Supporting Information

A Novel Reactive Turn-On Probe Capable of Selective Profiling and No-Wash Imaging of Bruton's Tyrosine Kinase in Live Cells

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1. General Information

All chemicals were purchased from commercial vendors and used without further purification, unless indicated otherwise. All reactions requiring anhydrous conditions were carried out under argon or nitrogen atmosphere using oven-dried glassware. AR-grade solvents were used for all reactions. Reaction progress was monitored by TLC on pre-coated silica plates (Merck 60 $F_{254\ nm}$, 0.25 μm) and spots were visualized by UV, iodine or other suitable stains. Flash column chromatography was carried out using silica gel (Qingdao Ocean company). All NMR spectra (^{1}H-NMR, ^{13}C-NMR) were recorded on Bruker 300 MHz/400 MHz NMR spectrometers. Chemical shifts were reported in parts per million (ppm) referenced with respect to appropriate internal standards or residual solvent peaks ($CDCl_3$ = 7.26 ppm, DMSO-d_6 = 2.50 ppm). The following abbreviations were used in reporting spectra, br s (broad singlet), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets). Mass spectra were obtained on Agilent LC-ESI-MS system. All analytical HPLC were carried out on Agilent system. Water with 0.1% TFA and acetonitrile with 0.1% TFA were used as eluents and the flow rate was 0.5 mL/min. Antibodies against BTK (ab137503) were purchased from Abcam. Click reagents were purchased from Click Chemistry Tools company (https://clickchemistrytools.com/). The recombinant human BTK protein was purchased from Sino Biological Inc (Cat: 10578-H08B).

2. Cell culture and Western blot

Cell lines were obtained from the National Cancer Institute Developmental Therapeutics Program (NCI-60). Cells were cultured in Dulbecco's modified Eagle medium (DMEM; Invitrogen, Carlsbad, CA) or RPMI 1640 Medium (Invitrogen, Carlsbad, CA) containing 10% heat-inactivated fetal bovine serum (FBS; Invitrogen), 100 units/mL penicillin, and 100 μg/mL streptomycin (Thermo Scientific) and maintained in a humidified 37 °C incubator with 5% CO_2. To generate protein lysates, cells were washed twice with cold phosphate-buffered saline (PBS), harvested with 1 × trypsin or by use of a cell scraper, and collected by centrifugation. Cell pellets were then washed with PBS and lysed with RIPA (Thermo Scientific™，#89900) lysis and extraction buffer (with Pierce™ Protease Inhibitor Tablets, Thermo Scientific™, #A32955). Protein concentration was determined by Pierce™ BCA Protein Assay Kit (Thermo Scientific™, #23252) and Synergy H1 Hybrid Multi-Mode Reader (BioTek). For Western blotting experiments, samples were resolved by SDS−polyacrylamide gels and transferred to poly membranes. Membranes were then blocked with 3% bovine serum albumin (BSA) in TBST (0.1% Tween in Tris-buffered saline) for 1 h at room temperature. After blocking, membranes were incubated with the corresponding primary antibody for another 1 hour. After incubation, membranes were washed with TBST (4 × 10 min) and then incubated with an appropriate secondary antibody. Finally, blots were washed again with TBST before being developed with SuperSignal West Dura Kit (Thermo Scientific), and finally imaged with Amersham Imager 600 (GE Healthcare). Cell Counting Kit-8 (CCK-8, DOJINDO, #CK04) was used for cell proliferation assay. Proteome labeling, in-gel fluorescence scanning and cellular imaging experiments were performed as previously reported.[1-4]

3. Table S1. Fluorescent probes and reporter tags used in current study

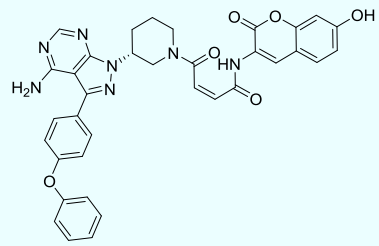
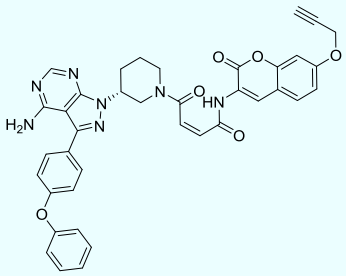
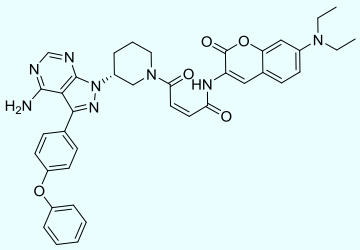
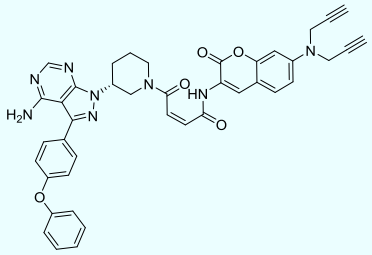
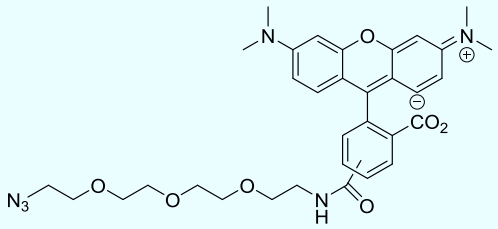
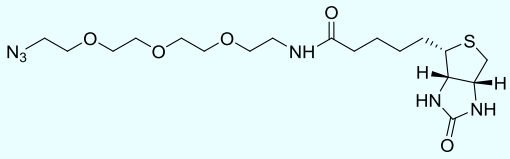
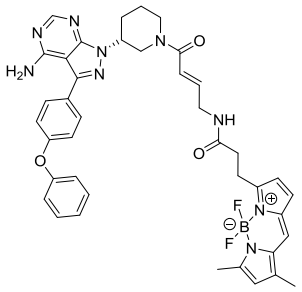
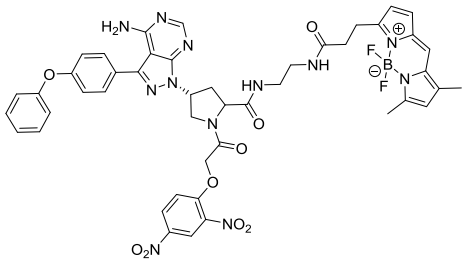
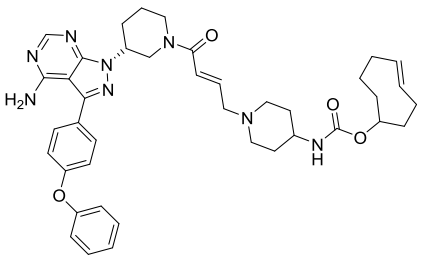
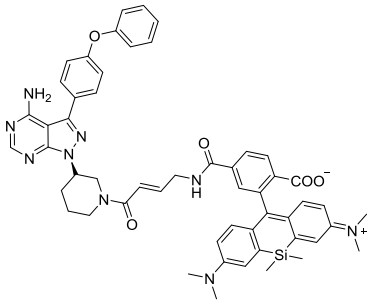
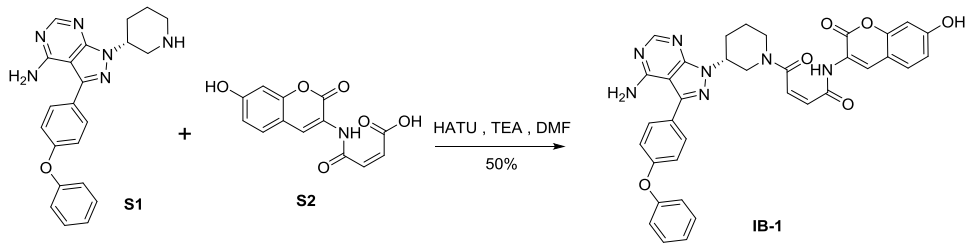
 **IB-1**	 **IB-2**
 **IB-3**	 **IB-4**
 **TAMRA-N_3**	 **Biotin-N_3**

Table S2. Previously reported ibrutinib-derived probes

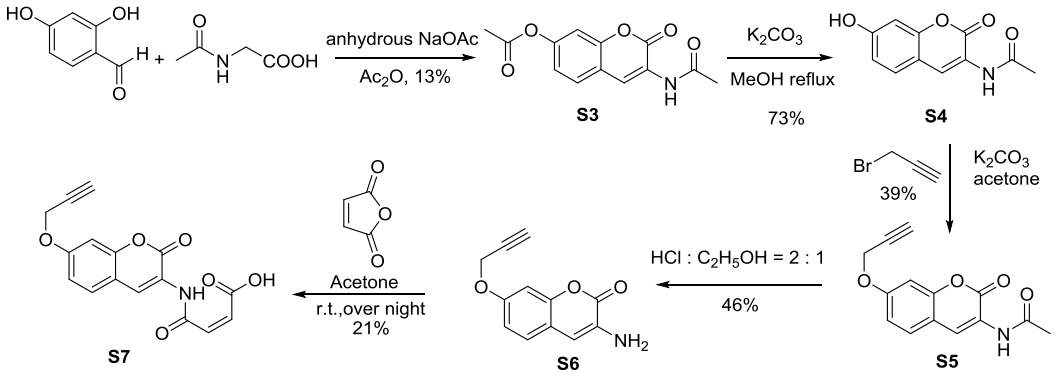
 *Bioconjugate. Chem.* **2015**, 26, 1513	 *Chem. Commun.*, **2014**, 50, 15319-15322
 *Chem. Commun. 2019, 55,834*	 *Sci. Rep*. **2014**, 4, 4782.

4. Chemical Synthesis



Scheme S1

(IB-1). The intermediate **S1** is commercially available and the **S2** was synthesized based on previously published procedures.[5] To a stirred solution of **S2** (28 mg, 0.1 mmol) in 5 mL DMF was added HATU (46 mg, 0.12 mmol), **S1** (39 mg, 0.1 mmol) and TEA (0.04 mL, 0.2 mmol). The reaction was stirred at room temperature overnight prior to addition of 10 mL water and then extracted with ethyl acetate (2 × 10 mL), the combined organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by flash column (methanol:CH_2Cl_2 = 1:50) to give **IB-1** as a light yellow solid (32 mg, 50%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.37 (s, 1H), 10.00 (s, 0.5H), 9.95 (s, 0.5H), 8.65 (s, 0.5H), 8.62 (s, 0.5H), 8.27 (s, 0.5H), 8.08 (s, 0.5H), 7.73 – 7.52 (m, 3H), 7.48 – 7.37 (m, 2H), 7.20 – 7.07 (m, 5H), 6.80 (d, *J* = 8.8 Hz, 1H), 6.74 (t, *J* = 2.3 Hz, 1H), 6.62 (d, *J* = 4.9 Hz, 1H), 6.52 (s, 1H), 4.91 – 4.71 (m, 1H), 4.60 (dd, *J* = 12.5, 4.3 Hz, 0.5H), 4.37 (d, *J* = 12.8 Hz, 0.5H), 3.89 (dd, *J* = 13.0, 4.2 Hz, 0.5H), 3.73 (d, *J* = 13.0 Hz, 0.5H), 3.56 (dd, *J* = 13.0, 10.4 Hz, 0.5H), 3.33 (s, 2H), 3.18 (t, *J* = 11.7 Hz, 0.5H), 3.10 (t, *J* = 12.1 Hz, 0.5H), 2.84 (t, *J* = 12.4 Hz, 0.5H), 2.31 – 2,16 (m, 1H), 2.16 – 2.05 (m, 1 H), 1.94 – 1.77 (m, 1H), 1.76 – 1.57 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.32, 159.79, 158.21, 157.77, 157.11, 156.29, 155.47, 153.9, 136.29, 136.03, 130.12, 130.10, 130.01, 129.26, 126.45, 125.14, 123.77, 120.68, 118.96, 113.66, 111.35, 101.95, 97.38, 52.29, 51.97, 45.64, 44.44, 24.15, 23.26. HR-MS (m/z) [M + H]$^+$ calcd: 644.2252; Found:644.2240. HPLC purity = 97.62%，Rt: 4.33 min (MeOH:H_2O = 90:10). NMR data showed that a pair of rotamers exists due to the amide bond rotation.



Scheme S2

(S3). A mixture of 2,4-dihydroxy benzaldehyde (5.52 g, 40 mmol), N-acetylglycine (4.68 g, 40 mmol), anhydrous sodium acetate (9.84 g, 120 mmol) in acetic anhydride (100 mL) was refluxed for 2 h. The reaction mixture was poured into ice (300 mL) to give a yellow precipitate **S3** which can be used in the next step directly (1.4 g, 13%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.76 (s, 1H), 8.62 (s, 1H), 7.74 (d, *J* = 8.5 Hz, 1H), 7.26 (d, *J* = 2.2 Hz, 1H), 7.13 (dd, *J* = 8.5, 2.2 Hz, 1H), 2.30 (s, 3H), 2.16 (s, 3H).

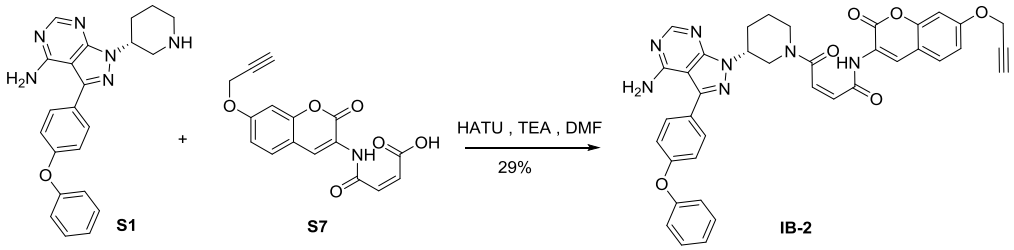
(S4). **S3** (1.31 g, 5 mmol) and K_2CO_3 (1.38 g, 15 mmol) in MeOH (25 mL) were refluxed for 2 h. The reaction mixture was evaporated, the residue was dissolved in water (25 mL), followed by acidification with 2N HCl (pH = 3), the resulting precipitate was filtered to yield a light brown solid **S4** (800 mg, 73%). ^{1}H NMR (400 MHz,

DMSO-d_6) δ 10.36 (s, 1H), 9.57 (s, 1H), 8.50 (s, 1H), 7.51 (d, *J* = 8.5 Hz, 1H), 6.79 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.73 (d, *J* = 2.2 Hz, 1H), 2.13 (s, 3H).

(S5). To a 100 mL round-bottomed flask was added **S4** (657 mg, 3 mmol), K_2CO_3 (622 mg, 4.5 mmol) and anhydrous acetone (20 mL), the resulting mixture was stirred at 60 ℃ for 1 h. Subsequently, 3-bromoprop-1-yne (0.388 mL, 4.5 mmol) was added to the mixture dropwisely and the mixture was stirred at 60 ℃ for 6 h. The reaction mixture was filtered and the solvent was removed to give a crude product. The crude product was purified by flash column (EA:PE = 1:3) to give the compound **S5** (298 mg, 39%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.65 (s, 1H), 8.56 (s, 1H), 7.65 (d, *J* = 8.7 Hz, 1H), 7.06 (d, *J* = 2.5 Hz, 1H), 6.99 (dd, *J* = 8.6, 2.5 Hz, 1H), 4.92 (d, *J* = 2.4 Hz, 2H), 3.65 (t, *J* = 2.3 Hz, 1H), 2.15 (s, 3H).

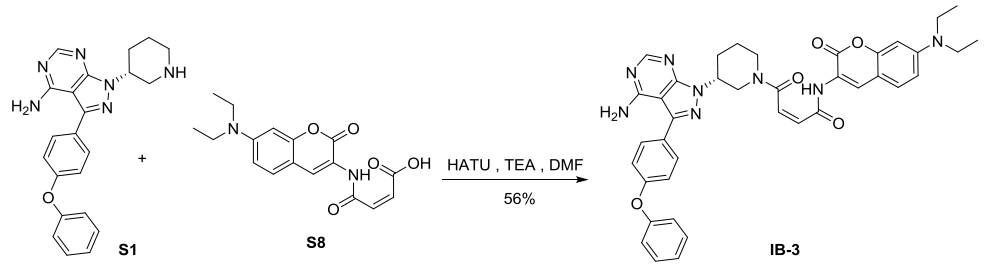
(S6). The compound **S5** (100 mg, 0.39 mmol) was refluxed in a solution of HCl (37%) and ethanol (2:1) for 2 h. Subsequently, the solution was poured into ice followed by addition of 30% NaOH aqueous solution until pH is 5~6, the solution was then concentrated to get the crude product, which was further recrystallized from EtOH to give **S6** (73.8 mg, 46%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.37 (dd, *J* = 8.6, 2.1 Hz, 1H), 6.97 (d, *J* = 2.4 Hz, 1H), 6.89 (dd, *J* = 8.6, 2.5 Hz, 1H), 6.72 (d, *J* = 2.1 Hz, 1H), 5.43 (s, 2H), 4.84 (d, *J* = 2.3 Hz, 2H), 3.60 (t, *J* = 2.4 Hz, 1 H).

