A supramolecular nano-delivery system based on AIE PARP inhibitor prodrug and glycosylated pillar[5]arene for drug-resistance therapy

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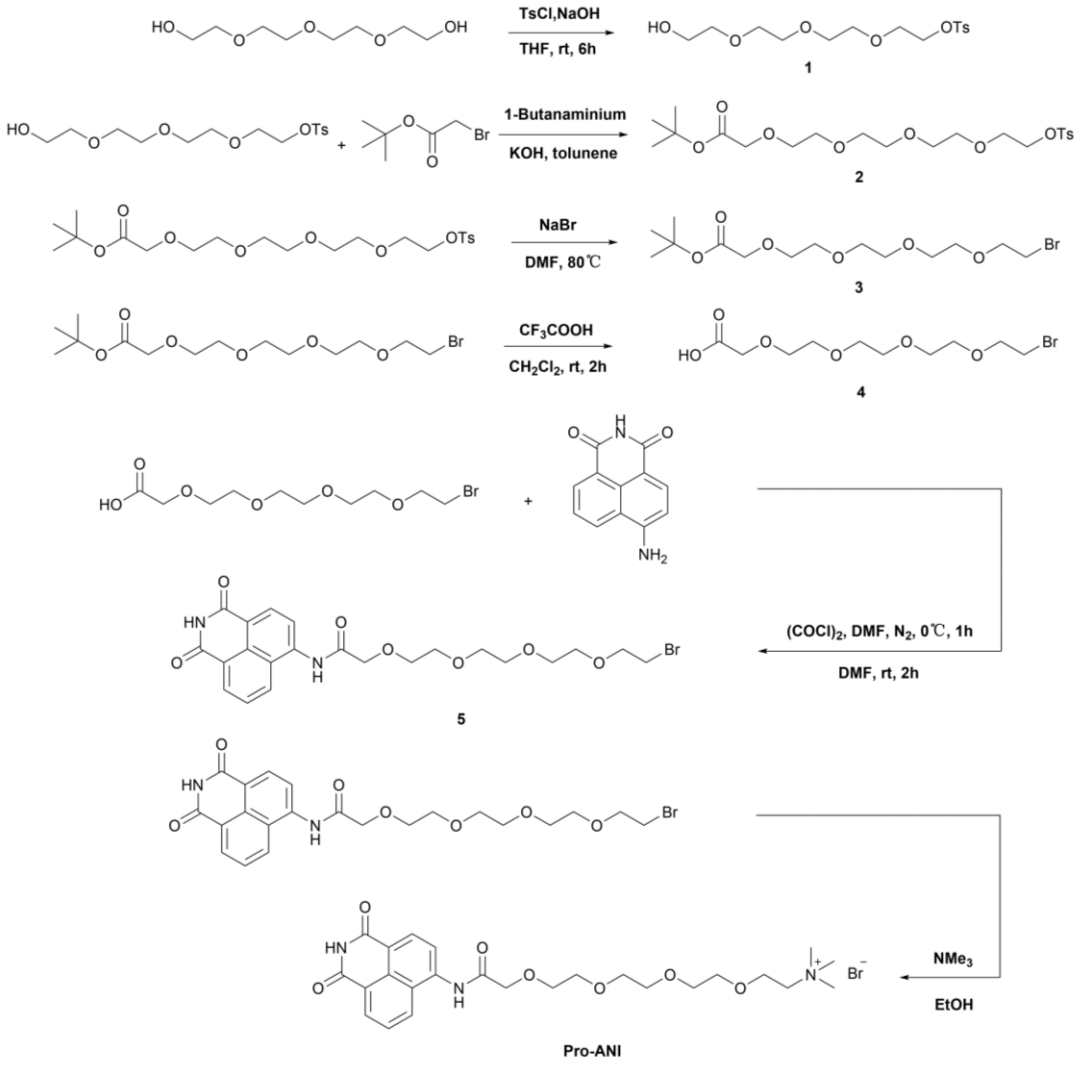
Supporting Information

1. Instrumentation and chemicals

All reagents were purchased from commercial suppliers and used without further purification unless specified. The water used in this work was triple distilled. ^{1}NMR spectra were recorded on a Bruker 400 or 500 MHz Spectrometer, with working frequencies of 400 or 500 MHz for ^{1}H nuclei (Bruker Corporation, Switzerland). DLS measurements were performed on ZEN3600 NANOPHOX (MALVERN INSTRUMENTS LIMITED, United Kingdom). UV-vis spectra were collected with (Shimadzu UV-2450, Japan) UV-visible spectrophotometer. Scanning electron microscopy (SEM) images were obtained from the Nano SEM-450 instrument (FEI Ltd. U.S.A.). Transmission electron microscopy (TEM) images were obtained from the TECNAI G2 SPIRIT BIO instrument (FEI Ltd. U.S.A.). Cell culture was carried out in an incubator with a humidified atmosphere of 5 % CO_2 at 37 ℃. The confocal laser microscope (CLSM) data were acquired using a Spectroscopic fluorescent lifetime Confocal Laser Scanning Microscopy STELLARIS8 FALCON (Lecia Instrument Co., LTD, Germany). The other fluorescent images were from the DMi8 Inverted fluorescence microscope (Leica MICROSYSTEMS, Germany). Flow cytometry data were obtained from BD FACSAria™ III Flow Cytometer (BD Biosciences, U.S.A.). Antibodies (anti-Cleaved-PARP1, anti-Caspase-3, anti-beta Beclin 1, anti-Bax, anti-p53, anti-Beclin 1 and anti-beta Actin) were from (HUABIO, China). Anti-mouse and anti-rabbit secondary antibodies (alkaline phosphatase and horseradish peroxidase conjugated) were from (HUABIO, China). The data of the western blot was obtained from ChemiDoc MP Chemiluminescence gel imaging system (Bio-Rad Laboratories, U.S.A.).

2. Synthesis and Characterization of the compounds



Scheme S1. Synthetic route of Pro-ANI[1]

GP5 and Pro-ANI was synthesized according to the previous reporters.[2, 3]

Compound 1: Tetra(ethylene)glycol (8.0 g, 41.2 mmol) was dissolved in an appropriate amount of tetrahydrofuran solution (THF) and sodium hydroxide (2 g, 50 mmol) in water. Mixing the system above. Next, the THF solution of Tosyl chloride (8.0 g, 42.1 mmol) was added. After 6 h under r.t, the solvent was removed under reduced pressure and the residue was dissolved in dichloromethane (DCM). The organic phase was washed with water (3 × 20 mL). Then the combined organic phases were dried with Na_2SO_4 and concentrated under reduced pressure to give 1 as a colorless liquid (3.4 g, 33%) by column chromatography (petroleum ether/ethyl acetate = 2:1, v/v). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.80 (d, J = 7.8 Hz, 2H), 7.35 (d, J = 7.7 Hz, 2H), 4.17 (s, 2H), 3.74 – 3.66 (m, 4H), 3.67 – 3.62 (m, 4H), 3.62 – 3.57 (m, 6H), 2.45 (s, 3H).

Compound 2: To the toluene solution of compound 1 (3.0 g, 8.62 mmol) was added 1-butanaminium (0.14 g,0.43 mmol), KOH (0.56 g, 10.0 mmol), and tert-butyl bromoacetate (1.98 g,10.17 mmol). Reaction for 12 h at r.t. Then toluene was removed under reduced pressure. The residue was dissolved in water and the aqueous phase was extracted with DCM (20 mL × 3). The combined organic phases were dried with Na_2SO_4 and concentrated under reduced pressure to give 2 as a colorless liquid (2.11 g, 53%) by column chromatography (petroleum ether/ethyl acetate = 4:1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (d, J = 8.3 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 4.02 (s, 2H), 3.72 – 3.66 (m, 8H), 3.66 – 3.61 (m, 4H), 3.58 (d, J = 8.0 Hz, 4H), 2.45 (s, 3H), 1.47 (s, 9H).

Compound 3: To the DMF solution of compound 2 (1.0 g, 2.2 mmol) was added sodium bromide (462 mg, 3.3 mmol). After heating and reflux for 6 h, DCM (100 mL) was added. The mixture was filtrated and concentrated under reduced pressure to give 3 as a colorless liquid (607 mg, 74%) by column chromatography (petroleum ether/ethyl acetate = 8:1, v/v).

Compound 4: The compound 3 (871 mg, 2.35 mmol) was dissolved in DCM and reacted with trifluoroacetic acid (4.7 g, 41.6 mmol) for 2 h at r.t. The solvent was removed and the residue was dissolved in water (5 mL), adjusting pH to 10 with 1 mol/L NaOH solution, washing with DCM for twice, then adjusting pH to 2 with 6 mol/L HCl solution. The aqueous phase was extracted three times with DCM. Combined organic phases were dried with Na_2SO_4. The solvent was removed under reduced pressure to give 4 as a faint colorless liquid (517 mg, 70%) by column chromatography (petroleum ether/ethyl acetate = 8:1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.89 (s, 1H), 4.19 (s, 2H), 3.82 (t, J = 6.3 Hz, 2H), 3.77 – 3.74 (m, 2H), 3.73 – 3.65 (m, 10H), 3.49 (t, J = 6.3 Hz, 2H).

