Supporting Information

Minimalist Linkers Suitable for Irreversible Inhibitors in Simultaneous Proteome Profiling, Live-Cell Imaging and Drug Screening

Cuiping Guo,Yu Chang, Xin Wang, Chengqian Zhang, Piliang Hao, Ke Ding and Zhengqiu Li**

School of Pharmacy, Jinan University, Guangzhou, China 510632
*Corresponding author (pharmlzq@jnu.edu.cn)

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). 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 EGFR (ab52894) and BTK (ab137503) were purchased from Cell Signaling Technology (CST). Click reagents were purchased from Click Chemistry Tools (https://clickchemistrytools.com/).

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 ℃ 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]

Table S1. The probes used in current study

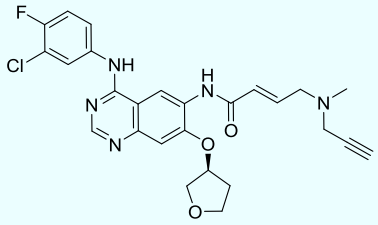
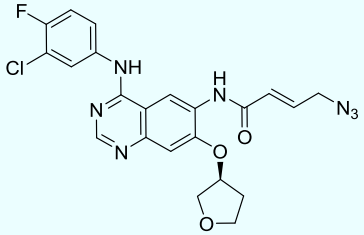
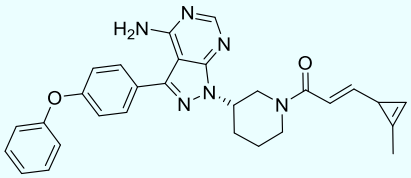
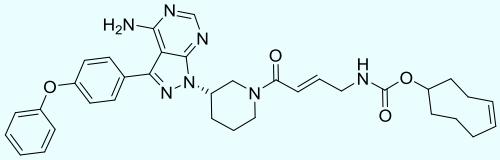
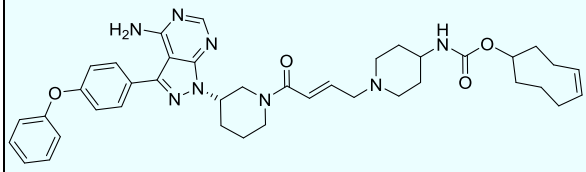
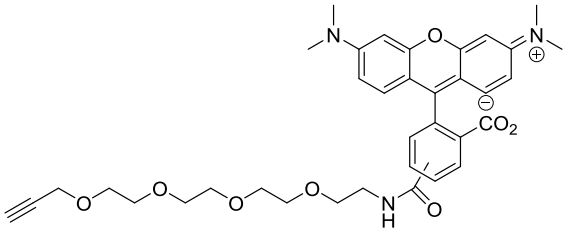
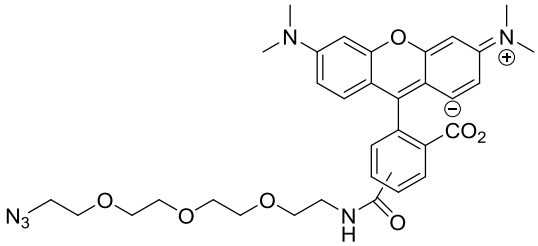
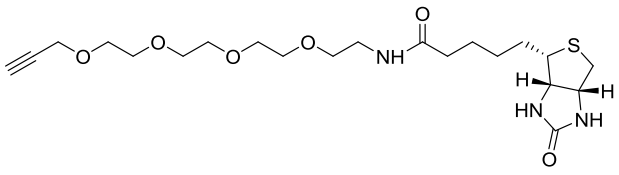
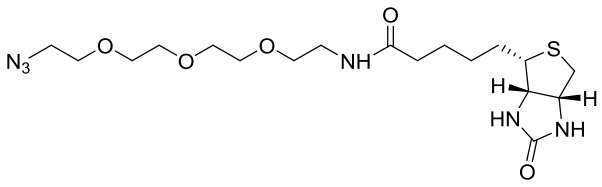
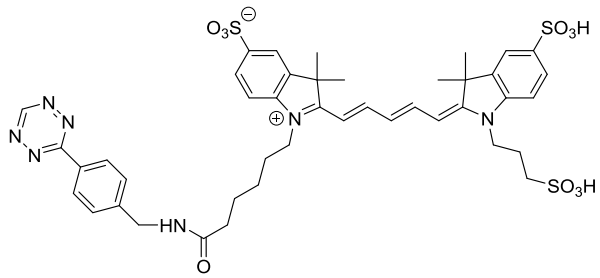
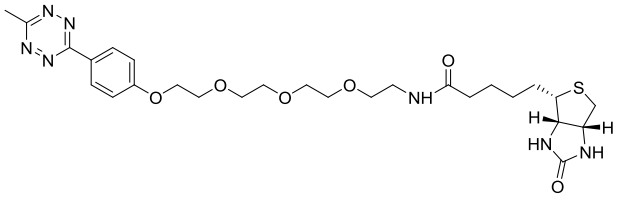
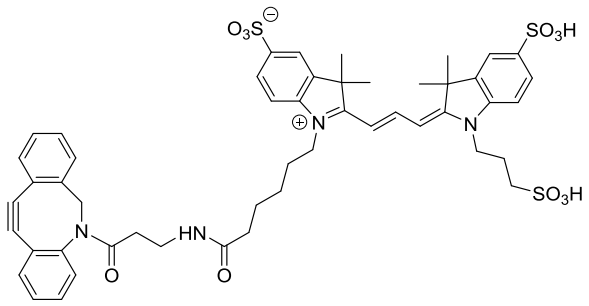
 AF-1	 AF-2
 IB-1	 IB-2
 IB-3	

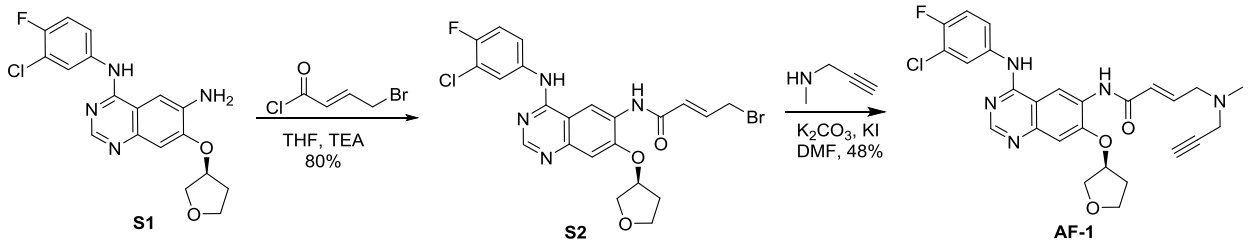
Table S2. Structures of the reporters

 TAMRA-alkyne	 TAMRA-N_3
 Biotin-alkyne	 Biotin-N_3
 Tetrazine-Cy5	 Methyltetrazine-biotin



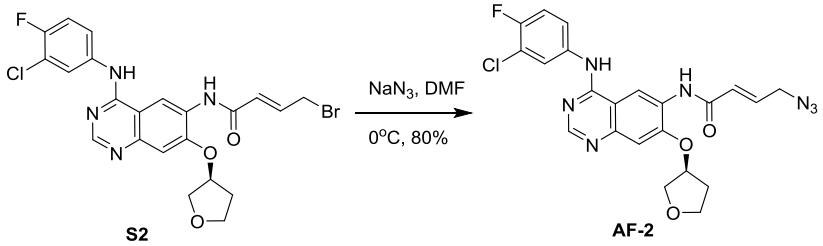
DBCO-Cy3

3. Chemical Synthesis



Scheme S1

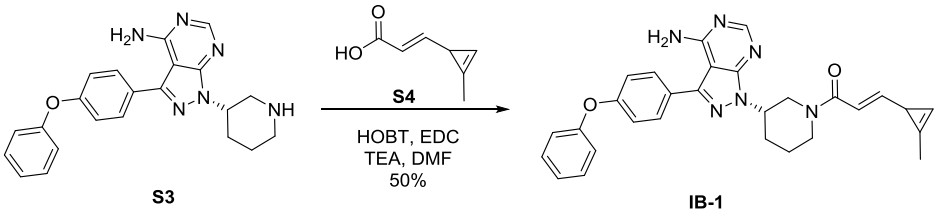
S2 was synthesized based on previously reported procedures from **S1**.[5] To a solution of compound **S2** (50 mg, 0.095 mmol) in 3 mL DMF was added *N*-methylpropargylamine (0.0317 mL, 0.38 mmol), potassium carbonate (26 mg, 0.19 mmol) and potassium iodide (27 mg, 0.17 mmol) at 0 ^{o}C. The mixture was stirred at 40 ^{o}C for 3 h under N_2 gas atomosphere and then quenched by addition of 10 mL water. The resulting mixture was extracted with ethyl acetate (3 × 20 mL), and the combined organic phase was washed with brine, dried over anhydrous Na_2SO_4. Upon solvent evaporation *in vacuo*, the residue was purified by flash column (methanol:ethyl acetate = 1:10) to give product **AF-1** as a yellow solid (23 mg, 48%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.00 (s, 1H), 8.56 (s, 1H), 8.35 (s, 1H), 8.10 (s, 1H), 7.79 (dd, *J* = 3.0, 6.0 Hz, 1H), 7.46 (m, 1H), 7.09 (s, 1H), 7.0 (m, 1H), 6.96 (m, 1H), 6.23 (d, *J* = 15.0 Hz, 1H), 5.10 (s, 1H), 4.15 (d, *J* = 12.0 Hz, 1H), 4.02 (m, 2H), 3.90 (m, 1H), 3.37 (s, 2H), 3.28 (d, *J* = 6.0 Hz，2H), 2.43 (m, 1H), 3.26 (s, 3H), 2.29 (t, *J* = 3.0 Hz, 1H), 2.22 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.98, 156.98, 154.63, 150.60, 148.16, 143.95, 135.46, 127.91, 125.63, 124.17, 121.89, 120.74, 116.49, 110.68, 109.61, 108.30, 79.48, 78.24, 77.23, 73.95, 73.12, 67.41, 56.55, 45.79, 42.11, 32.84. HR-MS (m/z) $[M + H]^+$ calcd: 510.1703; Found: 510.1666.



Scheme S2

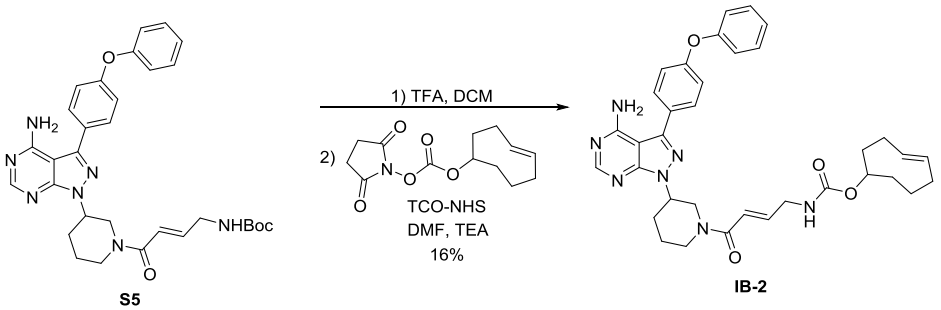
To a stirred solution of NaN_3 (6 mg, 0.095 mmol) in 3 mL DMF was added **S2** (50 mg, 0.095 mmol) at 0^{o}C. The resulting mixture was stirred for 30 min and then at room temperature for 4 h. Subsequently, 5 mL water was added and the mixture was extracted with ethyl acetate (2 × 15 mL). The combined organic phase was washed with brine and then dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column (methanol:CH_2Cl_2 = 3:50) to give **AF-2** as a white solid (37 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.10 (s, 1H), 8.65 (s, 1H), 8.13 (s, 1H), 7.93 (dd, *J* = 4.0, 8.0 Hz, 1H), 7.75 (s, 1H), 7.54 (m, 1H), 7.21 (s, 1H), 7.14 (t, *J* = 8.0 Hz, 1H), 7.01 (dt, *J* = 4.0 12.0 Hz, 1H), 6.32 (dt, *J* = 4.0, 12.0 Hz, 1H), 5.19 (t, *J* = 4.0 Hz, 1H), 4.20 (d, *J* = 12.0 Hz, 1H), 4.14 (d, *J* = 4.0 Hz, 2H), 4.11 (d, *J* = 4.0 Hz, 1H), 4.05 (m, 1H), 3.95 (m, 1H), 2.45 (m, 1H), 2.25 (m, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 163.29, 156.96, 154.81, 150.57, 148.34, 139.60, 128.24, 125.66, 124.43, 121.95, 121.89, 116.83, 116.61, 110.18, 109.60, 108.72, 79.70, 73.18, 67.48, 51.49, 32.98, 29.92. HR-MS (m/z) $[M + H]^+$ calcd: 484.1295; Found: 484.1290.



