A nanoplatform based on allylthiopurine bio-MOF and glycosylated AIE PARP inhibitor for cancer synthetic lethal therapy

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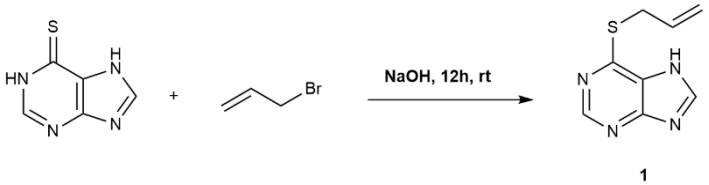
Supporting Information

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. NMR spectra were recorded on a Bruker 400 MHz Spectrometer (Bruker Corporation, Switzerland), with working frequencies of 400 MHz for ^{1}H and 101 MHz for ^{13}C nuclei. DLS measurements were performed on ZEN3600 NANOPHOX (MALVERN INSTRUMENTS LIMITED, United Kingdom). UV-vis spectra were collected with UV-visible spectrophotometer (Shimadzu UV-2450, Japan). 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 fluorescent images were from the DMi8 Inverted fluorescence microscope (Leica MICROSYSTEMS, Germany). The fluorescent intensity was detected from Synergy H1 Microplate Reader (BioTek, U.S.A). Flow cytometry data were obtained from BD FACSAria™ III Flow Cytometer (BD Biosciences, U.S.A.).

2. Synthesis and Characterization of the compounds

Synthesis of 6-Allylthiopurine (Compound 1)

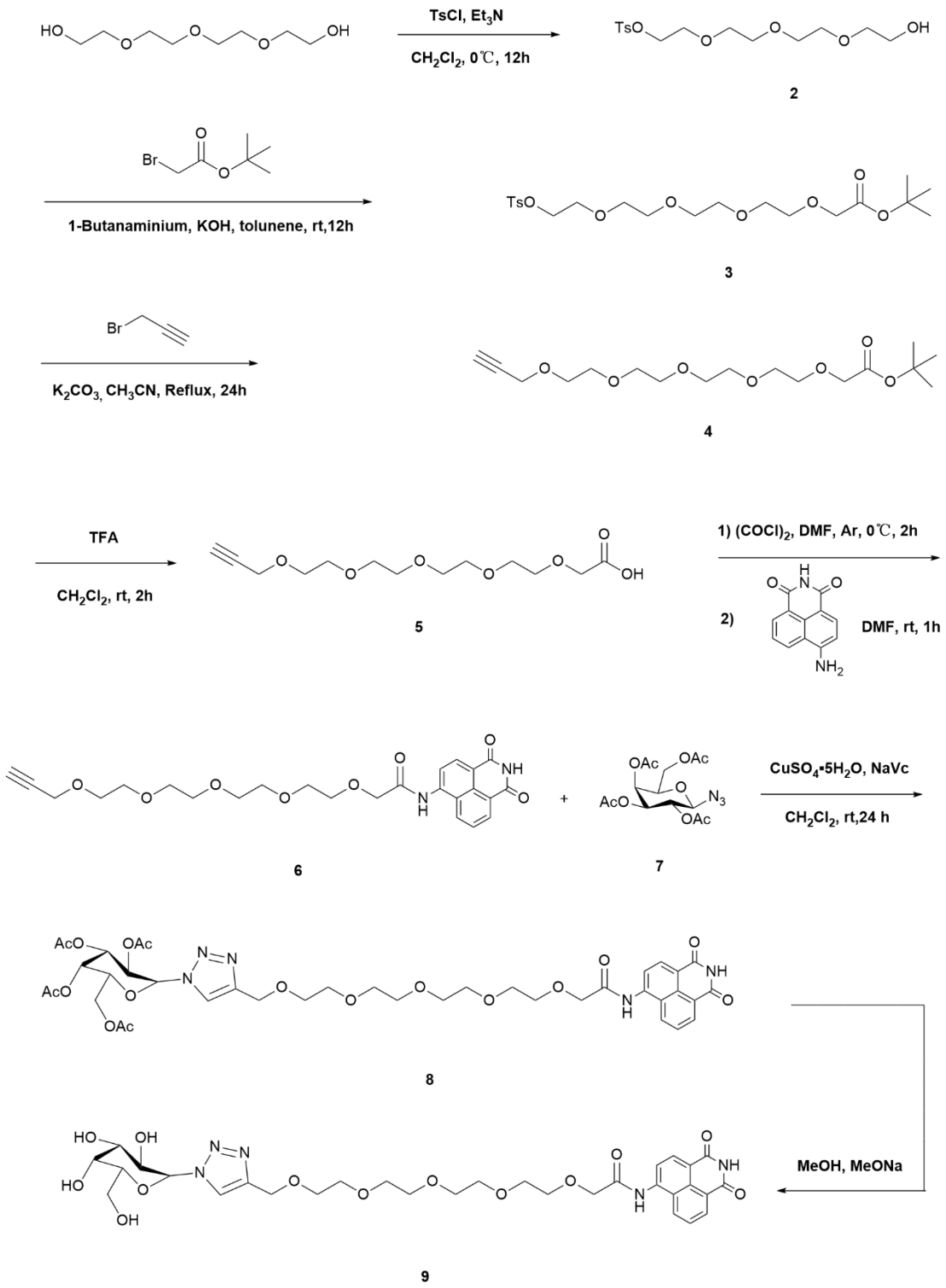


Scheme S1. Synthetic route of 6-Allylthiopurine (Compound 1)

Compound 1 was synthesized according to the previous reporter[1]

Compound 1: Allyl bromide dropwise (10.8 mmol, 1.305 g) was added to the NaOH solution (2 N, 30 ml) of 6-Mercaptopurine (9 mmol, 1.365 g). The mixture was stirred for 12 hours at room temperature. Then the mixture was acidified with acetic acid to a pH of 5 before filtering. The filter cake was washed with water (30 mL) and dried under vacuum to give compound 1 as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.71 (s, 1H), 8.46 (s, 1H), 6.00 (dt, J = 16.9, 8.4 Hz, 1H), 5.36 (d, J = 18.5 Hz, 1H), 5.14 (d, J = 8.4 Hz, 1H), 4.07 (d, J = 6.8 Hz, 2H).

Synthesis of Gal-ANI (Compound 9)



Scheme S2. Synthetic route of Gal-ANI (Compound 9)

Compound 2-6 were synthesized according to the previous reporter [2]

Compound 2: Tetra(ethylene)glycol (47.1 mmol, 5 g) was dissolved in DCM and Triethylamine (94.4 mmol, 9.5 g) was added. Then, Tosyl chloride (56.5 mmol, 10.78 g) was added at 0℃. The mixture was stirred for 12 h at room temperature. Then the mixture was acidified with HCl (6 mol/L) to a pH of 7 and extracted with DCM (20 mL×3). The collected organic phase was dried with Na_2SO_4, concentrated under reduced pressure and purified with column chromatography (petroleum ether/ethyl acetate = 2:1, v/v) to obtain compound 2.

Compound 3: 1-butanaminium (0.86 mmol, 0.28 g), KOH (20.0 mmol, 1.12 g) and tert-butyl bromoacetate (20.34 mmol, 3.96 g) was added to the toluene solution of compound 2 (17.24 mmol, 6.0 g). The solution was stirred for 12 h at room temperature and concentrated 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 to obtain the crude product, which was subsequently purified by column chromatography (petroleum ether/ethyl acetate = 4:1, v/v) to give compound 3.

Compound 4: Compound 3 (2.2 mmol, 1.0 g), 3-Bromopropyne (4.4 mmol, 1.05 g) and K_2CO_3 (4.4 mmol, 0.67 g) were added in Acetonitrile (CH_3CN, 8ml). The mixture was refluxed for 24 h under a nitrogen atmosphere. The suspension was filtrate and the filtrate was concentrated to give the residue. The residue was dissolved in DCM, washed with water (3×20 mL), dried with Na_2SO_4, concentrated under reduced pressure and finally purified via column chromatography (petroleum ether/ dichloromethane = 1:1, v/v) to obtain compound 4. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.18 (d, *J* = 2.4 Hz, 2H), 4.00 (s, 2H), 3.68-3.66 (m, 2H), 3.64 (d, *J*=1.0Hz, 14H), 2.43 (s, 1H), 1.45 (s, 9H).

Compound 5：Trifluoroacetic acid (41.6 mmol, 4.7 g) was added dropwise to the DCM solution of compound 4 (2.08 mmol, 0.72 g), and then the solution was stirred for 2h at room temperature. The mixture was concentrated and basified with NaOH (1 mol/L) to a pH of 10. The aqueous phase was washed with DCM (2×20 mL) and acidified with HCl (6 mol/L) to a pH of 2 to give the crude product. Subsequently, the crude product was extracted with ethyl acetate (3×15 mL). The combined organic phase was dried with Na_2SO_4 to give compound 5. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.19 (s, 2H), 4.15 (s, 2H), 3.68-3.65 (m, 16H), 2.44 (s, 1H).

