Supplementary Information

Discovery of the ERα-Targeting PROTACs with Intrinsic Fluorescence for Precision Theranostics of Breast Cancer

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1. Experimental Section

1.1 Materials and Instruments

Chemical reagents were purchased commercially and used directly without further purification. Thin layer chromatography was used to determine the reaction status, and 200-300 mesh silica gel column was used for purification. Bruker AVANCE NEO 600 (600 MHz) was utilized to obtain ^{1}H NMR and ^{13}C NMR data. An IonSpec 4.7 T FTMS mass spectrometer was used to get mass information. UV and fluorescence spectra were recorded on SHIMADZU UV-2600i and HITACHI F-4700 instruments, respectively. Cell imaging was recorded by a LeicaLCS-SP8 laser confocal microscope. Flow cytometry was analyzed by EPICS XL (Beckman Coulter, Brea, California, USA).

1.2 Chemistry

Critical intermediates were synthesized according to an established protocol.

Synthesis of ultimate products **W1**-**W5**. Compound **8a/b** (100 mg,1.05 eq.) and HATU (2.00 eq.) were dissolved in DMF (5 mL) and stirred at room temperature for 30 min. Then, **13a-c/15** (1.00 eq.) and DIPEA (6.00 eq.) were added into the solution and stirred at room temperature for 12 h. The mixture was extracted with water and EA (15 mL × 3). Finally, the organic phase was evaporated under reduced pressure, and the crude product was purified by flash column chromatography (DMC/MeOH = 80:1) on silica gel, affording the desired compounds **W1-W5** as yellow solid (yield: 42-83%).

2-(4-(((1S, 2R, 4S)-5,6-Bis(4-hydroxyphenyl)-N-(2, 2,2 -trifluoroethyl)-7-oxabicyclo[2.2.]hept-5-ene)-2-sulfonamido)phenoxy)-N-(4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-5-yl)oxy)butyl)acetamide (**W1**), yield: 58%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.03 (s, 1H), 9.69 (s, 1H), 9.63 (s, 1H), 8.40 – 8.25 (m, 2H), 8.18 (d, J = 4.9 Hz, 1H), 8.10 – 7.93 (m, 2H), 7.86 – 7.79 (m, 1H), 7.32 (dd, J = 9.1, 3.0 Hz, 2H), 7.19 – 7.10 (m, 4H), 6.97 – 6.87 (m, 2H), 6.80 – 6.73 (m, 2H), 6.72 – 6.68 (m, 2H), 5.84 (dt, J = 11.3, 5.3 Hz, 1H), 5.48 (s, 1H), 5.33 (d, J = 3.9 Hz, 1H), 4.55 – 4.47 (m, 4H), 4.22 (t, J = 6.3 Hz, 2H), 3.54 (dd, J = 7.6, 4.9 Hz, 1H), 3.27 – 3.22 (m, 2H), 2.95 (m, 1H), 2.63 – 2.57 (m, 2H), 2.06 (dd, J = 9.5, 4.5 Hz, 1H), 1.95 (dq, J

= 11.8, 6.0, 4.2 Hz, 2H), 1.81 (q, *J* = 7.0 Hz, 2H), 1.66 (q, *J* = 7.3 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.86, 170.24, 167.30, 163.46, 163.08, 162.68, 162.27, 157.41, 157.27, 157.11, 157.07, 140.58, 136.57, 133.50, 133.44, 133.15, 132.12, 131.58, 129.26, 129.21 (d, *J* = 286.90) 128.96, 128.81, 128.67, 127.77, 127.72, 125.22, 123.36, 123.25, 122.90, 122.74, 122.64, 121.99, 121.70, 121.32, 115.64, 115.46, 115.15, 114.79, 114.39, 83.72, 82.04, 68.07, 67.42, 67.08, 61.23, 59.77, 51.92, 51.70, 50.59, 50.56, 41.85, 38.10, 37.94, 30.87, 30.33, 29.81, 28.37, 25.87, 25.69, 23.26, 22.41, 21.47, 21.42, 20.75, 18.08, 16.73, 14.08, 13.89, 12.50, 10.80. HRMS (ESI) calcd for $[C_{49}H_{43}F_3N_4O_{12}SNa]^+$ $[M + Na]^+$, 991.2448; found 991.2454.

2-(4-(((1S, 2R, 4S)-5, 6-Bis(4-hydroxyphenyl)-N-(2, 2, 2-trifluoroethyl)-7-oxabicyclo[2.2.1]hept-5-ene)-2-sulfonamido)phenoxy)-N-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-5-yl)oxy)hexyl)acetamide (**W2**), yield, 83%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.03 (s, 1H), 9.68 (s, 1H), 9.63 (d, *J* = 2.6 Hz, 1H), 8.36 – 8.27 (m, 2H), 8.09 (d, *J* = 7.1 Hz, 1H), 7.95 (s, 1H), 7.81 (dd, *J* = 12.9, 6.3 Hz, 2H), 7.31 (d, *J* = 8.8 Hz, 2H), 7.12 (dd, *J* = 14.6, 8.3 Hz, 4H), 6.90 (d, *J* = 8.3 Hz, 2H), 6.75 (d, *J* = 8.1 Hz, 2H), 6.70 (d, *J* = 8.3 Hz, 2H), 5.85 – 5.82 (m, 1H), 5.48 (s, 1H), 5.32 (d, *J* = 4.0 Hz, 1H), 4.52 (dd, *J* = 15.2, 7.0 Hz, 2H), 4.19 (m, 3H), 3.54 (dd, *J* = 7.6, 4.9 Hz, 1H), 3.15 (m, 2H), 3.05 – 3.03 (m, 1H), 3.00 – 2.86 (m, 2H), 2.60 (m, 3H), 2.11 – 2.00 (m, 2H), 1.82 (d, *J* = 7.8 Hz, 2H), 1.50 – 1.46 (m, 4H), 1.25 (d, *J* = 7.6 Hz, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.30, 170.68, 169.37, 167.58, 157.37, 157.24, 140.55, 136.55, 133.21, 132.06, 129.2 (q, *J* = 279.35 Hz), 128.93, 123.22, 122.76, 122.62, 115.61, 115.43, 115.10, 83.67, 82.00, 68.39, 53.59, 51.85, 48.59, 41.84, 38.44, 38.21, 30.84, 29.09, 29.00, 28.41, 26.18, 26.04, 25.21, 25.14, 22.62, 18.08, 16.72, 14.08, 12.48. HRMS (ESI) calcd for $[C_{51}H_{47}F_3N_4O_{12}S]^+$ $[M + Na]^+$, 1019.2761; found 1019.2756.

2-(4-(((1S, 2R, 4S)-5,6-Bis(4-hydroxyphenyl)-N-(2, 2, 2-trifluoroethyl)-7-oxabicyclo[2.2.1]hept-5-ene)-2-sulfonamido)phenoxy)-N-(8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-5-yl)oxy)octyl)acetamide (**W3**), yield 42%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.01 (s, 1H), 9.67 (s, 1H), 9.62 (s, 1H), 8.31–8.26 (m, 2H), 8.10 – 7.96 (m, 3H), 7.86 – 7.80 (m, 1H), 7.29 (d, *J* = 8.6 Hz, 2H), 7.15

– 7.08 (m, 4H), 6.89 (d, *J* = 8.8 Hz, 2H), 6.76 – 6.72 (m, 2H), 6.70 – 6.67 (m, 2H), 5.82 (dt, *J* = 11.3, 5.3 Hz, 1H), 5.46 (d, *J* = 1.3 Hz, 1H), 5.33 – 5.30 (m, 1H), 4.54 – 4.47 (m, 2H), 4.45 (s, 2H), 4.20 (q, *J* = 6.1 Hz, 2H), 3.52 (dd, *J* = 7.7, 4.8 Hz, 1H), 3.13 – 3.08 (m, 2H), 2.96 – 2.90 (m, 1H), 2.58 (t, *J* = 4.9 Hz, 1H), 2.07 – 1.96 (m, 2H), 1.95 – 1.87 (m, 2H), 1.83 – 1.77 (m, 2H), 1.44 (dt, *J* = 20.1, 7.5 Hz, 4H), 1.29 (d, *J* = 3.2 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.82, 170.19, 167.06, 157.36, 157.23, 140.54, 136.54, 132.05, 129.64, 129.17(d, *J* = 279.35), 128.92, 127.72, 125.93, 123.21, 122.77, 122.60, 115.60, 115.42, 115.09, 114.74, 83.66, 81.99, 68.43, 67.02, 61.16, 53.56, 50.53, 38.24, 35.11, 31.53, 31.27, 30.83, 30.28, 29.41, 29.03, 28.82, 28.68, 28.65, 28.57, 28.45, 26.54, 26.27, 25.41, 18.07, 16.72, 12.45. HRMS (ESI) calcd for $[C_{53}H_{51}F_3N_4O_{12}S]^+$ [M + Na]$^+$, 1047.3074; found 1047.3073.

2-(4-(((1S, 2R, 4S)-5, 6-Bis(4-hydroxyphenyl)-N-(2, 2, 2-trifluoroethyl)-7-oxabicyclo[2.2.1]hept-5-ene)-2-sulfonamido)phenoxy)-N-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-5-yl)oxy)ethoxy)ethyl)acetamide (**W4**), yield 50%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.04 (s, 1H), 9.69 (s, 1H), 9.63 (s, 1H), 8.39 – 8.27 (m, 2H), 8.19 – 8.15 (m, 1H), 8.12 – 7.97 (m, 2H), 7.84 – 7.79 (m, 1H), 7.32 – 7.28 (m, 2H), 7.15 – 7.10 (m, 4H), 6.92 – 6.87 (m, 2H), 6.78 – 6.74 (m, 2H), 6.71 – 6.69 (m, 2H), 5.84 (dt, *J* = 11.4, 5.5 Hz, 1H), 5.48 (d, *J* = 1.2 Hz, 1H), 5.34 – 5.30 (m, 1H), 4.53 – 4.48 (m, 4H), 4.36 – 4.33 (m, 2H), 3.86 – 3.82 (m, 2H), 3.61 – 3.57 (m, 2H), 3.54 (dd, *J* = 7.8, 4.7 Hz, 1H), 3.37 (s, 2H), 2.97 – 2.91 (m, 1H), 2.63 – 2.58 (m, 2H), 2.05 (dd, *J* = 11.6, 5.9 Hz, 1H), 1.94 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.86, 170.24, 167.48, 163.46, 163.06, 162.67, 162.25, 157.40, 157.26, 157.22, 156.99, 140.58, 136.57, 133.57, 133.51, 133.12, 132.14, 131.61, 129.27, 129.26 (d, *J* = 273.31), 128.97, 128.87, 128.73, 128.68, 128.27, 127.79, 127.62, 125.21, 123.34, 123.25, 122.99, 122.85, 122.63, 121.94, 121.74, 121.36, 115.65, 115.46, 115.36, 115.27, 115.13, 114.79, 114.66, 83.70, 82.04, 69.04, 68.51, 68.07, 67.43, 66.99, 61.22, 51.91, 50.60, 50.57, 48.62, 38.28, 30.87, 30.31, 29.81, 28.38, 23.26, 22.42, 21.46, 10.81. HRMS (ESI) calcd for $[C_{49}H_{43}F_3N_4O_{13}S]^+$ [M + Na]$^+$, 1007.2397; found 1007.2401.

6-(4-(((1S, 2R, 4S)-5,6-Bis(4-hydroxyphenyl)-N-(2, 2, 2-trifluoroethyl)-7-oxabicyclo[2.2.1]hept-5-ene)-2-sulfonamido)phenoxy)-N-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-5-yl)oxy)hexyl)hexanamide (**W5**), yield 68%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.04 (s, 1H), 9.70 (s, 1H), 9.65 (s, 1H), 8.38 – 8.26 (m, 2H), 8.08 – 7.95 (m, 1H), 7.93 (dd, J = 5.1, 2.6 Hz, 1H), 7.82 – 7.76 (m, 2H), 7.27 (d, J = 8.5 Hz, 2H), 7.15 – 7.11 (m, 4H), 6.83 (d, J = 8.5 Hz, 2H), 6.77 (d, J = 8.4 Hz, 2H), 6.71 (dd, J = 8.7, 2.2 Hz, 2H), 5.84 (dt, J = 11.2, 5.2 Hz, 1H), 5.49 (d, J = 2.9 Hz, 1H), 5.33 (d, J = 4.2 Hz, 1H), 4.53 – 4.48 (m, 2H), 4.18 (q, J = 6.5 Hz, 2H), 3.92 (dt, J = 8.0, 4.0 Hz, 2H), 3.52 (dd, J = 8.1, 4.6 Hz, 1H), 3.19 (d, J = 3.6 Hz, 1H), 3.06 (t, J = 4.9 Hz, 2H), 2.60 (dt, J = 15.4, 4.4 Hz, 2H), 2.08 (d, J = 7.3 Hz, 4H), 1.98 – 1.92 (m, 2H), 1.81 (d, J = 7.9 Hz, 2H), 1.69 (d, J = 7.4 Hz, 2H), 1.54 (d, J = 7.6 Hz, 2H), 1.50 – 1.44 (m, 4H), 1.38 – 1.34 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.87, 171.93, 171.89, 170.25, 163.46, 163.09, 162.69, 162.28, 158.26, 157.42, 157.26, 157.13, 140.59, 136.58, 133.45, 133.17, 131.30, 129.36, 129.20 (d, J = 286.90) 129.04, 128.89, 128.81, 128.70, 128.55, 127.76, 125.24, 123.38, 123.27, 122.89, 122.73, 122.67, 121.99, 121.70, 121.32, 115.78, 115.64, 115.46, 114.71, 114.37, 83.73, 82.04, 68.37, 67.58, 61.06, 51.98, 50.60, 38.36, 35.40, 33.42, 31.54, 30.88, 30.39, 30.31, 29.44, 29.13, 28.42, 26.16, 25.21, 25.12, 25.01, 21.48, 21.43. HRMS (ESI) calcd for $[C_{55}H_{55}F_3N_4O_{12}S]^+$ $[M + Na]^+$, 1075.3387; found 1075.3391.

