Supporting Information

Harnessing affinity-based protein profiling to reveal a novel target of nintedanib

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Author Contributions

[#] These authors contributed equally to this work.

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1 Supplementary Figures and Tables

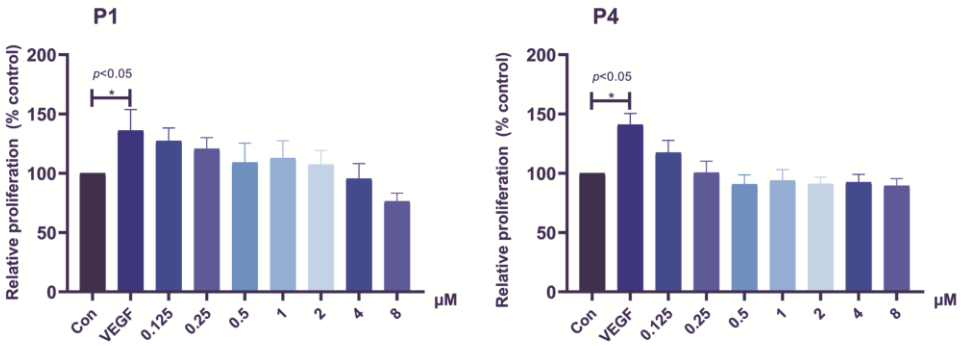


Figure S1. HUVEC 72 h proliferation inhibition treated with DMSO control, VEGF control or probe (0.125-8 μM) assessed by cell counting kit-8 (CCK8) assay: (left) activity evaluation of Probe P1, (right) activity evaluation of Probe P4. Data shown are average ± SEM, n = 5 biological replicates/group. Significance is expressed as *p < 0.05 compared with vehicle-treated controls. Con: control.

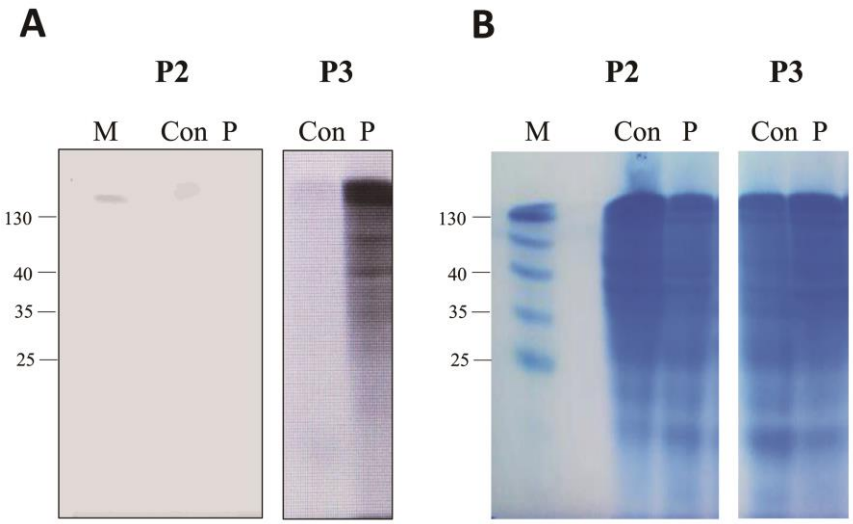


Figure S2. Protein labeling experiments of P2 and P3 in HUVEC cells. (A) For each probe, the experiment contains two groups, including control group (0.1% DMSO) and probe group (P2 or P3). HUVEC cells were treated with 0.1%DMSO, P2 (2 μM) or P3 (2 μM) for 2 h, followed by reaction with TAMRA-azide. The treated samples were analyzed by in-gel fluorescence scanning. (B) CBB-stained gels demonstrate equal loading. M: marker; Con: control.

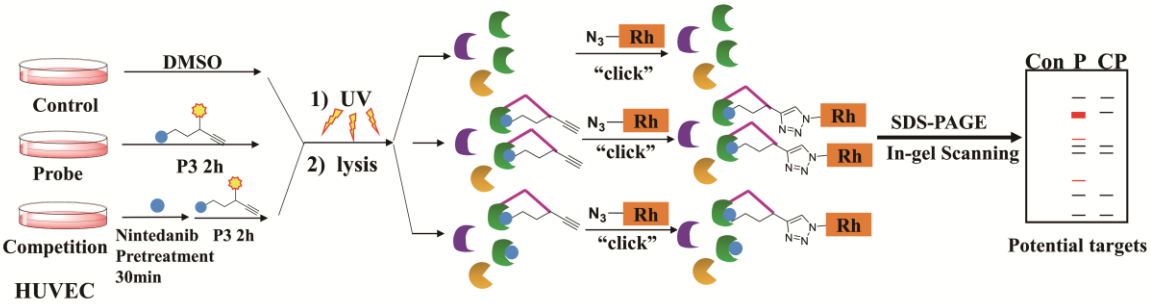


Figure S3. Schematic overview of *in situ* competitive-A*f*BPP experiment in living HUVEC cells.