Compound 6: Oxalyl chloride (10.9 mmol, 1.53 g) was added dropwise to compound 5 (2.2 mmol, 0.63 g) under an argon atmosphere at 0℃. The solution was stirred for 2h at room temperature. Then N,N-dimethylformamide (5 ml) and 4-amino-1,8-naphthalimide (2.08 mmol, 0.44 g) were added and stirred for 1h. The mixture was extracted with ethyl acetate (3×10 mL). The combined organic phase was washed with saturated brine, dried with Na_2SO_4 and finally purified via column chromatography to obtain compound 6. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.76 (s, 1H), 8.60 (dd, *J* = 11.3, 7.6 Hz, 2H), 8.53 (d, *J* = 8.1 Hz, 1H), 8.43 (d, *J* = 8.5 Hz, 1H), 7.82 (t, *J* = 7.8 Hz, 1H), 4.33 (s, 2H), 4.15 (d, *J* = 2.3 Hz, 2H), 3.93 (d, *J* = 4.3 Hz, 2H), 3.85 (d, *J* = 4.4 Hz, 2H), 3.75-3.72 (m, 2H), 3.63-3.57 (m, 6H), 3.51 (d, *J* = 4.1 Hz, 4H), 2.43 (s, 1H).

Compound 7-9 were synthesized according to the previous reporter [3]

Compound 7: NaN_3 (7.8 mmol, 0.51 g) was carefully added into the DMF solution of 2,3,4,6-Tetra-O-acetyl-alpha-D-glucopyranosyl bromide (2.5 mmol, 1.03 g), and the solution was stirred for 24 h at 78℃ away from light. The mixture was extracted with water. The combined organic phase was dried with Na_2SO_4, concentrated under reduced pressure and purified via column

chromatography (petroleum ether/ethyl acetate = 2:1, v/v) to obtain compound 7. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 5.43 (d, *J* = 2.6 Hz, 1H), 5.17 (t, *J* = 9.5 Hz, 1H), 5.05 (dd, *J* = 10.3 Hz, 3.3 Hz, 1H), 4.61 (d, *J* = 8.7 Hz, 1H), 4.18-4.11 (m, 2H), 4.03 (t, *J* = 6.3 Hz, 1H), 2.18 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H),1.99 (s, 3H).

Compound 8: Compound 6 (2 mmol, 0.99 g) and compound 7 (2 mmol, 0.74 g) were dissolved in CH_2Cl_2 (5 mL). Then, $CuSO_4 \cdot 5H_2O$ (0.3 mmol, 0.08 g) and NaVc (1.14 mmol, 0.16 g) were added in the solution. The mixture was stirred for 24 h at room temperature under a nitrogen atmosphere. The mixture was concentrated to obtain the crude product and DCM (20 mL) was added into it. The mixture was washed with water (3×10 mL) and saturated NaCl (10 mL). The organic phase was dried with $MgSO_4$, concentrated under reduced pressure and purified with flash column chromatography to give compound 8. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.71 (s, 1H), 9.34 (s, 1H), 8.57-8.50 (m, 2H), 8.48-8.45 (m, 1H), 8.38 (d, *J* = 8.3 Hz, 1H), 7.79-7.74 (m, 1H), 5.86 (d, *J* = 9.2 Hz, 1H), 5.54 (t, *J* = 6.4 Hz, 2H), 5.29 (dd, *J* = 10.0, 2.8 Hz, 1H), 4.62 (s, 2H), 4.30 (s, 2H), 4.17-4.13 (m, 2H), 3.90 (d, *J* = 4.5 Hz, 2H), 3.82 (d, *J* = 4.8 Hz, 2H), 3.72-3.70 (m, 2H), 3.57-3.54 (m, 6H), 3.46 (d, *J* = 4.5 Hz, 4H), 2.21 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.86 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 170.40, 170.02，169.86，169.08, 164.06, 138.93, 132.07, 131.03, 127.85, 126.74, 123.03, 119.06, 118.58, 74.00, 71.61, 70.82, 70.59, 70.35, 70.04 , 69.59, 67.94, 66.90, 64.41, 61.24, 20.60, 20.31. HRMS: m/z calcd for $[M + Na]^+$ $C_{39}H_{47}N_5O_{17}Na^+$, 880.2865, found 880.2842.

Compound 9: Compound 8 (0.25 mmol, 0.99 g) was dissolved in the methanolic solution of sodium methanolate (75 mmol, 0.99 g) and stirred for 12h at room temperature. The mixture was filtrate and the residue was washed with methanol (5×20 mL) to give compound 9. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm)：8.61 (d, *J* = 7.6 Hz, 1H), 8.37 (d, *J* = 7.3 Hz, 1H), 8.14 (d, *J* = 8.3 Hz, 1H), 7.64 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.41 (s, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 4.55 (s, 1H), 3.57-3.54 (m, 6H), 3.51 (d, *J* = 3.6 Hz, 4H), 3.39 (s, 20H). ^{13}C NMR (101 MHz, DMSO-d_6) δ(ppm): 172.33, 165.00, 164.19, 153.14, 144.41, 133.58, 131.52, 130.77, 129.77, 124.37, 123.30, 122.84, 120.20, 108.48, 88.54, 78.87, 74.14, 70.71, 70.03, 69.53, 68.95, 63.88, 60.92. HRMS: m/z calcd for $[M + Na]^+$ $C_{31}H_{39}N_5O_{13}Na^+$, 712.2442, found 712.2430.