Compound 5: To the solution of compound 4 (230 mg, 0.73 mmol) was added oxalyl chloride (395 mg, 3.11 mmol) under nitrogen protection and ice bath. Then the reaction was transferred to r.t for1 h. Removed the solvent, then adding DMF and 4-amino-1,8-naphthalimide (155 mg, 0.73 mmol) for 2 h at r.t. After the reaction, water was added and extracted three times with ethyl acetate (EA). The combined organic phase was washed with saturated salt water, dried with anhydrous Na_2SO_4, and purified by column chromatography to give 5 as a faint yellow soild (66 mg, 18%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.66 (s, 1H), 9.40 (s, 1H), 8.56 (d, J = 6.9 Hz, 1H), 8.51 (dd, J = 12.0, 8.0Hz, 2H), 8.37 (d, J = 8.0 Hz, 1H), 7.77 (dd, J = 8.4, 7.4 Hz, 1H), 4.32 (s, 2H), 3.98 – 3.83 (m, 4H), 3.77 – 3.73 (m, 2H), 3.71 (t, J = 6.2 Hz, 2H), 3.64 – 3.59 (m, 2H), 3.56 – 3.48 (m, 4H), 3.40 (t, J = 6.2 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.04, 164.19, 163.62, 138.77,

132.01, 130.96, 129.97, 127.54, 126.70, 124.11, 122.93, 118.69, 118.37, 71.62, 71.07, 70.78, 70.56, 70.40, 70.38, 70.37, 70.07, 30.36. HRMS: m/z calcd for $[M + Na]^+$ $C_{25}H_{39}BrN_3O_{13}Na^+$, 531.0743, found 531.0727.

Pro-ANI: Compound 5 (58 mg, 0.11 mmol) was added to 300 μL trimethylamine (33% ethanol solution) and 1.5 mL ethanol and stirred for 12 h at r.t. Pro-ANI as a faint yellow solid (20 mg, 40%) was gotten after the purification of column chromatography. ^{1}H NMR (400 MHz, D_2O): δ 7.61 (d, J = 7.2 Hz, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.48 – 7.37 (m, 2H), 7.15 (t, J = 7.8 Hz, 1H), 4.20 (s, 2H), 3.82 – 3.75 (m, 4H), 3.73 – 3.67 (m, 2H), 3.65 – 3.60 (m, 2H), 3.58 – 3.55 (m, 2H), 3.51 (t, J = 12.0 Hz , 4H), 3.45 – 3.41 (m, 2H), 3.04 (s, 9H). ^{13}C NMR (101 MHz, D2O) δ 170.88, 164.57, 163.90, 137.98, 131.01, 130.77, 127.97, 127.87, 126.62, 120.28, 119.05, 119.04, 116.55, 70.62, 69.75, 69.66, 69.53, 69.51, 69.44, 69.40, 64.24, 59.23, 53.84, 53.80, 53.77. HRMS: m/z calcd for $[M - Br]^+$ $C_{25}H_{34}O_7N_3^+$, 488.2391, found 488.2384.

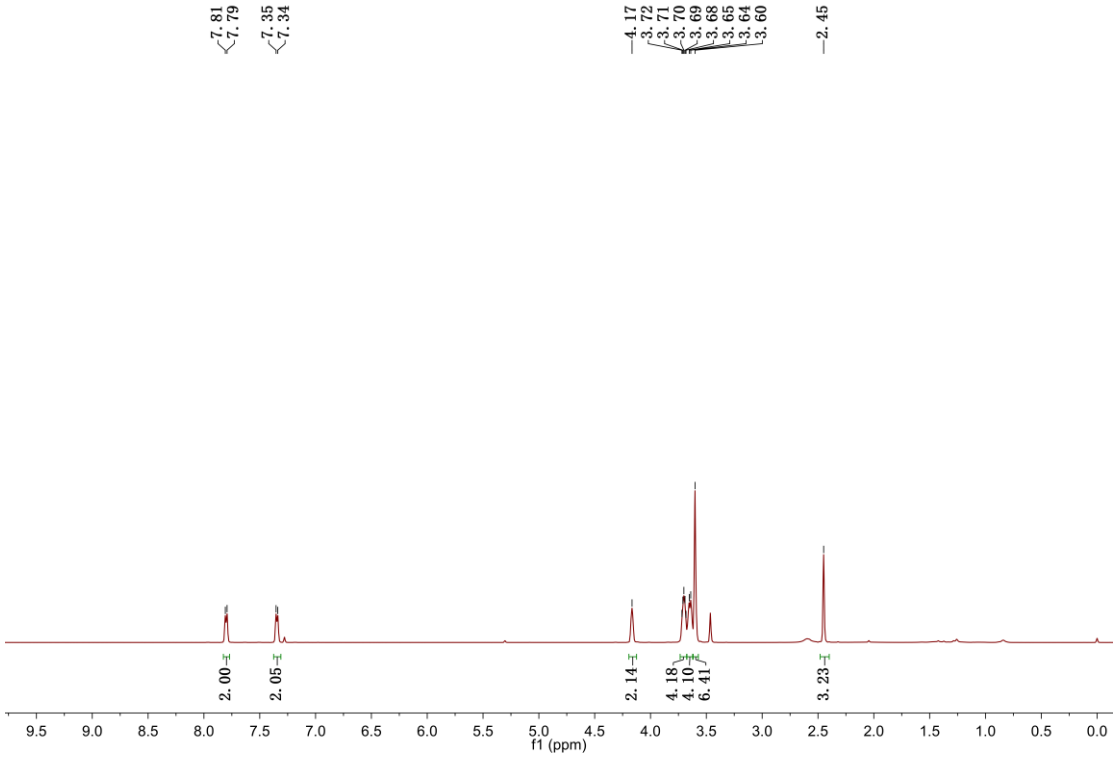


Fig S1: ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of Compound 1.

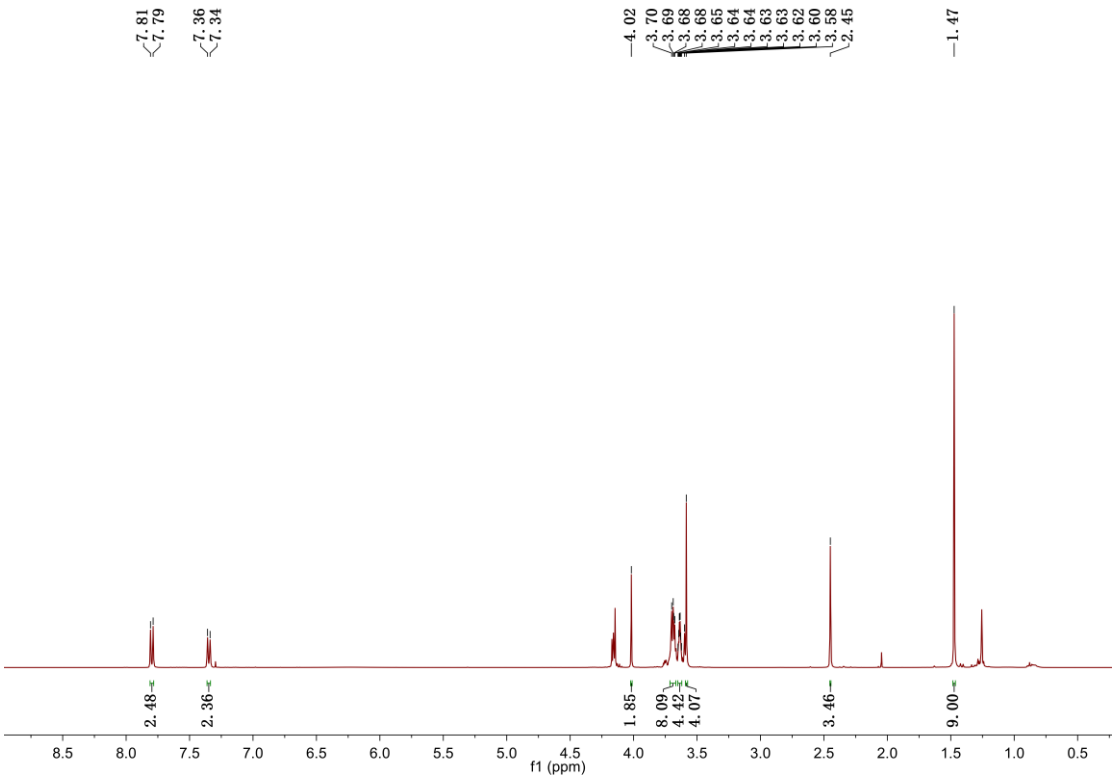


Fig S2: ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of Compound 2.