1.3 Fluorescence-Spectroscopy Test

The optical properties of **W1**-**W5** and **WUN29654** were tested in 10% DMSO/PBS (pH = 7.4) at 37 °C. For the anti-interference experiment, analytes, including cations, anions, various amino acids and reactive substances, were added to **W2** (10 μM) in 10% DMSO/PBS. In contrast to the procedures above, the optical properties and photostability for the coexistence of **W2** and ERα were tested in 10% DMSO/PBS at 37 °C. The response was measured using a HITACHI F-4700 spectrophotometer (slit: **W1** = 10/5 nm; **W2** = 5/5 nm; **W3** = 10/10 nm; **W4** = 10/5 nm; **W5** = 5/10 nm; **WUN29654** = 10/5 nm).

1.4 Molecular Docking

The crystal structure of ERα LBD (PDB: 5KCC) and CRBN (PDB: 5FQD) was obtained from the Protein Data Bank (PDB) and processed by SPDBV software (4.0.1) to remove initial ligand and water molecules. The probe was docked into the three-dimensional (3D) LBD of ERα using AutoDock software (version 4.2).

1.5 Cell Culture and *CCK-8* Assay

Human normal breast epithelial MCF-10A cells and human BC MCF-7 cells were obtained from ATCC. ERαMUT BC cell lines MCF-7^{D538G}, MCF-7^{Y537S}, and MCF-7^{EGFR} were purchased from Cyagen Biosciences. Cells were cultured in MEM (MCF-7^{D538G}, MCF-7^{Y537S}, and MCF-7^{EGFR}) containing 15% fetal bovine serum (FBS) and 1% penicillin streptomycin. Cells were plated in the 96-well plates with a seeding density of 7×10^3 cells/well, and cultured in MEM (MCF-7) and DMEM (MCF-10A、DU145) containing 10% FBS and 1% penicillin-streptomycin for 24 h. Then, compounds were treated for 96 h, and cell viability assays were determined with *CCK-8* assays.

1.6 Western Blotting

The capacity of **W1-W5** to induce ERα degradation in MCF-7 cells was evaluated through Western blotting (WB). MCF-7 cells were cultured in 6-well plates (Corning, China) at a density of 7×10^5 cells per well and exposed to **W1-W5** for predefined time intervals. After the designated treatments, protein extraction was performed employing SDS lysis buffer (Beyotime, China). These protein specimens were subsequently separated on 8 % SDS-PAGE gels and thereafter transferred onto PVDF membranes. Following an overnight incubation with primary antibodies at 4 ℃, and a subsequent 1 h incubation with HRP-tagged secondary antibodies at room temperature, the membranes were developed utilizing an ECL agent.

Cells were cultured in DMEM in PC9 cells. The other steps were same to those mentioned above.

1.7 Living Cell Imaging

MCF-7, MCF-10A and DU145 cells were adhered to confocal dishes for 24 h and then incubated with **W2** at times and concentrations required. For the competitive

experiment, an excess of estradiol (100 μM) and **W2** (50 μM) were concurrently applied to the cells for 1 h. Subsequently, images were acquired using a confocal laser scanning microscope (CLSM, Nikon-A1 system, Japan). **W2** was visualized using a 405 nm laser, and the emission wavelength was detected within the range of 430–550 nm and represented in blue fluorescence.

1.8 Flow Cytometry for Cell Cycle Arrest and Apoptosis

Flow cytometry was performed to evaluate the cell cycle and apoptosis using cell cycle staining kit (MultiSciences Biotech Co., Ltd, CCS012-01) and YF488-Annexin V-FITC/propidium iodide (PI) staining kit (BioSciences Biotech Co., Ltd, Y6002S) respectively. The cells were seeded into a 6-well plate with a density of 10^5 cells/well and incubated for 24 h, followed by starving with DMEM containing 2 % FBS for 24 h. After 48 h of treatment with different concentrations of drugs, all the cell samples were prepared by following the instructions, including cell collection, washing and incubation.

For the cell cycle arrest, the samples were incubated with 1 mL DNA staining solution and 10 μL permeabilization solution for 30 min, whereas for cell apoptosis analysis, the samples were incubated with 5 μL propidium iodide (PI) and 5 μL RNAase for 15 min. The samples were analyzed as soon as possible using flow cytometry (EPICS XL; Beckman Coulter, Brea, California, USA).

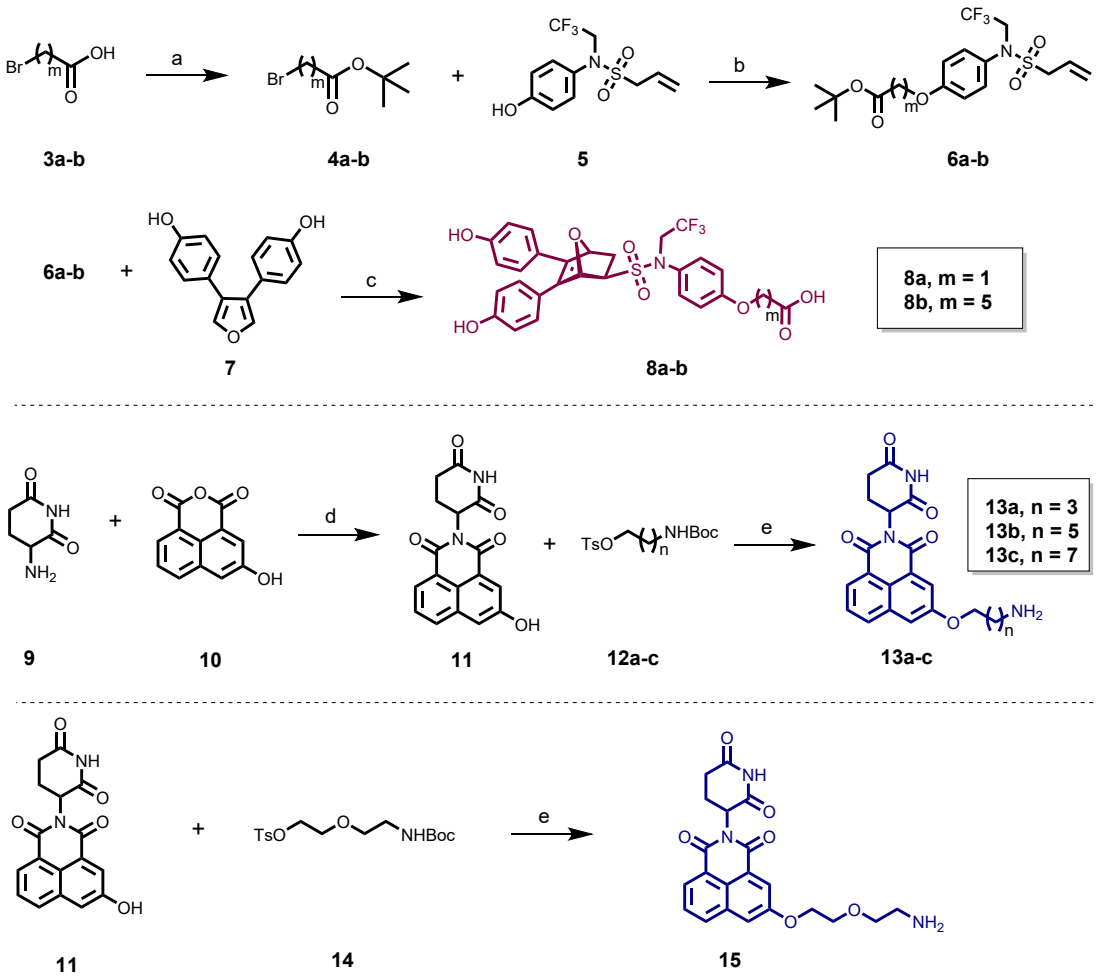
1.9 Acute Toxicity Experiment

Female BALB/C nude mice (5 weeks) were purchased from Beijing HFK Bioscience Co. Ltd. (Beijing, China). All operations described below were carried out following the guidelines of the Animal Experimentations Ethics Committee of Wuhan University. The composition of dosing vehicles was 5% DMSO, 40% PEG400, and 55% β-cyclodextrin (20% in water) for intraperitoneal injection. Dosage of 500 $mg•kg^{-1}$ was administered to 5 mice per group.

1.10 Hemolysis Assay

The hemolytic activity of **W2** was determined using freshly collected rats blood. Red blood cells were isolated *via* centrifugation (3000 rpm, 10 min, 4 °C), following by being washing with PBS immediately prior to the assay. This procedure was repeated until the supernatant was colorless, after which it was decanted. After resuspending 25 μL fresh rats blood in a 480 μL PBS solution, a 4% red blood cell suspension was obtained. The probe **W2** concentration (0.1, 1 10, 20 μM) was prepared *via* dilution of the stock solution using PBS. The erythrocyte suspension (500 μL) and 500 μL of the **W2** at each concentration were combined, incubated at 37 °C for 4 h, and centrifuged (3000 rpm, 10 min, 4 °C). A portion of each supernatant (200 μL) was transferred to a microtiter plate, and the absorbance was measured at 570 nm. PBS buffer and ddH_2O were used as negative and positive controls, respectively (n = 3).

Scheme S1. The synthetic routes for intermediates **8a-b**, **13a-c** and **15**. [a]



[a] Reagents and conditions: (a) Trifluoroacetic Anhydride, *Tert*-Butanol, THF, 25 ℃, 12 h; (b) K_2CO_3, KI, Acetone, 60 ℃, 12 h; (c) 1. THF, 90 ℃, 9 h; 2. TFA, DCM, 25 ℃, 0.5 h; (d) K_2CO_3, DMF, 85 °C, 12 h; (e) 1. Na_2CO_3, DMF, 50 °C, 12 h; 2. TFA, DCM, r.t., 4 h.

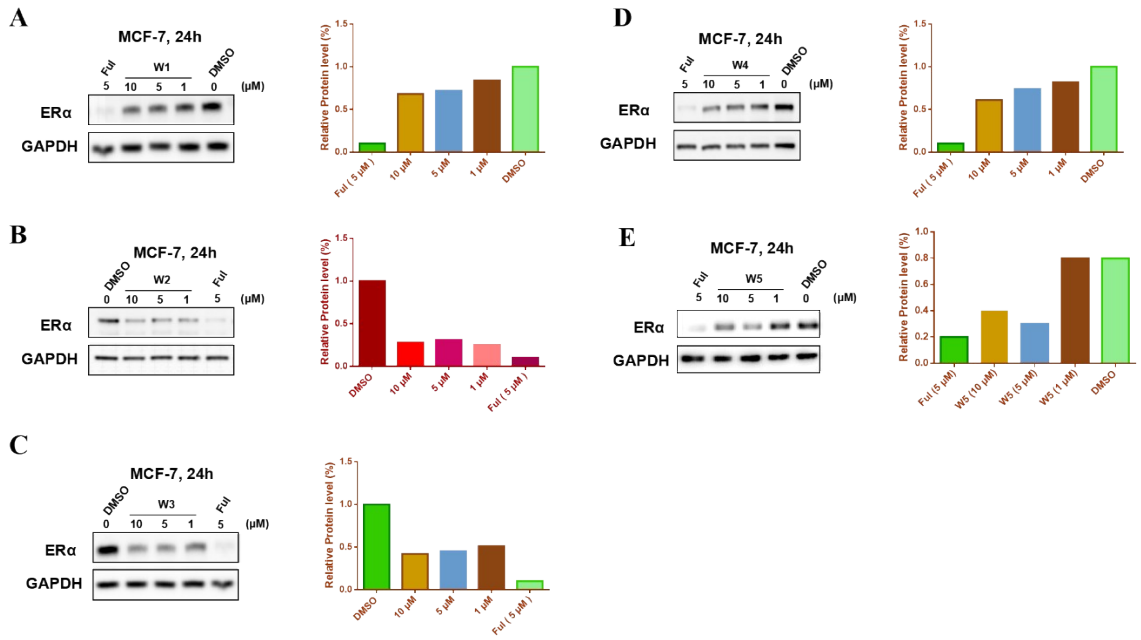


Fig. S1 Degradation activity of compounds **W1**-**W5** evaluated by western blotting assay. MCF-7 cells were individually pretreated with 1, 5 and 10 μM degraders for 24 h before protein level analysis.

