Electronic Supplementary Material (ESI) for RSC Advances

Novel Self-Assembled Lithocholic Acid Nanoparticles for Durg Delivery in Cancer

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Materials and Methods:

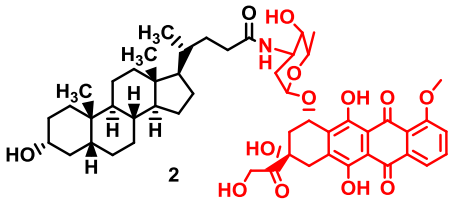
Material:

All reactions were performed under inert conditions unless otherwise indicated. All commercially obtained compounds were used without further purification. Ethyl acetate, petroleum ether, dry dichloromethane (DCM), methanol, dry dimethylformamide (DMF), lithocholic acid, chromium trioxide, acetic anhydride, sodium sulphate, pyridine, *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), O-(Benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU), L-α-phosphatidylcholine, sephadex G-25 and silicon wafer for FE-SEM were bought from Sigma-Aldrich. Paclitaxel, PI103 and doxorubicin were bought from Selleck Chemicals. 1,2-Distearoyl-*sn*-Glycero-3-Phosphoethanolamine-N-[Amino(Polythylene Glycol)2000] (DSPE-PEG) and the mini handheld Extruder kit (including 0.2 μm Whatman Nucleopore Track-Etch Membrane, Whatman filter supports and 1.0 mL Hamiltonian syringes) were bought from Avanti Polar Lipids Inc. Analytical thin-layer chromatography (TLC) was performed using precoated silica gel aluminium sheets 60 F_{254} bought from EMD Laboratories. DMEM media and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from HiMedia and HeLa cells were obtained from National Centre for Cell Science (NCCS), Pune. Spots on the TLC plates were visualized using alkanine permanganate or phosphomolybdic acid hydrate in methanol. ^{1}H and ^{13}C NMR (400 MHz) spectra were obtained on a Jeol-400 spectrophotometer. The chemical shifts are expressed in parts per million (ppm) using suitable deuterated NMR solvents with reference to TMS at 0 ppm. The release kinetic data, drug loading, nanoparticle size and cell viability assay were plotted using GraphPad Prism software. Each sample was done in triplicate. LysoSensor™ Green DND-153 was purchased from Invitrogen and Hoechst 33342 was purchased from Cell Signaling Technology. N-Propyl gallate was purchased from Sigma-Aldrich. The laser scanning

confocal microscopy was performed by Zeiss LSM 710 machine.

Methods:

Synthesis of lithocholic acid-doxorubicin conjugate (2):



Lithocholic acid (5 mg, 0.0132 mmol, 1 equiv) was dissolved in 1 mL dry DMF. After continuous stirring under inert condition at 0^0C for 5 min, HBTU (15 mg, 0.0396 mmol, 3 equiv) was added followed by addition of DIPEA (11.49 µL, 0.066 mmol, 5 equiv). The reaction mixture was cooled down for 10 min and doxorubicin (9.1 mg, 0.0158 mmol, 1.2 equiv) was added to it. The reaction was stirred at RT for 24 h. The reaction was then quenched using H_2O (40 mL). To remove dissolved salts present in the reaction mixture, wash with brine solution was given (2 X 30 mL). Anhydrous sodium sulphate (Na_2SO_4) was added to the organic layer to remove the trace amount of water present. Organic layer was then concentrated using rotary evaporator. Crude product was purified by silica gel column chromatography by using 2 % methanol in DCM to obtain product as a red colored solid.

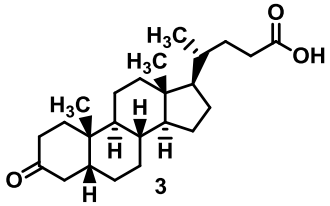
Yield: 74.7%.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.05-8.03 (dd, *J* = 7.6 Hz, 0.8 Hz, 1H), 7.80-7.76 (t, *J* = 8 Hz, 1H), 7.40-7.37 (dd, *J* = 6.8 Hz, 0.8 Hz, 1H), 6.65 (s, 1H), 5.82-5.80 (d, *J* = 8.4 Hz, 1H), 5.49-5.48 (m, 1H), 5.30-5.20 (m, 1H), 4.76-4.75 (m, 2H), 4.55 (bs, 1H), 4.20-4.14 (m, 2H), 4.07 (s, 3H), 3.63-3.57 (m, 2H), 3.30-3.25 (m, 1H), 3.04-2.99 (m, 2H), 2.35-2.31 (m, 1H), 2.21-2.14 (m, 2H), 2.06-1.98 (m, 1H), 1.93-1.60 (m, 8H), 1.55-1.31 (m, 7H), 1.29-1.23 (m, 9H), 1.13-0.97 (m, 4H), 0.89 (s, 3H), 0.88-0.86 (d, *J* = 6.4 Hz, 3H), 0.60 (s, 3H).

^{13}C NMR (100 MHz): δ = 214.1, 188.4, 187.3, 173.1, 171.1, 161.2, 156.8, 151.9, 143.8, 139.4, 133.7, 130.9, 130.1, 128.9, 124.2, 123.6, 120.0, 118.5, 116.0, 114.2, 100.9, 72.0, 69.9, 67.3, 65.7, 56.8, 56.6, 56.1, 55.1, 54.0, 52.8, 52.2, 51.0, 36.5, 35.9, 35.6, 33.9, 32.1, 31.7, 29.8, 29.6, 29.5, 29.3, 29.1, 27.3, 26.5, 23.5, 22.8, 18.5, 14.3, 12.2.

HRMS (ESI): m/z: for $C_{51}H_{67}NO_{13}Na^+$ [M+Na]+ : calculated = 924.4509, observed= 924.4525.

Synthesis of keto-lithocholic acid by Jones oxidation (3):



In 5 mL glass vial 0.350 mg CrO_3 and 0.350 mL concentrated H_2SO_4 were added to obtain reddish brown suspension. After 5 min 0.9 mL water was added to the suspension in a dropwise manner. Whole addition was done in 0°C. Prepared Jone's reagent (0.6 mL, 1 mmol, 7.7 equiv) was added dropwise to a solution of lithocholic acid (50 mg, 0.13 mmol, 1 equiv) in 6 mL dry acetone and stirred at 0°C under inert atmosphere for 1h. Reaction was monitored by TLC. After completion of reaction, isopropenol was added dropwise to destroy excess Jone's reagent, as indicated by the appearance of deep green color. The reaction mixture was then concentrated under reduced

pressure and diluted with DCM (20 mL), washed with saturated $NaHCO_3$ (2 X 20 mL) solution and water (2 X 20 mL), dried over anhydrous Na_2SO_4 and concentrated. The crude material was purified by flash chromatography through a plug of silica gel using EtOAc : pet ether = 1:4 to give compound **3** as colorless white solid.

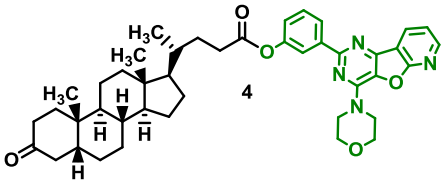
Yield: 53%.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.72-2.65 (t, *J* = 14 Hz, 1H), 2.42-2.21 (m, 3H), 2.04-1.99 (m, 3H), 1.91-1.76 (m, 4H), 1.62-1.40 (m, 6H), 1.38-1.32 (m, 4H), 1.28-1.25 (m, 4H), 1.13-1.08 (m, 3H), 1.10 (s, 3H), 0.93-0.92 (d, *J* = 6.4 Hz, 3H), 0.68 (s, 3H).

^{13}C NMR (100 MHz): δ = 213.8, 180.1, 56.5, 56.1, 44.4, 42.9, 42.5, 40.8, 40.2, 37.3, 35.6, 35.4, 35.0, 31.1, 30.8, 29.8, 28.2, 26.7, 25.9, 24.3, 22.8, 21.3, 18.7, 12.2.

HRMS (ESI): m/z: for $C_{24}H_{38}O_3Na^+$ $[M+Na]^+$: calculated = 397.2718; observed = 397.2714

Synthesis of keto-lithocholic acid-PI103 conjugate (4):



Keto-lithocholic acid (**3**) (5 mg, 0.013 mmol, 1 equiv) was dissolved into 2 mL dry N,N'-dimethylformamide (DMF) and cooled at 0°C added under inert atmosphere. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (5.06 mg, 0.0264 mmol, 2 equiv), 4-dimethylaminopyridine (DMAP) (1.61 mg, 0.013 mmol, 1 equiv) were added into the solution. After 10 min, PI103 (5.09 mg, 0.015 mmol, 1.1 equiv) was added into the reaction mixture then reaction mixture was stirred at room temperature for 24 h. After 24 h, the reaction was quenched with 0.1 N HCl (5 mL) and diluted with DCM (10 mL). The organic layer was extracted with DCM (2 X 20 mL) and washed with brine solution (10 mL). The organic layer was dried on anhydrous sodium sulphate (Na_2SO_4). Organic solvent was then evaporated using rotary evaporator and the crude product was purified using silica gel (100-200 mesh size) column chromatography EtOAC: Pet ether = 1:4 to obtain pure compound **4** as white solid powder.

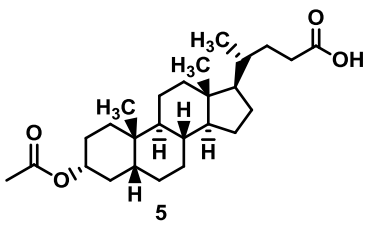
Yield: 96%.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.61-8.59 (m, 2H), 8.37-8.34 (m, 1H), 8.19-8.18 (m, 1H), 7.52-7.46 (m, 2H), 7.20-7.17 (m, 1H), 4.24-4.21 (m, 4H), 3.93-3.91 (m, 4H), 2.73-2.64 (m, 2H), 2.57-2.51 (m, 1H), 2.42-2.29 (m, 2H), 2.19-2.14 (m, 1H), 2.07-1.99 (m, 4H), 1.56-1.51 (m, 8H), 1.28-1.25 (m, 10H), 1.03 (s, 3H), 0.87 (s, 3H), 0.73 (s, 3H).

^{13}C NMR (100 MHz): δ = 213.5, 172.8, 162.7, 158.7, 151.0, 149.6, 148.8, 147.2, 139.9, 133.4, 131.8, 130.9, 129.3, 123.2, 121.4, 121.2, 120.3, 115.4, 114.1, 66.9, 56.5, 56.0, 45.7, 44.3, 42.4, 40.7, 40.1, 37.0, 35.6, 34.9, 31.9, 31.5, 29.7, 29.4, 28.0, 28.2, 26.6, 25.8, 24.2, 22.7, 21.2, 18.4, 14.1, 12.1.

HRMS (ESI): m/z: for $C_{43}H_{53}N_4O_5^+$ $[M+H]^+$: calculated = 705.4017, observed = 705.4026.

Synthesis of aceyl-lithocholic acid (5):



Lithocholic acid (50 mg, 0.1320 mmol, 1 equiv.) was dissolved in 500 µL anhydrous pyridine and cooled at 0^0C under inert atmosphere. Acetic anhydride (500 µL) was added into the reaction mixture. The reaction mixture was stirred for 5h at room temperature. Reaction was monitored by thin layer chromatography (TLC). After the reaction was over, pyridine was removed under vacuum. Then reaction mixture was diluted with 10 mL of DCM and quenched with 5 mL of 0.1 N HCl solutions. The organic layer was washed with 0.1M HCl (2 X 10 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and crude product was purified by silica gel flash column chromatography by using 10% ethyl acetate in petroleum ether to obtain 41.4 mg amorphous white colour compound **5**.