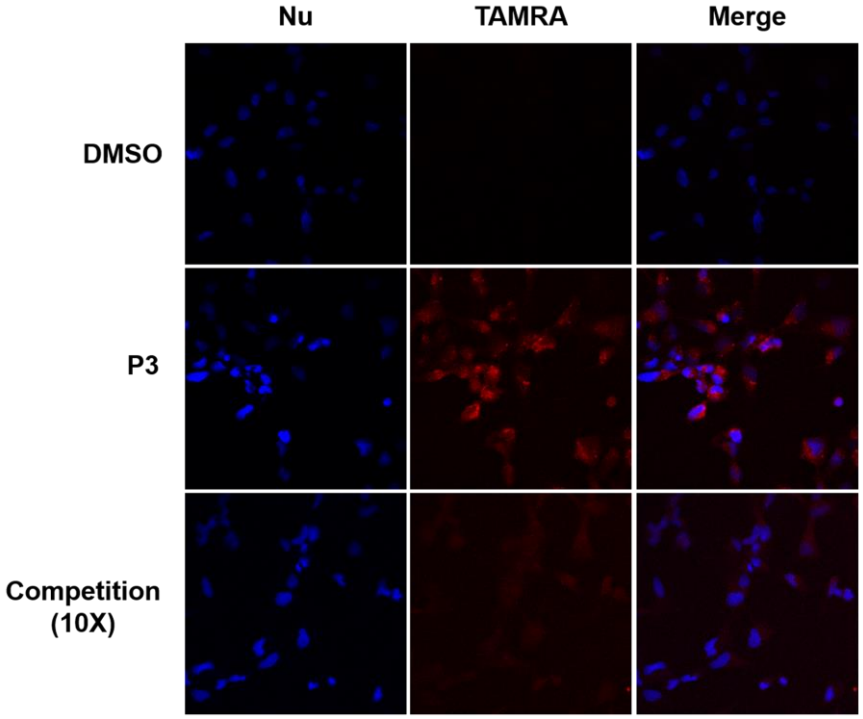


Figure S4. Fluorescence microscopy experiments of HUVEC cells treated with P3 (10 μM) with or without excessive nintedanib. Blue: DAPI nuclear staining, red: TAMRA channel. Competition (10x): 10 μM P3 + 100 μM nintedanib. Scale bar = 50 μm.

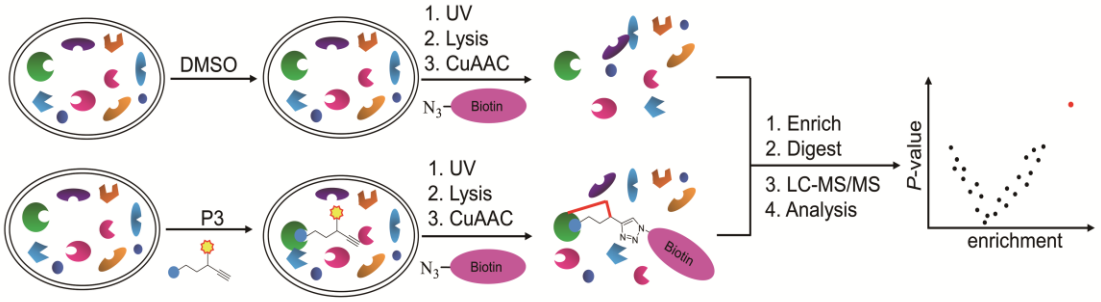


Figure S5. Workflow of A*f*BPP-Pull Down strategy for target identification of nintedanib in living HUVEC cells. For nano LC-MS/MS, label free quantification (LFQ) method was used. For each group, three biological replicates were performed.

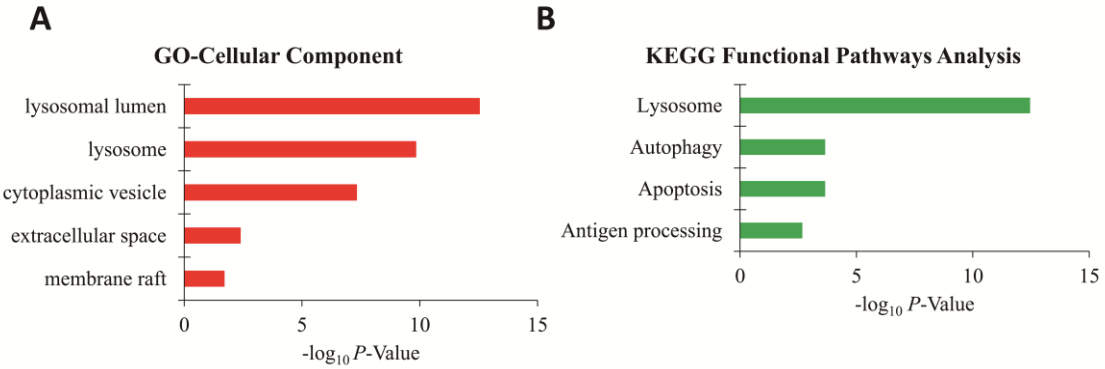


Figure S6. Bioinformatic analysis of highly reliable target proteins of nintedanib. (A) Cellular component analysis of potential target proteins of nintedanib by gene ontology (GO) (B) Functional pathways analysis of potential target proteins of nintedanib by Kyoto Encyclopedia of Genes and Genomes (KEGG). The enriched pathways are ranked based on their P values.