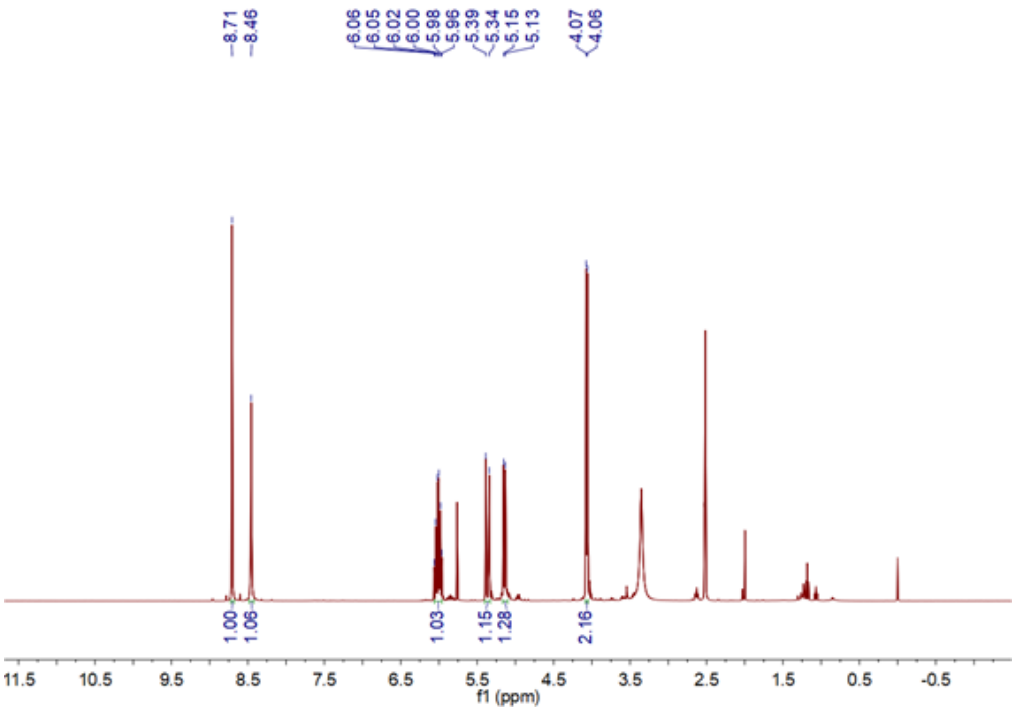


Fig S1: ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of Compound 1

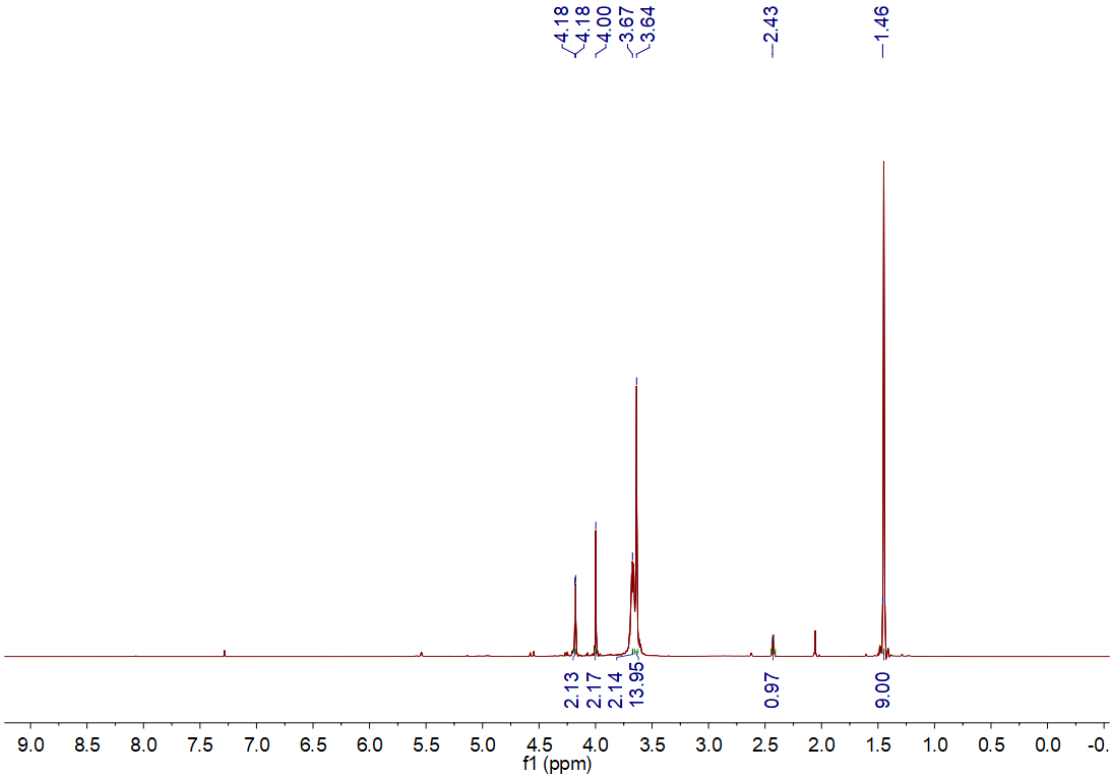


Fig S2: ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of Compound 4

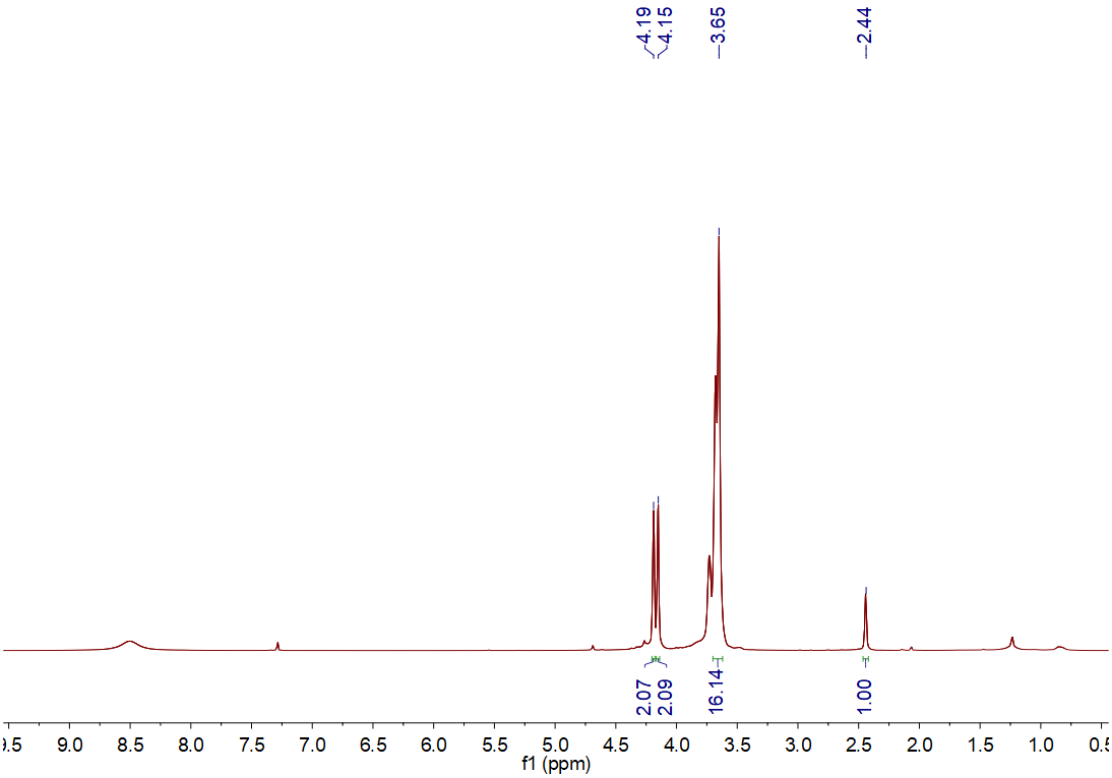


Fig S3: ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of Compound 5

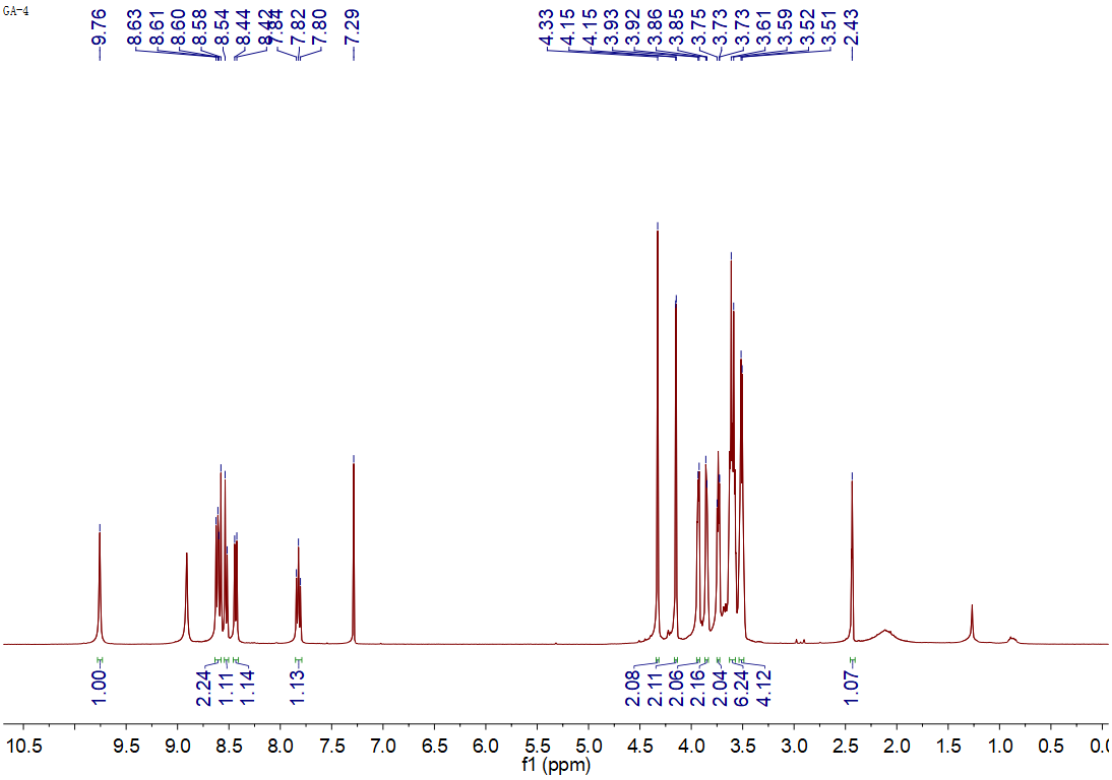


Fig S4: ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of Compound 6

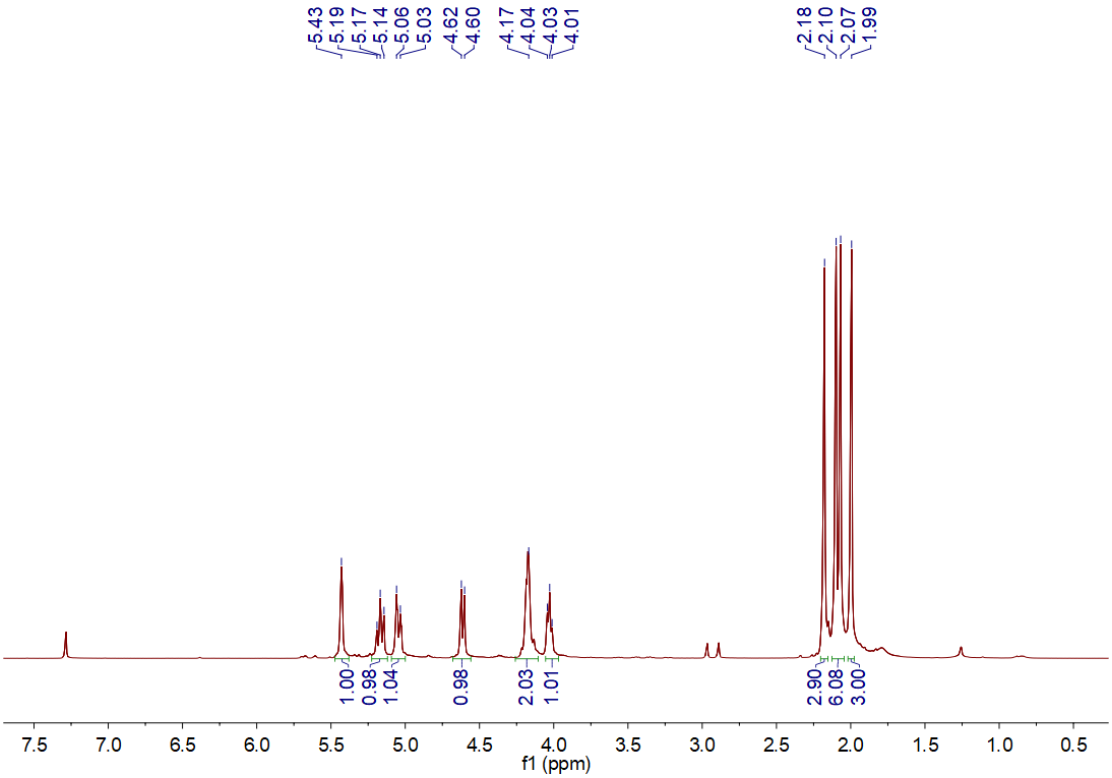


Fig S5: ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of Compound 7

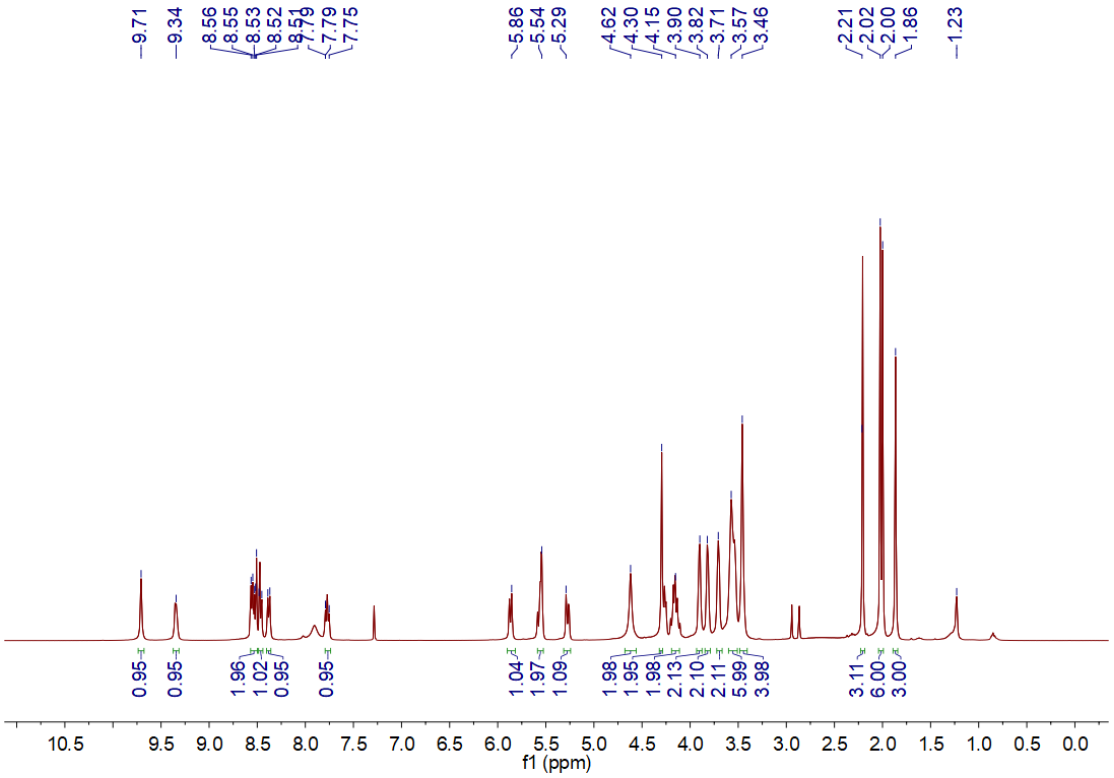


Fig S6: ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of Compound 8

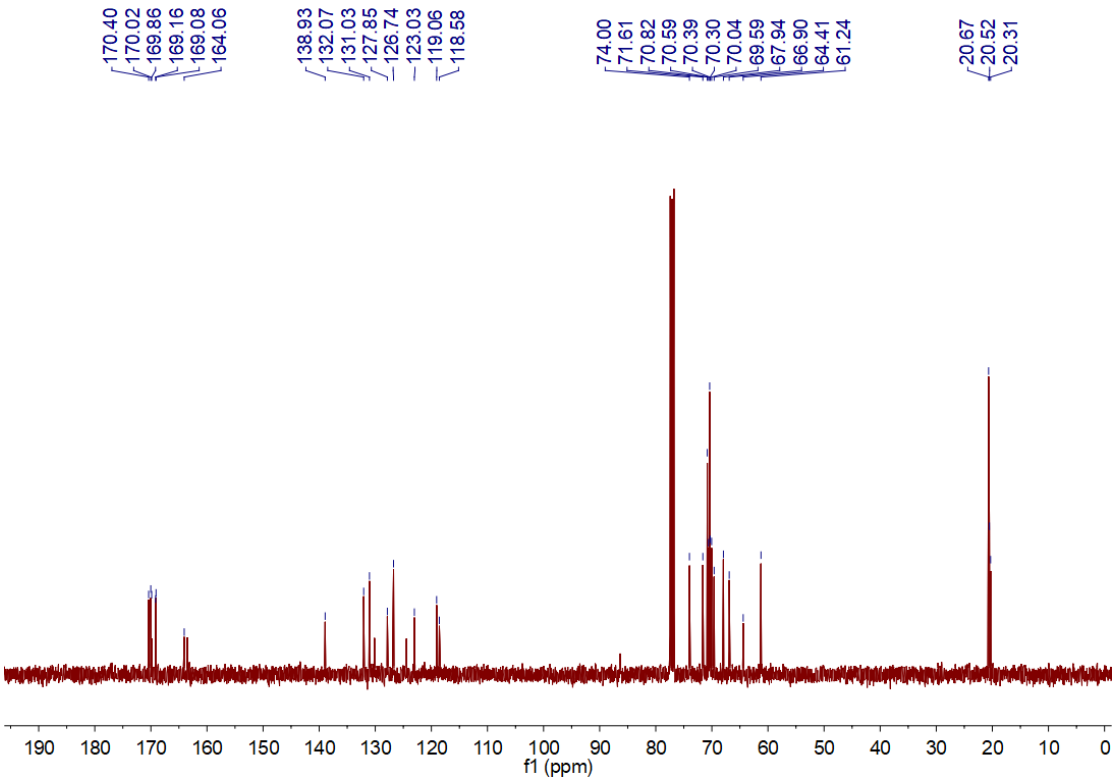


