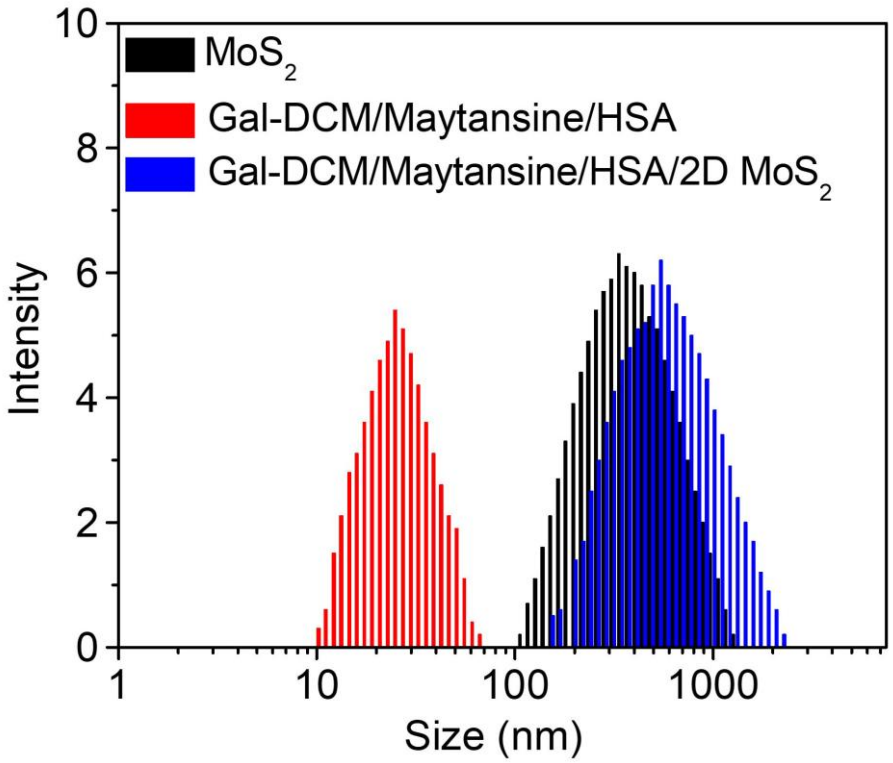


Fig. S3 Dynamic light scattering (DLS) of 2D MoS_2 (1 μg mL^{-1}) and glycoprotein (**Gal-DCM**/**Maytansine**/HSA = 1 μM/20 nM/1 μM) and 2D glycomaterial (**Gal-DCM**/**Maytansine**/HSA/2D MoS_2 = 1 μM/20 nM/1 μM/1 μg mL^{-1}).

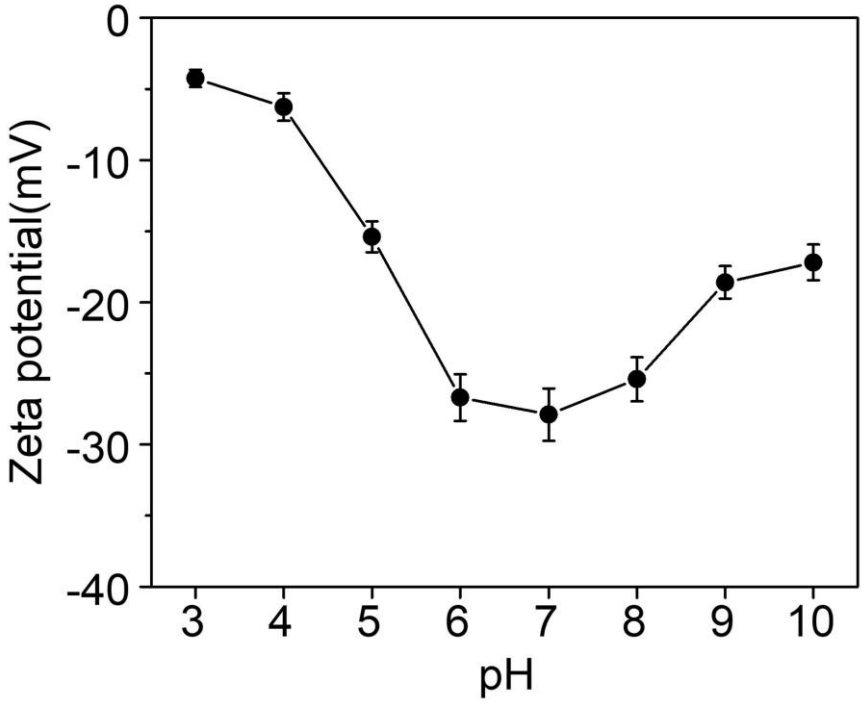


Fig. S4 Zeta potential of the 2D glycomaterial (**Gal-DCM/Maytansine/HSA/2D MoS_2** = 5 μM/0.1 μM/5 μM/5 μg mL^{-1}**)** at different indicated pH.