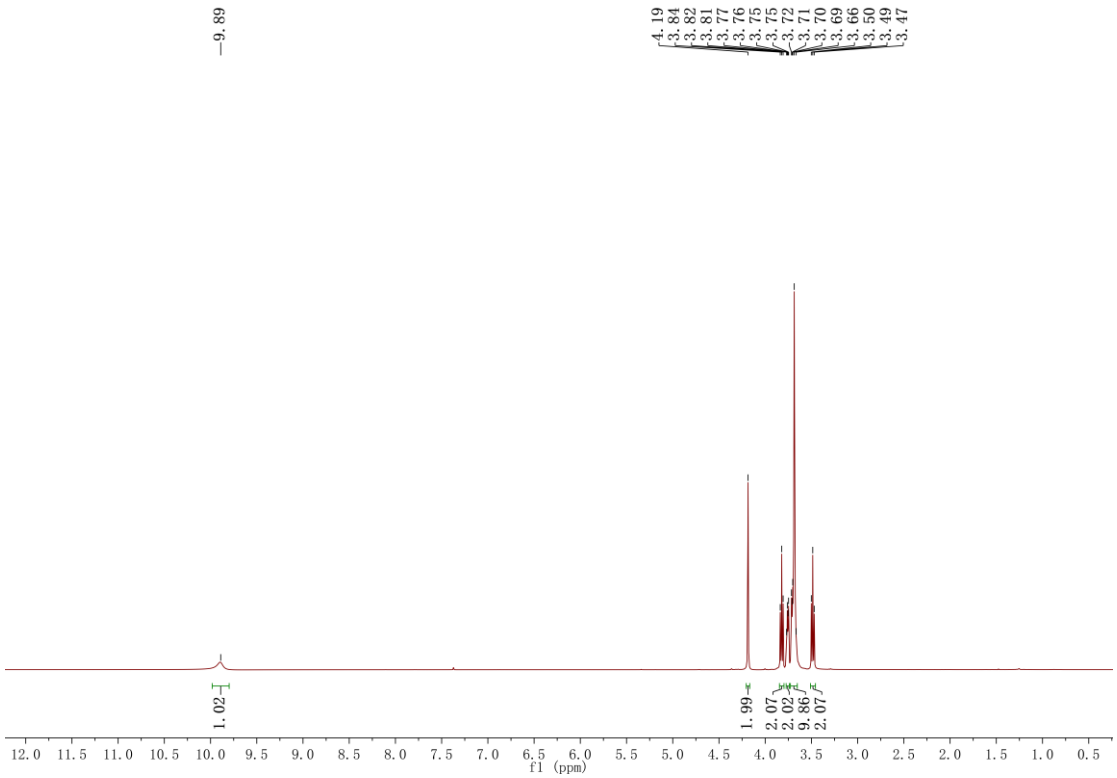
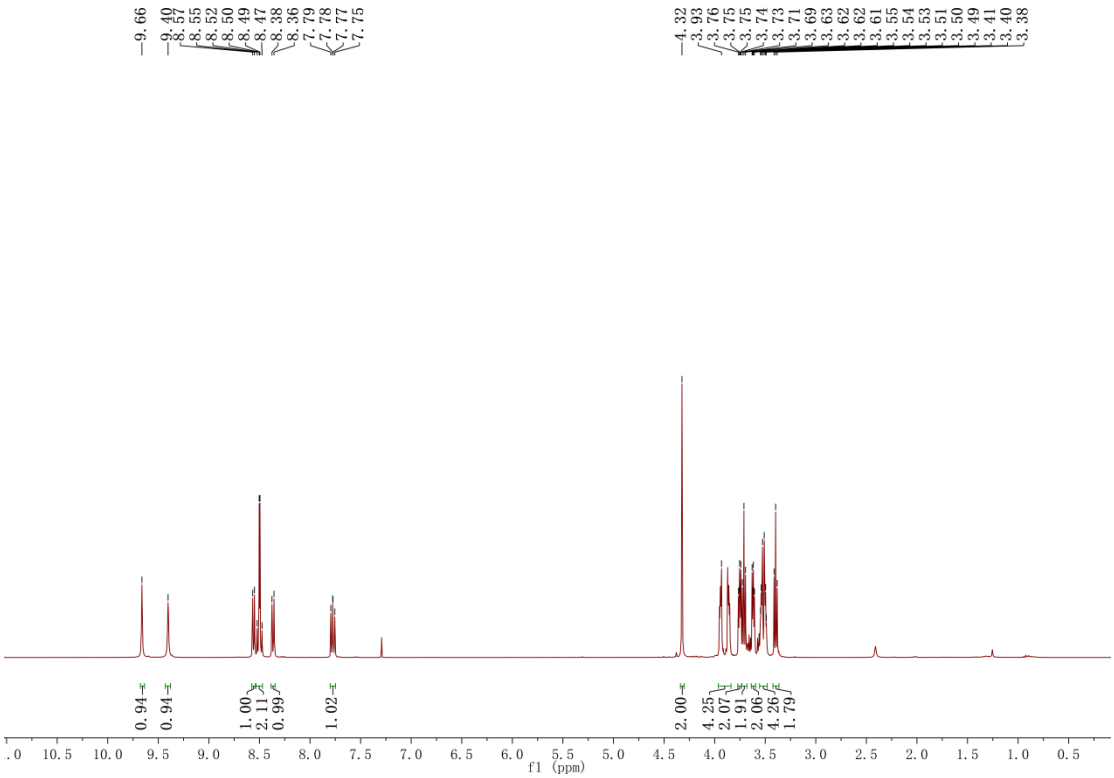


Fig S3: ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of Compound 4.



1Fig S4: ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of 5.

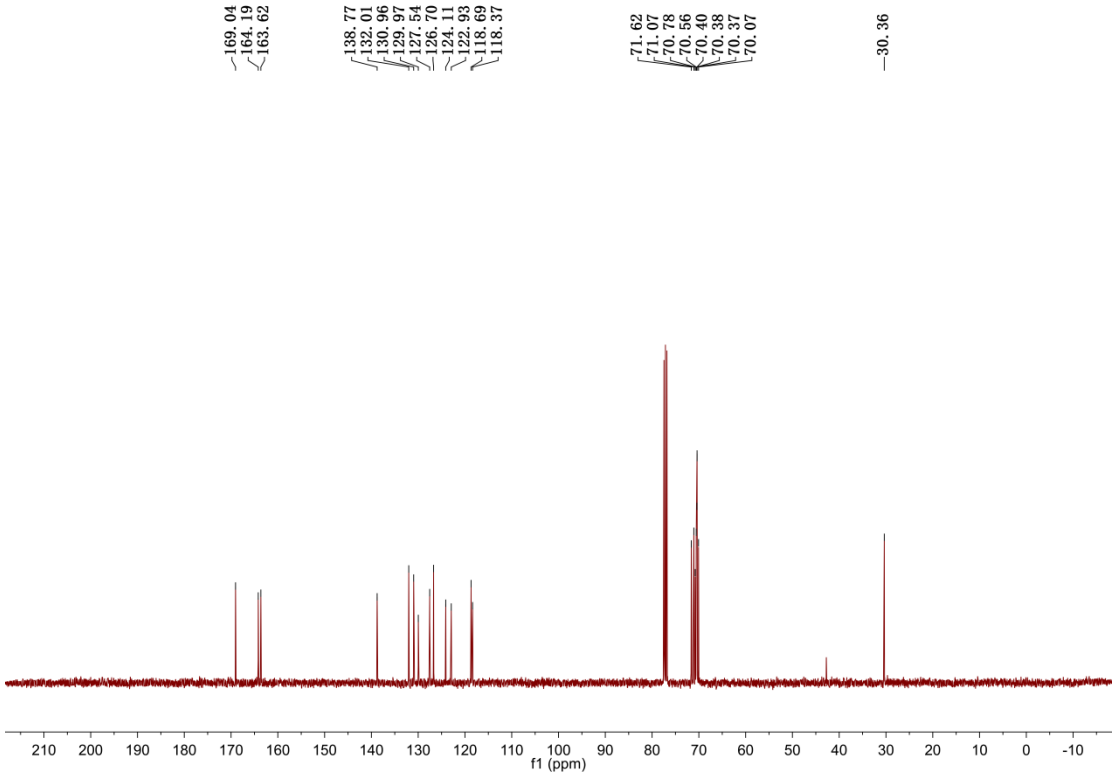


Fig S5: ^{13}C NMR spectrum (101 MHz, $CDCl_3$) of 5.

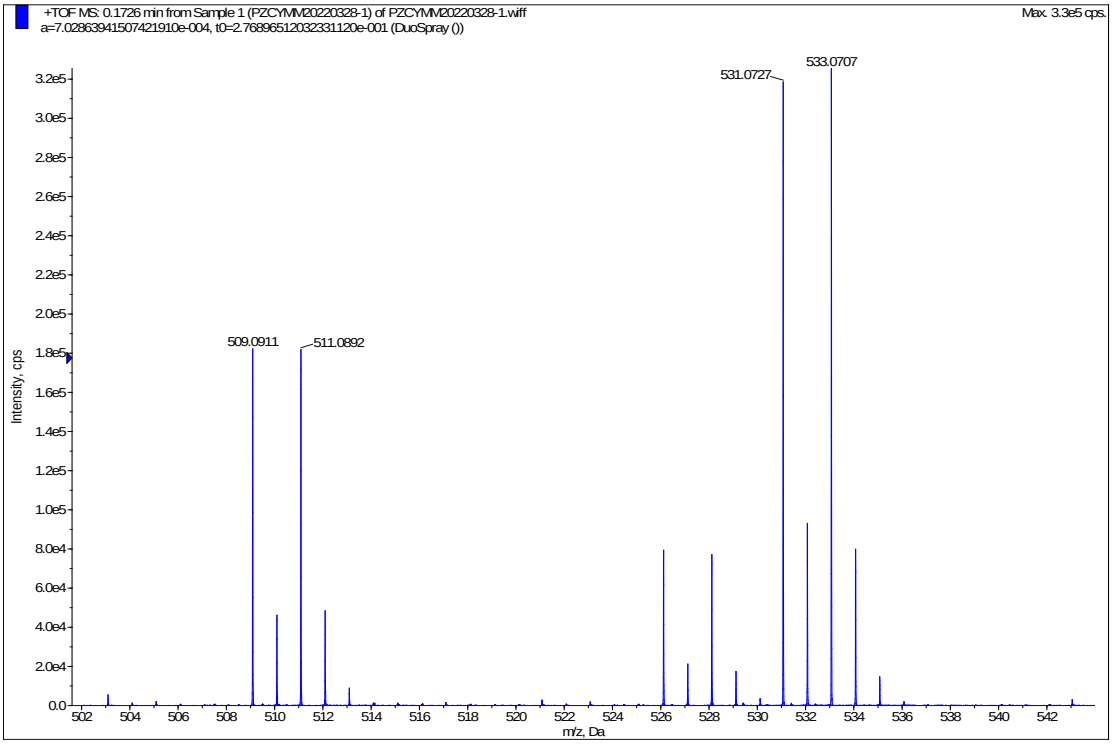


Fig S6: HRMS of 5.