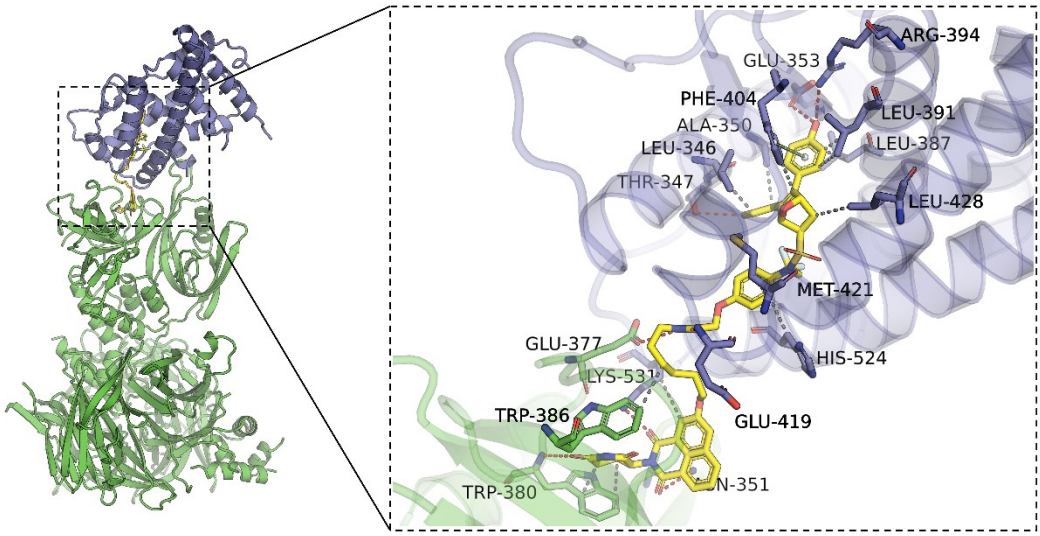


Figure **S2**. Prediction of ERα-**W2**-CRBN ternary complex. Docking pose of the ternary ERα-**W2**-CRBN complex. The ERα protein (PDB: 5KCC) is colored blue, whereas the CRBN E3 ligase (PDB: 5FQD) is colored green. The compound **W2** is represented by yellow sticks. Hydrogen bond is shown as red dash.

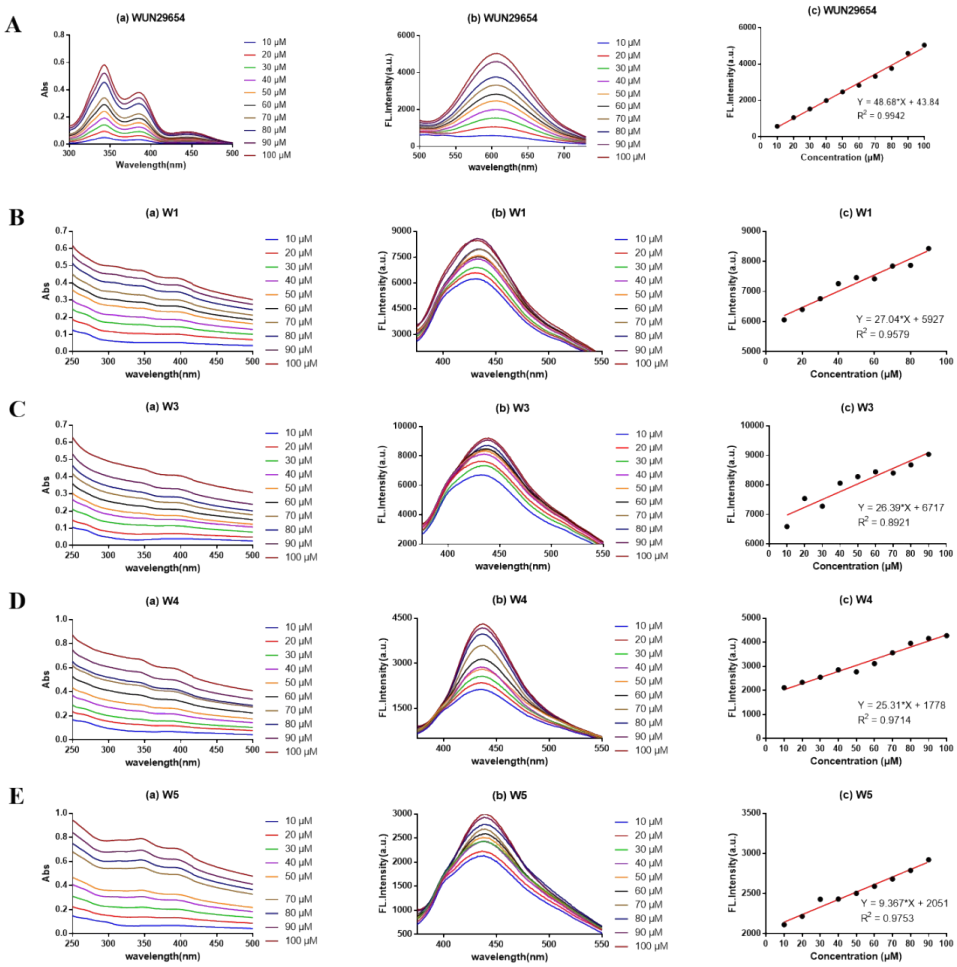


Fig. S3 Optical properties of **WUN29654** (A), **W1** (B), **W3** (C), **W4** (D) and **W5** (E). Relationship curve of (a) UV absorption and (b) fluorescence intensity at different concentrations. (c) Linear relationship derived from (b) (**WUN29654**: R^2 = 0.9942; **W1**: R^2 = 0.9579; **W3**: R^2 = 0.8921, **W4**: R^2 = 0.9714, **W5**: R^2 = 0.9753).

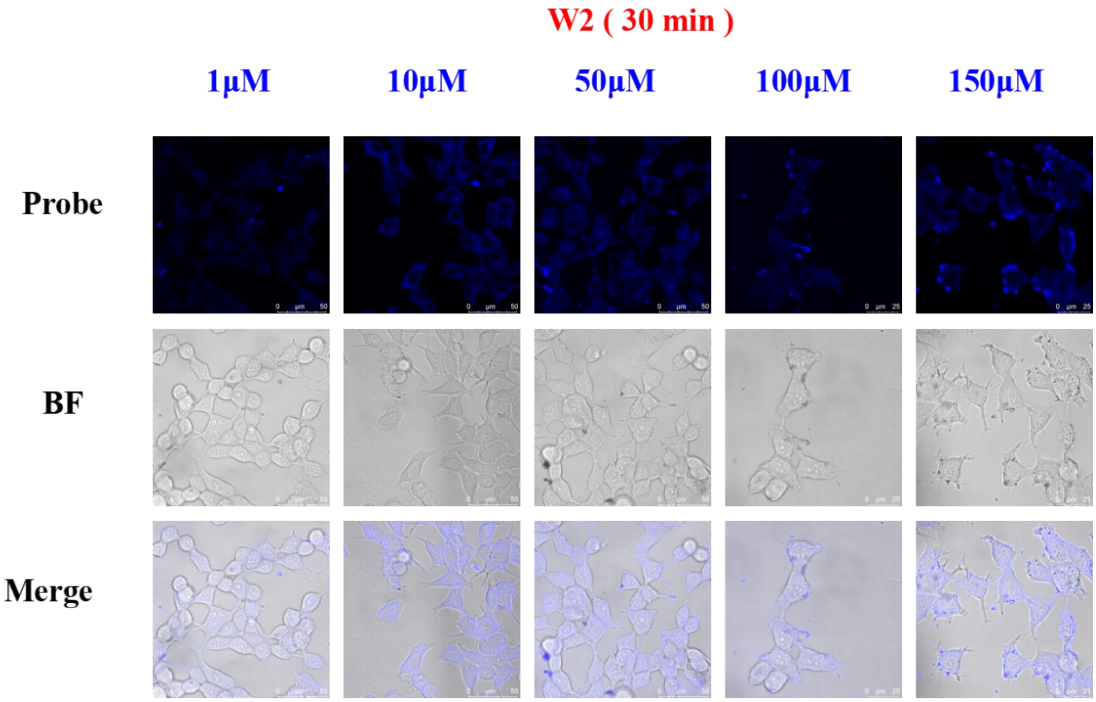


Fig. S4 Cellular imaging of **W2** in MCF-7 cells at different concentrations. Confocal images of living MCF-7 cells stained with **W2** (blue). Scale bar: 50 μm. Excitation: 405 nm.

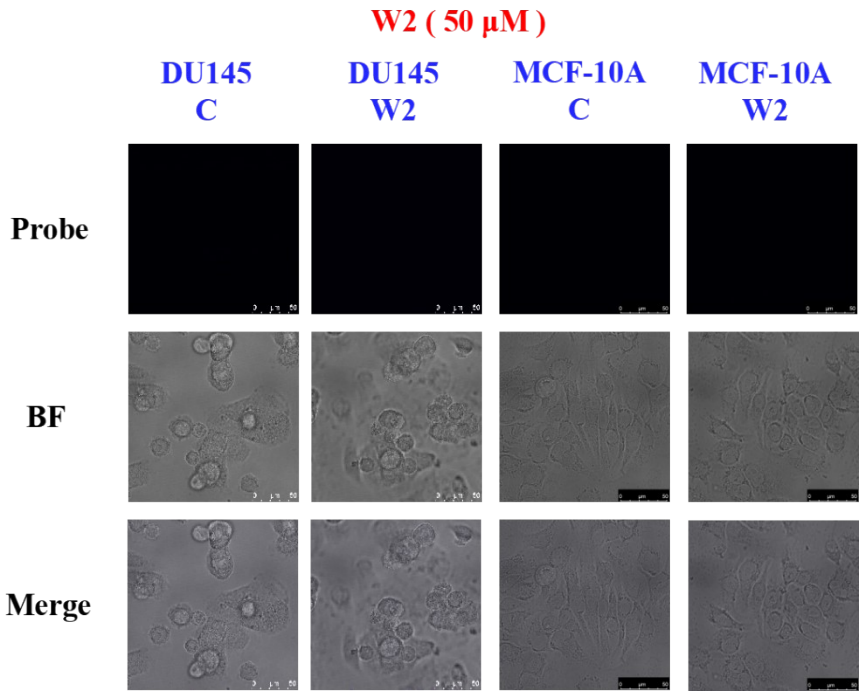


Fig. S5 Cellular imaging in DU145 and MCF-10A cells after treatment with 50 μM of **W2**. Confocal images of living cells stained with **W2** (blue). Scale bar: 50 μm. Excitation: 405 nm.