Fig S7: ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of Compound 8

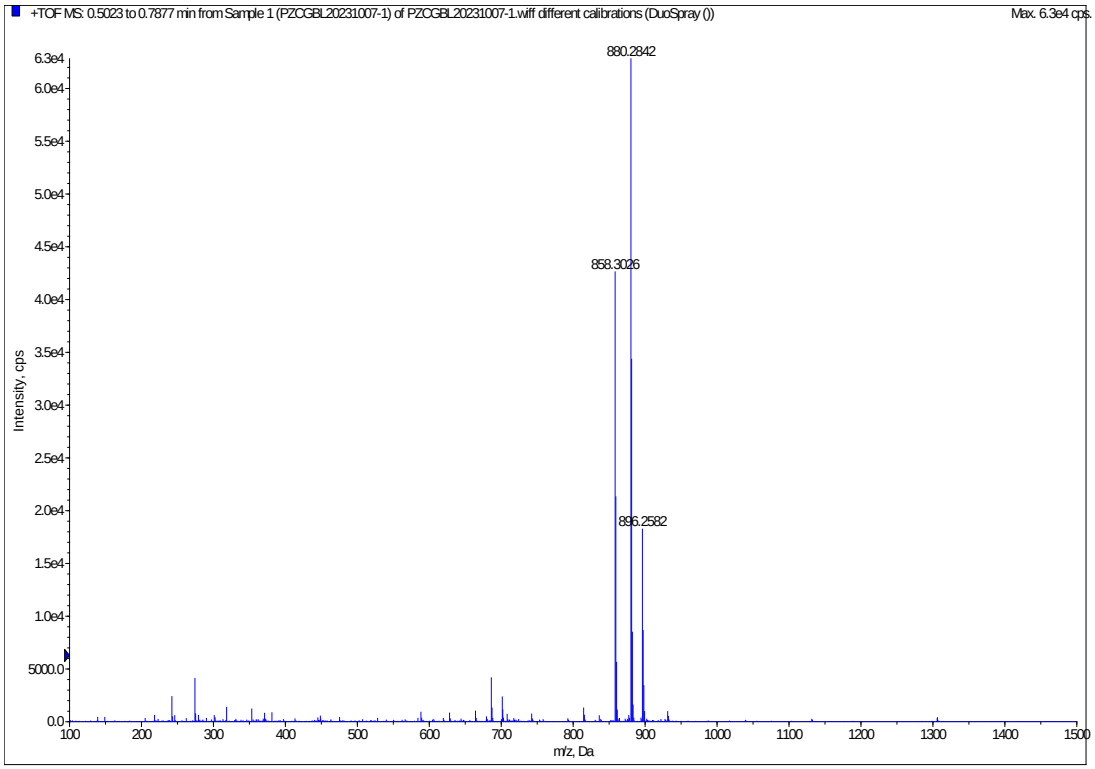


Fig S8: HRMS spectrum of compound 8

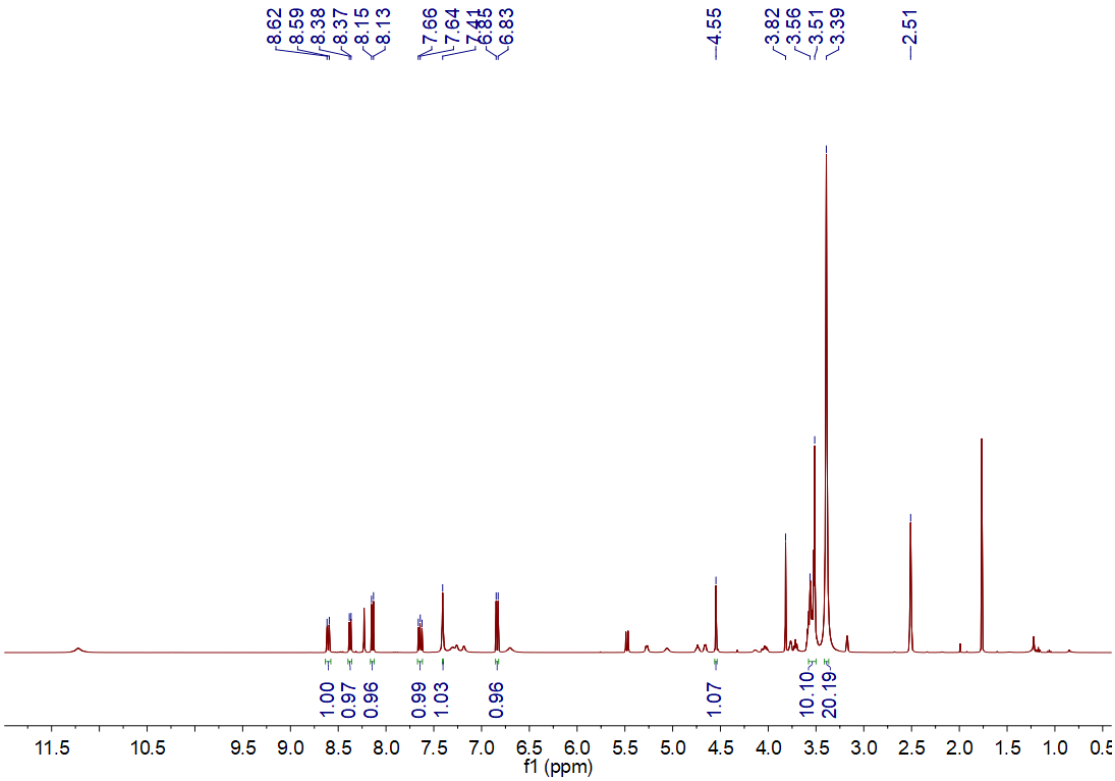


Fig S9: ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of Compound 9

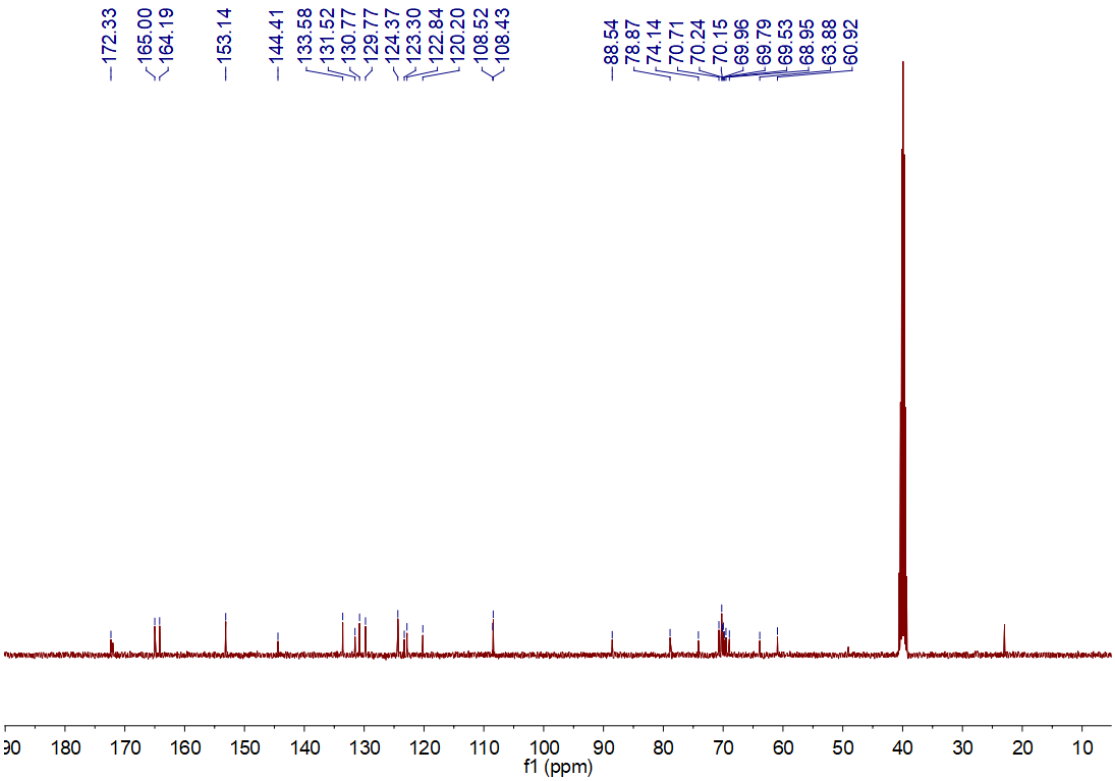


Fig S10: ^{13}C NMR (101 MHz, DMSO-d_6) spectrum of Compound 9

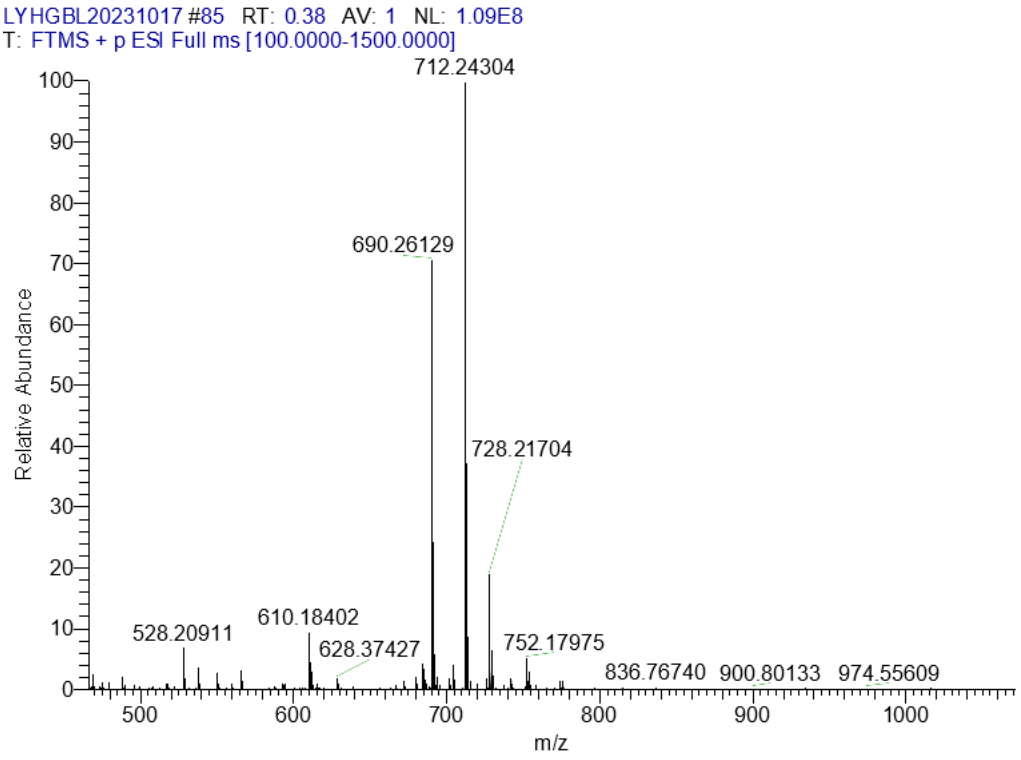


Fig S11: HRMS spectrum of compound 9

3. Preparation and Characterization of ZnAP and Gal-ANI@ZnAP NPs

ZnAP was prepared according to the previous reporter[4]. Firstly, $Zn(NO_3)_2 \cdot 6H_2O$ (0.1 mmol, 30 mg), 6-AP(0.1 mmol, 21.5 mg), biphenyl-4,4'-dicarboxylic acid (0.07 mmol, 17 mg) and nitric acid (2 M, 0.01 ml) were added in DMF (4 ml). The mixture was sonicated for 30 minutes and then transferred to a Teflon-lined autoclave (10 mL), which was subsequently heated to 130 °C for 24 h. After cooling to room temperature, the suitable size ZnAP nanoparticles were obtained by differential centrifugation and washed with DMF. As for Gal-ANI@ZnAP NPs, ZnAP (3 mg) was dispersed in ultrapure water (2 ml) to obtain the stock solution and Gal-ANI (1mg) was dissolved in ultrapure water (1 ml). The two stock solutions were mixed with sonication for 30 min and stirred for 24h at room temperature. Gal-ANI@ZnAP NPs were obtained by centrifugation and washed with ultrapure water. The nanoparticles above were characterized by FT-IR, XPS, ^{1}H NMR, PXRD, TEM and DLS.