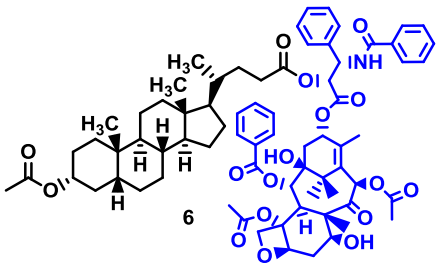
Yield: 75.30%.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.75-4.67 (m, 1H), 2.43-2.36 (m, 1H), 2.29-2.22 (m, 1H), 2.03 (s, 3H), 1.98-1.95 (m, 1H), 1.87-1.78 (m, 5H), 1.69-1.66 (m, 1H), 1.59-1.51 (m, 2H), 1.45-1.34 (m, 7H), 1.29-1.21 (m, 5H), 1.12-1.00 (m, 5H), 0.92 (s, 3H), 0.91 (s, 3H), 0.64 (s, 3H).

^{13}C NMR (100 MHz): δ = 180.3, 170.9, 74.6, 56.6, 56.1, 42.9, 42.0, 40.5, 40.3, 35.9, 35.4, 35.2, 34.7, 32.4, 30.9, 29.8, 28.3, 27.1, 26.8, 26.5, 24.3, 23.5, 21.6, 20.9, 18.4, 12.2.

HRMS (ESI): m/z: for $C_{26}H_{42}O_4Na^+$[M+Na]$^+$: calculated = 441.2980; observed = 441.9742.

Synthesis of acetyl-lithocholic acid-paclitaxel conjugate (6):



Acetylithocholic acid (**5**) (2.5 mg, 0.00597 mmol, 1 equiv) was dissolved in 1 mL dry dichloromethane (DCM) under inert atmosphere and cooled to 0^0C. Subsequently, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) (2.2 mg, 0.01194 mmol, 2 equiv) and 4-(dimethylamino) pyridine (DMAP) (0.7 mg, 0.00597 mmol, 1 equiv) were added to the reaction mixture with continuous stirring under inert condition. After 15 min, paclitaxel (6.11 mg, 0.007154 mmol, 1.2 equiv) was added into the reaction mixture. The reaction was monitored by TLC. After 24 hrs, the reaction was quenched with water (5 mL) and diluted with DCM (10 mL). The organic layer was extracted with DCM (2 X 15 mL) and washed with brine solution (10 mL). The organic layer was dried over anhydrous sodium sulphate (Na_2SO_4). Organic solvent was then evaporated using rotary evaporator and the crude product was purified using silica gel (100-200 mesh size) column chromatography with 17% ethyl acetate in petroleum ether to obtain 5.8 mg of pure compound **6**.

Yield: 73%.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.15-8.14 (m, 1H), 8.13-8.12 (m, 1H), 7.75-7.76 (m, 1H), 7.73-7.72 (m, 1H), 7.63-7.59 (m, 1H), 7.54-7.49 (m, 3H), 7.43-7.40 (m, 3H), 7.37-7.34 (m, 3H), 6.88-6.86 (m, 1H), 6.27-6.23 (m, 1H), 5.96-5.93 (m, 1H), 5.69-5.67 (m, 1H), 5.51-5.49 (m, 1H), 5.01-4.97 (m, 1H), 4.75-4.68 (m, 1H), 4.47-4.42 (m, 1H), 4.33-4.31 (m, 1H), 4.21-4.19 (m, 1H), 3.82-3.80 (m, 1H), 2.51-2.49 (m, 1H), 2.47-2.44 (m, 4H), 2.23 (s, 3H), 2.17-2.13 (m, 2H), 2.03 (s, 3H), 1.95-1.94 (m, 4H), 1.79-1.77 (m, 3H), 1.68 (s, 3H), 1.60-1.59 (m, 10H), 1.25-1.23 (m, 16H), 1.16-1.13 (m, 5H), 0.86 (s, 3H), 0.85 (s, 3H), 0.61 (s, 3H).

^{13}C NMR (100 MHz): δ = 203.9, 173.3, 171.4, 170.8, 169.9, 168.3, 167.2, 167.1, 143.0, 139.4, 137.2, 133.8, 132.8, 132.2, 131.0, 130.4, 129.3, 129.2, 128.9, 128.6, 127.2, 126.7, 124.6, 124.1, 114.2, 84.6, 81.2, 79.3, 75.7, 75.2, 74.5, 73.9, 72.3, 71.9, 58.7, 56.6, 56.1, 52.9, 45.7, 43.3, 42.9, 42.1, 37.2, 35.9, 35.6, 35.4, 34.7, 32.5, 32.4, 32.1, 31.6, 30.9, 30.8, 30.5, 29.8, 29.5, 28.3, 27.1, 27.0, 26.7, 26.4, 24.3, 24.2, 23.1, 22.8, 22.3, 21.6, 20.9, 18.3, 15.0, 14.3, 12.2, 9.7.

HRMS (ESI): m/z: for $C_{73}H_{92}NO_{17}$ $[M+H]^+$: calculated = 1254.6366, observed = 1254.6361, for $C_{73}H_{91}NO_{17}K^+$ $[M+K]^+$: calculated = 1292.5924, observed = 1292.5927.

General procedure of synthesizing self-assembled lithocholic acid-drug nanoparticles:

2.0 mg of L-α-phosphatidylcholine (PC), 1.0 mg of lithocholic acid-drug conjugates (**2**, **4** or **6**) and 0.2 mg of 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Amino(Polythylene Glycol)2000](DSPE-PEG) were dissolved in 5.0 mL DCM. Solvent was evaporated into a thin and uniform lipid-drug film with the help of a rotary evaporator. After thorough drying with vacuum pump lipid-drug film was hydrated with 1.0 mL H_2O for 2 h at 60˚C. It was passed though Sephadex G-25 column and extruded through 200 nm Whatmann polycarbonate membrane at 65˚C to obtain sub 200 nm particles. The self-assembled nanoparticles were stored at 4^{o}C for further use.

General procedure for quantification of drug loading:

A Calibration curve was plotted in the concentration range of 2.5 to 25 μM (for PI103), 10-40 μM (for paclitaxel) and 10-100 μM (for doxorubicin) by diluting the 1 mM standard stock solution of drugs in dimethyl sulfoxide (DMSO). The absorbance was measured at 293 nm, 273 nm and 480 nm for PI103, paclitaxel and doxorubicin respectively against the corresponding solvent blank. The linearity was plotted for absorbance (A) against concentration (C). For checking the drug loadings, PC, lithochholic acid-drug conjugates and DSPE-PEG were used in 1 mg, 0.5 mg and 0.1 mg scale and the lipid layer was hydrated with 1 mL of dd water. For checking drug loading in nanoparticles, prepared nanoparticles were dissolved in spectroscopic grade DMSO in 5%, 10% and 15% dilution. Absorbance was measured at characteristic wave length against the corresponding solvent blank in 400 μL quartz cuvette and from the calibration curve drug loading was measured in triplicates.

General procedure for determining the drug release profile:

Concentrated 250 µL drug loaded nanoparticles were suspended in 250 µL pH = 5.5 buffer and sealed in a dialysis membrane (MWCO= 500 Dalton for PI103 release and 1000 Dalton for paclitaxel and doxorubicin release). The dialysis bags were incubated in 30.0 mL PBS buffer at room temperature with gentle shaking. A 500 µL portion of the aliquot was collected from the incubation medium at predetermined time intervals, and the released drug was quantified by UV-VIS Spectrophotometer.

Determination of size distribution of nanoparticles by dynamic light scattering (DLS):

The mean particle size of the nanoparticles was measured by Dynamic Light Scattering method using Zetasizer Nano2590 (Malvern, UK). 10 µL of nanoparticles solution was diluted to 1ml using DI water and 3 sets of 10 measurements each were performed at 90 degree scattering angle to get the average particle size.

Field-Emission Scanning Electron Microscopy (FESEM) of nanoparticles:

5 µL of nanoparticle in water was placed on a silicon chip without any dopant and it was allowed to dry at room temperature under desiccator. The silicon chip was then gold coated (30-40 nm thickness) using Quorum, Q150T- E5. The FESEM measurements were done using Carl Zeiss, Ultra plus, scanning electron microscope at an operating voltage of 4.0 KV.

Atomic Force Microscopy (AFM) of nanoparticles:

5 µL of nanoparticle in water was placed on mica sheet and dried under the light bulb for 20 min. Shape and size of nanoparticals were determined using NanoWizard Atomic Force Microscopy (AFM).

Cell viability assay:

5000 HeLa cells/well (100 µL) in DMEM was seeded in a 96-well microtitre plate and allowed to attach overnight in 5% CO_2 incubator at 37^0C. Cells were then treated with 100 microlitres of either the free drug or drug conjugated nanoparticle with different concentrations (25, 12.5, 6.25, 3.2, 1.6, 0.8 0.4 and 0.2 µM) and incubated for 48h in 5% CO_2 at 37^0C. After 48 hours, media from all the wells was aspirated and 20 microlitres of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) from a stock solution of 5mg/ml in PBS was added to each well. After an incubation of 3 hours in the incubator, the purple formazan crystals formed were solubilized using acidified isopropanol (62.5 µL conc. HCl in 100 mL isoprpanol) and the absorbance was measured at 540nm. The amount of purple formazan produced by cells treated with free drugs and drug loaded nanoparticles was compared with the amount of formazan produced by untreated control cells to calculate the effectiveness of the test samples.

Cell internalization observed by confocal laser scanning microscopy (CLSM):

HeLa cells (5×10^4) were seeded on cover slip in 24 well plate and incubated overnight at 37 ^{0}C in a CO_2 incubator. The cells were then washed with PBS (pH = 7.4) and treated with free

Doxorubicin and Doxorubicin-NP with the content of Doxorubicin being equivalent to free Doxorubicin at 2 µg/mL for 1 h, 3 h and 6 h. After specified time intervals cells were washed twice with PBS and fixed with 500 µL paraformaldehyde (3.7 % in PBS, pH = 7.2) by incubating for 10 min at 4 ^{0}C. The paraformaldehyde was aspirated and cells were washed with PBS to remove the excess of paraformaldehyde. Low pH lysosomes were stained with 1 µM LysoSensor™ Green DND-153 (Invitrogen) by incubating the cells at 37 ^{0}C in CO_2 incubator for 45 min. The cells were then washed three times to remove the unbound LysoSensor™ Green followed by staining the cells for nuclei with 2 µg/mL Hoechst 33342 (Cell Signaling Technology) by incubating at 37 ^{0}C for 20 min. Then cells were washed three times with PBS buffer and mounted on glass slide using 5 µL antifed-mounting medium. The slides were subjected for fluorescence imaging by a laser scanning confocal microscope (Zeiss LSM 710). The fluorescence of LysoSensor Green, Hoechst 33342 and Doxorubicin was excited with argon laser at 458 nm, 405 nm and 488 nm, and the emission was collected through a 470-509 nm, 403-452 nm and 560-590 nm filters, respectively. Antifed mounting medium was prepared by dissolving 500 mg of *n*-propyl gallate in 10 mL of 0.1 M PBS and 90 mL of glycerol.

Absorbance vs concentration calibration graph of Paclitaxel, PI103 and Doxorubicin: Size and loading of lithocholic acid nanoparticles:

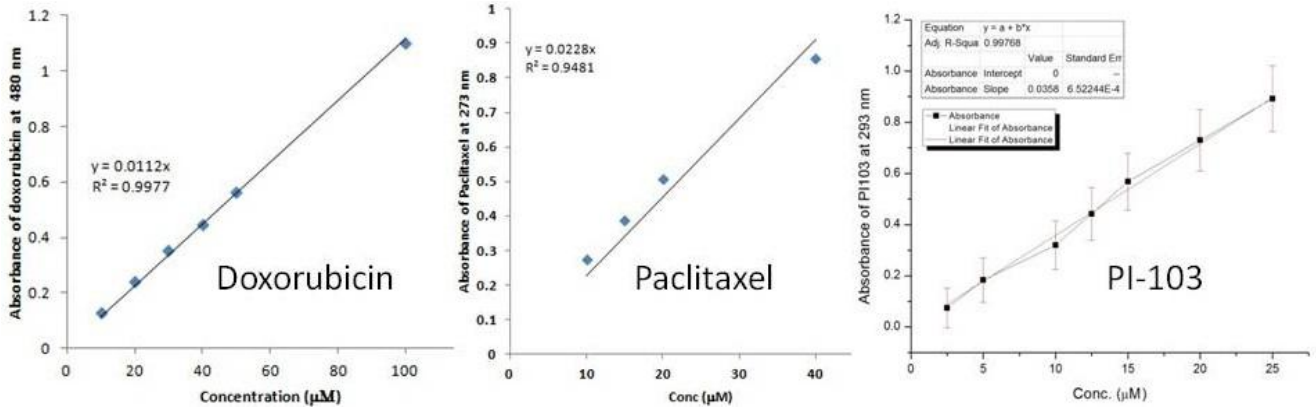


Fig. S1. Concentration vs absorbance calibration curve for doxorubicin, paclitaxel and PI103 at λmax = 480 nm, 273 nm and 293 nm respectively.