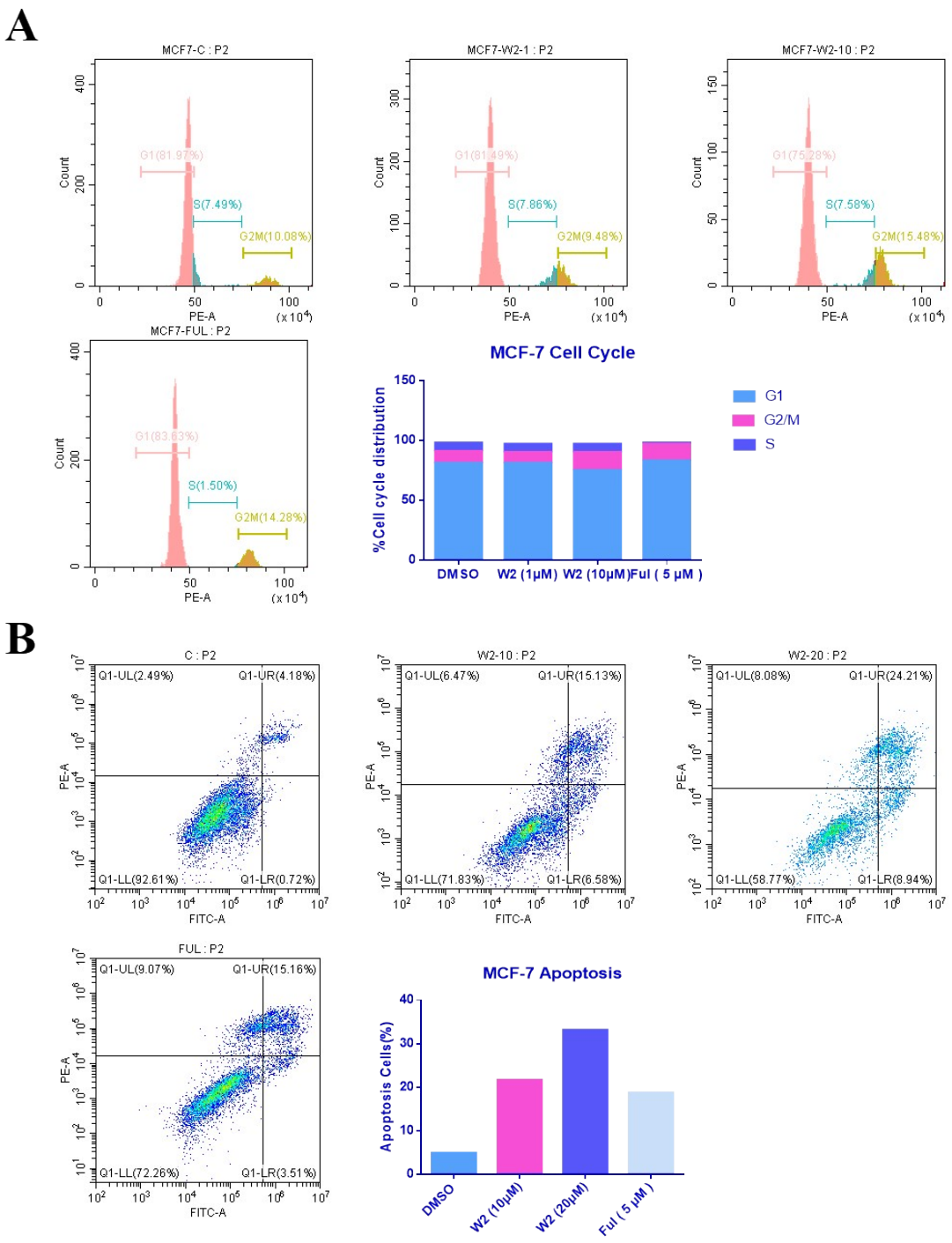


Fig. S6 Analysis of (A) cell-cycle arrest and (B) Flow cytometry and quantitative analysis of apoptotic cells induced by the indicated concentrations of compound **W2** for 48 h in MCF-7.

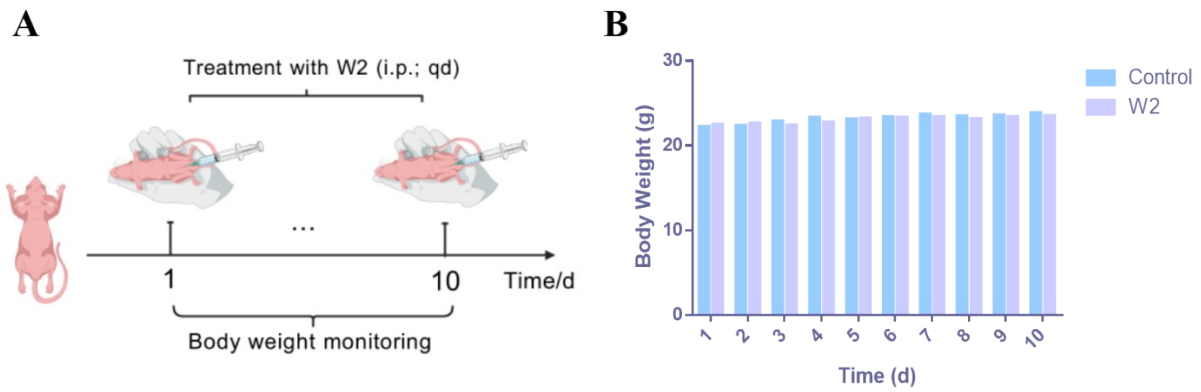


Fig. S7 Safety assessment of **W2** *in vivo*. (A) Schematic of acute toxicity test in athymic nude mice. (B) Relative body weight changes of athymic nude mice in different groups.

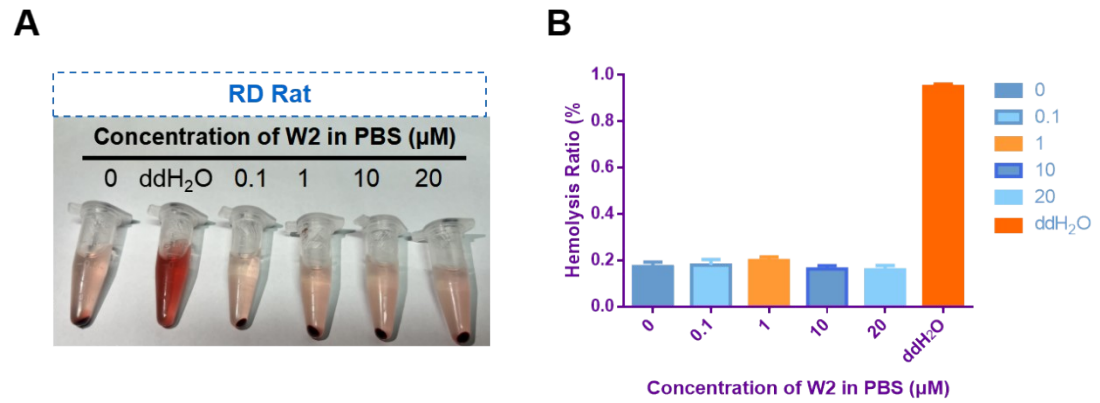
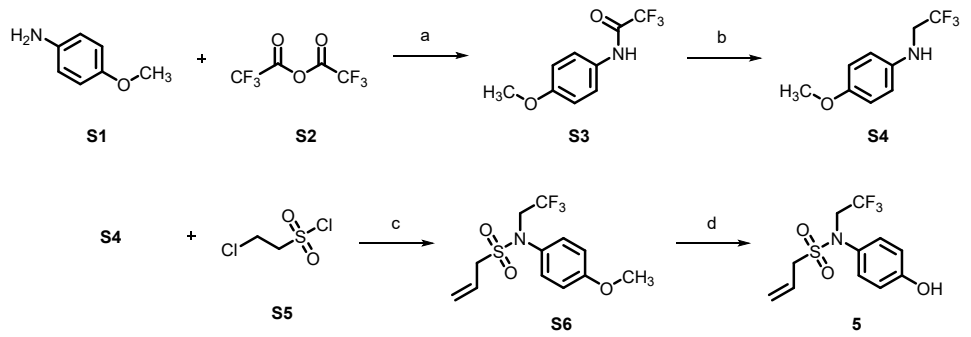


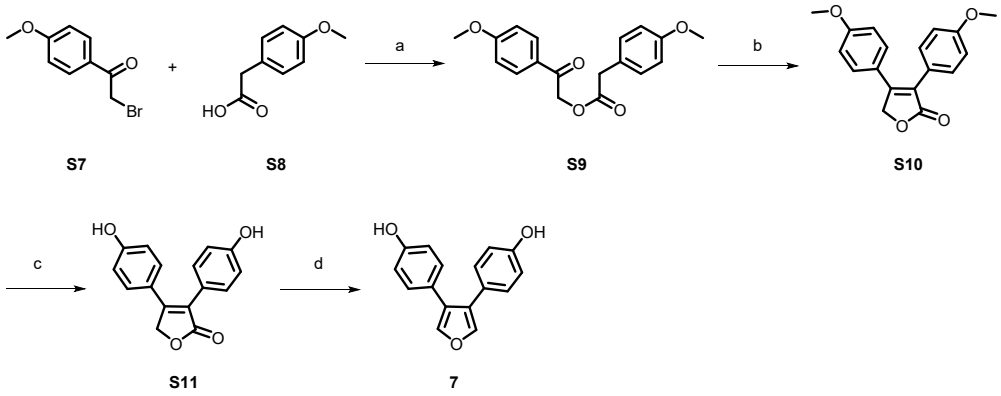
Fig. S8 Hemolytic toxicity of **W2**. (A) Hemolysis evaluation by **W2** at different concentrations against rats red blood cells. (B) Hemolytic radio (%) of **W2** against rats red blood cells.

Scheme S2. Synthesis of intermediate **5**.[a]



[a] Reagents and conditions: (a) DMAP, DCM, r.t., 3 h; (b) BH_3SMe_2, THF, 65 °C, 12 h; (c) TEA, DCM, r.t., 12 h; (d) BBr_3, DCM, Ar, -20 °C, 12 h.

Scheme S3. Synthesis of intermediate **7**.[a]



[a] Reagents and conditions: (a) TEA, ACN, r.t., 4 h; (b) DBU, ACN, 0 °C, 3 h; (c) BBr_3, DCM, Ar, -20 °C, 12 h; (d) DIBAL-H, THF, -78 °C, 12 h.

Synthesis of **S3**. 4-Methoxyaniline (0.50 g, 4.06 mmo1) and 2,2,2-trifluoroacetic anhydride (0.90 g, 4.26 mmo1) were dissolved in DCM. Then, 4-dimethylaminopyridine (DMAP, 99.10 mg, 0.81 mmol) was added into the solution and stirred at room temperature for 3 h. The mixture was adjusted to neutral pH with saturated $NaHCO_3$ solution and extracted with water and DCM (50 mL × 3). Finally, the organic phase was evaporated under reduced pressure, and the crude product was purified by flash column chromatography (PE/EA = 3:1) on silica gel, affording compound **S3** as a white solid (0.89 g, 4.06 mmol, yield: 86%).

Synthesis of **S4**. Compound **S3** (0.89 g, 4.06 mmol) and borane dimethyl sulfide (0.61 g, 8.12 mmo1) were dissolved in THF and stirred at 65 °C for 12 h. Then, the reaction was quenched by dropwise addition of anhydrous methanol at -20 °C. The mixture was evaporated under reduced pressure and used directly in the next reaction without further purification.

Synthesis of **S6**. Compound **S4** (1.00 g, 4.87 mmol) were dissolved in DCM. Dichloroethane sulphonyl chloride (0.95 g, 5.84 mmo1) were dissolved in DCM and added into the solution above at Ar atmosphere over ice bath. Then, TEA (1.48 g, 14.61 mmol) was added slowly into the solution and stirred at room temperature for 12 h. The mixture was extracted with water and DCM (50 mL × 3). Finally, the organic phase was evaporated under reduced pressure, and the crude product was purified by flash column chromatography (PE/EA = 3:1) on silica gel, affording compound **S6** as a white solid (0.98 g, 3.16 mmol, yield:65%).

Synthesis of **5**. Compound **S6** (1.00 g, 3.39 mmol) was dissolved in DCM. Then, BBr_3 (0.65 mL, 6.77 mmo1) was added slowly into the solution in the Ar atmosphere at -20 °C and stirred for 12 h. The reaction was quenched by dropwise addition of anhydrous methanol at -20 °C. The mixture was evaporated under reduced pressure, and the crude product was purified by flash column chromatography (PE/EA = 3:1) on silica gel, affording compound **5** as a yellow solid (0.69 g, 2.32 mmol, yield: 72%).

Synthesis of **S9**. 2-Bromo-1-(4-methoxyphenyl) ethan-1-one (1.59 g, 6.94 mmo1) and 2-(4-methoxyphenyl) acetic acid (1.15 g, 6.94 mmo1) were dissolved in ACN. TEA (70.2 mg, 0.69 mmo1) was added into the solution and stirred at room temperature for 4 h. The mixture was adjusted to neutral pH with 2M HCl and saturated sodium bicarbonate solution and extracted with water and EA (50 mL × 3). Finally, the organic phase was evaporated under reduced pressure, and the crude product was purified by flash column chromatography (PE/EA = 30:1) on silica gel, affording the desired compound **S9** as a white solid (1.88 g, 6.00 mmol, yield: 86%).

Synthesis of **S10**. Compound **S9** (1.00 g, 3.18 mmol) was dissolved in ACN. DBU (0.97 g, 6.36 mmol) was added slowly into the solution at 0 °C and stirred for 3 h. The mixture was extracted with water and DCM (50 mL × 3). Finally, the organic phase was evaporated under reduced pressure, and the crude product was purified by flash column chromatography (PE/EA = 10:1) on silica gel, affording the desired compound **S10** as a yellow solid (0.58 g, 1.97 mmol, yield: 62%).

Synthesis of **S11**. Compound **S10** (1.26 g, 4.25 mmo1) was dissolved in DCM. Then, BBr_3 (2.6 mL, 27.33 mmo1) was added slowly into the solution in the Ar atmosphere at -20 °C and stirred for 12 h. Then, the reaction was quenched by dropwise addition of anhydrous methanol at -20 °C. The solution was extracted with water and EA (50 mL × 3). Finally, the organic phase was evaporated under reduced pressure, and the crude product was purified by flash column chromatography (PE/EA = 3:1) on silica gel, affording the desired compound **S11** as a yellow solid (0.93 g, 3.47 mmol, yield: 82%).

