Discovery of Novel Indole Derivatives as Fructose-1,6-bisphosphatase Inhibitors and X-ray Cocrystal structures Analysis

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Content

1. Materials and Methods

1.1. Chemistry section——synthesis of target compounds

1.1.1. General

Melting points were measured on a Yanaco micro melting point apparatus and are uncorrected. ^{1}H-NMR (400 MHz or 500 MHz) on a Varian Mercury 400 or 500spectrometer was recorded in DMSO-*d6*, acetone-*d6* or $CDCl_3$. Chemical shifts are reported in δ (ppm) units relative to the internal standard tetramethylsilane (TMS). High resolution mass spectra (HRMS) were obtained on an Agilent Technologies LC/MSD TOF spectrometer. All chemicals and solvents used were of reagent grade without purified or dried before use. All the reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel G plates at 254 nm under a UV lamp. Column chromatography separations were performed with silica gel (200-300 mesh).

Note: The preparation of intermediate compounds **2a-4a, 2b-4b, 8, 9, 11-15, 17-20** was described in the part 2 (see pages S17-S23).

1.1.2. General procedure for synthesis of 5-substituted indole-2-carboxylate derivatives (5a-e, 16)

Taking **5a** as an example: To a solution of **4a** (220 mg, 0.53 mmol) in toluene (12 mL), $Pd(OAc)_2$ (12 mg, 0.053 mmol), (t-Bu)$_3$P.HBF$_4$ (31 mg, 0.11 mmol), K_3PO_4 (564 mg, 2.66 mmol) and propylboronic acid (140 mg, 1.60 mmol) were sequentially added. The reaction mixture was heated to 90 ℃ for 4 h under argon atmosphere, and then concentrated under reduced pressure. The residue was dissolved in EtOAc (30 mL), and washed with brine (20 mL × 3) and water (20 mL × 3). The crude product obtained after concentration was purified by silica gel column chromatography to afford **5a** as a yellow solid (157 mg, 63.6%).

1.1.2.1. Ethyl 3-(3-ethoxy-3-oxopropyl)-7-nitro-5-propyl-1*H*-indole-2-carboxylate (5a)

Starting from compound **4a** (220 mg, 0.53 mmol), compound **5a** was afforded as yellow solid (157 mg, 63.8%). m.p. 79-80 ℃; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 10.09 (s, 1H), 8.14 (s, 1H), 7.90 (s, 1H), 4.47 (q, J = 7.2 Hz, 2H), 4.09 (q, J = 7.2 Hz, 2H), 3.42 (t, J = 7.6 Hz, 2H), 2.78 (t, J = 7.6 Hz , 2H), 2.69 (t, J = 7.6 Hz , 2H), 1.74

(m, 2H), 1.45 (m, 3H), 1.20 (t, *J* = 7.2 Hz, 3H), 0.99 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): m/z, calcd.for $C_{19}H_{25}N_2O_6$ $[M+H]^+$: 377.1707, found 377.1697.

1.1.2.2. Ethyl 5-cyclopropyl-3-(3-ethoxy-3-oxopropyl)-7-nitro-1*H*-indole-2-carboxylate (5b)

Starting from compound **4a** (200 mg, 0.48 mmol), compound **5b** was afforded as a yellow solid (181.3 mg, 92.5%). m.p. 101-102℃; ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 10.78 (s, 1H), 7.99 (s, 1H), 7.98 (s, 1H), 4.37 (q, *J* = 7.0 Hz, 2H), 4.01 (q, *J* = 7.0 Hz, 2H), 3.31 (t, *J* = 7.5 Hz, 2H), 2.62 (t, *J* = 7.5 Hz, 2H), 2.15-2.20 (m, 1H), 1.37 (t, *J* = 7.5 Hz, 3H), 1.12 (t, *J* = 7.0 Hz, 3H), 1.01-1.05 (m, 2H), 0.80-0.84 (m, 2H); HRMS (ESI): m/z, calcd.for $C_{19}H_{23}N_2O_6$ $[M+H]^+$: 375.1551, found 375.1542.

1.1.2.3. Ethyl 7-chloro-3-(3-ethoxy-3-oxopropyl)-5-ethyl-1*H*-indole-2-carboxylate (5c)

Starting from compound **4b** (100 mg, 0.25 mmol), compound **5c** was afforded as a white solid (56 mg, 64.4%). m.p. 80-81 ℃; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.76 (s, 1H), 7.40 (s, 1H), 7.20 (s, 1H), 4.44 (q, *J* = 7.6 Hz, 2H), 4.11 (q, *J* = 7.6 Hz, 2H), 3.38 (t, *J* = 7.6 Hz, 2H), 2.73 (q, *J* = 7.6 Hz, 2H), 2.66 (t, *J* = 7.6 Hz, 2H), 1.44 (t, *J* = 7.2 Hz, 3H), 1.28 (t, *J* = 7.6 Hz, 3H), 1.21 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): m/z, calcd.for $C_{18}H_{23}ClNO_4$ $[M+H]^+$: 352.1310, found 352.1301.

1.1.2.4. Ethyl 7-chloro-3-(3-ethoxy-3-oxopropyl)-5-propyl-1*H*-indole-2-carboxylate (5d)

Starting from compound **4b** (150 mg, 0.37 mmol), compound **5d** was afforded as a yellow solid (162 mg, 89.5%). m.p. 68-69 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.76 (s, 1H), 7.38 (s, 1H), 7.18 (s, 1H), 4.44 (q, *J* = 7.2 Hz 2H), 4.11 (q, *J* = 6.8 Hz, 2H), 3.38 (t, *J* = 7.6 Hz, 2H), 2.64-2.68 (m, 4H), 1.64-1.70 (m, 2H), 1.44 (t, *J* = 6.8 Hz, 3H), 1.21 (t, *J* = 7.2 Hz, 3H), 0.96 (t, *J* = 7.2 Hz, 3H).

1.1.2.5. Ethyl 7-chloro-5-cyclopropyl-3-(3-ethoxy-3-oxopropyl)-1*H*-indole-2-carboxylate (5e)

Starting from compound **4b** (100 mg, 0.25 mmol), compound **5e** was afforded as a light yellow solid (60 mg, 66%). m.p. 84-85 ℃; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.74 (s, 1H), 7.34 (s, 1H), 7.07 (s, 1H), 4.43 (q, *J* = 7.2 Hz, 2H), 4.11 (q, *J* = 7.2 Hz,

2H), 3.36 (t, *J* = 8.0 Hz, 2H), 2.65 (t, *J* =8.0 Hz, 2H), 1.95-2.00 (m, 1H), 1.44 (t, *J* = 7.2 Hz, 3H), 1.22 (t, *J* = 7.2 Hz, 3H), 0.95-0.98 (m, 2H), 0.70-0.73 (m, 2H); HRMS (ESI): m/z, calcd. for $C_{19}H_{23}ClNO_4[M+H]^+$: 364.1237, found 364.1298

1.1.2.6. Ethyl 3-(3-ethoxy-3-oxopropyl)-5-ethyl-7-methyl-1*H*-indole-2-carboxylate (16)

Starting from compound **15** (100 mg, 0.25 mmol), compound **16** was afforded as a light yellow solid (50.6 mg, 55.5%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 8.56 (s, 1H), 7.34 (s, 1H), 6.99 (s, 1H), 4.43 (q, *J* = 7.0 Hz, 2H), 4.13 (q, *J* = 7.0 Hz, 2H), 3.39 (t, *J* = 7.5 Hz, 2H), 2.73 (q, *J* = 7.5 Hz, 2H), 2.66 (t, *J* = 8.0 Hz, 2H), 2.47 (s, 3H), 1.44 (t, *J* = 7.0 Hz, 3H), 1.28 (t, *J* = 7.0 Hz, 3H),1.22 (m, *J* = 7.0 Hz, 3H); HRMS (ESI): m/z, calcd for $C_{19}H_{26}NO_4$ $[M+H]^+$: 332.1856, found 332.1847.

1.1.3. General procedure for synthesis of indole-2-carboxylic acid derivatives (6a-i)

Taking **6a** as an example: A solution of NaOH (150 mg, 3.7 mmol) in water (2 mL) was added dropwise to a solution of compound **5a** (150 mg, 0.37 mmol) in THF (4 mL) and ethanol (2 mL). The reaction mixture was stirred at room temperature for overnight and concentrated under vacuum. The residue was dissolved in water (10 mL), which was acidified to pH = 3-4 with diluted HCl aqueous solution. After filtration, the title compound was obtained.

1.1.3.1. 3-(2-Carboxyethyl)-7-nitro-5-propyl-1*H*-indole-2-carboxylic acid (6a)

Starting from compound **5a** (150 mg, 0.37 mmol), compound **6a** was afforded as a white solid (118 mg, 83.1%). m.p. 261-262 ℃; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm):10.55 (s, 1H), 8.11 (d, *J* = 4.8 Hz, 2H), 3.29 (t, *J* = 7.6 Hz , 2H), 2.76 (t, *J* = 7.6 Hz , 2H), 2.56 (t, *J* = 7.6 Hz , 2H), 1.68(q, *J* = 7.6 Hz, 2H), 0.93(t, *J* = 7.2 Hz, 3H)；^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 173.75, 162.21, 133.99, 132.54, 131.31, 128.96, 126.97, 126.79, 122.59, 122.39, 36.49, 34.77, 24.44, 19.51, 13.49; HRMS (ESI): m/z, calcd. for $C_{15}H_{15}N_2O_6[M-H]^-$:319.1008, found 319.0685.

1.1.3.2. 3-(2-Carboxyethyl)-5-cyclopropyl-7-nitro-1*H*-indole-2-carboxylic acid (6b)

Starting from compound **5b** (97.2 mg, 0.26 mmol), compound **6b** was afforded as a

light yellow solid (68.6 mg, 83%). m.p. >300 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm)： 10.54 (s, 1H), 7.99 (s, 2H), 3.28 (t, *J* = 7.6 Hz , 2H), 2.55 (t, *J* = 7.6 Hz, 2H), 2.15-2.22 (m, 1H), 1.03 (m, 2H), 0.82 (m, 2H)； ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 173.81, 162.20, 135.59, 132.61, 131.38, 127.07, 126.64, 125.84, 122.56, 119.99, 34.79, 19.44, 14.71, 9.48; HRMS (ESI): m/z, calcd. for $C_{15}H_{13}N_2O_6$[M-H]$^-$:317.0852, found 317.0526.

1.1.3.3. 3-(2-Carboxyethyl)-7-chloro-5-ethyl-1*H*-indole-2-carboxylic acid (6c)

Starting from compound **5c** (120 mg, 0.32 mmol), compound **6c** was afforded as a light yellow solid (90 mg, 90.1%) m.p. 236-237 ℃; 1H-NMR (500 MHz, DMSO-d_6) *δ* (ppm): 11.42 (s, 1H), 7.47 (s, 1H), 7.21 (s, 1H), 3.23 (t, J = 6.0 Hz , 2H), 2.68 (q, J = 7.5 Hz , 2H), 2.48-2.50 (m, 2H), 1.22 (t, J = 7.5 Hz , 3H);^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.93, 162.82, 136.24, 131.67, 129.04, 125.80, 124.79, 122.12, 117.49, 116.33, 34.95, 27.98, 20.00, 16.12; HRMS (ESI): m/z, calcd. for $C_{14}H_{13}ClNO_4$[M-H]$^-$: 294.0368, found 294.0317.

1.1.3.4. 3-(2-Carboxyethyl)-7-chloro-5-propyl-1*H*-indole-2-carboxylic acid (6d)

Starting from compound **5d** (120 mg, 0.33 mmol), compound **6d** was afforded as a white solid (76.3 mg, 75.5%). m.p. 212-213 ℃; ^{1}H-NMR (400MHz, acetone-d_6) δ (ppm): 11.01 (s, 1H), 10.35 (s, 1H), 7.60 (s, 1H), 7.26 (s, 1H), 3.41 (t, *J* = 8.0 Hz, 2H), 2.70 (q, *J* = 7.6 Hz, 4H), 1.64-1.74 (m, 2H), 0.96 (t, *J* = 7.6 Hz, 3H)； ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 173.92, 162.82, 134.55, 131.69, 129.00, 125.76, 125.17, 122.08, 118.19, 116.24, 36.98, 34.93, 24.50, 19.99, 13.60; HRMS (ESI): m/z, calcd. for $C_{15}H_{15}ClNO_4$[M-H]$^-$: 308.0768, found 308.0469.

1.1.3.5. 3-(2-Carboxyethyl)-7-chloro-5-cyclopropyl-1*H*-indole-2-carboxylic acid (6e)

Starting from compound **5e** (90.7 mg, 0.25 mmol), compound **6e** was afforded as an off-white solid (61.7 mg, 80.4%). m.p. 238-239 ℃; ^{1}H-NMR (400 MHz, acetone-d_6) δ (ppm): 10.33 (s, 1H), 7.51 (s, 1H), 7.12 (s, 1H), 3.39 (t, *J* = 8.0 Hz, 2H), 2.67 (t, *J* = 7.6 Hz, 2H), 2.04-2.06 (m, 1H), 0.94-0.99 (m, 2H), 0.72-0.77 (m, 2H); ^{13}C -NMR (100 MHz, DMSO-d_6) δ (ppm): 173.98, 162.84, 135.97, 131.60, 129.01, 126.00, 122.56, 121.94, 116.46, 115.59, 34.97, 19.94, 15.00, 9.04; HRMS (ESI): m/z, calcd.

for $C_{15}H_{13}ClNO_4$[M-H]$^-$: 306.0611, found 306.0315.

1.1.3.6. 3-(2-Carboxyethyl)-7-chloro-5-bromo-1*H*-indole-2-carboxylic acid (6f)

Starting from compound **4b** (90 mg, 0.26 mmol), compound **6f** was afforded as a white solid (68 mg, 90%). m.p. 270-271 ℃; ^{1}H-NMR (400MHz, acetone-d_6) δ (ppm): 11.88 (s, 1H), 7.93 (s, 1H), 7.51 (s, 1H), 3.23 (t, *J* = 7.6 Hz, 2H), 2.51 (t, *J* = 7.6 Hz, 2H)； HRMS (ESI): m/z, calcd. for $C_{12}H_8BrClNO_4$[M-H]$^-$:344.9403, found 344.9010.

1.1.3.7. 7-Acetamido-3-(2-carboxyethyl)-5-ethyl-1*H*-indole-2-carboxylic acid (6g)

Starting from compound **9** (43 mg, 0.11 mmol), compound **6h** was afforded as a white solid (33.6 mg, 94.4%). m.p. 248-249 ℃; ^{1}H-NMR (500 MHz, acetone-d_6) δ (ppm): 10.54 (s, 1H), 9.39 (s, 1H), 7.36 (s, 1H), 7.34 (s, 1H), 3.38 (t, *J* = 7.5 Hz, 2H), 2.63-2.73 (m, 4H), 2.20 (s, 3H), 1.24 (t, *J* = 7.5 Hz, 3H); HRMS (ESI): m/z, calcd. for $C_{16}H_{17}N_2O_5$[M-H]$^-$: 317.1216, found 317.0892.

1.1.3.8. 7-Bromo-3-(2-carboxyethyl)-5-ethyl-1*H*-indole-2-carboxylic acid (6h)

Starting from compound **15** (30 mg, 0.11 mmol), compound **6i** was afforded as white solid (22.4 mg, 86.8%). m.p. 227-228 ℃; ^{1}H-NMR (500 MHz, acetone-d_6) δ (ppm): 10.06 (s, 1H), 7.64 (s, 1H), 7.42 (s, 1H), 3.40 (t, *J* = 7.5 Hz, 2H), 2.75 (q, *J* = 7.5 Hz, 2H), 2.68 (t, *J* = 7.5 Hz, 2H), 1.28 (t, *J* = 7.5 Hz, 3H); HRMS (ESI): m/z, calcd. for $C_{14}H_{13}BrNO_4$[M-H]$^-$:338.0106, found 337.9784.

1.1.3.9. 3-(2-Carboxyethyl)-5-ethyl-7-methyl-1*H*-indole-2-carboxylic acid (6i)

Starting from compound **16** (23 mg, 0.07 mmol), compound **6j** was afforded as a white solid (15.3 mg, 80.5%). m.p. 207-208 ℃; ^{1}H-NMR (500 MHz, acetone-d_6) δ (ppm): 10.13 (s, 1H), 7.41 (s, 1H), 6.99 (s, 1H), 3.40 (t, *J* = 7.8 Hz, 2H), 2.64-2.73 (m, 4H), 2.54 (s, 3H), 1.26 (t, *J* = 7.5 Hz, 3H); HRMS (ESI): m/z, calcd for $C_{15}H_{16}NO_4$ [M-H]$^-$: 274.1158, found 274.0871.

1.1.4. General procedure for synthesis of indole derivatives (21a-i)

Taking **21a** as an example, compound **20** (300 mg, 0.9 mmol) was dissolved in anhydrous dichloromethane (18 mL) at 0 ℃18 mL) Et_3N (455 mg, 4.5 mmol), HATU (616 mg, 1.62 mmol), DMAP (55 mg, 0.45 mmol), ammonium hydroxide (45.5 mg, 2.7 mmol) were added. The mixture was stirred at r.t. for 5 h. The crude product was purified by flash chromatography to obtain the light yellow product **21a**.

1.1.4.1. Ethyl 3-(3-amino-3-oxopropyl)-5-ethyl-7-nitro-1*H*-indole-2-carboxylate (21a)

Starting from compound **20** (300 mg, 0.9 mmol), compound **21a** was afforded as a light yellow solid (135.2 mg, 45.2%). m.p. 224-225 ℃; ^{1}H-NMR ($CDCl_3$) δ (ppm): 10.16 (s, 1H), 8.16 (d, *J* = 8.1 Hz, 2H), 4.42 (q, *J* = 7.6 Hz, 2H), 3.38 (t, *J* = 7.6 Hz, 2H), 2.86 (q, *J* = 7.6 Hz, 2H), 2.47 (t, *J* = 7.6 Hz, 2H), 1.41 (t, *J* = 7.6 Hz, 3H), 1.32 (t, *J* = 7.6 Hz, 3H).

1.1.4.2. Ethyl 5-ethyl-3-(3-(methylamino)-3-oxopropyl)-7-nitro-1*H*-indole-2-carboxylate (21b)

Starting from compound **20** (200 mg, 0.6 mmol), compound **21b** was afforded as a light yellow solid (69.8 mg, 33.6%). ^{1}H-NMR (500 MHz, acetone-d_6) δ (ppm): 10.28 (s, 1H), 8.16 (s, 1H), 8.15 (s, 1H), 4.44 (q, *J* = 7.0 Hz, 2H), 3.42 (t, *J* = 7.5 Hz, 2H), 2.88 (q, *J* = 7.5 Hz, 2H), 2.60 (s, 3H), 2.51 (t, *J* = 7.5 Hz, 2H), 1.43 (t, *J* = 7.0 Hz, 3H), 1.34 (t, *J* = 7.5 Hz, 3H); HRMS (ESI): m/z, calcd.for $C_{17}H_{22}N_3O_5$ $[M+H]^+$: 348.1554, found 348.1544.

1.1.4.3. Ethyl 5-ethyl-3-(3-(methylsulfonamido)-3-oxopropyl)-7-nitro-1*H*-indole-2-carboxylate (21c)

Starting from compound **20** (200 mg, 0.6 mmol), compound **21c** was afforded as a light yellow solid (89 mg, 72.4%). m.p. 231-232 ℃; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 10.10 (s, 1H), 8.46 (s, 1H), 8.19 (s, 1H), 7.89 (s, 1H), 4.49 (q, *J* = 7.0 Hz, 2H), 3.46 (t, *J* = 7.5 Hz, 2H), 3.21 (s, 3H), 2.85 (q, *J* = 7.5 Hz, 2H), 2.75 (t, *J* = 7.5 Hz, 2H), 1.47 (t, *J* = 7.0 Hz, 3H), 1.34 (t, *J* = 7.5 Hz, 3H); HRMS (ESI): m/z, calcd. for $C_{17}H_{22}N_3O_7S[M+H]^+$: 412.1173, found 412.1161.

1.1.4.4. Ethyl 5-ethyl-3-(3-(1-methylethylsulfonamido)-3-oxopropyl)-7-nitro-1*H*-indole-2-carboxylate (21d)

Starting from compound **20** (200 mg, 0.6 mmol), compound **21d** was afforded as a light yellow solid (65 mg, 50%). m.p. 176-177 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 10.10 (s, 1H), 8.18 (s, 2H), 7.90 (s, 1H), 4.49 (q, *J* = 7.0 Hz, 2H), 3.66-3.72 (m, 1H), 3.46 (t, *J* = 7.0 Hz, 2H), 2.85 (q, *J* = 7.5 Hz, 2H), 2.76 (t, *J* = 7.5 Hz, 2H), 1.47 (t, *J* = 7.0 Hz, 3H), 1.34 (t, *J* = 7.5 Hz, 3H), 1.30 (d, *J* = 7.0 Hz, 6H); HRMS (ESI): m/z,

calcd.for $C_{19}H_{26}N_3O_7S$ $[M+H]^+$:440.1486, found 440.1475.