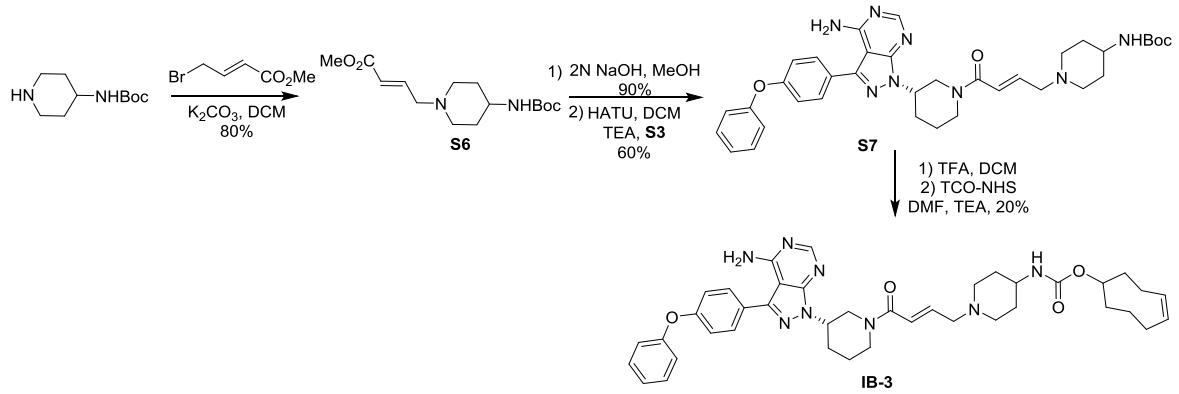
Scheme S3

(IB-1). The intermediate **S3** is commercially available and the cyclopropene-containing linker (**S4**) was synthesized based on previously published procedures.[6] To a solution of compound **S4** (20.0 mg, 0.05 mmol) in 4 mL DMF was added HOBT (10.49 mg, 0.075 mmol), EDC (14.37 mg, 0.075 mmol), TEA (10 mg, 0.1 mmol) and **S8** (6.2 mg, 0.05 mmol), successively. The reaction was stirred at room temperature overnight prior to addition of 3 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 (12.3 mg, 50 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.62 (d, *J* = 12.0 Hz, 2H), 7.37 (t, *J* = 6.0, 9.0 Hz, 2H), 7.14 (t, *J* = 9.0 Hz, 3H), 7.06 (d, *J* = 6.0 Hz, 2H), 6.53 (m, 1H), 6.32 (d, *J* = 3.0 Hz, 1H), 5.66 (s, 1H), 4.84 (m, 1H), 4.13 (d, *J* = 6.0 Hz, 1H), 3.67 (d, *J* = 6.0 Hz, 1H), 3.31 (m, 1H), 2.24 (m, 3H), 2.11 (s, 3H), 1.95 (m, 2H), 1.69 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.07, 158.53, 157.84, 156.35, 156.18, 155.79, 154.25, 143.83, 129.97, 127.75, 124.05, 119.54, 119.30, 119.14, 115.54, 101.24, 98.59, 53.62, 52.70, 49.87, 46.16, 45.72, 42.13, 35.96, 31.91, 30.38, 29.26, 27.22, 22.46. ESI-MS (m/z) $[M + H]^+$ calcd: 493.2; Found: 493.5. HR-MS (m/z) $[M + H]^+$ calcd: 493.2347; Found: 493.2331.



Scheme S4

(IB-2). Synthesis of **S5** was based on previously published procedures from commercially available intermediate **S3**.[7] To a solution of 2 mL TFA and 6 mL DCM was added compound **S5** (17.1 mg, 0.03 mmol), the reaction was then stirred at room temperature for 30 minutes. Upon solvent evaporation *in vacuo*, the crude product was dissolved in 3 mL DMF followed by addition of TCO-NHS (5.3 mg, 0.02 mmol) and TEA (10 mg, 0.1 mmol), the resulting mixture was stirred at room temperature for 1 hour and then quenched by addition of 3 mL water. Upon extraction with ethyl acetate (2 × 10 mL) and concentration *in vacuo*, the residue was purified by flash column (MeOH:DCM = 1:20) to afford **IB-2** (3 mg, 16% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (d, *J* = 16.4 Hz, 1H), 7.63 (d, *J* = 8.2 Hz, 2H), 7.40 (dd, *J* = 8.5, 7.4 Hz, 2H), 7.21 – 7.13 (m, 3H), 7.11 – 7.06 (m, 2H), 6.75 (s, 1H), 6.37 (t, *J* = 18.0 Hz, 1H), 5.88 (d, *J* = 65.7 Hz, 1H), 5.51 (s, 2H), 4.80 (d, *J* = 42.7 Hz, 2H), 4.56 (d, *J* = 12.9 Hz, 1H), 4.34 (d, *J* = 25.6 Hz, 1H), 4.15 (d, *J* = 11.6 Hz, 1H), 3.92 (d, *J* = 22.3 Hz, 3H), 3.74 (s, 1H), 3.35 (t, *J* = 12.0 Hz, 1H), 3.15 (d, *J* = 14.8 Hz, 1H), 2.87 (s, 1H), 2.31 (d, *J* = 30.9 Hz, 4H), 2.04 – 1.81 (m, 12H). ^{13}C NMR (101 MHz,CDCl3) δ 158.77 , 156.22 , 134.90 , 133.00 , 130.01 , 129.92 , 127.31 , 124.17 , 119.63 , 119.15 , 41.08 , 41.00 , 38.65 , 34.26 , 32.49 , 31.92 , 30.91 , 29.79 , 29.71 , 29.61 , 29.33 , 29.25 , 27.22 , 25.55 , 22.70 , 14.14 .HR-MS (m/z) $[M + H]^+$ calcd: 622.3136; Found: 622.3126.



Scheme S5

(S6). To 30 mL DCM was added *tert*-butyl piperidin-4-ylcarbamate (400 mg, 2 mmol) and K_2CO_3 (552.8 mg, 4 mmol), the resulting mixture was stirred for 1 hour followed by addition of methyl (*E*)-4-bromobut-2-enoate (254 mg, 2 mmol) and further stirred at 60℃ for 20 hours. Upon solvent evaporation, the residue was purified by flash column to afford **S6** (238.5 mg, 80% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.87 – 6.70 (m, 2H), 5.98 (dt, *J* = 15.7, 1.6 Hz, 1H), 3.65 (s, 3H), 3.18 (dd, *J* = 7.5, 3.7 Hz, 1H), 3.08 (dd, *J* = 5.9, 1.7 Hz, 2H), 2.72 (dt, *J* = 11.8, 3.7 Hz, 2H), 1.95 (td, *J* = 12.0, 2.8 Hz, 2H), 1.67 (dd, *J* = 12.7, 4.2 Hz, 2H), 1.37 (s, 9H).

(S7). To a stirred solution of **S6** (149 mg, 0.5 mol) in 10 mL MeOH was added 2 mL NaOH solution (2N). The reaction was stirred overnight at room temperature and then diluted with 10 mL H_2O. The mixture was then acidified with 1N HCl followed by extraction with ethyl acetate (3 × 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 to give a crude product (127.8 mg, 90 %.), which can be used in next step directly. ^{1}H NMR (400 MHz, Methanol-d_4) δ 6.86 (dd, *J* = 15.2, 7.3 Hz, 1H), 6.20 (d, *J* = 15.4 Hz, 1H), 3.88 (d, *J* = 6.9 Hz, 2H), 3.59 (s, 1H), 3.49 – 3.35 (m, 2H), 3.17 – 3.01 (m, 2H), 2.14 – 1.98 (m, 2H), 1.85 – 1.68 (m, 2H), 1.37 (s, 9H).

To 10 mL DCM was added the crude product (99 mg, 0.35 mmol), HATU (160 mg, 0.42 mmol), TEA (70 mg, 0.7 mmol) and **S3** (135 mg, 0.35 mmol), successively. The reaction was stirred for 10 hours and then quenched by addition of 5 mL water, the resulting mixture was extracted with EtOAc (3×10 mL). The combined organics were washed with 5% $NaHCO_3$ (2×10 mL), dried over anhydrous Na_2SO_4, concentrated *in vacuo* to give compound **S7** (137 mg, 60%), which can be used in next step directly. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.26 (d, *J* = 9.4 Hz, 1H), 7.71 – 7.64 (m, 2H), 7.45 – 7.37 (m, 2H), 7.22 – 7.04 (m, 5H), 6.93 – 6.57 (m, 2H), 4.58 (dd, *J* = 12.9, 4.1 Hz, 1H), 4.21 – 4.02 (m, 2H), 3.95 (dd, *J* = 13.7, 8.5 Hz, 1H), 3.69 – 3.61 (m, 1H), 3.61 – 3.40 (m, 3H), 3.19 – 3.06 (m, 1H), 2.69 (d, *J* = 56.6 Hz, 2H), 2.36 (dt, *J* = 16.1, 6.5 Hz, 1H), 2.22 (dt, *J* = 13.3, 4.6 Hz, 1H), 2.18 – 2.06 (m, 1H), 1.99 (dd, *J* = 27.9, 11.4 Hz, 2H), 1.80 – 1.54 (m, 3H), 1.44 (d, *J* = 4.4 Hz, 9H).

(IB-3). To a solution of 2 mL TFA and 6 mL DCM was added **S7** (19.6 mg, 0.03 mmol), the reaction was stirred at room temperature for 30 minutes followed by solvent evaporation *in vacuo*. The crude product was dissolved in 2 mL DMF followed by addition of TCO-NHS (5.3 mg, 0.02 mmol) and TEA (10 mg, 0.1 mmol), the reaction was stirred at room temperature for 1 hour. After that the mixture was quenched by addition of 3 mL water, extracted with EtOAc (2×10 mL), concentrated and then purified by flash column (MeOH:DCM = 1:50) to give compound **IB-3** (2.8 mg, 20% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.25 (s, 1H), 7.69 – 7.62 (m, 2H), 7.48 – 7.40 (m, 2H), 7.22 – 7.09 (m, 5H), 6.65 (s, 1H), 6.54 (s, 1H), 5.57 (ddd, *J* = 14.9, 10.1, 4.1 Hz, 1H), 5.42 (t, *J* = 13.5 Hz, 1H), 4.69 (d, *J* = 16.8 Hz, 1H), 4.52 (d, *J* = 12.6 Hz, 1H), 4.25 – 4.00 (m, 3H), 3.74 (t, *J* = 11.3 Hz, 1H), 2.21 (d, *J* = 31.4 Hz, 4H), 2.11 (s, 1H), 2.01 – 1.78 (m, 6H), 1.58 (d, *J* = 24.2 Hz, 6H). HR-MS (m/z) $[M + H]^+$ calcd: 705.3871; Found: 705.3866.

4. Crystal structures of various kinase inhibitors with the corresponding main target proteins

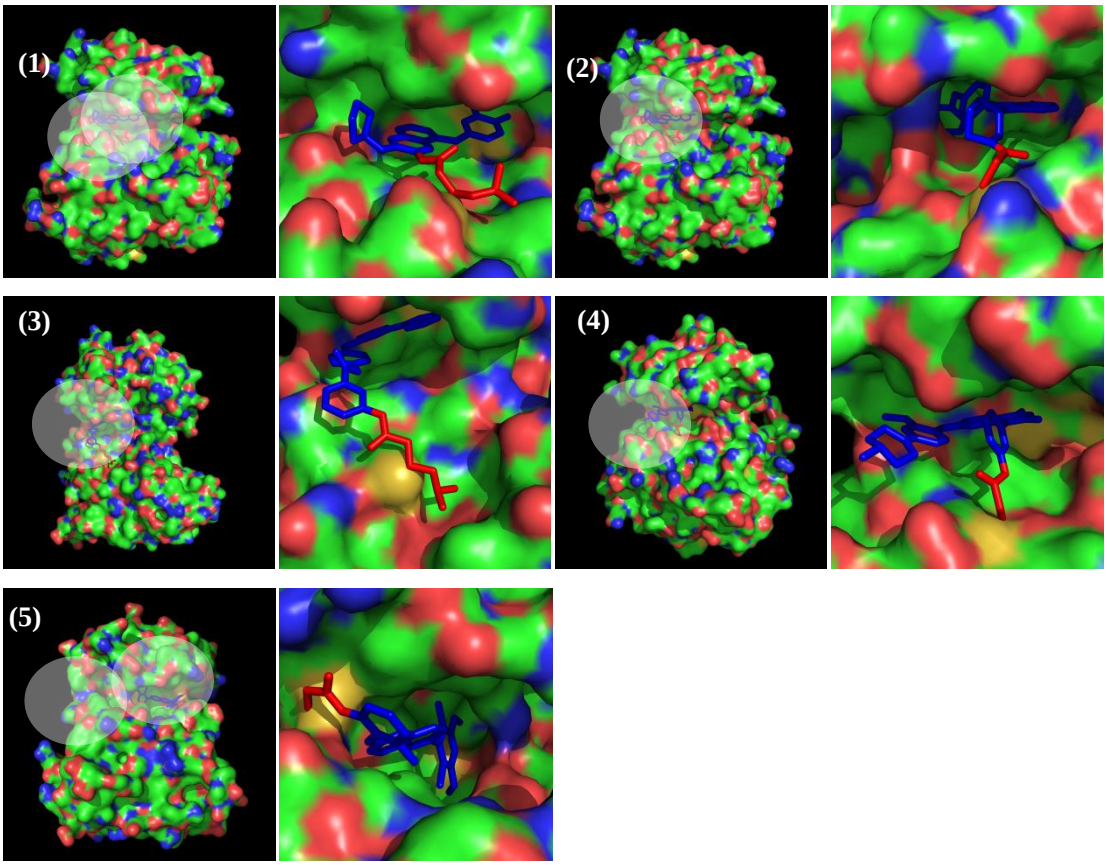


Figure S1. Crystal structure view of (1) **Afatinib**/EGFR (4g5p), (2) **Ibrutinib**/BTK (5p9j), (3) **JNK-IN-8**/JNK3 (3v6s), (4) **XTF262**/EGFR^{T790M} (5gmp), (5) **BLU9931**/FGFR4 (4XCU), the amenable site of the parent inhibitors was red-colored.