Size and loading of drugs in lithocholic acid nanoparticles:

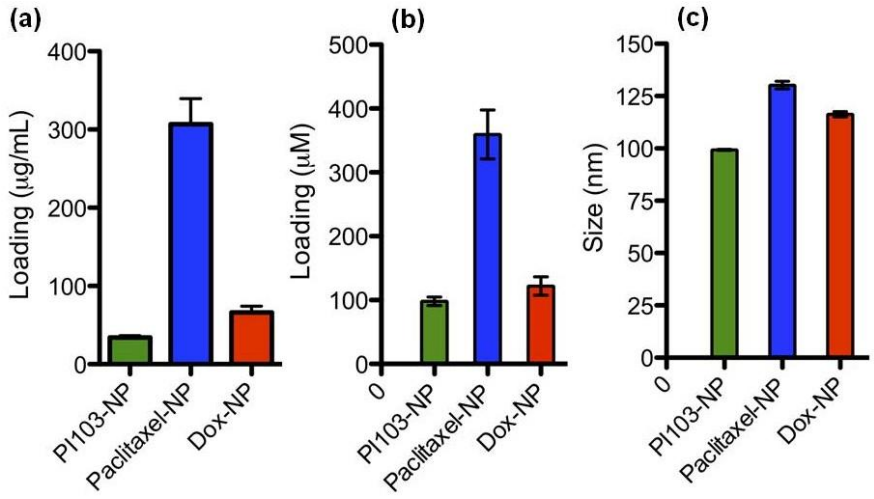


Fig. S2: Size and loading of different drug-loaded nanoparticles.

Size and morphology of the self-assembled nanoparticles by atomic force microscopy (AFM):

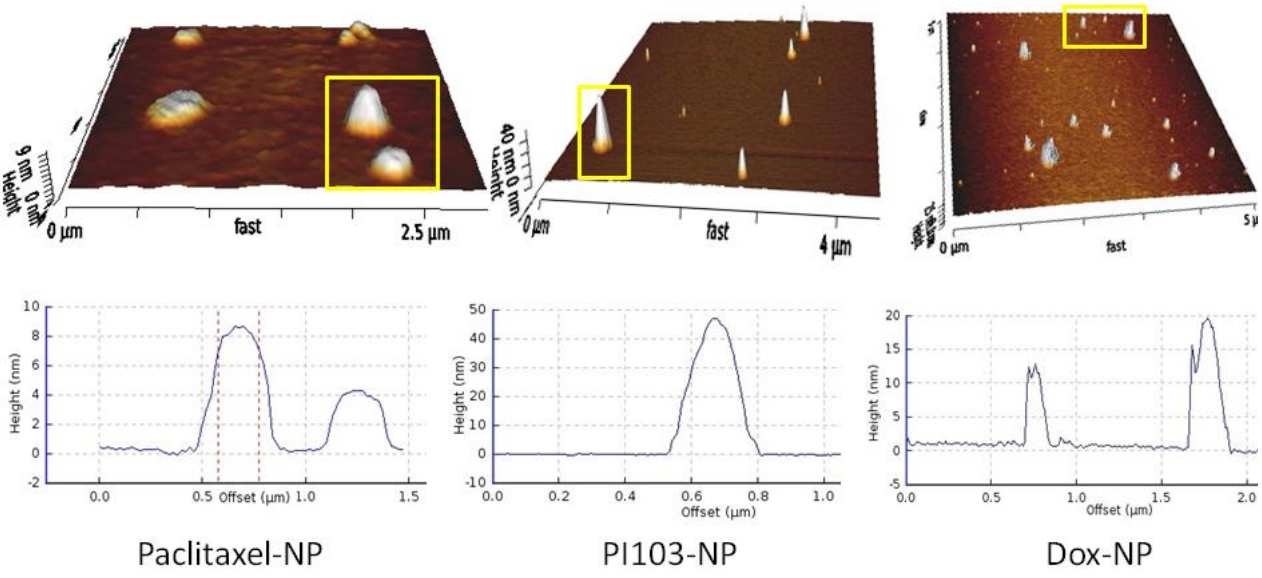


Fig. S3: Size and morphology determination of self-assembled lithocholic acid-NPs by AFM.

Size and morphology of the self-assembled nanoparticles by FE-SEM:

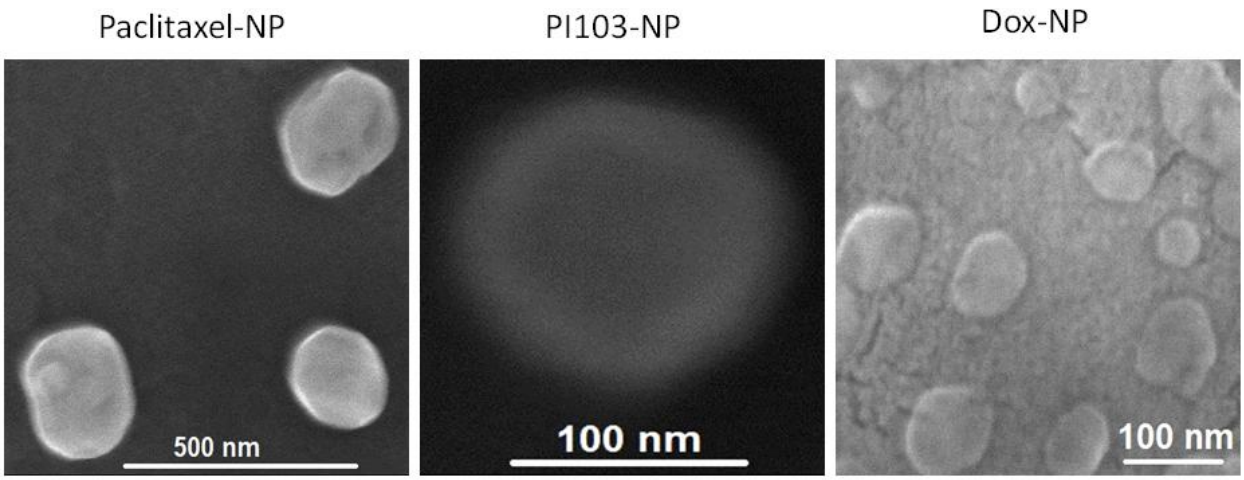


Fig. S4: Size and shape determination of lithocholic acid-NPs by FE-SEM.

Size distribution of nanoparticle synthesis without lithocholic acid:

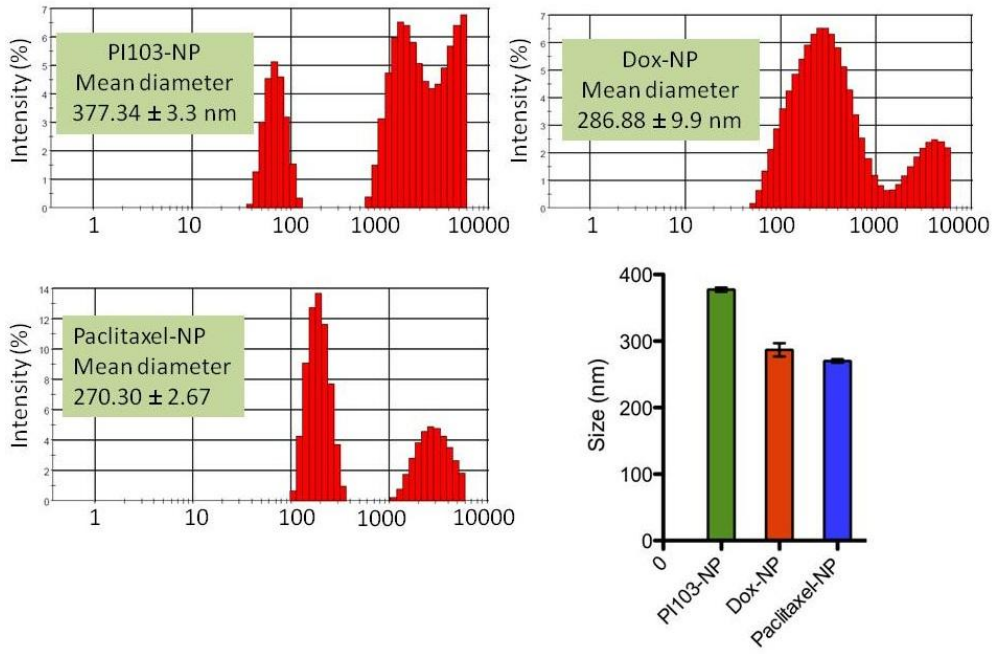


Fig. S5: Size distribution of nanoparticles synthesized from PC and DSPE-EPG without any lithocholic acid.

Stability of the drug loaded nanoparticles in FBS over 5 days:

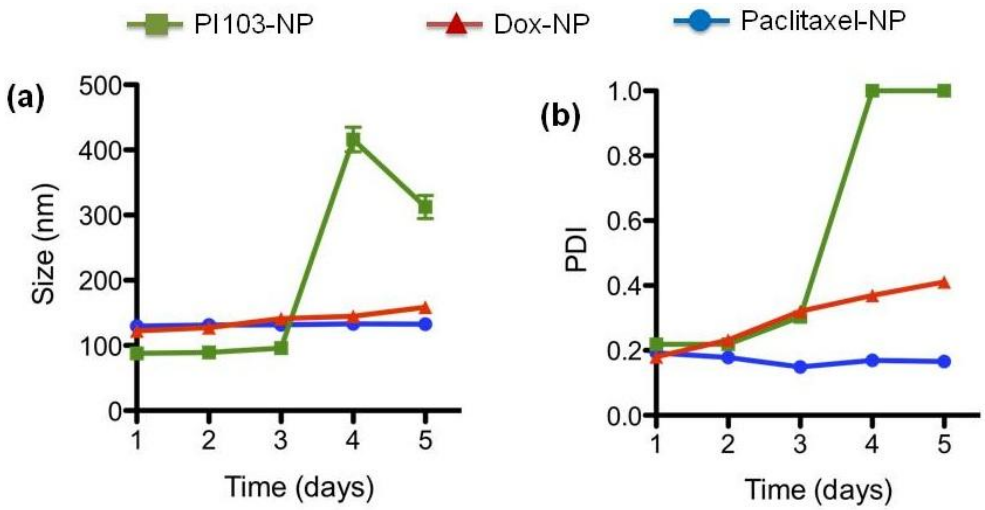


Fig. S6: Size and PDI of different drug loaded nanoparticles in FBS over 5 days.