(S7). The mixture of **S6** (65 mg, 0.3 mmol) and maleic anhydride (36 mg, 0.36 mmol) in 5 mL acetone was stirred at room temperature overnight. After filtration, the yellow solid was washed by acetone to give product **S7** (21 mg, 21%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.27 (s, 1H), 8.68 (s, 1H), 7.70 (d, *J* = 8.7 Hz, 1H), 7.08 (d, *J* = 2.5 Hz, 1H), 7.02 (dd, *J* = 8.7, 2.5 Hz, 1H), 6.62 (d, *J* = 12.1 Hz, 1H), 6.42 (d, *J* = 12.0 Hz, 1H), 4.93 (d, *J* = 2.4 Hz, 2H), 3.65 (q, *J* = 2.1 Hz, 1H). MS (ESI) *m/z*: 312.1[M-H]$^+$



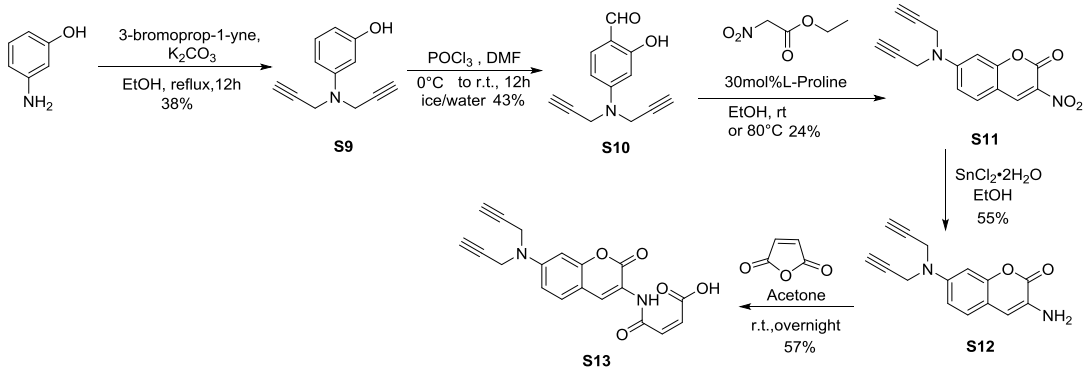
Scheme S3

(IB-2). The intermediate **S1** is commercially available. To a stirred solution of **S7** (17 mg, 0.05 mmol) in 2 mL DMF was added HATU (23 mg, 0.06 mmol), **S1** (20 mg, 005 mmol) and TEA (0.02 mL, 0.1 mmol). The reaction was stirred at room temperature overnight prior to addition of 5 mL water and then extracted with ethyl acetate (2 × 5 mL), the combined organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by flash column (methanol : CH_2Cl_2 = 1:30) to give **IB-2** as a yellow solid (10 mg, 29%).
^{1}H NMR (400 MHz, DMSO-d_6) δ10.08 (s, 0.5H), 10.03 (s, 0.5H), 8.71 (s, 0.5H), 8.68 (s, 0.5H), 8.27 (s, 0.5H), 8.09 (s, 0.5H), 7.77 – 7.65 (m, 2H), 7.61 (d, *J* = 8.6 Hz, 1H), 7.42 (q, *J* = 8.1 Hz, 2H), 7.21 – 7.08 (m, 5H), 7.06 (t, *J* = 2.9 Hz, 1H), 7.00 (d, *J* = 8.6 Hz, 1H), 6.65 (d, *J* = 3.1 Hz, 1H), 6.54 (d, *J* = 1.6 Hz, 1H), 4.92 (s, 2H), 4.88 – 4.71 (m, 1H), 4.61 (d, *J* = 8.6 Hz, 0.5H), 4.37 (d, *J* = 12.7 Hz, 0.5H), 3.90 (d, *J* = 9.4 Hz, 0.5H), 3.74 (d, *J* = 12.6 Hz, 0.5H), 3.64 (q, *J* = 2.2 Hz, 1H), 3.61 – 3.52 (m, 0.5H), 3.23 – 3.16 (m, 0.5H), 3.10 (t, *J* = 11.7 Hz, 0.5H), 2.84 (t, *J* = 11.3 Hz, 0.5H), 2.35 – 2.17 (m, 1H), 2.17 – 2.03 (m, 1H), 1.96 – 1.76 (m, 1H), 1.77 – 1.51 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.47, 158.70, 157.57, 157.11, 156.28, 155.67, 153.96, 151.16, 136.46, 136.23, 130.11, 130.09, 130.0, 129.03, 127.83, 125.41, 125.12, 123.77, 121.94, 118.96, 113.30, 101.61, 97.39, 78.84, 78.61, 56.06, 52.30, 49.85, 44.46, 23.27. HR-MS (mz) [M + H]$^+$ calcd: 682.2409; Found: 682.2396. HPLC purity = 100%, Rt: 4.79 min (MeOH : H_2O = 90 : 10). NMR data showed that a pair of rotamers exists due to the amide bond rotaion.



Scheme S4

(IB-3). The intermediate **S1** is commercially available and the **S8** was synthesized based on previously published procedures.[6] To a stirred solution of **S8** (33 mg, 0.1 mmol) in 5 mL DMF was added HATU (46 mg, 0.12 mmol), **S1** (39 mg, 0.1 mmol) and TEA (0.03 mL, 0.2 mmol). The reaction was stirred at room temperature overnight prior to addition of 3 mL water and then extracted with ethyl acetate (2 × 5 mL), the combined organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by flash column (methanol:CH_2Cl_2 = 1:50) to give **IB-3** as a yellow solid (39 mg, 56%). ^{1}H NMR (400 MHz, DMSO-d_6) δ9.89 (s, 0.5H), 9.83 (s, 0.5H), 8.59 (s, 0.5H), 8.54 (s, 0.5H), 8.27 (s, 0.5H), 8.10 (s, 0.5H), 7.69 (d, *J* = 8.6 Hz, 1H), 7.61 (d, *J* = 8.6 Hz, 1H), 7.51 – 7.38 (m, 3H), 7.21 – 7.07 (m, 5H), 6.70 (d, *J* = 8.9 Hz, 1H), 6.59 (d, *J* = 3.2 Hz, 1H), 6.53 (t, *J* = 2.9 Hz, 1H), 6.49 (d, *J* = 2.7 Hz, 1H), 4.83 (m, 1H), 4.60 (dd, *J* = 12.5, 4.4 Hz, 0.5H), 4.37 (dd, *J* = 10.2, 6.7 Hz, 0.5H), 3.90 (dd, *J* = 13.2, 4.2 Hz, 0.5H), 3.74 (d, *J* = 13.2 Hz, 0.5H), 3.57 (dd, *J* = 13.0, 10.4 Hz, 0.5H), 3.40 (q, *J* = 5.7, 5.0 Hz, 4H), 3.18 (t, *J* = 11.7 Hz 0.5H), 3.09 (t, *J* = 11.6 Hz, 0.5H), 2.83 (t, *J* = 12.2 Hz, 0.5H), 2.30 – 2.17 (m, 1H), 2.16 – 2.04 (m, 1H), 1.94 – 1.76 (m, 1H), 1.76 – 1.58 (m, 1H). 1.14 – 1.06 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.03, 158.21, 158.13, 156.29, 155.67, 153.96, 152.71, 149.27, 135.84, 135.58, 130.11, 130.08, 130.00, 128.98, 127.91, 127.59, 125.29, 123.76, 118.95, 118.38, 109.48, 107.44, 97.38, 96.58, 52.29, 51.97, 49.85, 43.99, 24.14, 23.27, 12.31. HR-MS (m/z) $[M + H]^+$ calcd: 699.3038; Found: 699.3026. HPLC purity = 100%，Rt: 17.95 min (MeOH:H_2O = 80:20). NMR data showed that a pair of rotamers exists due to the amide bond rotation.



Scheme S5

(S9). To a solution of 3-aminophenol (11 g, 100 mmol) in 500 mL ethanol was added 3-bromoprop-1-yne (34.4 mL, 400 mmol), potassium carbonate (28 g, 200 mmol) and refluxed for 3 h. Upon completion of the reaction, the reaction mixture was extracted with ethyl acetate and water. The organic layer was dried with anhydrous Mg_2SO_4 and evaporated. The product **S9** (7.3 g, 38%) was purified by flash column (PE:EA = 50:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (s, 1H), 7.00 (t, *J* = 8.1 Hz, 1H), 6.36 (dd, *J* = 7.9, 2.1 Hz, 1H), 6.32 (t, *J* = 2.3 Hz, 1H), 6.23 (dd, *J* = 7.9, 1.5 Hz, 1H), 4.06 (d, *J* = 2.4 Hz, 4H), 3.15 (t, *J* = 2.3 Hz, 2H).

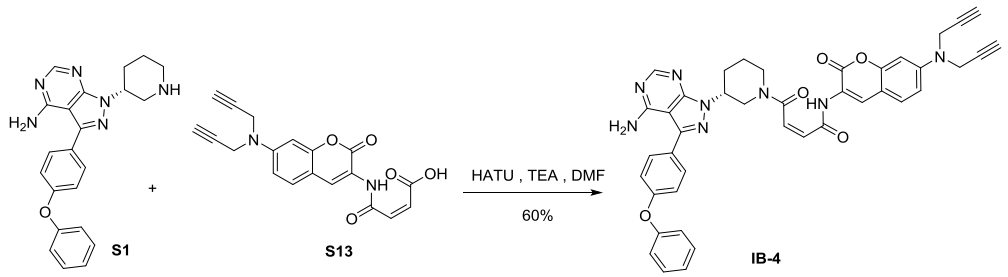
(S10). $POCl_3$ (3.26 mL, 35 mM) was added slowly to anhydrous DMF (30 mL) and stirred for 30 min. After that compound **S9** (6.5 g, 35 mmol) in DMF (10 mL) was added dropwisely. The mixture was slowly warmed to room temperature and stirred overnight. The reaction solution was poured into ice and stirred for a few minutes and then

filtered to give **S10** (3.17 g, 43%) as a brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.82 (s, 1H), 7.55 (d, *J* = 8.9 Hz, 1H), 6.53 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.32 (d, *J* = 2.4 Hz, 1H), 4.28 (d, *J* = 2.5 Hz, 4H), 3.27 – 3.24 (m, 2H).

(S11). To the solution of **S10** (2.13 g, 10 mmol) and ethyl nitroacetate (1.1 mL, 10 mmol) in 50 ml ethanol was added L-proline (30 mol %). After completion of the reaction (monitored by TLC), the solvent was evaporated and the product was dissolved in 20 mL $CHCl_3$ and then washed with water (3 × 20 mL). The organic layer was washed with 20 mL brine, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The product **S11** (675 mg, 24%) was recrystallized from ethanol. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (s, 1H), 7.87 (d, *J* = 9.0 Hz, 1H), 7.07 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.86 (d, *J* = 2.4 Hz, 1H), 4.45 (d, *J* = 2.5 Hz, 4H), 3.32 (t, *J* = 2.4 Hz, 2H).

(S12). To a 10 mL round-bottomed flask was added $SnCl_2$ $2H_2O$ (3.38 g, 15 mmol), 20 mL 37% HCl, and compound **S11** (564 mg, 2 mmol). The resulting solution was further stirred at room temperature for 6 h. After that, a solution of 5 M NaOH was added followed by extraction with ethyl acetate. The organic layer was dried with anhydrous Na_2SO_4 and evaporated. The crude product was obtained as a brown solid (139 mg, 55%) which was used in the next step directly. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.30 (d, *J* = 8.6 Hz, 1H), 6.93 – 6.76 (m, 2H), 6.71 (s, 1H), 5.27 (s, 2H), 4.20 (d, *J* = 2.5 Hz, 4H), 3.26 – 3.13 (m, 2H).

(S13). The mixture of **S12** (126 mg, 0.5 mmol) and maleic anhydride (59 mg, 0.6 mmol) in 8 mL acetone was stirred at room temperature overnight. After filtration, the yellow solid was washed by acetone to give product **S13** (100 mg, 57%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.99 (s, 1H), 10.19 (s, 1H), 8.62 (s, 1H), 7.60 (d, *J* = 8.8 Hz, 1H), 6.96 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.85 (d, *J* = 2.5 Hz, 1H), 6.63 (d, *J* = 12.1 Hz, 1H), 6.41 (d, *J* = 12.0 Hz, 1H), 4.30 (d, *J* = 2.5 Hz, 4H), 3.23 (t, *J* = 2.3 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.87, 163.60, 157.88, 151.72, 148.85, 132.48, 128.67, 128.56, 126.65, 120.3, 111.95, 110.13, 100.24, 79.52, 75.37. MS (ESI) *m/z*: 349.1[M-H]$^+$



Scheme S6

(IB-4). The intermediate **S1** is commercially available. To a stirred solution of **S13** (11 mg, 0.03 mmol) in 2 mL DMF was added HATU (14 mg, 0.036 mmol), **S1** (12 mg, 0.03 mmol) and TEA (0.01 mL, 0.06 mmol). The reaction was stirred at room temperature overnight prior to addition of 3 mL water and then extracted with ethyl acetate (2 × 5 mL), the combined organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by flash column (methanol:CH_2Cl_2 = 1:50) to give **IB-4** as a yellow solid (13 mg, 60%). ^{1}H NMR (400 MHz, DMSO-d_6) δ10.02 (s, 0.5H), 9.97 (s, 0.5H), 8.67 (s, 0.5H), 8.64 (s, 0.5H), 8.28 (s, 0.5H), 8.10 (s, 0.5H), 7.69 (d, *J* = 8.7 Hz, 1H), 7.67 – 7.57 (m, 2H), 7.49 – 7.37 (m, 2H), 7.23 – 7.08 (m, 5 H), 6.95 (dd, *J* = 8.7, 2.5 Hz, 1H), 6.85 (t, *J* = 2.4 Hz, 1H), 6.62 (d, *J* = 3.0 Hz, 1H), 6.52 (d, *J* = 1.7 Hz, 1H), 4.93 – 4.72 (m, 1H), 4.60 (dd, *J* = 12.5, 4.4 Hz, 0.5H), 4.37 (d, *J* = 12.8 Hz, 0.5H), 4.29 (s, 4H), 3.90 (d, *J* = 10.1 Hz, 0.5H), 3.74 (d, *J* = 12.9 Hz, 0.5H), 3.56 (dd, *J* = 13.1, 10.4 Hz, 0.5H), 3.22 (q, *J* = 2.3 Hz, 2H), 3.17 (d, *J* = 11.7 Hz, 0.5H), 3.10 (t, *J* = 11.6 Hz, 0.5H), 2.84 (t, *J* = 11.7 Hz, 0.5H), 2.32 – 2.18 (m, 1H), 2.12 (dd, *J* = 13.0, 3.9 Hz, 1H), 1.94 – 1.76 (m, 1H), 1.76 – 1.58 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.25, 157.87, 157.10, 156.29, 153.96, 151.60, 148.74, 143.23, 136.17, 135.95, 130.12, 130.10, 130.02, 128.60, 126.37, 125.19, 123.77, 120.36, 118.96, 111.89, 110.17, 100.20, 97.38, 79.42, 75.27, 54.91, 52.27, 51.97, 44.45, 24.15, 23.27. HR-MS (m/z) [M +

H]$^+$ calcd: 719.2725; Found: 719.2691. HPLC purity = 99.61%，Rt: 9.88 min (MeOH : H_2O = 90:10). NMR data showed that a pair of rotamers exists due to the amide bond rotation.

5. The reaction mechanism between the probe and BTK.

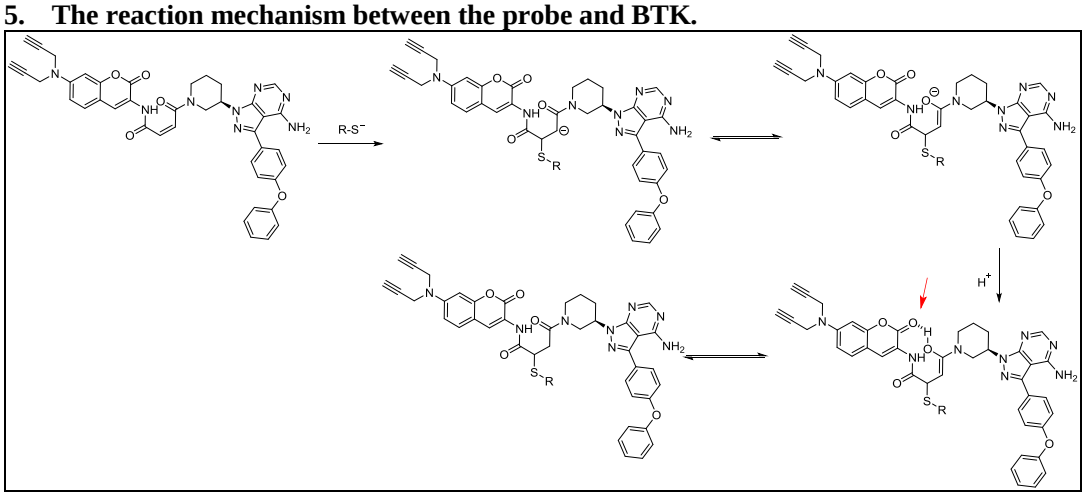


Figure S1. Proposed reaction mechanism between the probe and BTK protein, an intramolecular hydrogen bond was formed in the intermediate (red arrow indicated).