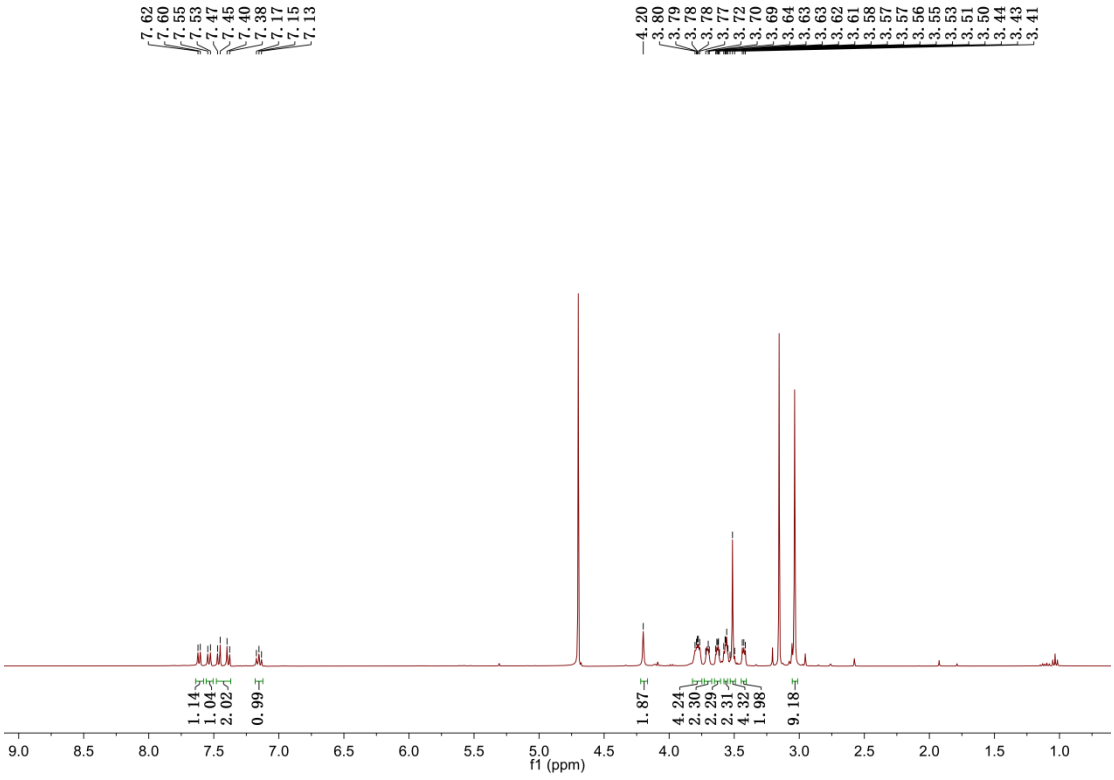


Fig S7: ^{1}H NMR (400 MHz, D_2O) spectrum of Pro-ANI.

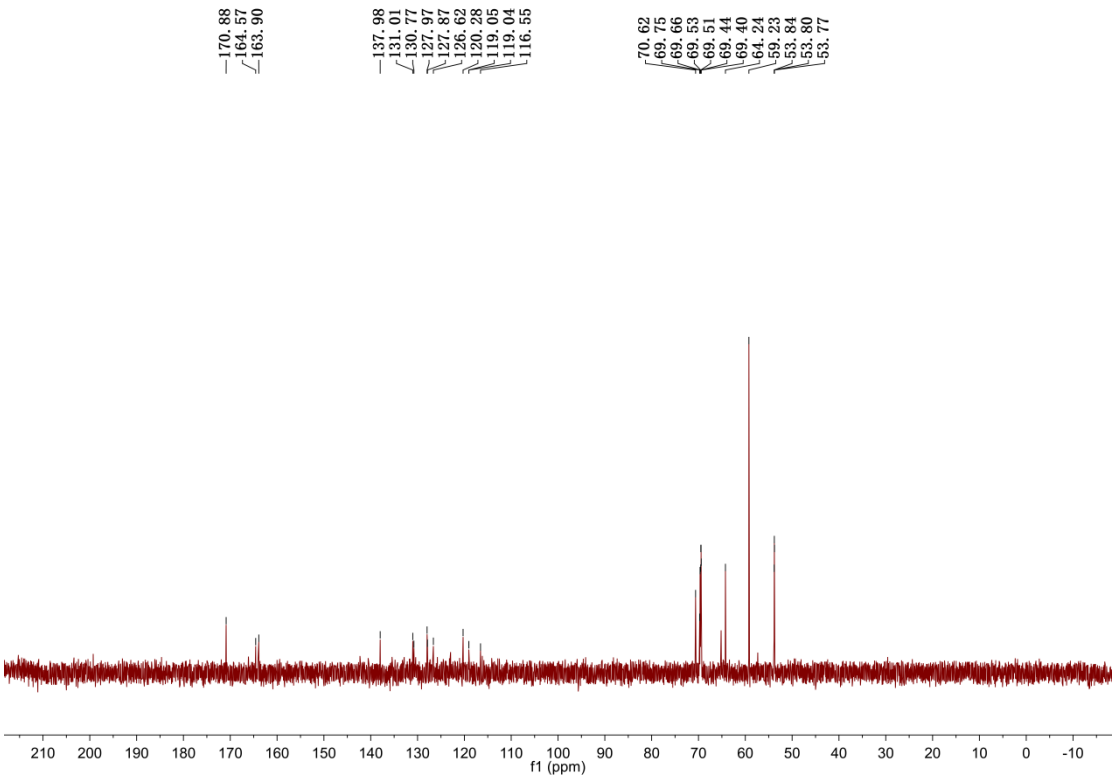


Fig S8: ^{13}C NMR spectrum (101 MHz, D_2O) of Pro-ANI.

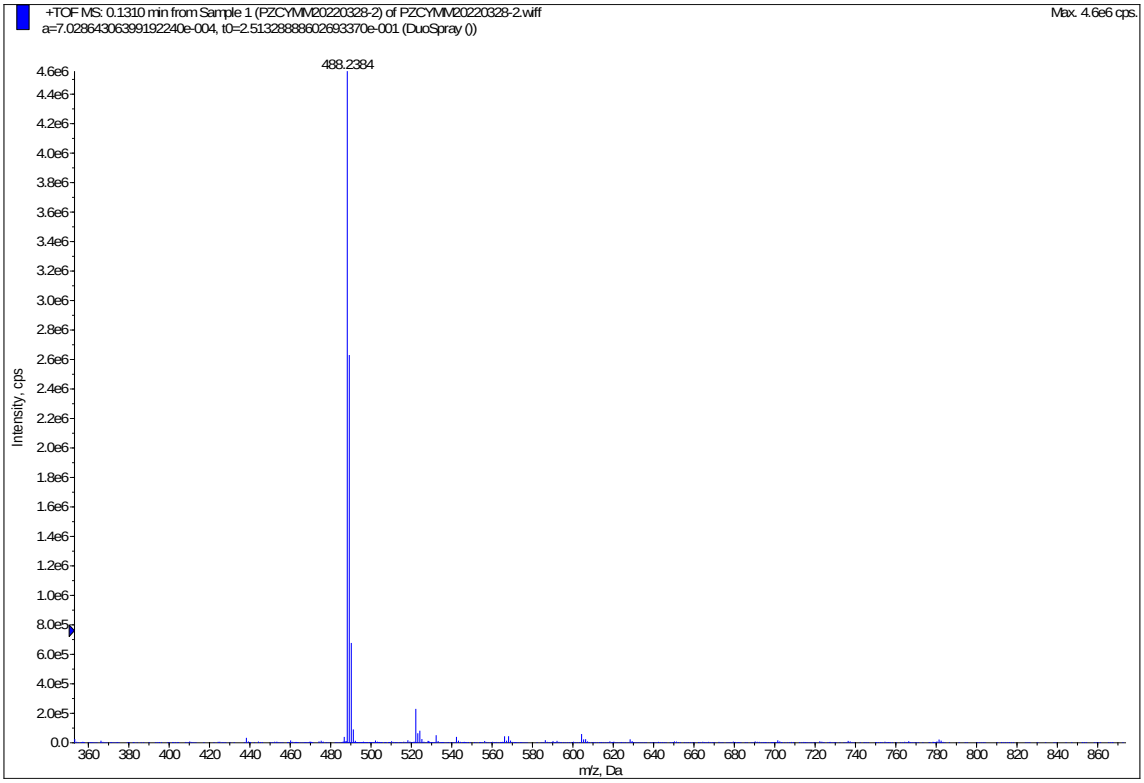


Fig S9: HRMS of Pro-ANI.

3. Preparation and characterization of GP5⊃Pro-ANI vesicles

To the pure water (1 mL) was added GP5 (6 mg) and Pro-ANI (1 mg). Then the mixture was

subjected to ultrasonication for 30 min and left to stand still overnight to obtain GP5⊃Pro-ANI vesicles (1.84 mM, 1 mL). Accordingly, DOX (1 mg, 1.84 mM) were added to the mixture, repeating the steps above to obtain DOX-loaded GP5⊃Pro-ANI vesicles (DOX@GP5⊃Pro-ANI). The vesicles above were characterized by SEM, TEM and DLS.

4. DOX loading and release profile of vesicles

The prepared DOX-loaded GP5⊃Pro-ANI vesicles solution was dialysed in distilled water for 24 h, and the amount of unloaded DOX in the dialysate was quantitatively measured by Ultraviolet and visible spectrophotometry (UV-Vis). According to the formula below, the DOX encapsulation efficiency was calculated to be 30%.

Encapsulation Efficiency (%) = $(m_{DOX\text{-}loaded}/m_{DOX})100\%$, $m_{DOX\text{-}loaded}$, and m_{DOX} are masses of DOX encapsulated in vesicles, DOX added.

The prepared DOX-loaded GP5⊃Pro-ANI vesicles solution (1.84 mM, 1 mL) was dialysed in H_2O, and aqueous solution containing esterase (100 U/L), away from light at r.t. At specified time intervals, the concentration of released DOX was determined by UV-vis.

5. Cell Culture

HL7702 cells (normal human liver), HepG2 cells (DOX-sensitive) and HepG2-ADR cells (DOX-resistant) were cultured in complete Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin in an atmosphere with 5% CO_2 at 37 ℃.

6. Cell Uptake observed by Confocal laser scanning microscopy (CLSM)

For the details of the uptake characterization experiment, HepG2-ADR cells were cultured in two 35 mm confocal dishes at a density of 2×10^5 cells/well and co-cultured with 10 μM GP5⊃Pro-ANI. After 24 h, discarded the old medium and washed with PBS and cells were immobilized with 4% paraformaldehyde for 10-15 min. Finally, the cells were washed with PBS ~~for~~ twice and the fluorescence images were captured by a confocal microscope with different channels under a Laser Scanning Confocal Microscope (Andor REVOLUTION WD).