Characterization of free PI103 released from PI103-NP in release kinetics by MALDI-TOF:

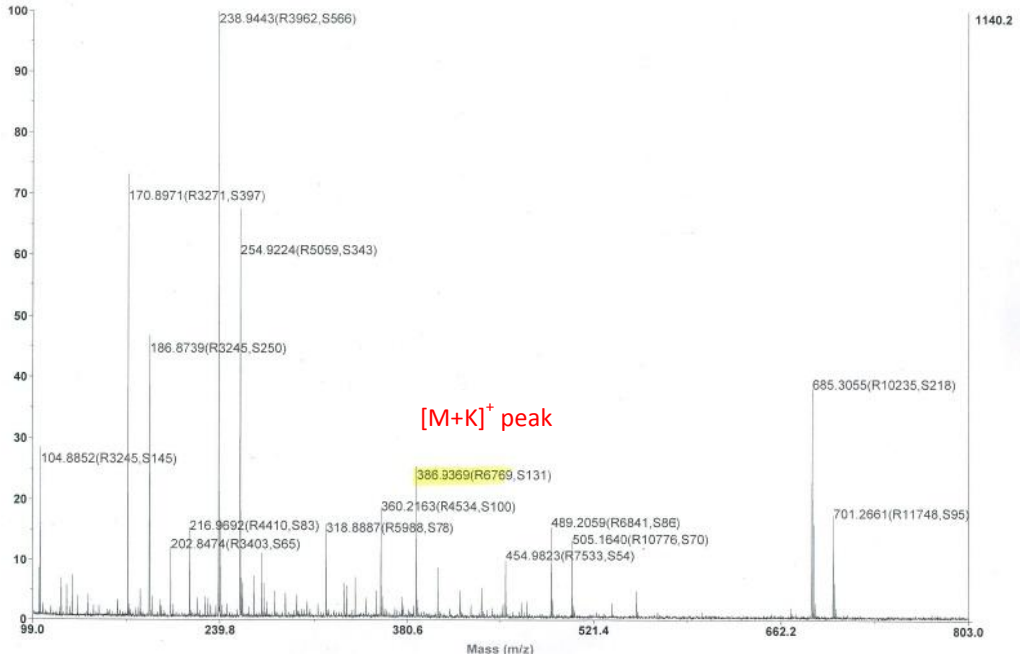


Fig. S7: MALDI-TOF of released PI103 from PI103-NP showing the presence of free PI103.

Characterization of free Paclitaxel released from Paclitaxel-NP in release kinetics by MALDI-TOF:

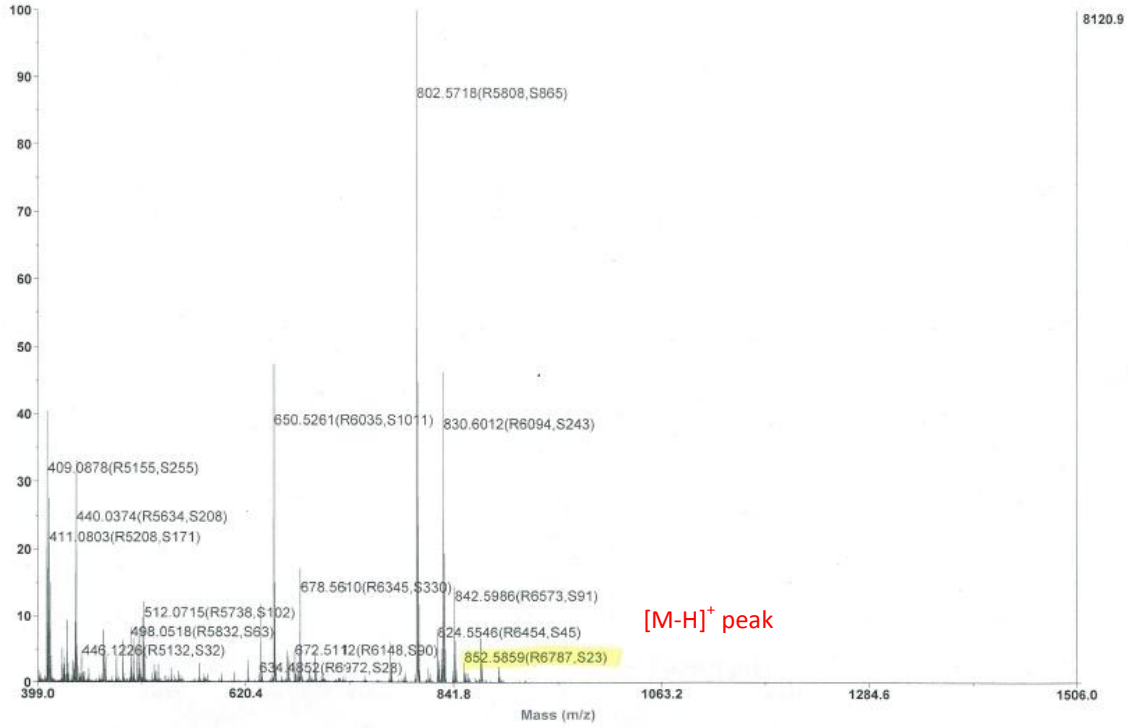


Fig. S8: MALDI-TOF of released Paclitaxel from Paclitaxel-NP showing the presence of free Paclitaxel.

Characterization of free Doxorubicin released from Dox-NP in release kinetics by MALDI-TOF:

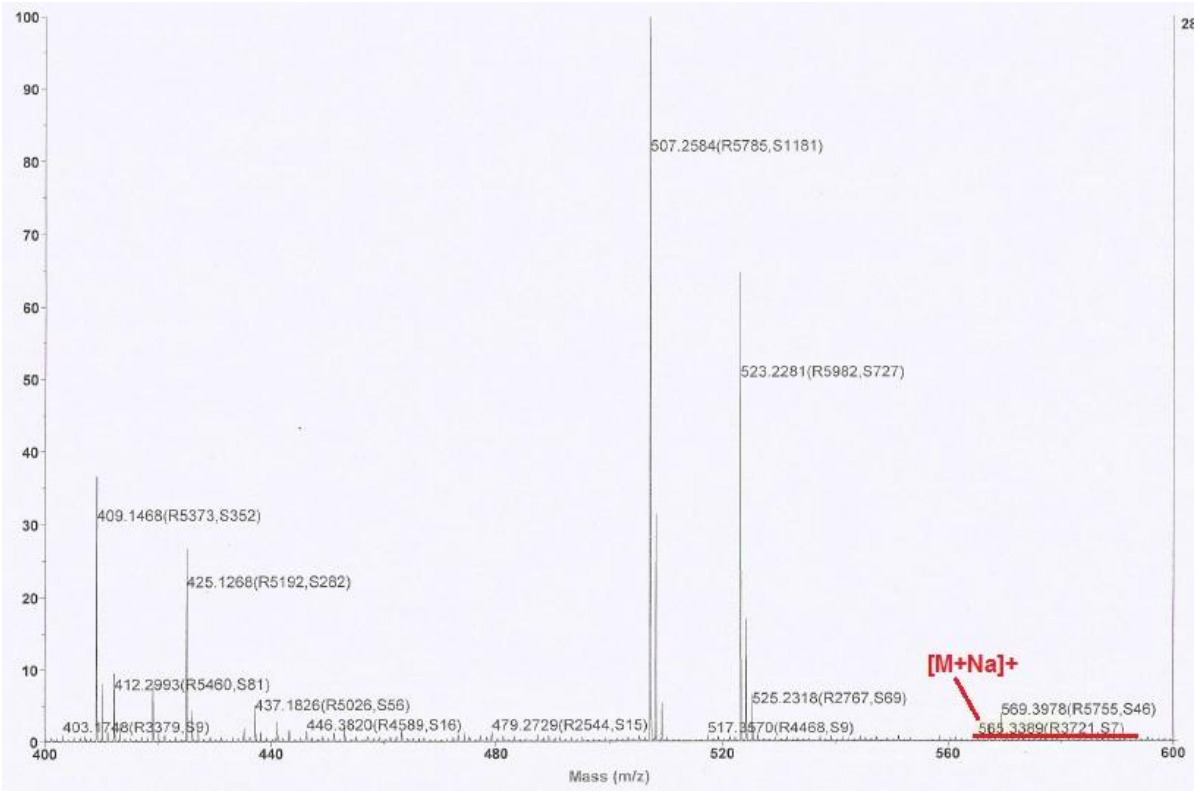


Fig. S9: MALDI-TOF of released Doxorubicin from Dox-NP showing the presence of free Doxorubicin.

In vitro dose dependent cytotoxicity assay of different nanoparticles at 24h:

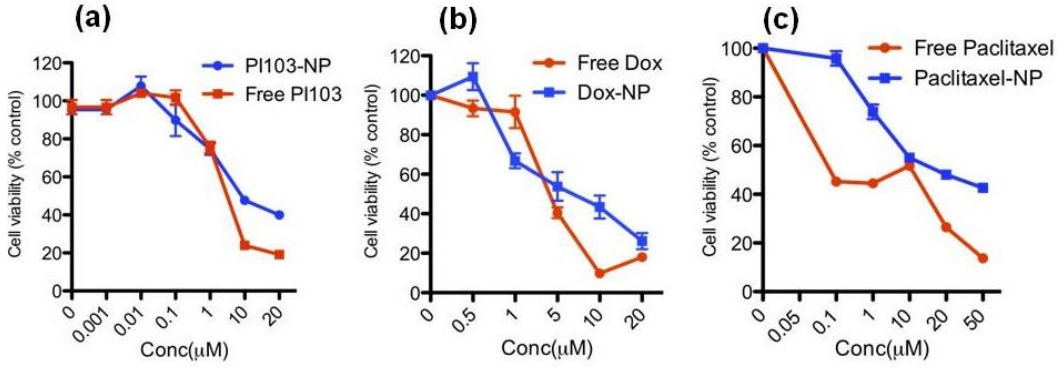


Fig. S10: *In vitro* dose dependent cytotoxicity assay of different drug-loaded NPs against HeLa cells at 24h.

Confocal Laser Scanning Microscopy (CLSM) images of time dependent internalization of free Doxorubicin in HeLa cells:

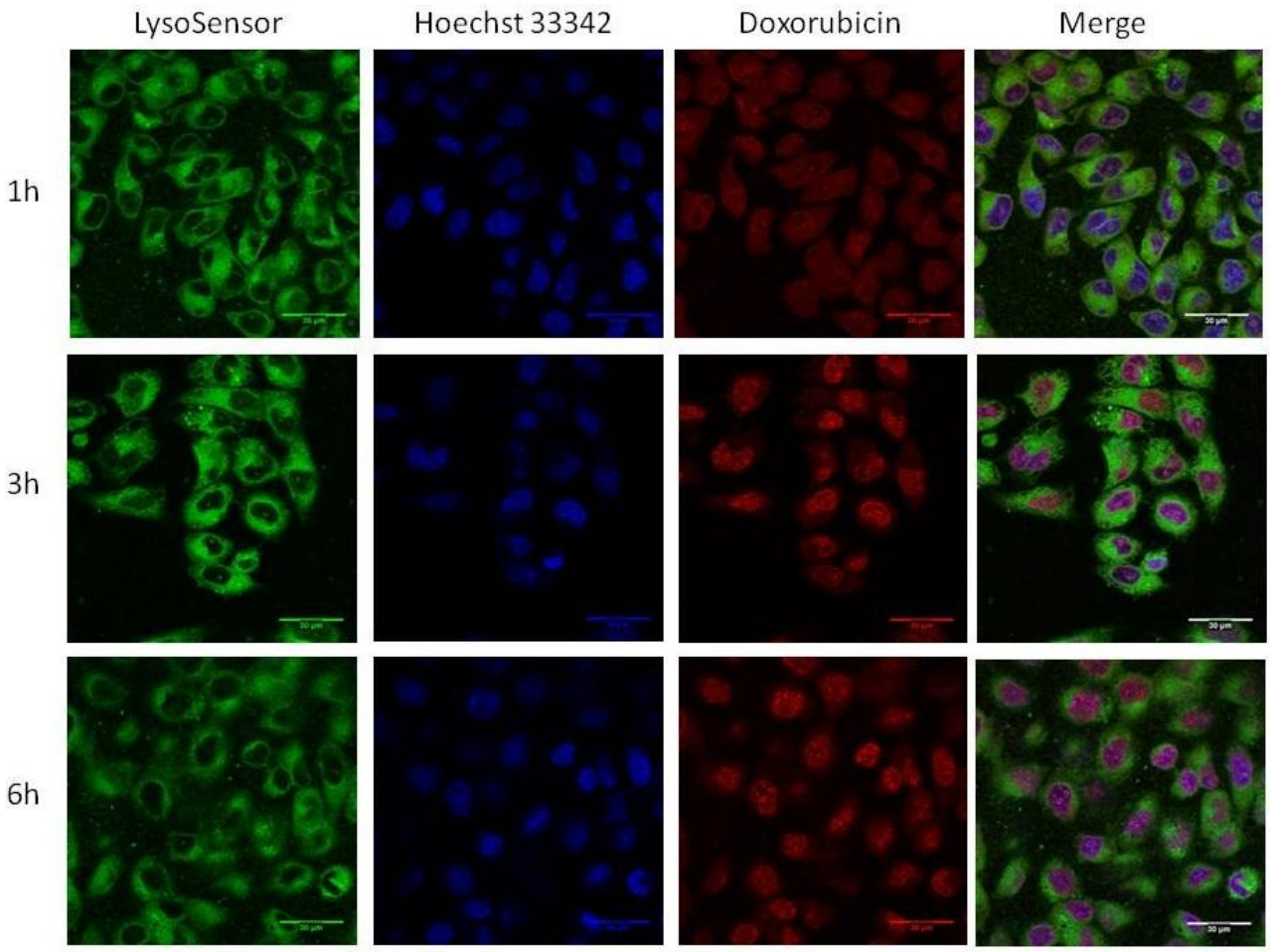


Fig. S11: Confocal laser scanning microscopy (CLSM) images of free doxorubicin internalization in HeLa cells in 1h, 3h and 6h time points. Low pH lysosomal compartments and nucleus were stained with LysoSensor^{TM} Green DND-153 (green) and Hoechst 33342 (blue) respectively. In merged images purple color showed the colocalization of free doxorubicin (red) in nucleus in a time dependent manner.