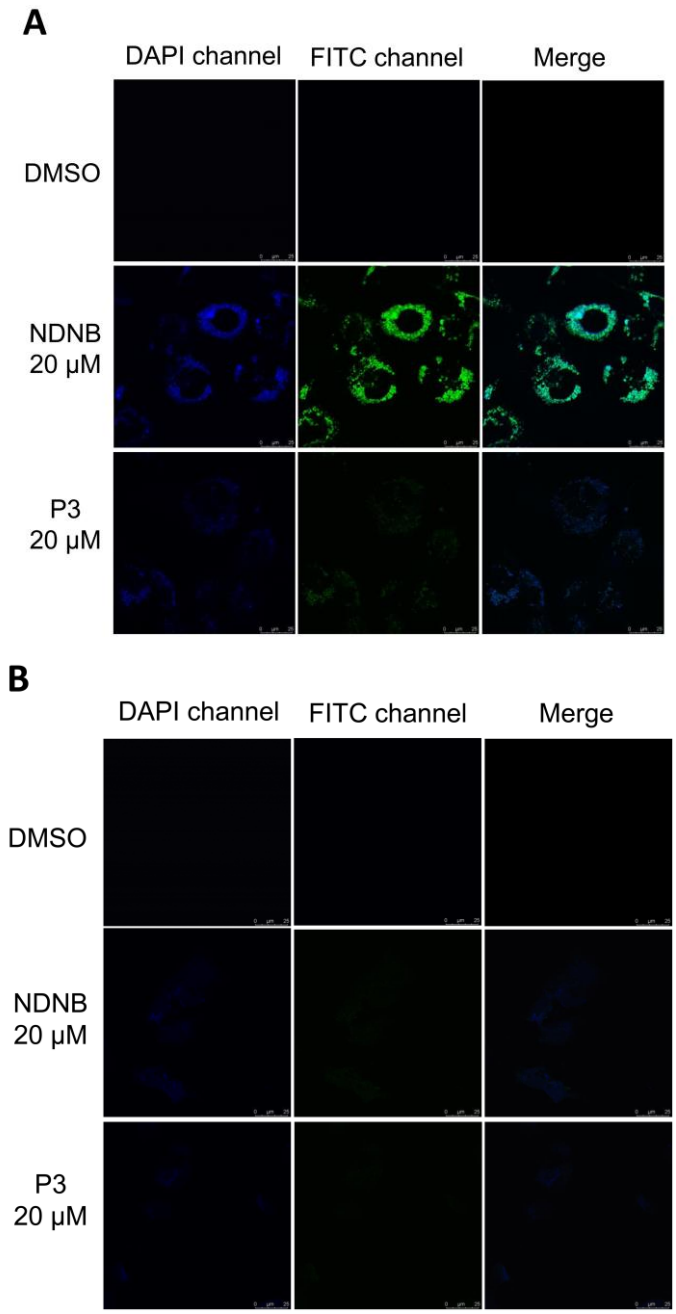


Figure S7. Live-cell imaging experiment after treatment of nintedanib (20 μM) or probe P3 (20 μM) (A) HUVEC cells were incubated with nintedanib (20 μM) or probe P3 (20 μM) for 1 h, then the fluorescence microscopy experiment was performed immediately at DAPI (405 nm) and FITC (488 nm) channel. (B) The imaging result after incubation with nintedanib (20 μM) or probe P3 (20 μM) for 1 h, followed by immunofluorescence staining procedure.

Table S1. Related information of the 10 identified and highly reliable targets of nintedanib.