Synthesis of **7**. Diisobutylaluminum hydride (DIBAL-H, 8.00 mL, 7.93 mmo1) was added into a 50 mL round-bottomed flask with compound **S11** (1.12 g, 4.17 mmol) in the Ar atmosphere at -78 °C for 12 h. The reaction was quenched with 2M HCl. The

solution was extracted with water and EA (50 mL × 3). Finally, the organic phase was evaporated under reduced pressure, and the crude product was purified by flash column chromatography (PE/EA = 3:1) on silica gel, affording the desired compound **7** as a yellow solid (0.44 g, 1.75 mmol, yield: 42%).

Synthesis of **6a/b**. Compounds **4a/b** (1.20 eq.) and KI (56 mg, 0.10 eq., 0.34 mmol) were dissolved in DMF and stirred at room temperature for 30 min. Then, compound **5** (1.00 g, 1.00 eq., 3.39 mmol) and K_2CO_3 (0.94 g, 2.00 eq., 6.78 mmol) were added into the solution and stirred at 85 °C for 12 h. The mixture was extracted with water and EA (50 mL × 3). Finally, the organic phase was evaporated under reduced pressure, and the crude product was purified by flash column chromatography (PE/EA = 5:1) on silica gel, affording the desired compounds **6a/b** as yellow solid (yield: 70-80%).

Synthesis of **8a/b**. Compounds **6a/b** (1.05 eq.) and **7** (200 mg, 1.00 eq., 0.79 mmol) were placed in a 25 mL round-bottomed flask with Ar atmosphere. Then, 1 mL THF was added into the flask and stirred at 90 °C for 12 h. The mixture was extracted with water and EA (15 mL × 3). The organic phase was evaporated under reduced pressure, and the crude product was purified by flash column chromatography (PE/EA = 3:1) on silica gel, affording the intermediate products. Then, the intermediate products were dissolved in DCM (1 mL), and TFA (1 mL) was added slowly over ice. The solution was stirred at room temperature for 30 min. Finally, the solution was evaporated under reduced pressure to get compounds **8a/b** as yellow solid. The products **8a/b** were used directly in the next reaction without further purification (yield: 40-70%).

Synthesis of **11**. 5-Hydroxy-1*H*,3*H*-benzo[*de*]isochromene-1,3-dione (1.21 g, 5.65 mmo1) and 3-aminopiperidine-2,6-dione (0.72 g, 5.65 mmo1) were dissolved in DMF (10 mL). K_2CO_3 (1.56 g, 11.3 mmo1) was added into the solution and stirred at 85 °C for 12 h. The mixture was extracted with water and EA (50 mL × 3). Finally, the organic phase was evaporated under reduced pressure, and the crude product was purified by flash column chromatography (PE/EA = 1:1) on silica gel, affording the desired compound **11** as a yellow solid (yield: 70%).

Synthesis of **13a-c**. Compound **11** (0.30 g, 1.00 eq., 0.93 mmol) and K_2CO_3 (0.25 g, 2.00 eq., 1.86 mmol) were dissolved in DMF (5 mL) and stirred at room temperature for 30 min. Then, **12a-c** (1.20 eq.) was added into the solution and stirred at 50 °C for 12 h. The mixture was extracted with water and EA (15 mL × 3). Finally, the organic phase was evaporated under reduced pressure, and the crude product was purified by flash column chromatography (PE/EA = 1:1) on silica gel, affording the intermediate products. The intermediate products were dissolved in DCM (1 mL), and TFA (1 mL) was added slowly over ice. Then, the solution was stirred at room temperature for 30 min. Finally, the solution was evaporated under reduced pressure to get compounds **13a-c** as yellow solid. The products **13a-c** were used directly in the next reaction without further purification (yield: 40-60%).

5 *N*-(4-Hydroxyphenyl)-*N*-(2,2,2-trifluoroethyl) prop-2-ene-1-sulfonamide, yield 42%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.82 (s, 1H), 7.20 – 7.14 (m, 2H), 6.94 (dd, J = 16.4, 9.9 Hz, 1H), 6.81 – 6.76 (m, 2H), 6.14 (d, J = 9.9 Hz, 1H), 6.02 (d, J = 16.4 Hz, 1H), 4.37 (q, J = 8.9 Hz, 2H).

6b *Tert*-butyl 6-(4-(*N*-(2,2,2-trifluoroethyl) allylsulfonamido) phenoxy) hexanoate, yield 78%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.20 (d, J = 8.5 Hz, 2H), 6.85 (d, J = 8.7 Hz, 2H), 6.57 (dd, J = 16.5, 9.9 Hz, 1H), 6.14 (d, J = 16.5 Hz, 1H), 5.97 (d, J = 9.8 Hz, 1H), 4.16 (q, J = 8.5 Hz, 2H), 3.92 (t, J = 6.4 Hz, 2H), 3.09 (t, J = 6.8 Hz, 2H), 1.76 (p, J = 6.7 Hz, 2H), 1.51 – 1.47 (m, 2H), 1.42 (s, 9H), 1.39 – 1.33 (m, 2H), 1.31 – 1.18 (m, 2H).

7 4,4'-(Furan-3,4-diyl) diphenol, yield 72%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.51 (s, 2H), 7.73 (s, 2H), 7.02 (d, J = 8.2 Hz, 4H), 6.72 (d, J = 8.2 Hz, 4H).

8b 6-(4-((5,6-Bis(4-hydroxyphenyl)-*N*-(2,2,2-trifluoroethyl)-7-oxabicyclo [2.2.1]hept-5-ene)-2-sulfonamido) phenoxy) hexanoic acid, yield 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.68 (s, 2H), 7.26 (d, J = 8.6 Hz, 2H), 7.13 (dd, J = 12.5, 8.3 Hz, 4H), 6.83 (d, J = 8.6 Hz, 2H), 6.75 (d, J = 8.2 Hz, 2H), 6.70 (d, J = 8.3 Hz, 2H), 5.49 (s, 1H), 5.33 (d, J = 3.6 Hz, 1H), 4.50 (dt, J = 12.1, 7.8 Hz, 2H), 3.92 (t, J = 6.3 Hz, 2H), 3.51 (dd, J = 7.6, 4.9 Hz, 1H), 2.23 (t, J = 7.2 Hz, 2H), 2.01 – 1.86 (m, 2H), 1.69 (t, J = 7.4 Hz, 2H), 1.54 (q, J = 5.7, 3.8 Hz, 2H), 1.43 – 1.37 (m, 2H).

11 2-(2,6-Dioxopiperidin-3-yl)-5-hydroxy-1*H*-benzo[de]isoquinoline-1,3(2H)-dione, yield 70%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.04 (s, 1H), 10.63 (s, 1H), 8.32 – 8.19 (m, 2H), 8.02 (dd, J = 43.6, 2.4 Hz, 1H), 7.75 (q, J = 7.6 Hz, 1H), 7.68 (t, J = 3.0 Hz, 1H), 5.82 (ddd, J = 12.0, 5.7, 3.0 Hz, 1H), 3.00 – 2.87 (m, 1H), 2.59 (dq, J = 15.1, 4.6 Hz, 2H), 2.04 (dd, J = 12.2, 6.5 Hz, 1H).

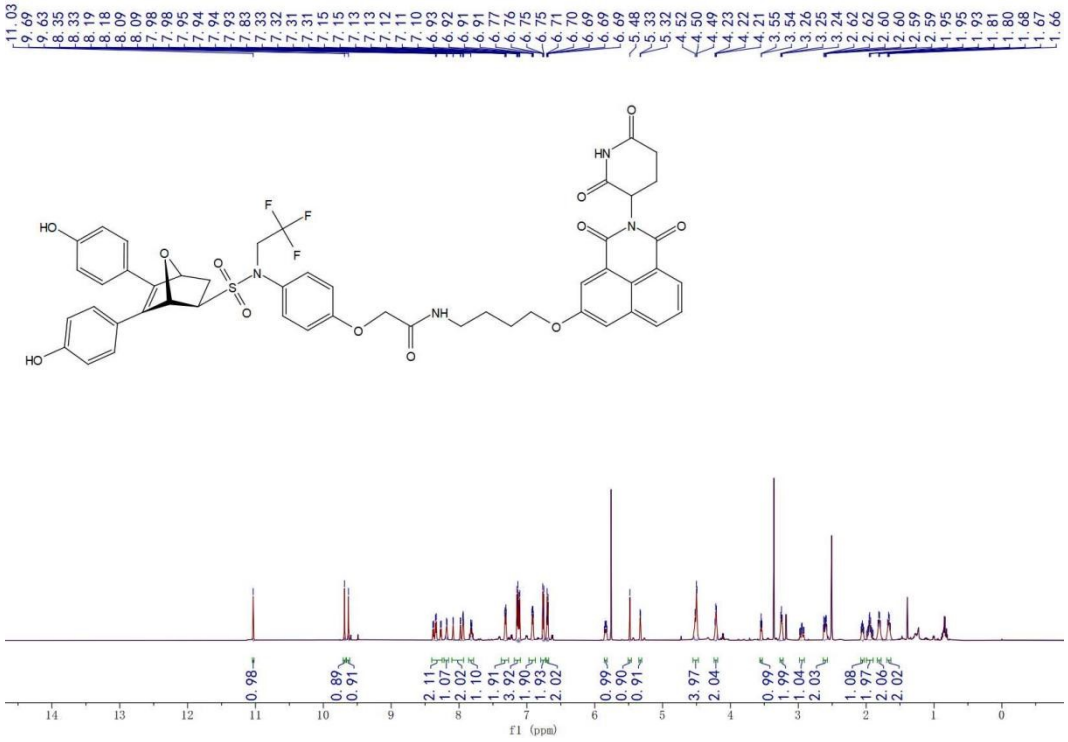
13b 5-((6-Aminohexyl)oxy)-2-(2,6-dioxopiperidin-3-yl)-1*H*-benzo[de]isoquinoline-1,3(2H)-dione, yield 52%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.06 (s, 1H), 8.42 – 8.26 (m, 2H), 8.04 (dd, J = 45.3, 2.6 Hz, 2H), 7.84 (q, J = 7.7 Hz, 1H), 5.89 – 5.81 (m, 1H), 4.22 (t, J = 5.9 Hz, 2H), 3.01 – 2.88 (m, 3H), 2.60 (dd, J = 13.7, 6.6 Hz, 2H), 2.08 – 2.01 (m, 2H), 1.82 (s, 2H), 1.48 (d, J = 7.1 Hz, 4H), 1.29 (t, J = 8.2 Hz, 2H).

14 2-(2-((*Tert*-butoxycarbonyl) amino) ethoxy) ethyl 4-methylbenzenesulfonate, yield 88%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.83 (d, J = 8.1 Hz, 2H), 7.38 (d, J = 8.1 Hz, 2H), 4.84 (s, 1H), 4.18 (dd, J = 5.7, 3.6 Hz, 2H), 3.65 (dd, J = 5.6, 3.7 Hz, 2H), 3.47 (t, J = 5.1 Hz, 2H), 3.26 (q, J = 5.3 Hz, 2H), 2.48 (s, 3H), 1.47 (s, 9H).

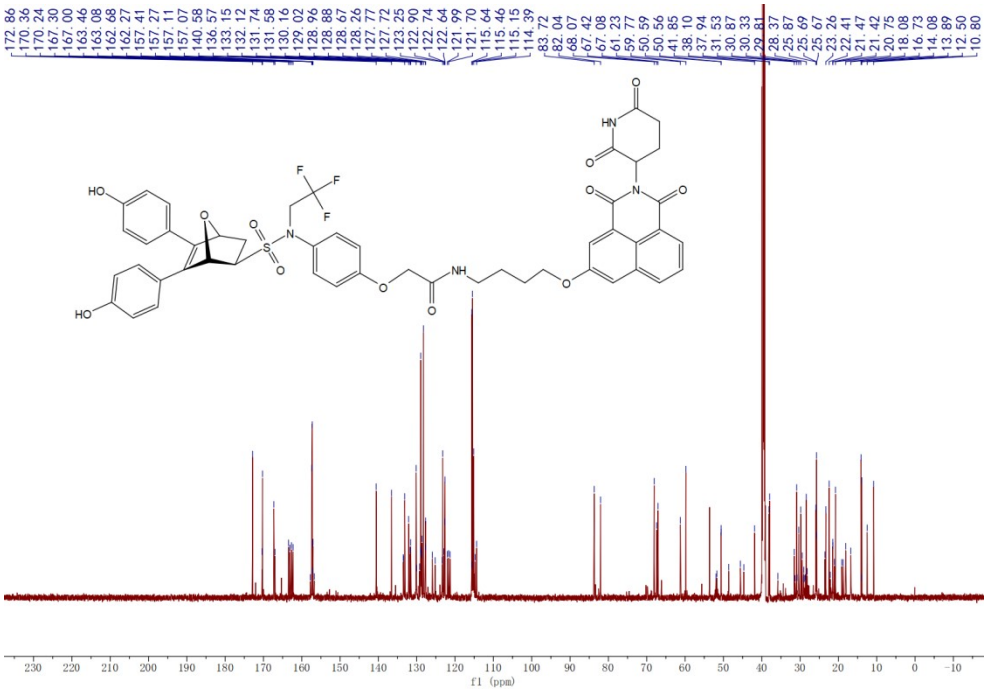
OBHSA 5,6-Bis(4-hydroxyphenyl)-*N*-phenyl-*N*-(2,2,2-trifluoroethyl)-7-oxabicyclo[2.2.1]hept-5-ene-2-sulfonamide, yield 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (s, 3H), 7.07 – 7.20 (m, 6H), 6.75 (d, J = 8.5 Hz, 2H), 6.72 – 6.64 (m, 4H), 5.52 – 5.46 (m, 1H), 5.33 (d, J = 3.9 Hz, 1H), 4.54 – 4.39 (m, 2H), 3.49 (dd, J = 8.0, 4.7 Hz, 1H), 1.98 – 1.87 (m, 2H).