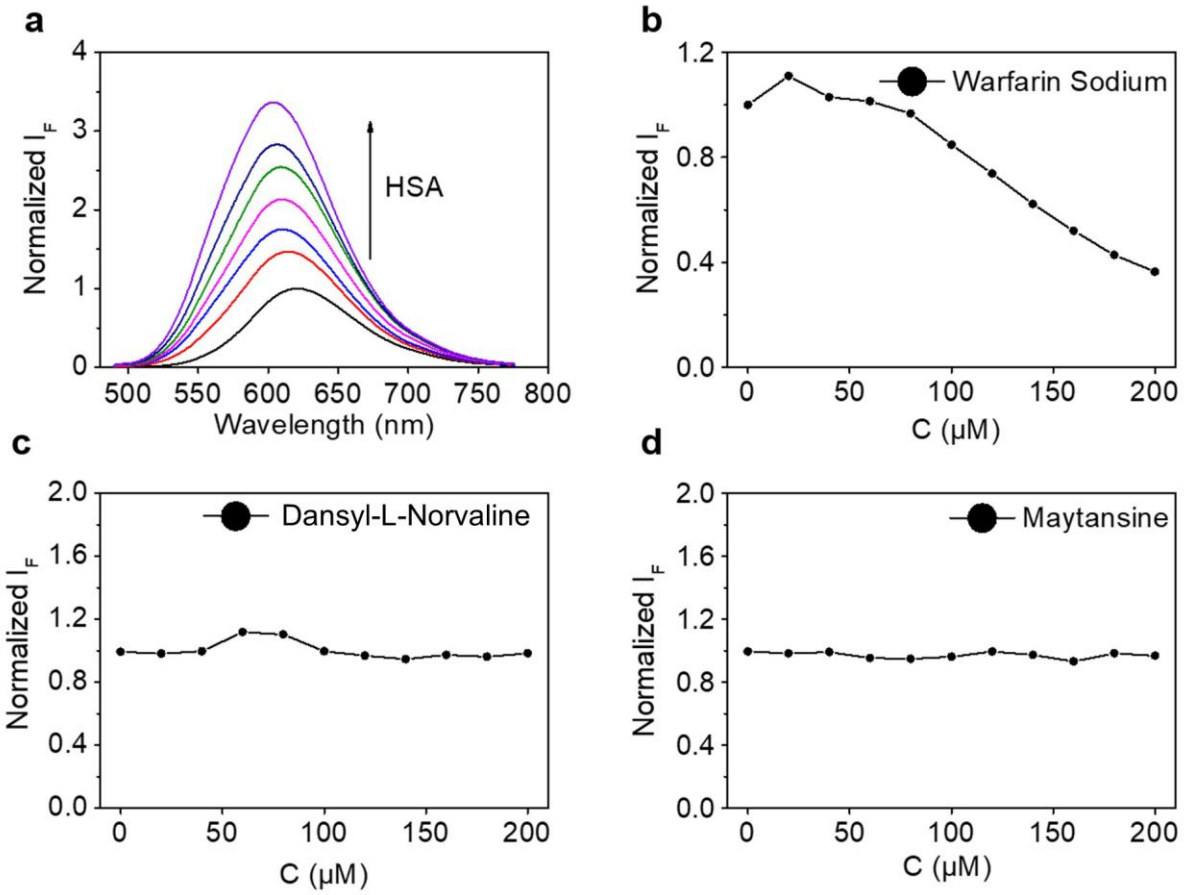


Fig. S5. (a) Fluorescence emission spectra of **Gal-DCM** (1 μM) with increasing concentrations of HSA (0-18 μM; interval: 3 μM). Fluorescence changes of **Gal-DCM** (1 μM) with HSA (18 μM) preincubated with increasing concentrations of (b) warfarin sodium (0-200 μM; interval: 20 μM), (c) dansyl-l-norvaline (0-200 μM; interval: 20 μM), and (d) **Maytansine** (0-200 μM; interval: 20 μM). All measurements were carried out in Tris-HCl (0.01 M, pH 7.4) with an excitation of 460 nm.