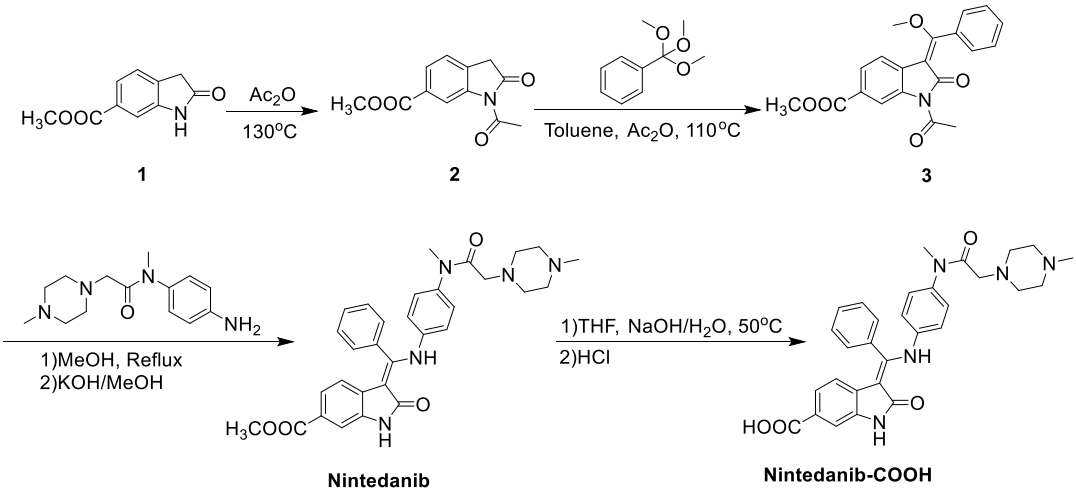
Gene names	Protein names	MW（KDa）	Fold change（P3/Competition）
USP5	Ubiquitin carboxyl-terminal hydrolase 5	95.8	3.5
APLP2	Amyloid-like protein 2	87.0	3.4
GLB1	Beta-galactosidase	76.1	2.2
PSAP	Prosaposin	58.4	4.7
TPP1	Tripeptidyl-peptidase 1	50.9	7.9
CTSD	Cathepsin D	44.2	3.2
CTSB	Cathepsin B	37.8	2.2
CTSL	Cathepsin L1	37.6	4.0
PPT1	Palmitoyl-protein thioesterase 1	37.1	4.0
PPT1	Palmitoyl-protein thioesterase 1 (Fragment)	8.7	37.7

2 Synthetic Methods of Chemistry

2.1 Methods and General Materials

All chemicals were obtained from commercial suppliers and were used without further purification. All experiments were monitored by analytical thin layer chromatography (TLC). TLC was performed on pre-coated silica gel plates (60 F-254, 0.25 mm, Merck KGaA) with detection by UV (λ = 254 or 365 nm). Flash chromatography was performed on silica gel 60 (35 – 70 μm, mesh 60 Å, Merck KGaA) with the indicated eluent. Reversed-phase HPLC was performed by HPLC-grade acetonitrile. NMR spectra (1H-NMR, 13C-NMR) were recorded at room temperature on Bruker DRX-600 instruments. Chemical shifts were recorded in ppm relative to tetramethylsilane (TMS) or residual solvent peaks ($CDCl_3$ = 7.26 ppm, DMSO-d_6 = 2.50 ppm for ^1H NMR; $CDCl_3$ =77.06 ppm, DMSO-d_6 = 39.53 ppm for ^{13}C NMR) with peaks being reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant (Hz). High-resolution mass spectra (HRMS) were obtained on an Agilent Q-TOF 6550 mass spectrometer by electrospray ionization – time-off light (ESI-TOF).

2.2 Synthesis of Intermediate Compound Nintedanib-COOH.



Scheme S1. Synthetic route of intermediate compound Nintedanib-COOH

Methyl 1-acetyl-2-oxoindoline-6-carboxylate (2)

Starting material **1** (1.91 g, 10 mmol) was suspended in acetic anhydride (20 mL) and stirred at 130 ℃ for 8 h[1,2]. After completion of the reaction, the mixture was cooled to room temperature naturally. The precipitate was filtered off and the filter cake was washed with petroleum ether (20 mL), followed by cold methanol (20 mL). to afford product **2** (2.11 g, 91% yield) as a light red solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.83 (d, *J* = 1.0 Hz, 1H), 7.91 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.36 – 7.32 (m, 1H), 3.92 (s, 3H), 3.75 (s, 2H), 2.68 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.6, 170.7, 166.6, 141.5, 130.4, 128.5, 126.7, 123.9, 117.5, 52.3, 36.7, 26.7. ESI-HRMS (m/z): calcd. for $C_{12}H_{10}NO_4$ $[M-H]^-$ 232.0688; found, 232.0617.

Methyl (E)-1-acetyl-3-(methoxy(phenyl)methylene)-2-oxoindoline-6-carboxylate (3)

To a solution of compound **2** (2.10 g, 9 mmol, 1.0 equiv) in toluene (25 mL) was added acetic anhydride (3 mL). The mixture was stirred at 110 ℃ and trimethyl orthobenzoate (4.10 g, 22.5 mmol, 2.5 equiv) was added dropwise. Subsequently, the mixture was stirred at this temperature for 8 h. After completion of the reaction, the mixture was cooled to room temperature naturally. The precipitate was filtered off and the filter cake was washed with toluene (20 mL), followed by mixed solution (toluene: ethyl acetate =1:1, 20 mL). to afford product **3** (2.79 g, 88% yield) as a yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.90 (d, *J* = 1.2 Hz, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 7.95 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.61 – 7.55 (m, 3H), 7.39 (m, 2H), 3.93 (s, 3H), 3.76 (s, 3H), 2.57 (s, 3H). ^{13}C

NMR (150 MHz, $CDCl_3$) δ 171.6, 171.2, 167.2, 167.1, 136.60, 130.8, 130.8, 129.0, 128.7, 128.4, 128.0, 126.4, 122.5, 116.6, 106.1, 57.9, 52.2, 26.9. ESI-HRMS (m/z): calcd. for $C_{20}H_{18}NO_5$ $[M+H]^+$ 352.1185; found, 352.1188.