5. *In Vitro* Enzymatic Activity Assay and Cell Growth Inhibition Assay[8]

All the probes were evaluated with the EGFR, BTK kinase inhibition using Z'-LYTE™ fluorescence resonance energy transfer (FRET) method, parent inhibitors were 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 A431 and Raji cells by CCK8 assay. 3000 cells per well were seeded in a 96-well plate (100 μL medium/well) and incubated for 24 h 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 31.2, 15.6, 7.8, 3.9, 1.95, 0.97, 0.48, 0.24, 0.12, 0.06, 0.03 and 0.015 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.

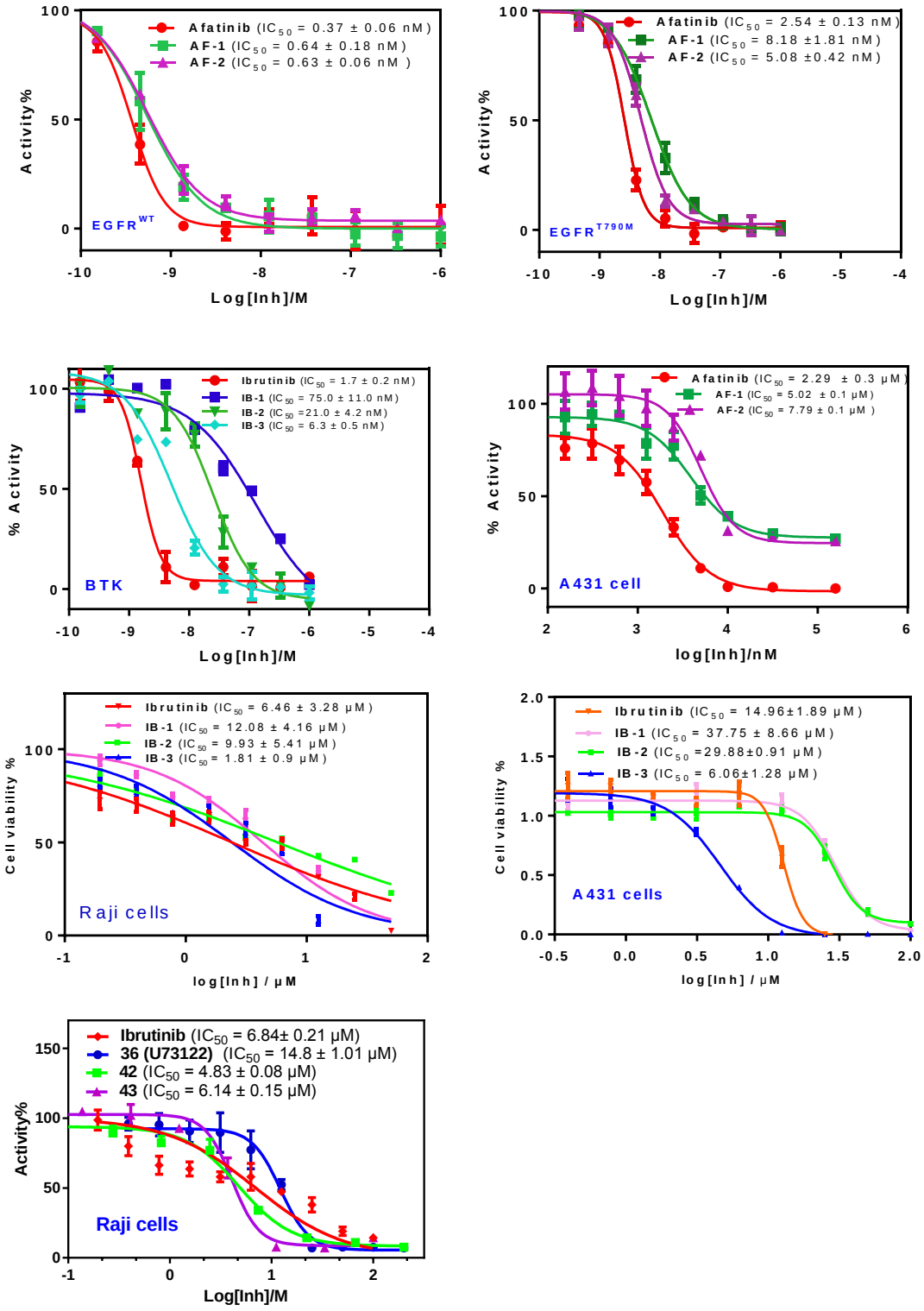


Figure S2. IC_{50} values of the probes against recombinant kinases and cancer cells with corresponding parent inhibitors as positive controls.

6. *In Vitro and In Situ* Proteome Labeling

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 (50 μM TAMRA-N_3 or TAMRA-alkyne from 30 mM stock solution in DMSO, 100 μM THPTA from 100 mM freshly prepared stock solution in DMSO, 1 mM TCEP from 1 M freshly prepared stock solution in deionized water, and 1 mM $CuSO_4$ from 1 M freshly prepared stock solution in deionized water) or Tetrazine-Cy5 (1 equivalent) 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 ℃), 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). (see Figures 3A-C, 4A for representative examples).

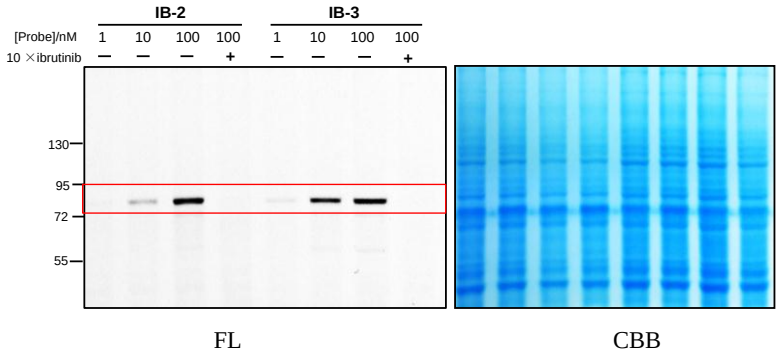


Figure S3. Concentration-dependent labeling of Toledo cells with **IB-2/3**, in the presence or absence of ibrutinib. FL = in-gel fluorescence scanning, CBB = Coomassie gel.

7. Pull down and Targets Validation

To identify the interacting cellular targets of **AF-1/2** and **IB-2/3** 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. A431, Toledo and Raji 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 2-4 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 (50 μM Biotin-N_3 or Biotin-alkyne from 30 mM stock solution in DMSO, 100 μM THPTA from 100 mM freshly prepared stock solution in DMSO, 1 mM TCEP from 1 M freshly prepared stock solution in deionized water, and 1 mM $CuSO_4$ from 1 M freshly prepared stock solution in deionized water) or Tetrazine-biotin (5 equivalent). 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 2 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 were carried out concurrently with live cells. WB 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°C 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, the supernatant was removed and the beads were washed by 1 mL PBS once. For the digestion, 150 μL 2 M urea in PBS, 1 mM $CaCl_2$ in 50 mM NH_4HCO_3 and 1 μg of trypsin were added. The reaction was incubated at 37 °C 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 1000 system coupled to a Q Exactive HF (both - Thermo Scientific). About 1 μg of peptides were separated in an home-made column (75 μm x 15 cm) packed with C18

AQ (5 μm, 300Å, Michrom BioResources, Auburn, CA, USA) at a flow rate of 300 nL/min. Mobile phase A (0.1% formic acid in 2% ACN) and mobile phase B (0.1% formic acid in 98% ACN) were used to establish a 60 min gradient comprised of 2 min of 5% B, 40 min of 5-26% B, 5 min of 26-30% B, 1 min of 30-35% B, 2 min of 35-90% B and 10 min of 90% B. Peptides were then ionized by electrospray at 1.9 kV. A full MS spectrum (375-1400 m/z range) was acquired at a resolution of 120,000 at m/z 200 and a maximum ion accumulation time of 20 ms. Dynamic exclusion was set to 30 s. Resolution for HCD MS/MS spectra was set to 30,000 at m/z 200. The AGC setting of MS and MS^2 were set at 3E6 and 1E5, respectively. The 20 most intense ions above a 1.0E3 counts threshold were selected for fragmentation by HCD with a maximum ion accumulation time of 60 ms. Isolation width of 1.6 m/z units was used for MS^2. Single and unassigned charged ions were excluded from MS/MS. For HCD, normalized collision energy was set to 25%.

The raw data were processed and searched with MaxQuant 1.5.4.1 with MS tolerance of 4.5 ppm, and MS/MS tolerance of 20 ppm. The UniProt human protein database (release 2016_07, 70630 sequences) and database for proteomics contaminants from MaxQuant were used for database searches. Reversed database searches were used to evaluate false discovery rate (FDR) of peptide and protein identifications. Two missed cleavage sites of trypsin were allowed. Carbamidomethylation (C) was set as a fixed modification, and oxidation (M), Acetyl (Protein N-term) and deamidation (NQ) were set as variable modifications. The FDR of both peptide identification and protein identification is set to be 1%.[9] The options of "Second peptides", "Match between runs" and "Dependent peptides" were enabled. Label-free quantification was used to quantify the difference of protein abundances between different samples. [10,11]

8. Cellular Imaging

To demonstrate the utility of the cell-permeable probes for imaging of cellular targets, we performed fluorescence microscopy. The general procedures were similar to what was previously reported.[1-4] For fixed cells, A431 cells seeded in glass bottom dishes and grown until 70−80% confluency were treated with 0.2 mL of DMEM with a probe (**AF-1**) or DMSO at different indicated concentrations. After incubation for 2-5 h, the medium was removed and cells were gently washed twice with PBS. 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 treated with a freshly premixed click chemistry reaction solution in a 200 μL volume (final concentration of reagents : 50 μM TAMRA-N_3 from 2.5 mM stock solution in DMSO, 50 μM THPTC from 2.5 mM freshly prepared stock solution in deionized water, 0.5 mM TCEP from 25 mM freshly prepared stock solution in deionized water, and 0.5 mM CuSO4 from 25 mM freshly prepared stock solution in deionized water) for 2 h at room temperature with vigorous shaking. Cells were washed with PBS at least three times and 0.1% Tween 20 in PBS for once. Cells were then blocked with 2-5% BSA in PBS for 30 min, washed twice with PBS, and further incubated with anti-EGFR antibody (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. Finally, the cells were stained with Hoechst (1:5000 dilution in PBS) for 10 min at room temperature prior to image.

For live-cell imaging, A431 and Toledo cells seeded in glass bottom dishes and grown until 70−80% confluency were treated with 0.2 mL of DMEM with **AF-2** or **IB-2/3** at different indicated concentrations, DMSO was performed as a control. After incubation for 2-4 h, the medium was removed and cells were gently washed twice with PBS. Cells were then incubated in DMEM containing DBCO-Cy3 or Tetrazine-Cy5 for 2h at 37 ℃, and washed with fresh DMEM medium 2 h before being imaged. In the last 20 min of incubation, Hochest nuclear stain (1:5000 dilution) was added to the incubation medium. 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-EGFR antibody or 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 Leica TCS SP8 confocal microscope system and images were processed as previously described.