7. Target Ability Assay

Cells were cultured with a density of 8000 cells/well in 96-well plates. HL7702 cells ~~was~~ were used as control, and HepG2-ADR cells were untreated or pre-treated with lactose (2 mg/mL) for 4 h. After 4 h, all of the cells were co-cultured with DOX@GP5⊃Pro-ANI for another 4 h. Hoechst 33342 was used to stain the nucleus and the fluorescence images were taken by the Inverted Fluorescence Microscope.

8. Cytotoxicity Evaluation

The relative cytotoxicities of different corresponding compounds (DOX, GP5⊃Pro-ANI and

DOX@GP5⊃Pro-ANI) were evaluated *in vitro* by Cell Counting Kit-8 (CCK-8) assay (Beyotime Biotechnology). The cells were cultured with complete DMEM for 24 h with a density of 8000 cells/well in 96-well plates (100 μL medium/well). The compounds above in different concentrations were added and co-cultured for 24 h and 48 h, respectively. Then, discarded the old medium, 10 μL of CCK-8 reagent per well was added away from the light and cultured for 1 h. Finally, the optical density (OD) at 450 nm was recorded with a microplate reader. Each assay has 3 repetitions.[4]

9. Colony Forming Assay

Colony formation assay is aimed at measuring the proliferation ability of HepG2 cells after treatment with single drug or drug-loaded vesicles. Cells were seeded in 6-well plates with a density of 1500 cells/well and incubated with DOX and DOX@GP5⊃Pro-ANI (DOX concentrations: 0, 0.5, 1 μM) respectively for one week to allow the formation of colonies. The obtained colonies were fixed with 10% methanol for 1 min and stained with 0.1% crystal violet for 15 min. Subsequently, the stained cells were rinsed several times with pure water. The plate was inverted and air-dried at r.t. Images of the colonies were taken by a camera and colonies were counted by Image J analysis software.[5]

10. Cell Scratch Test

Cell scratch test was used to evaluate cell migration capacity. HepG2 cells were seeded in a 6-well plate with a density of 5×10^5 cells/well. When the cells had reached 90% fusion, lines were made vertically in the cell monolayer with 200 μL tip, the cells were washed with PBS for 3 times. Then, the experimental group was treated with GP5⊃Pro-ANI (10 μM) and DOX@GP5⊃Pro-ANI (GP5⊃Pro-ANI 10 μM) and the control group was cultured in serum-free medium. The relative distance of cell migration was observed and photographed at 0 and 24h with the Inverted Fluorescence Microscope.

11. Mitochondrial and Lysosomal Co-location Assay

HepG2 cells were co-cultured with DOX and DOX@GP5⊃Pro-ANI (DOX concentration: 5 μM) for 4 and 16 h. Then the cells were co-cultured with Lyso-Tracker Red (50 nM) and Mito-Tracker Green (200 nM) for 30 min at 37 ℃ and washed with DPBS twice to avoid deep background. Fluorescent images were taken ~~by~~ with the Inverted Fluorescence Microscope.

12. ROS and Mitochondrial membrane potential Detection

ROS level and Mitochondrial membrane potential were evaluated with a Reactive Oxygen Species Assay Kit and an Enhanced mitochondrial membrane potential assay kit (JC-1) (Beyotime Biotechnology).[6] JC-1 buffer solution was prepared according to the method provided by

Beyotime Biotechnology. HepG2 cells were seeded in 96-well plates with a density of 8000 cells/well and incubated for 12 h. Replaced the old culture with new culture containing DOX and DOX@GP5⊃Pro-ANI (DOX concentrations: 5 and 10 μM) and co-cultured for 4 and 16 h, respectively. For ROS detection, the cells were treated with DCFH-DA (10 μM). For MMP detection, the JC-1 working solution was gotten by the mixture of JC-1 buffer solution and DMEM in a ratio of 1:1. The cells were treated with the JC-1 working solution (100 μL) for 20 min at 37 ℃. Discarded the solution of the probes above and washed with basal DMEM, the fluorescence of DCF and JC-1 monomer/aggregates were detected by a microplate reader at 488 and 525 nm.

13. Apoptosis Assay by Annexin V-FITC/PI

HepG2-ADR cells were inoculated into 6-well plates with a density of 2×10^5 cells/well and treated with DOX and DOX@GP5⊃Pro-ANI (DOX concentration: 2 μM), respectively. After 48 h, the cells were collected, washed with PBS for twice, centrifuged at 300 ×g for 5 min at 4 ℃, and resuspended in Binding Buffer. Cells staining was performed according to the method provided by the Annexin V-FITC/PI apoptosis detection kit (YEASEN Biotech); apoptosis was detected by flow cytometer (Becton Dickinson-LSR, USA). Each assay has 3 repetitions.[7]

14. Wetsern Blot Analysis

After the treatment of DOX and DOX@GP5⊃Pro-ANI (DOX concentration: 15 μM) for 18 h to HepG2 cells, respectively, proteins were collected after cell lysis by RIPA Lysis Buffer (Beyotime Biotechnology). Protein samples (80 μg) were separated by 10% and 12% SDS polyacrylamide gel, transferred onto Immun-Blot PVDF membranes, blocked with 5% milk powder for 1 h, incubated overnight with antibodies anti-Cleaved-PARP 1, anti-Caspase 3, anti-Bax, anti-Beclin 1, anti-p-Beclin 1 and anti-β-actin and incubated for 2 h with appropriate secondary antibodies. Bands were visualized by Gel-Capture and pictures were taken by its imaging system.[8]

15. Fig S10-S26

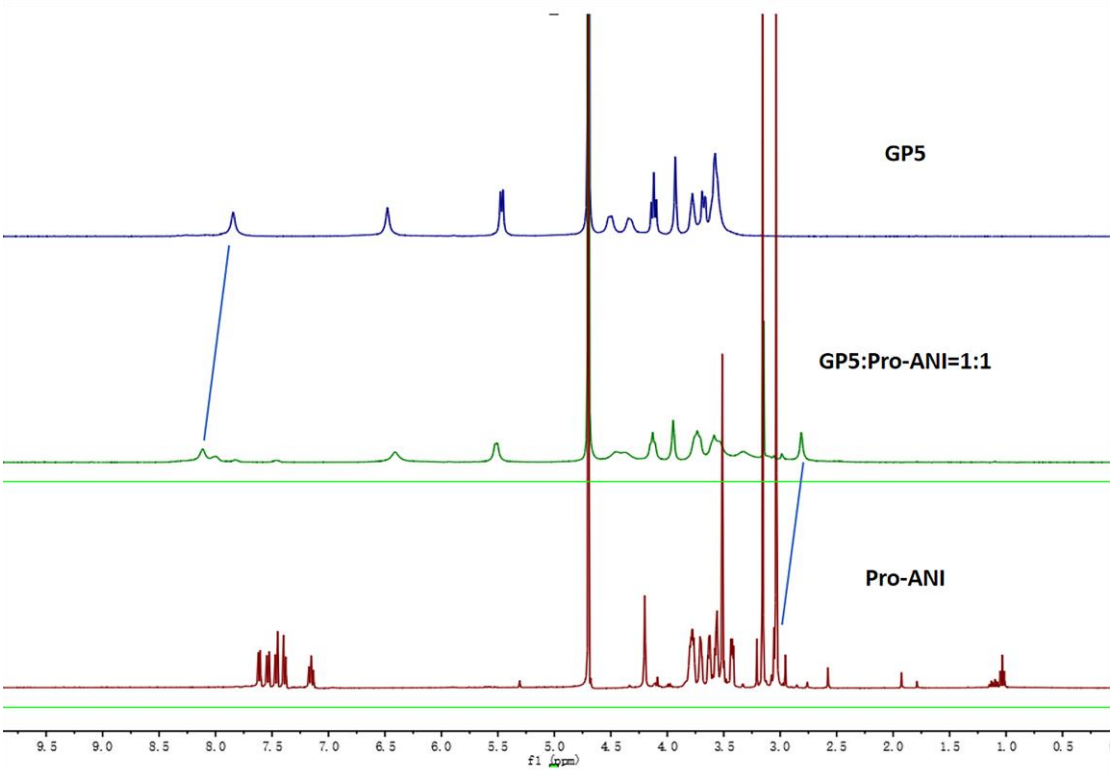


Fig S10. ^{1}H NMR spectra (400 MHz, D_2O): (top) GP5 (1.84 mM); (middle) GP5 : Pro-ANI = 1:1; (bottom) Pro-ANI (1.84 mM).

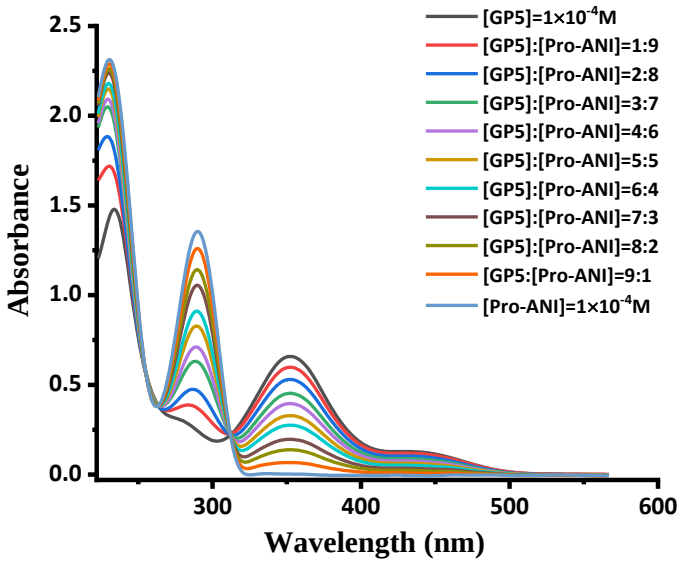


Fig S 11. UV-Vis absorption of the mixture of GP5 and Pro-ANI in water at different molar ratios while GP5 + Pro-ANI = 1.0×10^{-4} M.