1.1.4.5. Ethyl 3-(3-(cyclopropanesulfonamido)-3-oxopropyl)-5-ethyl-7-nitro-1*H*-indole-2-carboxylate (21e)

Starting from compound **20** (300 mg, 0.9 mmol), compound **21e** was afforded as a light yellow solid (158 mg, 80.6%). m.p. 131-132 ℃; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 10.10 (s, 1H), 8.18 (s, 1H), 7.91 (s, 1H), 4.49 (q, *J* = 7.0 Hz, 2H), 3.47 (t, *J* = 7.5 Hz, 2H), 2.85 (t, *J* = 7.5 Hz, 2H), 2.75 (t, *J* = 7.5 Hz, 2H), 2.56-2.58 (m, 1H), 1.47 (t, *J* = 7.5 Hz, 3H), 1.34 (t, *J* = 7.5 Hz, 3H), 0.96-1.05 (m, 4H); HRMS (ESI): m/z, calcd. for $C_{19}H_{24}N_3O_7S[M+H]^+$: 438.1330, found 438.1316.

1.1.4.6. Ethyl 5-ethyl-7-nitro-3-(3-oxo-3-(thiophene-2-sulfonamido)propyl)-1*H*-indole-2-carboxylate (21f)

Starting from compound **20** (200 mg, 0.6 mmol), compound **21f** was afforded as a light yellow solid (85 mg, 45.5%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 10.09 (s, 1H), 8.83 (s, 1H), 8.20 (s, 1H), 8.05 (s, 1H), 7.85 (d, *J* = 4.0 Hz, 1H), 7.66 (d, *J* = 5.0 Hz, 1H), 7.09 (t, *J* = 4.5 Hz, 1H), 4.47 (q, *J* = 7.0 Hz, 2H), 3.39 (t, *J* = 7.0 Hz, 2H), 2.82 (q, *J* = 7.5 Hz, 2H), 2.72 (t, *J* = 7.5 Hz, 2H), 1.45 (t, *J* = 7.5 Hz, 3H), 1.33 (t, *J* = 7.5 Hz, 3H); HRMS (ESI): m/z, calcd. for $C_{20}H_{22}N_3O_7S_2[M+H]^+$:480.0894, found 480.0877.

1.1.4.7. Ethyl 5-ethyl-7-nitro-3-(3-oxo-3-(phenylsulfonamido)propyl)-1*H*-indole-2-carboxylate (21g)

Starting from compound **20** (200 mg, 0.6 mmol), compound **21g** was afforded as a light yellow solid (104 mg, 73.4%). m.p.132-133 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 10.05 (s, 1H), 8.58 (s, 1H), 8.15 (s, 1H), 7.98 (d, *J* = 7.5 Hz, 2H), 7.81 (s, 1H), 7.60 (t, *J* = 7.5 Hz, 2H), 7.48 (t, *J* = 7.5 Hz, 1H), 4.47 (q, *J* = 7.5 Hz, 2H), 3.36 (t, *J* = 7.5 Hz, 2H), 2.81 (q, *J* = 7.5 Hz, 2H), 2.67 (t, *J* = 7.5 Hz, 2H), 1.44 (t, *J* = 7.5 Hz, 3H), 1.32 (t, *J* = 7.5 Hz, 3H); HRMS (ESI): m/z, calcd.for $C_{22}H_{24}N_3O_7S$ $[M+H]^+$: 474.1330, found 474.1317.

1.1.4.8. Ethyl 5-ethyl-3-(3-(4-methoxyphenylsulfonamido)-3-oxopropyl)-7-nitro-1*H*-indole-2-carboxylate (21h)

Starting from compound **20** (167 mg, 0.5 mmol), compound **21h** was afforded as a

light yellow solid (137.8 mg, 54.8%). m.p. 100-101 ℃; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 10.04 (s, 1H), 8.36 (s, 1H), 8.15 (s, 1H), 7.90 (d, *J* = 9.0 Hz, 2H), 7.81 (s, 1H), 6.91 (d, *J* = 9.0 Hz, 2H), 4.47 (q, *J* = 7.5 Hz, 2H), 3.87 (s, 3H), 3.35 (t, *J* = 7.5 Hz, 2H), 2.81 (q, *J* = 7.5 Hz, 2H), 2.65 (t, *J* = 7.5 Hz, 2H), 1.45 (t, *J* = 7.5 Hz, 3H), 1.32 (t, *J* = 7.5 Hz, 3H); HRMS (ESI): m/z, calcd. for $C_{23}H_{26}N_3O_8S[M+H]^+$: 504.1435, found 504.1422.

1.1.4.9. Ethyl 3-(3-(3-chlorophenylsulfonamido)-3-oxopropyl)-5-ethyl-7-nitro-1*H*-indole-2-carboxylate (21i)

Starting from compound **20** (167 mg, 0.5 mmol), compound **21i** was afforded as a light yellow solid (123.2 mg, 48.6%). ^{1}H NMR ($CDCl_3$) δ (ppm): 10.02 (s, 1H), 8.12(s, 1H), 8.04 (s, 1H), 7.78 (m, 2H), 7.52 (s, 1H), 7.64 (s, 1H), 4.45 (q, *J* = 7.6 Hz, 2H), 3.35 (t, *J* = 7.6 Hz, 2H), 2.81 (q, *J* = 7.6 Hz, 2H), 2.65 (t, *J* = 7.6 Hz, 2H), 1.45 (t, *J* = 7.6 Hz, 3H), 1.32 (t, *J* = 7.6 Hz, 3H); HRMS (ESI): m/z, calcd. for $C_{22}H_{23}ClN_3O_7S[M+H]^+$: 507.9440, found 507.9488.

1.1.5. General procedure for indole acid derivatives (22a-i)

Following the precedure of 4.1.3 and starting from compounds **21a-i**, compounds **22a-i** were prepared.

1.1.5.1. 3-(3-Amino-3-oxopropyl)-5-ethyl-7-nitro-1*H*-indole-2-carboxylic acid (22a)

Starting from compound **21a** (150 mg, 0.45 mmol), compound **22a** was afforded as a light yellow solid (102.1 mg, 74.3%). m.p. 255-256 ℃; ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 13.56 (s, 1H), 10.52 (s, 1H), 8.13 (s, 2H), 7.27 (s, 1H), 6.75 (s, 1H), 3.28 (t, *J* = 7.5 Hz, 2H), 2.81 (q, *J* = 7.5 Hz, 2H), 2.40 (t, *J* = 7.5 Hz, 2H), 1.28 (t, *J* = 7.5 Hz, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 173.52, 162.30, 135.49, 132.54, 131.48, 128.54, 126.88, 126.79, 123.30, 121.88, 36.17, 27.65, 19.91, 15.95; HRMS (ESI): m/z, calcd. for $C_{14}H_{14}N_3O_5[M-H]^-$: 304.1012, found 304.0703.

1.1.5.2. 5-Ethyl-3-(3-(methylamino)-3-oxopropyl)-7-nitro-1*H*-indole-2-carboxylic acid (22b)

Starting from compound **21b** (38 mg, 0.11 mmol), compound **22b** was afforded as a light yellow solid (23.3 mg, 66.5%). m.p.: 150-151 ℃; ^{1}H NMR (500 MHz,

DMSO-d_6) δ (ppm): 10.53 (s, 1H), 8.13 (s, 1H), 8.08 (s, 1H), 7.71 (s, 1H), 3.29(t, *J* = 7.5 Hz, 2H), 2.82(q, *J* = 8.0 Hz, 2H), 2.50 (s, 3H), 2.39 (t, *J* = 7.5 Hz, 2H), 1.27 (t, *J* = 7.5 Hz, 3H); HRMS (ESI): m/z, calcd. for $C_{15}H_{16}N_3O_5$[M-H]$^-$: 318.1168, found 318.0855.

1.1.5.3. 5-Ethyl-3-(3-(methylsulfonamido)-3-oxopropyl)-7-nitro-1*H*-indole-2-carboxylic acid (22c)

Starting from compound **21c** (60 mg, 0.15 mmol), compound **22c** was afforded as a light yellow solid (49.3 mg, 88.2%). m.p. 240-241 ℃; ^{1}H-NMR (500 MHz, acetone-d_6) δ (ppm): 10.28 (s, 1H), 8.18 (s, 2H), 3.49 (t, *J* = 7.5 Hz, 2H), 3.13 (s, 3H), 2.89 (q, *J* = 7.5 Hz, 2H), 2.84 (t, *J* = 7.5 Hz, 2H), 1.34 (t, *J* = 7.5 Hz, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.85, 162.25, 135.66, 132.62, 131.30, 128.35, 127.28, 126.72, 121.93, 40.90, 36.49, 27.66, 19.01, 15.91; HRMS (ESI): m/z, calcd. for $C_{15}H_{16}N_3O_7S$[M-H]$^-$: 382.0787, found 382.0421.

1.1.5.4. 5-Ethyl-3-(3-(1-methylethylsulfonamido)-3-oxopropyl)-7-nitro-1*H*-indole-2-carboxylic acid (22d)

Starting from compound **21d** (56 mg, 0.13 mmol), compound **22d** was afforded as a light yellow solid (38.1 mg, 72.6%). m.p. 209-210 ℃; ^{1}H-NMR (500 MHz, acetone-d_6) δ (ppm): 10.31 (s, 1H), 10.06 (s, 1H), 8.19 (d, *J* = 3.5 Hz, 2H), 3.54-3.60 (m, 1H), 3.49 (t, *J* = 7.5 Hz, 2H), 2.86-2.92 (m, 4H), 1.34 (t, *J* = 7.5 Hz, 3H), 1.15 (d, *J* = 7.0 Hz, 6H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.89, 162.18, 135.67, 132.62, 131.28, 128.48, 127.18, 126.75, 122.09, 122.01, 52.09, 36.47, 27.67, 19.18, 16.01, 15.21; HRMS (ESI): m/z, calcd. for $C_{17}H_{20}N_3O_7S$[M-H]$^-$: 410.1100, found 410.0721.

1.1.5.5. 3-(3-(Cyclopropanesulfonamido)-3-oxopropyl)-5-ethyl-7-nitro-1*H*-indole-2-carboxylic acid (22e)

Starting from compound **21e** (80 mg, 0.18 mmol), compound **22e** was afforded as a light yellow solid (65.9 mg, 88%). m.p. 181-182 ℃; ^{1}H-NMR (500 MHz, acetone-d_6) δ (ppm): 10.30 (s, 1H), 8.21 (s, 1H), 8.19 (s, 1H), 3.50 (t, *J* = 7.5 Hz, 2H), 2.85-2.91 (m, 5H), 1.34 (t, *J* = 7.5 Hz, 3H), 0.94-0.99 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6)

δ (ppm): 171.38, 162.19, 135.69, 132.62, 131.30, 128.47, 127.09, 126.77, 122.13, 121.98, 36.41, 30.61, 27.65, 19.04, 15.98, 5.31, 4.98; HRMS (ESI): m/z, calcd. for $C_{17}H_{18}N_3O_7S[M-H]^-$: 408.0944, found 408.0552.

1.1.5.6. 5-Ethyl-7-nitro-3-(3-oxo-3-(thiophene-2-sulfonamido)propyl)-1*H*-indole-2-carboxylic acid (22f)

Starting from compound **21f** (80 mg, 0.17 mmol), compound **22f** was afforded as a light yellow solid (63.6 mg, 84.4%). m.p. 218-219 ℃; ^{1}H-NMR (500 MHz, acetone-d_6) δ (ppm): 10.74 (s, 1H), 10.23 (s, 1H), 8.17 (s, 1H), 8.13 (s, 1H), 7.87 (d, *J* = 4.0 Hz, 1H), 7.74 (s, 1H), 7.12 (brs, 1H), 3.42 (t, *J* = 7.0 Hz, 2H), 2.87 (q, *J* = 7.5 Hz, 2H), 2.82 (t, *J* = 7.0 Hz, 2H), 1.33 (t, *J* = 7.5 Hz, 3H); HRMS (ESI): m/z, calcd. for $C_{18}H_{16}N_3O_7S_2[M-H]^-$: 450.0508, found 450.0065.

1.1.5.7. 5-Ethyl-7-nitro-3-(3-oxo-3-(phenylsulfonamido)propyl)-1*H*-indole-2-carboxylic acid (22g)

Starting from compound **21g** (80 mg, 0.17 mmol), compound **22g** was afforded as a light yellow solid (67.3 mg, 89.4%). m.p. 156-157 ℃; ^{1}H NMR (500 MHz, acetone-d_6) δ (ppm): 10.20 (s, 1H), 8.10 (s, 1H), 8.04 (s, 1H), 7.93 (d, *J* = 7.5 Hz, 2H), 7.61 (brs, 1H), 7.50 (t, *J* = 6.5 Hz, 2H), 3.39 (t, *J* = 7.0 Hz, 2H), 2.84 (q, *J* = 7.0 Hz, 2H), 2.80 (t, *J* = 7.5 Hz, 2H),, 1.31 (t, *J* = 7.0 Hz, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.71, 162.47, 140.29, 135.44, 132.95, 132.44, 131.44, 128.77, 128.35, 127.22, 126.49, 121.52, 121.26, 36.94, 27.64, 18.97, 15.96; HRMS (ESI): m/z, calcd. for $C_{20}H_{18}N_3O_7S[M-H]^-$: 444.0944, found 444.0535.

1.1.5.8.5-Ethyl-3-(3-(4-methoxyphenylsulfonamido)-3-oxopropyl)-7-nitro-1*H*-indole-2-carboxylic acid (22h)

Starting from compound **21h** (40 mg, 0.08 mmol), compound **22h** was afforded as a light yellow solid (32 mg, 84.7%). m.p. 132-133 ℃; ^{1}H-NMR (500 MHz, acetone-d_6) δ (ppm): 8.13 (s, 1H), 8.06 (s, 1H), 7.82 (d, *J* = 8.5 Hz, 2H), 6.98 (d, *J* = 9.0 Hz, 2H), 3.89 (s, 3H), 3.38 (t, *J* = 7.5 Hz, 2H), 2.86 (q, *J* = 7.5 Hz, 2H), 2.78 (t, *J* = 7.5 Hz, 2H), 1.32 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 171.92, 162.81, 162.37, 135.44, 132.43, 131.38, 131.13, 129.66, 128.44, 127.97, 126.51, 121.57, 121.31, 113.99, 55.68, 36.42, 27.63, 18.42, 15.94; HRMS (ESI): m/z, calcd. for

$C_{21}H_{22}N_3O_8S[M+H]^+$:476.1122, found 476.1114.

1.1.5.9. 3-(3-(3-Chlorophenylsulfonamido)-3-oxopropyl)-5-ethyl-7-nitro-1*H*-indole-2-carboxylic acid (22i)

Starting from compound **21i** (78 mg, 0.15 mmol), compound **22i** was afforded as a light yellow solid (52.2 mg, 72.7%). ^{1}H-NMR (500 MHz, acetone-d_6) δ (ppm): 10.21 (s, 1H), 8.12 (s, 1H), 8.04 (s, 1H), 7.83-7.87 (m, 2H), 7.63-7.65 (m, 1H), 7.53-7.55 (m, 1H), 3.29 (s, 2H), 2.84 (s, 4H), 1.31 (s, 3H); HRMS (ESI): m/z, calcd. for $C_{20}H_{19}ClN_3O_7S[M+H]^+$: 479.8908, found 479.9026.

1.1.6. Synthesis of diethyl 2-((5-bromo-2-(ethoxycarbonyl)-7-nitrobenzofuran-3-yl)methyl)malonate(27)

To a suspension of sodium hydride (21 mg, 0.885 mmol) in dry DMF (0.5 mL) was slowly added diethyl malonate (142 mg，0.885 mmol) in THF (1.5 mL). After stirring for 1 h, **26** (253 mg, 0.737 mmol) in DMF (1.5 mL) and THF (4.5 mL) was added, The mixture was heated at 70 ℃ for 2 h. After cooling, water (20 mL) and ethyl acetate (15 mL) were added. The organic layer was separated, washed twice with water and brine, dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified by silica gel chromatography to afford **27** (162 mg, 53.6%) as a white powder. m.p. 72-73 ℃, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.39 (s, 1H), 8.24 (s, 1H), 4.50 (q, *J* = 7.2 Hz, 2H), 4.12-4.16 (m, 4H), 3.91 (t, *J* = 7.6 Hz, 1H), 3.56 (d, *J* = 7.6 Hz, 2H), 1.45 (t, *J* = 7.2 Hz, 3H), 1.20 (t, *J* = 7.2 Hz, 6H); HRMS (ESI): m/z, calcd. for $C_{19}H_{21}BrNO_9[M+H]^+$: 486.0321, found 486.0363.

1.1.7. General procedure for synthesis of benzofuran derivatives (28a-b)

To a solution of compound **27** (325 mg, 0.67 mmol) in toluene (25 mL), $Pd(OAc)_2$ (15 mg, 0.067 mmol), *t*-$Bu_3P.HBF_4$ (39 mg, 0.134 mmol), ethyl boric acid (148 mg, 2 mmol), and potasium phosphate (578 mg, 2.72 mmol) in water (3 mL) were sequentially added. The reaction mixture was strirred at 90 ℃ under the Ar atomosphere for 10 h. After comcentration under reduced pressure, the residue was dissolved in EtOAc (30 mL), and washed with brine (20 mL x 3) and water (20 mL x 3). The crude product obtained after concentration was purified by silica gel column chromatography to give compound **28a** as a white solid (50 mg, 17.2%), and **28b** as a

white solid (85 mg, 28.9%). Compound **28a**: ^{1}HNMR ($CDCl_3$) δ (ppm): 8.14 (s, 1H), 7.90 (s, 1H), 4.49 (q, *J* = 7.2 Hz, 2H), 4.10 (q, *J* = 7.2 Hz, 4H), 3.94 (t, *J* = 7.6 Hz, 1H), 3.61 (d, *J* = 7.6 Hz, 2H), 2.85 (q, *J* = 7.2 Hz, 2H), 1.47 (t, *J* = 7.2 Hz, 3H), 1.35 (t, *J* = 7.6 Hz, 3H), 1.26 (t, *J* = 6.8 Hz, 3H), 1.16 (t, *J* = 6.8 Hz, 3H); HRMS (ESI): m/z, calcd.for $C_{21}H_{26}NO_9$ $[M+H]^+$: 436.1602, found 436.1592; Compound **28b**: ^{1}H NMR ($CDCl_3$) δ (ppm): 8.30 (d, *J* = 8.0 Hz, 1H), 8.12 (d, *J* = 8.0 Hz, 1H), 7.47 (t, *J* = 8.0 Hz, 1H), 4.50 (q, *J* = 7.2 Hz, 2H), 4.11 (q, *J* = 7.2 Hz, 4H), 3.94 (t, *J* = 8.0 Hz, 1H), 3.62 (d, *J* = 7.6 Hz, 2H), 1.47 (t, *J* = 6.8 Hz, 3H), 1.17 (t, *J* = 7.2 Hz, 6H); HRMS (ESI): m/z, calcd.for $C_{19}H_{22}NO_9$ $[M+H]^+$: 408.1289, found 408.1276.

1.1.8. Synthesis of benzofuran derivatives (29a-c)

Compound **27** (150 mg, 0.1 mmol) dissolved in HCl (7.5 mL), HOAc(30 mL) and H_2O(30 mL), The mixture was heated at 100 ℃ for 10 h. After cooling, the reaction mixture was diluted with ethyl acetate. The organic layer was seperated, dried over sodium sulfate and evaporated under reduced pressure. Compound **29c** was obtained without further purification as a white powder (73 mg, 66.4%); m.p. > 300 ℃, ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.63 (s, 1H), 8.42 (s, 1H), 3.27 (t, *J* = 7.2 Hz, 2H), 2.61 (t, *J* = 7.2 Hz, 2H); HRMS (ESI): m/z, calcd for $C_{12}H_7BrNO_7$ $[M-H]^-$: 355.9484, found 355.9070.

1.1.8.1. 3-(2-Carboxyethyl)-5-ethyl-7-nitrobenzofuran-2-carboxylic acid (29a)

Following the procedure of 4.1.13, starting from compound **28a** (48 mg，0.11 mmol), **29a** was afforded as a light yellow solid (24 mg, 70.8%). m.p. > 300 ℃, ^{1}HNMR (400 MHz, acetone-d_6) δ (ppm): 8.23 (s, 1H), 8.22 (s, 1H), 3.43 (t, *J* = 7.2 Hz, 2H), 2.93 (q, *J* = 7.6 Hz, 2H), 2.78 (t, *J* = 7.2 Hz, 2H), 1.35 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): m/z, calcd for $C_{14}H_{12}NO_7$$[M-H]^-$: 306.0962, found 306.0369.

1.1.8.2. 3-(2-Carboxyethyl)-7-nitrobenzofuran-2-carboxylic acid (29b)

Following the procedure of 4.1.13, starting from compound **28b** (68 mg, 0.167 mmol), **29b** was afforded as a light yellow solid (24 mg, 51.6%). m.p. 239-240 ℃, ^{1}HNMR (400 MHz, DMSO-d_6) δ (ppm): 8.34 (d, *J* = 4.4 Hz, 2H), 7.57 (t, *J* = 6.0 Hz, 1H), 3.29 (t, *J* = 6.0 Hz, 2H), 2.62 (t, *J* = 6.0 Hz, 2H); HRMS (ESI): m/z, calcd for $C_{12}H_8NO_7$ $[M-H]^-$:278.0379, found 278.0081.

1.2. The enzymatic assay for evaluation of FBPase activity

The expression and purification of the human liver FBPase, and the measurement of inhibitory activity for the target molecules were conducted according to the protocol in our previous work [1,2].