4. ANI and 6-AP release from Gal-ANI@ZnAP NPs

Firstly, the standard curves between absorbance and concentration of ANI and 6-AP were determined via UV/Vis. Gal-ANI@ZnAP NPs (3 mg) were dispersed in 5 ml of different PBS buffer solution (pH:7.4, pH:5.0, pH:7.4+esterase:100U/L, pH:5.0+esterase:100U/L) and stirred for different times (0.5, 1, 2, 4, 6, 8, 12, 24, 36 and 48 h). Next, the supernates were collected by centrifugation and corresponding absorbance were determined by spectrophotometer to evaluate the release efficiency of ANI and 6-AP respectively.

5. The AIE property of Gal-ANI and Gal-ANI@ZnAP NPs

Gal-ANI and Gal-ANI@ZnAP were dissolved in different proportion of ethanol-water

system respectively and fluorescence intensity changes of Gal-ANI and Gal-ANI@ZnAP were monitored by fluorescence spectrophotometer. During this process their concentration maintained 10 μg/ml. Besides that, the fluorescence behavior of Gal-ANI@ZnAP in the solid state was observed under the irradiation of a UV lamp.

6. Cell Culture

HepG2 cells (human hepatoma carcinoma cell line) and HL7702 cells (human normal liver cell line) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 1% penicillin/streptomycin and 10% fetal bovine serum (FBS) at 37 ℃ with 5% CO_2.

7. Cell Uptake observed by inverted fluorescence microscope

HepG2 cells were treated with Gal-ANI@ZnAP (45 μg/ml) for 3h in a 35mm cell culture dish. Thereafter, the cells were washed with PBS and stained with DAPI (1 μg/ml) for 30 min. Finally, the cells were washed with PBS and the fluorescence images were captured by an inverted fluorescence microscope.

8. Target Ability Assay

HepG2 cells and HL7702 cells were seeded in 6-well plates at a density of 2×10^5 cells/well and experiment was designed as three groups: HL7702 cells, HepG2 cells and HepG2 cells pretreated with lactobionic acid (2 mg/ml) for 4 h. After 4 h, the cells were cultured with Gal-ANI@ZnAP (45 μg/ml) for 3 h and subsequently stained with DAPI (1 μg/ml) for 30 min. Finally, the cells were washed with PBS and the fluorescence images were captured by an inverted fluorescence microscope.

9. In vitro Cytotoxicity Evaluation

The *in vitro* cytotoxicity of different administrations (Gal-ANI, ZnAP, Gal-ANI@ZnAP) was evaluated by Cell Counting Kit-8 (CCK-8) assay with HL7702 cells and HepG2 cells. The cells were seeded in 96-well plates for 24 h at a density of 5×10^3 cells/well. Subsequently, the samples above in different concentrations were co-cultured with cells for 24 h and 48 h respectively. After removing the medium, CCK-8 reagent (10 μl/well) was added away from light and cultured for 30 min. Finally, the optical density (OD) was tested by a microplate reader at the wavelength of 450 nm. Each assay has 5 repetitions.

10. Colony Forming Assay

The antiproliferation ability of different administrations (Gal-ANI, ZnAP, Gal-ANI@ZnAP) was evaluated by colony forming assay with HepG2 cells. The cells were seeded in 6-well plates for 48 h at a density of 1000 cells/well. After 48 h, samples above were co-cultured with cells for 10 days (Gal-ANI@ZnAP concentration: 2 μg/ml). The obtained colonies were fixed with cold methanol for 5 min, stained with 0.5% crystal violet for 15 min, rinsed with pure water and dried naturally. Finally, the images were taken by a camera and the number of colonies were analyzed by Image J analysis software.

11. Combination index of ANI and 6-AP

To evaluate the Synergistic effect between ANI and 6-AP, the combination index (CI) was

determined by the Chou-Talalay method using the CompuSyn software. Firstly, Cell viabilities were detected by CCK-8 assay after the cells were treated with free ANI, free 6-AP and the combination (C_{ANI}:$C_{6\text{-}AP}$=4:1) respectively. CI values were further calculated by CompuSyn software based on cell viabilities and drug concentrations. The classifications of synergy are synergistic (CI < 1), additive (CI =1), and antagonistic (CI > 1).[5]

12. Mitochondrial membrane potential Detection

HepG2 cells were seeded in 96-well plates at a density of 5×10^3 cells/well and treated with different conditions for 6 h: Control, Gal-ANI, ZnAP and Gal-ANI@ZnAP (Gal-ANI@ZnAP concentration:45 μg/ml) respectively. Mitochondrial membrane potential was measured by membrane potential detection kit (JC-1) and the JC-1 working solution was prepared by mixing JC-1 buffer solution and DMEM in a ratio of 1:1. The cells were cultured with the JC-1 working solution (100 μl/well) for 20 min at 37 ℃. After washing with DMEM twice, the fluorescence images were captured by an inverted fluorescence microscope and the fluorescence intensity was detected by a microplate reader at the wavelength of 488 nm and 525 nm. Each assay has 3 repetitions.

13. Cellular ROS Detection

HepG2 cells were seeded in 96-well plates at a density of 5×10^3 cells/well and treated with different conditions for 6 h: Control, Gal-ANI, ZnAP and Gal-ANI@ZnAP (Gal-ANI@ZnAP concentration:45 μg/ml) respectively. Cellular ROS level was measured by Dihydroethidium (DHE), which is a fluorescent probe for the detection of ROS generation in live cells. The cells were cultured with the DHE solution (10 μM) for 30 min at 37℃. After washing with PBS twice, the fluorescence images were captured by an inverted fluorescence microscope and the fluorescence intensity was detected by a microplate reader at the wavelength of 610 nm. Each assay has 3 repetitions.

14. Apoptosis Assay by Annexin V-FITC/PI

HepG2 cells were seeded in 6-well plates at a density of 2×10^5 cells/well and treated with different conditions for 48 h: Control, Gal-ANI, ZnAP and Gal-ANI@ZnAP (Gal-ANI@ZnAP concentration: 90 μg/ml) respectively. Subsequently, the cells were collected, washed with PBS, resuspended in Binding Buffer (400 μl) and stained with Annexin V-FITC (5 μl) or PI (10 μl) for 10 min away from light. Finally, the fluorescence intensity was detected by flow cytometer.

15. Fig S12-S22

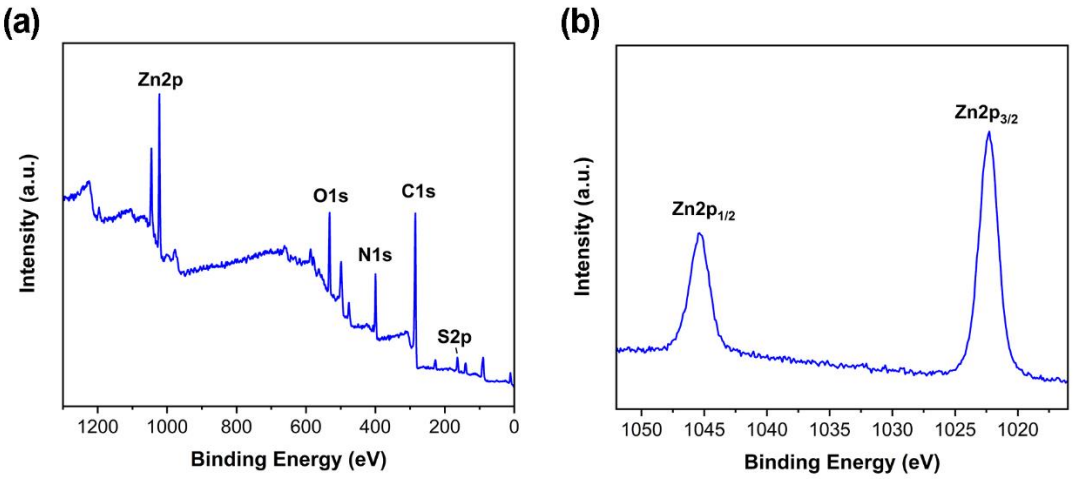


Fig S12: (a) XPS survey scan of ZnAP; (b) the high-resolution XPS spectra of the Zn2p orbitals.