6. *In Vitro* Enzymatic Activity Assay and Cell Growth Inhibition Assay[7]

All the probes were evaluated with the BTK kinase inhibition using Z'-LYTE™ fluorescence resonance energy transfer (FRET) method, ibrutinib was used as the reference compounds. The Z'-LYTE™ biochemical assay employs a FRET-based, coupled-enzyme format and is based on the differential sensitivity of phosphorylated and non-phosphorylated peptides to proteolytic cleavage. The peptide substrate is labeled with two fluorophores-one at each end-that make up a FRET pair. The compounds were diluted three-fold from $5.1x10^{-9}$ M to $1x10^{-4}$ M in DMSO. Plate was measured on EnVision Multilabel Reader (Perkin Elmer). Curve fitting and data presentations were performed using Graph Pad Prism version 4.0. Cytotoxicity assays were carried out using Raji cells by CCK-8 assay. 9000 cells per well were seeded in a 96-well plate (100 μL medium/well) and incubated in a humidified incubator for adherence. The probes and parent inhibitors in DMSO were added to cells at the final concentrations (DMSO never exceeded 1‰) of 100, 50, 25, 12.5 6.25, 3.12, 1.56, 0.78, 0.39 and 0.19 μM and further incubated for 48 h. CCK-8 reagent (10 μL) was added to each well and incubated for 2 h. Following that, the absorbance was measured at 450 nm and 650 nm on a plate reader (Synergy HI, BioTek Instruments, Inc. Vermont, US). Cell viability rate was determined as VR = $(A - A_0)/(As - A_0) \times 100\%$, where A is the absorbance of the experimental group, As is the absorbance of the control group (DMSO was used as the control) and A_0 is the absorbance of the blank group (no cells). IC_{50} values were calculated using GraphPad Prism

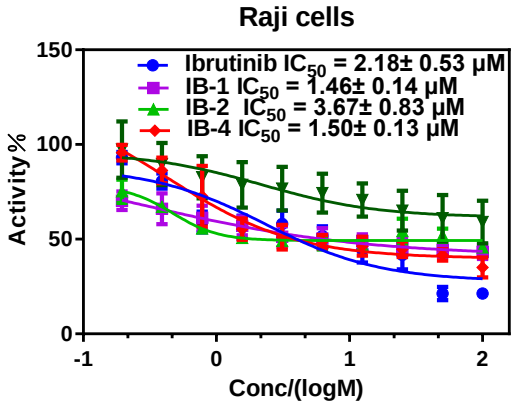


Figure S2. IC_{50} values of **IB-1/2/3/4** against Raji cancer cells.

7. Fluorescent Properties Measurements

For the measurement of fluorescence turn-on, purified recombinant BTK, BSA, Cys and GSH were diluted to the desired concentrations in PBS buffer (50 μL). Subsequently, **IB-1/2/3/4** in DMSO (1 μM final concentration) was added to the mixture. The fluorescence intensity was monitored and recorded by EnVision Multilabel Reader (Perkin Elmer) (Ex = 405 nm, Em = 475 nm) using OptiPlate™ –384F at 25 ℃. For competition experiments, the competitor ibrutinib was diluted to desired concentrations in PBS (50 μL) and pre-incubated with BTK at room temperature for 30 min, **IB-4** was then added. Probe **IB-4** in DMSO (1 μL) was added to PBS buffer (50 μL) as a control (final probe concentration is 1 μM **IB-4** with 0.5 μM BTK), the emission spectra of the reaction system was recorded by EnVision Multilabel Reader (Perkin Elmer) (Ex = 405 nm, Em = 475 nm) at different time points (0 min, 5min, 10 min, 30 min, 60 min).

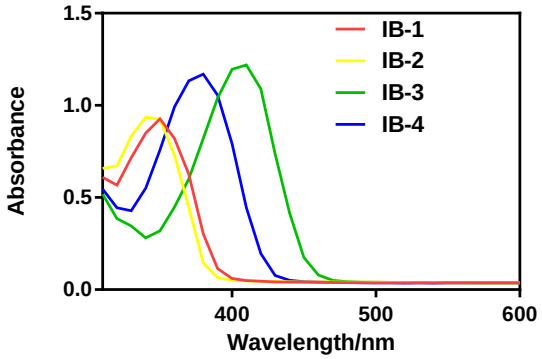


Figure S3. Absorbance spectra of **IB-1/IB-2/IB-3/IB-4** in the presence of recombinant BTK protein.

8. *In Vitro and In Situ* Proteome Labeling

For gel-based recombinant protein labeling, **IB-4** (1 μM final probe concentration) was incubated with purified BTK protein at different final concentrations in PBS buffer for 30 min at 37 ℃ with gentle shaking. Subsequently, the labeled proteins were subjected to click reaction with TAMRA-azide under standard click chemistry conditions (20 μM TAMRA-N_3 from 1 mM stock solution in DMSO, 50 μM THPTA from 2.5 mM freshly prepared stock solution in DMSO, 0.5 mM TCEP from 25 mM freshly prepared stock solution in deionized water, and 0.5 mM $CuSO_4$ from 25 mM freshly prepared stock solution in deionized water). After 2 h, 2×SDS loading dye was added and the mixture was heated to 90 ℃ for 2-5 min. The resulting proteins were resolved by SDS-PAGE. In-gel fluorescence scanning was used to visualize the labeled protein bands. Both in-gel fluorescence scanning (FL) and coomassie staining (CBB) were always carried out on the gels upon SDS-PAGE separation of labeled samples.

For in situ proteome labeling, cells were grown to 80−90% confluency in 6-well plates under conditions as described above. The medium was removed and washed twice with PBS and then treated with 2 mL probe-containing medium in the presence or absence of excessive competitors (diluted from DMSO stocks whereby DMSO never exceeded 1% in the final solution). After 2-5 h of incubation, the medium was aspirated and cells were washed twice with PBS to remove excessive probe. The cells were lysed with 200 μL RIPA lysis buffer (Thermo Scientific™ #89900) containing phosphatase inhibitor (Thermo Scientific™ #88669) on ice for 30 min. A soluble protein solution was obtained by centrifugation for 10 min (14000 rpm, 4 ℃). Eventually, the protein concentrations were determined by using the BCA protein assay (Pierce™ BCA protein assay kit) and diluted to 1 mg/mL with PBS. A freshly pre-mixed click chemistry reaction cocktail (20 μM TAMRA-N_3 from 1mM stock solution in DMSO, 50 μM THPTA from 2.5 mM freshly prepared stock solution in DMSO, 0.5 mM TCEP from 25 mM freshly prepared stock solution in deionized water, and 0.5 mM $CuSO_4$ from 25 mM freshly prepared stock solution in deionized water) was added to the labeled proteome. The reaction was further incubated for 2 h prior to addition of pre-chilled acetone (-20 ℃). The precipitated proteins were subsequently collected by centrifugation

(14000 rpm, 10 min at 4 °C), and washed with 200 μL of prechilled methanol. The samples were dissolved in 1×SDS loading buffer and heated for 10 min at 95 °C. 20 μg proteins for each lane were loaded on SDS−PAGE (10% gel) and then visualized by in-gel fluorescence scanning (Typhoon FLA 9500).

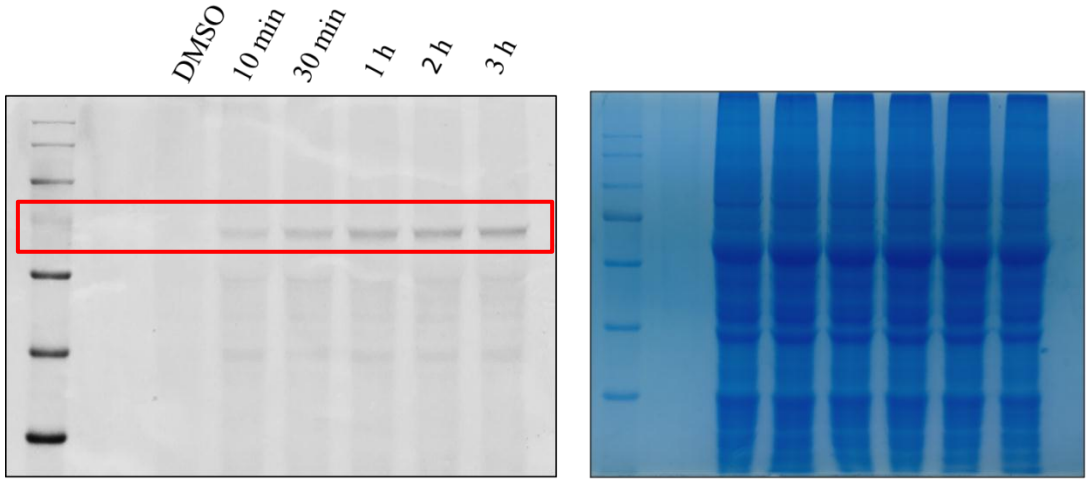


Figure S4. Time-dependent labelling profiles of **IB-4** (1 μM) and with Namalwa cells.

9. Pull down and Targets Validation

To identify the interacting cellular targets of **IB-4**, pull-down (PD) experiments were carried out, and followed by Western blotting (WB) and LC-MS/MS, where applicable. The general pull-down experiments were based on previously reported procedures,[1-4] with the following optimizations. Namalwa and Toledo cells were grown to 80−90% confluency under the conditions described above. The medium was removed and the cells were treated with probe-containing medium in the presence or absence of corresponding competitors. After 3 h of incubation, the medium was aspirated, and cells were washed twice with PBS to remove excessive probe. The cells were lysed with RIPA buffer and centrifuged for 10 min (14000 rpm, 4 ℃) to get a soluble protein solution. Eventually, the protein concentrations were determined by BCA protein assay and then diluted to 1 mg/mL with PBS. A freshly premixed click chemistry reaction cocktail was added (20 μM Biotin-N_3 from 1 mM stock solution in DMSO, 50 μM THPTA from 2.5 mM freshly prepared stock solution in DMSO, 0.5 mM TCEP from 25 mM freshly prepared stock solution in deionized water, and 0.5 mM $CuSO_4$ from 25 mM freshly prepared stock solution in deionized water). The reaction was further incubated for 2 h with gentle mixing prior to precipitation by addition of pre-chilled acetone (−20 °C). Precipitated proteins were subsequently collected by centrifugation (13000 rpm, 10 min at 4 ℃) and dissolved in PBS containing 1% SDS. Upon incubation with streptavidin beads for 4 hours at rt, the beads were washed with PBS containing 0.5% SDS (3 × 1 mL) and PBS (3 × 1 mL). The enriched proteins was eluted by 1 × loading buffer at 95 ℃ for 10 min and separated by SDS-PAGE (10%). Control pull-down experiments using the DMSO or in the presence of competitors were carried out concurrently with live cells. Western blotting experiments were carried out as previously described using the corresponding antibodies.

Subsequently, beads were resuspended in 500 μL 6 M urea in PBS, 25 μL of 200 mM DTT in 25 mM NH_4HCO_3 buffer was added and the reaction was incubated for 37 ℃ for 30 min. For alkylation, 25 μL of 500 mM IAA in 25 mM NH_4HCO_3 buffer was added and incubated for 30 min at room temperature in dark. Then, remove supernatant and wash bead by 1 mL PBS once. For the digestion, 150 μL 2 M urea in PBS, 150 μL 1 mM $CaCl_2$ in 50 mM NH_4HCO_3 and 1 μg of trypsin were added. The reaction was incubated at 37 ℃ overnight. The supernatants containing the digested peptides were collected, desalted with Waters C18 Tips and dried by vacuum centrifugation. The peptides were separated and analyzed on an Easy-nLC 1200 nano-HPLC system coupled to an

Orbitrap Fusion Lumos mass spectrometer (both - Thermo Fisher Scientific, USA). The raw data were processed and searched with MaxQuant.

10. Cellular Imaging

To validate the utility of the probes for imaging of potential cellular targets, fluorescence microscopy was further performed. The general procedures were similar to what was previously reported. [1-4] For no-wash imaging in live-cells, Namalwa and Jurkat cells seeded in glass bottom dishes and grown until 70−80% confluency.The cells were treated with 0.2 mL medium (1640 for Namalwa and Jurkat cells, IMDM for Toledo cells) with 1 μM **IB-3/4**, DMSO was performed as a control, and then imaged directly. For immunofluorescence (IF) experiments, after live-cell imaging, the cells were fixed for 1 h at room temperature with 3.7% formaldehyde in PBS, washed twice with cold PBS again, and permeabilized with 0.1% Triton X-100 in PBS for 30 min. Cells were then blocked with 2-5% BSA in PBS for 30 min, washed twice with PBS, and further incubated with anti-BTK (1:100 dilution) for 1 h at room temperature, washed twice with PBS, and then incubated with goat anti-rabbit IgG H&L (Alexa Fluor ® 488, 1:500 dilution) for 1 h, following by washing again with PBS before imaging. All imaging data were collected on a Zeiss LSM880 NLO (2+1 with BIG) Confocal Microscope System.

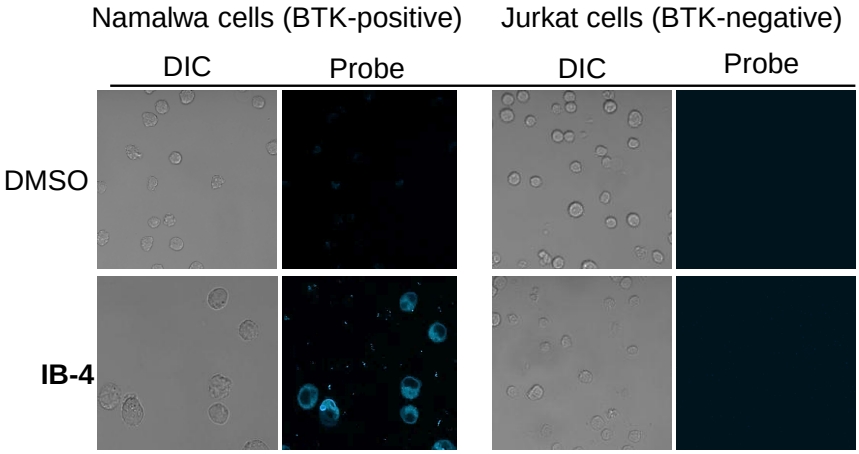


Figure S5. Cellular imaging of IB-4 with BTK-positive and BTK-negative cells.

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Table S3. Protein hits identified by LC-MS/MS with IB-4 in the presence or absence of its competitor Ibruitinb. "NaN" means "not available".

Protein names	Gene names	Mol.weight [kDa]	SILAC ratios(IB-4/IB-4+IB)			SILAC ratios(IB-4/DMSO)		
			1	2	3	1	2	3
Tyrosine-protein kinase BTK;Non-specific protein-tyrosine kinase	BTK	76.28	10.57	10.415	9.6632	12.972	12.066	13.386
Non-specific protein-tyrosine kinase;Tyrosine-protein kinase Blk	BLK	49.75	NaN	NaN	8.0766	12.493	11.923	11.553
60S ribosomal protein L35a	RPL35A	10.645	NaN	0.91728	1.4091	NaN	NaN	NaN
Acetyl-CoA carboxylase 1;Biotin carboxylase;Acetyl-CoA carboxylase 2;Biotin carboxylase	ACACA;ACACB	265.55	1.3962	1.563	1.3726	1.2368	1.1333	1.1701
Serine/arginine-rich splicing factor 7	SRSF7	27.366	1.4047	1.4517	1.3454	0.96801	0.99183	1.0796
Polypyrimidine tract-binding protein 1;Polypyrimidine tract-binding protein 3	PTBP1;PTBP3	9.1322	NaN	NaN	1.3231	1.8961	0.89069	1.6815
Dihydropyrimidinase-related protein 5	DPYSL5	20.867	1.2653	1.3581	1.3194	1.3014	NaN	NaN
Pre-mRNA-splicing factor 38B	PRPF38B	52.487	1.4863	1.0324	1.253	1.592	1.2462	2.119
Cytochrome b-c1 complex subunit 8	UQCRQ	9.9062	1.184	1.0857	1.2286	1.1137	NaN	NaN
Phosphate carrier protein, mitochondrial	SLC25A3	36.161	NaN	1.0465	1.2234	1.0599	1.0925	1.2136
60S ribosomal protein L10a	RPL10A	24.831	1.0242	1.2184	1.2232	0.87665	0.89074	1.1487
Histone H4	HIST1H4A	11.367	1.1868	1.2035	1.2104	1.0931	1.0853	1.1176
Serine/arginine repetitive matrix protein 1	SRRM1	93.435	0.97887	1.0008	1.1866	0.98895	0.91287	0.91964
Bcl-2-associated transcription factor 1	BCLAF1	83.231	1.2473	1.2182	1.1757	0.95908	1.0543	1.0322
60S ribosomal protein L22	RPL22	5.0827	0.85605	0.95499	1.1739	NaN	1.0618	1.2701
RNA-binding protein 39	RBM39	26.698	1.0608	1.0556	1.1714	1.5666	1.1605	0.92441
Transformer-2 protein homolog beta	TRA2B	33.665	1.4427	1.3398	1.171	0.94776	1.0298	1.132
Exportin-2	CSE1L	110.42	NaN	1.1279	1.155	1.0217	NaN	1.0357