9. Gel-based ABPP screening assay

For gel-based ABPP screening, toledo cells were grown to 80-90% confluency in 6-well plates. The medium was removed and treated with 2 mL free medium with DMSO, **IB-3** (2 μL, stock concentration was 1 μM in DMSO) and/or screening compounds (2 μL, stock concentration was 10 μM). After 4 h incubation at 37 ℃, the medium was removed. The cells were washed twice with PBS, followed by lysis with 100 μL RIPA lysis buffer (Thermo Scientific™ #89900) on ice for 30 min. A protein solution was obtained by centrifugation (14000 rmp, 4℃) for 5 min. The protein concentrations were further determined using the BCA protein assay (Pierce™ BCA protein assay kit) and diluted to 1 mg/mL with RIPA lysis buffer. Subsequently, 2 μL Tz-Cy5 (stock concentration was 100 μM in DMSO) was added to react for 1 h at room temperature. Then click reactions were stopped by 1 mL cold acetone at 4 ℃ for 30 min and the solutions were removed through centrifugation (14000 rmp, 4℃) for 10 min. The proteins were dissolved in 30 μL 2×SDS loading dye and heated to 95℃ for 10 min. Finally, the proteins were separated by SDA-PAGE and visualized by in-gel fluorescence scanning (Typhoon FLA 9500). * marked band was BTK produced by **IB-3**.

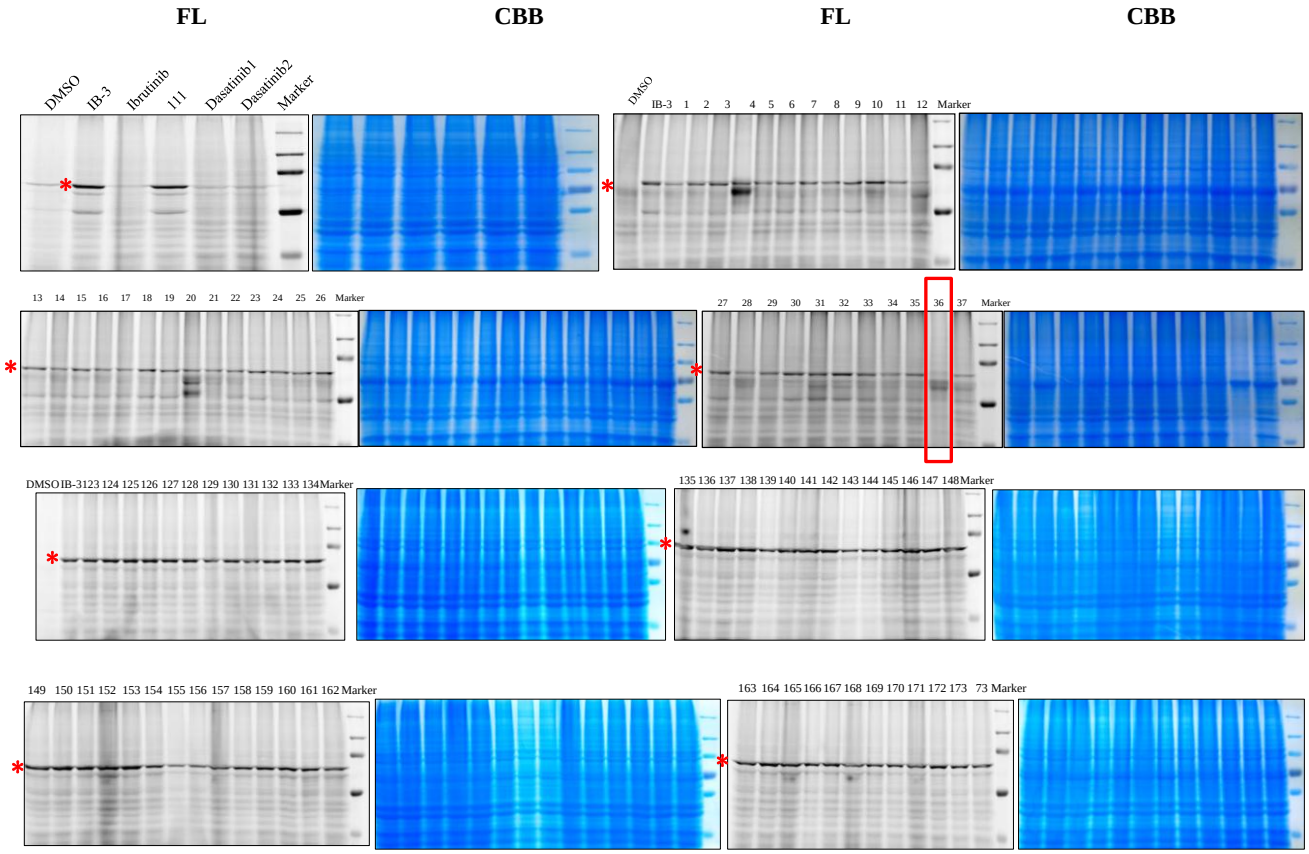


Figure S4. Gel-based ABPP assay for screening the inhibitor library against BTK in live cells, FL = in-gel fluorescence scanning, CBB = Coomassie gel.

Table S3. Protein hits identified by pull-down/LC-MS/MS with AF-1/2 (100 nM final concentration) in A431 cells.

Protein names	Gene names	Mol. weight [kDa]	LFQ ratio	
			AF-1/(AF-1+5×afatinib)	AF-2/(AF-2+5×afatinib)
Receptor protein-tyrosine kinase;Epidermal growth factor receptor	EGFR	120.69	3.60617845	1.905804752
Lymphocyte antigen 6D	LY6D	13.286	10	10
Ras-related protein Rab-1B;Putative Ras-related protein Rab-1C	RAB1B;RAB1C	18.483	10	10

Pyrroline-5-carboxylate reductase 1, mitochondrial;Pyrroline-5-carboxylate reductase	PYCR1	30.213	3.392584515	2.438440182
DNA topoisomerase 3-alpha	TOP3A	112.37	2.322429387	10
40S ribosomal protein S17	RPS17	15.919	1.708557364	10
Adenosylhomocysteinase	AHCY	47.716	1.522028783	10
40S ribosomal protein S20	RPS20	7.2473	2.092920354	1.744558294
Keratin, type I cytoskeletal 10	KRT10	59.51	3.019208492	1.609142724
Keratin, type II cytoskeletal 1	KRT1	66.038	1.875190529	2.364751504
Zinc finger protein 736	ZNF736	49.868	2.231588354	2.806366414
Keratin, type II cytoskeletal 5	KRT5	62.378	1.984438984	1.682878847
SURP and G-patch domain-containing protein 2	SUGP2	93.859	0	10
40S ribosomal protein S16	RPS16;ZNF90	11.075	0	10
Probable ATP-dependent RNA helicase DDX5	DDX5	11.026	0	10
Aconitate hydratase, mitochondrial	ACO2	87.819	0	10
MAP kinase-activating death domain protein	MADD	183.3	0	9.611363791
Probable tumor suppressor protein MN1	MN1	136	0.619632967	4.060301508
Pre-mRNA-splicing factor 38A	PRPF38A	37.476	0	3.786776 86
Keratin, type I cytoskeletal 9	KRT9	62.129	1.099919641	3.527376375
Heterogeneous nuclear ribonucleoprotein U	HNRNPU	90.583	0.688223865	2.834791489
40S ribosomal protein S23	RPS23	15.807	0.553289622	2.751570825
Putative 60S ribosomal protein L39-like 5;60S ribosomal protein L39	RPL39P5;RPL39	6.3225	0.588904093	2.286373 77
60S ribosomal protein L11	RPL11	20.252	0	2.205329974
Glycogen phosphorylase, brain form;Alpha-1,4 glucan phosphorylase	PYGB	96.695	0.845306274	1.992052066
Keratin, type I cytoskeletal 14;Keratin, type I cytoskeletal 16;Keratin, type I cytoskeletal 15;Keratin, type I cytoskeletal 17	KRT14;KRT16;KRT15;KRT17	51.621	0.838810067	1.961300445
60S ribosomal protein L27a	RPL27A	12.201	0.913939564	1.748651079
40S ribosomal protein S3	RPS3	13.069	1.27880801	1.690309431
Phosphoglycerate mutase 1;Phosphoglycerate mutase 2	PGAM1;PGAM2	28.804	0	1.619120707
Keratin, type II cytoskeletal 6A;Keratin, type II cytoskeletal 6C	KRT6A;KRT6C	60.044	1.040833514	1.593584868
Histone H4	HIST1H4A	11.367	1.254950618	1.592071283
Keratin, type II cytoskeletal 2 epidermal	KRT2	65.865	2.833994738	1.461059037

Dynein light chain 1, cytoplasmic;Dynein light chain 2, cytoplasmic	DYNLL1;DYNLL2	10.366	1.435034509	1.434656972
Cytochrome b-c1 complex subunit 8	UQCRQ	9.9062	1.351513833	1.424787263
Paxillin	PXN	64.232	2.480225989	1.420278159
Arginine/serine-rich coiled-coil protein 2	RSRC2	50.559	0.759970609	1.384040159
Spectrin beta chain, non-erythrocytic 1	SPTBN1	274.83	1.657235955	1.382478 17
40S ribosomal protein S8	RPS8	21.879	0.640729637	1.374486408
Actin, alpha skeletal muscle;Actin, alpha cardiac muscle 1;Actin, gamma-enteric smooth muscle;Actin, aortic smooth muscle	ACTA1;ACTC1;ACTG2;ACTA2	42.051	1.050129944	1.299008843
60S ribosomal protein L35a	RPL35A	12.538	1.291750791	1.285350154
60S ribosomal protein L30	RPL30	12.656	1.889164861	1.277069755
Guanine nucleotide-binding protein subunit beta-2-like 1;Guanine nucleotide-binding protein subunit beta-2-like 1, N-terminally processed	GNB2L1	25.869	0.943618317	1.27171
Leucine-twenty homeobox	LEUTX	18.626	0	1.269530378
ATP synthase subunit alpha, mitochondrial	ATP5A1	21.339	1.162437774	1.241360174
Lysophospholipid acyltransferase 5	LPCAT3	56.034	2.326233308	1.204748 64
Splicing regulatory glutamine/lysine-rich protein 1	SREK1	59.38	0	1.189639851
Hepatocyte growth factor-regulated tyrosine kinase substrate	HGS	32.433	0	1.188177302
Heterogeneous nuclear ribonucleoprotein L	HNRNPL	58.362	0	1.182557904
ADP/ATP translocase 2;ADP/ATP translocase 2, N-terminally processed;ADP/ATP translocase 3;ADP/ATP translocase 3, N-terminally processed	SLC25A5;SLC25A6	32.852	1.185207348	1.165266 39
40S ribosomal protein S9	RPS9	22.591	0.982964574	1.163561403
Beta-glucuronidase	GUSB	74.731	0.828959366	1.161316242
ATP synthase subunit a	MT-ATP6	24.817	0.549038 03	1.160408866
40S ribosomal protein S11	RPS11	18.431	0.967120836	1.154511487
Zinc finger protein 160	ZNF160	1.5498	0.950850219	1.139731641
60S ribosomal protein L14	RPL14	14.558	1.0705756	1.118913 68
60S ribosomal protein L31	RPL31	14.463	0.992501802	1.101564753
Eukaryotic translation initiation factor 2A;Eukaryotic translation initiation factor 2A, N-terminally processed	EIF2A	26.11	0.935088491	1.101446727
60S ribosomal protein L34	RPL34	13.293	1.727953125	1.099489087

Heat shock 70 kDa protein 1B;Heat shock 70 kDa protein 1A	HSPA1B;HSPA1A	70.108	0.99156608	1.099311666
Cell cycle and apoptosis regulator protein 2	CCAR2	102.9	1.118828346	1.098768035
60S ribosomal protein L15;Ribosomal protein L15	RPL15	24.146	1.389278978	1.097319206
40S ribosomal protein S3a	RPS3A	22.572	2.495949792	1.095882168
Tubulin--tyrosine ligase-like protein 12	TTLL12	74.403	2.206052489	1.092348478
Eukaryotic translation initiation factor 5A-1;Eukaryotic translation initiation factor 5A	EIF5A	16.832	0	1.090144113
Nucleosome assembly protein 1-like 4	NAP1L4	12.25	1.452961097	1.089669517
Serine hydroxymethyltransferase;Serine hydroxymethyltransferase, mitochondrial;Serine hydroxymethyltransferase, cytosolic	SHMT2;SHMT1	23.965	0.833773507	1.057230157
Filamin-A	FLNA	245.85	1.22359436	1.056422594
Myosin-9	MYH9	226.53	1.216571552	1.055273426
60S ribosomal protein L32	RPL32	15.616	0.969666775	1.052043774
Fatty acid synthase;[Acyl-carrier-protein] S-acetyltransferase;[Acyl-carrier-protein] S-malonyltransferase;3-oxoacyl-[acyl-carrier-protein] synthase;3-oxoacyl-[acyl-carrier-protein] reductase;3-hydroxyacyl-[acyl-carrier-protein] dehydratase;Enoyl-[acyl-carrier-protein] reductase;Oleoyl-[acyl-carrier-protein] hydrolase	FASN	273.42	0.887993889	1.039148362
40S ribosomal protein S4, X isoform	RPS4X	29.597	0.942620938	1.036065085
60S ribosomal protein L21	RPL21	18.565	1.046093618	0.998379544
60S ribosomal protein L23	RPL23	14.865	1.436699627	0.991312073
60S ribosomal protein L36a-like;60S ribosomal protein L36a	RPL36A;RPL36AL;RPL36A-HNRNPH2	16.378	0.722761029	0.990224804
Serpin B5	SERPINB5	42.1	0.932768617	0.975887728
60S ribosomal protein L13a;Putative 60S ribosomal protein L13a protein RPL13AP3	RPL13A;RPL13a;RPL13AP3	23.577	1.368066066	0.969691915
Caveolin;Caveolin-1	CAV1	19.177	1.003551194	0.965814608
Splicing factor, proline- and glutamine-rich	SFPQ	76.149	0.653068054	0.961595734
Tubulin beta chain;Tubulin beta-4B chain;Tubulin beta-2B chain;Tubulin beta-2A chain;Tubulin beta-4A chain;Tubulin beta-3 chain	TUBB;TUBB4B;TUBB2B;TUBB2A;TUBB4A;TUBB3	47.766	0.611003712	0.954651081
60S ribosomal protein L3	RPL3	46.108	1.139352037	0.948192689
Pre-mRNA-processing-splicing factor 8	PRPF8	273.6	1.366900055	0.94652428