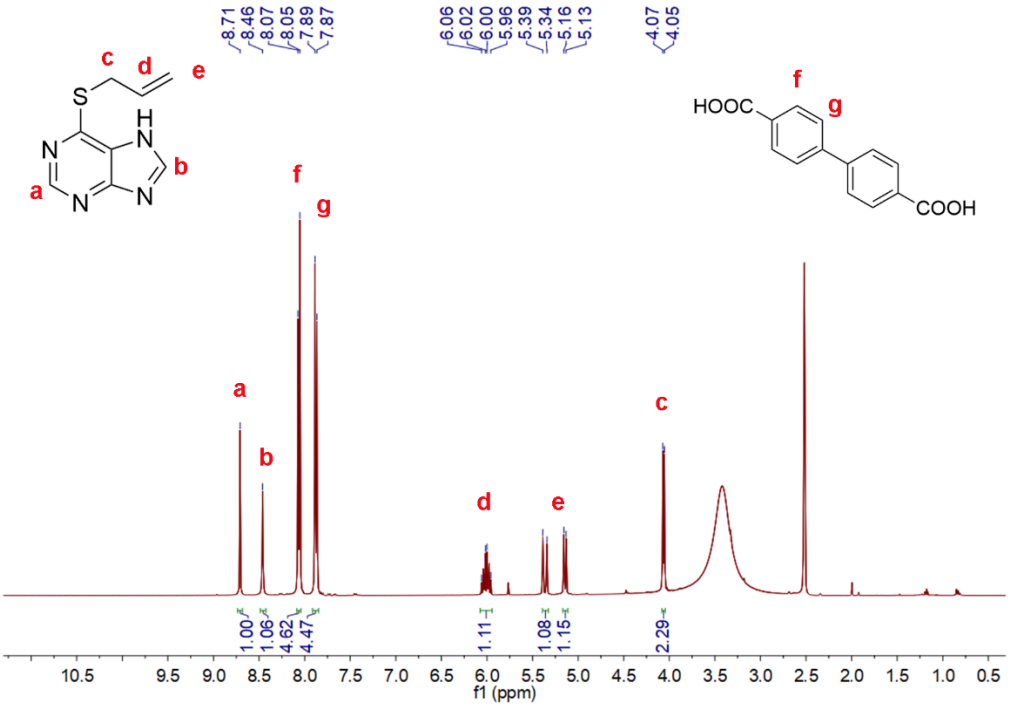


Fig S13: ^{1}H NMR(400 MHz, DMSO-d_6) spectra of acid-digested ZnAP. Peaks at a, b, c, d, e are related to 6-AP and peaks at f, g correspond to 4-BPA.

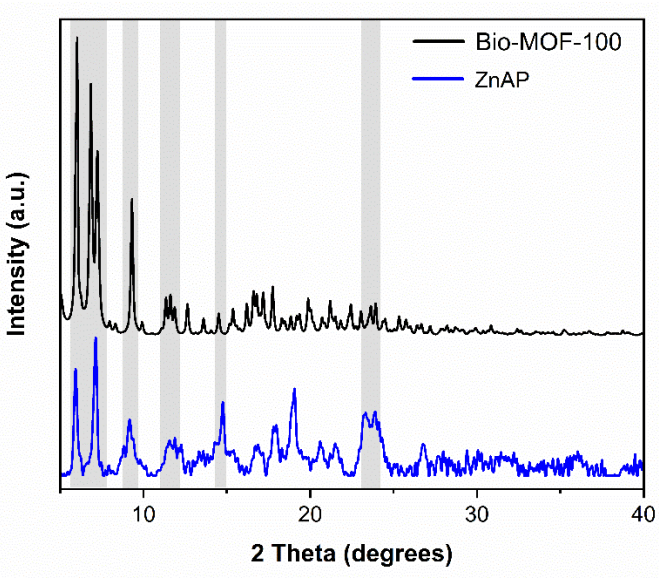


Fig S14: The PXRD patterns of ZnAP.

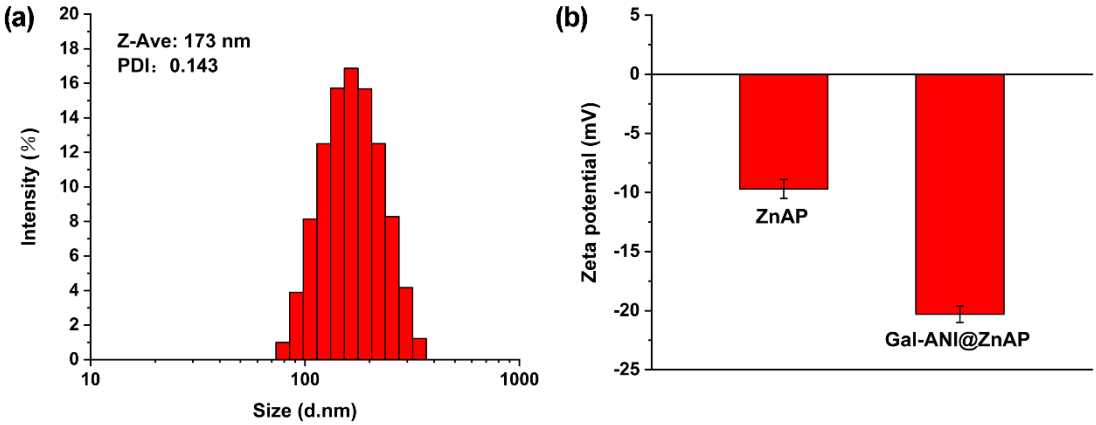


Fig S15: (a) DLS of ZnAP; (b) zeta potential of ZnAP and Gal-ANI@ZnAP. Datas represent mean ± SD (n=3).

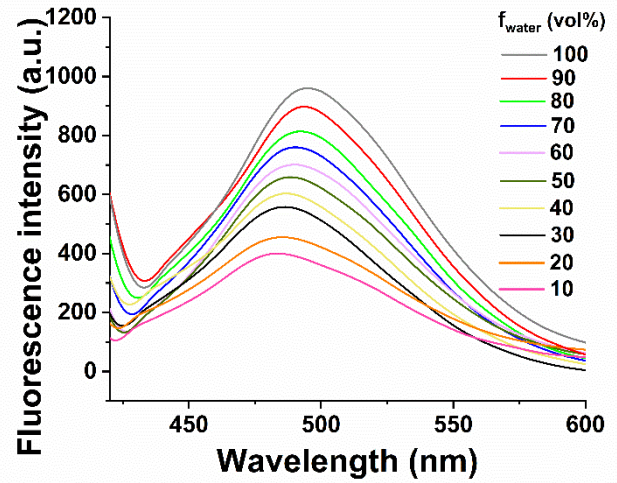


Fig S16: Fluorescence spectra of Gal-ANI@ZnAP in ethanol-water mixture (λem: 492 nm).

Fig S17: Fluorescent images of Gal-ANI@ZnAP NPs in their solid state.

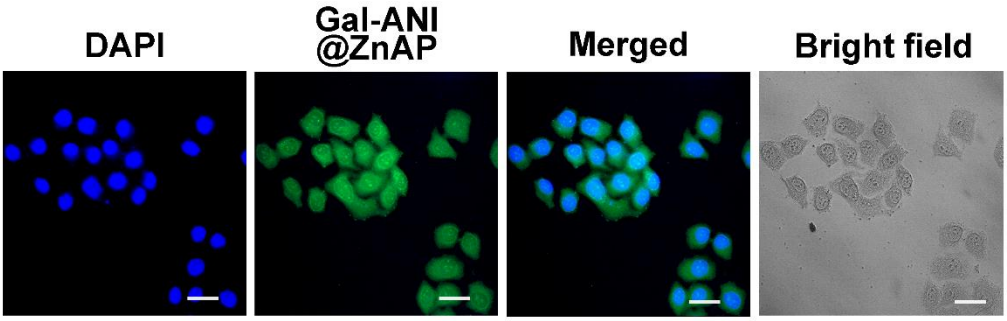


Fig S18: Fluorescent images of HepG2 cells incubated with Gal-ANI@ZnAP (45 μg/ml) for 3h (Scale bar: 40 μm).

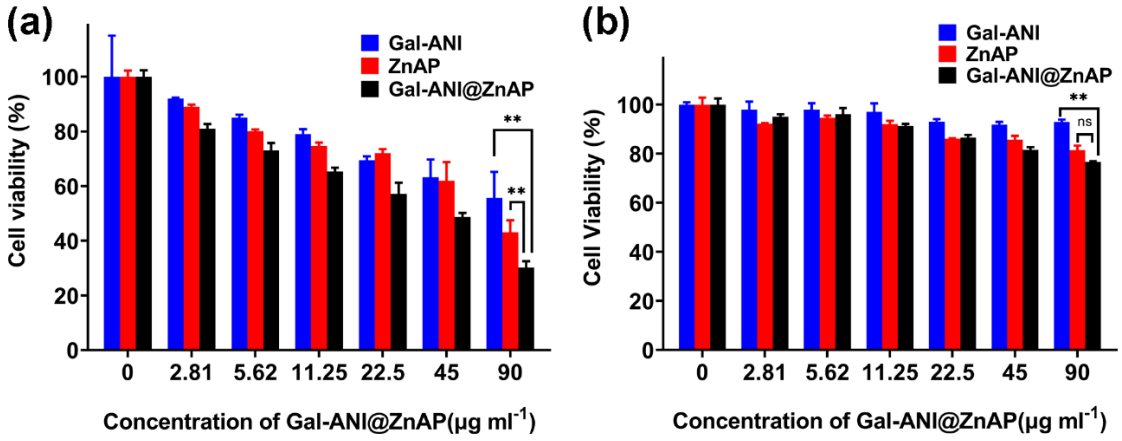


Fig S19: Cell viability of (a) HepG2 cells and (b) HL7702 cells after different treatments for 24h. Datas represent mean ± SD (n＝5), and ns means $p > 0.05$, **means $p < 0.01$.