Methyl (Z)-3-(((4-(N-methyl-2-(4-methylpiperazin-1-yl)acetamido)phenyl)amino) (phenyl)methylene)-2-oxoindoline-6-carboxylate (Nintedanib)

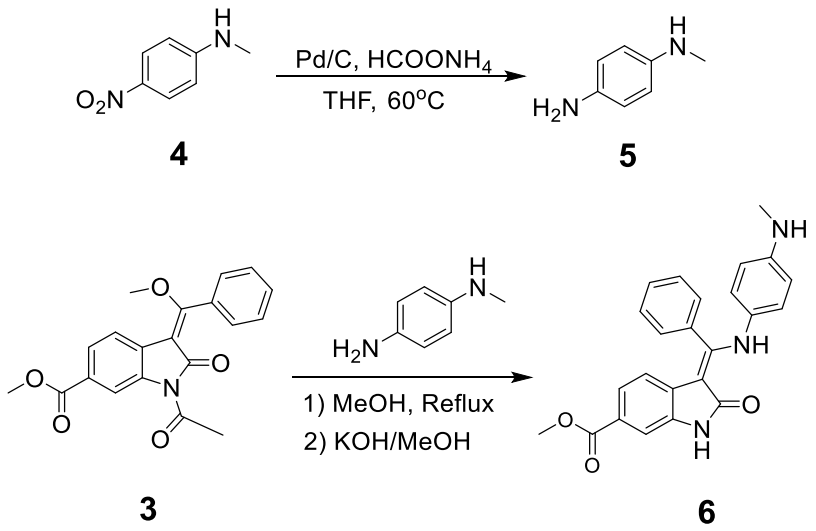
To a solution of compound **3** (1.40 g, 4 mmol, 1.0 equiv) in MeOH (25 mL) was added N-(4-aminophenyl)-N-methyl-2-(4-methylpiperazin-1-yl) acetamide (1.15 g, 4.4 mmol, 1.1 equiv). The mixture was stirred and the reaction was refluxed for 5 h. Subsequently, the deacetylation reaction was continued by addition of KOH (134 mg, 2.4 mmol, 0.6 equiv) dissolved in MeOH (2 mL) and the mixture was stirred for 20 min. The mixture was cooled to 10 ℃ and stirred for 1 h. The precipitate was filtered off and washed with cold methanol (2 x 20 mL). After air drying, we obtained **Nintedanib** (1.82 g, 84% yield) as yellow powder crystal. The purity detected by HPLC is 99%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 12.18 (s, 1H), 9.55 (s, 1H), 7.62 (d, *J* = 1.2 Hz, 1H), 7.56 (m, 1H), 7.52 (t, *J* = 7.2 Hz, 2H), 7.41 (d, *J* = 7.2 Hz, 2H), 7.37 (dd, *J* = 8.4, 1.2 Hz, 1H), 6.97 (d, *J* = 8.4 Hz, 2H), 6.80 (d, *J* = 8.4 Hz, 2H), 5.98 (d, *J* = 8.4 Hz, 1H), 3.83 (s, 3H), 3.17 (s, 3H), 2.79 (s, 2H), 2.43 (m, 8H), 2.27 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.1, 169.4, 167.4, 158.3, 139.9, 138.0, 135.7, 132.3, 130.6, 129.6, 129.1, 128.6, 127.8, 125.1, 124.0, 122.7, 118.2, 110.4, 98.5, 59.5, 54.8, 53.1, 51.9, 45.8, 37.3. ESI-HRMS (m/z): calcd. for $C_{31}H_{34}N_5O_4$ $[M+H]^+$ 540.2611; found, 540.2620.

(Z)-3-(((4-(N-methyl-2-(4-methylpiperazin-1-yl)acetamido)phenyl)amino) (phenyl)methylene)-2-oxoindoline-6-carboxylic acid (Nintedanib-COOH)