Probable ATP-dependent RNA helicase DDX5;Probable ATP-dependent RNA helicase DDX17	DDX5;DDX17	5.9768	1.1059	1.2111	1.1492	NaN	1.0455	1.224
Heterogeneous nuclear ribonucleoprotein U	HNRNPU	90.583	1.1489	1.0888	1.1464	1.1984	0.83652	1.0329
60S ribosomal protein L4	RPL4	47.697	1.1897	1.1295	1.1215	1.1367	1.0527	1.0634
Nucleolin	NCL	76.613	1.0523	1.1055	1.1188	1.075	1.0469	0.97993
Histone H3;Histone H3.2;Histone H3.1t;Histone H3.3;Histone H3.1;Histone H3.3C	H3F3B;HIST2H3A;HIST3H3;H3F3A;HIST1H3A;HIST2H3PS2;H3F3C	14.914	1.2291	1.2111	1.1173	1.0326	1.0416	1.1027
60S ribosomal protein L15;Ribosomal protein L15	RPL15	24.146	1.1472	1.1216	1.1069	1.0386	1.1179	1.0597
Serine/arginine-rich splicing factor 1	SRSF1	28.329	0.9997	0.87112	1.1026	0.95783	0.84696	0.91252
60S ribosomal protein L3;60S ribosomal protein L3-like	RPL3;RPL3L	40.267	1.1056	1.0767	1.0969	1.1122	0.7794	1.0839
60S ribosomal protein L37;Ribosomal protein L37	RPL37	11.078	1.0839	1.0319	1.0968	0.97242	0.9729	1.0009
60S ribosomal protein L13a	RPL13a;RPL13A	16.731	1.0863	1.1223	1.0942	1.0938	1.0518	0.98054
Probable ATP-dependent RNA helicase DDX46	DDX46	117.46	1.0845	1.0755	1.0873	0.99087	0.9622	1.1507
Putative high mobility group protein B1-like 1;High mobility group protein B1	HMGB1;HMGB1P1	18.311	0.93886	0.88674	1.0845	0.80018	0.947	0.95605
Putative 40S ribosomal protein S26-like 1;40S ribosomal protein S26	RPS26P11;RPS26	13.002	1.0338	1.0716	1.0793	0.90446	0.83081	1.1416
60S ribosomal protein L27a	RPL27A	10.127	0.91412	0.94522	1.0762	NaN	0.89611	0.80051
60S ribosomal protein L18a	RPL18A	16.714	1.1454	1.0731	1.0758	1.1279	1.0729	0.94447
Luc7-like protein 3	LUC7L3	58.22	1.153	1.1384	1.0705	1.0858	1.1637	1.0827
Heat shock cognate 71 kDa protein	HSPA8	68.805	1.0732	1.0967	1.0646	1.1716	1.1521	1.0649
	C11orf98	14.234	1.0917	1.0809	1.0635	1.0747	1.0705	1.0028
60S ribosomal protein L35	RPL35	14.551	0.98799	0.96313	1.0604	1.0637	0.96515	1.0429

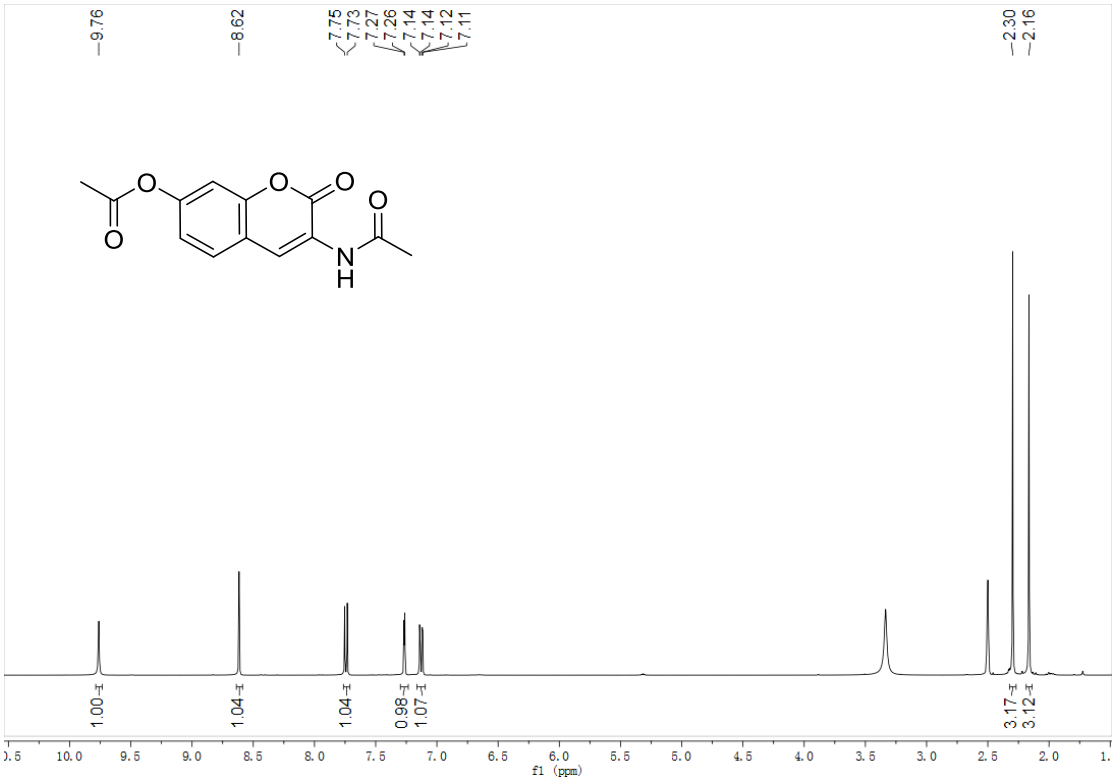
40S ribosomal protein S8	RPS8	21.879	1.0885	1.0513	1.0562	1.0507	1.0596	1.0927
60S ribosomal protein L6	RPL6	32.728	1.0211	1.0545	1.0557	1.0163	0.99919	1.0415
L-lactate dehydrogenase B chain;L-lactate dehydrogenase	LDHB	36.638	0.94539	1.0429	1.0543	1.0342	0.92971	0.9955
60S ribosomal protein L36	RPL36	12.254	1.0467	1.0853	1.0536	1.0303	1.0939	1.0535
60S ribosomal protein L34	RPL34	13.293	1.0044	1.0655	1.0529	1.0175	1.0354	1.0162
40S ribosomal protein S2	RPS2	21.154	0.91212	1.0342	1.0469	0.8542	1.0004	0.99841
Heat shock protein HSP 90-beta	HSP90AB1	83.263	1.0933	1.109	1.0466	1.1309	1.1169	1.1737
60S ribosomal protein L28	RPL28	15.747	1.0131	1.0358	1.0435	1.0637	0.98026	1.026
Heterogeneous nuclear ribonucleoprotein K	HNRNPK	50.976	0.85794	1.0519	1.0429	2.4962	0.85541	1.1624
60 kDa heat shock protein, mitochondrial	HSPD1	61.054	0.88835	1.2076	1.0421	1.1183	0.90089	1.0819
Serine/arginine-rich splicing factor 6;Serine/arginine-rich splicing factor 4	SRSF6;SRSF4	39.586	1.2769	1.1242	1.04	1.0586	0.94964	0.95153
60S ribosomal protein L13	RPL13	24.261	1.0164	1.0113	1.0384	1.0223	0.96713	1.0199
60S ribosomal protein L31	RPL31	14.463	1.0171	0.98944	1.0292	1.0221	1.0139	0.98353
60S ribosomal protein L32	RPL32	17.962	1.0056	1.0288	1.0274	1.027	0.97743	1.0432
40S ribosomal protein S16	RPS16;ZNF90	11.075	NaN	0.87628	1.0254	NaN	0.94205	1.0818
Serine/arginine-rich splicing factor 3	SRSF3	10.32	0.98923	1.0198	1.0226	0.93686	0.89144	0.94022
40S ribosomal protein S6	RPS6	28.68	0.93125	0.96818	1.0193	0.98051	0.97741	1.1315
40S ribosomal protein S25	RPS25	13.742	1.0085	0.96084	1.0136	1.1493	0.99507	1.1312
Ribosomal protein L19;60S ribosomal protein L19	RPL19	23.134	0.88246	0.96151	1.0074	1.0184	0.93435	1.0227
60S ribosomal protein L18	RPL18	21.728	1.0106	1.0132	1.0033	0.98969	1.0015	0.97247
Putative elongation factor 1-alpha-like 3;Elongation factor 1-alpha 1;Elongation factor 1-alpha	EEF1A1P5;EEF1A1	50.184	0.96546	0.96091	1.0028	0.94114	0.93441	1.0217
Serine/arginine repetitive matrix protein 2	SRRM2	299.61	0.9998	0.99455	1.002	1.0265	1.0274	1.051
40S ribosomal protein S9	RPS9	22.591	0.97866	1.0531	1.0011	0.94632	0.9697	1.0056

60S ribosomal protein L21	RPL21	18.565	1.0536	1.0008	0.99981	0.99664	1.0684	0.92387
Heterogeneous nuclear ribonucleoproteins A2/B1	HNRNPA2B1	37.429	1.0084	1.0122	0.99606	0.86355	0.88407	1.0567
Tubulin beta-4B chain;Tubulin beta-4A chain	TUBB4B;TUBB4A	49.83	0.93572	0.94416	0.99557	1.0048	0.98777	0.89975
40S ribosomal protein S23	RPS23	15.807	0.99736	0.96976	0.99405	1.1444	1.1179	0.908
Phosphoglycerate kinase 1	PGK1	44.614	0.7568	0.62583	0.99168	0.73637	0.73753	0.82405
Nucleophosmin	NPM1	32.575	0.99505	1.0406	0.99102	0.94729	1.01	0.98075
Elongation factor 2	EEF2	95.337	1.0142	0.98314	0.989	0.97982	1.0097	1.0593
Putative heat shock 70 kDa protein 7;Heat shock 70 kDa protein 6;Heat shock 70 kDa protein 1-like	HSPA7;HSPA6;HSPA1L	40.244	0.94494	0.8426	0.98821	1.0126	1.0875	1.1743
Heterogeneous nuclear ribonucleoprotein A1;Heterogeneous nuclear ribonucleoprotein A1, N-terminally processed	HNRNPA1	33.155	0.96661	1.0531	0.98545	0.99201	0.92253	1.0366
U1 small nuclear ribonucleoprotein 70 kDa	SNRNP70	51.556	0.99063	1.0152	0.98344	0.96248	0.99	0.97895
Serine/arginine-rich splicing factor 2;Serine/arginine-rich splicing factor 8	SRSF2;SRSF8	15.527	1.0286	0.97443	0.98057	0.98936	0.93688	0.94138
40S ribosomal protein S4, X isoform;40S ribosomal protein S4, Y isoform 2	RPS4X;RPS4Y2	29.597	1.0682	1.0429	0.98052	1.2231	1.0781	1.0031
60S ribosomal protein L11	RPL11	20.252	0.91033	1.0513	0.97759	0.96104	1.0396	0.93101
Propionyl-CoA carboxylase alpha chain, mitochondrial	PCCA	80.058	0.85486	0.89575	0.97728	0.83188	0.87548	0.93037
60S ribosomal protein L29	RPL29	17.752	0.97765	0.97182	0.97663	NaN	0.93606	0.91612
60S ribosomal protein L17	RPL17;RPL17-C18orf32	19.586	1.0115	1.0853	0.97652	0.94633	1.0494	0.98632
Multifunctional protein ADE2;Phosphoribosylaminoimidazole-succinocarboxamide synthase;Phosphoribosylaminoimidazole carboxylase	PAICS	45.651	0.9345	1.039	0.97539	1.0171	1.3091	1
Plasminogen activator inhibitor 1 RNA-binding protein	SERBP1	44.965	1.3619	1.1331	0.97502	1.1172	1.004	1.0593

Malate dehydrogenase;Malate dehydrogenase, mitochondrial	MDH2	24.594	0.87816	0.97527	0.97324	0.99852	0.92898	0.94478
L-lactate dehydrogenase A chain	LDHA	36.688	0.98402	0.98156	0.97233	1.0104	1.0112	1.0053
60S ribosomal protein L8	RPL8	28.024	1.0674	1.0908	0.96361	1.0107	1.0118	1.0143
60S ribosomal protein L24	RPL24	14.369	1.0584	1.0183	0.96146	1.0573	0.98533	0.99648
60S ribosomal protein L7	RPL7	29.225	1.0464	1.0149	0.96026	1.0183	1.0097	0.9967
Heterogeneous nuclear ribonucleoprotein H2;Heterogeneous nuclear ribonucleoprotein H;Heterogeneous nuclear ribonucleoprotein H, N-terminally processed	HNRNPH1;HNRNPH2	11.181	NaN	NaN	0.95984	NaN	NaN	1.1399
Transgelin-2	TAGLN2	21.086	0.90833	1.02	0.95894	0.98651	0.94833	1.0205
60S ribosomal protein L10-like;60S ribosomal protein L10	RPL10;RPL10L	10.028	0.99993	0.95353	0.95727	1.3744	NaN	1.0238
40S ribosomal protein S14	RPS14	16.273	1.1058	1.0357	0.95423	0.96904	1.0641	0.95668
GTP-binding nuclear protein Ran	RAN	26.224	0.91123	0.94072	0.95294	0.83527	0.92793	1.0119
Stress-70 protein, mitochondrial	HSPA9	6.8096	0.99926	0.94679	0.94837	1.1466	1.0693	1.0367
Putative RNA-binding protein Luc7-like 2	LUC7L2;C7orf55-LUC7L2	46.513	0.90799	0.98798	0.94795	0.98649	1.0437	1.0447
40S ribosomal protein S3	RPS3	17.407	1.094	0.95613	0.94629	0.96092	0.80536	0.95
14-3-3 protein sigma	SFN	27.774	NaN	0.86083	0.94511	0.93763	0.90921	0.914
Elongation factor 1-gamma	EEF1G	50.118	1.0931	1.01	0.94044	1.0636	NaN	1.2365
Heat shock protein HSP 90-alpha	HSP90AA1	84.659	1.0681	0.96486	0.92149	0.9146	1.0501	1.0233
Putative 60S ribosomal protein L39-like 5;60S ribosomal protein L39	RPL39P5;RPL39	6.3225	0.89129	0.88864	0.91371	0.92119	1.1358	0.8538
Fructose-bisphosphate aldolase;Fructose-bisphosphate aldolase A	ALDOA	39.817	0.91151	0.94598	0.91139	0.84347	0.89765	0.9362

Tubulin alpha-1C chain;Tubulin alpha-3C/D chain;Tubulin alpha-1A chain;Tubulin alpha-1B chain;Tubulin alpha-4A chain;Tubulin alpha-8 chain;Tubulin alpha-3E chain	TUBA1C;TUBA3C;TUBA1A;TUBA1B;TUBA4A;TUBA8;TUBA3E	57.73	0.93778	0.9195	0.90706	0.95027	0.94243	0.90247
40S ribosomal protein S13	RPS13	17.222	0.90759	0.91278	0.89375	0.83881	0.83132	0.89918
14-3-3 protein gamma;14-3-3 protein gamma, N-terminally processed	YWHAG	28.302	0.89284	0.84302	0.8881	1.011	0.92747	0.9403
Methylcrotonoyl-CoA carboxylase subunit alpha, mitochondrial	MCCC1	80.472	0.8991	0.90323	0.88737	1.226	1.1619	1.1907
Coronin-1A;Coronin	CORO1A	51.026	0.89675	0.88492	0.88526	0.90544	0.82587	0.89263
Heterogeneous nuclear ribonucleoprotein C-like 4;Heterogeneous nuclear ribonucleoprotein C-like 1;Heterogeneous nuclear ribonucleoprotein C-like 3;Heterogeneous nuclear ribonucleoprotein C-like 2;Heterogeneous nuclear ribonucleoproteins C1/C2	HNRNPC;HNRNPCL4;HNRNPCL1;HNRNPCL3;HNRNPCL2	12.78	1.0689	NaN	0.88426	0.84072	NaN	0.90384
Transketolase	TKT	49.91	0.9924	0.9271	0.87791	1.2695	0.92269	0.98479
14-3-3 protein beta/alpha;14-3-3 protein beta/alpha, N-terminally processed;14-3-3 protein eta	YWHAB;YWHAE;YWHAH	8.4284	1.0125	0.83651	0.87034	0.91225	0.91458	0.85058
Actin, cytoplasmic 2;Actin, cytoplasmic 2, N-terminally processed;Actin, cytoplasmic 1;Actin, cytoplasmic 1, N-terminally processed;Actin, alpha skeletal muscle;Actin, alpha cardiac muscle 1;Actin, gamma-enteric smooth muscle;Actin, aortic smooth muscle	ACTG1;ACTB;ACTA1;ACTC1;ACTG2;ACTA2	41.792	0.85985	0.85683	0.86869	0.87924	0.8436	0.88154
Voltage-dependent anion-selective channel protein 2	VDAC2	30.348	1.0928	1.022	0.8525	1.3037	1.3645	1.4666
Pyruvate kinase;Pyruvate kinase PKM	PKM	53.045	0.8613	0.87061	0.84887	0.85679	0.82813	0.93395
Tubulin beta chain;Tubulin beta-2B chain;Tubulin beta-2A chain	TUBB;TUBB2B;TUBB2A	47.766	0.92384	0.87288	0.8465	NaN	0.95294	0.84812