1.3. X-ray Crystallization Experiment

1.3.1. FBPase Production for Crystallization

The crystallization method was similar to previously publication [3], FBPase gene (UniprotKB entry P09467) was inserted into a pET-28a vector and expressed in *E. coli* BL21 (DE3). Before purification, the IPTG induced culture resuspension was lysed with ultrasonication and the cell lysis was centrifugated at 12,000 rpm for 1 h to harvest supernatant. The prepared crude supernatant was then purified steps-wisely by Ni^{+} affinity (HisTrap HP, GE healthcare), anion exchange (Hitrap Q HP, GE healthcare) and gel filtration (Superdex G75 increase, GE healthcare). The FBPase was finally eluted in stock buffer (20 mM KH_2PO_3−KOH, 20% v/v glycerol, 1 mM di-threitol, 0.1 mM EDTA, pH 7.5) and stored at −80 °C.

Compounds were dissolved at 100 mM in DMSO, and each compound along with fructose-1,6-diphosphate was diluted with 2 × stock buffer which was further adjusted to 1 × stock buffer containing 1 mM compound and 2 mM fructose-1,6-diphosphate. Before incubating on ice for 1 h, the compound and fructose-1,6-diphosphate dissolved stock buffer was mixed with FBPase containing stock buffer in a 1:1 ratio. Then, protein concentration of the mixed solution was concentrated to 25 mg/mL using an Amicon Ultra-4 10K Centrifugal Filter Device (Millipore). 2 μL of 25 mg/mL protein solution and 1 μL of precipitant solution (200 mM ammonium acetate, 20% w/v polyethylene glycol 3350, 100 mM HEPES, pH 7.0) were mixed, and the crystal appeared at 18 ℃ through the hanging drop vapor diffusion method as previous publication mentioned.

1.3.2. Crystal Data Collection and Analysis.

The cryo protectant buffer consists of 70% (v/v) reservoir solution and 30% (v/v) glycerol. FBPase crystals were separately soaked in the cryo protectant buffer, then frozen in liquid nitrogen and diffracted at the Shanghai Synchrotron Research Facility

(SSRF) using the beam-line BL19U1. Raw data were indexed and integrated with XDS [4] or iMosflm [5]. The processed data were then scaled with AIMLESS and phasing by molecular replacement with 2FIE as a search model. After refining by Refmac5, Coot [6], and Phenix.refine [7] with ligand restrains, the final coordinate of the structure was deposited to the Protein Data Bank. PDB structure figures were generated with PyMol-open-source [8].

1.4. Chemoinfomatics section

The protein–ligand interaction fingerprint was calculated with Open Drug Discovery Toolkit (ODDT) package [9], and protein–ligand extended connectivity (PLEC) interaction fingerprint was used. To handle the PLEC fingerprint vectors with different lengths, each fingerprint was padded with zero at the end so that tanimoto factor could be calculated to evaluate similarity. Cluster heat map was generated with Seaborn package [10].

1.5. References

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2. Experimental procedures and spectra data for intermediate compounds

General procedure for synthesis of phenylhydrazones (2a-b, 13, 19)

Taking **2b** as an example: A solution of 2-chloro-4-bromoaniline (3 g, 14.5 mmol) in 29 mL hydrochloric acid (1.5 mol/L) was cooled to 0 ℃. A solution of $NaNO_2$ (1.1 g, 15.8 mmol) in water (7.2 mL) was then added to the above solution slowly so as to the reaction temperature was maintained below 0 ℃. After the addition was complete, a solution of sodium acetate (11 g, 81 mmol) in water (18 mL) was added. Then ethyl 2-oxocyclopentanecarboxylate (2.6 mL, 17.4 mmol) was added and stirred vigorously for 15 minutes at 0 ℃ and 60 minutes at room temperature. The mixture was extracted with ethyl acetate (30 mL × 2). The combined organic layers were concentrated in vacuo and the crude material was added to a solution of boiling Na_2CO_3 (1.6 g) in water (24 mL). The solution was refluxed for 10 minutes and cooled to room temperature, and the pH was adjusted to 2 with 6 mol/L HCl aqueous solution. The yellow solid was precipitated and recrystallized from ethyl ether to give the title compound (5 g, 88%).

5-(2-(4-Bromo-2-nitrophenyl)hydrazono)-6-ethoxy-6-oxohexanoic acid (2a)

Starting from 4-bromo-2-nitroaniline (6.5 g, 30 mmol), compound **2a** was afforded as a yellow solid (1.8 g, 15%). mp: 159-161 ℃; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 8.34 (s, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 4.40 (q, *J* = 7.2 Hz, 2H), 2.68 (t, *J* = 8.0 Hz, 2H), 2.47 (t, *J* = 8.0 Hz, 2H), 2.02 (m, 2H), 1.40 (t, *J* = 7.2 Hz, 3H). HRMS (ESI): *m/z*, calcd. for $C_{14}H_{17}N_3O_6Br$ $[M+H]^+$: 402.0295, found 402.0289.

5-(2-(4-Bromo-2-chlorophenyl)hydrazono)-6-ethoxy-6-oxohexanoic acid (2b)

Starting from 2-chloro-4-bromoaniline (3 g, 14.5 mmol), compound **2b** was afforded as a yellow solid (5 g, 88%); mp: 135-136℃; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 12.38 (s, 1H), 7.47 (t, *J* = 7.6 Hz, 2H), 7.32 (d, *J* = 7.6 Hz, 1H), 4.32 (q, *J* = 7.6 Hz, 2H), 2.61 (t, *J* = 7.6 Hz, 2H), 2.46 (t, *J* = 7.6 Hz, 2H), 2.00 (m, 2H), 1.38 (t, *J* = 7.6 Hz, 3H). HRMS (ESI): *m/z*, calcd. for $C_{14}H_{17}BrClN_2O_4$[M+H]$^+$: 390.9982, found 390.9556.

(Z)-5-(2-(2-Bromo-4-ethylphenyl)hydrazono)-6-ethoxy-6-oxohexanoic acid (13)

Starting from **12** (944 mg, 4.72 mmol), compound **13** was afforded as colorless oil(443.5 mg, 24.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 12.34 (s, 1H), 7.50 (d, *J*

= 8.4 Hz, 1H), 7.30 (d, *J* = 1.6 Hz, 1H), 7.11 (dd, J_1= 8.4 Hz, J_2= 1.6 Hz, 1H), 4.32 (q, *J* = 7.6 Hz, 2H), 2.56-2.62 (m, 4H), 2.46 (t, *J* = 7.6 Hz, 2H), 1.99 (quint, *J* = 7.6 Hz, 2H), 1.37 (t, *J* = 7.2 Hz, 3H), 1.20 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): m/z, calcd. for $C_{16}H_{20}BrN_2O_4[M\text{-}H]^-$: 383.0685, found 383.0267

(Z)-6-Ethoxy-5-(2-(4-ethyl-2-nitrophenyl)hydrazono)-6-oxohexanoic acid (19)

Starting from **18** (4.9 g, 29.5 mmol), compound **19** was afforded as yellow solid (6.67 g, 64.4%). mp: 104-105℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 11.11(s, 1H), 7.99 (s, 1H), 7.94 (d, *J* = 9.0 Hz, 1H), 7.46 (d, *J* = 8.0 Hz, 1H), 4.36 (q, *J* = 7.0 Hz, 2H), 2.77 (t, *J* = 8.0 Hz, 2H), 2.66 (q, *J* = 7.5 Hz, 2H), 2.50 (t, *J* = 7.5 Hz, 2H), 1.88-2.03 (m, 2H), 1.39 (t, *J* = 7.0 Hz, 3H), 1.25 (t, *J* = 7.5 Hz, 3H); HRMS (ESI): m/z, calcd. for $C_{16}H_{20}N_3O_6[M\text{-}H]^-$: 350.1430, found 350.1117

General procedure for synthesis of diethyl derivatives (3a-b, 14)

Taking **3b** as an example: To a solution of **2b** (1.66 g, 4.24 mmol) in ethanol (20 mL), concentrated H_2SO_4 (0.1 mL) was added dropwise. The reaction mixture was heated to reflux for 2 hours, and then poured into the ice-water. The mixture was filtered to afford the crude product, which was dried and recrystallized from ethyl acetate to give the desired product **3b** (1.74 g, 95%) as yellow solid.

Diethyl 2-(2-(4-bromo-2-nitrophenyl)hydrazono)hexanedioate (3a)

Starting from compound **2a** (1.70 g, 3.95 mmol), compound **3a** was afforded as light yellow solid (1.57 g, 92%). mp: 80-82 ℃; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 13.91 (s, 1H), 8.34 (s, 1H), 7.90 (d, *J* = 8.4 Hz, 1H), 7.61-7.65 (m, 1H), 4.39 (q, *J* = 6.9 Hz, 2H), 4.41 (q, *J* = 7.2 Hz, 2H), 2.65 (t, *J* = 7.2 Hz, 2H), 2.41 (t, *J* = 7.2 Hz, 2H), 2.01 (m, 2H), 1.40 (t, *J* = 7.2 Hz, 3H), 1.25 (t, *J* = 7.2 Hz, 3H).

Diethyl 2-(2-(4-bromo-2-chlorophenyl)hydrazono)hexanedioate (3b)

Starting from compound **2b** (1.66 g, 4.24 mmol), compound **3b** was afforded as white solid (1.74 g, 95%). mp: 49-50℃; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 12.37 (s,1H), 7.48 (t, *J* = 7.6 Hz, 1H), 7.33 (d, *J* = 7.6 Hz, 2H), 4.32 (q, *J* = 7.6 Hz, 2H), 4.12 (q, *J* = 7.6 Hz, 2H), 2.58 (t, *J* = 7.6 Hz, 2H), 2.39 (t, *J* = 7.6 Hz, 2H), 1.97 (m, 2H), 1.37 (t, *J* = 7.6 Hz, 3H), 1.24 (t, *J* = 7.6 Hz, 3H).

Ethyl 3-(3-ethoxy-3-oxopropyl)-5-ethyl-7-nitro-1*H*-indole-2-carboxylate (14)

Starting from compound **13** (250 mg, 0.75 mmol), compound **14** was afforded as white solid (133.9 mg, 49.4%). m.p.: 95-96℃; ^{1}H NMR (400 $CDCl_3$) δ (ppm): 12.33(s, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.30 (d, *J* = 1.2 Hz, 1H), 7.24-7.25 7.10 (dd, J_1 = 8.4 Hz, J_2 = 1.2 Hz, 1H), 4.32 (q, *J* = 6.8 Hz, 2H), 4.13 (q, *J* = 7.2 Hz, 2H), 2.53-2.58 (m, 4H), 2.39 (t, *J* = 7.2 Hz, 2H), 1.97 (t, *J* = 7.2 Hz, 2H), 1.39 (t, *J* = 7.2 Hz, 3H), 1.19-1.24 (m, 6H); HRMS (ESI): m/z, calcd. for $C_{18}H_{26}BrN_2O_4[M+H]^+$: 413.1071, found 413.1047.

General procedure for synthesis of indole-2-carboxylate derivatives (4a-b, 15)

Taking **4b** as an example: To a solution of **3b** (4.57 g, 10.89 mmol) in toluene (80 mL), *p*-toluenesulfonic acid (3.11 g, 16.34 mmol) was added. The resulting mixture was heated to reflux for 8 h and then concentrated under vacuum. The residue was dissolved in ethyl acetate (120 mL), washed with saturated $NaHCO_3$ aqueous solution (60 mL×2) and dried over anhydrous $MgSO_4$. The crude product obtained after concentration was purified by column chromatography to afford **4b** (2.02 g, 48.2%) as white solid.

Ethyl 5-bromo-3-(3-ethoxy-3-oxopropyl)-7-nitro-1*H*-indole-2-carboxylate (4a)

Starting from compound **3a** (1.47 g, 3.4 mmol), compound **4a** was afforded as an off-white solid (690 mg, 49%). m.p. 120-121℃; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 10.18(s, 1H), 8.40(s, 1H), 8.24(s, 1H), 4.48(q, *J* = 7.6 Hz, 2H), 4.10(q, *J* = 7.6 Hz, 2H), 3.39(t, *J* = 7.6 Hz, 2H), 2.69(t, *J* = 7.6 Hz, 2H), 1.46(t, *J* = 7.6 Hz, 3H), 1.22(t, *J* = 7.6 Hz, 3H); HRMS (ESI): m/z, calcd. for $C_{16}H_{18}N_2O_6Br[M+H]^+$: 413.0343, found 413.0338

Ethyl 5-bromo-7-chloro-3-(3-ethoxy-3-oxopropyl)-1*H*-indole-2-carboxylate (4b)

Starting from compound **3b** (4.57 g, 10.89 mmol), compound **4b** was afforded as a white solid (2.02 g, 48.2%). m.p. 128-129℃; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm)：8.88 (s, 1H), 7.77 (s, 1H), 7.45 (s, 1H), 4.45 (q, *J* =6.8 Hz, 2H), 4.10 (q, *J* =6.8 Hz, 2H), 3.34 (t, *J* = 7.6 Hz, 2H), 2.65 (t, *J* =8.0 Hz, 2H), 1.44 (t, *J* = 7.2 Hz, 3H), 1.23 (t, *J* = 6.8 Hz, 3H).

Ethyl 7-bromo-3-(3-ethoxy-3-oxopropyl)-5-ethyl-1*H*-indole-2-carboxylate (15)

Starting from compound **14** (180 mg, 0.44 mmol), compound **15** was afforded as a light yellow solid (95.7 mg, 55.5%). m.p. 78-79℃; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 8.71 (s, 1H), 7.44 (s, 1H), 7.35 (s, 1H), 4.44 (q, *J* = 7.5 Hz, 2H), 4.11 (q, *J* = 7.5 Hz, 2H), 3.37 (t, *J* = 7.5 Hz, 2H), 2.73 (q, *J* = 7.5 Hz, 2H), 2.66 (t, *J* = 7.8 Hz, 2H), 1.44 (t, *J* = 7.5 Hz, 3H) 1.28 (t, *J* = 7.5 Hz, 3H), 1.21 (t, *J* = 7.0 Hz, 3H); HRMS (ESI): m/z, calcd. for $C_{18}H_{23}BrNO_4[M+H]^+$: 396.0805, found 396.0787.

Ethyl 7-amino-3-(3-ethoxy-3-oxopropyl)-5-ethyl-1H-indole-2-carboxylate (8)

To a solution of iron powder (185 mg, 3.3 mmol) in acetic acid (5 mL), compound **7** (124 mg, 0.33 mmol) was added after 0.5 h. The reaction mixture was heated to 40℃ for 1 h, and then ice water (20 mL) and ethyl acetate (20 ml) were added. The mixture was filtered and the filtrate was extracted with EtOAc (30 mL x 3). The organic layer was washed with brine (20 mL x 3) and water (20 mL x 3). The crude product obtained after concentration was purified by silica gel column chromatography to afford **8** as a light pink solid (99.4 mg, 87.4%). m.p. 99-100℃; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 8.78 (s, 1H), 6.99 (s, 1H), 6.55 (s, 1H), 4.42 (q, *J* = 7.0 Hz, 2H), 4.13 (q, *J* = 7.5 Hz, 2H), 3.79 (br, 2H), 3.37 (t, *J* = 8.0 Hz, 2H), 2.63-2.69 (m, 4H), 1.43 (t, *J* = 7.5 Hz, 3H), 1.21-1.28 (m, 6H); HRMS (ESI): m/z, calcd. for $C_{18}H_{25}N_2O_4[M+H]^+$:333.1809, found 333.1795.

Ethyl 7-acetamido-3-(3-ethoxy-3-oxopropyl)-5-ethyl-1*H*-indole-2-carboxylate (9)

To a solution of compound **8** in EtOAc, anhydrous pyridine was added and then acetic anhydride was added dropwise. The reaction mixture was stirred at room temperature for 3.5 h, and concentrated under vacuum. The crude product was recrystallized with ether to afford **9** as a solid (64.4%).^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 9.96 (s, 1H), 7.44 (s, 1H), 7.34 (s, 1H), 6.79 (s, 1H), 4.41 (q, *J* = 7.0 Hz, 2H), 4.12 (q, *J* = 7.0 Hz, 2H), 3.39 (t, *J* = 7.5 Hz, 2H), 2.64-2.73 (m, 4H), 2.28 (s, 3H), 1.42 (t, *J* = 7.0 Hz, 3H), 1.26 (t, *J* = 7.5 Hz, 3H), 1.22 (t, *J* = 7.5 Hz, 3H); HRMS (ESI): m/z, calcd. for $C_{20}H_{27}N_2O_5[M+H]^+$: 375.1915, found 375.1912.

General procedure for synthesis of 4-ethyl acetanilide (10)

To a solution of 4-ethyl-aniline (**6g**, 24.8 mmol) in ethyl acetate(30 mL), anhydrous pyridine(4.4 mL) was added, under stirring, acetic anhydride(5.2 mL) was added

dropwise. The rection mixture was stirred at room temperature for 3 hours, e vaporated under reduced pressure, recrystalized with diethyl ether, compound **10** was afforded as white solid (4.58 g, 65.5%). m.p.:94℃-95℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.39 (d, *J* = 9.0 Hz, 2H), 7.14 (d, *J* = 9.0 Hz, 2H), 2.61 (q, *J* = 7.5 Hz, 2H), 2.15 (s, 3H), 1.21 (t, *J* = 7.5 Hz, 3H)

General procedure for synthesis of 2-substituted-4-ethyl acetanilide (11, 17)

***N*-(4-Ethyl-2-chlorophenyl)acetamide (11)**

Compound **10** (4 g, 24.5 mmol) was slowly dissolved in 1 M bromine in acetic acetate (32 mL) at -5 ℃, the mixture was stirred at 45 ℃ for 2 h. The mixture was poured into H_2O (120 mL), filtered the precipitate and the solid was purified with column chromatography to give the title compound (2.7 g, 92.3%); m.p. 93℃-94℃. ^{1}H NMR ($CDCl_3$) δ (ppm): 8.19 (d, *J* = 7.6 Hz, 1H), 7.51 (s, 1H), 7.37 (s, 1H), 7.14 (dd, *J* = 7.6 Hz, 1H), 2.59 (q, *J* = 7.6 Hz, 2H), 2.22 (s, 3H), 1.21 (t, *J* = 7.6 Hz, 3H); HRMS (ESI): m/z, calcd. for $C_{10}H_{13}BrNO[M+H]^+$: 242.0175, found 242.0169.

***N*-(4-Ethyl-2-nitrophenyl)acetamide (17)**

Compound **10** (5.08 g, 31.1 mmol) was dissolved in acetic acid (5.5 mL) and acetic anhydride (12.5 mL) at -5 ℃, HNO_3(2.5 mL) was dropwise added under vigorous stirring. After complete addition, the mixture was stirred at ambient temperature for 1.5 h. The mixture was poured into H_2O (50 mL) and the mixture was stirred for 0.5 h, filtered the precipitate, the solid was recrystallized with ester acetate to give the title compound (6 g, 93.1%); m.p. 50℃-51℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 10.21 (s, 1H), 8.64 (d, *J* = 6.8 Hz, 1H), 8.02 (s, 1H), 7.49 (d, *J* = 6.8 Hz, 1H), 2.70 (q, *J* = 6.0 Hz, 2H), 2.31 (s, 3H), 1.27 (t, *J* = 6.0 Hz, 3H); HRMS (ESI): m/z, calcd. for $C_{10}H_{13}N_2O_3[M+H]^+$: 209.0921, found 209.0908.

Synthesis of 2-substituted-4-ethyl aniline (12, 18)

2-Bromine-4-ethyl aniline (**12**)

Starting from compound **11** (2.48 g, 10.24 mmol), the title compound **12** was obtained as a white solid (1.80 g, 87.2%). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.93 (s, 1H), 7.23 (d, *J* = 8.1 Hz, 1H), 6.74 (d, *J* = 8.1 Hz, 1H), 5.93 (s, 2H), 2.58 (q, *J* = 7.6 Hz, 2H), 1.22 (t, *J* = 7.6 Hz, 3H); HRMS (ESI): m/z, calcd. for $C_8H_{11}BrN[M+H]^+$:200.0069, found

200.0066.

2-Nitro-4-ethyl aniline (**18**)

To a solution of **17** (1.57 g, 7.54 mmol) in ethanol (36 mL), a solution of KOH (973 mg, 17.34 mmol) in water (6 mL) was added dropwise. The resulting mixture was heated to 80 ℃ for 5 h, and then concentrated under reduced pressure to give the yellow solid (1.33 g, 99%), m.p. 46℃-47℃; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.93 (s, 1H), 7.23 (d, *J* = 8.1 Hz, 1H), 6.74 (d, *J* = 8.1 Hz, 1H), 5.93 (s, 2H), 2.58 (q, *J* = 7.6 Hz, 2H), 1.22 (t, *J* = 7.6 Hz, 3H); HRMS (ESI): m/z, calcd. for $C_8H_{11}N_2O_2[M+H]^+$: 167.0815, found 167.0811.

3-(2-(Ethoxycarbonyl)-5-ethyl-7-nitro-1*H*-indol-3-yl)propanoic acid (20)

The reaction mixture of compound **19** (6.3 g, 17.9 mmol) in PPA (12.6 g) was heated at 80℃ for 4 h. The reaction mixture was cooled to room temperature and water (30 mL) was added to the mixture to destroy the PPA. The resulting solution was extracted with EtOAc (20 mL×2). The crude product obtained after concentration was purified by column chromatography to afford the title compound **20** (3.85 g, 64.2%) as a yellow solid. m.p.157-158℃, ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 10.11 (s, 1H), 8.17 (s, 1H), 7.91 (s, 1H), 4.47 (q, *J* = 7.0 Hz, 2H), 3.43 (t, *J* = 7.5 Hz, 2H), 2.84 (q, *J* = 7.5 Hz, 2H), 2.76 (t, *J* = 7.5 Hz, 2H), 1.46 (t, *J* = 7.0 Hz, 3H), 1.34 (t, *J* = 7.5 Hz, 3H).