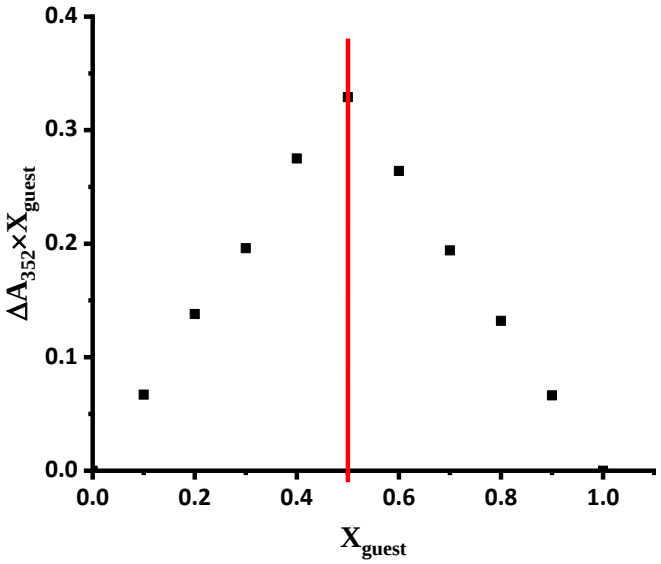


Fig S12. Binding stoichiometry of GP5 and Pro-ANI.

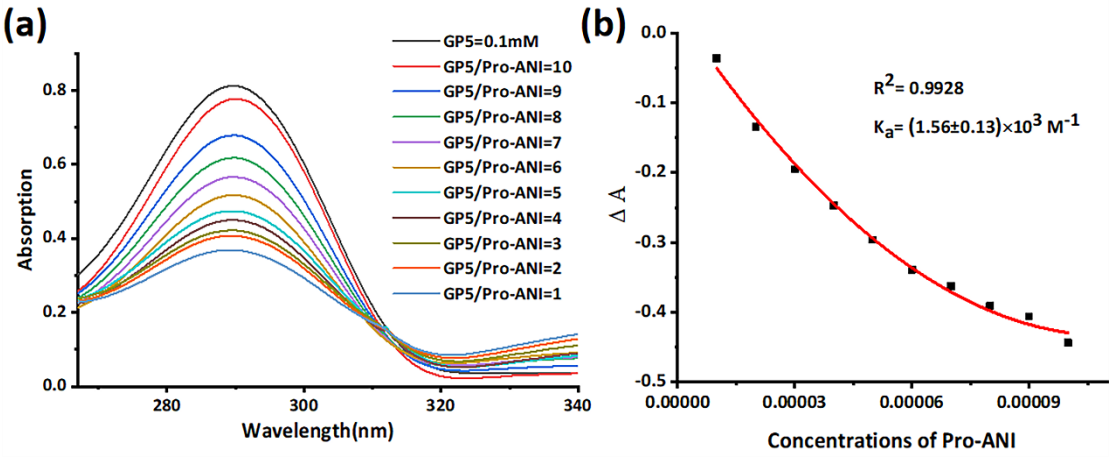


Fig S13. Determination of the association constants between GP5 and Pro-ANI: UV−vis absorption changes of GP5 with varied concentrations of Pro-ANI (a); Dependence of the UV−vis absorption at 290 nm on GP5 with varied concentrations of Pro-ANI (b). The non-linear curve-fittings were based on the equation:

$\Delta A = (\Delta A_\infty/[H]_0)\ (0.5[G]_0 + 0.5([H]_0 + 1/K_a) - (0.5\ ([G]_0^2 + (2[G]_0(1/K_a - [H]_0)) + (1/K_a + [H]_0)^2)^{0.5}))$; Where ΔA is the UV-vis absorption changes at 290 nm at $[H]_0$, ΔA_∞ is the UV-vis absorption changes at 290 nm when Pro-ANI is completely complex, $[G]_0$ is the initial concentration of Pro-ANI, and $[H]_0$ is the fixed initial concentration of GP5.

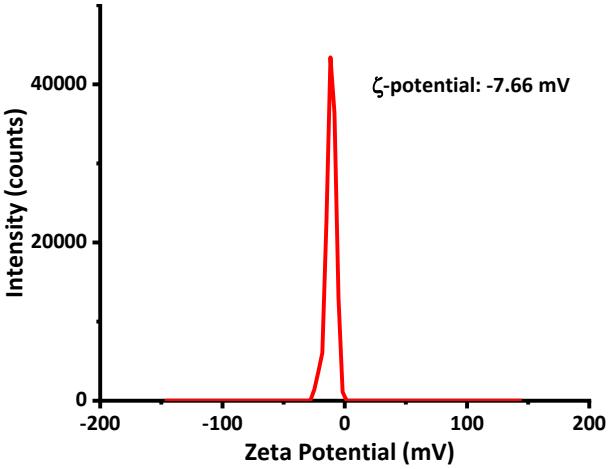


Fig S14. Zeta potential of GP5⊃Pro-ANI.

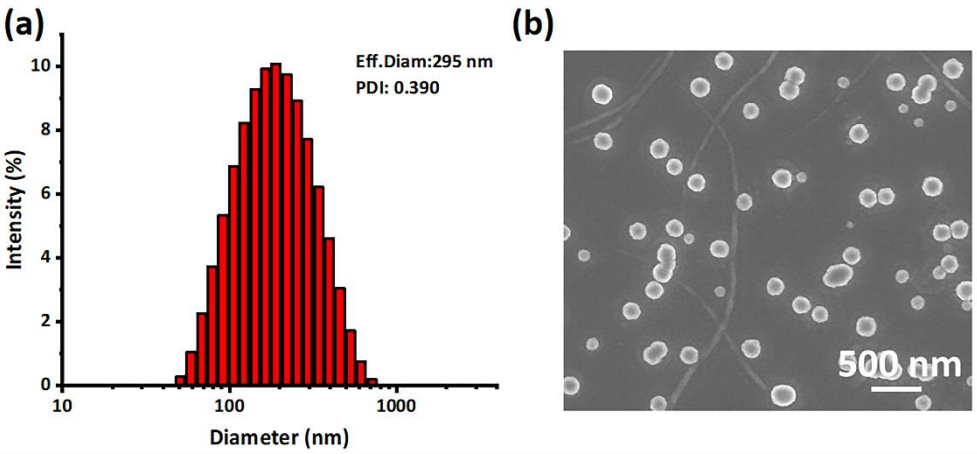


Fig S15. (a) DLS of DOX@GP5⊃Pro-ANI; (b) SEM image of DOX@GP5⊃Pro-ANI.

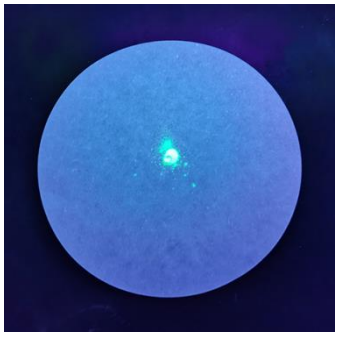


Fig S16. Fluorescent images of GP5⊃Pro-ANI in its solid state.

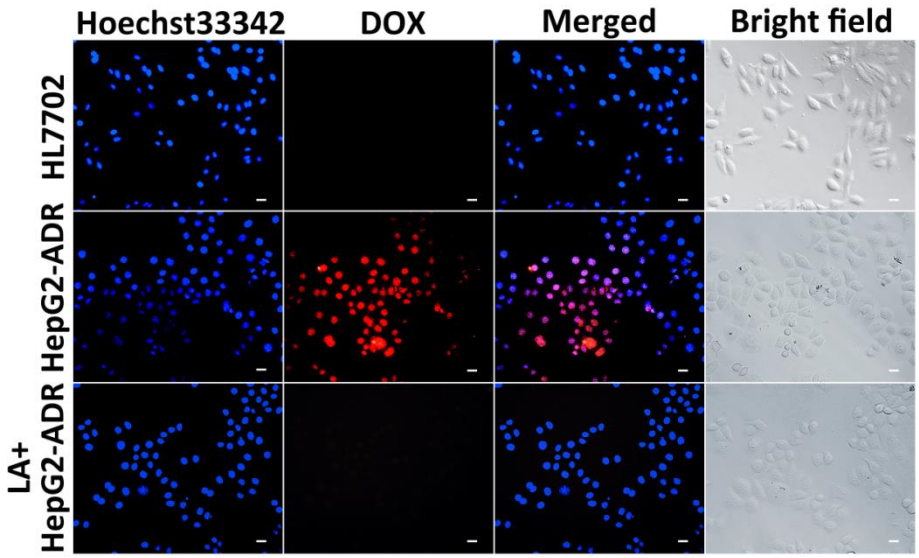


Fig S17. Fluorescent images of HL7702 and HepG2-ADR cells cultured with DOX@GP5⊃Pro-ANI (DOX concentration: 10 μM) for 4 h. Images of HepG2-ADR cells cultured with DOX@GP5⊃Pro-ANI for another 4 h after the preincubation with lactose (LA) for 4 h. The nucleus was stained with Hoechst 33342. Images were taken from the Hoechst 33342 channel, the DOX channel, the bright channel and the overlapped images. (Scar bars 20 μm).