Protein SREK1IP1	SREK1IP1	11.405	1.476403665	0.933968479
60S ribosomal protein L7	RPL7	29.225	1.576658259	0.933230558
Eukaryotic translation initiation factor 2 subunit 3	EIF2S3	51.109	0.93289568	0.927632939
Tubulin alpha-1B chain;Tubulin alpha-4A chain	TUBA1B;TUBA4A	50.151	0.520799119	0.923675072
60S ribosomal protein L10a	RPL10A	24.831	1.603610266	0.914980641
60S ribosomal protein L5	RPL5	34.362	1.434072172	0.914924864
60S ribosomal protein L7a	RPL7A	29.995	1.577257946	0.912683723
Actin, cytoplasmic 2;Actin, cytoplasmic 2, N-terminally processed;Actin, cytoplasmic 1;Actin, cytoplasmic 1, N-terminally processed	ACTG1;ACTB	41.792	0.941189706	0.909293777
Elongation factor 2	EEF2	95.337	2.199524161	0.907574921
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	36.053	0.857302213	0.905073124
Hemicentin-1	HMCN1	613.38	0.642164272	0.904313047
Transaldolase	TALDO1	37.54	1.116536764	0.900007886
Serine/arginine-rich splicing factor 11	SRSF11	42.316	0.781878428	0.899461914
Nucleoside diphosphate kinase;Nucleoside diphosphate kinase A;Nucleoside diphosphate kinase B;Putative nucleoside diphosphate kinase	NME1;NME2;NME1-NME2;NME2P1	15.261	0.990891702	0.88920755
60S ribosomal protein L13	RPL13	24.261	1.537585421	0.885586279
Ribosomal protein L19;60S ribosomal protein L19	RPL19	23.134	0.679142122	0.884239343
60S ribosomal protein L9	RPL9	20.874	0.771730626	0.876784147
40S ribosomal protein S2	RPS2	25.211	1.052087243	0.875738797
60S ribosomal protein L4	RPL4	47.697	1.395341013	0.869662492
Pyruvate kinase PKM;Pyruvate kinase	PKM	57.936	0.615988889	0.867146732
60S ribosomal protein L36	RPL36	12.254	1.345646956	0.852333018
U1 small nuclear ribonucleoprotein 70 kDa	SNRNP70	51.556	1.327476283	0.849986373
Cell division control protein 42 homolog	CDC42	21.258	1.055514577	0.84742248
Phosphoglycerate kinase 1;Phosphoglycerate kinase 2	PGK1;PGK2	44.614	1.101754841	0.837281244
Alpha-enolase	ENO1	47.168	0.814671228	0.830763419
Heterogeneous nuclear ribonucleoprotein A1;Heterogeneous nuclear ribonucleoprotein A1, N-terminally processed	HNRNPA1	19.471	1.164220811	0.823985516
Acetyl-CoA carboxylase 1;Biotin carboxylase	ACACA	265.55	0.9731506	0.823162

			38	778
Uncharacterized protein C11orf98	C11orf98	14.234	1.20904446	0.819102921
60S ribosomal protein L10	RPL10	18.565	0.779583682	0.817941977
40S ribosomal protein S6	RPS6	24.968	0.551381155	0.814327705
Probable ATP-dependent RNA helicase DDX46	DDX46	117.46	1.014852924	0.807980109
Putative elongation factor 1-alpha-like 3;Elongation factor 1-alpha 1;Elongation factor 1-alpha;Elongation factor 1-alpha 2	EEF1A1P5;EEF1A1;EEF1A2	50.184	0.741717046	0.806496215
Neuroblast differentiation-associated protein AHNAK	AHNAK	629.09	0.644958832	0.802088782
60S ribosomal protein L8	RPL8	22.389	1.485411671	0.793866231
Serine/arginine repetitive matrix protein 1	SRRM1	103.39	0.542324905	0.793763727
Heterogeneous nuclear ribonucleoproteins A2/B1	HNRNPA2B1	37.429	0.903548748	0.793742227
Neutral alpha-glucosidase AB	GANAB	106.87	0.937140286	0.790784058
L-lactate dehydrogenase B chain;L-lactate dehydrogenase	LDHB	36.638	0.853273868	0.782956567
NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 11, mitochondrial	NDUFB11	17.316	0.472497254	0.780861262
Serine/threonine-protein kinase PAK 7	PAK7	80.744	0.414875209	0.779566487
Pyruvate carboxylase, mitochondrial	PC	129.63	0.775374806	0.778674474
GTP-binding nuclear protein Ran	RAN	26.224	0.935602454	0.776573305
14-3-3 protein zeta/delta	YWHAZ	28.036	1.075088202	0.774134698
Fascin	FSCN1	54.529	0	0.772639156
Annexin A2;Annexin;Putative annexin A2-like protein	ANXA2;ANXA2P2	38.604	0.783689024	0.764164957
60S ribosomal protein L17	RPL17;RPL17-C18orf32	14.894	0.852973939	0.758839345
Elongation factor 1-gamma	EEF1G	50.118	1.363331376	0.75623998
Macrophage migration inhibitory factor	MIF	12.476	0	0.754439179
Nucleophosmin	NPM1	32.575	0.899499444	0.751632788
Heat shock protein HSP 90-beta	HSP90AB1	83.263	0.904590907	0.738279698
Fructose-bisphosphate aldolase A;Fructose-bisphosphate aldolase	ALDOA	39.42	0.873079771	0.738147981
60S ribosomal protein L28	RPL28	9.657	0.857483182	0.737696347
Serine/arginine repetitive matrix protein 2	SRRM2	299.61	1.070757594	0.734022556
Peroxiredoxin-1	PRDX1	22.11	0.919689449	0.732031418

Ezrin	EZR	69.371	4.543886117	0.729875669
60S ribosomal protein L6	RPL6	32.728	1.203275227	0.727107914
60 kDa heat shock protein, mitochondrial	HSPD1	61.054	1.245660162	0.724244359
DDB1- and CUL4-associated factor 13	DCAF13	21.134	1.486272417	0.712142595
Methylcrotonoyl-CoA carboxylase subunit alpha, mitochondrial	MCCC1	68.331	0.763740341	0.702918297
60S ribosomal protein L18	RPL18	18.756	1.626488493	0.681175106
L-lactate dehydrogenase A chain	LDHA	36.688	0.677359201	0.659412055
ADP-ribosylation factor-like protein 6-interacting protein 4	ARL6IP4	23.687	0.501357773	0.657781619
Transgelin-2	TAGLN2	21.086	0.761803498	0.657534989
Small ubiquitin-related modifier 4;Small ubiquitin-related modifier 2;Small ubiquitin-related modifier 3	SUMO3;SUMO4;SUMO2	15.317	0.947796797	0.652933797
Creatine kinase U-type, mitochondrial	CKMT1B;CKMT1A	11.197	1.565384037	0.645531005
Profilin-1	PFN1	17.517	0.339842881	0.645440573
Propionyl-CoA carboxylase alpha chain, mitochondrial	PCCA	80.058	0.820663887	0.640997943
Clathrin heavy chain;Clathrin heavy chain 1;Clathrin heavy chain 2	CLTC;CLTCL1	192.06	1.460812384	0.637033127
Heat shock 70 kDa protein 6;Putative heat shock 70 kDa protein 7	HSPA6;HSPA7	71.027	1.331248017	0.627984795
Rho GDP-dissociation inhibitor 1	ARHGDIA	9.9439	1.912383926	0.601035496
60S ribosomal protein L35	RPL35	14.551	0.270622729	0.592135028
RNA-binding protein 39	RBM39	26.698	0.924623447	0.590165487
Histone H2A type 1-J;Histone H2A type 1-H;Histone H2A.J;Histone H2A type 2-C;Histone H2A type 1-C;Histone H2A type 3;Histone H2A type 2-A;Histone H2A type 1-D;Histone H2A type 1;Histone H2A type 1-B/E;Histone H2A;Histone H2A.V;Histone H2A.Z;Histone H2A type 2-B;Histone H2A type 1-A;Histone H2AX	HIST1H2AJ;HIST1H2AH;H2AFJ;HIST2H2AC;HIST1H2AC;HIST3H2A;HIST2H2AA3;HIST1H2AD;HIST1H2AG;HIST1H2AB;H2AFV;H2AFZ;HIST2H2AB;HIST1H2AA;H2AFX	18.481	0.465782548	0.588274695
Dual specificity protein phosphatase 19	DUSP19	24.194	2.038900149	0.570566336
40S ribosomal protein S26	RPS26	13.015	0.462392851	0.56880581
Signal peptidase complex subunit 1	SPCS1	9.2748	1.930451075	0.534371629

Myosin light polypeptide 6;Myosin light chain 6B	MYL6;MYL6B	14.436	0.990391866	0.525419068
TERF1-interacting nuclear factor 2	TINF2	15.693	0	0.266750388
Heat shock cognate 71 kDa protein;Heat shock-related 70 kDa protein 2	HSPA8;HSPA2	68.805	10	0
Very-long-chain enoyl-CoA reductase	TECR	36.034	10	0
Splicing factor, arginine/serine-rich 19	SCAF1	139.27	10	0
Eukaryotic peptide chain release factor subunit 1	ETF1	47.475	10	0
40S ribosomal protein S5;40S ribosomal protein S5, N-terminally processed	RPS5	14.763	10	0
Nucleolar GTP-binding protein 1	GTPBP4	73.964	5.959417681	0
Histone H1.2;Histone H1.4;Histone H1.3	HIST1H1C;HIST1H1E;HIST1H1D	21.364	3.575224678	0
Luc7-like protein 3	LUC7L3	58.22	3.346046532	0
LIM and SH3 domain protein 1	LASP1	18.98	3.344096141	0
Keratinocyte proline-rich protein	KPRP	64.135	3.045673211	0
Aspartate aminotransferase, mitochondrial	GOT2	47.517	2.738517688	0
Elongation of very long chain fatty acids protein 1	ELOVL1	32.662	2.352836257	0
40S ribosomal protein S18	RPS18	17.718	2.272032517	0
Endothelin-2	EDN2	19.96	1.878088962	0
Pleiotropic regulator 1	PLRG1	57.193	1.659937192	0
40S ribosomal protein S27	RPS27	7.3564	1.640086405	0
Ubiquitin-40S ribosomal protein S27a;Ubiquitin;40S ribosomal protein S27a	RPS27A	17.965	1.534862963	0
Cofilin-1;Cofilin-2	CFL1;CFL2	9.0904	1.432606395	0
Cystatin-B	CSTB	11.139	1.365634776	0
40S ribosomal protein S13	RPS13	17.222	1.346501026	0
Heat shock protein beta-1	HSPB1	20.406	1.300665613	0
40S ribosomal protein S14	RPS14	16.273	1.126671976	0
Putative RNA-binding protein Luc7-like 2	LUC7L2;C7orf55-LUC7L2	46.513	0.995328626	0
Pre-mRNA-splicing factor 38B	PRPF38B	64.467	0.975081423	0
Pyrroline-5-carboxylate reductase;Pyrroline-5-carboxylate reductase 2	PYCR2	25.868	0.963806199	0
Annexin;Annexin A1	ANXA1	12.642	0.854159988	0
60S ribosomal protein L24	RPL24	14.369	0.79582491	0

Eosinophil cationic protein	RNASE3	18.385	0.65695603	0
Protein S100-A10	S100A10	11.203	0.610564865	0
E3 ubiquitin-protein ligase TRIM41	TRIM41	21.533	0.577023734	0
Heterogeneous nuclear ribonucleoprotein K	HNRNPK	50.976	0.571121013	0
Voltage-dependent anion-selective channel protein 1	VDAC1	30.772	0.566140183	0
Elongation factor Tu, mitochondrial	TUFM	49.541	0.43261637	0
Caskin-1	CASKIN1	149.81	0.352930065	0
Protein disulfide-isomerase A3	PDIA3	56.782	0.201177168	0