Synthesis of ethyl 2-(2-acetyl-4-bromo-6-nitrophenoxy)acetate (24)

To a stirred solution of 1-(5-bromo-2-hydroxy-3-nitrophenyl)ethan-1-one (1.22 g, 4.7 mmol) in acetone (25 mL) and DMF (6 mL) were added potassium carbonate (1.3 g, 9.4 mmol) and ethyl bromoacetate (3 mL, 14 mmol). The reaction mixture was heated to 60℃ for 5 h. After cooling , the reation mixture was evaporated under vacuum, water (30 mL) and ethyl acetate (30 mL) were added. The organic layer was separated, washed twice with water and brine, dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified by silica gel chromatography to afford **24** (2 g, 62%) as a white powder. m.p. 61-62℃; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.11 (d, *J* = 2.4 Hz, 1H), 7.82 (d, *J* = 2.0 Hz, 1H), 4.65 (s, 2H), 4.25 (q, *J* = 7.2 Hz, 2H), 2.68 (s, 3H), 1.28 (t, *J* = 7.2 Hz, 3H).

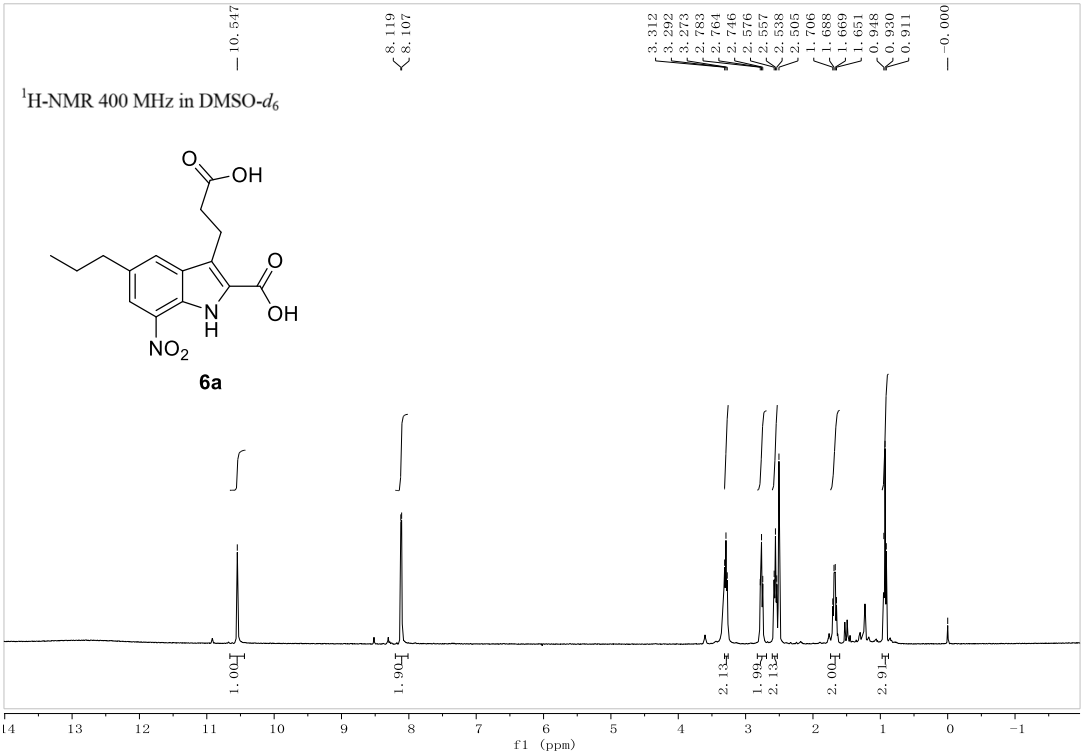
Synthesis of ethyl 5-bromo-3-methyl-7-nitrobenzofuran-2-carboxylate (25)

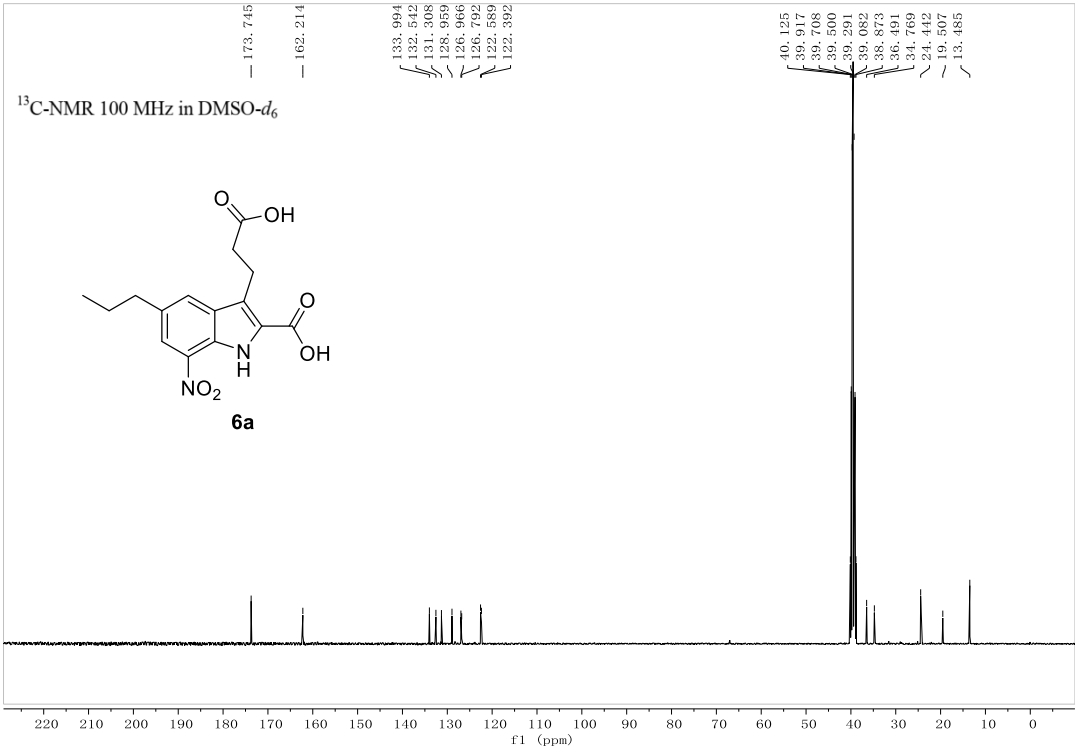
To a stirred solution of compound **24** (425 mg, 1.22 mmol) in toluene (10 mL) were added DBU (280 mg，1.84 mmol). The mixture was heated at 80℃ for 2 h. After cooling, water (20 mL) and ethyl acetate (15 mL) were added. The organic layer was separated, washed twice with water and brine, dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified by silica gel chromatography to afford **25** (211 mg, 52.4%) as a white powder. m.p. 169-170℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.40 (d, *J* = 1.6 Hz, 1H), 8.07 (d, *J* = 1.6 Hz, 1H), 4.49 (q, *J* = 7.2 Hz, 2H), 2.62 (s, 3H), 1.45 (t, *J* = 7.2 Hz, 3H).

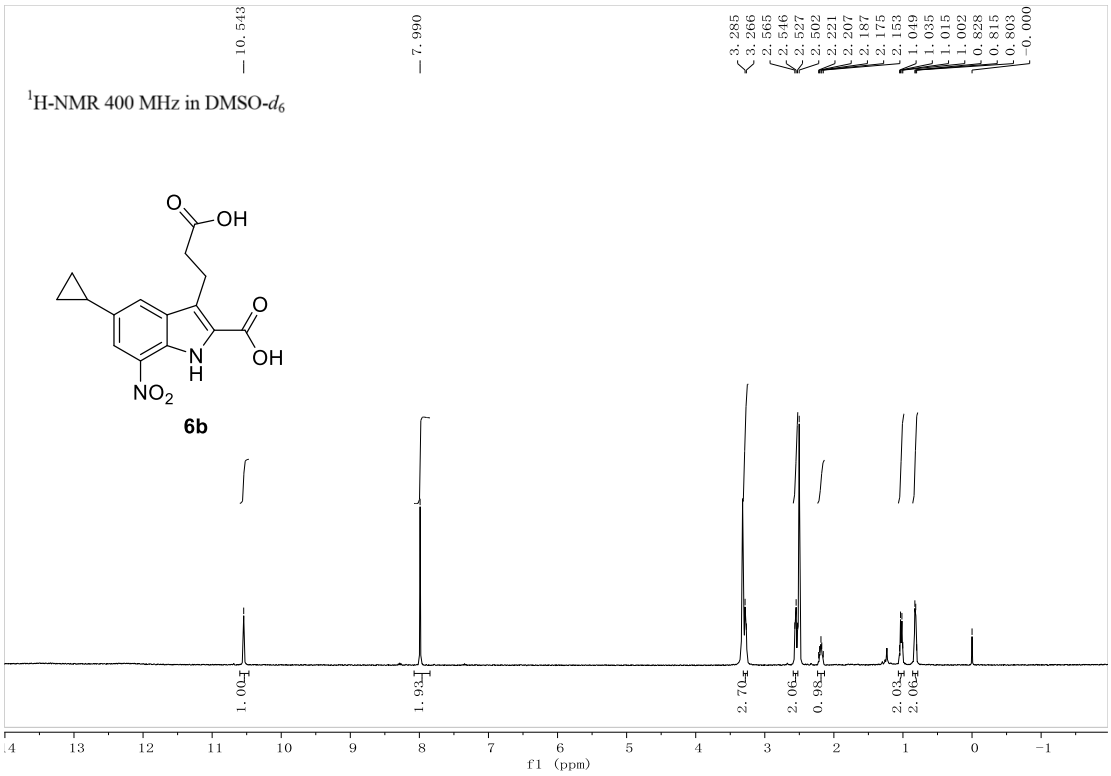
Synthesis of ethyl 5-bromo-3-(bromomethyl)-7-nitrobenzofuran-2-carboxylate (26)

To a stirred solution of compound **25** (191 mg, 0.58 mmol) in tetrachloromethane (15 mL) were added NBS (206.5 mg, 1.16 mmol) and AIBN (10 mg，0.06 mmol). The mixture was heated at 90 ℃ for 12 h. The crude product was purified by silica gel chromatography to afford **26** (225.1 mg, 95%) as a white powder. m.p. 168-169℃; ^{1}H NMR (400 $CDCl_3$) δ (ppm): 8.44 (s, 1H), 8.26 (s, 1H), 4.98 (s, 2H), 4.53 (q, *J* = 7.2 Hz, 2H), 1.49 (t, *J* = 7.2 Hz, 3H).

3. ^{1}H NMR and ^{13}C NMR of the target compounds



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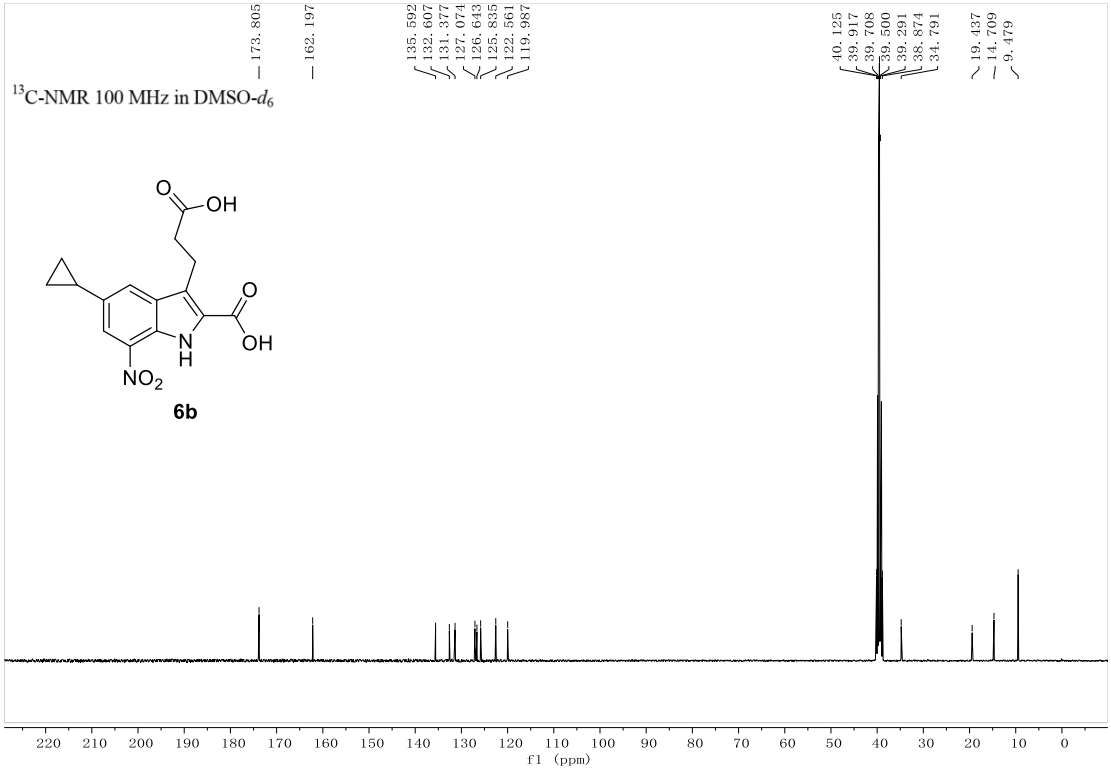
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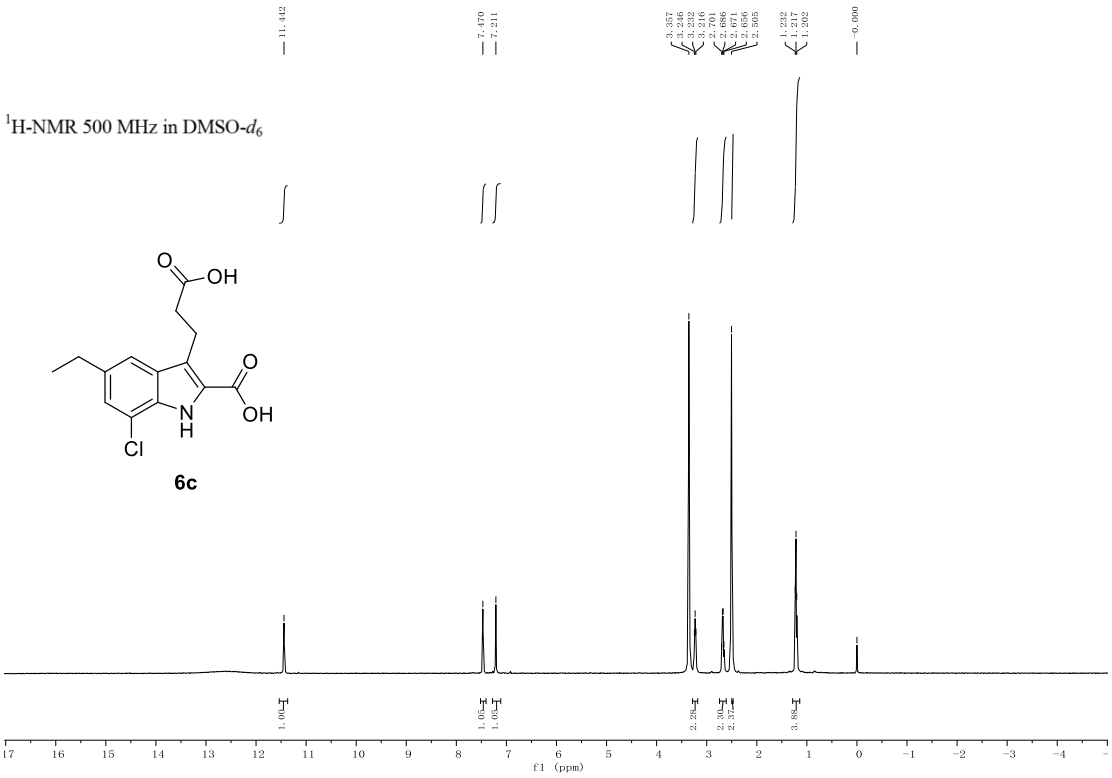

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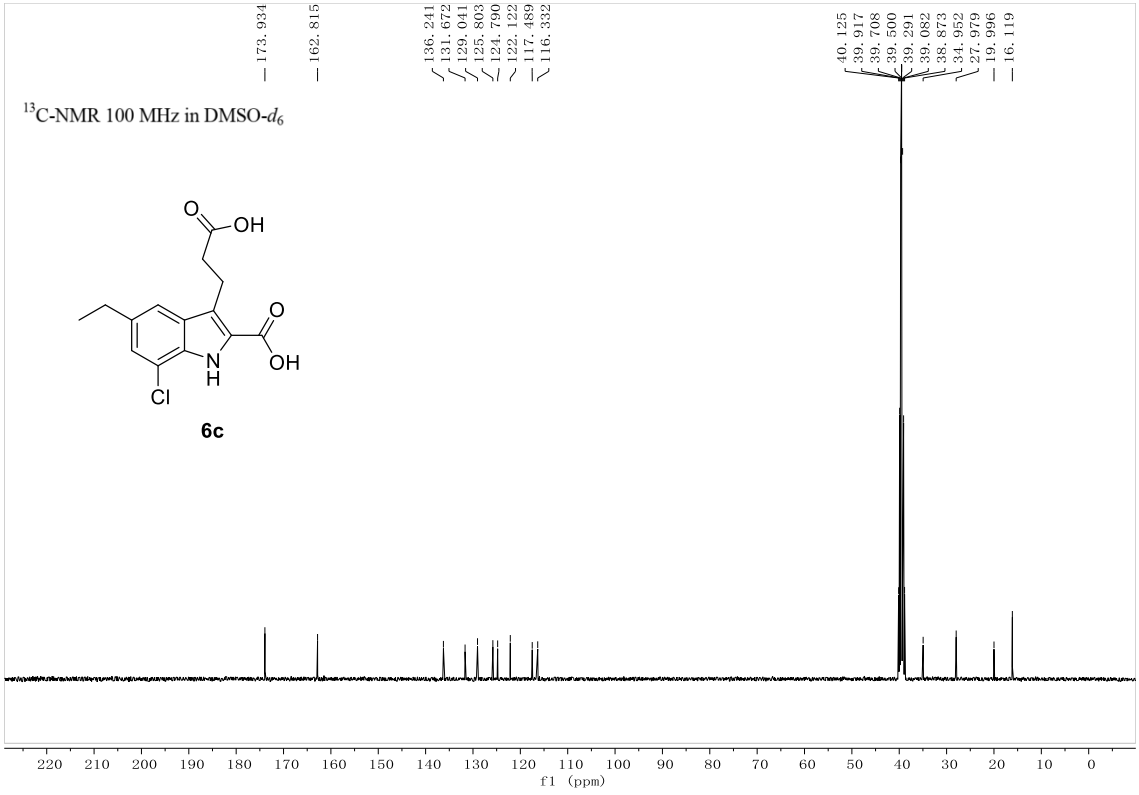