Characterization of the products 2, 3, 4, 5 and 6 by ^{1}H, ^{13}C NMR and HR-MS:

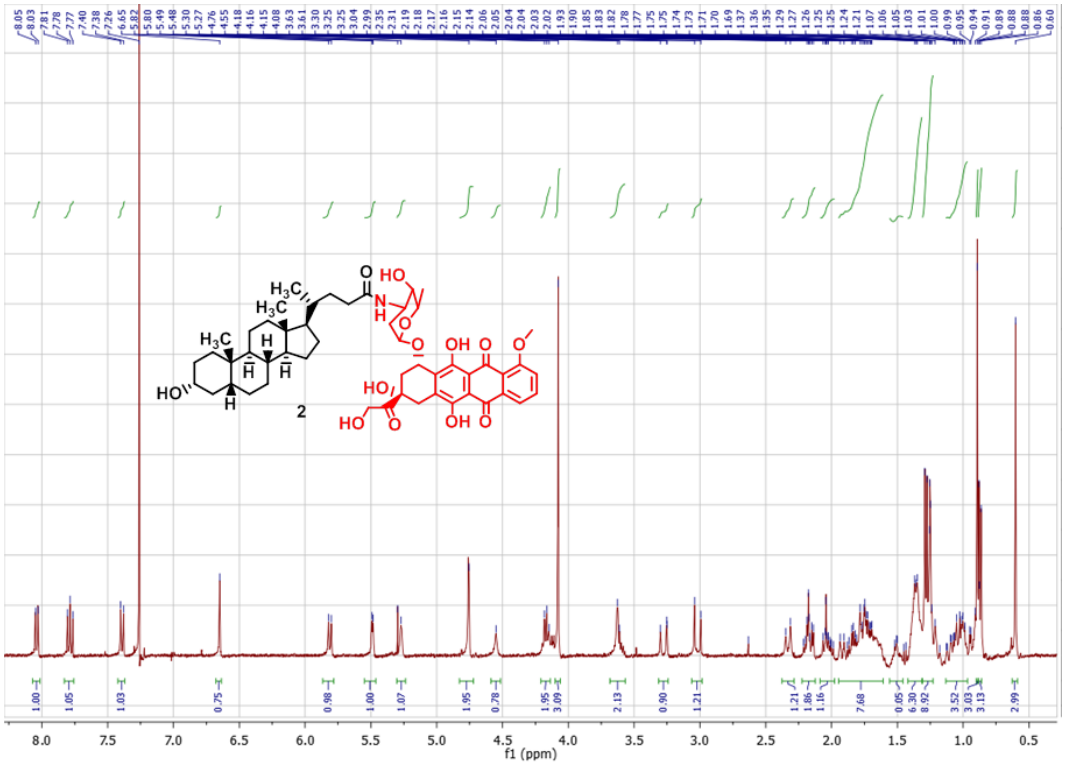


Fig. S12: ^{1}H NMR spectra of lithocholic acid-doxorubicin conjugate (**2**).

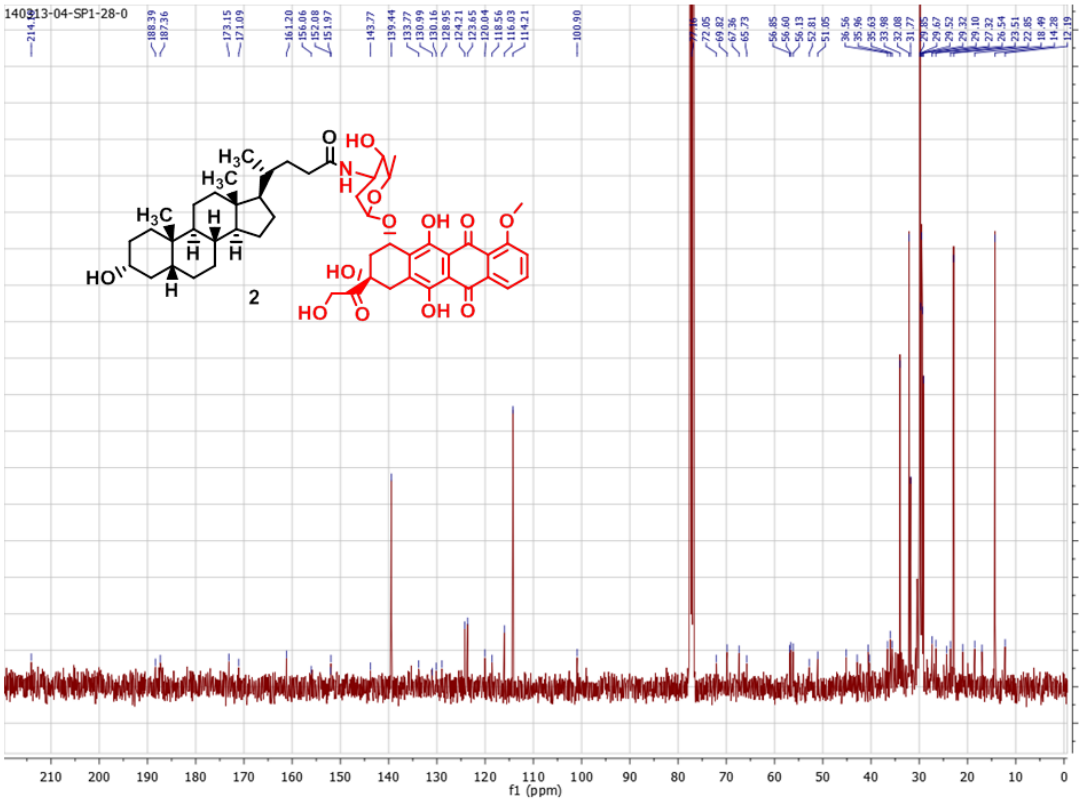


Fig. S13: ^{13}C NMR spectra of lithocholic acid-doxorubicin conjugate (**2**).

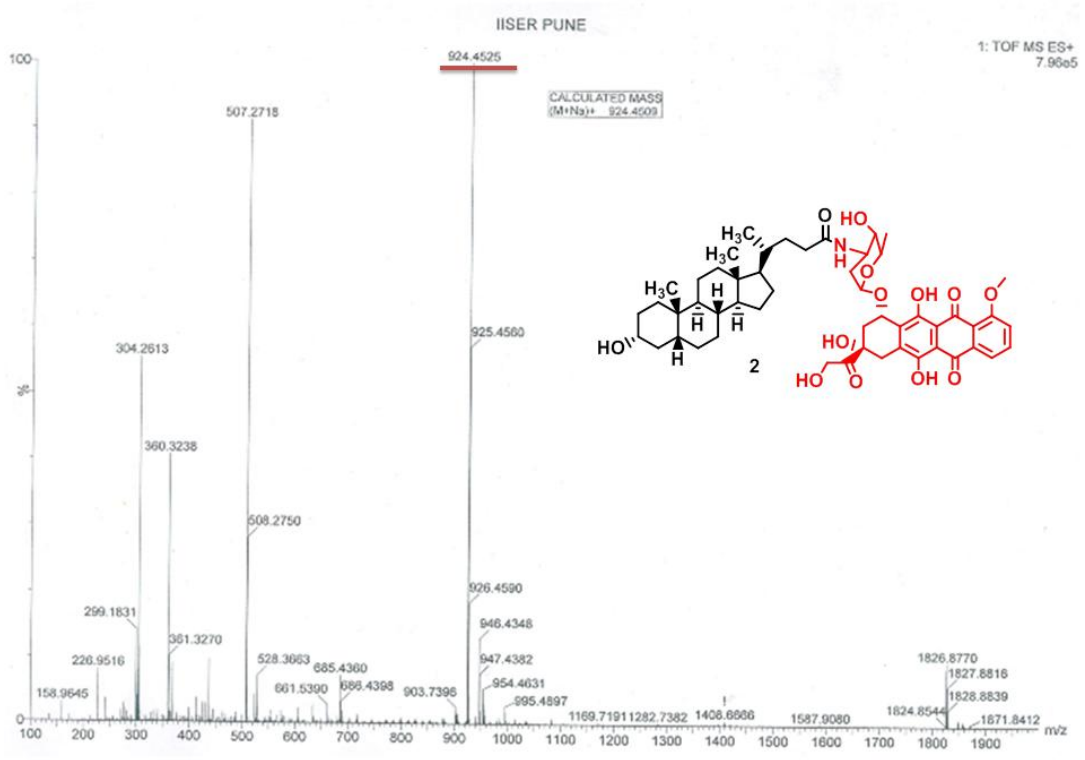


Fig. S14: HR-MS of lithocholic acid-doxorubicin conjugate (**2**).

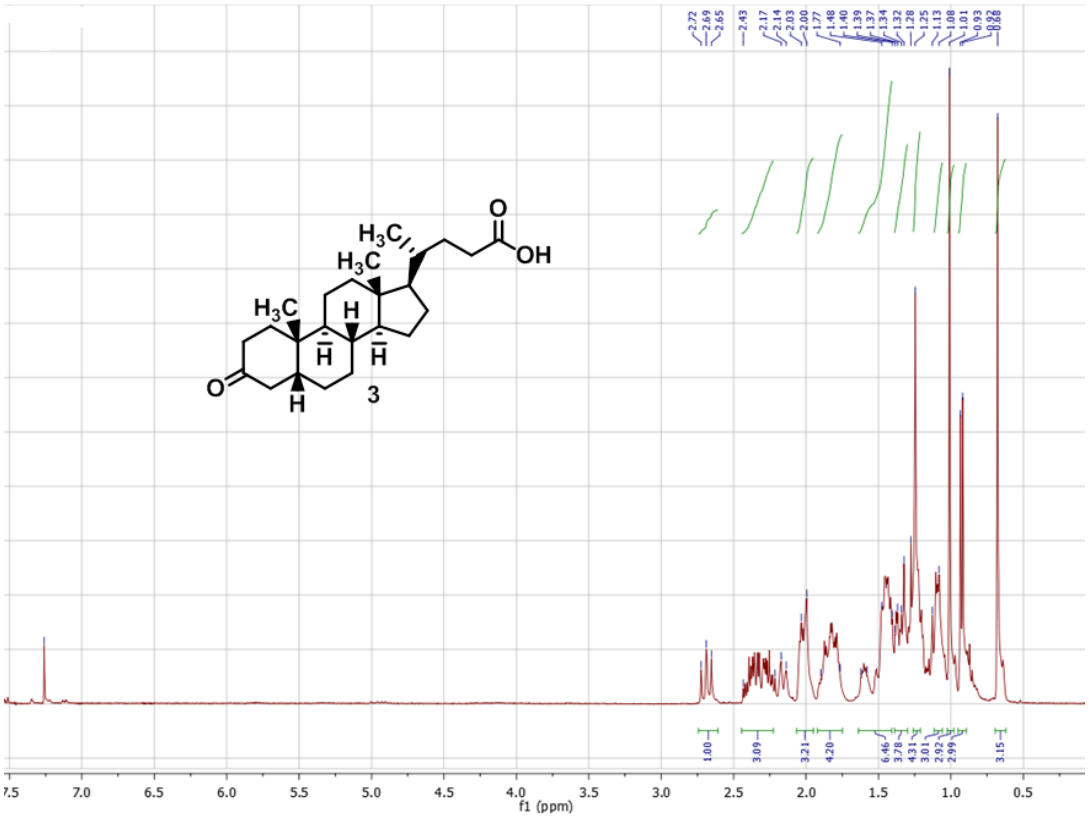


Fig. S15: ^{1}H NMR spectra of keto-lithocholic acid (**3**).