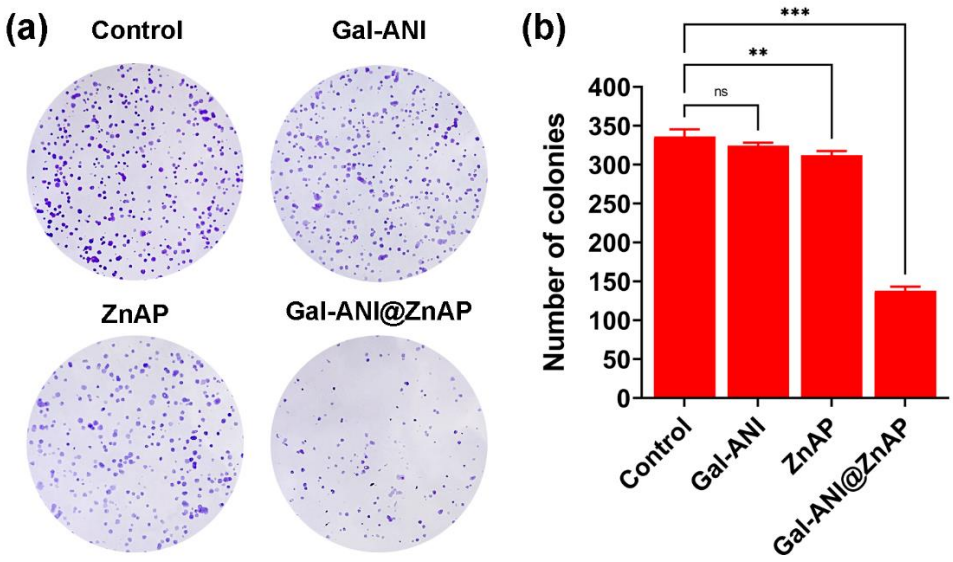


Fig S20: (a) Colony formation assay of HepG2 cells incubated with Gal-ANI, ZnAP and Gal-ANI@ZnAP for 10 days. (b) Bar graphs plotting the number of colonies analyzed by Image J. Datas represent mean ± SD (n＝3), and ns means $p>0.05$, **means $p<0.01$, *** means $p<0.001$.

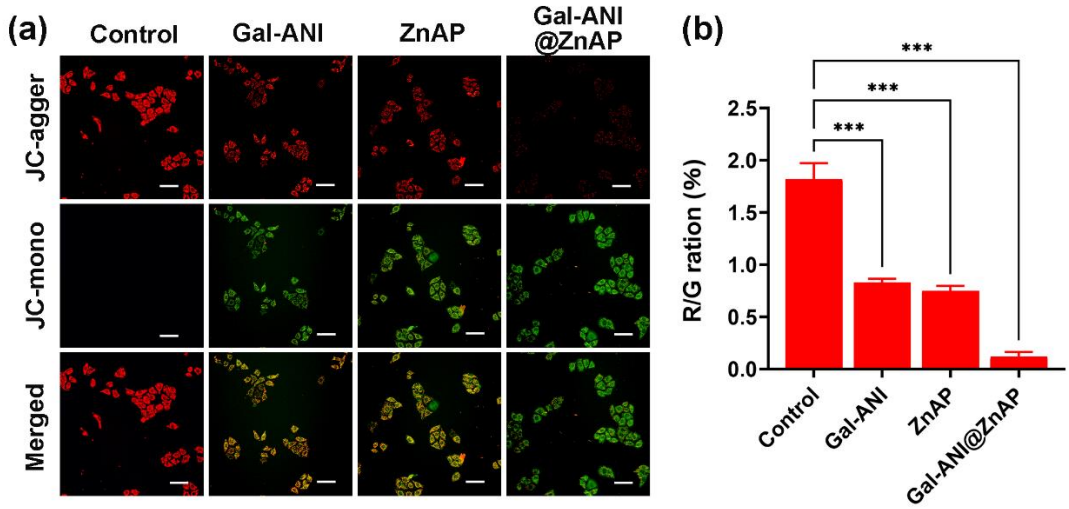


Fig S21: (a) Fluorescent images of HepG2 cells after various treatments of Gal-ANI, ZnAP and Gal-ANI@ZnAP for 6 h and stained with JC-I probe (Scale bar: 20 μm). (b) Quantification of the fluorescence ratio of JC-1 agger/JC-1 mono (R/G) detected by a microplate reader. Datas represent mean ± SD (n＝3), and *** means $p<$ 0.001.

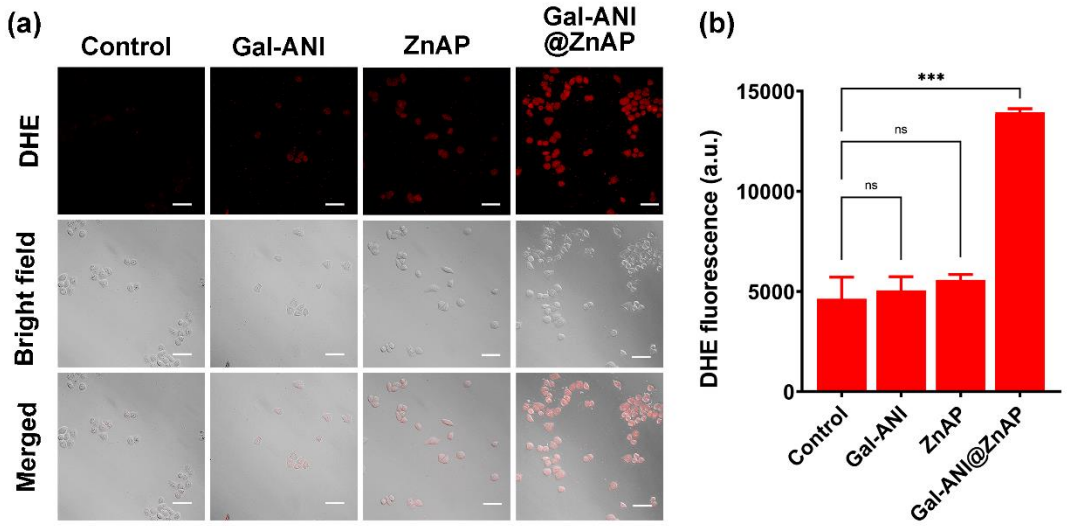


Fig S22: (a) Fluorescent images of HepG2 cells after various treatments of Gal-ANI, ZnAP and Gal-ANI@ZnAP for 6 h and stained with DHE (Scale bar: 20 μm). (b) Quantification of the red fluorescence detected by a microplate reader. Data represent mean ± SD (n＝3), and ns means $p>0.05$, *** means $p<0.001$.

16. List of Abbreviations

SL: synthetic lethality;
MOFs: metal-organic frameworks;
AIE: aggregation-induced emission;
PARP: Poly(ADP-ribose)polymerase;
ANI: 4-Amino-1,8-Naphthimide;
6-AP: 6-Allylthiopurine;
ZnAP: MOFs constructed by Zn ion and 6-AP;
Gal-ANI: Glycosylated ANI;
Gal-ANI@ZnAP: ZnAP coated with Gal-ANI
4-BPA: biphenyl-4, 4'-dicarboxylic acid;
FT-IR: fourier Transform Infrared Spectoscopy;
TEM: transmission electron microscopy;
DLS: dynamic light scattering;
ASGPR: asialoglycoprotein receptor;
CCK-8: Cell Counting Kit-8;
CI: combination index
MMP: Mitochondrial membrane potential;
ROS: reactive oxygen species;
DHE：dihydroethidium
PI: phosphatidylinositol;
FCM: flow cytometry;
DCM: dichloromethane;
UV-Vis: Ultraviolet and visible spectrophotometry;
DMEM: Dulbecco’s modified Eagle’s medium;
FBS: fetal bovine serum

17. References

1. R. Hu, W.-L. Wang, K.-J. Xiao and N.-Y. Wang, *Pharmaceutical Chemistry Journal*, 2021, **55**, 36-45.
2. X.Zhang, K. He and W.Wang, ChinaPat., CN111574498A,2020.
3. K. Yang, K. Ma, M. Yang, Y. Lv, Y. Pei and Z. Pei, *Chemical Communications*, 2023, **59**, 3779-3782.
4. J. An, S. J. Geib and N. L. Rosi, *Journal of the American Chemical Society*, 2009, **131**, 8376-8377.
5. T.-C. Chou, *Cancer Research*, 2010, **70**, 440-446.