To a solution of **Nintedanib** (1.62 g, 3 mmol, 1.0 equiv) in THF (15 mL) was added NaOH (aq) (1 mol/L, 6 mL, 2.0 equiv). The mixture was stirred at 50 ℃ for 8 h. After completion of the reaction, most of the THF was removed under reduced pressure and water (10 mL) was added into the flask. Then, HCl (1 mol/L) was used to adjust PH value to 2-3. The precipitate was filtered off and washed with EtOH (20 mL) to afford product **Nintedanib-COOH** (1.45 g, 92% yield) as a yellow solid. ^{1}H NMR (600 MHz, DMSO-*d*$_6$) δ 12.23 (s, 1H), 10.99 (s, 1H), 7.66 – 7.62 (m, 1H), 7.59 (t, *J* = 7.2Hz, 2H), 7.53 – 7.50 (m, 2H), 7.40 (d, *J* = 1. 8 Hz, 1H), 7.19 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.15 (d, *J* = 7.8, 2H), 6.89 (d, *J* = 7.8 Hz, 2H), 5.83 (d, *J* = 7.8 Hz, 1H), 3.17 (s, 3H), 3.50-2.90 (m, 4H), 2.83 (s, 2H), 2.65 (s, 6H), 2.61-2.53 (m, 2H). ^{13}C NMR (150 MHz, DMSO-*d*$_6$) δ 170.7, 168.5, 167.9, 163.5, 158.4, 140.0, 138.0, 136.8, 132.6, 131.0, 130.0, 128.9,

128.3, 125.9, 124.3, 122.1, 117.7, 110.3, 98.3, 58.3, 52.9, 49.5, 49.0, 42.7, 40.5, 37.1. ESI-HRMS (m/z): calcd. for $C_{30}H_{32}N_5O_4$ $[M+H]^+$ 526.2454; found, 526.2473.

2.3 Synthesis of Intermediate Compound 6.



Scheme S2. Synthetic route of intermediate compound 6

N^1-methylbenzene-1,4-diamine (5)

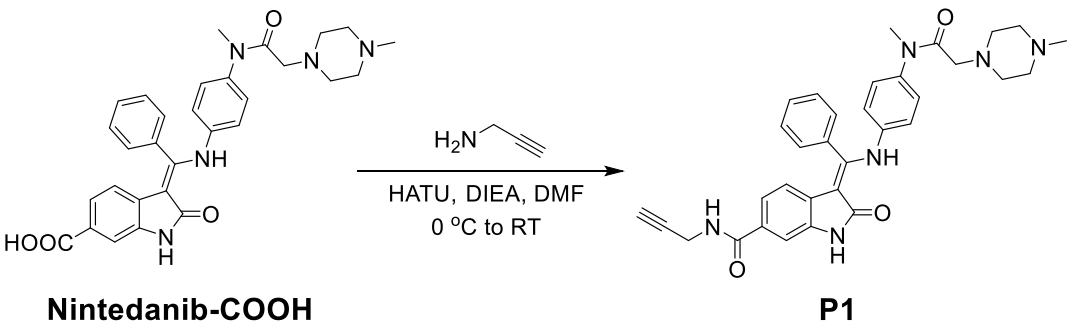
To a solution of compound **4** (1.52 g, 10 mmol, 1.0 equiv) in THF (30 mL) was added 10% Pd/C (300 mg) and $HCOONH_4$ (aq) (1.26 g, 20 mmol, 2.0 equiv). The mixture was heated to 60 ℃ for 1.5 h, additional $HCOONH_4$ (aq) (1.26 g, 20 mmol, 2.0 equiv) was added to continue hydrogenation for another 1.5 h. After completion of the reaction, Pd/C was removed by filtration using diatomite and the filtrate was concentrated under reduced pressure to remove THF. The liquid was added to a separatory funnel and extracted with DCM (3 x 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. Finally, we obtained product **5** (1.15 g, 94% yield) as a brown solid. The solid was used for the next step without further purification.

Methyl (Z)-3-(((4-(methylamino)phenyl)amino)(phenyl)methylene)-2-oxoindoline -6-carboxylate (6)

To a solution of compound **3** (1.05 g, 3 mmol, 1.0 equiv) in MeOH (20 mL) under reflux was added dropwise a solution of compound **5** (403 mg, 3.3 mmol, 1.1 equiv) in MeOH (8 mL). The mixture was refluxed for 3 h. Subsequently, the deacetylation reaction was

continued by addition of KOH (101 mg, 1.8 mmol, 0.6 equiv) dissolved in MeOH (2 mL) and the mixture was stirred for 20 min. After completion of the reaction, the mixture was cooled to room temperature naturally. The precipitate was filtered off and the filter cake was washed with cold methanol (20 mL) The product **6** was obtained as an orange solid (1.02 g, 85% yield).^{1}H NMR (600 MHz, DMSO-d_6) δ 12.08 (s, 1H), 10.83 (s, 1H), 7.53-7.49 (m, 3H), 7.44 – 7.38 (m, 3H), 7.15 (dd, J = 8.4, 1.2 Hz, 1H), 6.69 (d, J = 8.4Hz, 2H), 6.30 (d, J = 8.4 Hz, 2H), 5.72 (d, J = 8.4 Hz, 1H), 5.65 (q, J = 4.8 Hz, 1H), 3.75 (s, 3H), 2.56 (d, J = 4.8 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.6, 167.0, 160.5, 148.3, 136.0, 132.9, 130.4, 130.0, 129.7, 128.9, 126.4, 125.8, 123.4, 121.7, 117.0, 111.8, 109.7, 95.9, 52.2, 30.1. ESI-HRMS (m/z): calcd. for $C_{24}H_{22}N_3O_3$ $[M+H]^+$ 400.1661; found, 400.1655.