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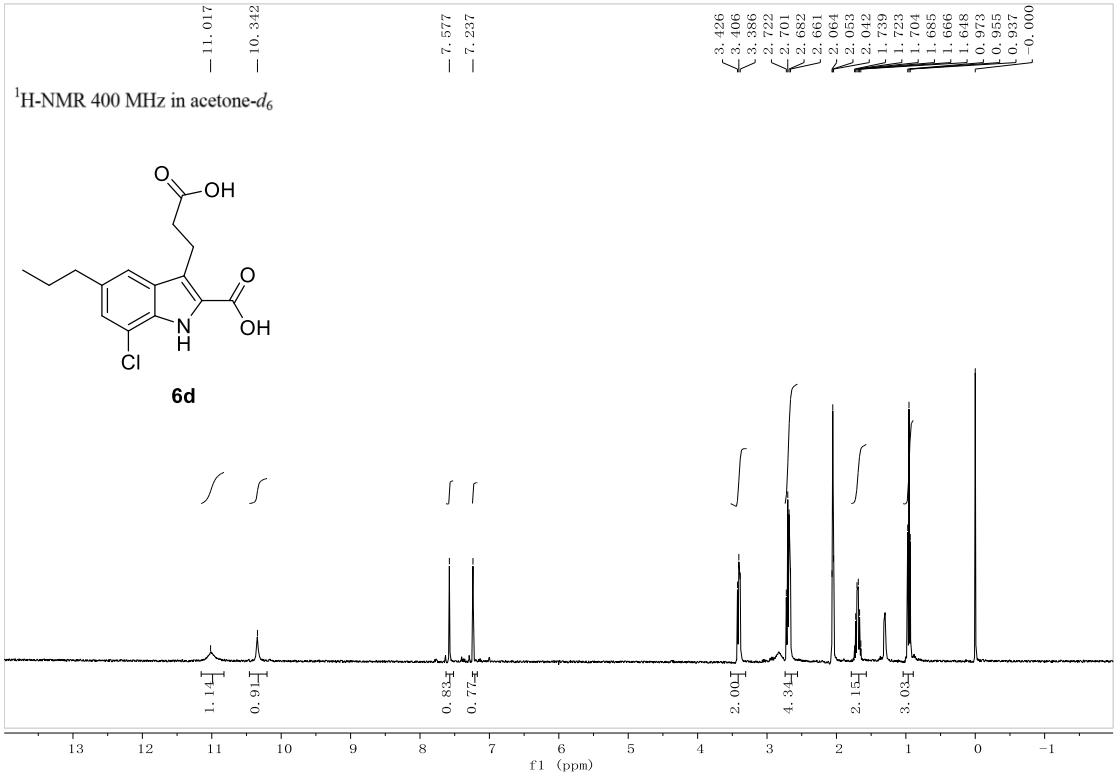
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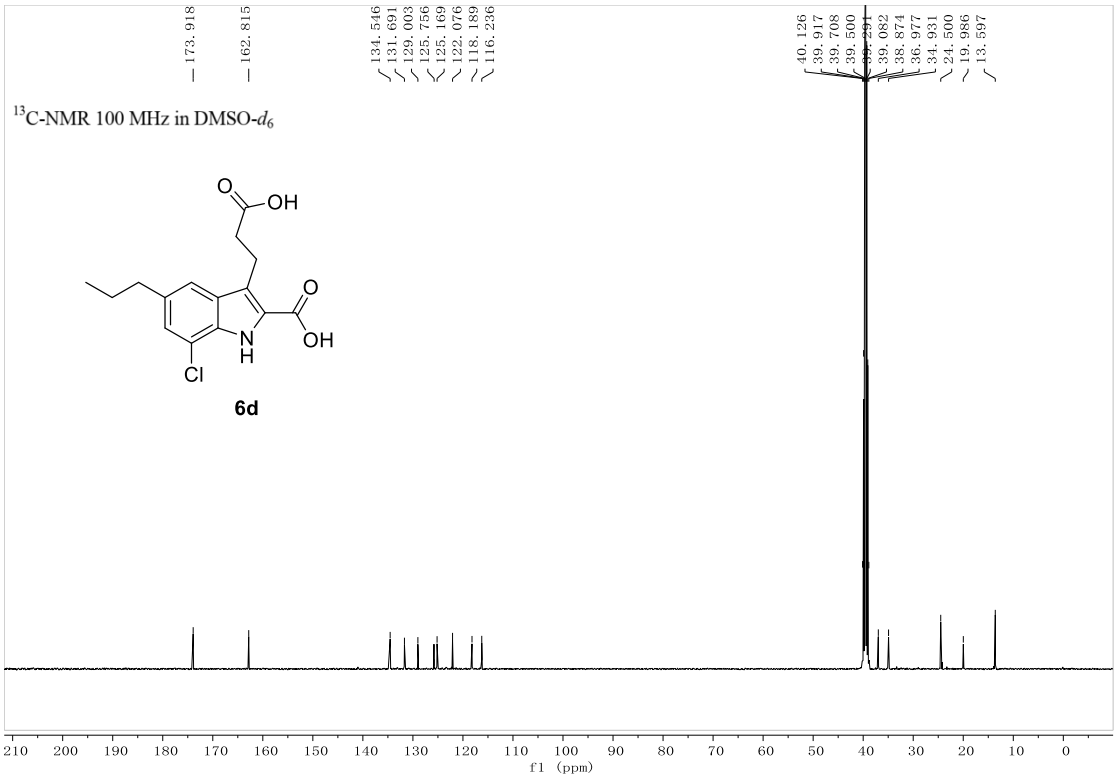
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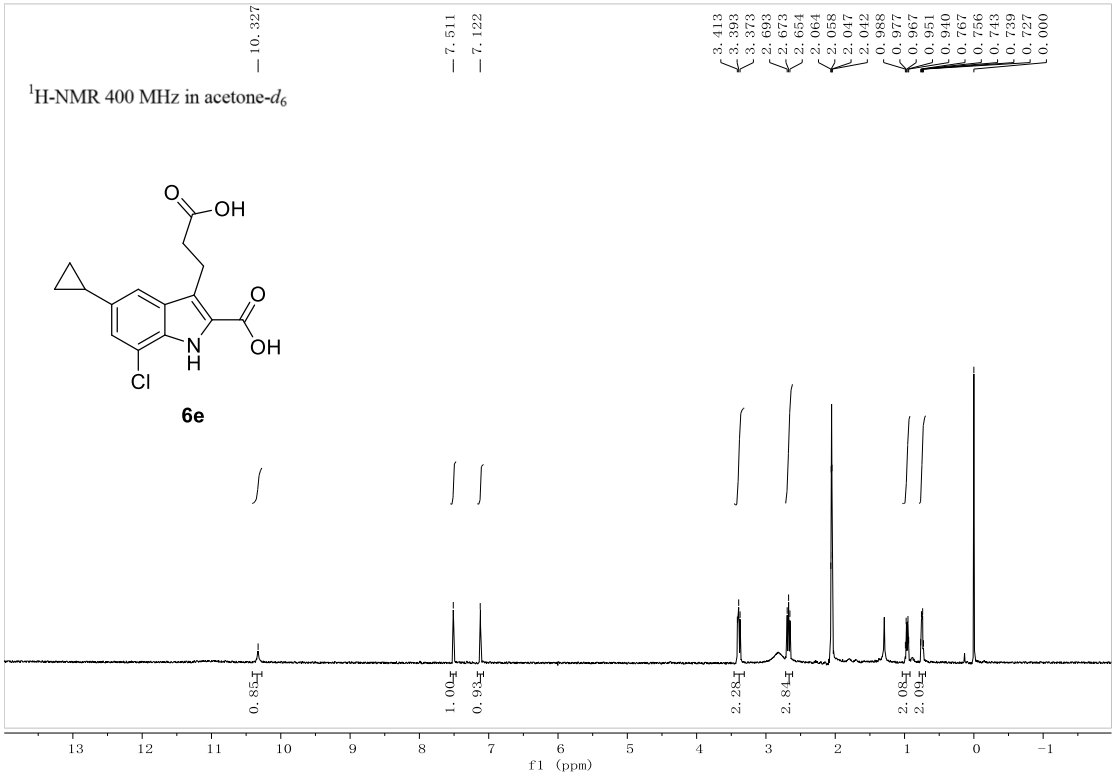
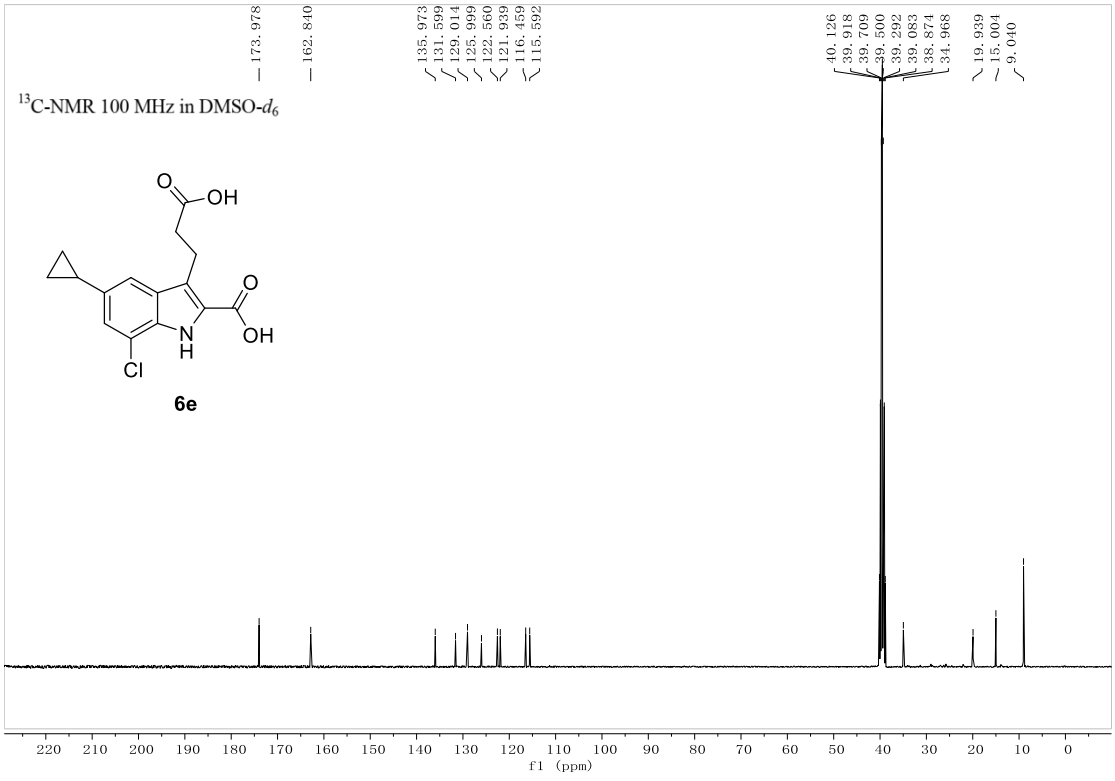
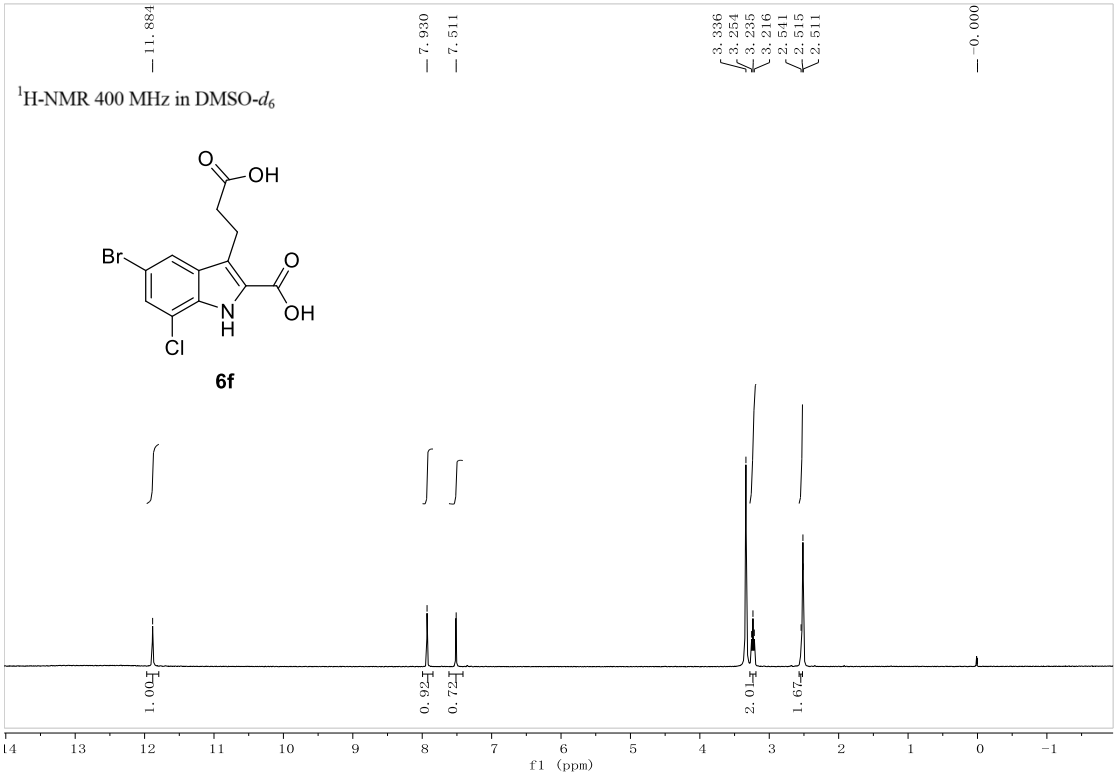
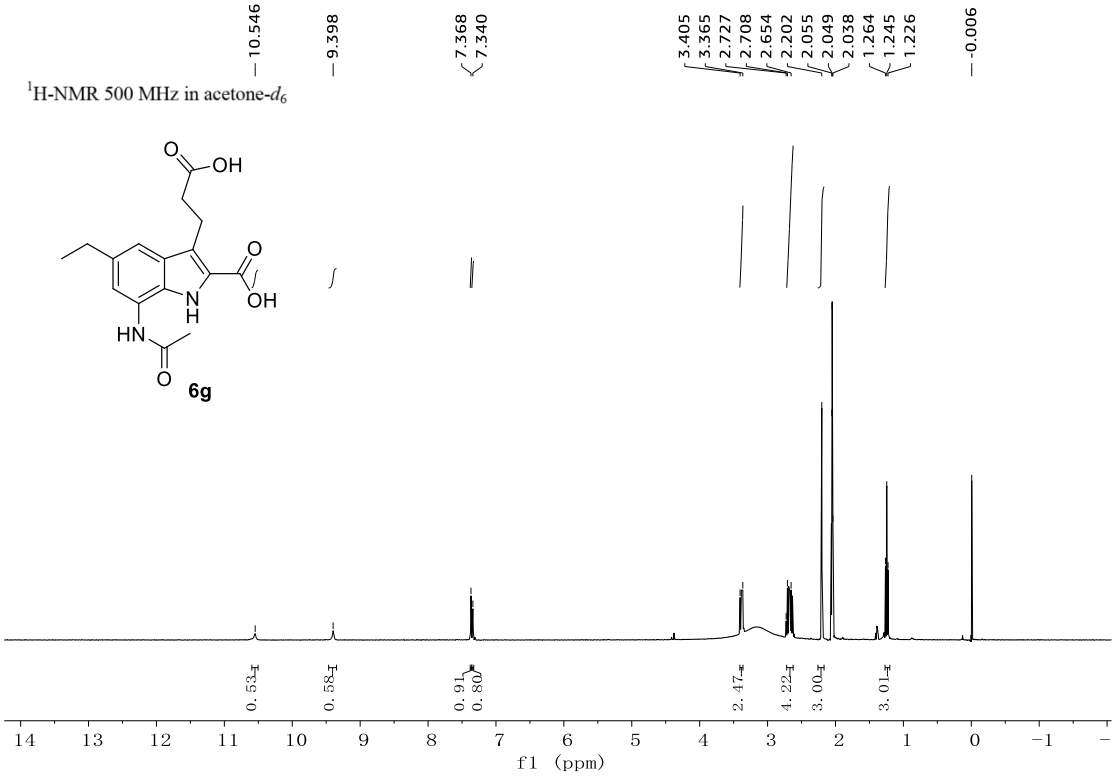
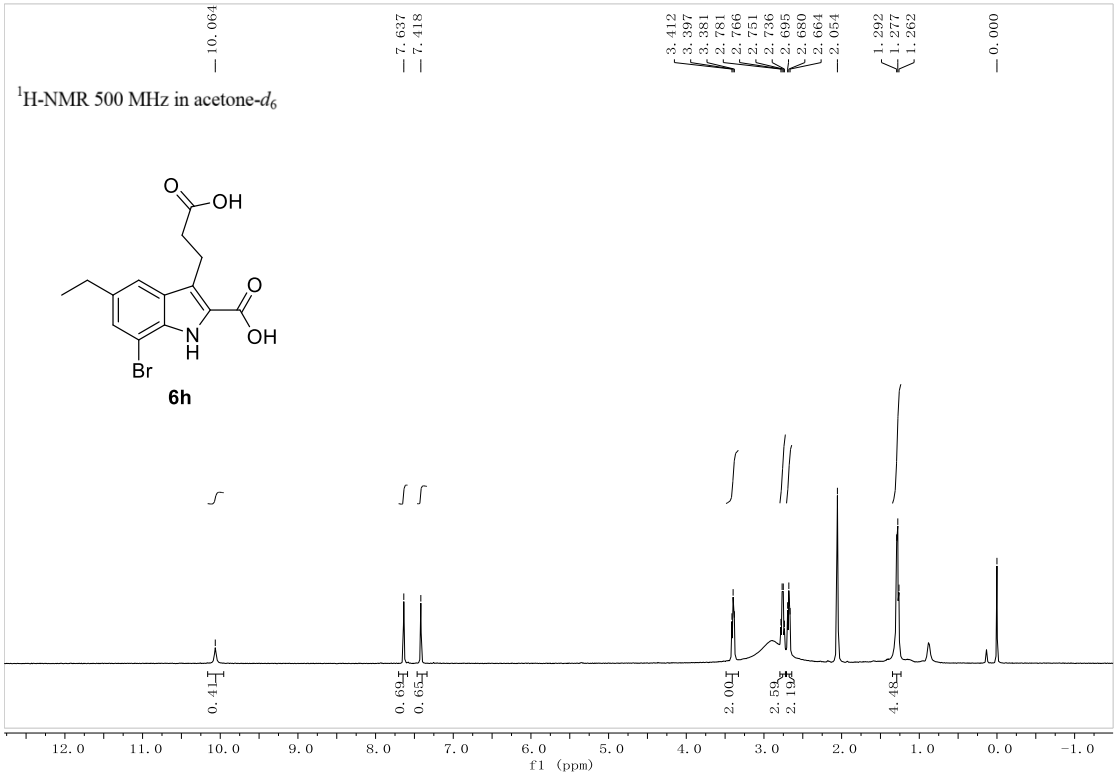
38.874
36.977
34.931
24.500
19.986
13.597
13C-NMR 100 MHz in DMSO-d6
O
OH
O
OH
N
H
Cl
6d
f1 (ppm)

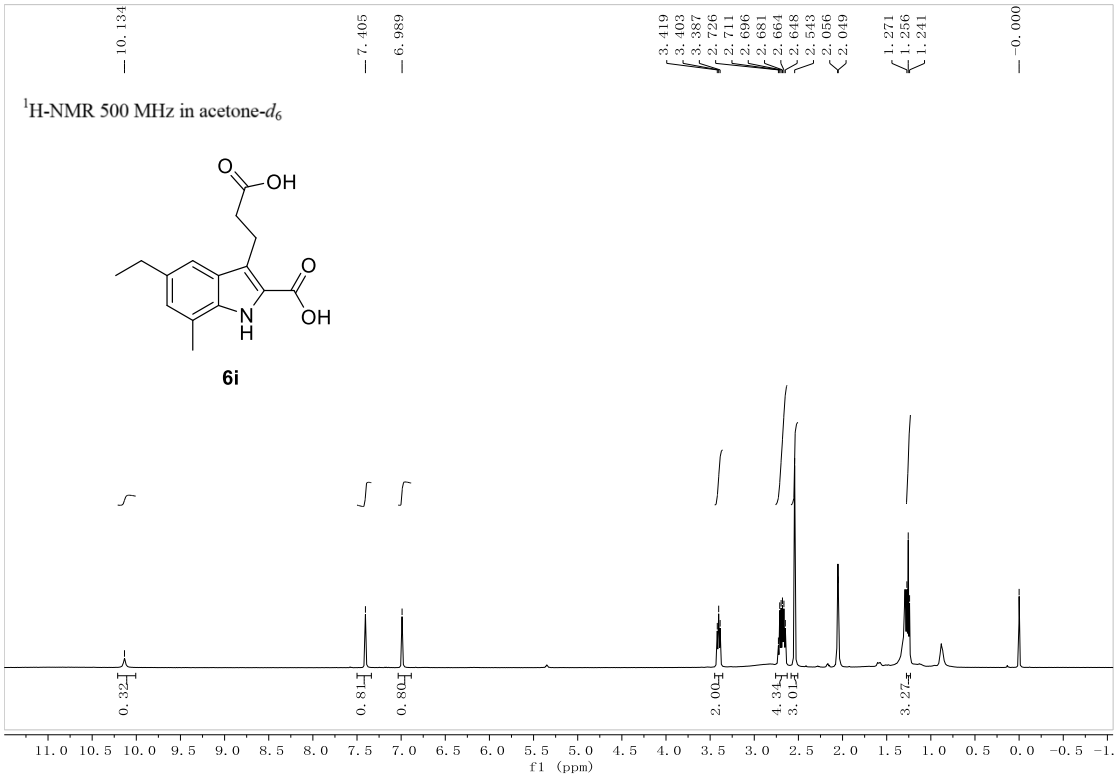

10.327
7.511
7.122
3.413
3.393
3.373
2.693
2.673
2.654
2.064
2.058
2.047
2.042
0.988
0.977
0.967
0.951
0.940
0.767
0.756
0.743
0.739
0.727
0.000
1H-NMR 400 MHz in acetone-d6
O
OH
O
OH
N
H
Cl
6e
0.85
1.00
0.93
2.28
2.84
2.08
2.09
13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1
f1 (ppm)

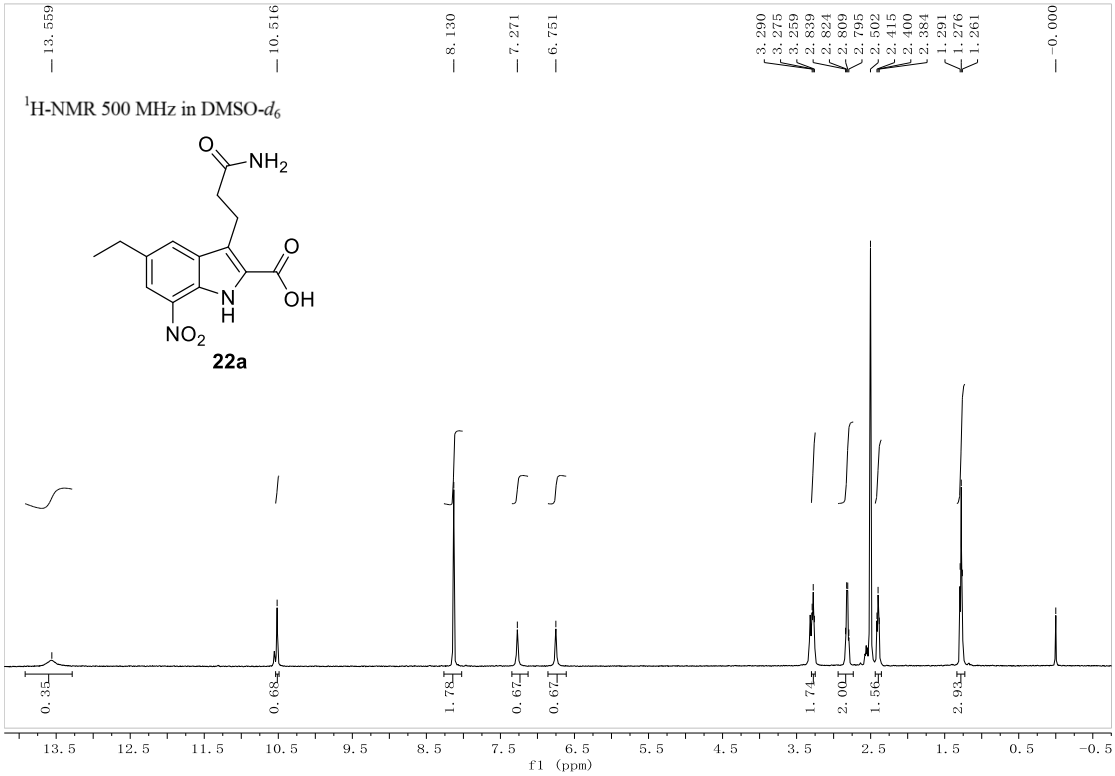

173.978
162.840
135.973
131.599
129.014
125.999
122.560
121.939
116.459
115.592
40.126
39.918
39.709
39.500
39.292
39.083
38.874
34.968
19.939
15.004
9.040
13C-NMR 100 MHz in DMSO-d6
O
OH
O
OH
N
H
Cl
6e
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