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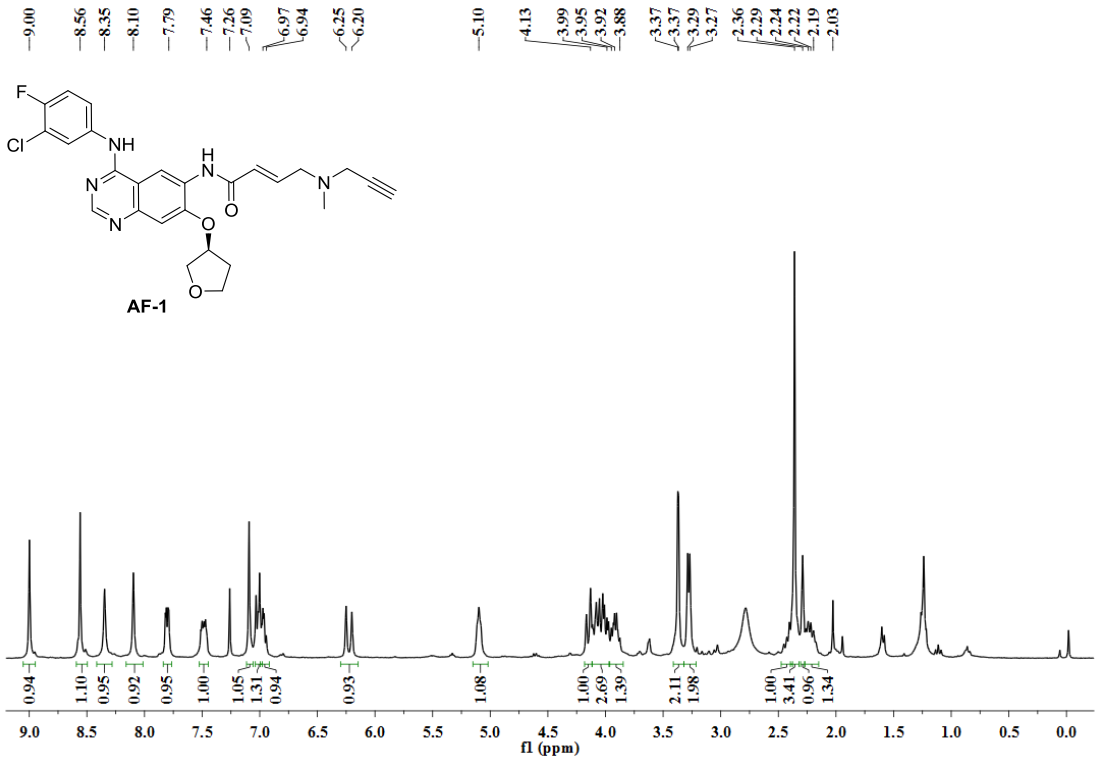
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5.10
4.13
3.99
3.95
3.92
3.88
3.37
3.37
3.29
3.27
2.36
2.29
2.24
2.22
2.19
2.03
F
Cl
NH
N
N
H
N
O
N
O
O
AF-1
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1.10
0.95
0.92
0.95
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1.31
0.94
0.93
1.08
1.00
2.69
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1.98
1.00
3.41
0.96
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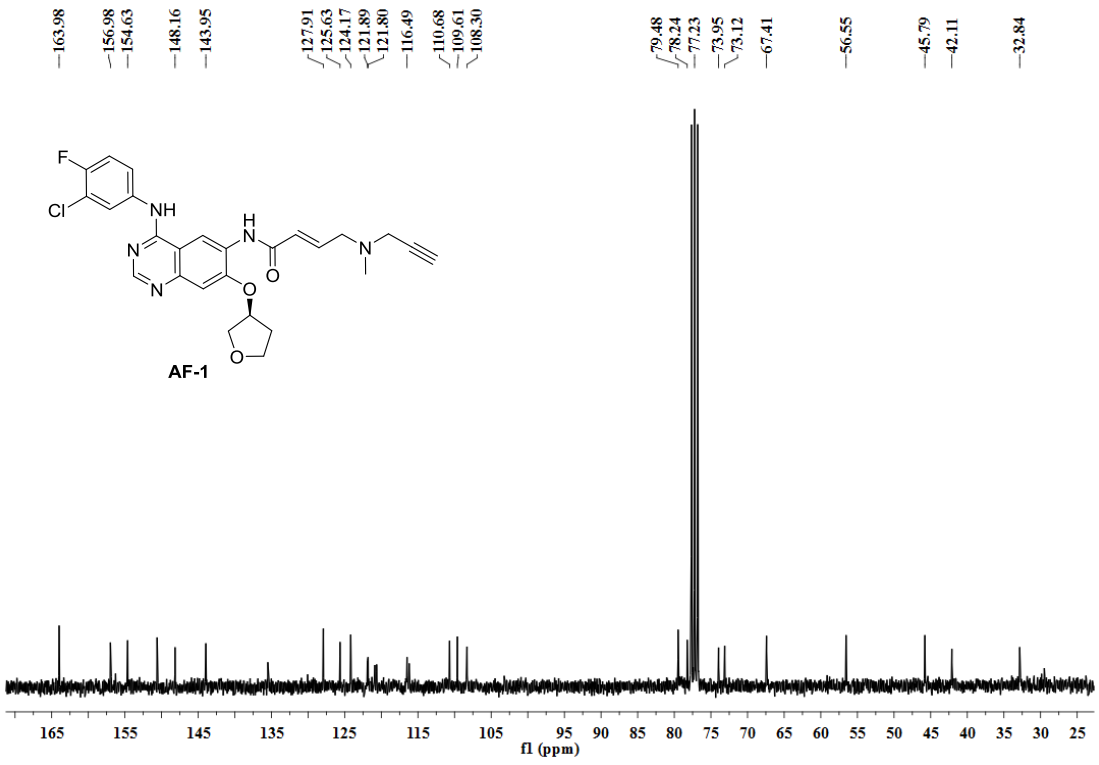
f1 (ppm)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108.30
79.48
78.24
77.23
73.95
73.12
67.41
56.55
45.79
42.11
32.84
F
Cl
NH
N
N
H
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O
N
O
O
AF-1
165
155
145
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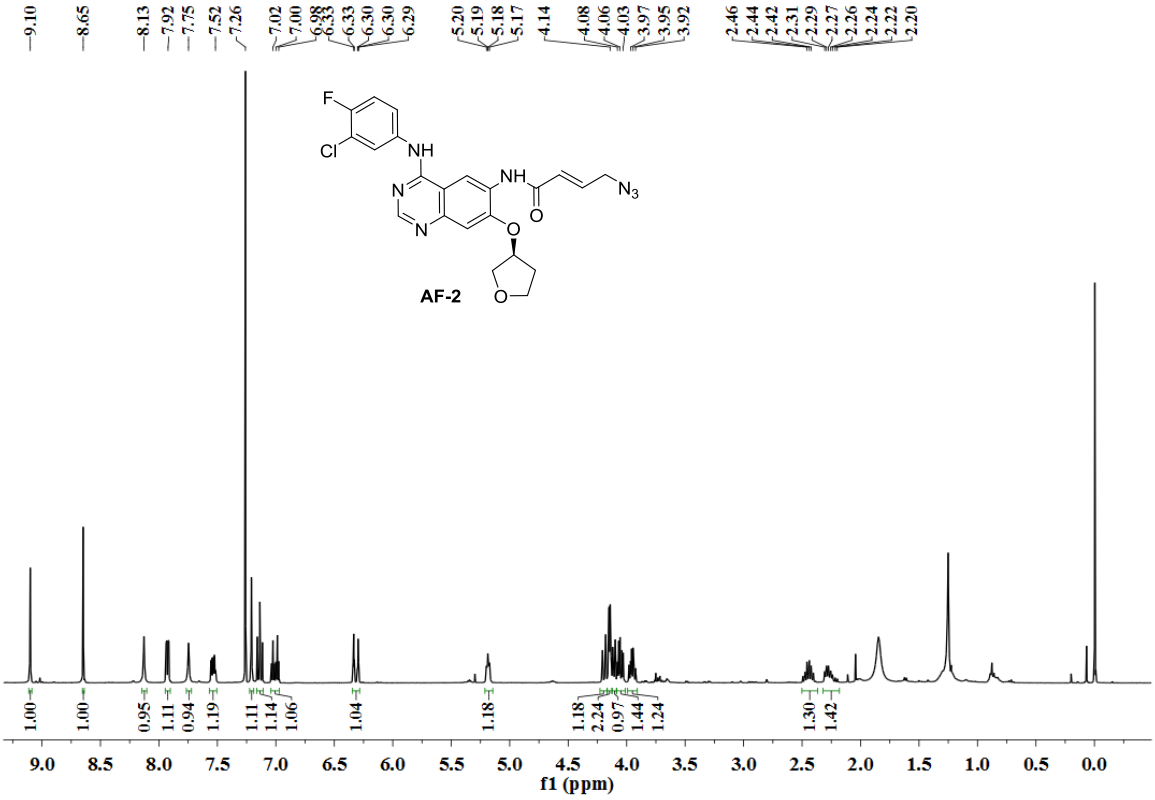
f1 (ppm)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6.29
5.20
5.19
5.18
5.17
4.14
4.08
4.06
4.03
3.97
3.95
3.92
2.46
2.44
2.42
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2.24
2.22
2.20
F
Cl
NH
N
N
NH
O
N3
O
AF-2
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1.00
0.95
1.11
0.94
1.19
1.11
1.14
1.06
1.04
1.18
1.18
2.24
0.97
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1.42