2. NMR spectra of probes.

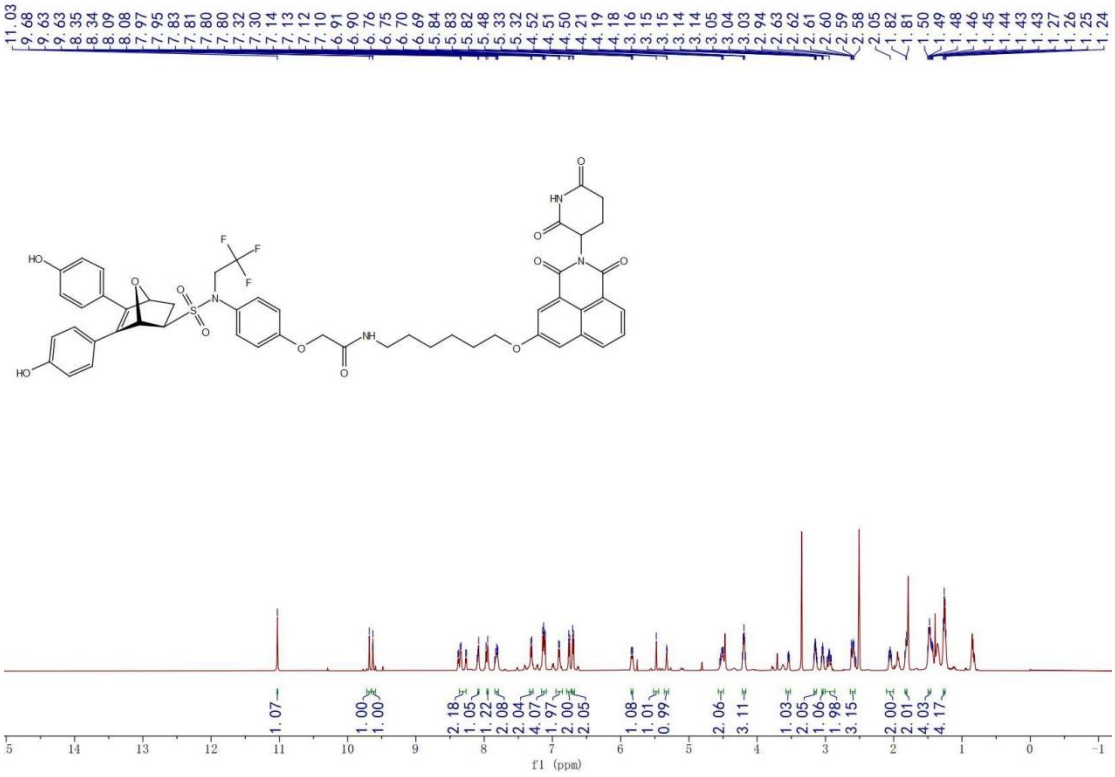
^{1}H NMR of **W1**.



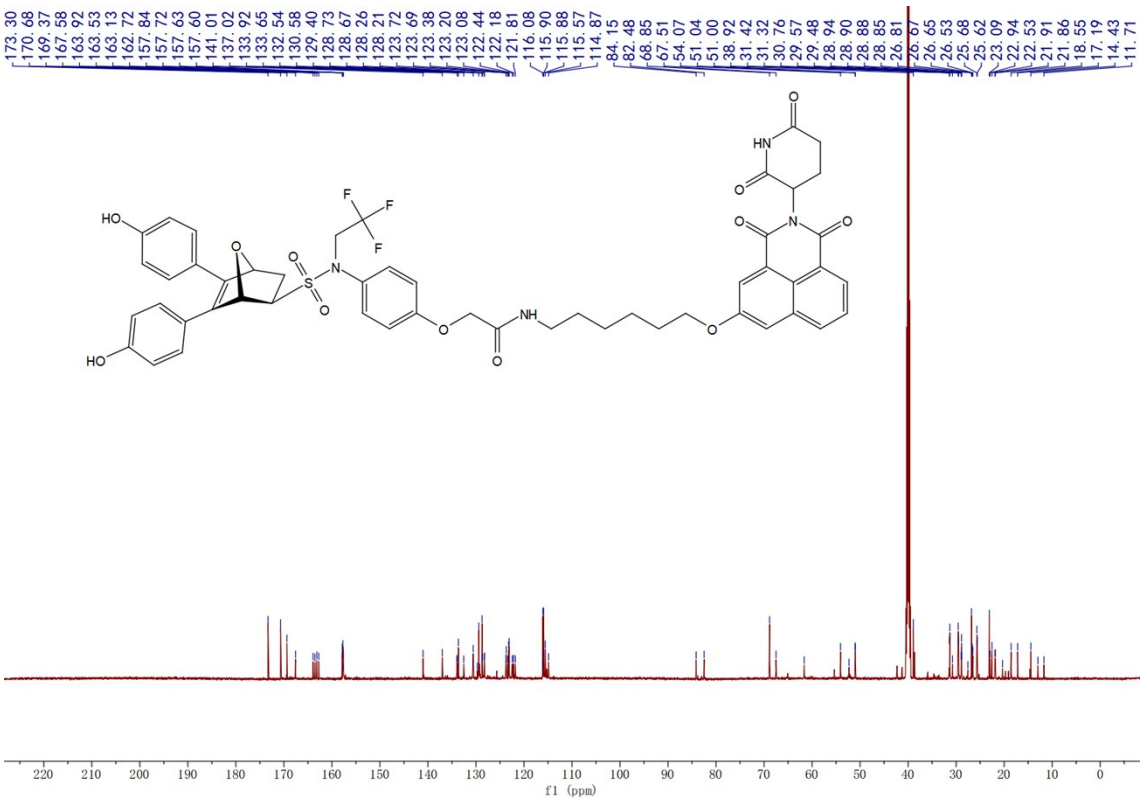
^{13}C NMR of **W1**.



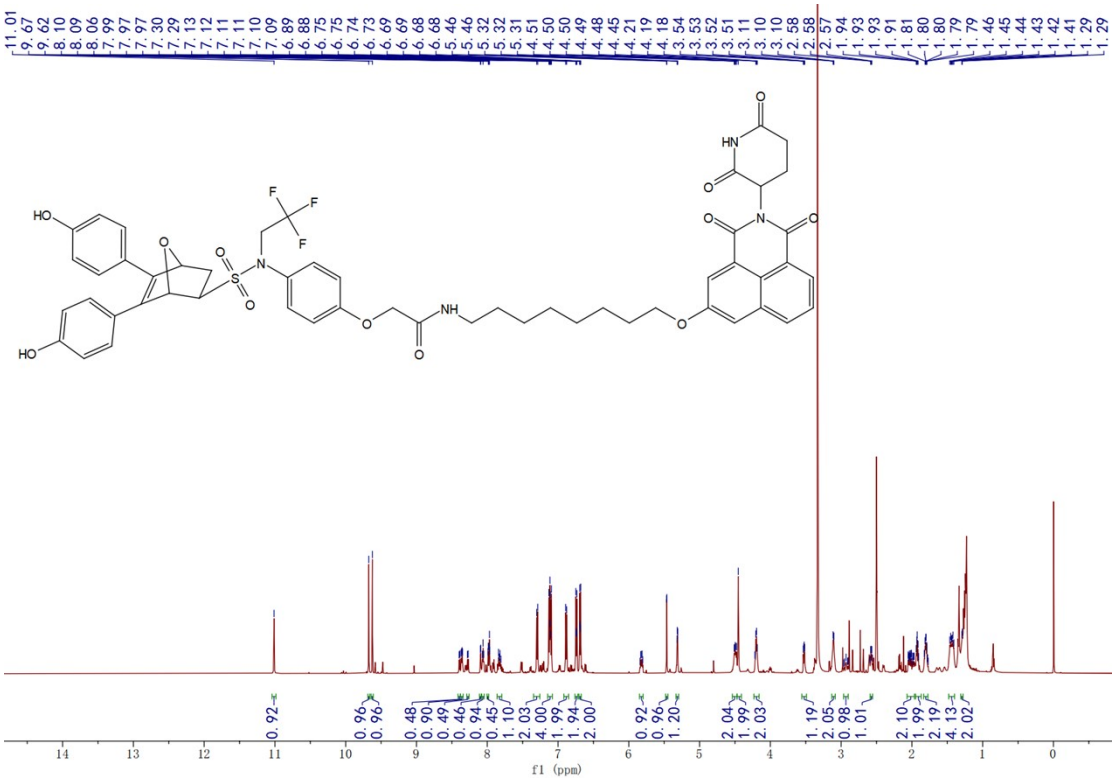
^{1}H NMR of **W2**.



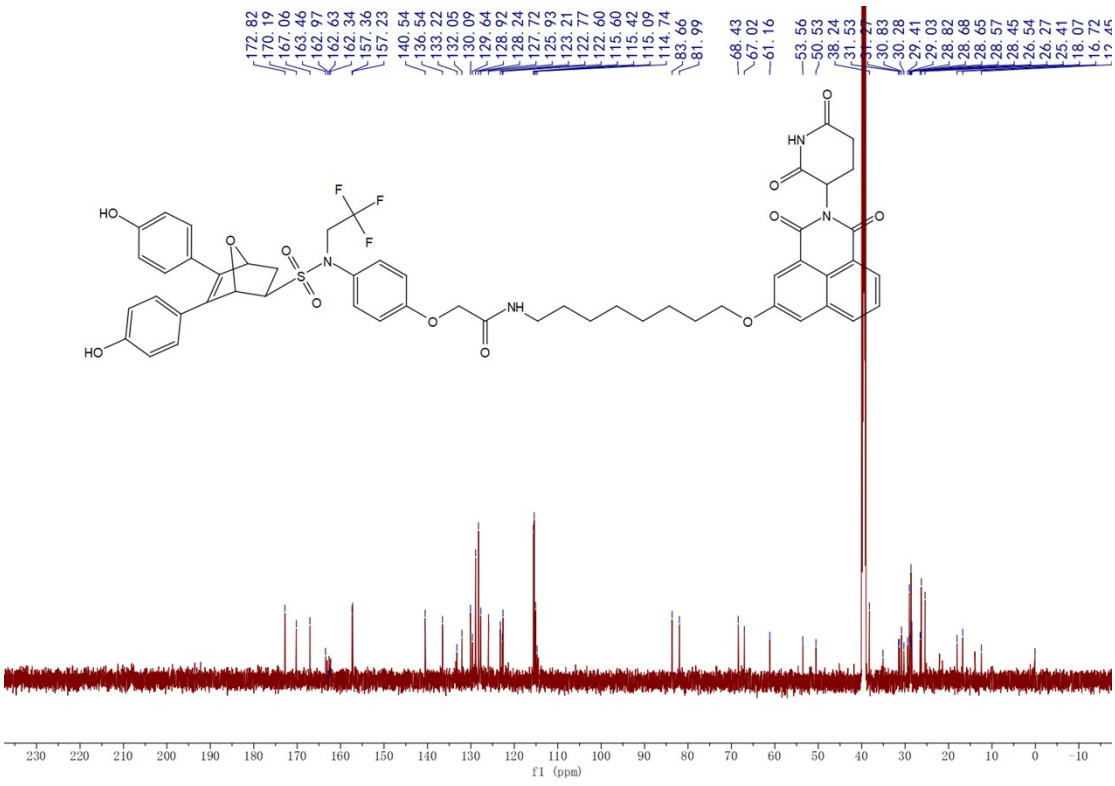
^{13}C NMR of **W2**.