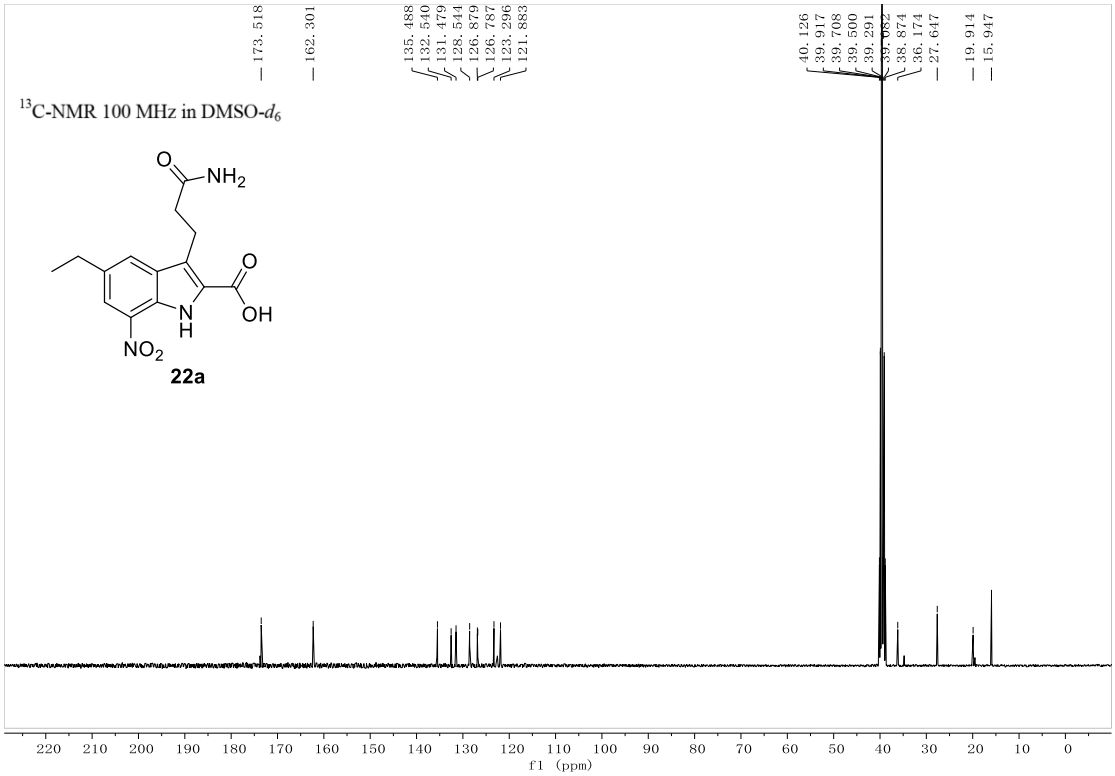

11.884
7.930
7.511
3.336
3.254
3.235
3.216
2.541
2.515
2.511
-0.000
1H-NMR 400 MHz in DMSO-d6
Br
Cl
N
H
O
OH
6f
1.00
0.92
0.72
2.01
1.67
f1 (ppm)

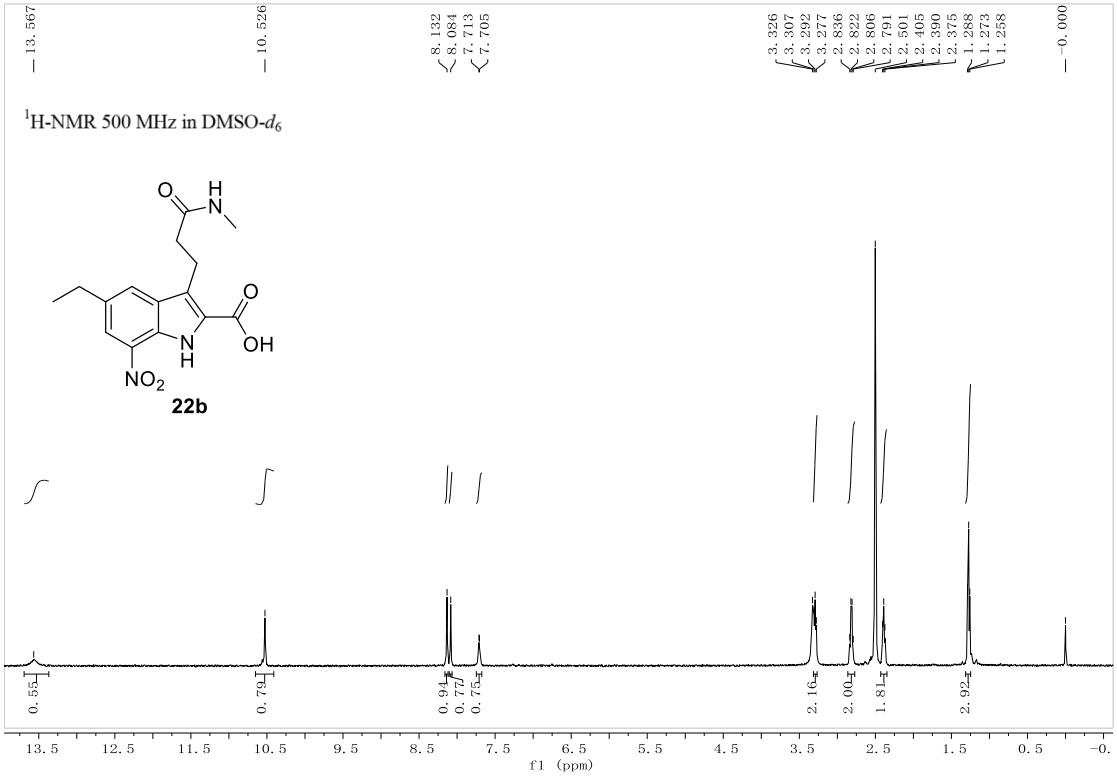

10.546
9.398
7.368
7.340
3.405
3.365
2.727
2.708
2.654
2.202
2.055
2.049
2.038
1.264
1.245
1.226
-0.006
1H-NMR 500 MHz in acetone-d6
HN
N
H
O
OH
6g
0.53
0.58
0.91
0.80
2.47
4.22
3.00
3.01
f1 (ppm)

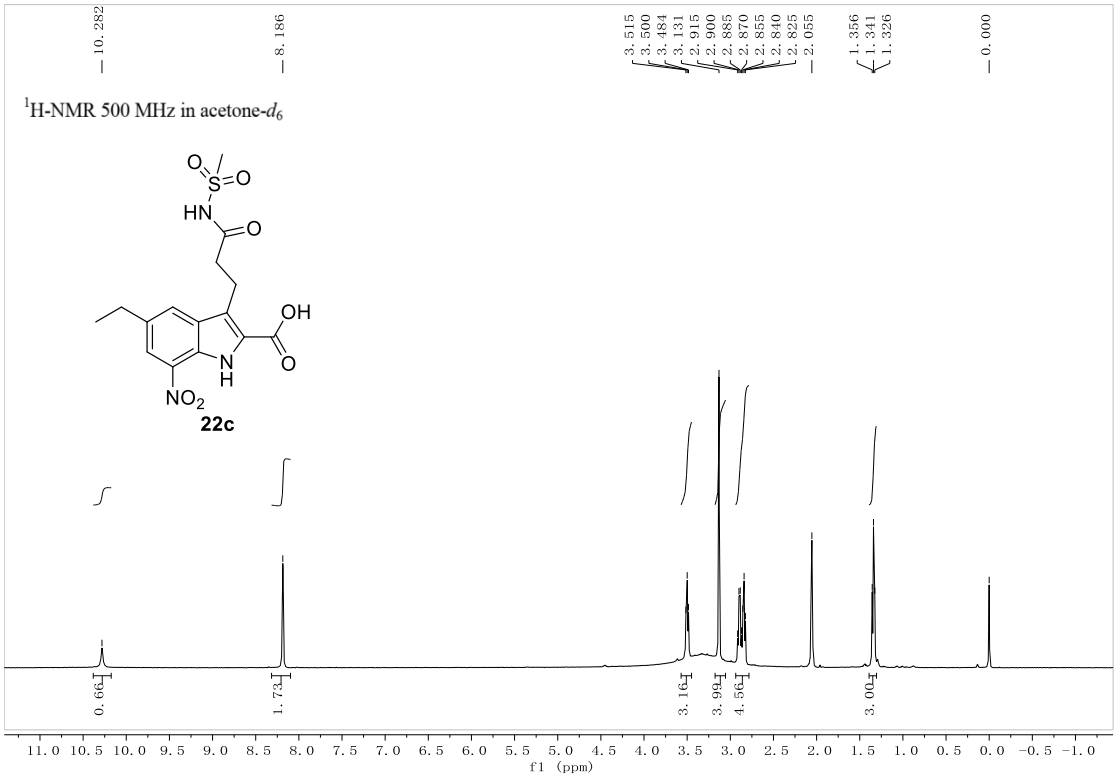

10.064
7.637
7.418
3.412
3.397
3.381
2.781
2.766
2.751
2.736
2.695
2.680
2.664
2.054
1.292
1.277
1.262
0.000
^{1}H-NMR 500 MHz in acetone-d_6
O
OH
O
OH
N
H
Br
6h
0.41
0.69
0.65
2.00
2.59
2.19
4.48
12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0 −1.0
f1 (ppm)

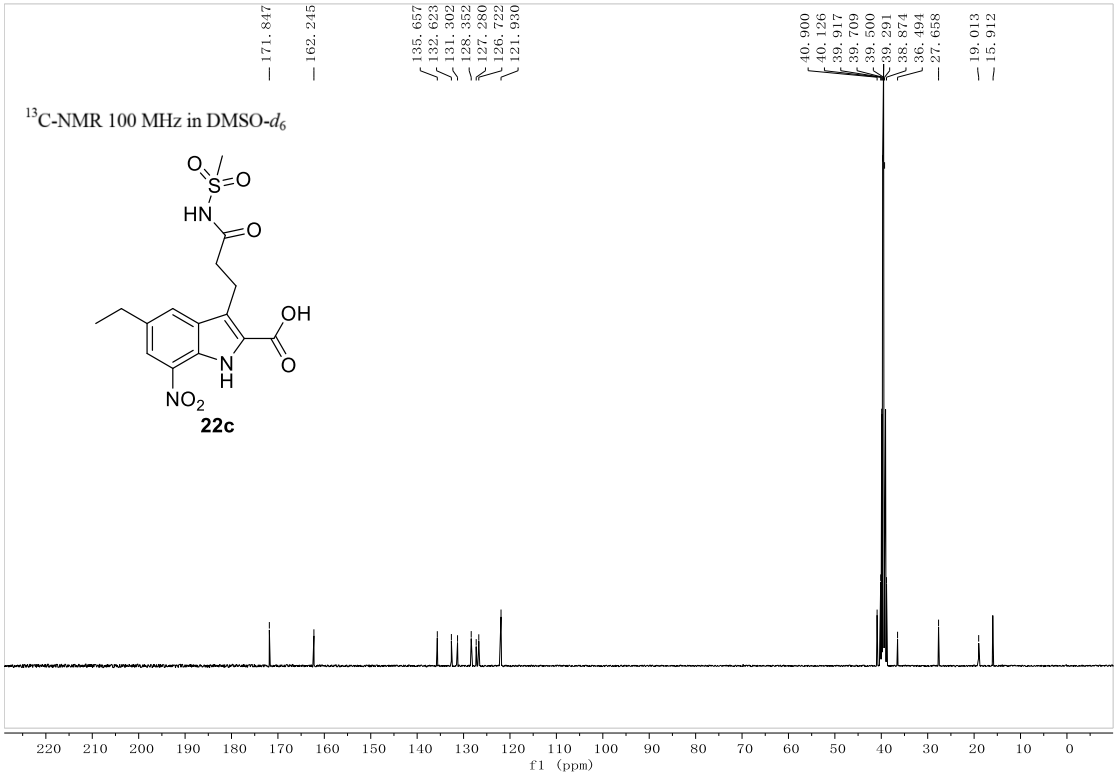

10.134
7.405
6.989
3.419
3.403
3.387
2.726
2.711
2.696
2.681
2.664
2.648
2.543
2.056
2.049
1.271
1.256
1.241
−0.000
^{1}H-NMR 500 MHz in acetone-d_6
O
OH
O
OH
N
H
6i
0.32
0.81
0.80
2.00
4.34
3.01
3.27
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 −0.5 −1.
f1 (ppm)

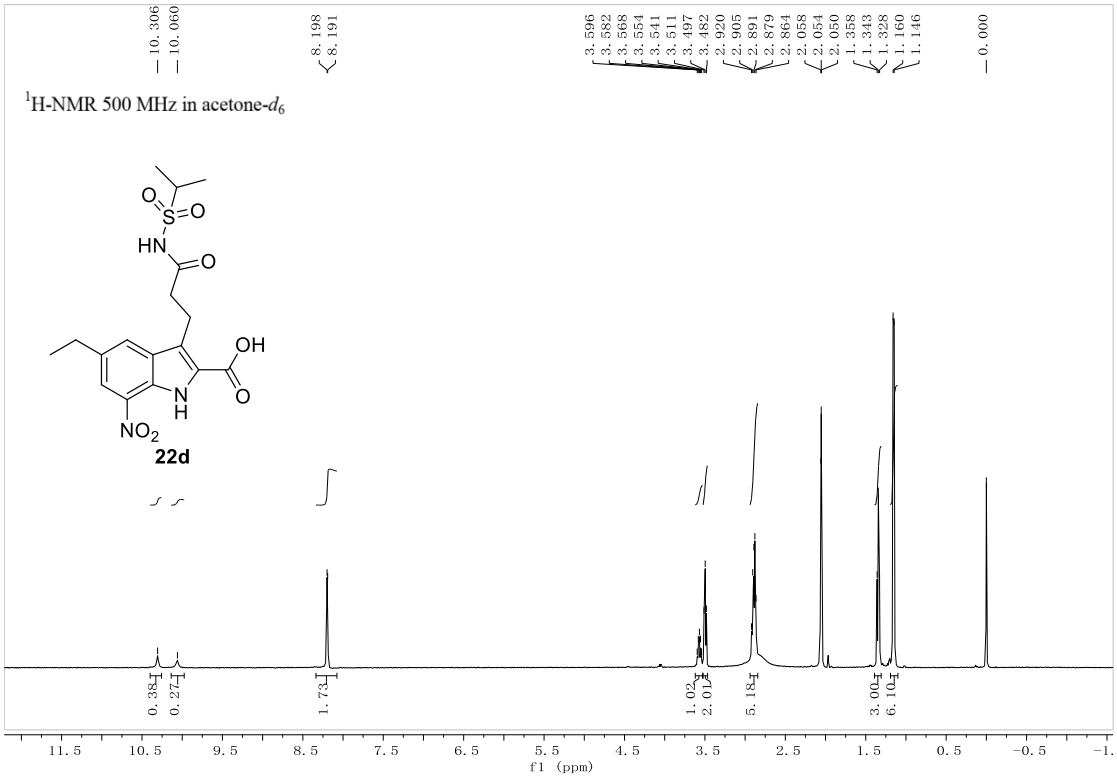

13.559
10.516
8.130
7.271
6.751
3.290
3.275
3.259
2.839
2.824
2.809
2.795
2.502
2.415
2.400
2.384
1.291
1.276
1.261
0.000
1H-NMR 500 MHz in DMSO-d6
O
NH2
O
OH
N
H
NO2
22a
0.35
0.68
1.78
0.67
0.67
1.74
2.00
1.56
2.93
13.5
12.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
−0.5
f1 (ppm)

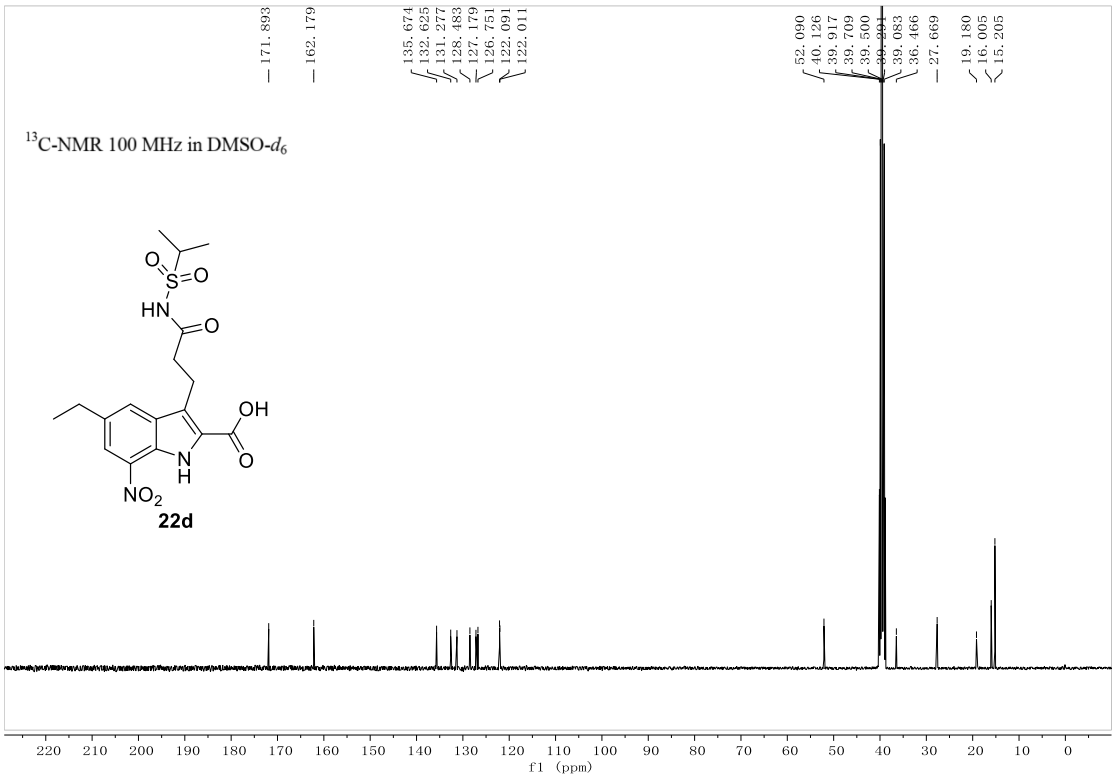

173.518
162.301
135.488
132.540
131.479
128.544
126.879
126.787
123.296
121.883
40.126
39.917
39.708
39.500
39.291
39.082
38.874
36.174
27.647
19.914
15.947
13C-NMR 100 MHz in DMSO-d6
O
NH2
O
OH
N
H
NO2
22a
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

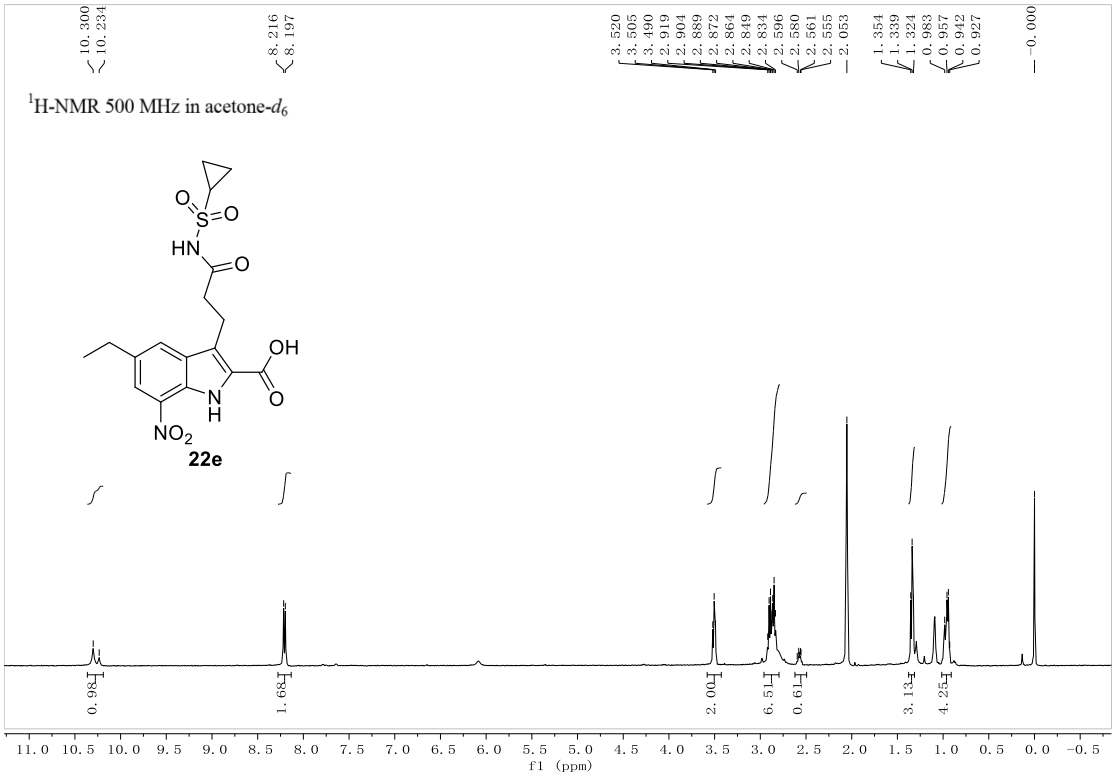

13.567
10.526
8.132
8.084
7.713
7.705
3.326
3.307
3.292
3.277
2.836
2.822
2.806
2.791
2.501
2.405
2.390
2.375
1.288
1.273
1.258
0.000
1H-NMR 500 MHz in DMSO-d6
22b
0.55
0.79
0.94
0.77
0.75
2.16
2.00
1.81
2.92
f1 (ppm)