Hornerin	HRNR	282.39	NaN	NaN	0.84422	NaN	NaN	NaN
Cofilin-1	CFL1	17.865	0.83508	0.91046	0.84197	0.95107	0.86345	0.91991
Macrophage migration inhibitory factor	MIF	12.476	0.90409	0.86979	0.84038	NaN	0.8152	NaN
Profilin-1	PFN1	15.054	0.8809	0.89295	0.83147	1.0084	0.86847	0.80498
Plastin-2	LCP1	70.288	0.8035	0.78569	0.83025	0.88094	0.87851	0.85332
Heat shock protein beta-1	HSPB1	22.782	0.81149	0.82052	0.82379	0.90604	0.86781	0.93319
Aspartate aminotransferase, mitochondrial	GOT2	47.517	1.012	0.83328	0.81282	0.79184	NaN	0.86666
60S ribosomal protein L7a	RPL7A	29.995	0.93866	1.267	0.81272	NaN	NaN	NaN
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	36.053	0.82774	0.81541	0.79971	0.86038	0.87545	0.8965
Heterogeneous nuclear ribonucleoprotein D0	HNRNPD	23.076	0.85242	1.1165	0.79461	0.89269	0.74401	0.94896
14-3-3 protein zeta/delta	YWHAZ	5.8626	0.82054	0.69871	0.78754	0.9679	0.80121	0.87974
14-3-3 protein theta	YWHAQ	17.048	0.77649	NaN	0.78714	NaN	0.99074	1.0453
Peroxiredoxin-1	PRDX1	18.976	0.70814	0.78884	0.75303	0.82812	0.78734	0.74517
Arginine/serine-rich coiled-coil protein 2	RSRC2	50.559	1.0559	0.68397	0.70568	1.2451	0.81878	0.99926
Alpha-enolase	ENO1	47.168	0.72127	0.72782	0.69719	0.9168	0.86168	0.88936
40S ribosomal protein S3a	RPS3A	14.446	1.0496	0.93416	0.69433	1.1714	0.94029	0.9787
Deoxyuridine 5-triphosphate nucleotidohydrolase, mitochondrial	DUT	14.306	NaN	NaN	0.67892	0.76095	NaN	NaN
Ubiquitin-60S ribosomal protein L40;Ubiquitin;60S ribosomal protein L40;Ubiquitin-40S ribosomal protein S27a;Ubiquitin;40S ribosomal protein S27a;Polyubiquitin-B;Ubiquitin;Polyubiquitin-C;Ubiquitin	UBA52;UBB;RPS27A;UBC	7.1321	0.42897	0.43721	0.46342	NaN	0.53615	0.5731
Pinin	PNN	81.613	1.2382	NaN	0.3818	NaN	1.3129	0.63668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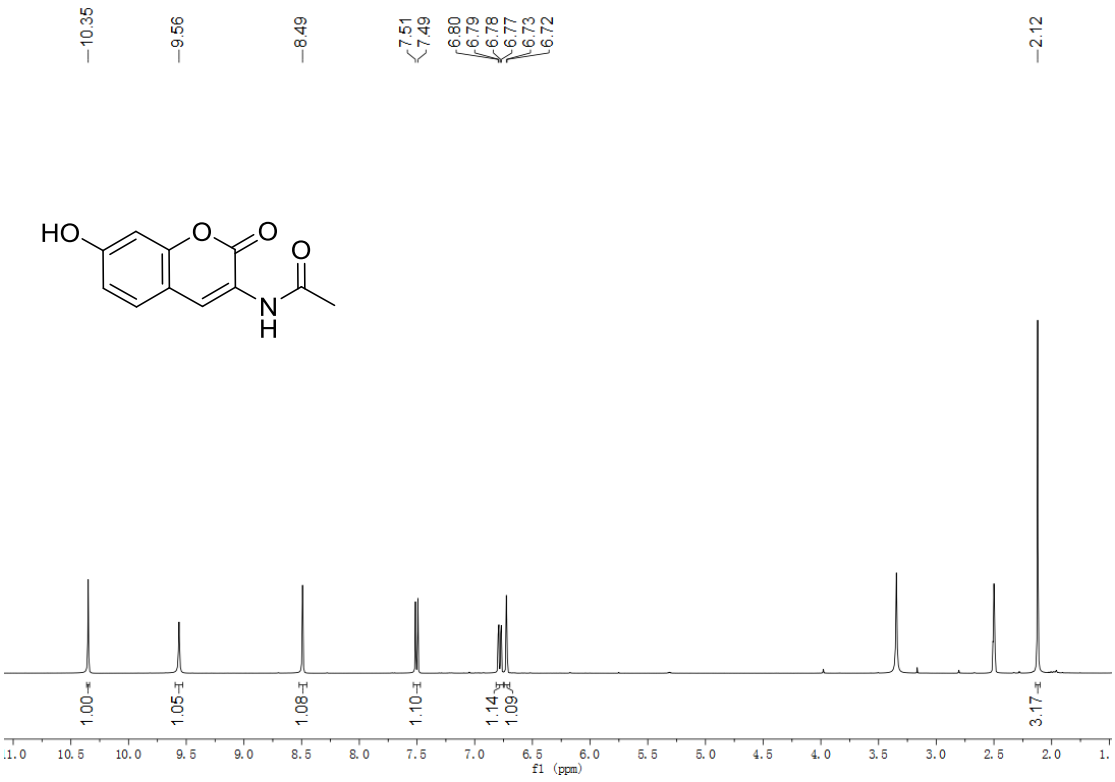