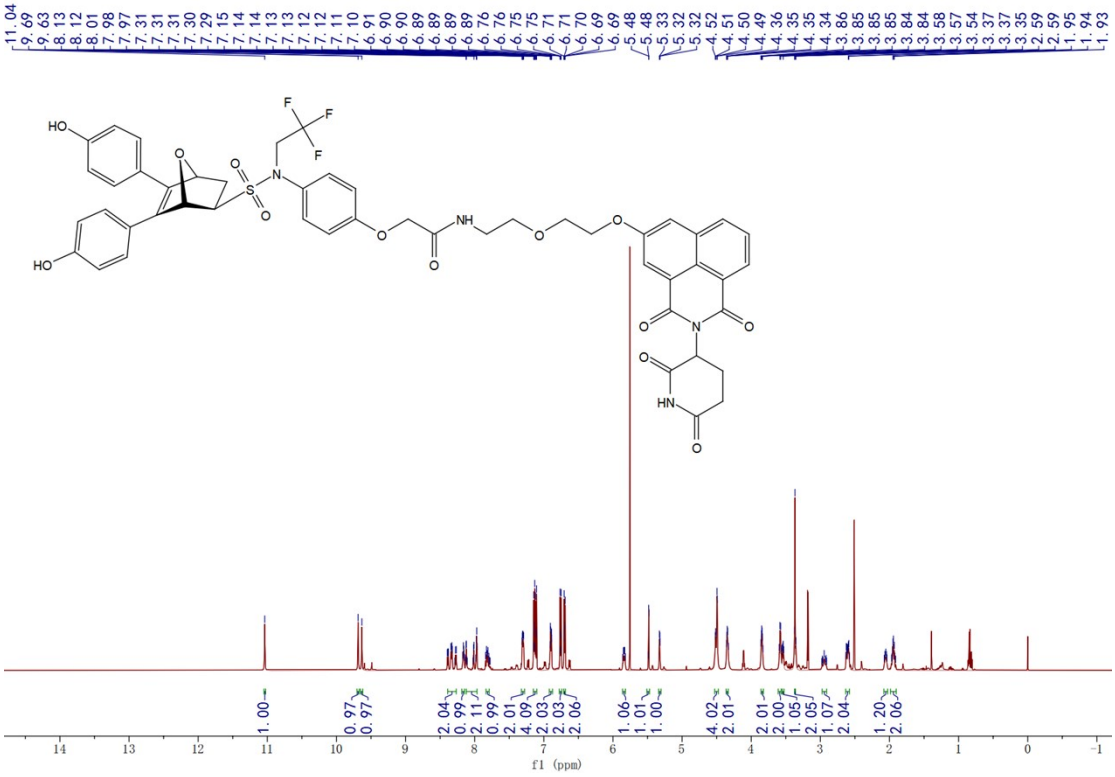
^{1}H NMR of **W3**.



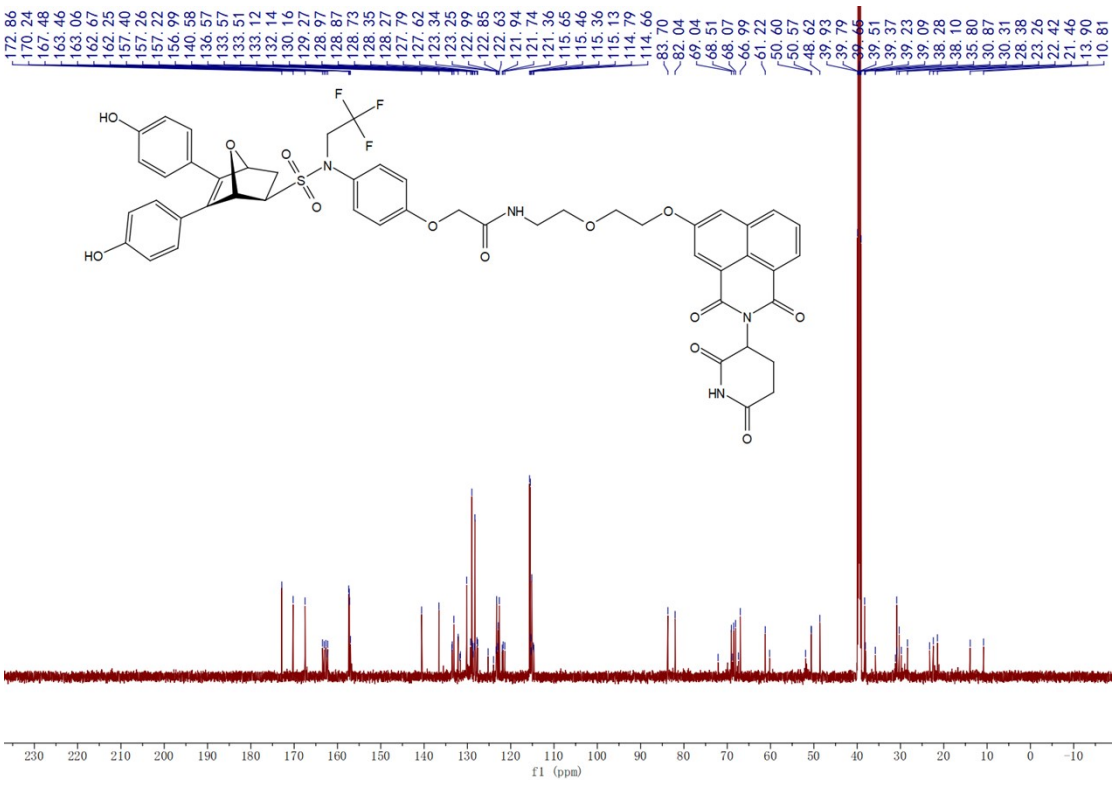
^{13}C NMR of **W3**.



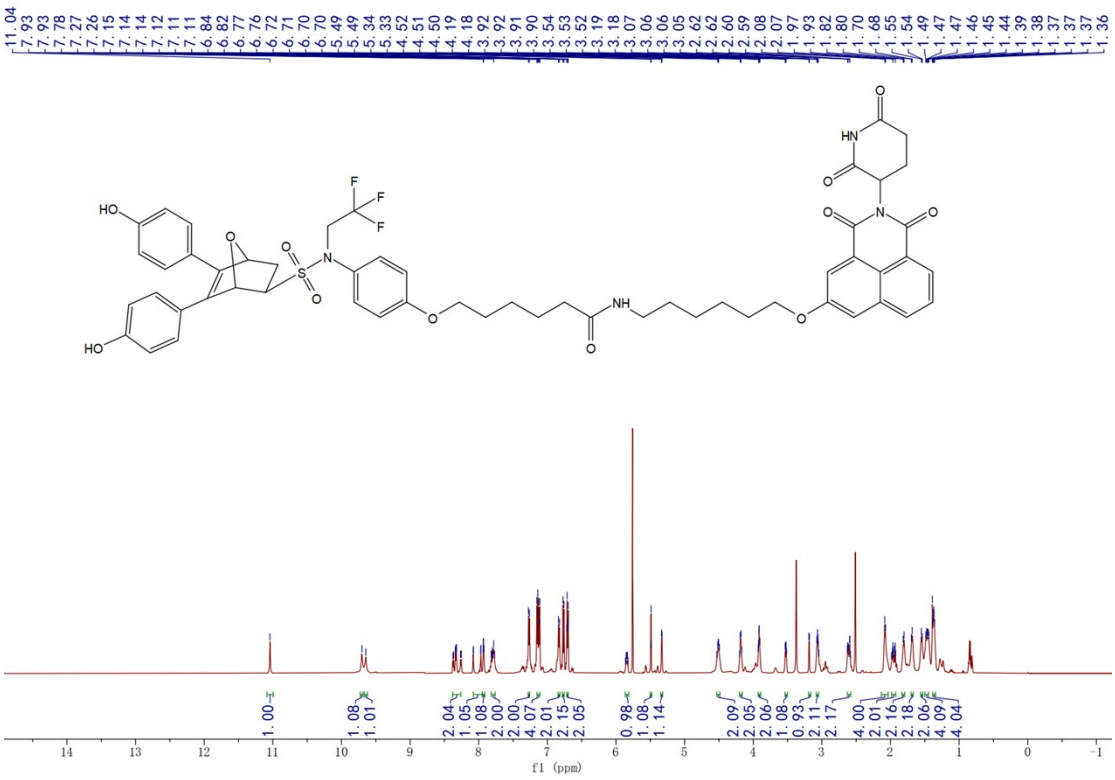
^{1}H NMR of **W4**.



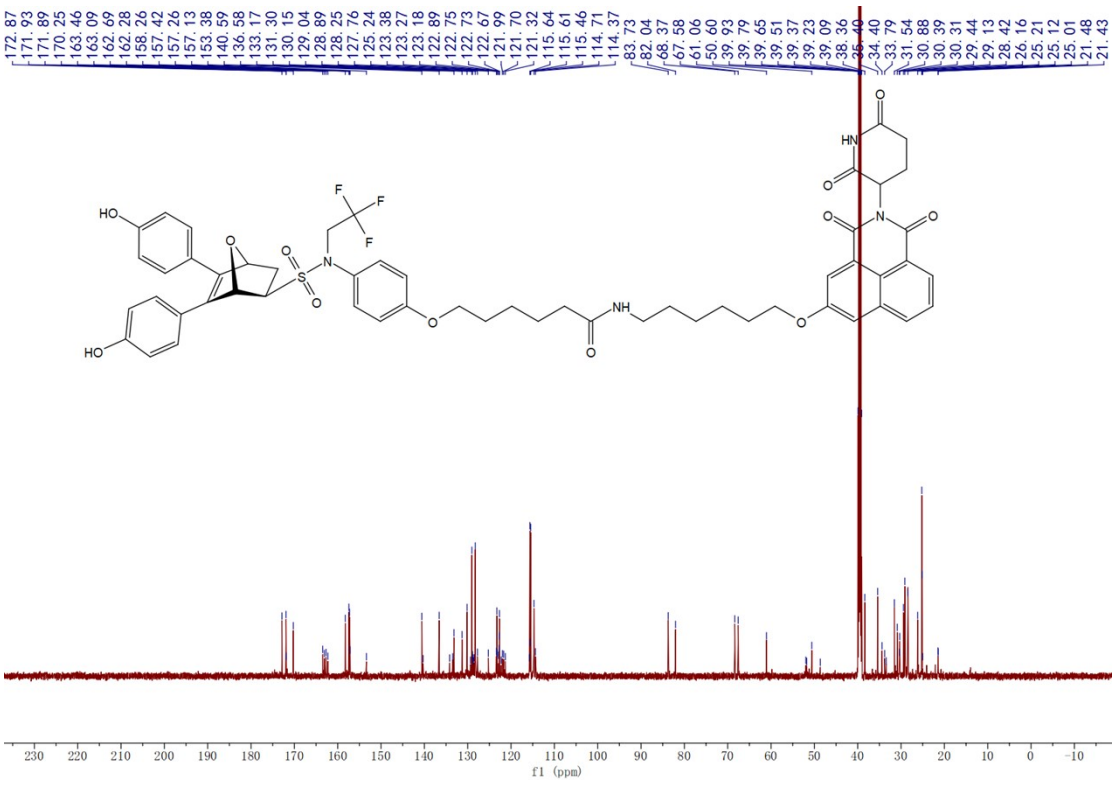
^{13}C NMR of **W4**.



^{1}H NMR of **W5**.

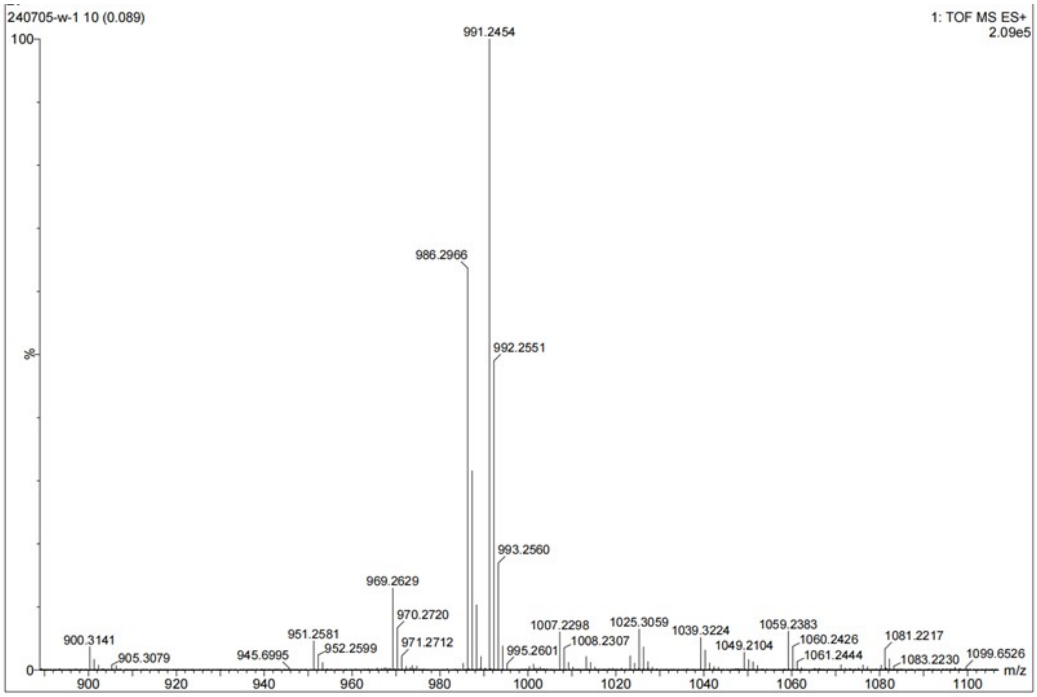


^{13}C NMR of **W5**.