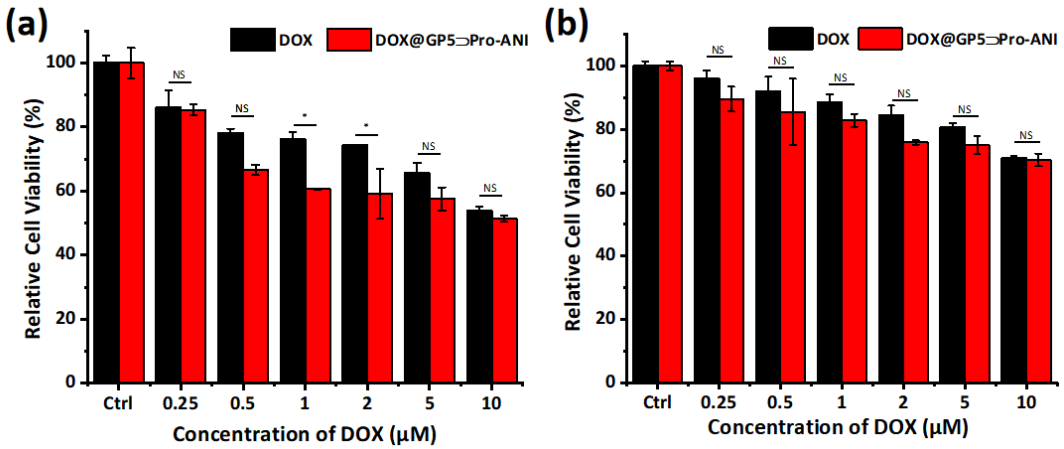


Fig S18. Cell viability of HepG2 cells (a) and HepG2-ADR cells (b) incubated with DOX and DOX@GP5⊃Pro-ANI for 24 h. Statistical analysis comparisons between two groups were analyzed using unpaired Student's t-tests, with $p < 0.05$ considered to be statistically significant (NS: not significantly different, *$p < 0.05$, **$p < 0.01$).

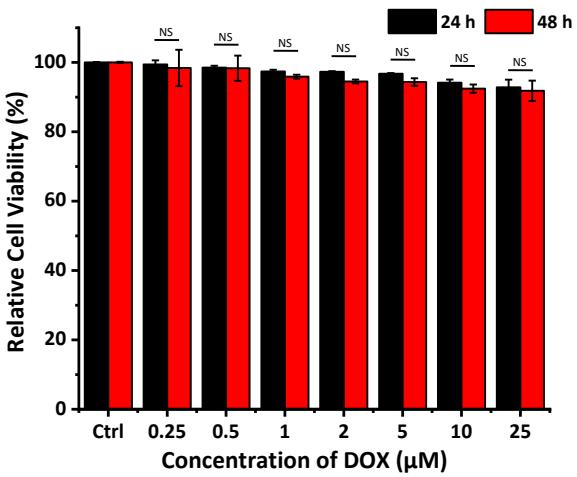


Fig S19. Cell viability of HL7702 cells incubated with GP5⊃Pro-ANI for 24 h and 48 h. Data are presented as means ± s.d. (n = 3). Statistical analysis comparisons between two groups were analyzed using unpaired Student's t-tests, with $p < 0.05$ considered to be statistically significant (NS: not significantly different, *$p < 0.05$, **$p < 0.01$).

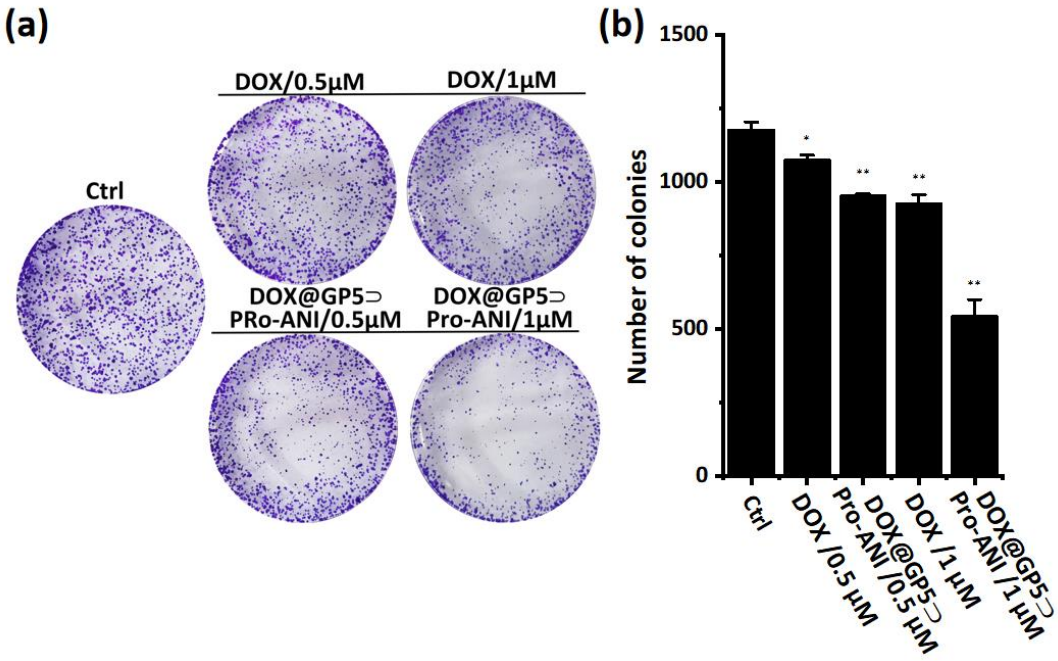


Fig S20. (a) Colonies formed by HepG2 cells incubated with DOX and DOX@GP5⊃Pro-ANI (DOX concentrations: 0.5, 1 μM) for one week; (b) bar graphs plotting the number of colonies analysed by Image J, when compared with the Ctrl group alone. Statistical analysis compared with the Ctrl group were analyzed using unpaired Student's t-tests, with $p < 0.05$ considered to be statistically significant (NS: not significantly different, *$p < 0.05$, **$p < 0.01$).

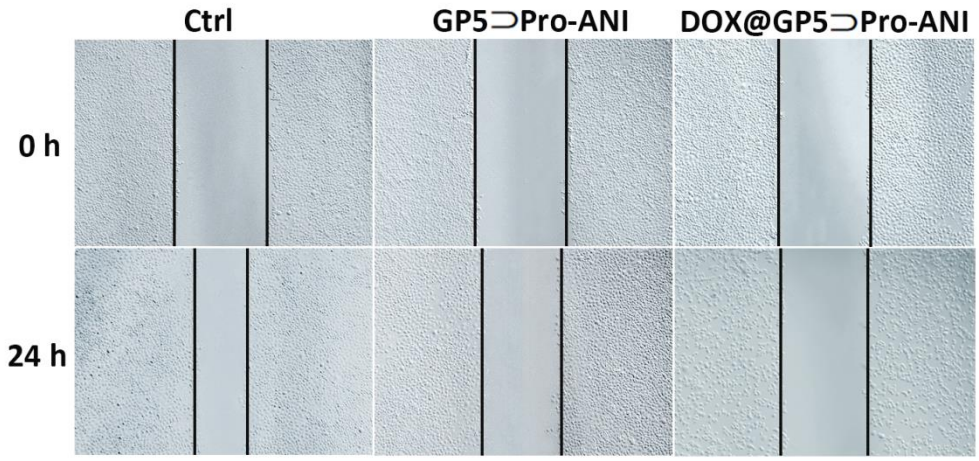


Fig S21. Evaluation of cell migration after the treatment with GP5⊃Pro-ANI (10 μM) and DOX@GP5⊃Pro-ANI (GP5⊃Pro-ANI 10 μM) for 24 h.

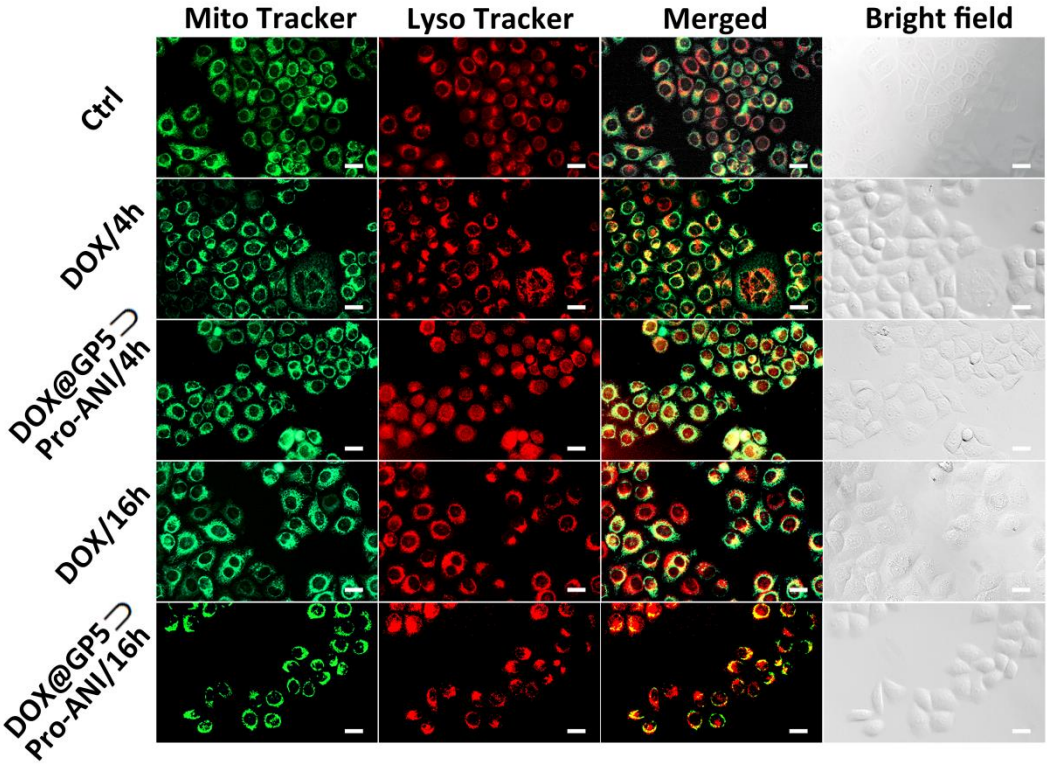


Fig S22. Fluorescent images of HepG2-ADR cells incubated with DOX and DOX@GP5⊃Pro-ANI for 4 and 16 h, respectively. (DOX concentration 5 μM; Scar bars 20 μm); Mitochondria were stained with Mito-Tracker Green and Lysosome were stained Lyso-Tracker Red.