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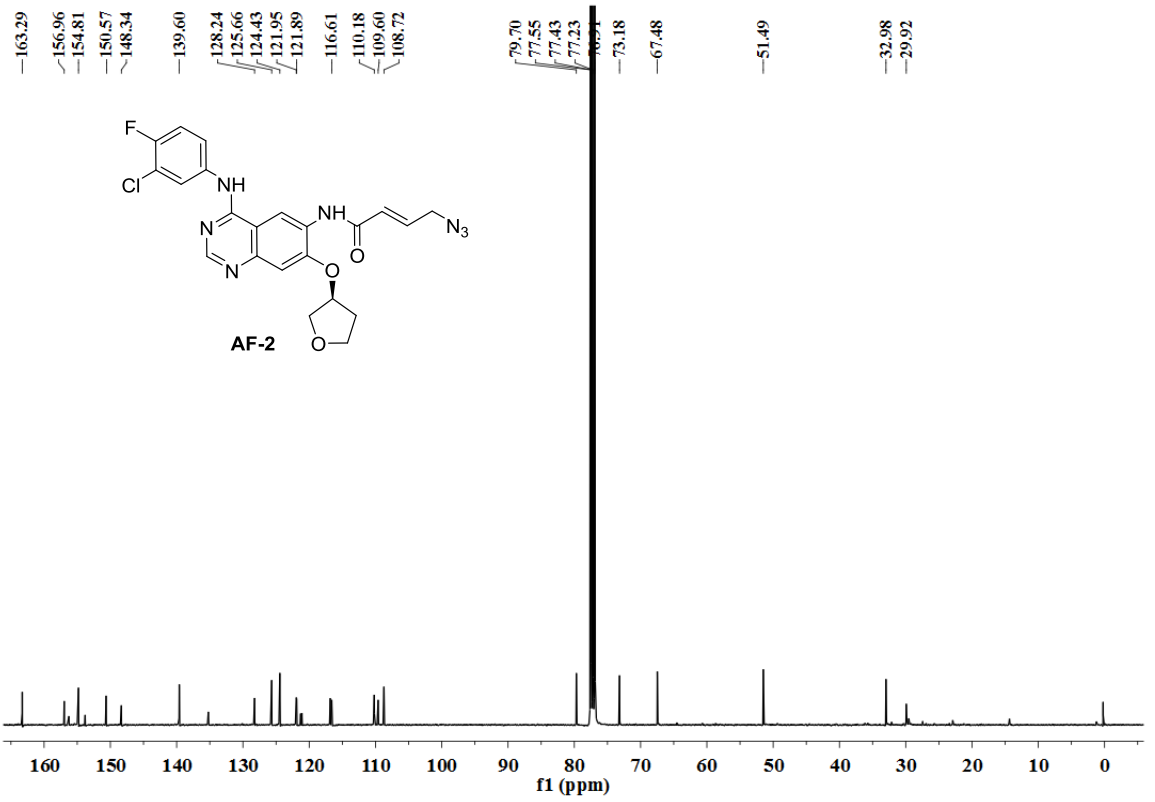
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108.72
79.70
77.55
77.43
77.23
73.18
67.48
51.49
32.98
29.92
F
Cl
NH
N
N
NH
O
N3
O
AF-2
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120
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90
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50
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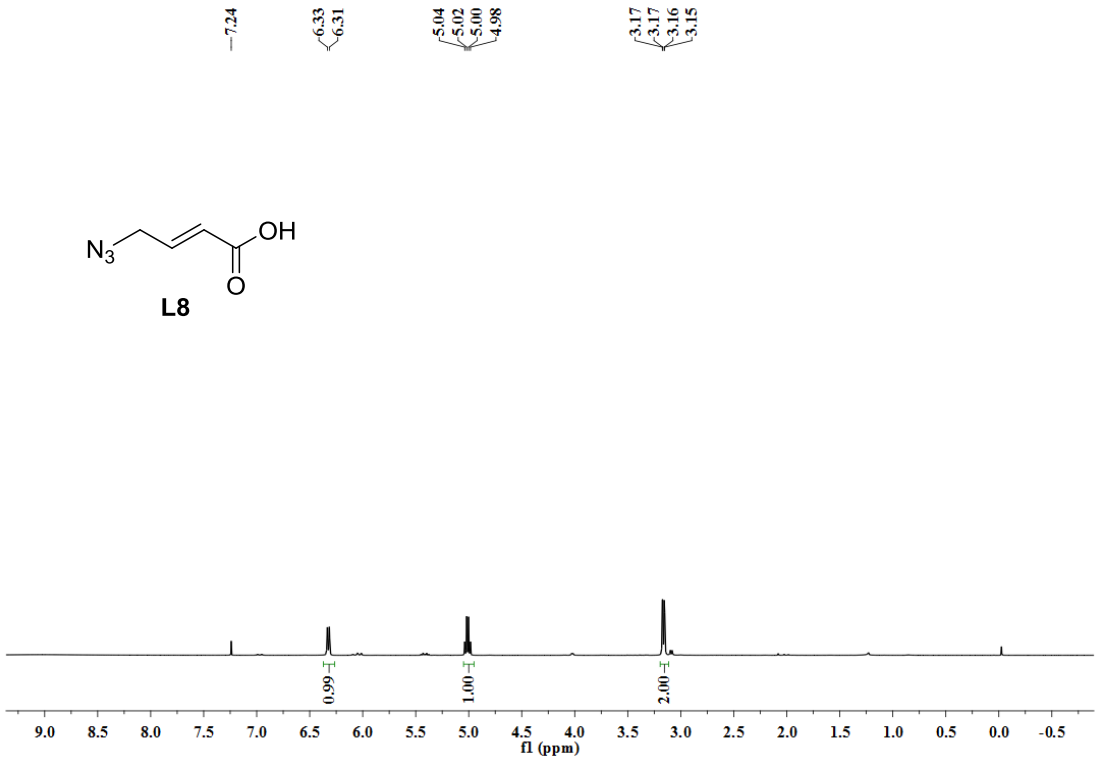
30
20
10
0
f1 (ppm)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5.00
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OH
O
L8
0.99
1.00
2.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)
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128.93
110.65
77.55
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OH
O
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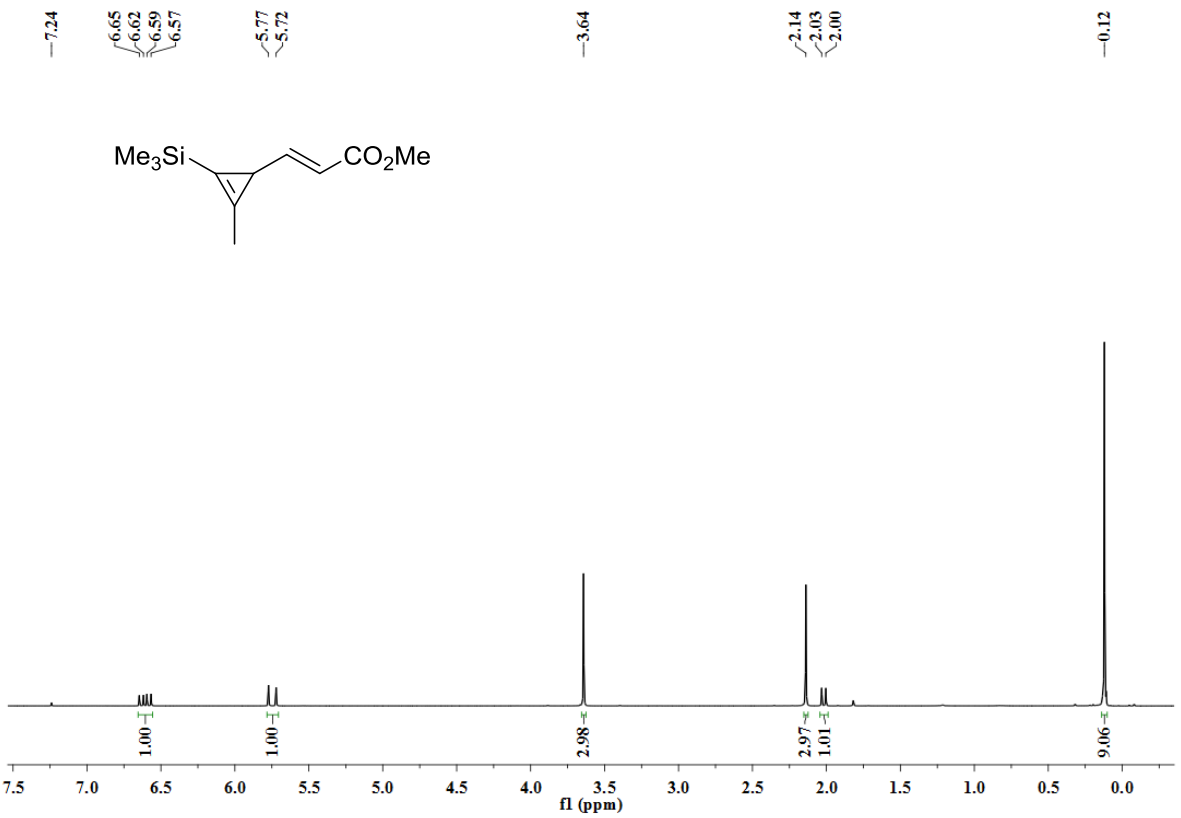
f1 (ppm)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CO2Me
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1.00
2.98
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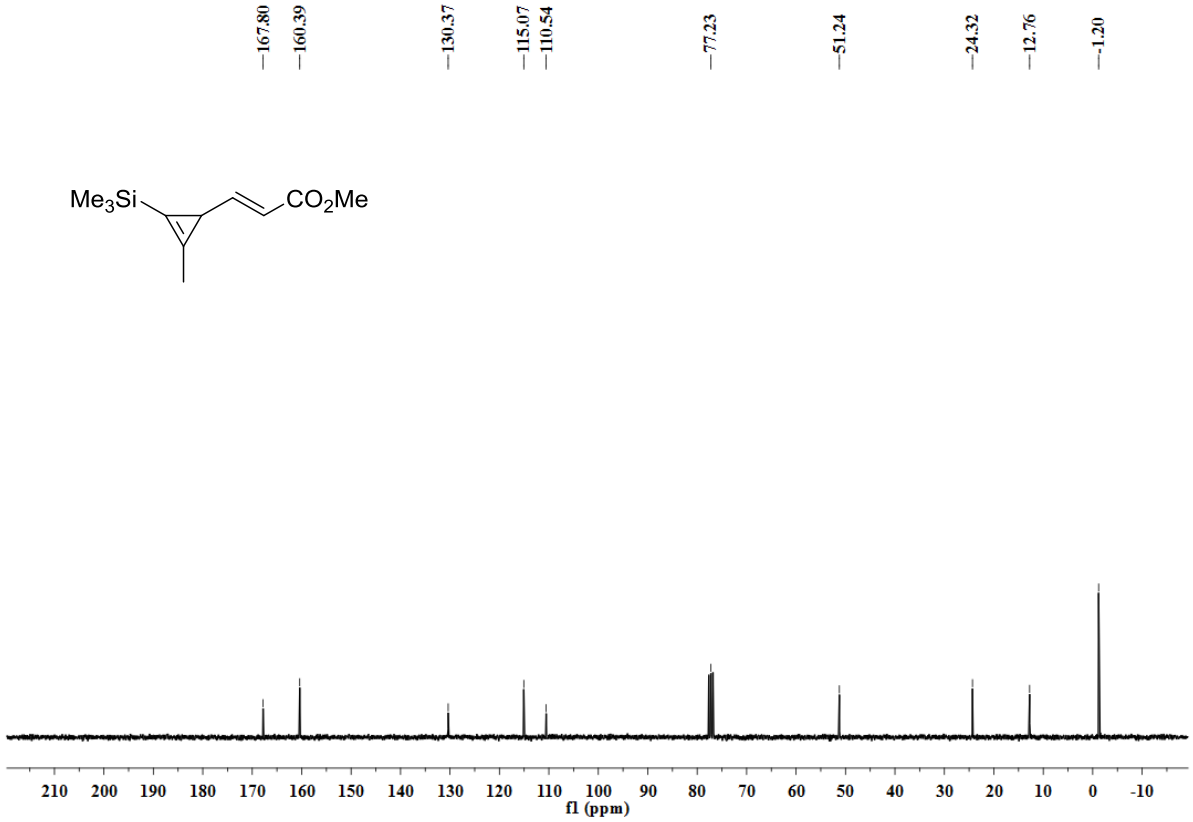
1.01
9.06
fl (ppm)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24.32
12.76
-1.20
Me3Si
CO2Me
fl (ppm)