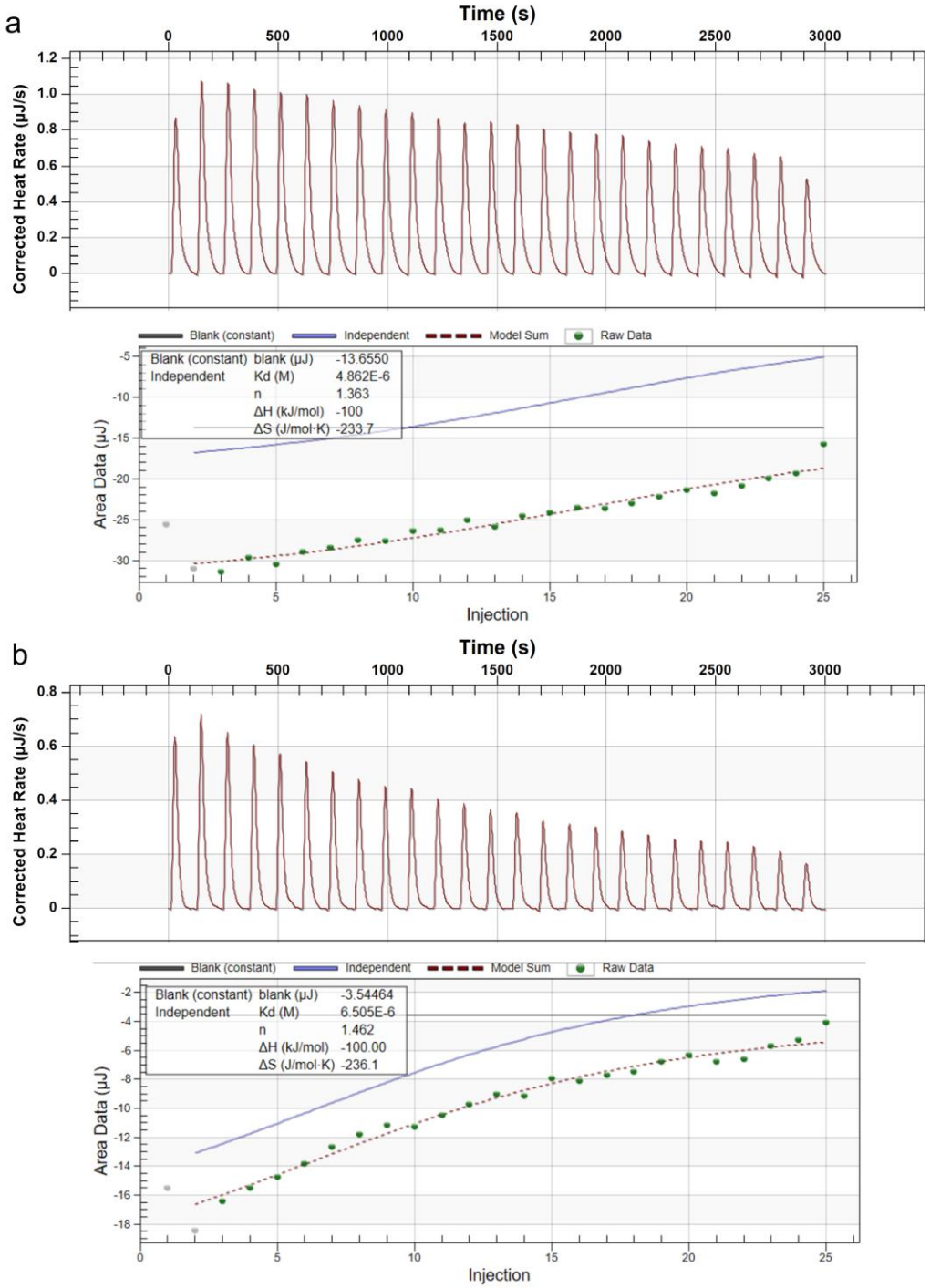


Fig. S6. Isothermal titration calorimetric (ITC) analysis for determining the association constant of **Gal-DCM** (100 nM) and **Maytansine** (100 nM) with HSA (1 μM) at 25 °C in H_2O (containing 1% DMSO, v/v). The first two titration points marked in grey was discarded during data fitting.