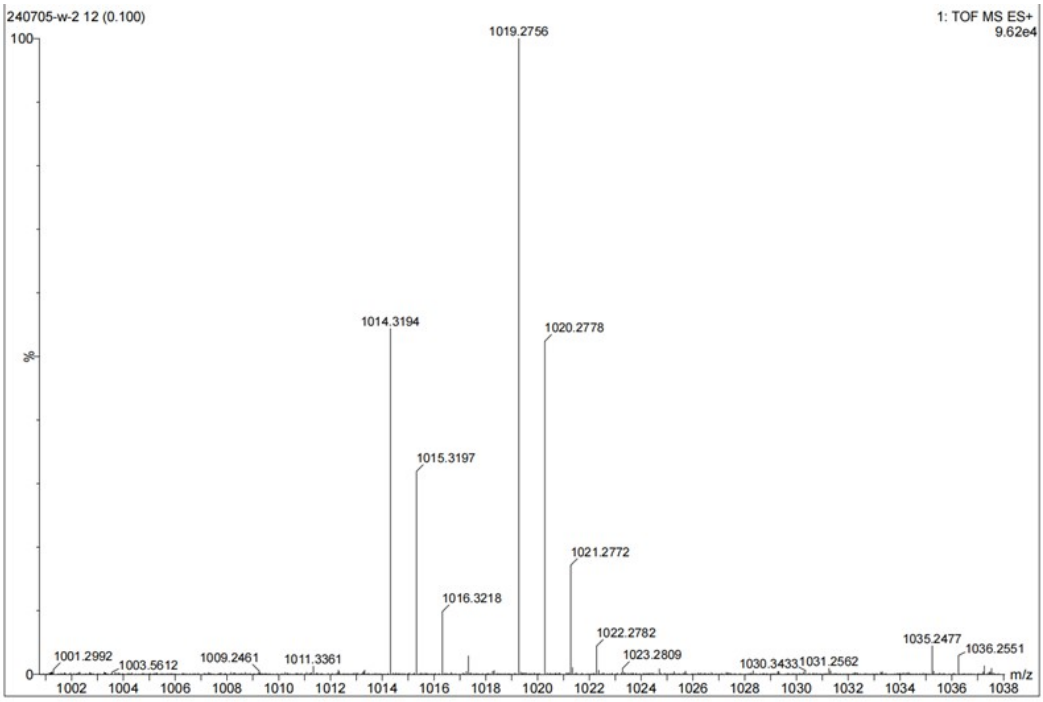


3. High-resolution mass spectra of probes W1-5.

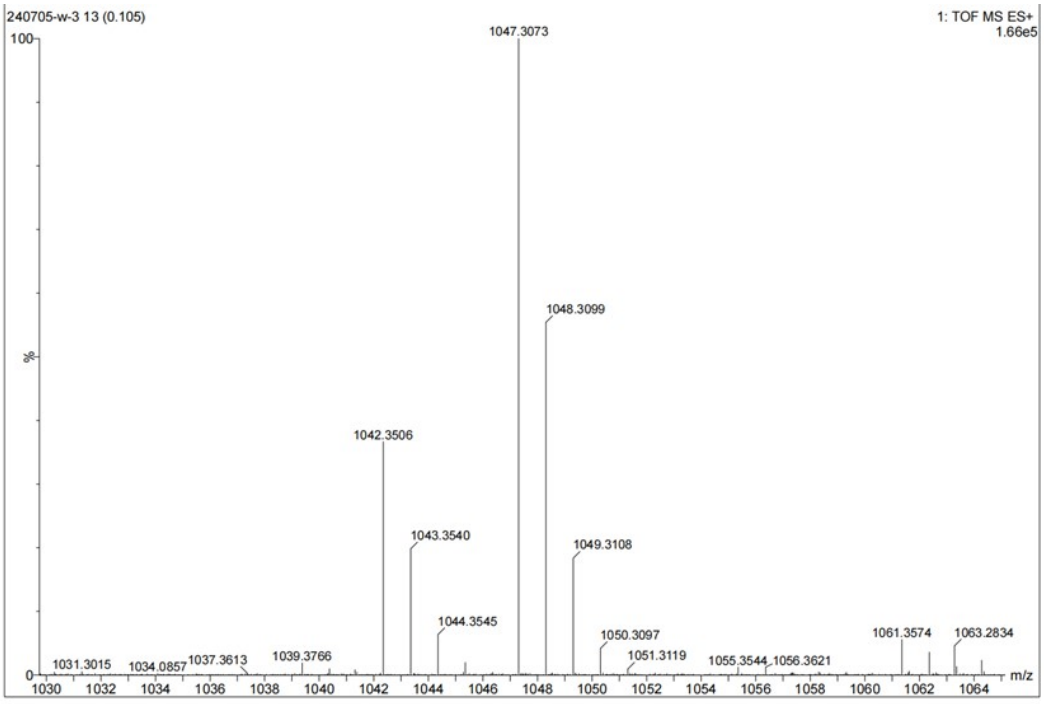
HMRS spectra of **W1**.



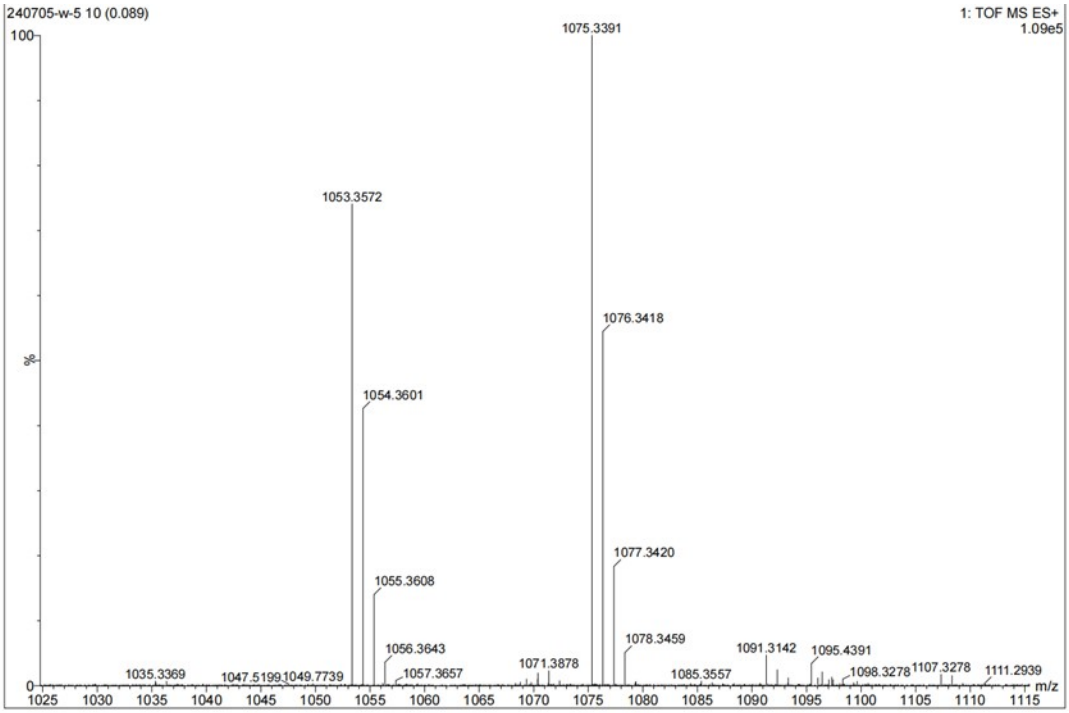
HMRS spectra of **W2**.



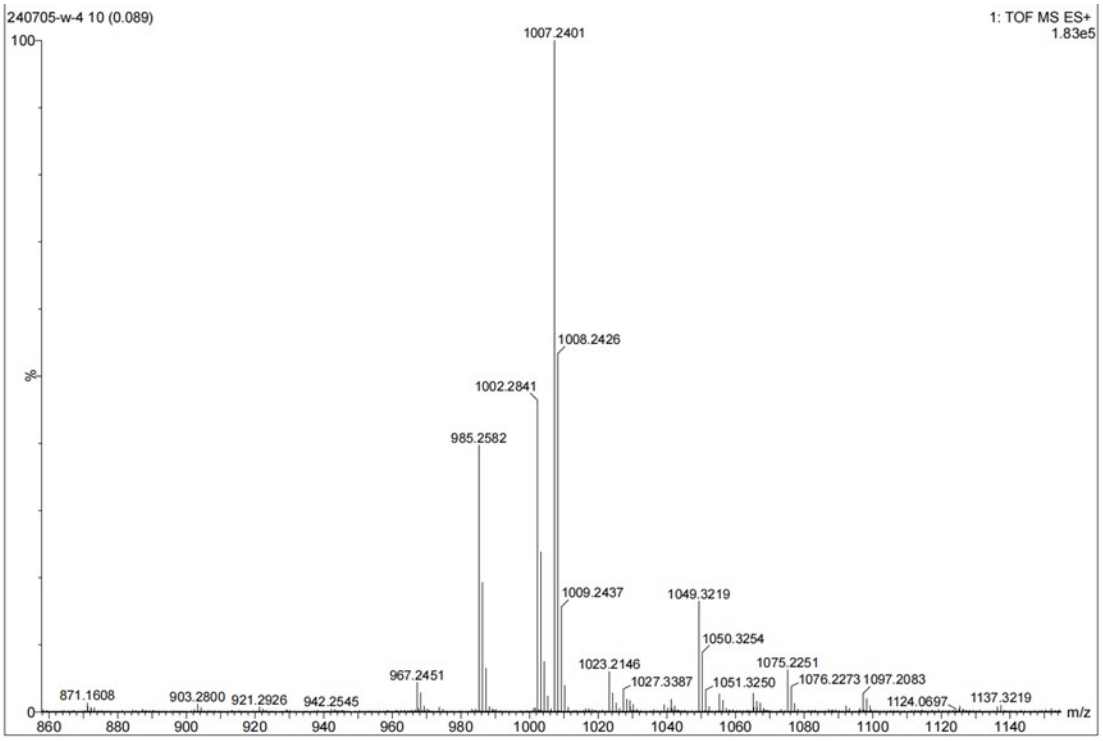
HMRS spectra of **W3**.



HMRS spectra of **W4**.



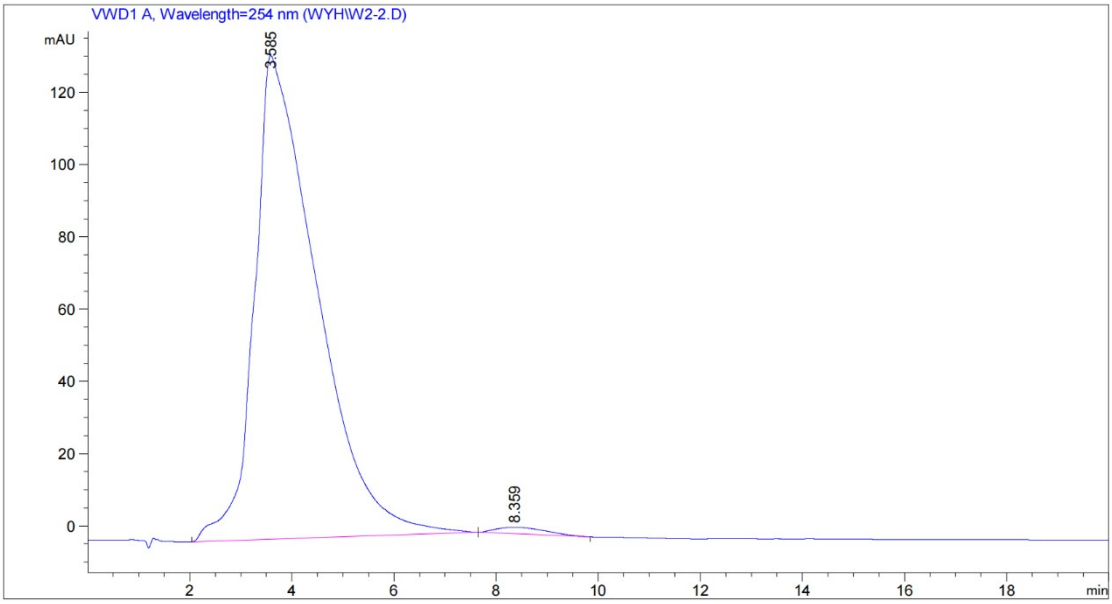
HMRS spectra of **W5**.



HPLC spectra of representative compound

Compound **W2**

(Reverse Phase Method 85:15 MeOH: H_2O, Flow rate 1.0 mL/min)



No.	Retention Time (min)	Peak height	Area	Percent
1	3.585	133.86	1.38621e5	98.91
2	8.359	2	1.80	1.09
Tota l				100