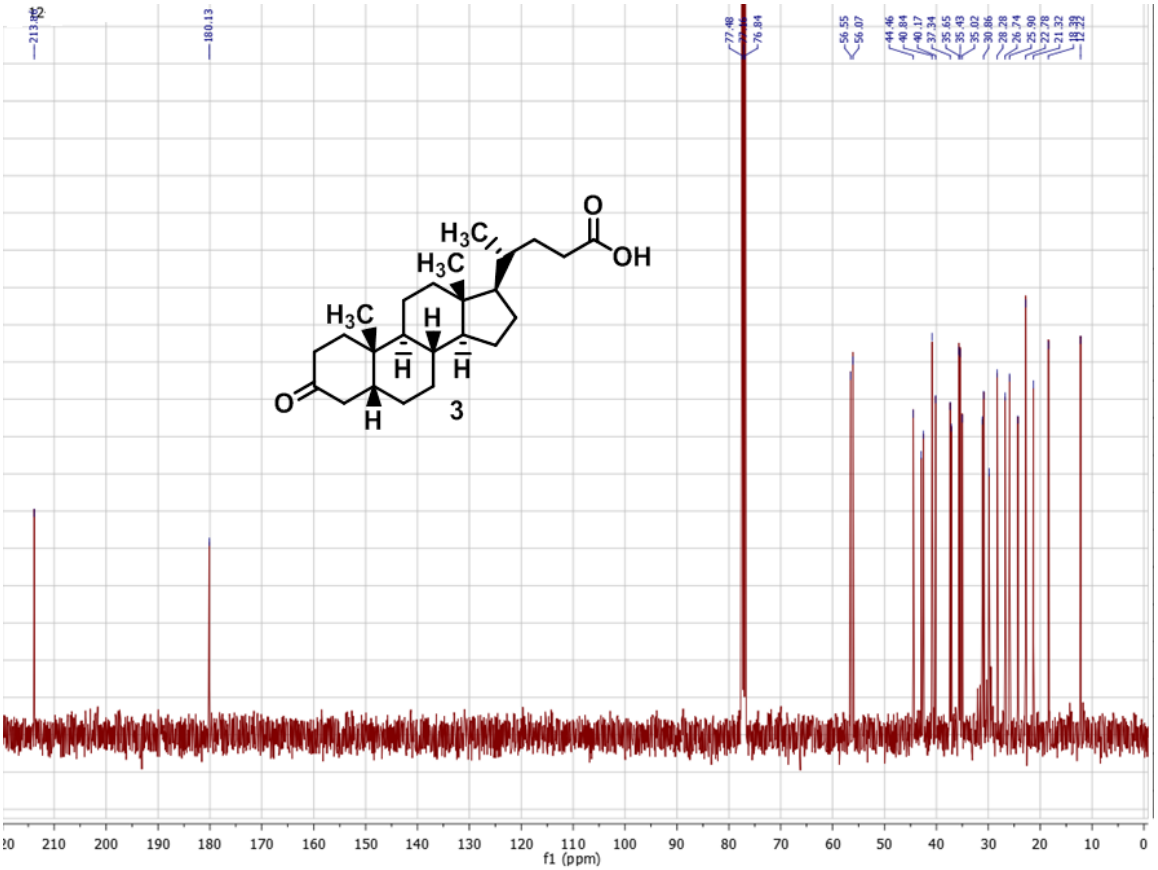


Fig. S16: ^{13}C NMR spectra of keto-lithocholic acid (**3**).

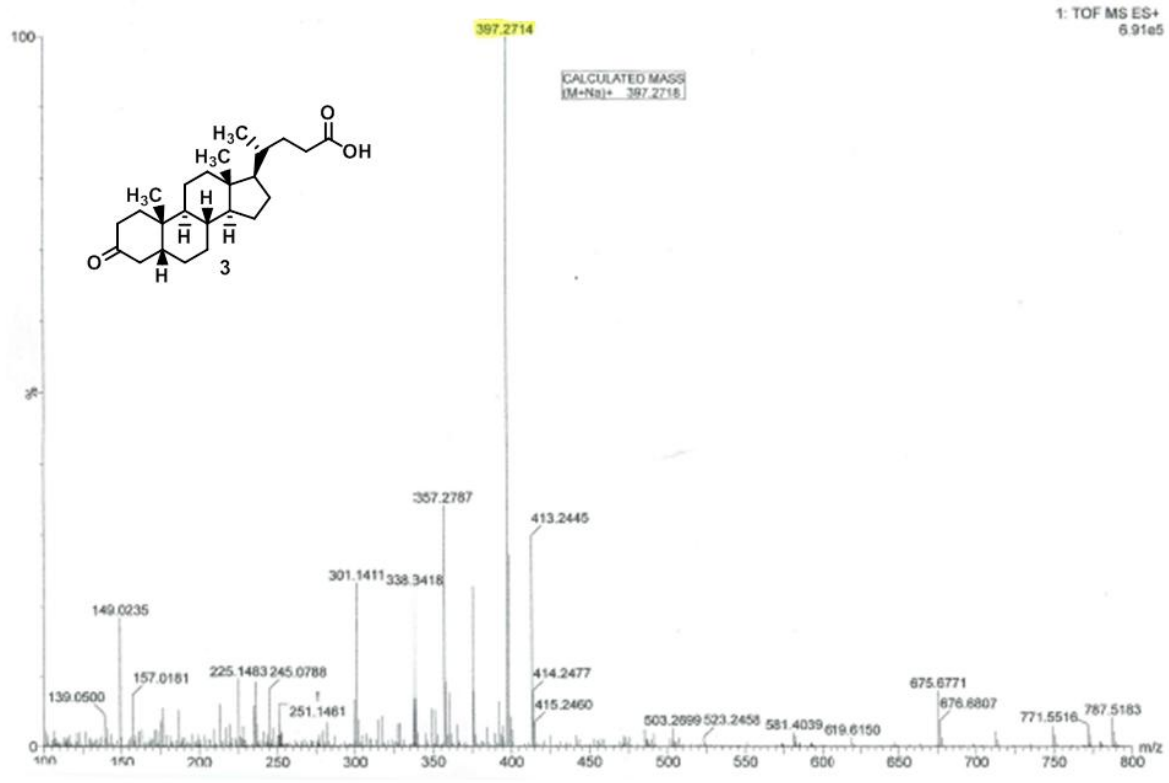


Fig. S17: HR-MS spectra of keto-lithocholic acid (**3**).

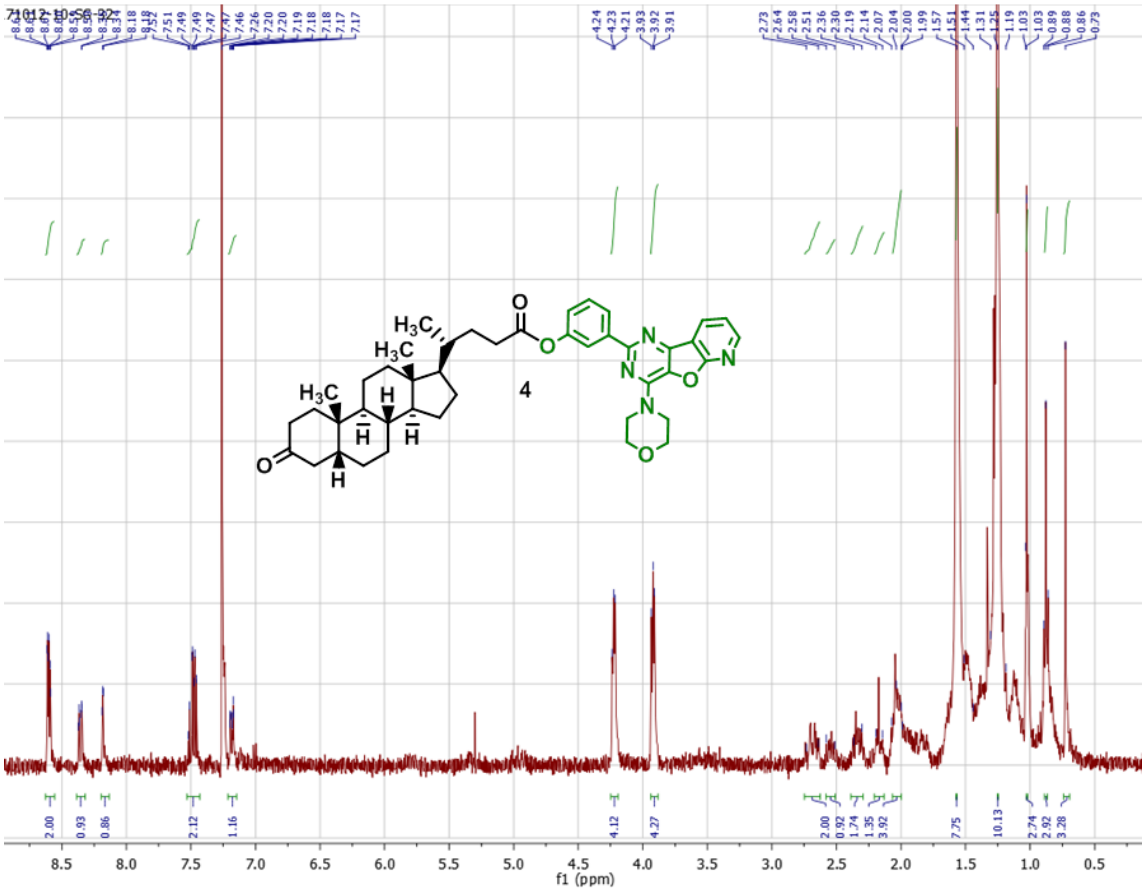


Fig. S18: ^{1}H NMR spectra of keto-lithocholic acid-PI103 conjugate (**4**).

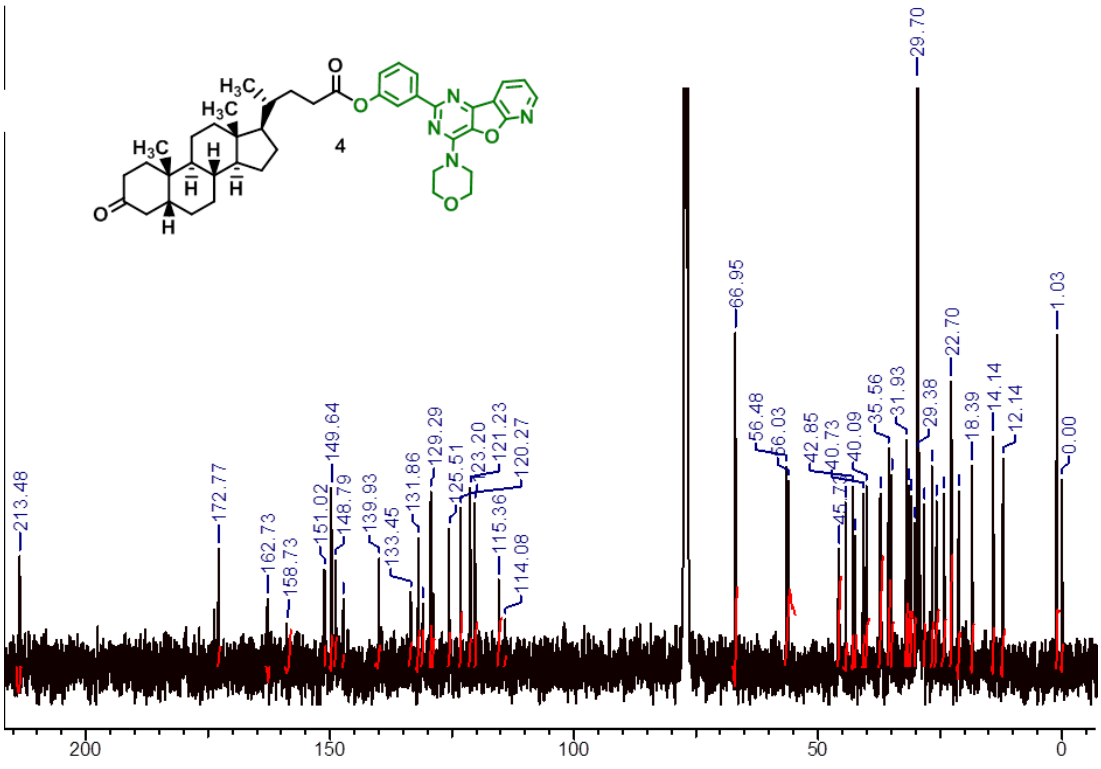


Fig. S19: ^{13}C NMR spectra of keto-lithocholic acid-PI103 conjugate (**4**).