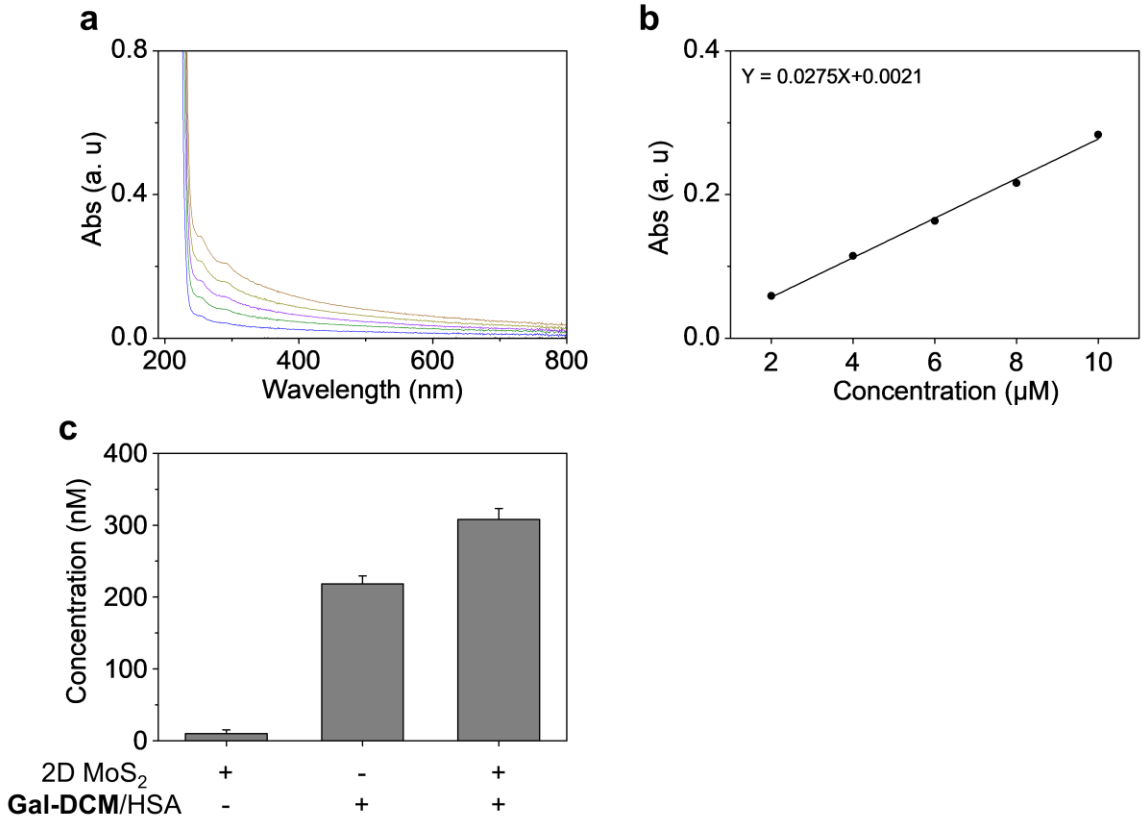


Fig. S7. (a) UV-vis absorption spectra of different concentrations of **Maytansine** (from bottom to top curve: 2, 4, 6, 8 and 10 μM) in Tris-HCl (0.01 M, pH 7.4). (b) Plotting the UV-vis absorption intensity at 252 nm as a function of concentration of **Maytansine** in 0.01 M Tris-HCl (pH 7.4). (c) Quantification of the **Maytansine**-loading capacity of 2D MoS_2 (5 μg mL^{-1}), **Gal-DCM**/HSA (5 μM/5 μM) and **Gal-DCM**/2D MoS_2/HSA (5 μM/5 μg mL^{-1}/5 μM). **Maytansine** (0.1 μM) was incubated with different materials for 30 min. The resulting mixtures were centrifuged (10000 rpm) for 5 min, and the supernatants were collected, in which the remaining **Maytansine** concentrations were determined through UV-vis spectroscopy.

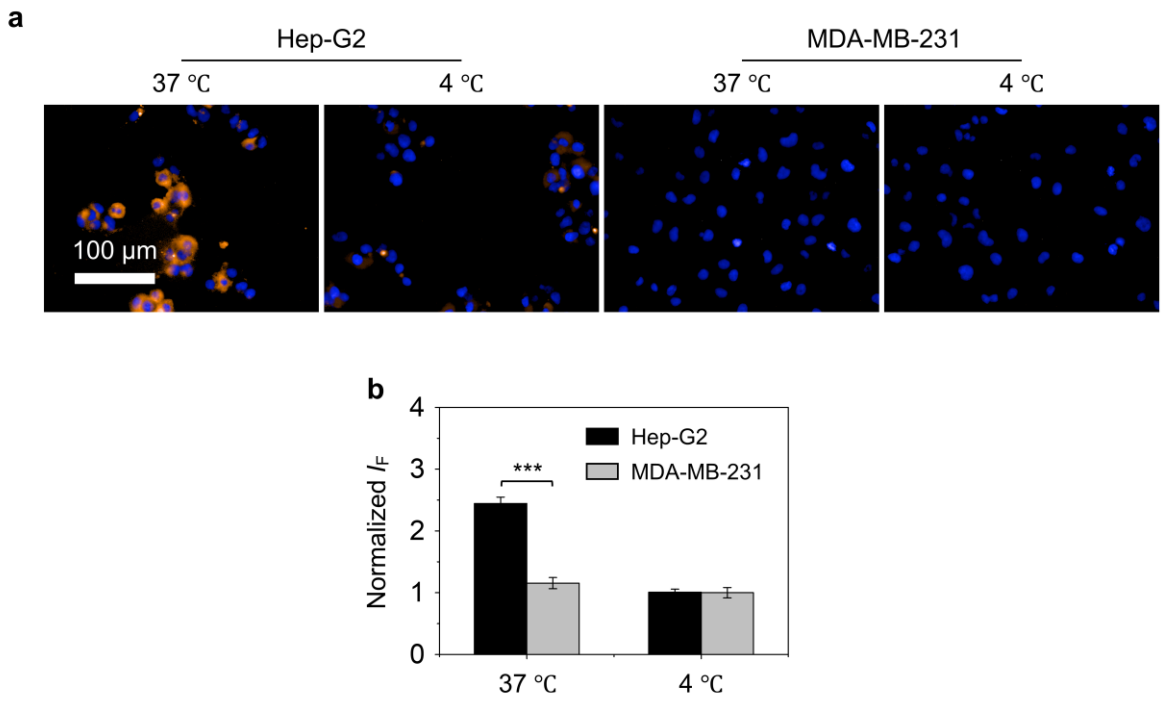
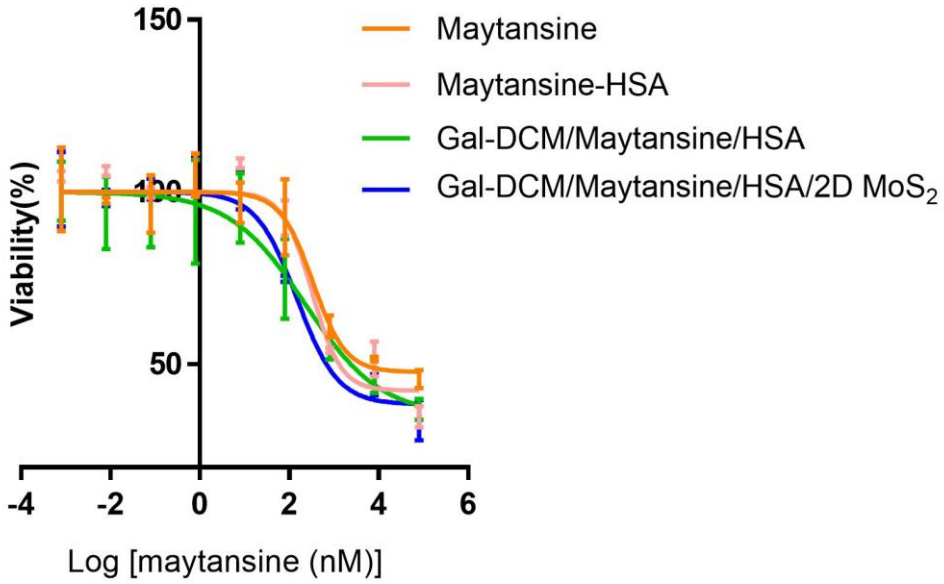