2.4 Synthesis of Probe P1.



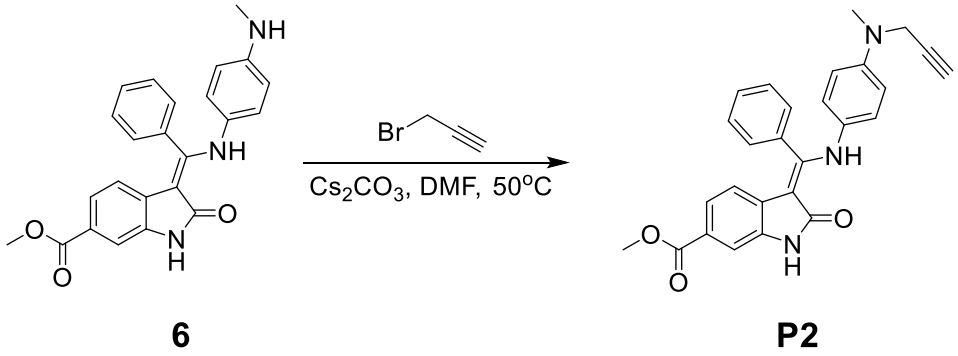
Scheme S3. Synthetic route of P1

(Z)-3-(((4-(N-methyl-2-(4-methylpiperazin-1-yl)acetamido)phenyl)amino)(phenyl) methylene)-2-oxo-N-(prop-2-yn-1-yl)indoline-6-carboxamide (Probe P1)

Similar to what was reported,[3] to a suspension of HATU (380 mg, 1 mmol, 1.0 equiv) in anhydrous DMF (3 mL) was added a solution of **Nintedanib-COOH** (525 mg,1 mmol, 1.0 equiv) in anhydrous DMF (2 mL) at 0 ℃, followed by the addition of DIEA (346 μL, 2 mmol, 2.0 equiv). The reaction mixture was stirred for 10 min followed by addition of propargylamine (128 μL, 2 mmol, 2.0 equiv), and the stirring was continued for 24 h at room temperature. After completion of the reaction, the mixture was added to a separatory funnel and water (30 mL) was added to the resulting mixture. Then, the aqueous phase was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with water (3 x 20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography on silica (DCM: MeOH =10:1) to get product **P1 (**518 mg, 92% yield) as a yellow solid.

^{1}H NMR (600 MHz, DMSO-d_6) δ 12.13 (s, 1H), 10.95 (s, 1H), 8.65 (t, J = 6.0, 1H), 7.58 (m, 3H), 7.52 – 7.47 (m, 2H), 7.36 (d, J = 1.8 Hz, 1H), 7.12 (d, J = 7.8 Hz, 2H), 7.07 (dd, J =8.4, 1.2 Hz, 1H), 6.87 (d, J = 7.8 Hz, 2H), 5.76 (m, 1H), 3.97 (m, 2H), 3.05 (m, 4H), 2.49-2.24 (m, 8H), 2.24 (s, 3H). ^{13}C NMR (150MHz, DMSO-d_6) δ 170.8, 168.9, 166.4, 166.36, 157.8, 140.3, 136.8, 132.8, 130.8, 129.9, 129.3, 129.0, 128.2, 127.5, 124.1, 119.8, 117.6, 108.7, 98.4, 82.0, 73.1, 59.3, 55.4, 54.5, 52.0, 45.3, 37.2, 28.8, 28.8. ESI-MS (m/z): calcd. for $C_{33}H_{35}N_6O_3$ $[M+H]^+$ 563.2771; found, 563.2755.

2.5 Synthesis of Probe P2.



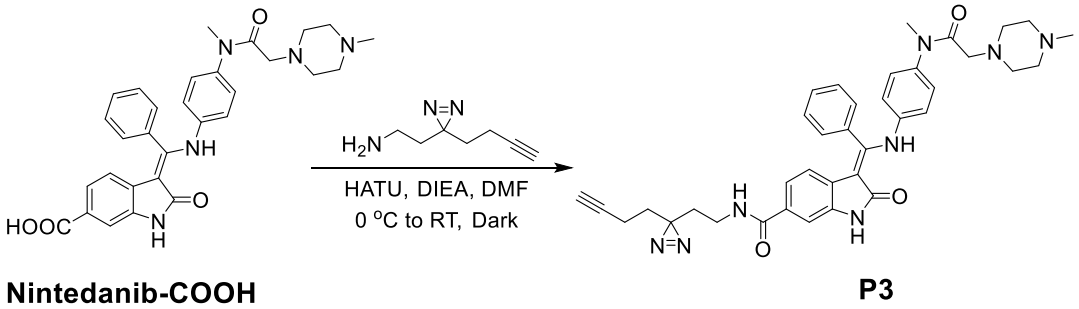
6 **P2**

Scheme S4. Synthetic route of P2

Methyl (Z)-3-(((4-(methyl(prop-2-yn-1-yl)amino)phenyl)amino)(phenyl)methyl -ene)-2-oxoindoline-6-carboxylate (Probe P2)