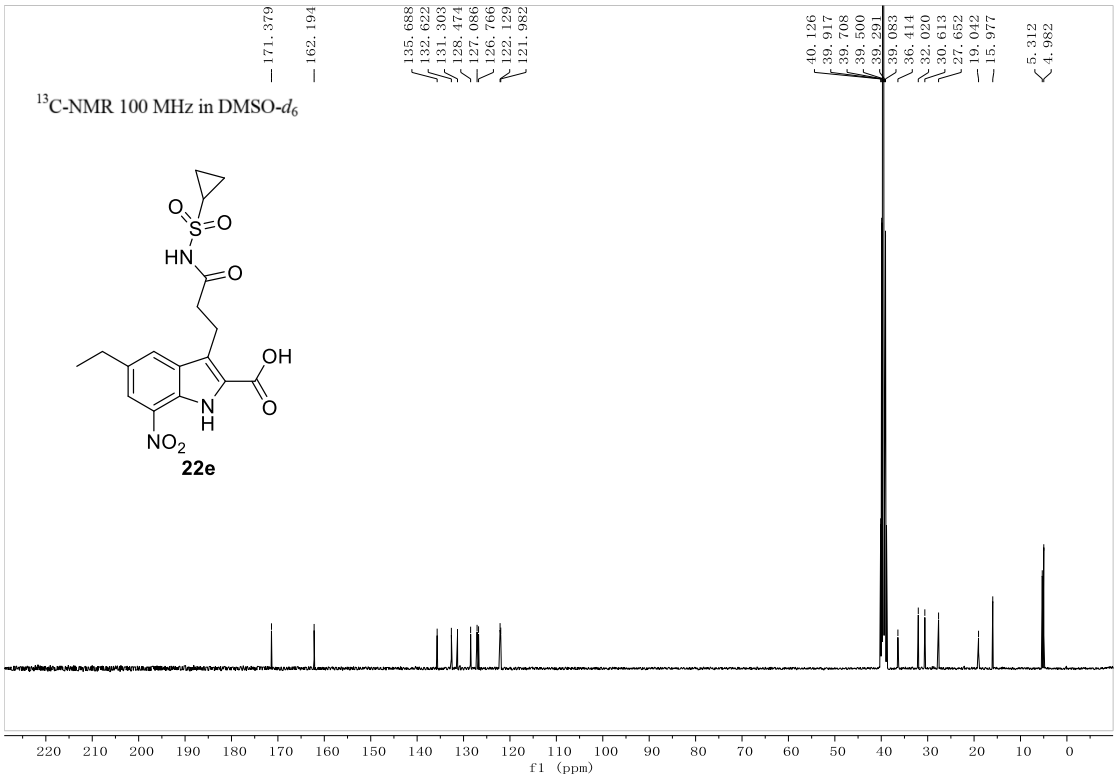

10.282
8.186
3.515
3.500
3.484
3.131
2.915
2.900
2.885
2.870
2.855
2.840
2.825
2.055
1.356
1.341
1.326
0.000
1H-NMR 500 MHz in acetone-d6
22c
0.66
1.73
3.16
3.99
4.56
3.00
f1 (ppm)

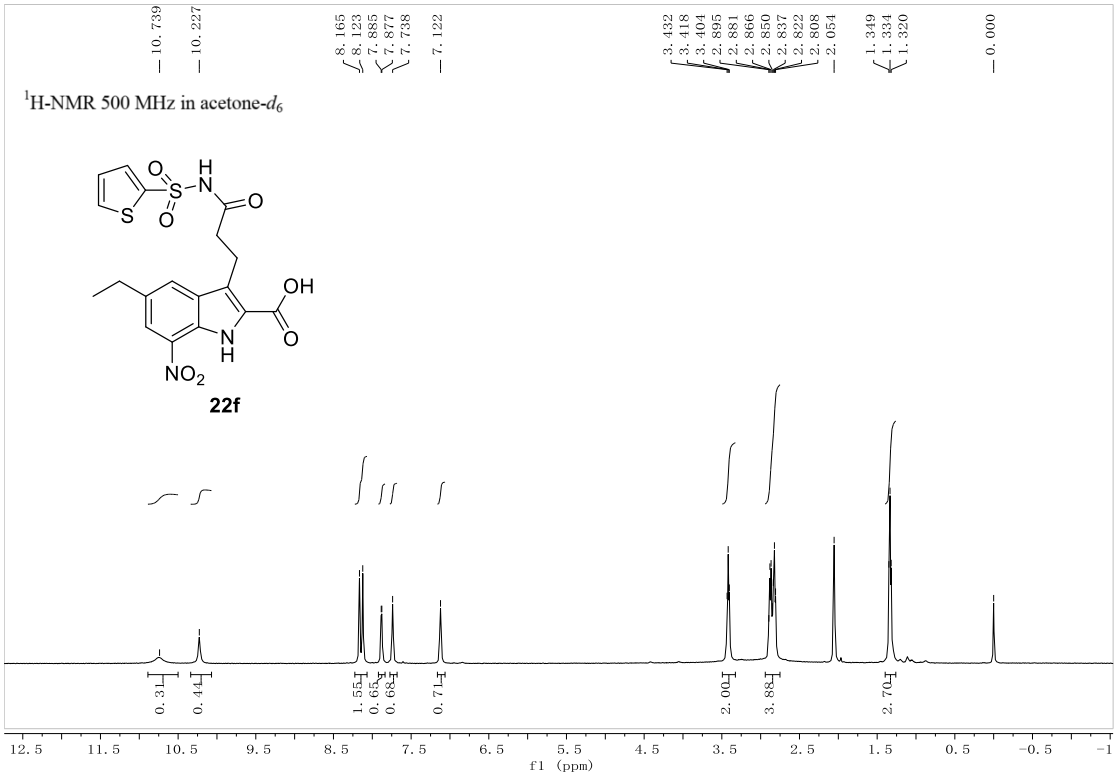

171.847
162.245
135.657
132.623
131.302
128.352
127.280
126.722
121.930
40.900
40.126
39.917
39.709
39.500
39.291
38.874
36.494
27.658
19.013
15.912
13C-NMR 100 MHz in DMSO-d6
O
S
O
HN
O
OH
O
N
H
NO2
22c
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

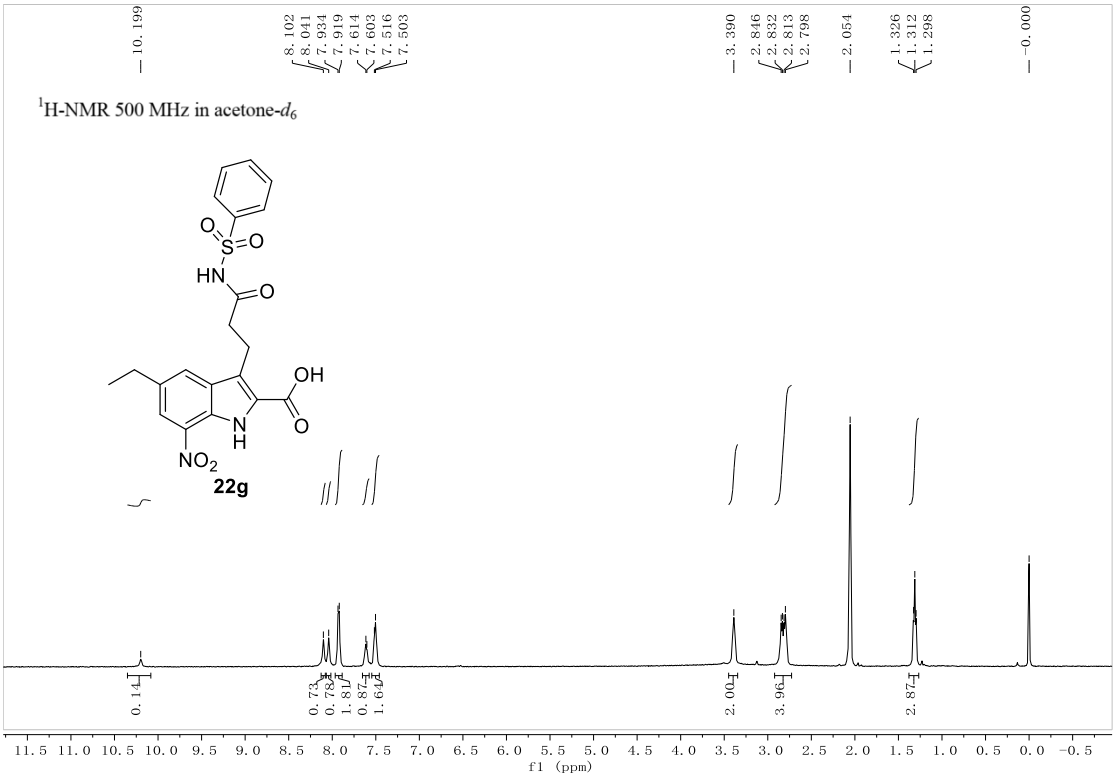

10.306
10.060
8.198
8.191
3.596
3.582
3.568
3.554
3.541
3.511
3.497
3.482
2.920
2.905
2.891
2.879
2.864
2.058
2.054
2.050
1.358
1.343
1.328
1.160
1.146
0.000
1H-NMR 500 MHz in acetone-d6
O
S
O
HN
O
OH
O
N
H
NO2
22d
0.38
0.27
1.73
1.02
2.01
5.18
3.00
6.10
11.5 10.5 9.5 8.5 7.5 6.5 5.5 4.5 3.5 2.5 1.5 0.5 −0.5 −1.
f1 (ppm)

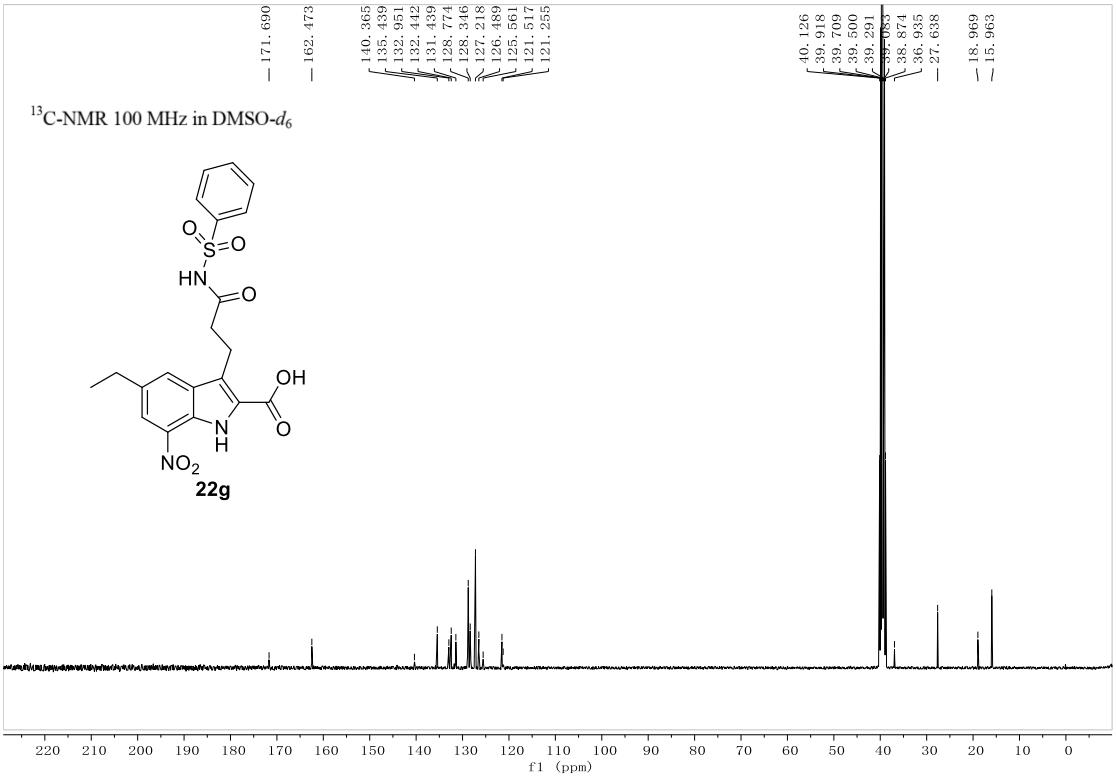

171.893
162.179
135.674
132.625
131.277
128.483
127.179
126.751
122.091
122.011
52.090
40.126
39.917
39.709
39.500
39.291
39.083
36.466
27.669
19.180
16.005
15.205
13C-NMR 100 MHz in DMSO-d6
O
S
O
HN
O
OH
O
N
H
NO2
22d
f1 (ppm)

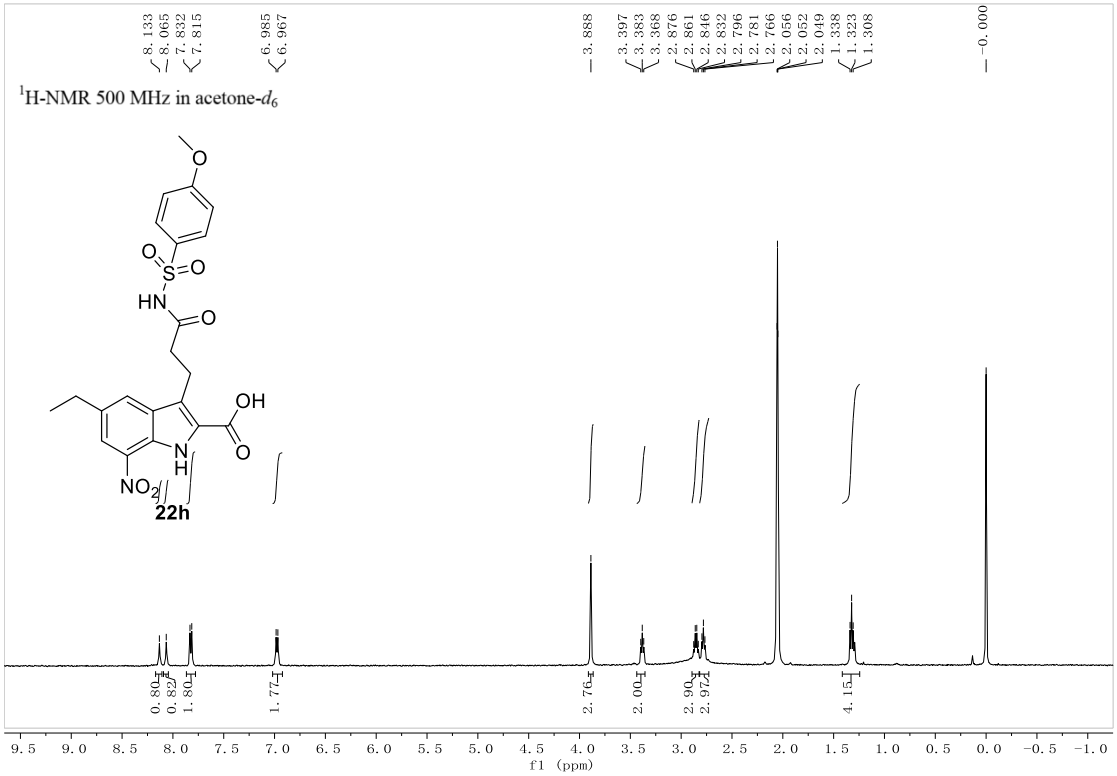

10.300
10.234
8.216
8.197
3.520
3.505
3.490
2.919
2.904
2.889
2.872
2.864
2.849
2.834
2.596
2.580
2.561
2.555
2.053
1.354
1.339
1.324
0.983
0.957
0.942
0.927
-0.000
1H-NMR 500 MHz in acetone-d6
O
S
O
HN
O
OH
O
N
H
NO2
22e
0.98
1.68
2.00
6.51
0.61
3.13
4.25
f1 (ppm)

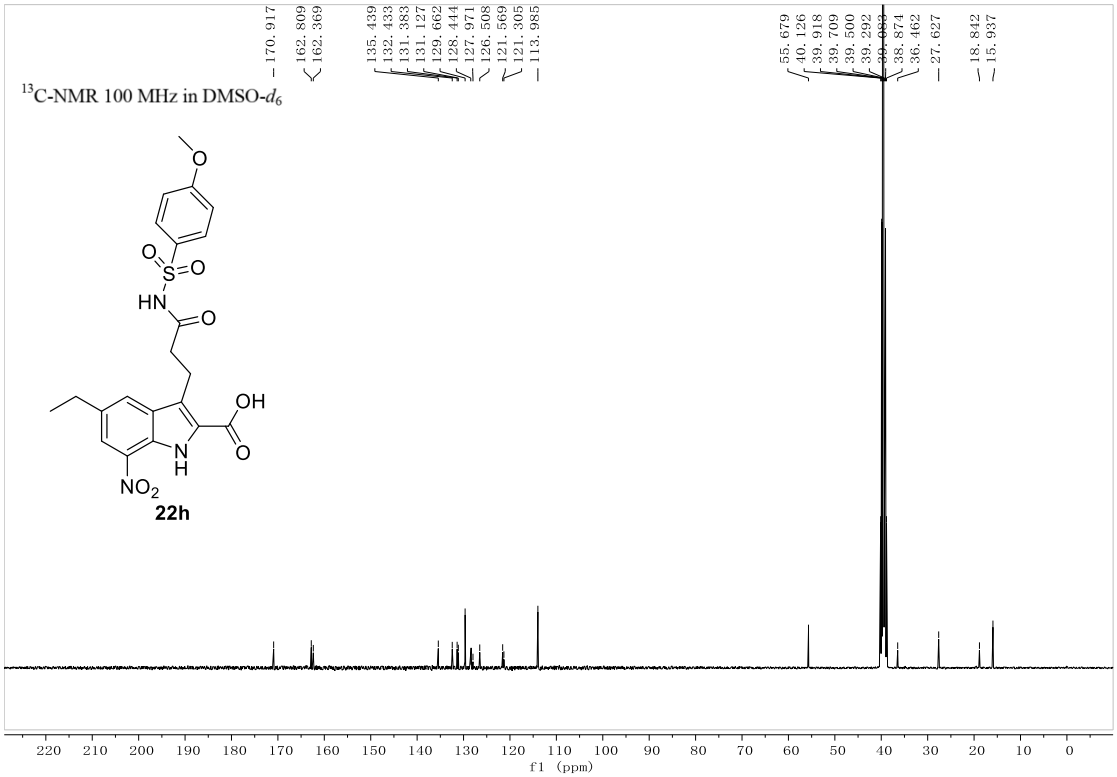

171.379
162.194
135.688
132.622
131.303
128.474
127.086
126.766
122.129
121.982
40.126
39.917
39.708
39.500
39.291
39.083
36.414
32.020
30.613
27.652
19.042
15.977
5.312
4.982
13C-NMR 100 MHz in DMSO-d6
22e
f1 (ppm)