Fig. S8. (a) Fluorescence imaging and (b) quantification of Hep-G2 (human liver cancer) and MDA-MB-231 (human breast cancer) cells after incubation with the 2D glycomaterial (**Gal-DCM**/HSA/2D MoS_2 = 5 μM/5 μM/5 μg mL^{-1}) at different temperatures. ***P<0.001. S. D. means standard deviation (n = 3). The excitation and emission channel used is 460-490 nm and 580-650 nm, respectively.

a



b

	IC_{50} (μM)
Maytansine	0.34 ±0.11
Maytansine-HSA	0.32 ±0.09
Gal-DCM/Maytansine/HSA	0.23 ±0.17
Maytansine-loaded 2D glycomaterial	0.15 ±0.05

Fig. S9. (a) Dose-response curves of the viability of Hep-G2 cells exposed to free **Maytansine**, **Maytansine**-HSA, **Gal-DCM**/**Maytansine**/HSA and **Maytansine**-loaded 2D glycomaterial. (b) IC_{50} values of free free **Maytansine**, **Maytansine**-HSA, **Gal-DCM**/**Maytansine**/HSA and **Maytansine**-loaded 2D glycomaterial in Hep-G2 cells as determined *via* MTS assay. All the values are expressed as mean ± standard deviation; n = 3.