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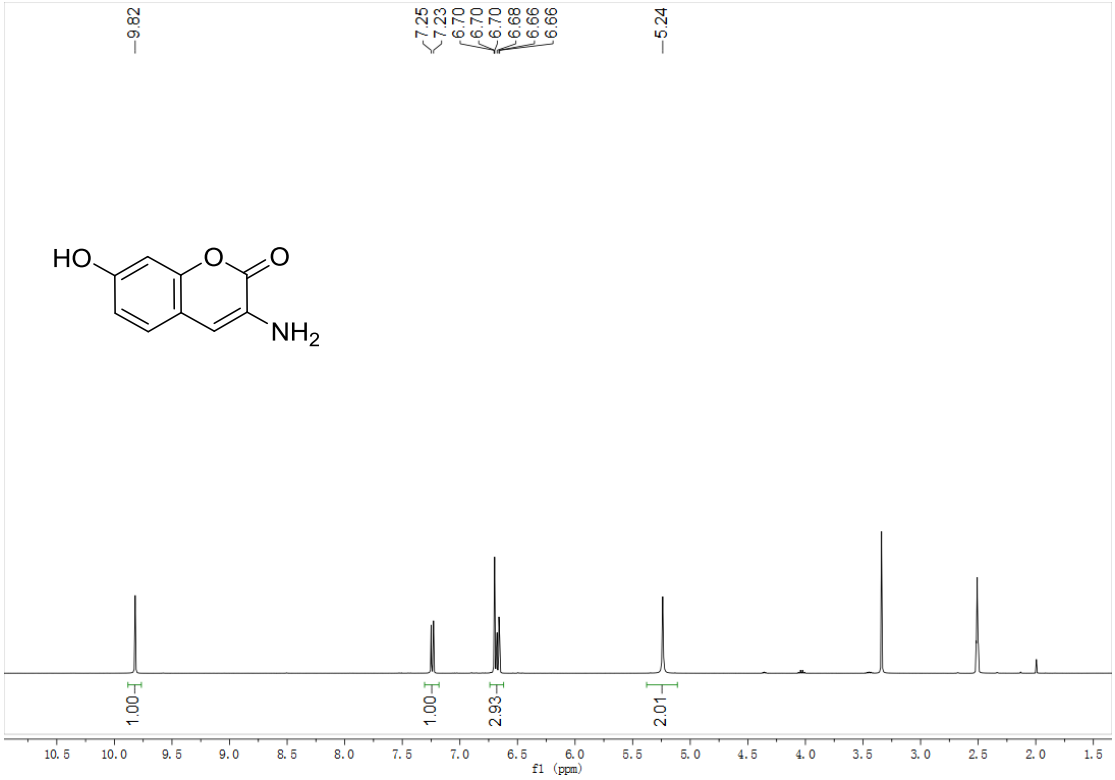
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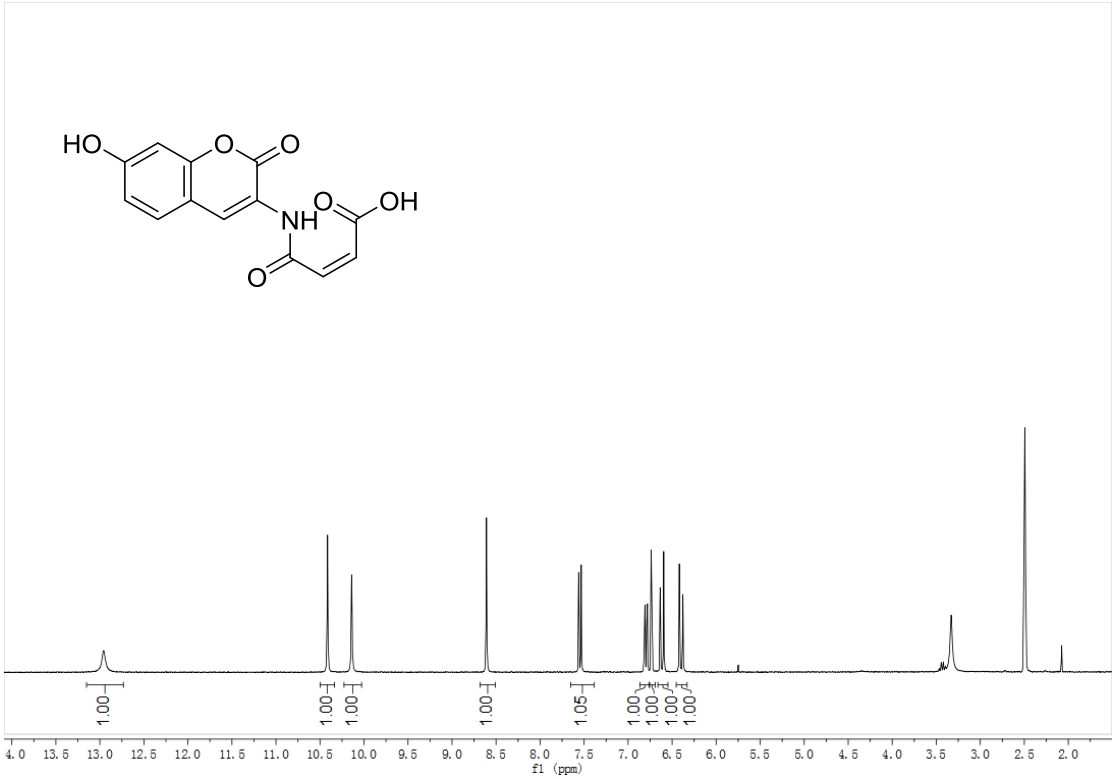
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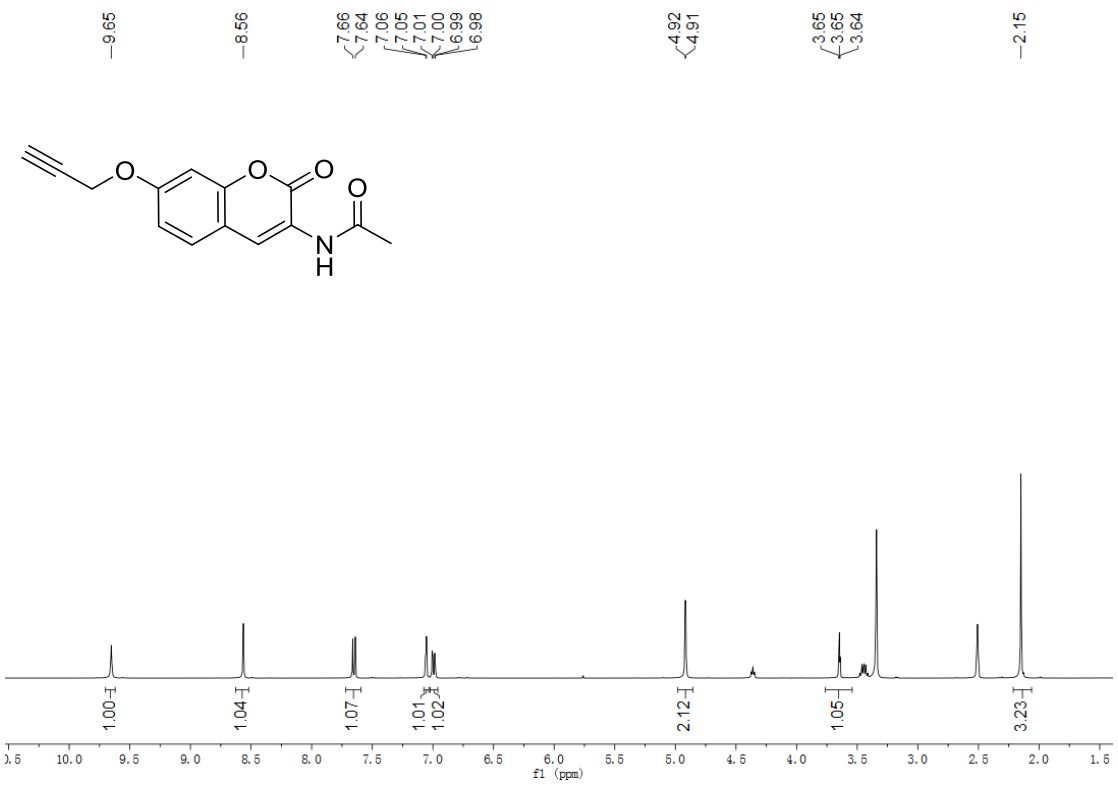
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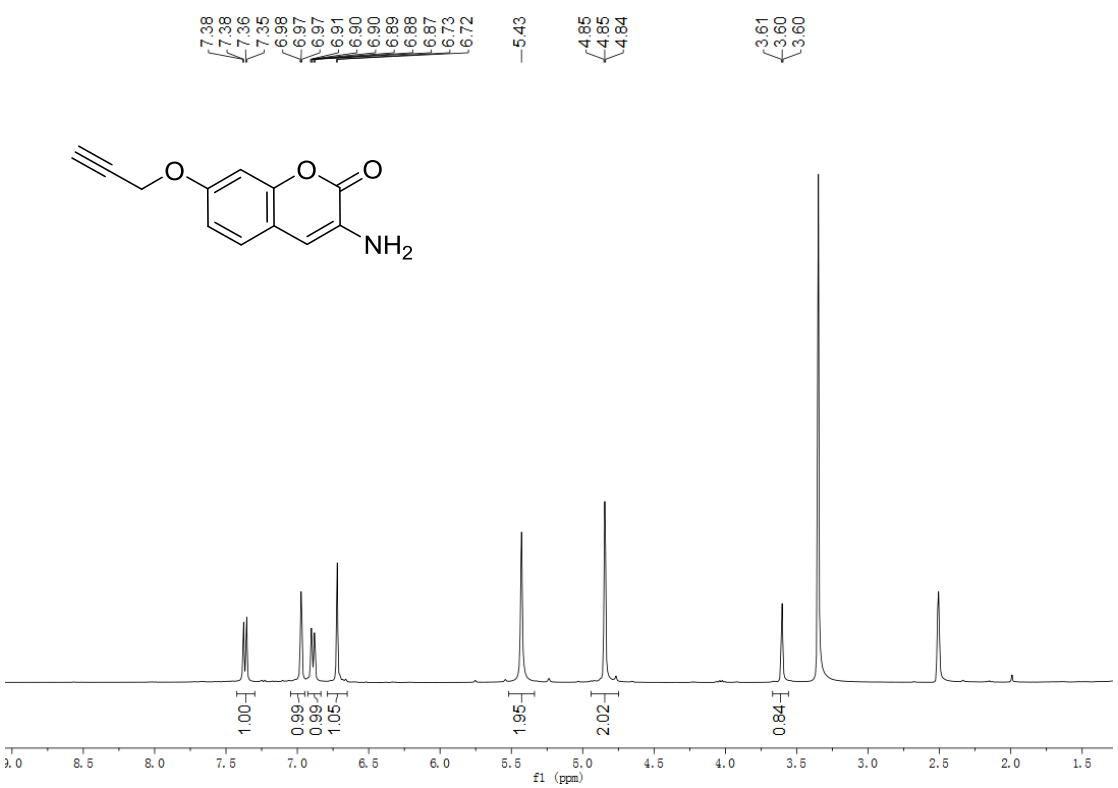
f1 (ppm)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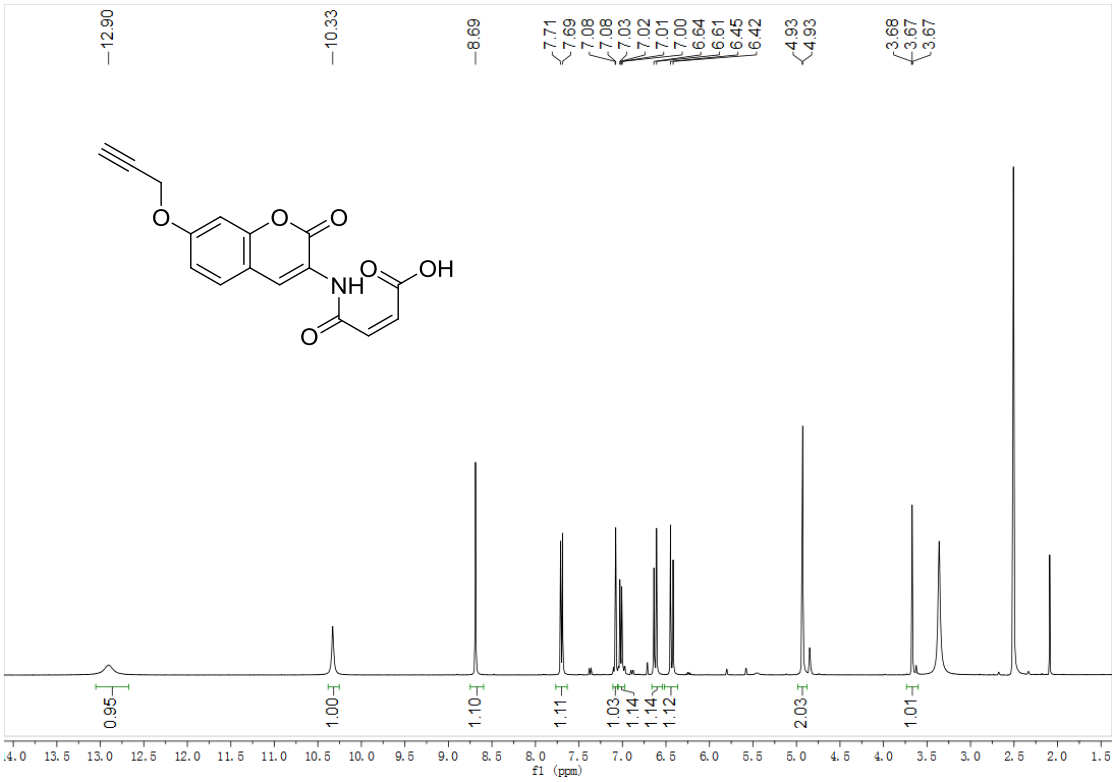
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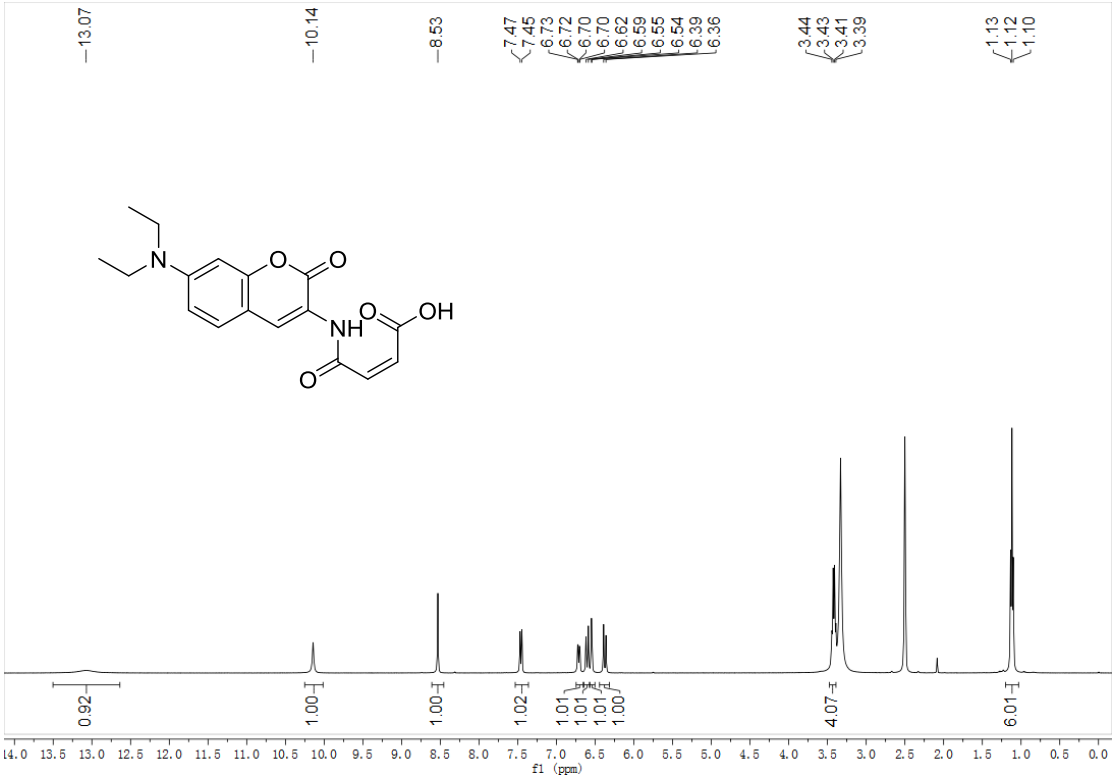
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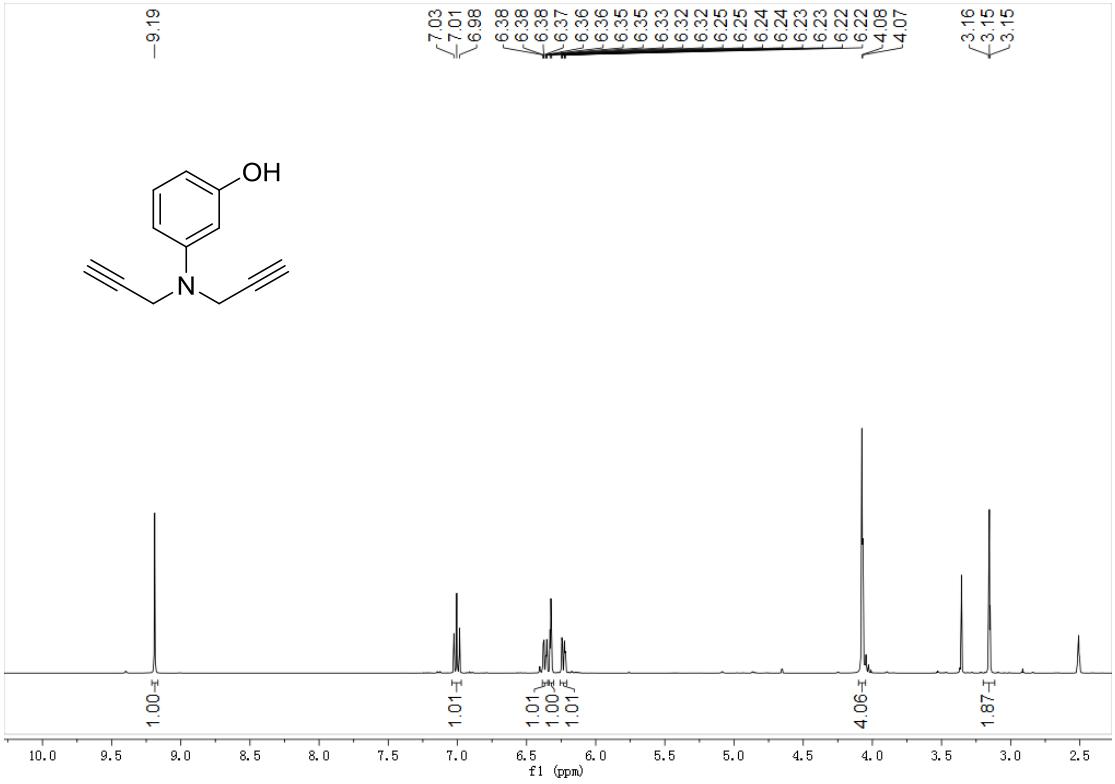
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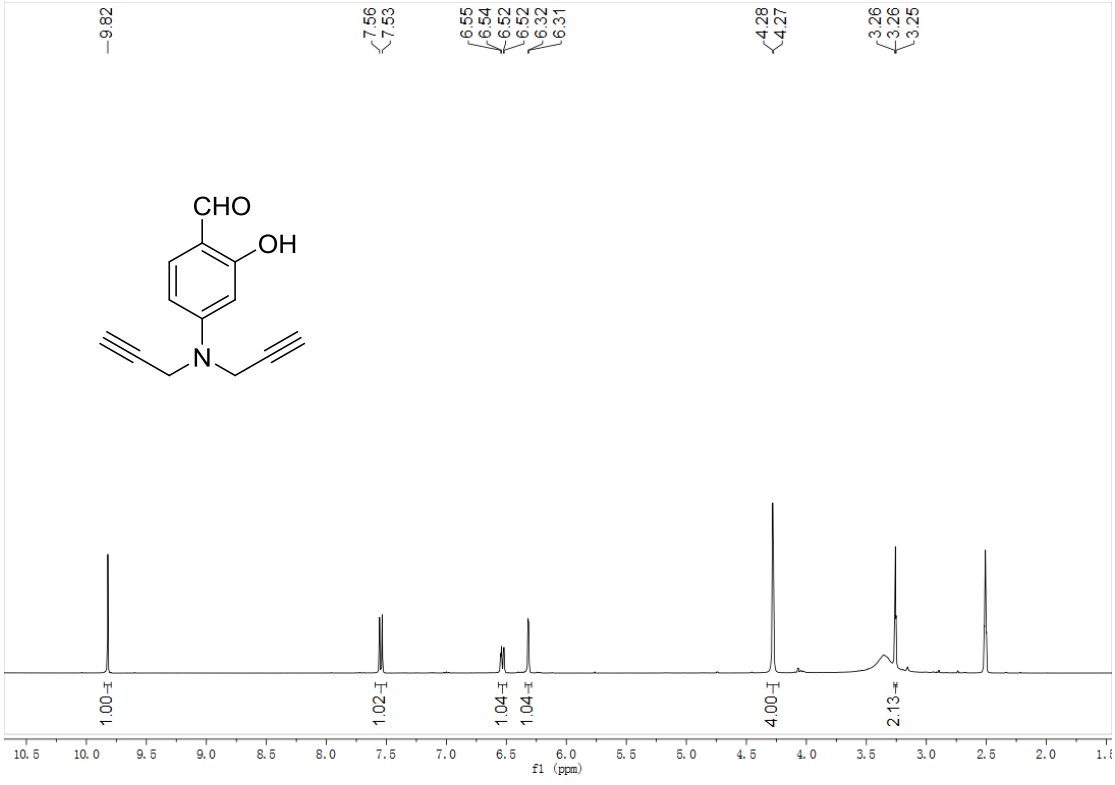
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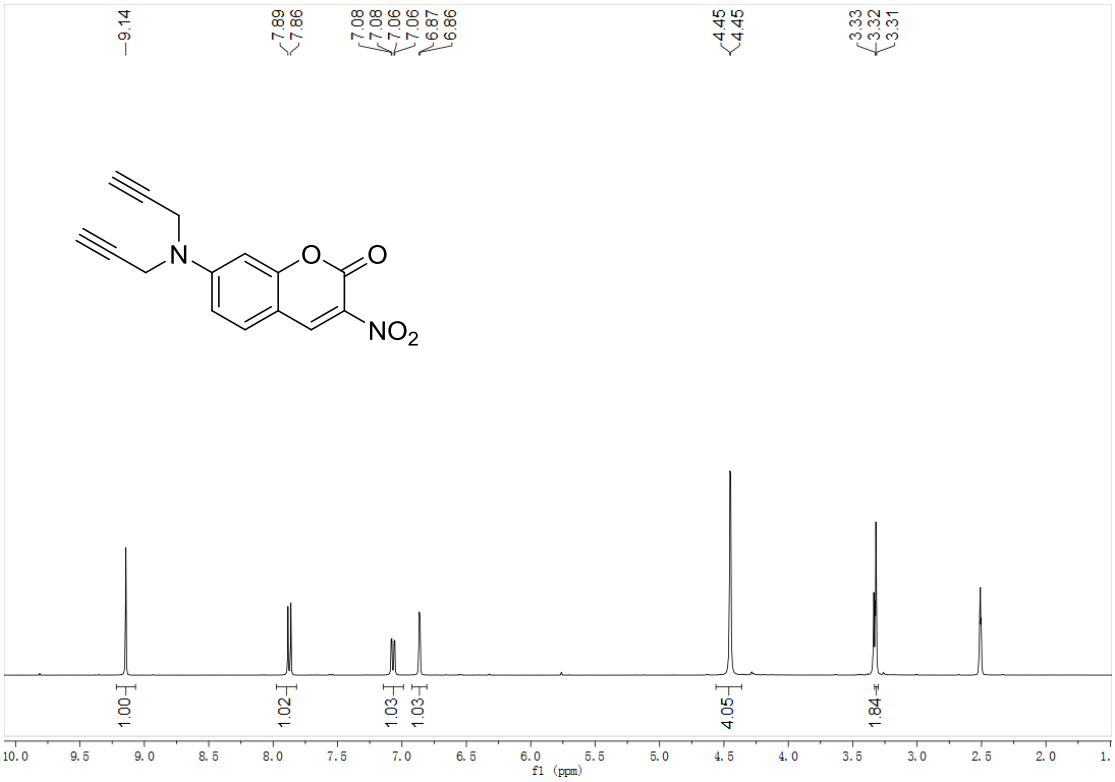
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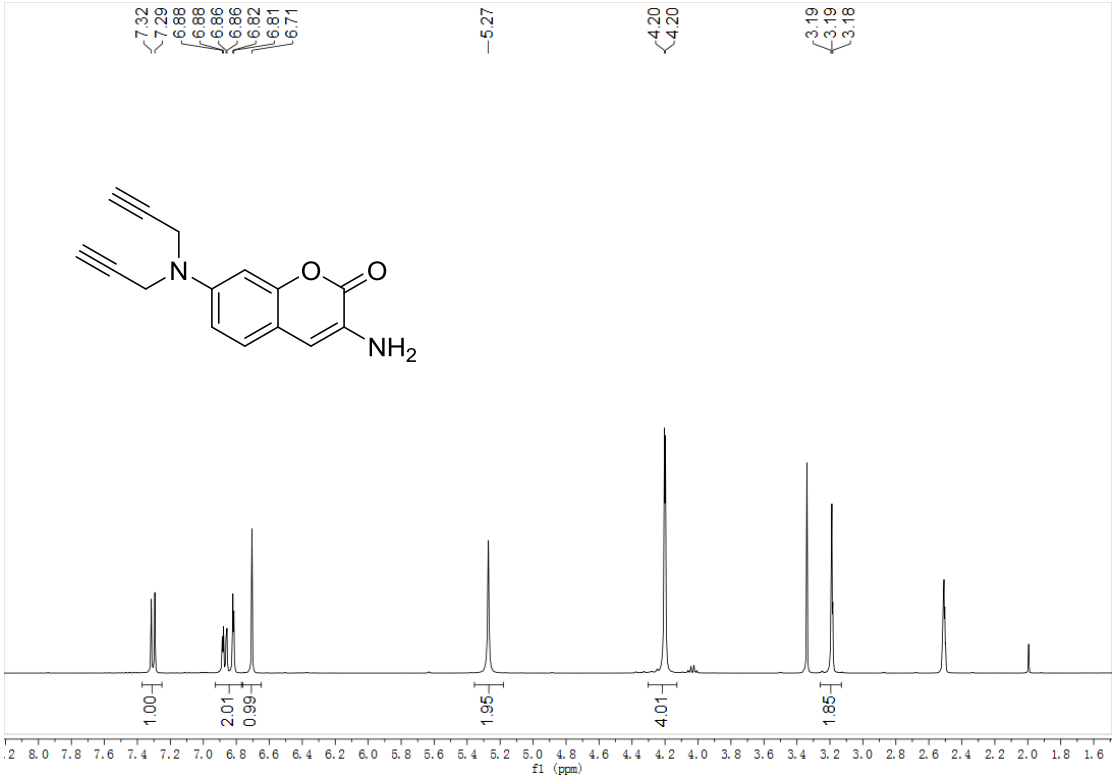
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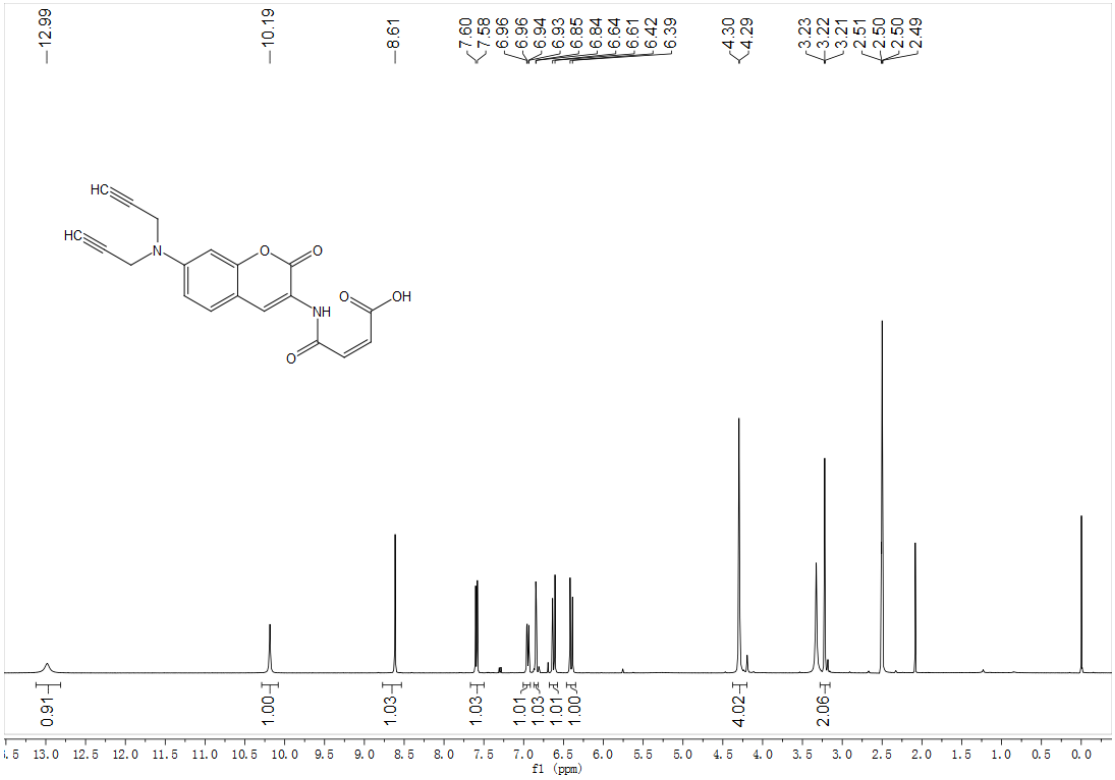
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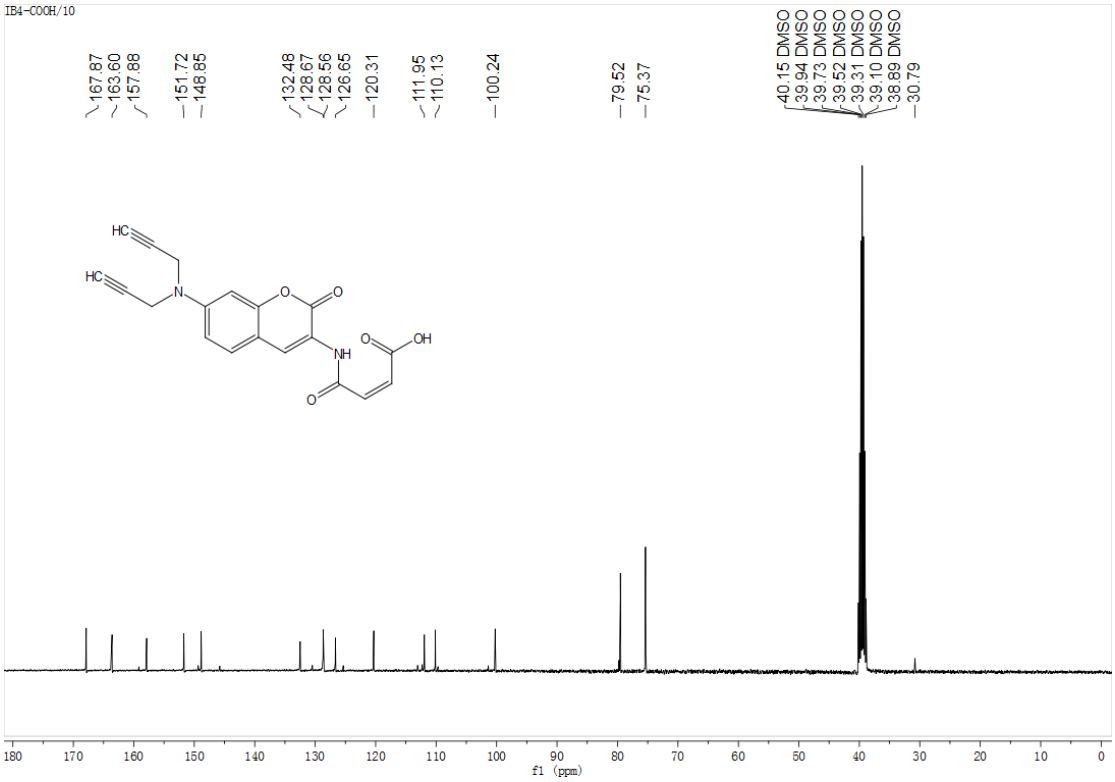
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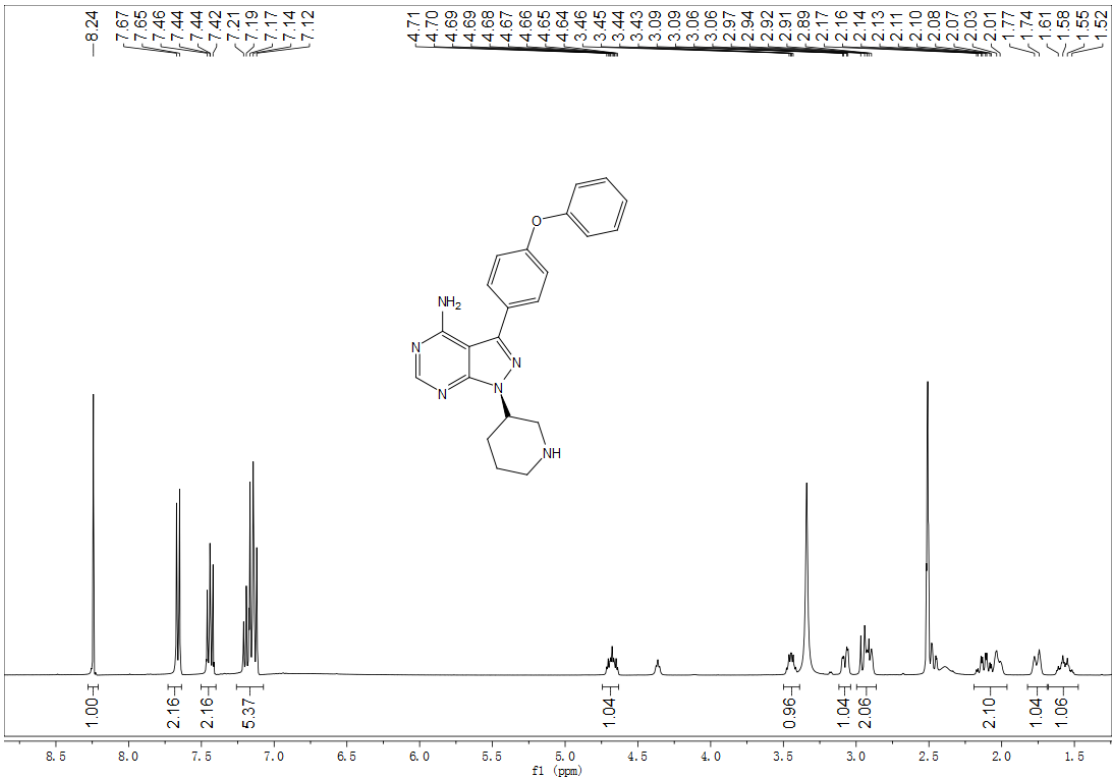
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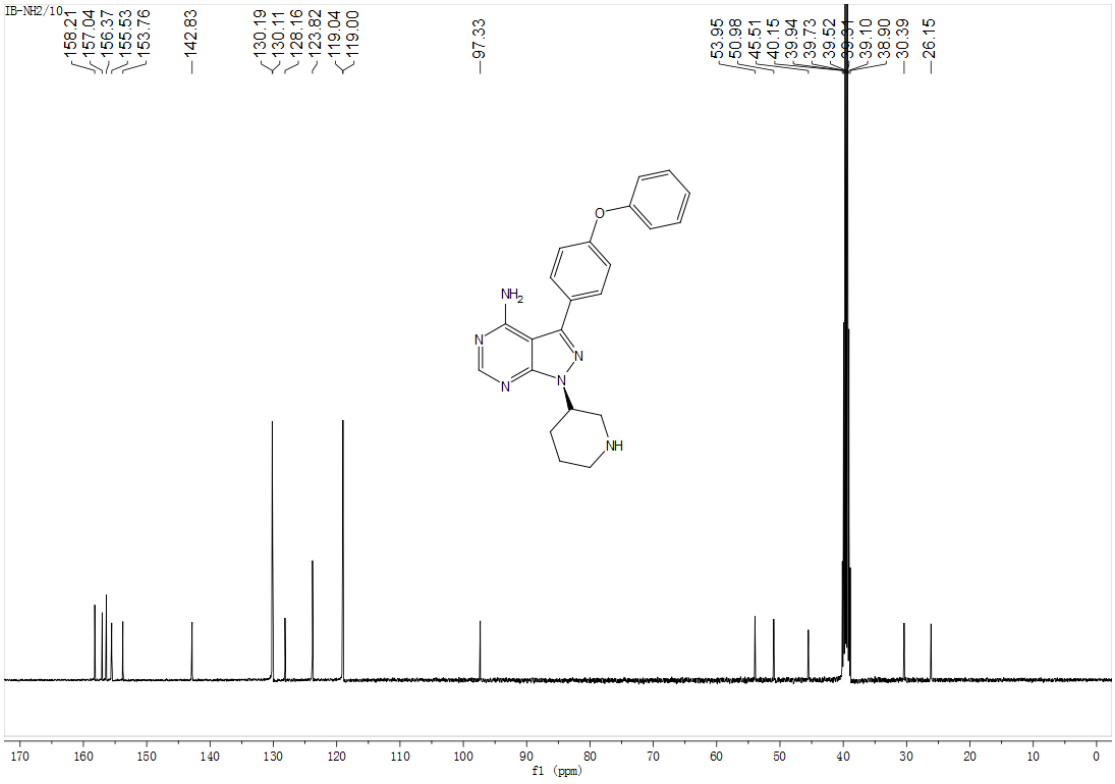
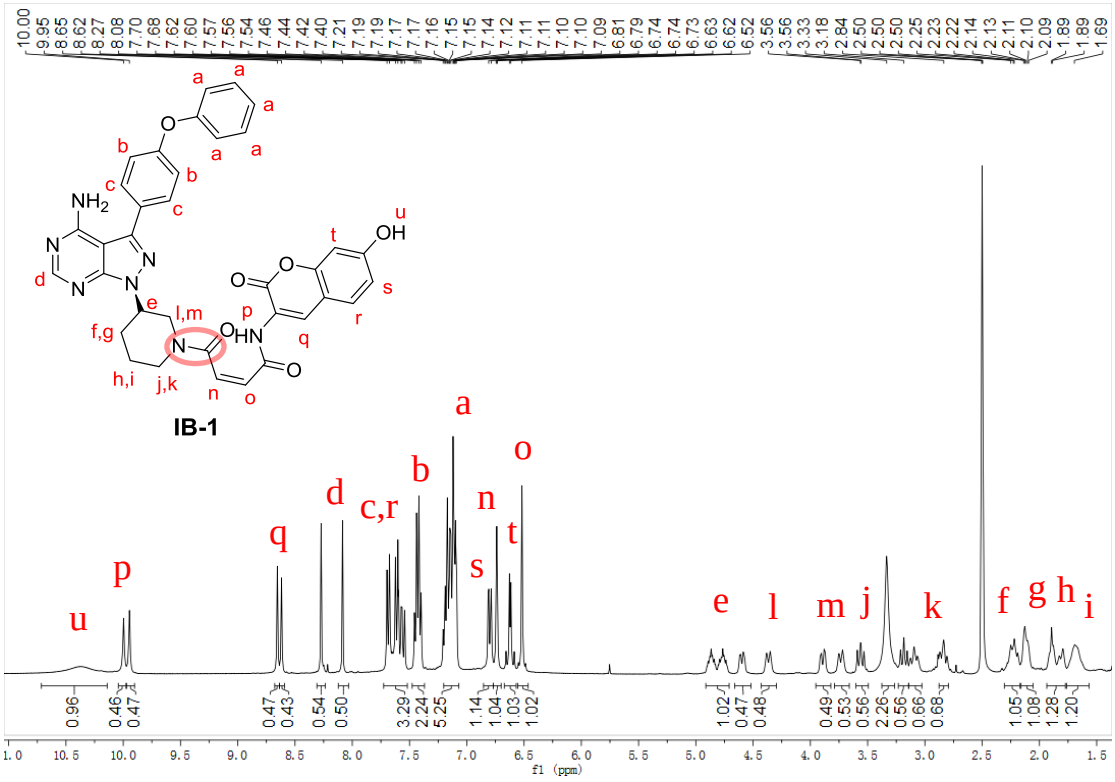
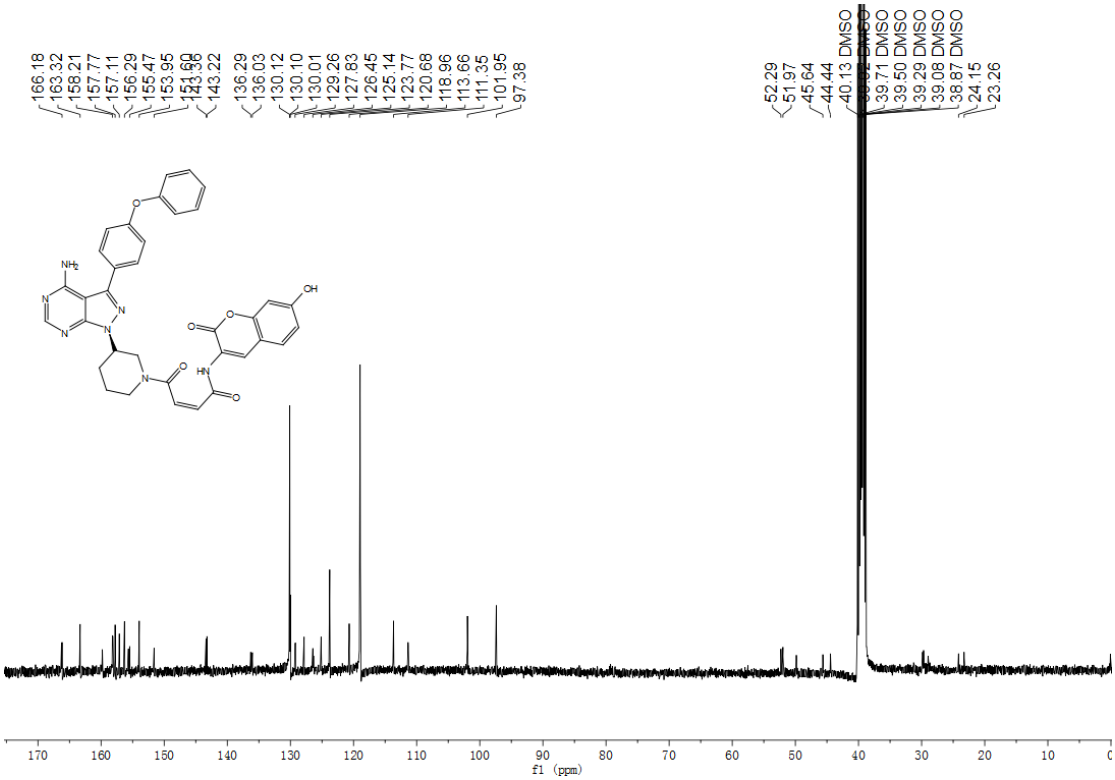
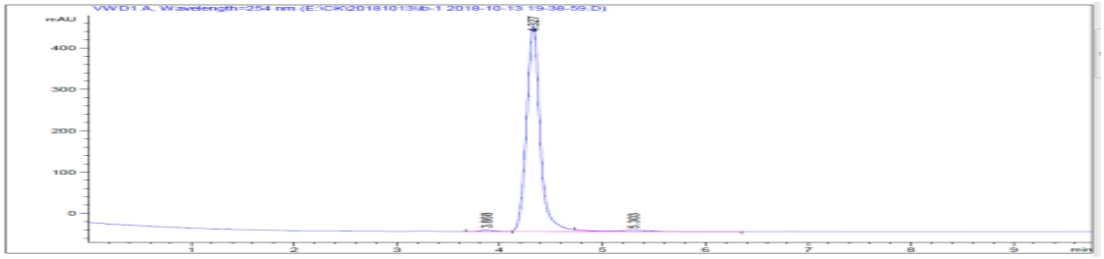
4.69
4.68
4.67
4.66
4.65
4.64
3.46
3.45
3.44
3.43
3.09
3.09
3.06
3.06
2.97
2.94
2.92
2.91
2.89
2.17
2.16
2.14
2.13
2.11
2.10
2.08
2.07
2.03
2.01
1.77
1.74
1.61
1.58
1.55
1.52
1.00
2.16
2.16
5.37
1.04
0.96
1.04
2.06
2.10
1.04
1.06
f1 (ppm)