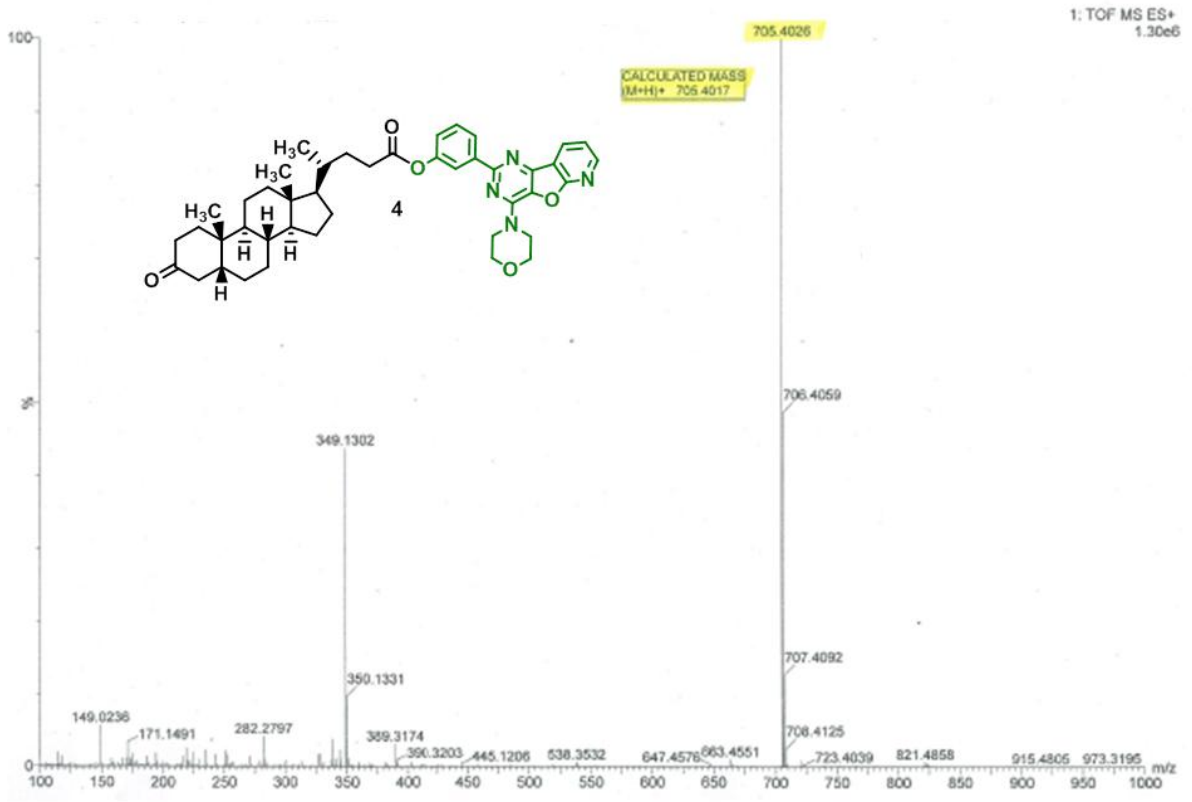


Fig. S20: HR-MS spectra of keto-lithocholic acid-PI103 conjugate (**4**).

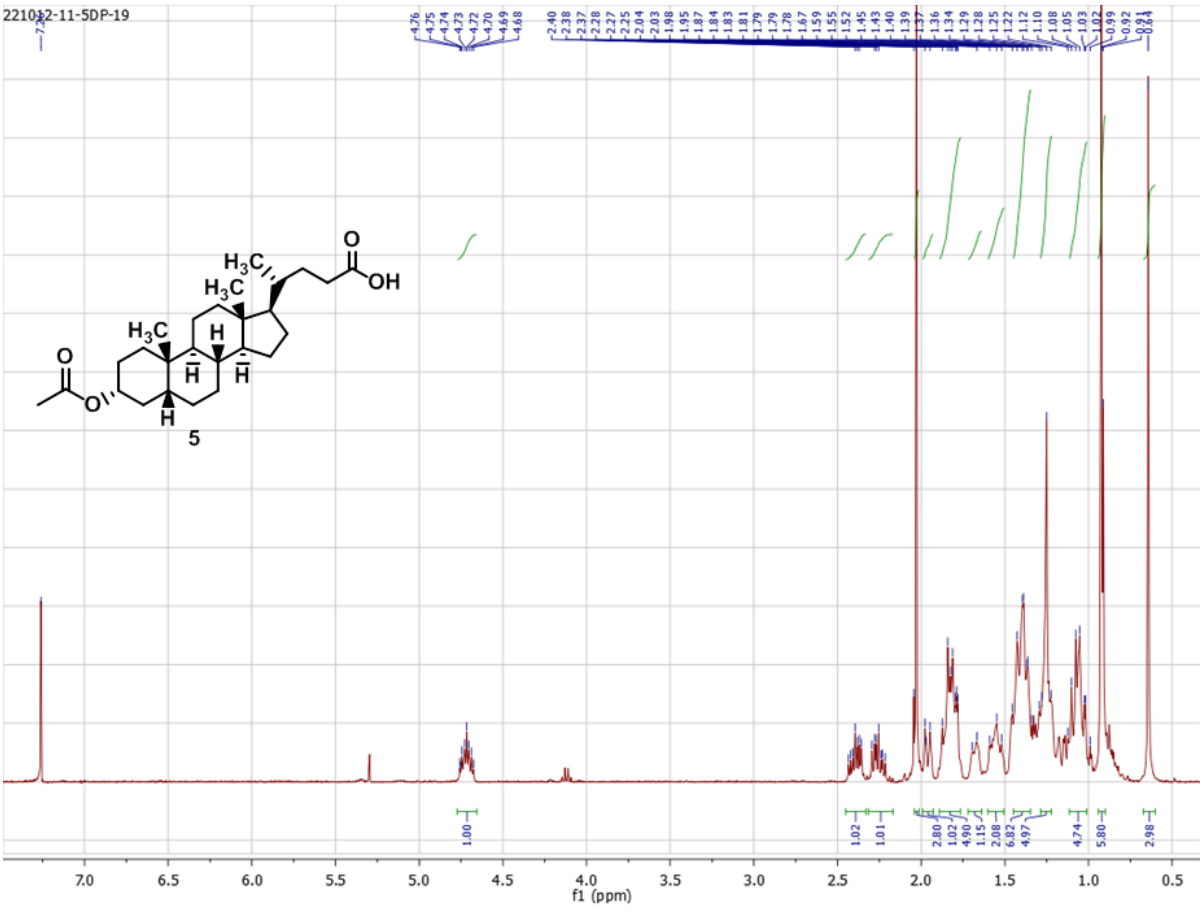


Fig. S21: ^{1}H NMR spectra of acetyl-lithocholic acid (**5**).

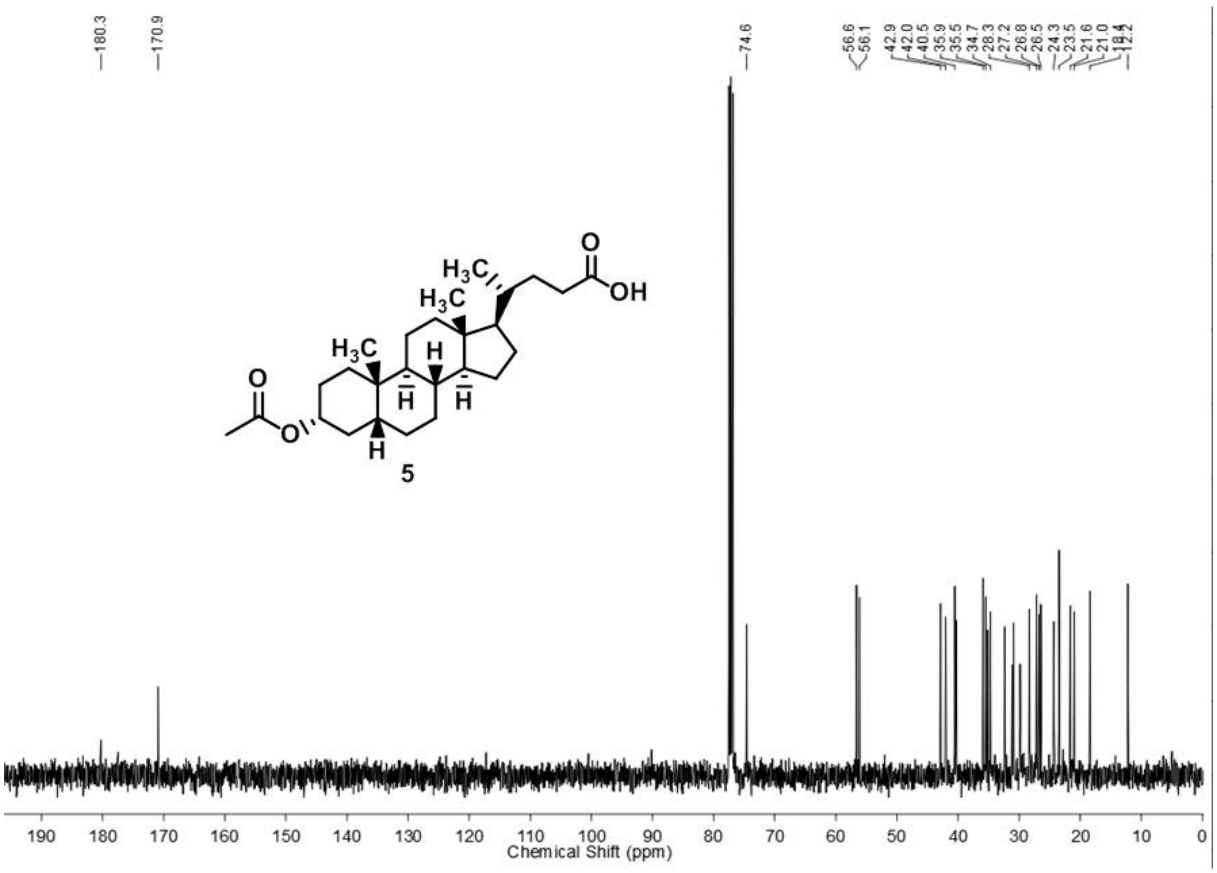


Fig. S22: ^{13}C NMR spectra of acetyl-lithocholic acid (**5**).