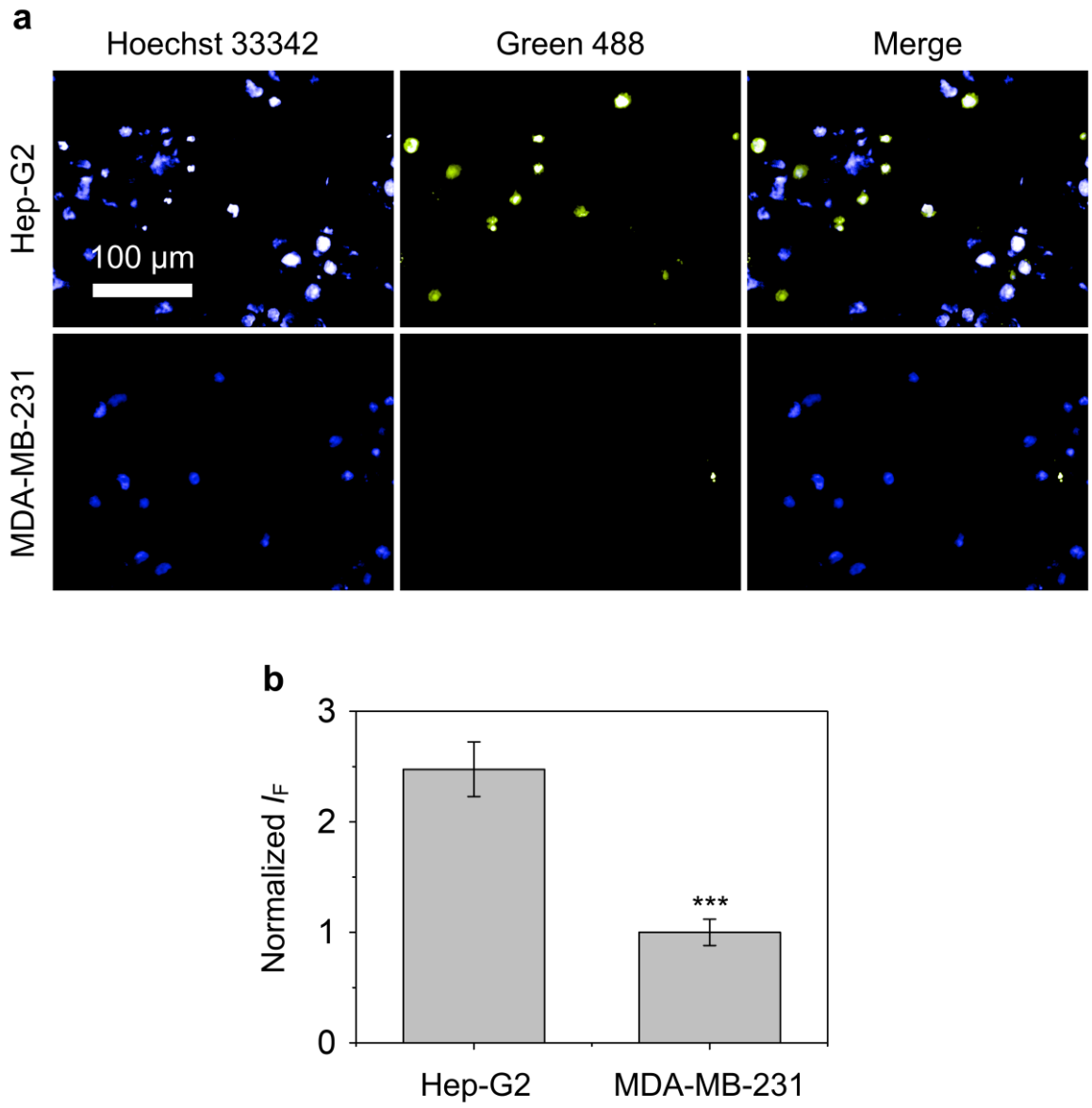


Fig. S10. (a) Fluorescence imaging and (b) quantification of EasyProbe Green 488 in Hep-G2 (human liver cancer) and MDA-MB-231 (human breast cancer) cells treated with the **Maytansine**-loaded 2D glycomaterial (**Gal-DCM**/**Maytansine**/HSA/2D MoS_2 = 5 μM/0.1 μM/5 μM/5 μg mL^{-1}). ***P<0.001. S. D. means standard deviation (n = 3). Hoechst 33342 (λ_{ex} = 360–400 nm, λ_{em} = 410–480 nm) and EasyProbe Green 488 (λ_{ex} = 460-490 nm, λ_{em} = 500-550 nm) were used to stain the total and dead cells, respectively.

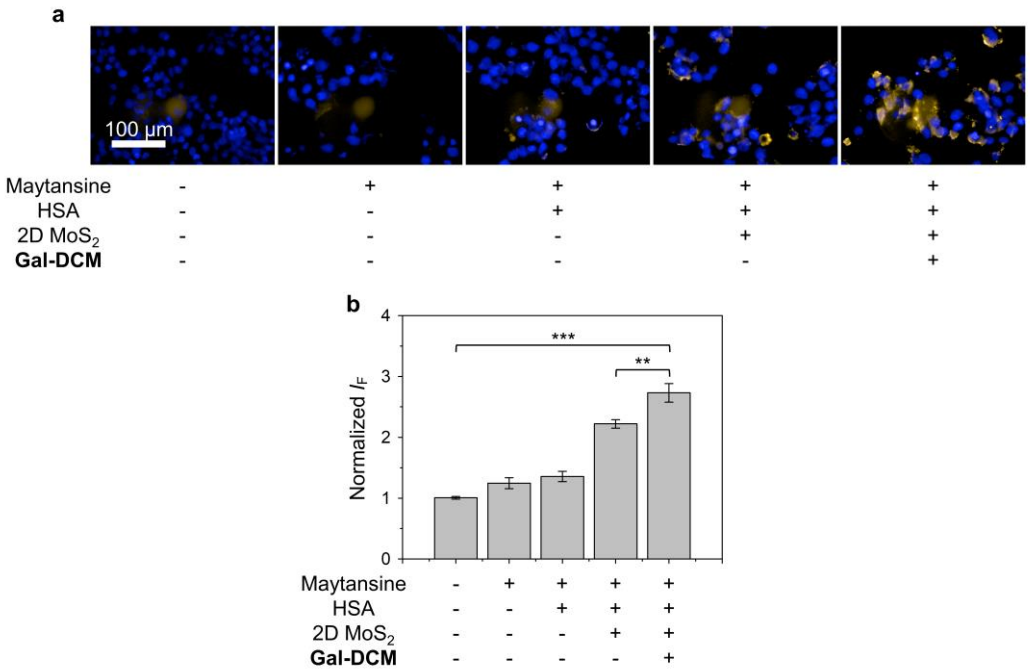
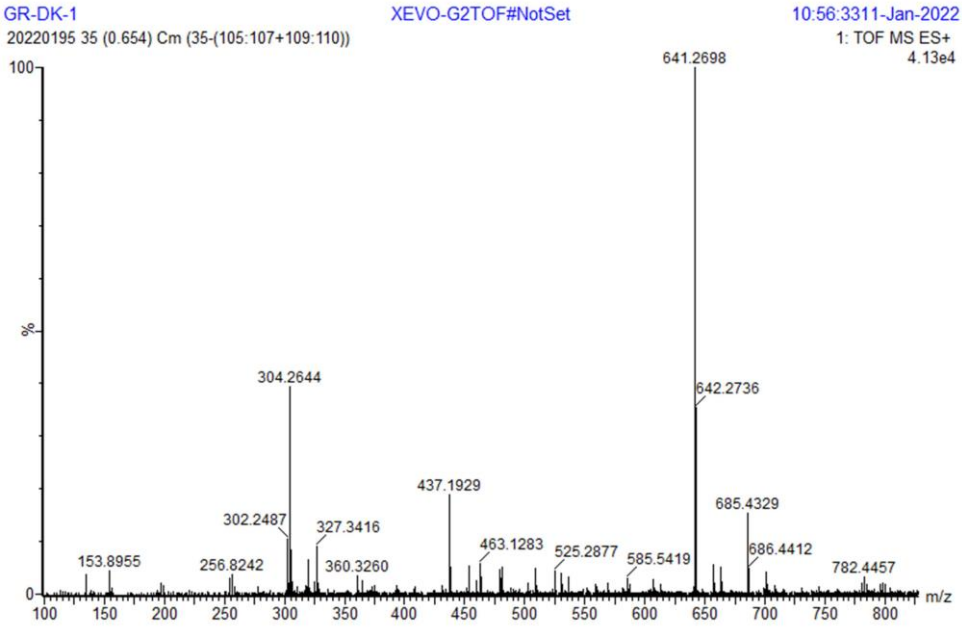