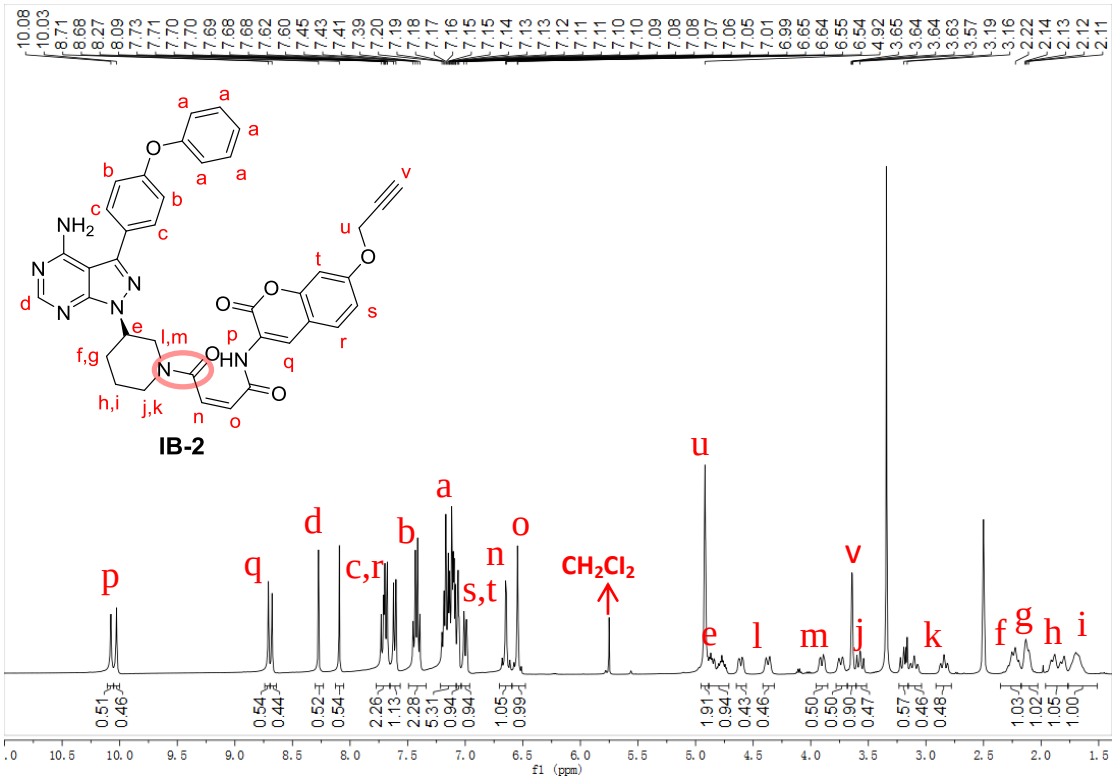

IB-NH2/10
158.21
157.04
156.37
155.53
153.76
142.83
130.19
130.11
128.16
123.82
119.04
119.00
97.33
53.95
50.98
45.51
40.15
39.94
39.73
39.52
39.31
39.10
38.90
30.39
26.15
f1 (ppm)