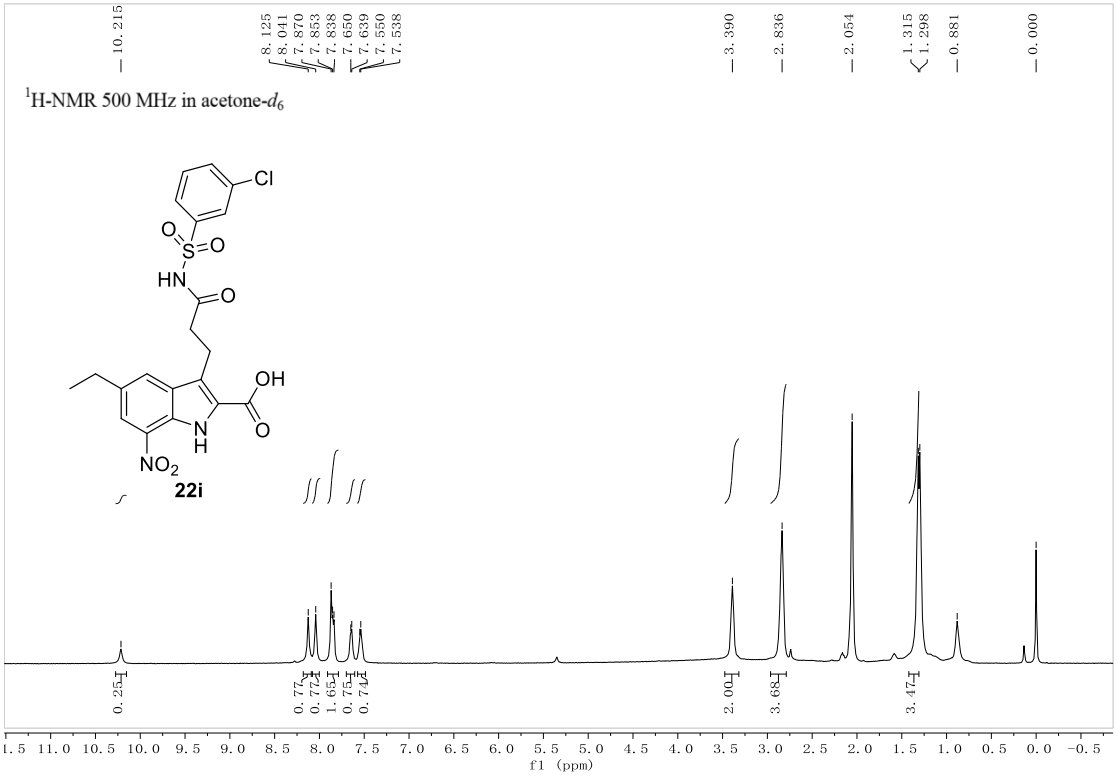

10.739
10.227
8.165
8.123
7.885
7.877
7.738
7.122
3.432
3.418
3.404
2.895
2.881
2.866
2.850
2.837
2.822
2.808
2.054
1.349
1.334
1.320
0.000
1H-NMR 500 MHz in acetone-d6
22f
0.31
0.44
1.55
0.65
0.68
0.71
2.00
3.88
2.70
f1 (ppm)

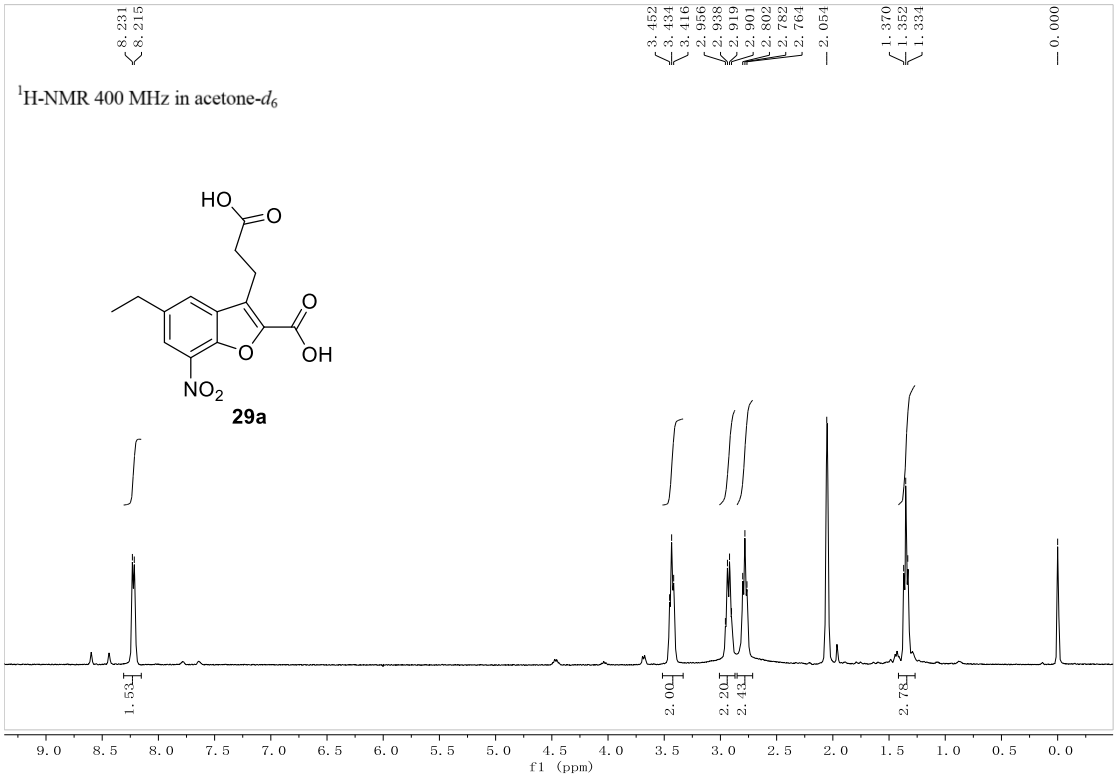

10.199
8.102
8.041
7.934
7.919
7.614
7.603
7.516
7.503
3.390
2.846
2.832
2.813
2.798
2.054
1.326
1.312
1.298
0.000
1H-NMR 500 MHz in acetone-d6
22g
0.14
0.73
0.78
1.81
0.87
1.64
2.00
3.96
2.87
f1 (ppm)

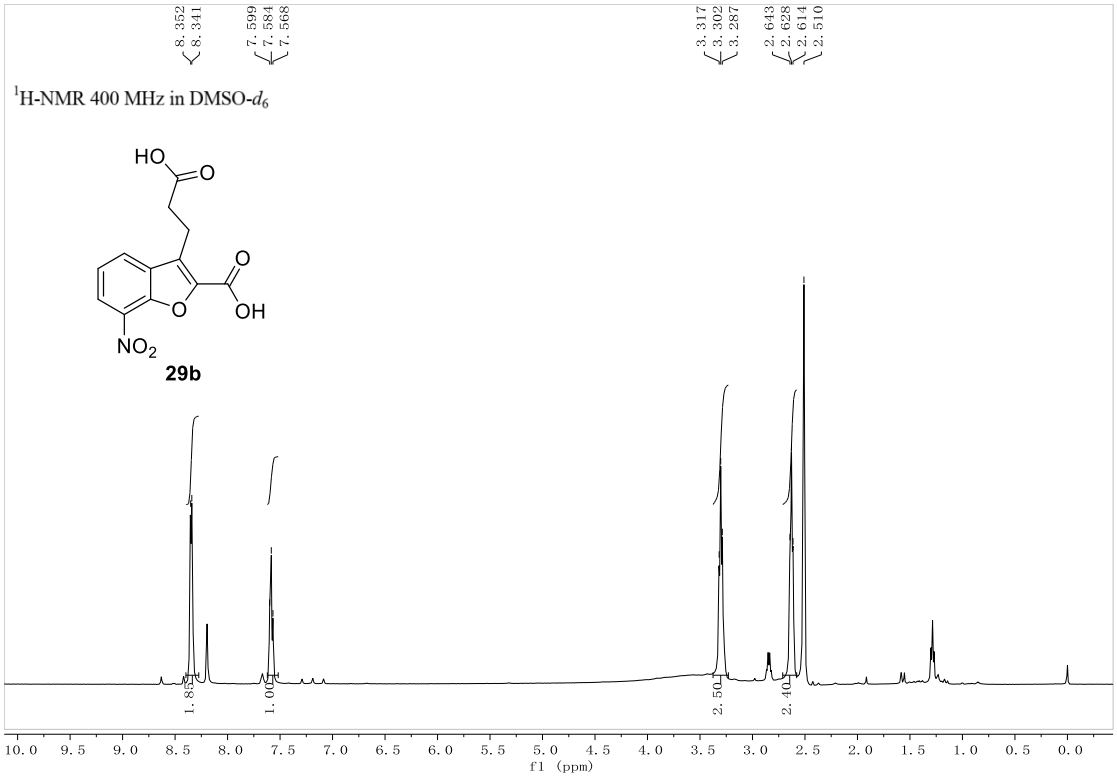

171.690
162.473
140.365
135.439
132.951
132.442
131.439
128.774
128.346
127.218
126.489
125.561
121.517
121.255
40.126
39.918
39.709
39.500
39.291
39.083
38.874
36.935
27.638
18.969
15.963
13C-NMR 100 MHz in DMSO-d6
22g
f1 (ppm)

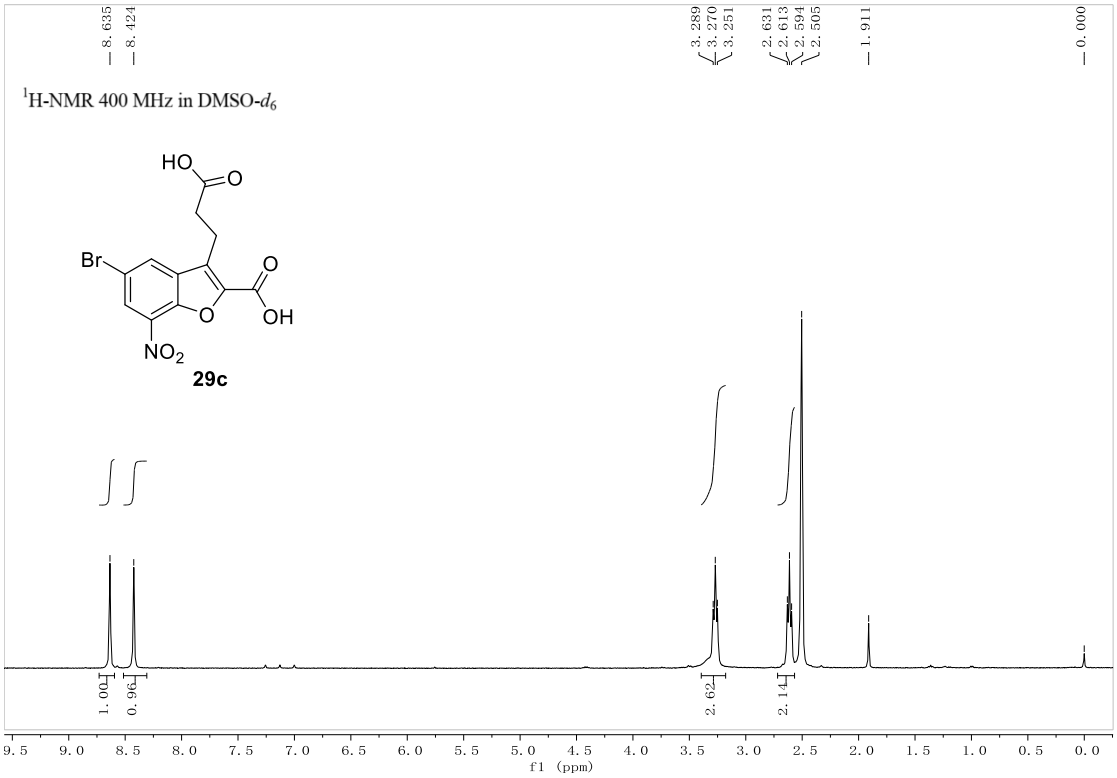

8.133
8.065
7.832
7.815
6.985
6.967
3.888
3.397
3.383
3.368
2.876
2.861
2.846
2.832
2.796
2.781
2.766
2.056
2.052
2.049
1.338
1.323
1.308
0.000
^{1}H-NMR 500 MHz in acetone-d_6
22h
0.80
0.82
1.80
1.77
2.76
2.00
2.90
2.97
4.15
f1 (ppm)


170.917
162.809
162.369
135.439
132.433
131.383
131.127
129.662
128.444
127.971
126.508
121.569
121.305
113.985
55.679
40.126
39.918
39.709
39.500
39.292
39.083
38.874
36.462
27.627
18.842
15.937
^{13}C-NMR 100 MHz in DMSO-d_6
22h
f1 (ppm)


10.215
8.125
8.041
7.870
7.853
7.838
7.650
7.639
7.550
7.538
3.390
2.836
2.054
1.315
1.298
0.881
0.000
1H-NMR 500 MHz in acetone-d6
Cl
O
S
O
HN
O
OH
O
N
H
NO2
22i
0.25
0.77
0.77
1.65
0.75
0.74
2.00
3.68
3.47
f1 (ppm)


8.231
8.215
3.452
3.434
3.416
2.956
2.938
2.919
2.901
2.802
2.782
2.764
2.054
1.370
1.352
1.334
0.000
1H-NMR 400 MHz in acetone-d6
HO
O
O
O
OH
NO2
29a
1.53
2.00
2.20
2.43
2.78
f1 (ppm)


8.352
8.341
7.599
7.584
7.568
3.317
3.302
3.287
2.643
2.628
2.614
2.510
1H-NMR 400 MHz in DMSO-d6
HO
O
O
O
OH
NO2
29b
1.83
1.00
2.50
2.40
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


8.635
8.424
3.289
3.270
3.251
2.631
2.613
2.594
2.505
1.911
0.000
1H-NMR 400 MHz in DMSO-d6
HO
O
Br
O
O
OH
NO2
29c
1.00
0.96
2.62
2.14
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

4. The data collection and refinement statistics of co-crystal structure of FBPase in complex with compounds *14b, 6d and 22f

Data Collection			
PDB ID	7EZP	7EZF	7EZR
Ligand	*14b	6d	22f
Wavelength (Å)	0.979	0.979	0.979
Resolution range[a] (Å)	71.5-2.8 (2.95-2.8)	47.78-2.76 (2.89-2.76)	47.54-3.27 (3.53-3.27)
Unique reflections[a]	32570	33781	20178
Completeness (%)[a]	99.9	99.4	99.8
$<I/\sigma>$[a]	9.9	13.6	12.4
Space group	P 1 21 1	P 1 21 1	P 1 21 1
Cell dimensions	a=67.1 Å b=143 Å c=73.6 Å α=90° β=107.74° γ=90°	a=67.076 Å b=143.329 Å c=73.491 Å α=90° β=108.14° γ=90°	a=66.902 Å b=142.591 Å c=73.575 Å α=90° β=108.66° γ=90°
Refinement			
Resolution range (Å)	70.2-2.8	47.67-2.76	40.84-3.27
R_{work}[b]	0.2102	0.1923	0.2106
R_{free}[c]	0.2555	0.2359	0.2528
RMS Deviations			
Bond length (Å)	0.008	0.007	0.004
Bond angles (°)	1.306	1.404	0.911

[a] Values in parentheses refer to the highest resolution shell

[b] $R_{work}=\Sigma|F_o-F_c|/\Sigma F_o$ where F_o is the observed and F_c is the calculated structure factor amplitudes.

[c] R_{free} is the same as R_{work}, but calculated on 5% reflections not used in refinement.

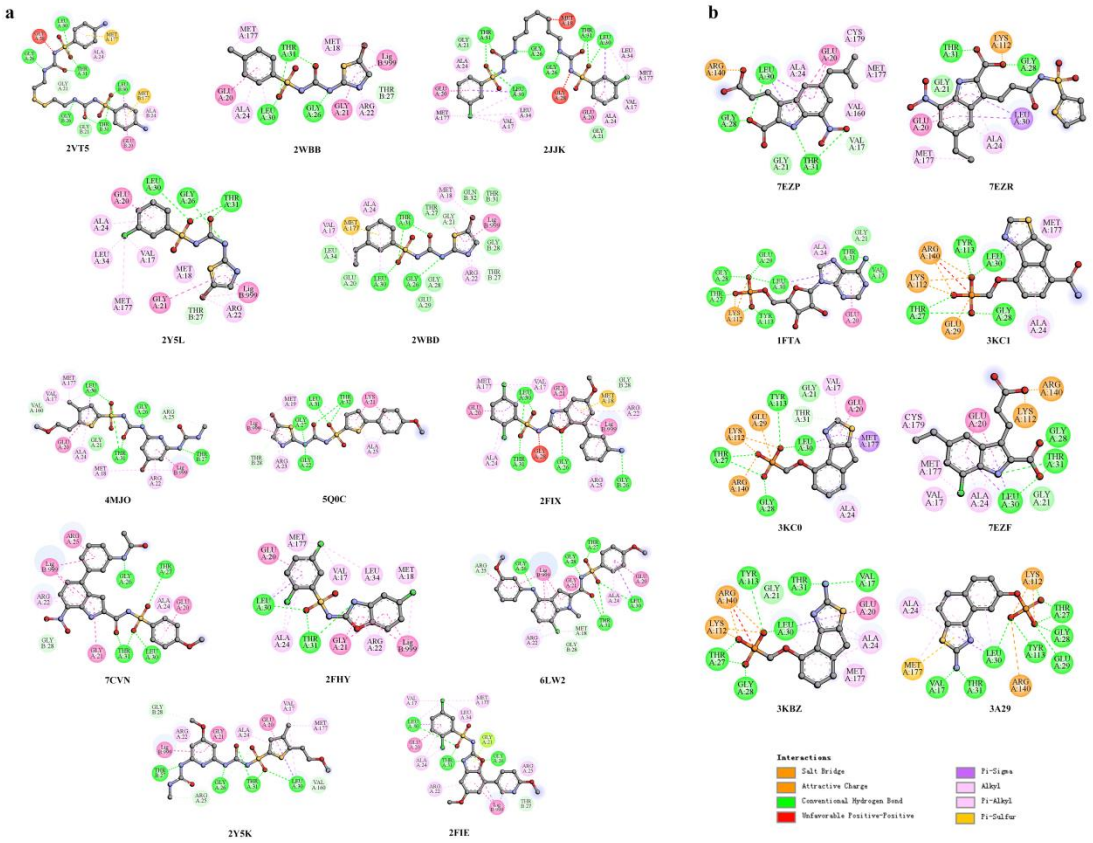


Figure S-1. The ligand structures in co-crystal structures of FBPase and their interactions with key amino acids.

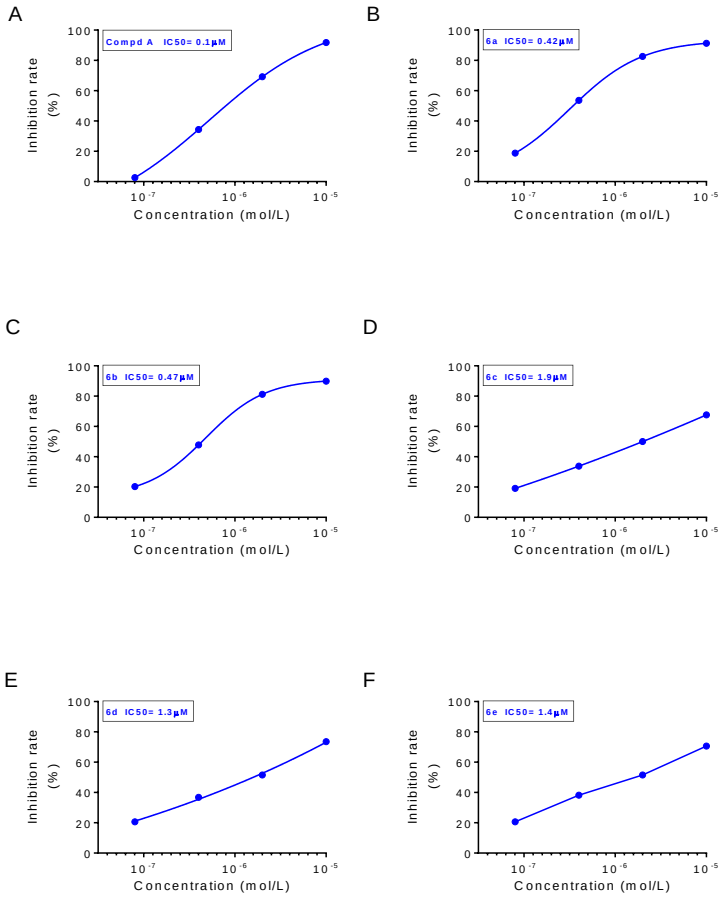


Figure S-2. IC_{50} curves of Compd A and compounds **6a-6i.** (A) Compd A, (B) 6a, (C) 6b, (D) 6c, (E) 6d, (F) 6e.

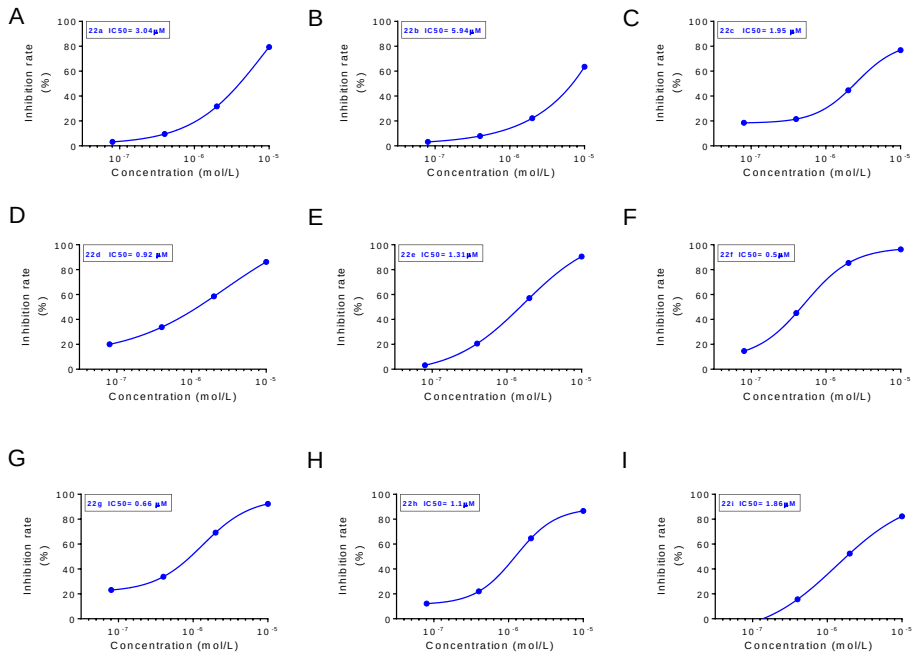


Figure S-3. IC_{50} curves of compounds **22a-22i.** (A) 22a, (B) 22b, (C) 22c, (D) 22d, (E) 22e, (F) 22f, (G) 22g, (H) 22h, (I) 22i.