Fig. S11. (a) Fluorescence imaging and (b) quantification of Annexin V-mCherry in Hep-G2 (human liver cancer) cells in the absence and presence of **Maytansine** (0.1 μM), **Maytansine**/HSA = 0.1 μM:5 μM, **Maytansine**/HSA/2D MoS_2 = 0.1 μM:5 μM:5 μg mL^{-1}, or **Gal-DCM**/**Maytansine**/HSA/2D MoS_2 = 5 μM/0.1 μM/5 μM/5 μg mL^{-1}. ***P* < 0.01, ****P*< 0.001. S. D. means standard deviation (n = 3). Hoechst 33342 (λ_{ex} = 360– 400 nm, λ_{em} = 410–480 nm) and Annexin V-mCherry (λ_{ex} = 520– 550 nm, λ_{em} = 580– 650 nm) were used to stain the total cells and to assess the presence of apoptosis in cells, respectively.

S2. Experimental section

General. All purchased chemicals and reagents are of analytical grade. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM 400 MHz spectrometer with tetramethylsilane (TMS) as internal reference. UV-vis Absorption spectra were measured on a Varian Cary 500 UV-Vis spectraphotometer. High resolution mass spectra (HRMS) were recorded with a Waters Micromass LCT mass spectrometer. Dynamic light scattering (DLS) and zeta potential were carried out on a Horiba LB-550 Nano-Analyzer. Isothermal titration calorimetry (ITC) was performed with Affinity ITC.

Gal-DCM. HR-ESI-MS *m/z*: $[M + Na]^+$ calcd for $C_{32}H_{38}N_6O_7Na^+$ 641.2694, found 641.2698.



Preparation of thin-layer MoS_2. Thin-layer MoS_2 was obtained through sonication-assisted exfoliation of commercially available bulk MoS_2 crystals in an EtOH/H_2O mixture according to a previous literature report (*Adv. Mater.*, 2016, **28**, 9356-9363).

High-resolution transmission electron microscope (HRTEM). A droplet of 2D MoS_2 (1 μg mL^{-1}) or 2D glycomaterial (**Gal-DCM**/**Maytansine**/HSA/MoS_2 = 1 μM:20 nM:1 μM:1 μg mL^{-1}) was dropped onto 200 mesh holey carbon copper grids. Then, the images were recorded with JEOL 2100 equipped with a Gatan Orius charged-coupled device camera and Tridiem energy filter operating at 200 kV.

Scanning electron microscope (SEM). A droplet of 2D MoS_2 (1 μg mL^{-1}) or 2D glycomaterial (**Gal-DCM**/**Maytansine**/HSA/MoS_2 = 1 μM:20 nM:1 μM:1 μg mL^{-1}) was cast onto a freshly

cleaved mica surface, followed by drying at room temperature. Then, SEM of the materials was carried out with S-3400N (HITACHI, Japan).