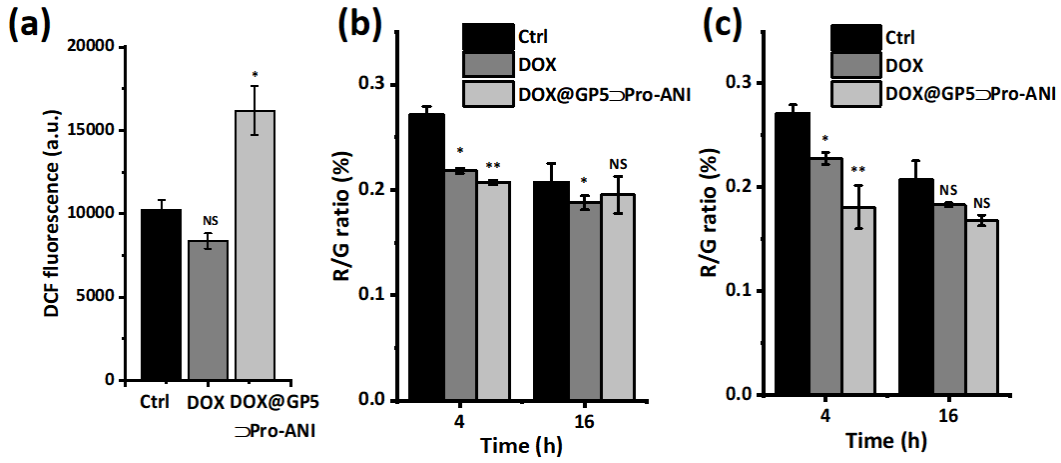


Fig S23. (a) ROS levels are expressed in terms of the relative intensity of DCF fluorescence,. HepG2 cells were treated with DOX and DOX@GP5⊃Pro-ANI (DOX concentration: 10 μM) for 4 h; Changes in mitochondrial membrane potential of HepG2 cells seeded in 96-well microplates and incubated with DOX and DOX@GP5⊃Pro-ANI (DOX concentrations: 5 μM (b) and 10 μM (c)) for 4 and 16 h. The fluorescence ratio of JC-1 dimmers/JC-1 monomers (R/G) is expressed as the change of mitochondrial membrane potential. Statistical analysis compared with the Ctrl group were analyzed using unpaired Student's t-tests, with $p < 0.05$ considered to be statistically significant (NS: not significantly different, *$p < 0.05$, **$p < 0.01$).

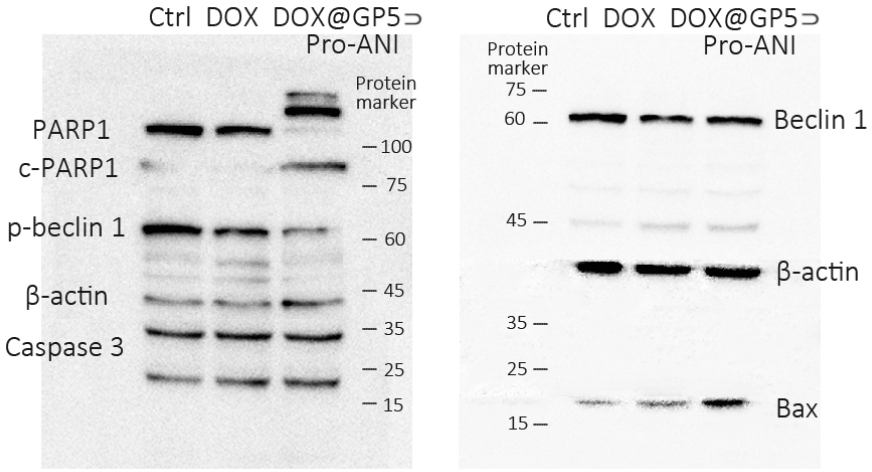


Fig S24. Original Electrophoretic gels and blots images. After the treatment of DOX and DOX@GP5⊃Pro-ANI (DOX concentration: 15 μM) for 18 h to HepG2 cells, respectively. Proteins were collected and detected by Western blotting.

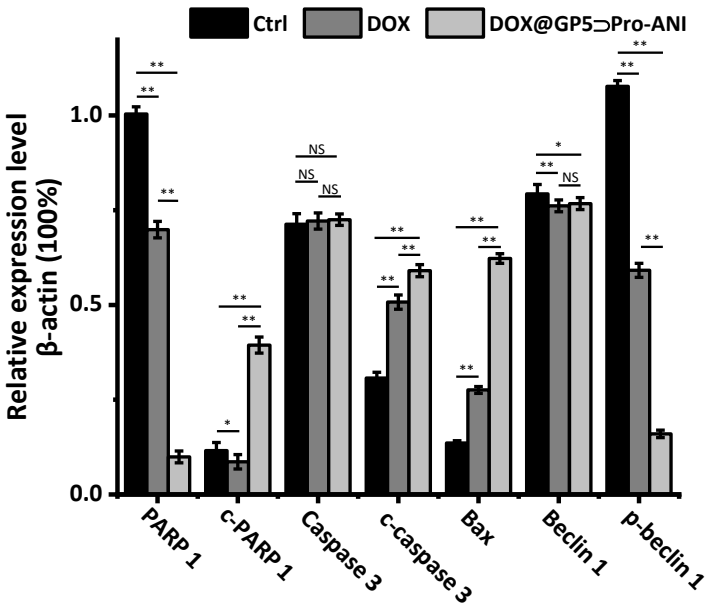


Fig S25. Western Blot analysis of PARP-1 and other apoptosis and autophagy-associated proteins are quantified. Statistical analysis comparisons between two groups were analyzed using unpaired Student's t-tests, with $p < 0.05$ considered to be statistically significant (NS: not significantly different, *$p < 0.05$, **$p < 0.01$).

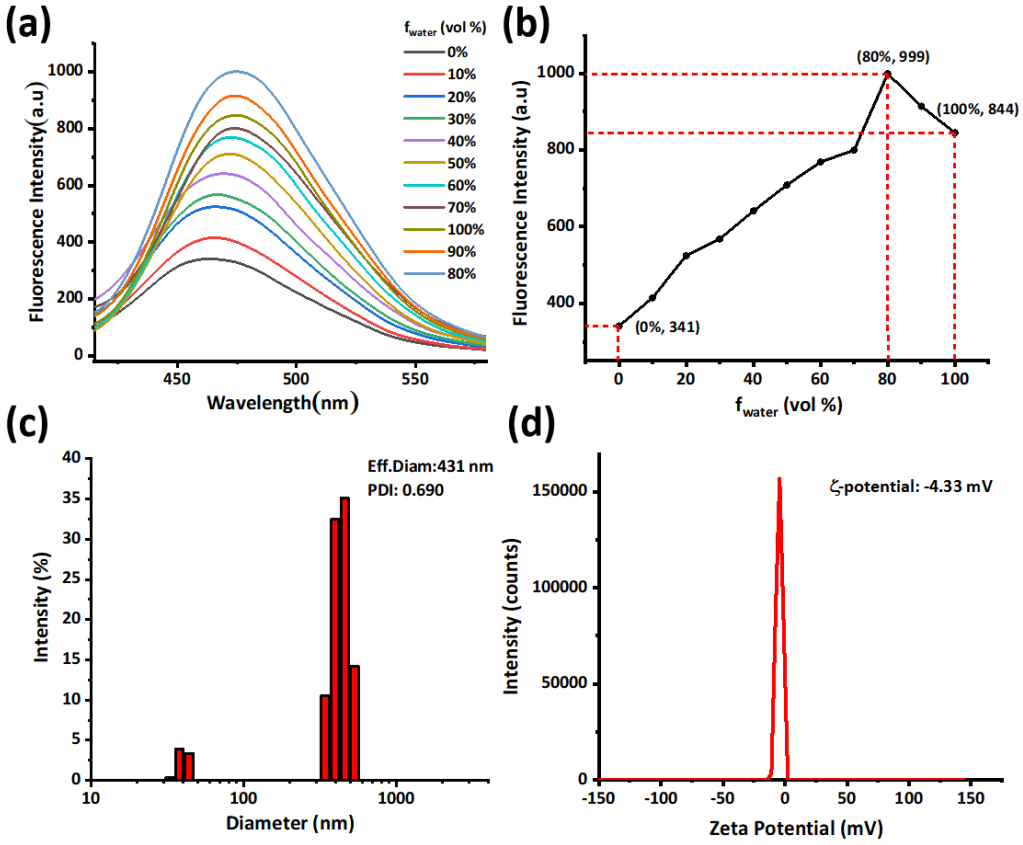


Fig S26. (a) Fluorescence spectra of Pro-ANI in the water-EtOH mixture (λex = 350 nm); (b) Plot of maximum emission intensity of Pro-ANI. [Pro-ANI] = 1×10^{-5} M. (c) DLS of Pro-ANI; (d) zeta potential of Pro-ANI.

16. List of Abbreviations

SNDS: supramolecular nano-delivery system;

AIEgens: aggregation-induced emission luminogens;

AIE: aggregation-induced emission;

GP5⊃Pro-ANIsupramolecular nano-delivery system;

GP5: Glycosylated pillar[5]arene;

ANI: 4-Amino-1,8-Naphthimide;

PARP: Poly(ADP-ribose)polymerase;

DOX@GP5⊃Pro-ANI: DOX-loaded GP5⊃Pro-ANI vesicles;

Pro-ANI: PARP inhibitor prodrug;

DOX: doxorubicin;

HepG2 cells (HepG2-ADR) cells HL7702 cells

CAC: critical aggregation concentration;

SEM: scanning electron microscopy;

TEM: transmission electron microscopy;

DLS: dynamic light scattering;

EtOH: ethanol;

CLSM: confocal laser scanning microscope;

ASGPR: asialoglycoprotein receptor;

CCK-8: Cell Counting Kit-8;

AL: autophagic lysosome;

ROS: reactive oxygen species;

MMP: Mitochondrial membrane potential;

PI: phosphatidylinositol;

FCM: flow cytometry;

THF: tetrahydrofuran solution;

DCM: dichloromethane;

UV-Vis: Ultraviolet and visible spectrophotometry;

DMEM: Dulbecco's modified Eagle's medium;

FBS: fetal bovine serum.

17. References

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