7.24
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6.68
6.54
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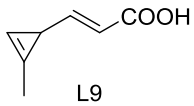
2.18
2.16
2.16
2.14
2.14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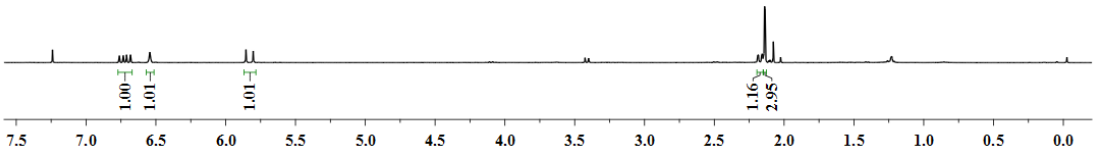
L9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2.5
2.0
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0.0


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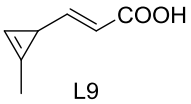
11.45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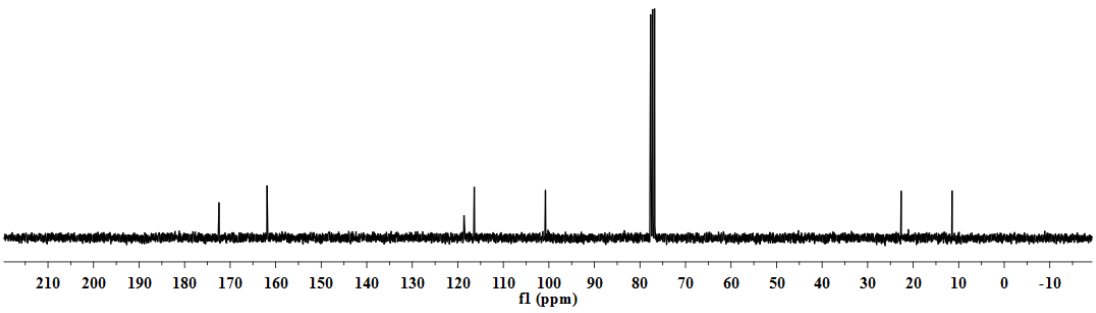
L9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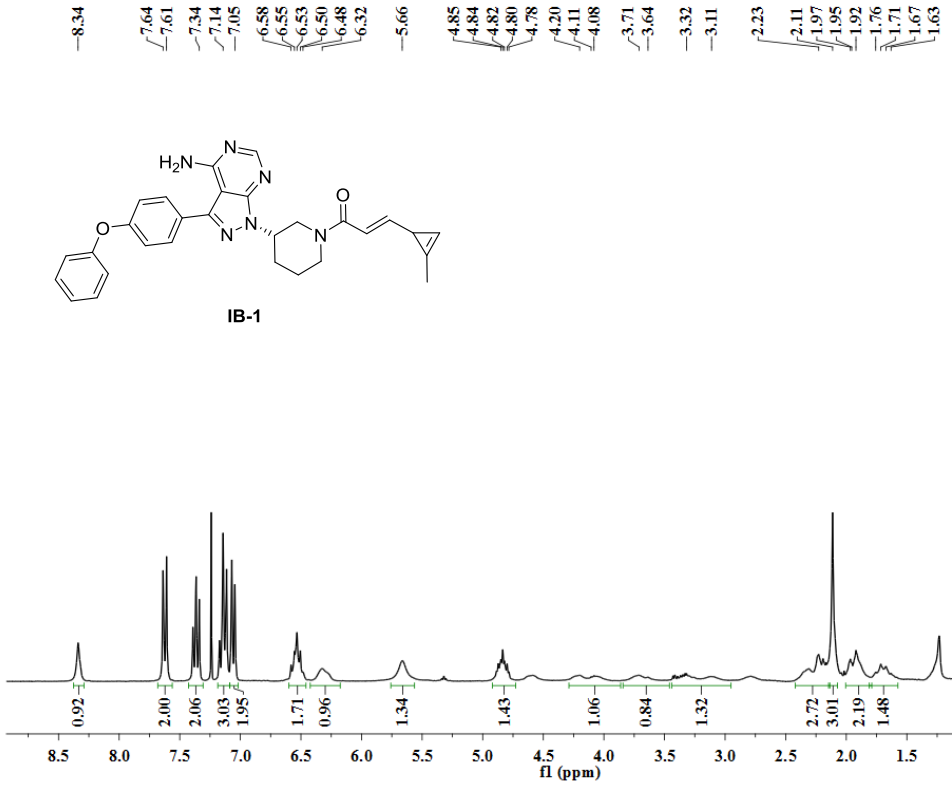
10
0
-10
f1 (ppm)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4.11
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3.71
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3.32
3.11
2.23
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1.76
1.71
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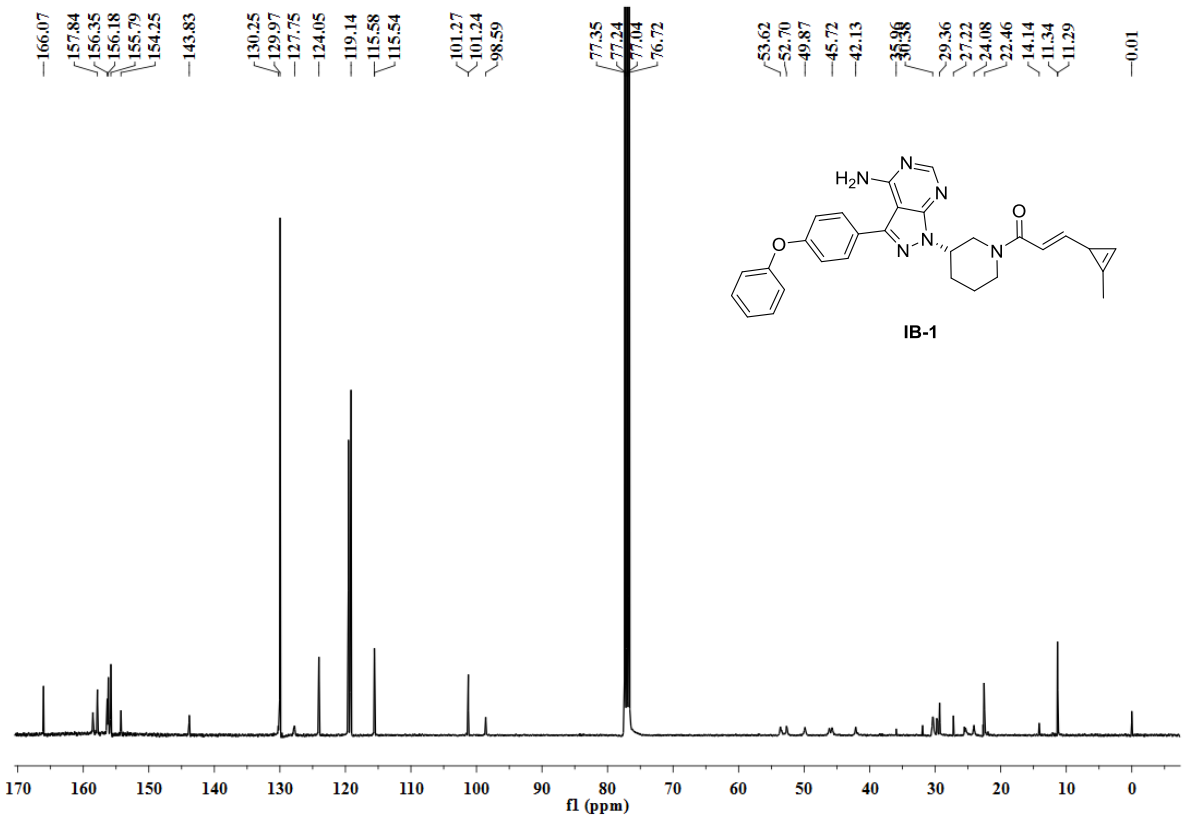
fl (ppm)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77.04
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49.87
45.72
42.13
35.86
30.38
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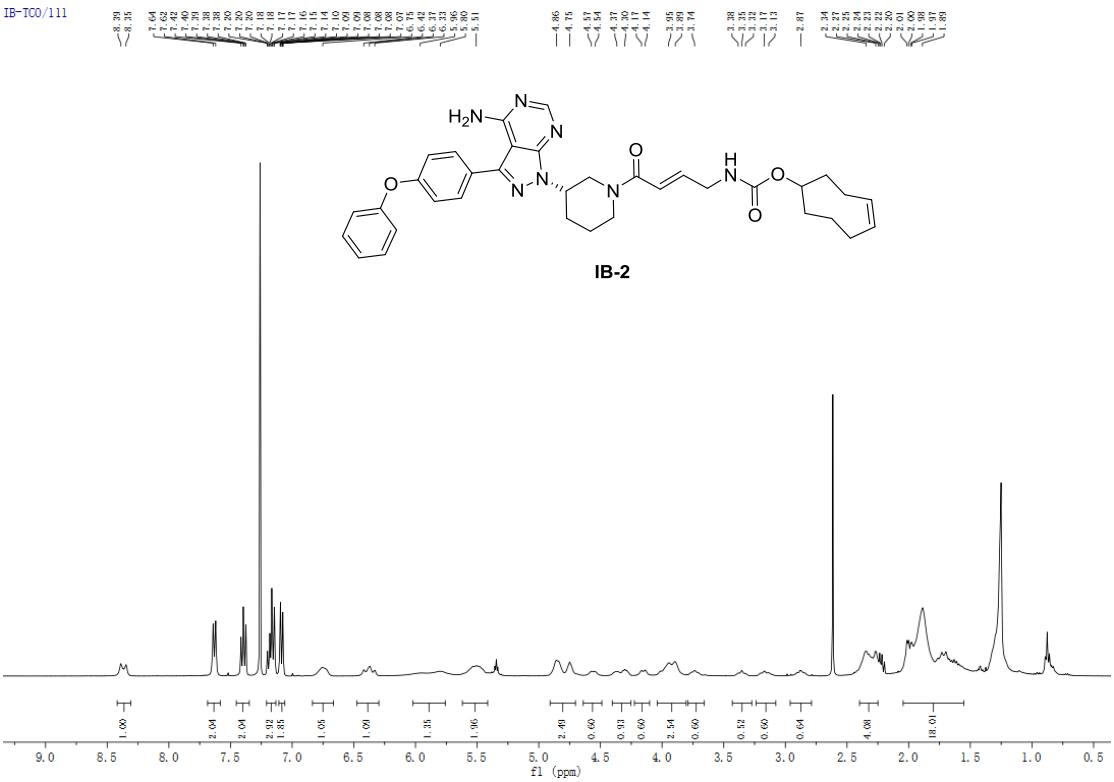
IB-1
fl (ppm)

IB-TCO/111



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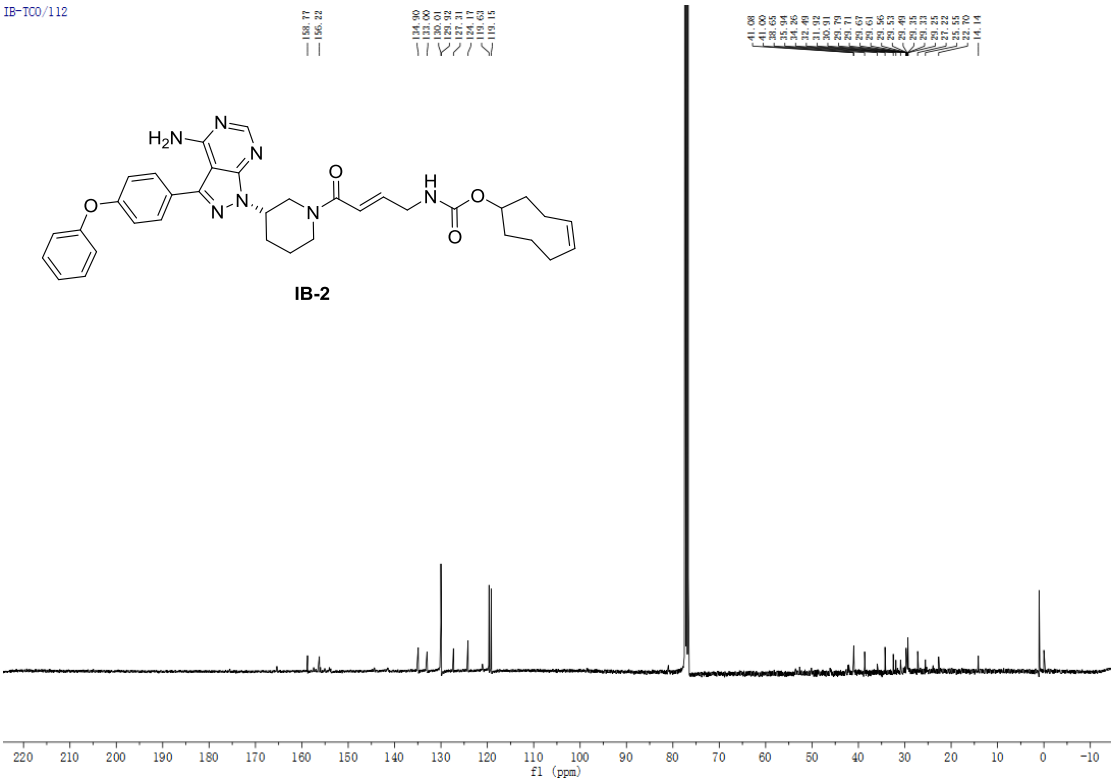

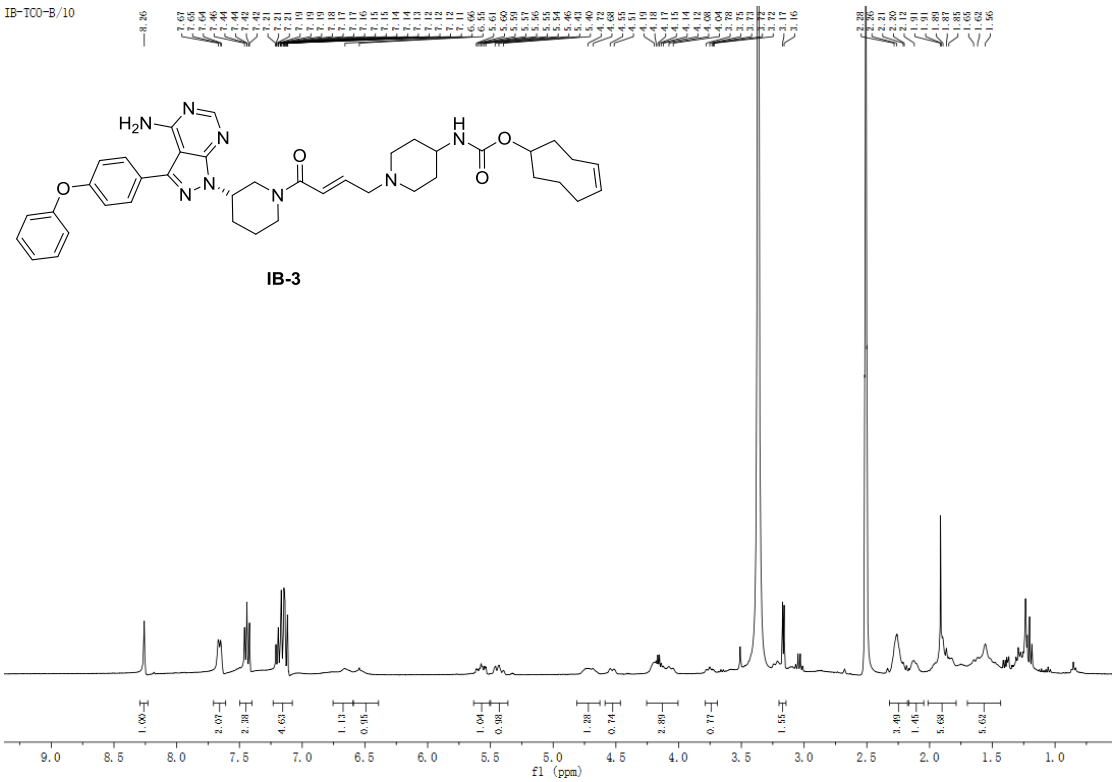
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