Self-assembly of 2D glycomaterial. To a phosphate buffered saline (PBS, 0.05 M, pH 7.4) solution of HSA (1 mL, 5 μM), galactosyl glycoprobe **Gal-DCM** (10 μL, 5 μM) and **Maytansine** (10 μL, 100 nM) were added successively. The resulting mixture was sonicated for 20 min. Then, the mixture (**Gal-DCM**/**Maytansine**/HSA) was further added to a 2D MoS_2 water suspension (1 mL, 5 μg mL^{-1}) and sonicated for 20 min. The resulting mixture was used as prepared.

Raman spectroscopy. Raman spectra of 2D MoS_2 (1 μg mL^{-1}; Tris-HCl buffer), 2D glycomaterial (**Gal-DCM**/**Maytansine**/HSA/MoS_2 = 1 μM :20 nM:1 μM: 1 μg mL^{-1}) were obtained using a Renishaw In Via Reflex Raman system (Renishaw plc, Wotton-under-Edge, UK) employing a grating spectrometer with a Peltier-cooled charge-coupled device detector coupled to a confocal microscope. The raw data obtained were processed with Renishaw WiRE 3.2 software. The Raman scattering was excited by an argon ion laser (I = 514.5 nm).

Cell culture. Hep-G2 cells were maintained in a Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10 % fetal bovine serum (Gibco, Gland Island, NY, USA). MDA-MB-231 cells were cultured in RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 5 % fetal bovine serum (Gibco, Gland Island, NY, USA). All cells were cultured in a humidified atmosphere of 5% CO_2 and 95% air at 37℃ and split when the cells reached 90% confluency.

Fluorescence imaging of cells. Cells were cultured in growth medium supplemented with 10% FBS. Then, cells were seeded on a black 96-well microplate with optically clear bottom (Greiner bio-one, Germany) overnight, and then incubated with **Gal-DCM**/HSA/2D MoS_2 for 30 min. For the competition experiment, Hep-G2 cells were preincubated with free D-galactose for 2 h, followed by incubation with **Gal-DCM**/HSA/2D MoS_2 for 30 min. The cells nuclei were stained with Hoechst 33342 (5 μg mL^{-1}) for 10 min. Then, cells were washed with PBS three times. The fluorescence images were recorded using an Operetta high-content imaging system (Perkinelmer, US), and was quantified and plotted by Columbus analysis system (Perkinelmer, US).

Cell viability assay. Hep-G2 cells were seeded on a 96-well cell culture plate and incubated in growth medium supplemented with 10 % FBS overnight at 37°C. Then, different concentrations of free **Maytansine**, **Maytansine**-HSA, **Gal-DCM**/**Maytansine**/HSA

Maytansine-loaded 2D glycomaterial were added, followed by incubation for 30 min. Cells were then washed three times with PBS, followed by addition of fresh medium. Subsequently, the cells were then cultured at 37 °C for 48 h. Then, an MTS/PMS (20:1, Promega, Corp.) solution (10 μL per well) was added to each well. After incubation at 37 °C under 5% CO_2 for 3 h, the absorption of the solutions was measured at 490 nm using an M5 microplate reader (Molecular Device, USA).

Double-staining assay. Hep-G2 and MDA-MB-231 cells were seeded on a 96-well cell culture plate and incubated in growth medium supplemented with 10 % FBS overnight at 37°C. Then, **Gal-DCM**/**Maytansine**/HSA/2D MoS_2 = 5 μM: 0.1 μM: 5 μM: 5 μg mL^{-1} were added, followed by incubation for 30 min. Cells were then washed three times with PBS, followed by addition of fresh medium. Subsequently, the cells were then cultured at 37 °C for 48 h. Thereafter, the cells were incubated with EasyProbes Green 488 (1:100 diluted by PBS) for 30 min and Hoechst 33342 (5 μg mL^{-1}) for 10 min, and then washed by PBS. The fluorescence images were recorded using an Operetta high-content imaging system (Perkinelmer, US), and was quantified and plotted by Columbus analysis system (Perkinelmer, US).

Annexin V-mCherry apoptosis assay. Hep-G2 cells were seeded on a 96-well cell culture plate and incubated in growth medium supplemented with 10 % FBS overnight at 37°C. Then, **Maytansine** (0.1 μM), **Maytansine**/HSA = 0.1 μM: 5 μM, **Maytansine**/HSA/2D MoS_2 = 0.1 μM: 5 μM: 5 μg mL^{-1}, or **Gal-DCM**/**Maytansine**/HSA/2D MoS_2 = 5 μM: 0.1 μM: 5 μM: 5 μg mL^{-1} were added, followed by incubation for 30 min. Cells were then washed three times with PBS, followed by addition of fresh medium. Subsequently, the cells were then cultured at 37 °C for 48 h. Thereafter, the cells were incubated with Annexin V-mCherry for 20 min, and then washed by PBS. The cells nuclei were stained with Hoechst 33342 (5 μg mL^{-1}) for 10 min. Then, cells were washed with PBS three times. The fluorescence images were recorded using an Operetta high-content imaging system (Perkinelmer, US), and was quantified and plotted by Columbus analysis system (Perkinelmer, US).