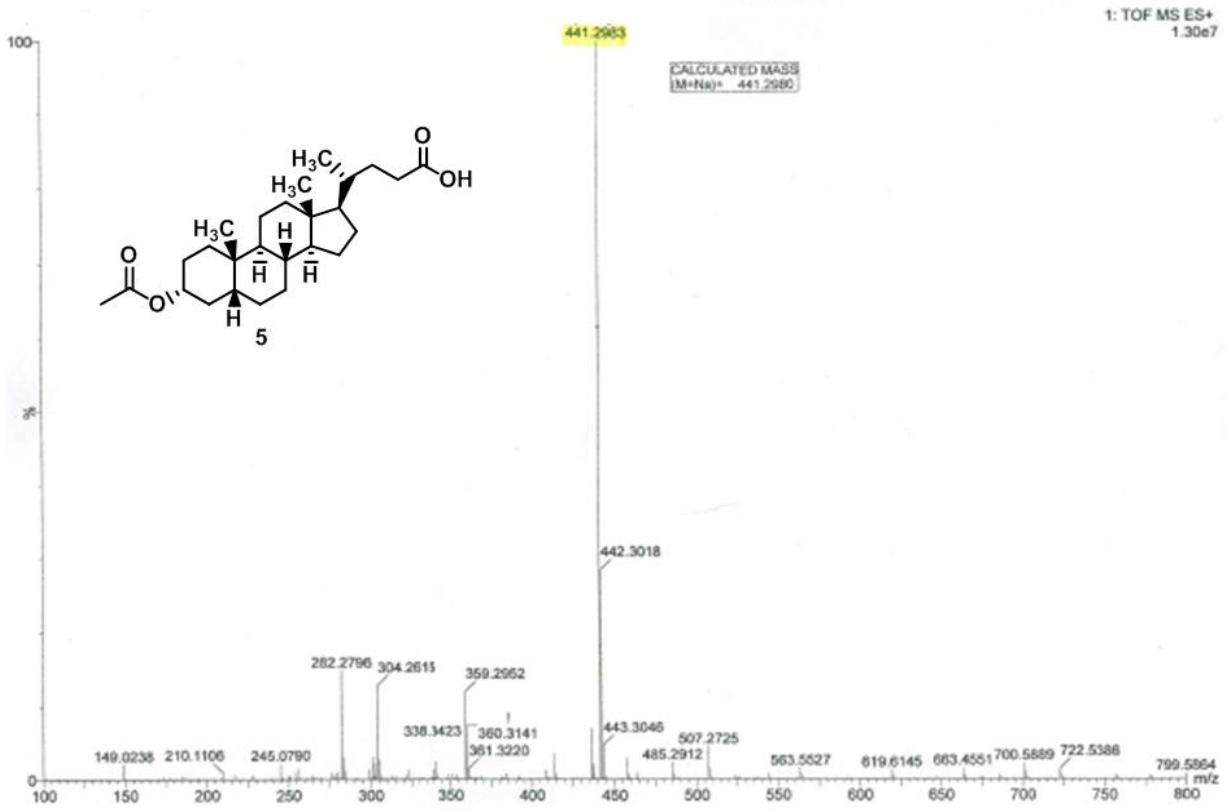


Fig. S23: HR-MS spectra of acetyl-lithocholic acid (**5**).

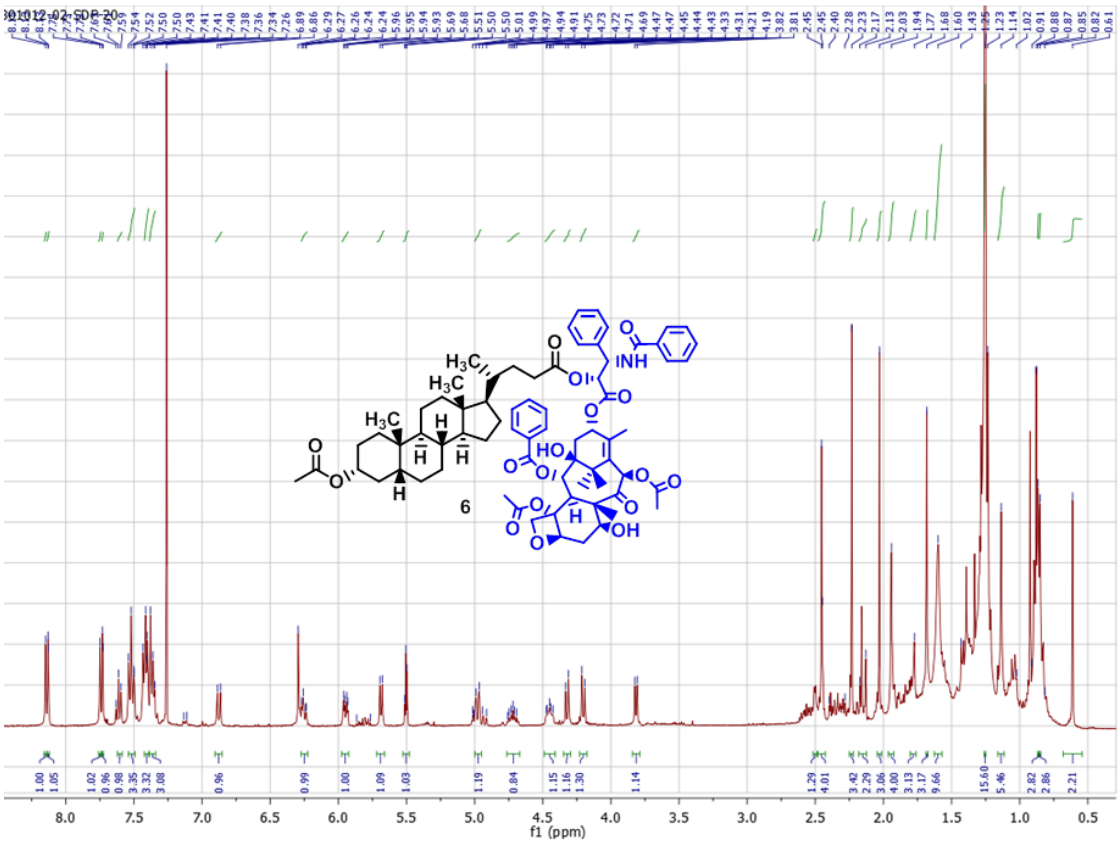


Fig. S24: ^{1}H NMR spectra of acetyl-lithocholic acid-paclitaxel conjugate (**6**).

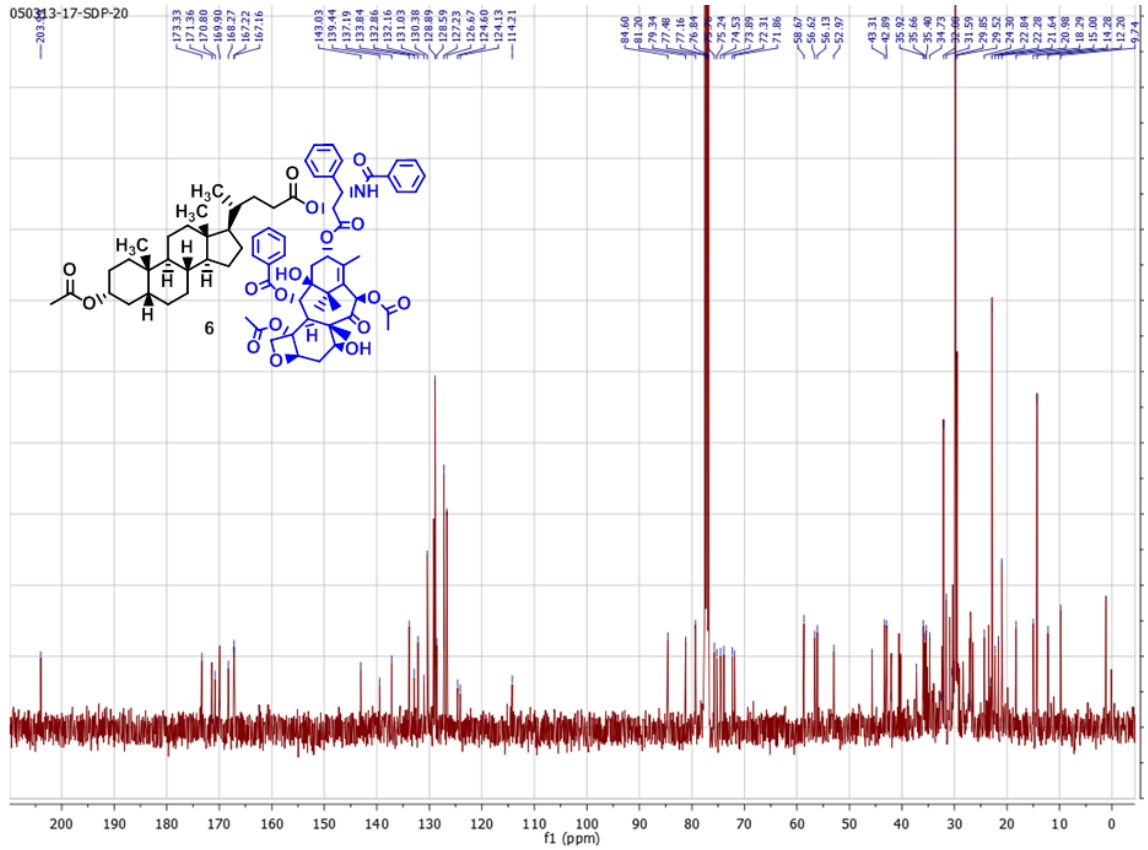


Fig. S25: ^{13}C NMR spectra of acetyl-lithocholic acid-paclitaxel conjugate (**6**).

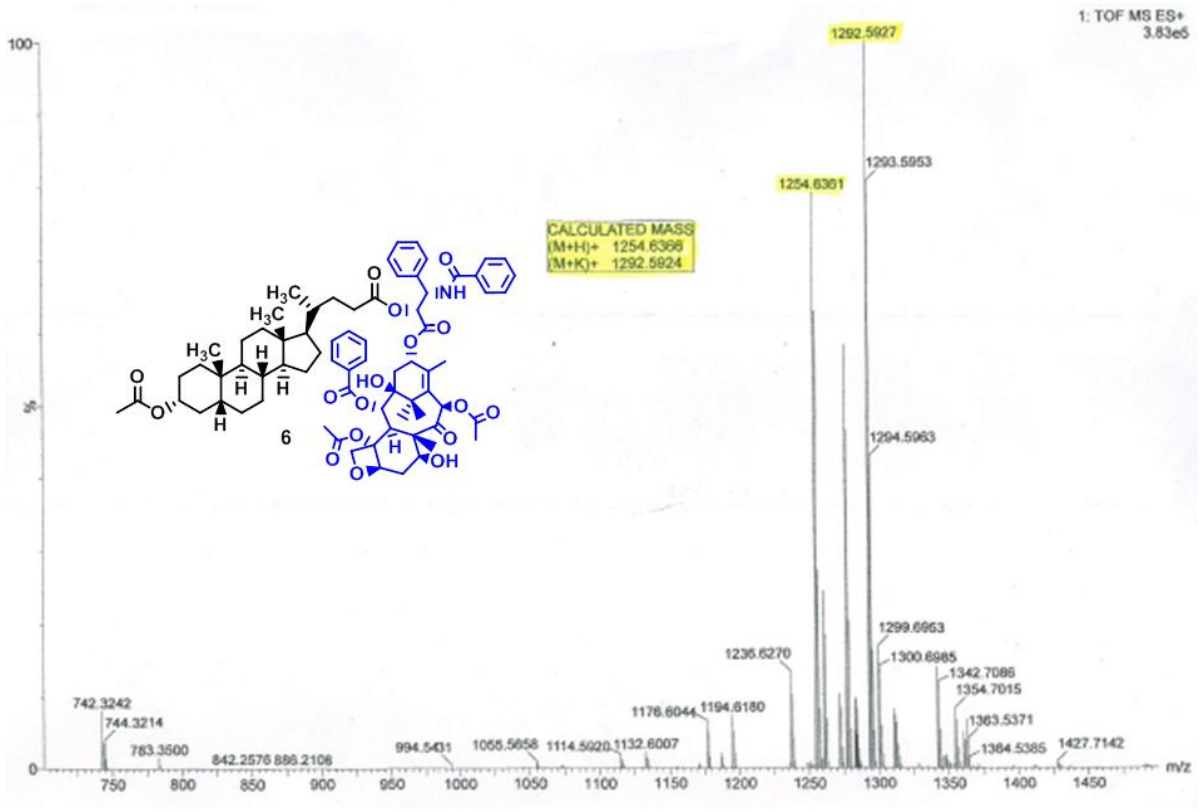


Fig. S26: HR-MS spectra of acetyl-lithocholic acid-paclitaxel conjugate (**6**).