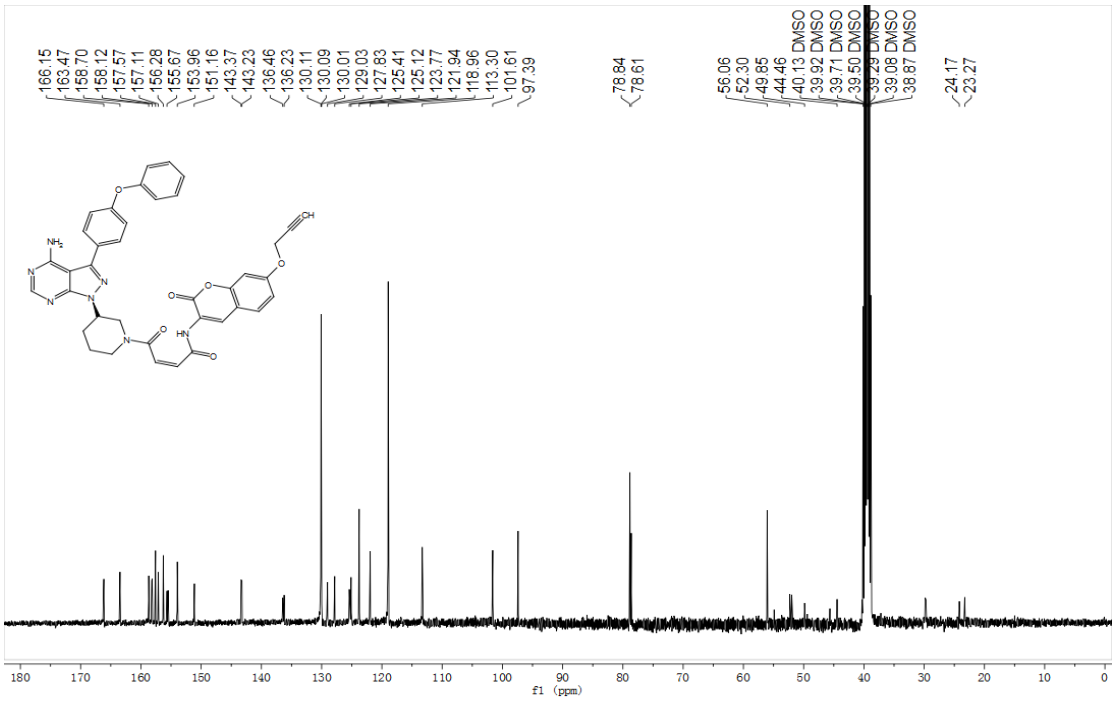




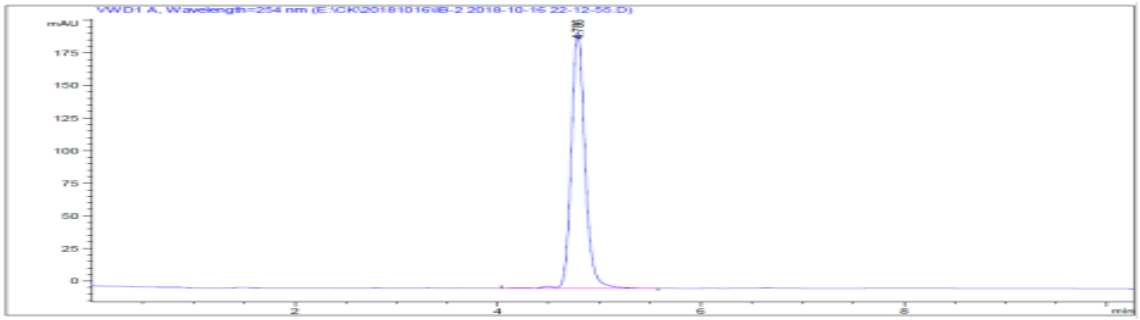
HPLC analysis of IB-1 (254 nm)

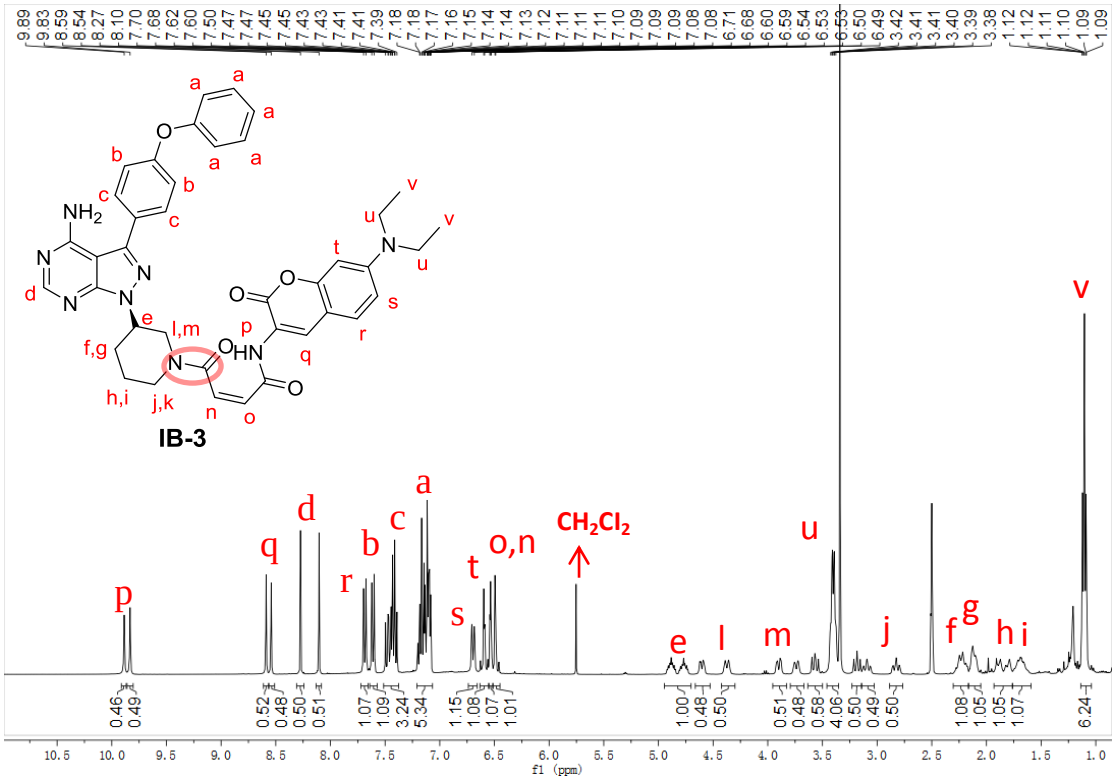


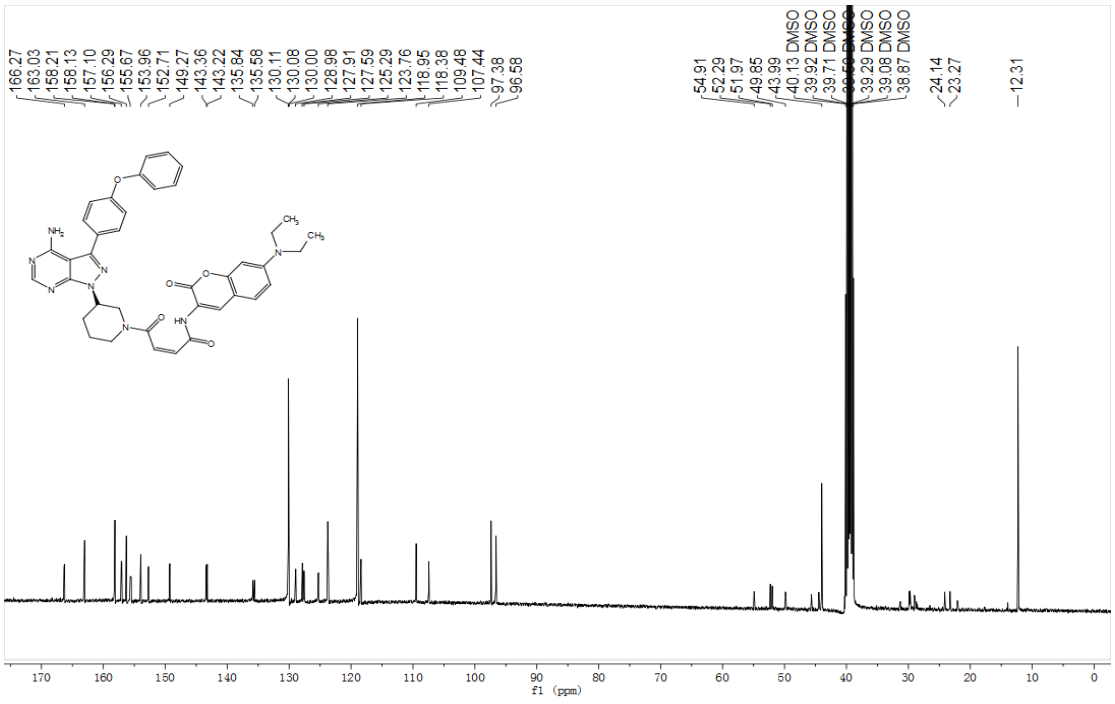




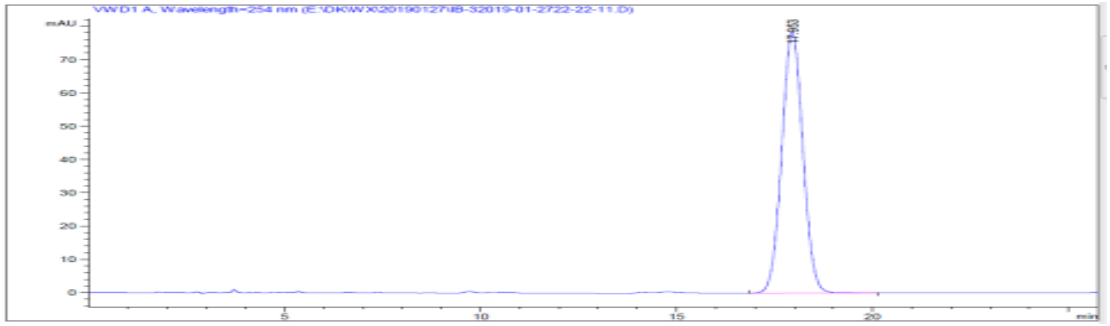
HPLC analysis of IB-2 (254 nm)

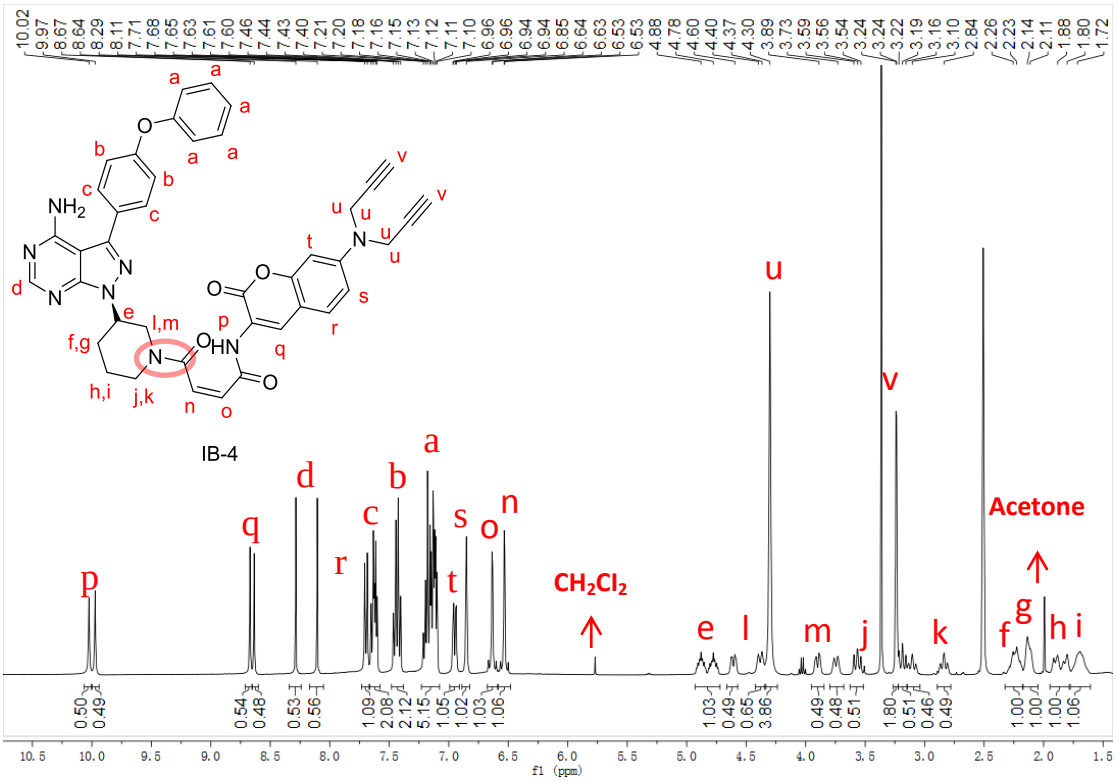


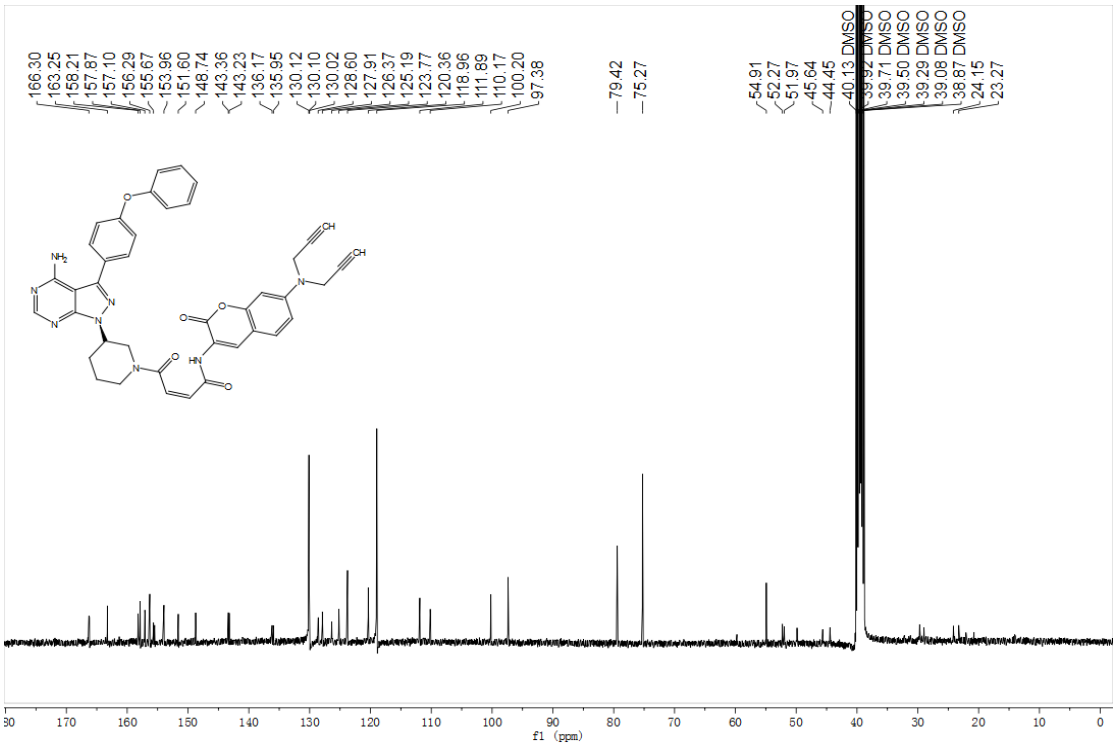




HPLC analysis of IB-3 (254 nm)







HPLC analysis of IB-4 (254 nm)