To a solution of compound **6** (160 mg, 0.4 mmol, 1.0 equiv) in anhydrous DMF (3 mL) was added Cs_2CO_3 (260 mg, 0.8 mmol, 2.0 equiv) followed by 3-bromopropyne (52 μL, 0.48 mmol, 1.2 equiv). The mixture was stirred at 50 ℃ for 8 h. After completion of the reaction, water (30 ml) was added and the resulting mixture was extracted with DCM (3 x 10 mL). The combined organic layers were washed with water (3 x 20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by reversed-phase HPLC (Acetonitrile / H_2O elution) to give product **P2** (116 mg, 66% yield) as a brown solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 11.98 (s, 1H), 7.71 (d, J = 1.8 Hz, 1H), 7.52-7.49 (m, 1H), 7.48-7.45 (m, 1H), 7.41 (dd, J = 8.4, 1.8 Hz, 1H), 7.36-7.35 (m, 2H), 6.67 (d, J = 8.4 Hz, 2H), 6.37 (d, J = 8.8 Hz, 2H), 5.94 (d, J = 8.4 Hz, 1H), 4.75 (d, J = 3.0 Hz, 2H), 3.87 (s, 3H), 2.75 (s, 3H), 2.25 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.3, 167.5, 159.9, 146.7, 135.5, 132.7, 130.0, 129.3, 128.6, 128.1, 125.3, 124.1, 123.1, 117.5, 112.47, 108.9, 95.5, 77.8, 71.8, 51.9, 30.8, 28.8. ESI-MS (m/z): calcd. for $C_{27}H_{24}N_3O_3$ $[M+H]^+$ 438.1818; found, 438.1792.

2.6 Synthesis of Probe P3.

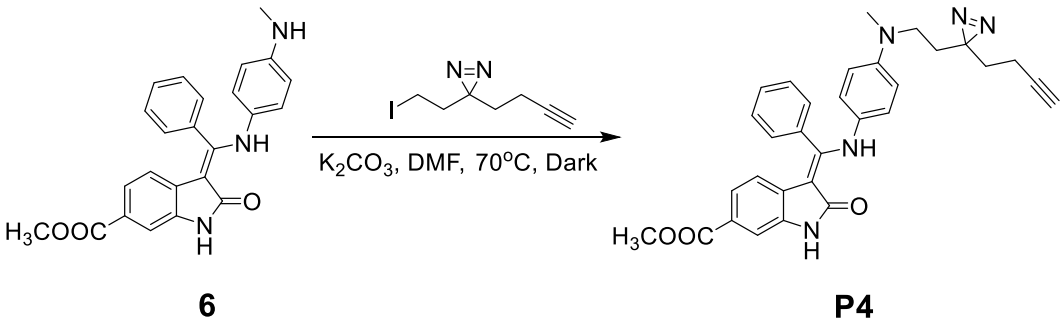


Scheme S5. Synthetic route of P3

(Z)-N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)-3-(((4-(N-methyl-2-(4-methyl-piperazin-1-yl)acetamido)phenyl)amino)(phenyl)methylene)-2-oxoindoline-6-carboxamide (Probe P3)

To a suspension of HATU (114 mg, 0.3 mmol, 1.0 equiv) in anhydrous DMF (2 mL) was added a solution of **Nintedanib-COOH** (158 mg, 0.3 mmol, 1.0 equiv) in anhydrous DMF (2 mL) at 0 ℃, followed by the addition of DIEA (104 μL, 0.6 mmol, 2.0 equiv). The reaction mixture was stirred for 10 min, followed by addition of 2-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)ethan-1-amine (76.5 μL, 0.6 mmol, 2.0 equiv), and stirring was continued for 24 h in dark condition at room temperature. After completion of the reaction, the mixture was added to a separatory funnel and water (30 mL) was added to the resulting mixture. Then, the aqueous phase was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with water (3 x 20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography on silica (DCM: MeOH =10:1) to get product **P3 (**174 mg, 90% yield) as a yellow solid. ^{1}H NMR (600 MHz, DMSO-*d*$_6$) δ 12.10 (s, 1H), 10.93 (s, 1H), 8.18 (t, *J* = 6.0, 1H),7.61 – 7.5-5 (m, 3H), 7.52 – 7.47 (m, 2H), 7.33 (d, *J* = 1.2 Hz, 1H), 7.11 (d, *J* = 8.4 Hz, 2H), 7.05 (dd, *J* = 7.8, 1.2 Hz, 1H), 6.86 (d, *J* = 8.4 Hz, 2H), 5.74 (d, *J* = 7.8 Hz, 1H), 3.12 – 2.98 (m, 5H), 2.80 (t, *J* = 2.4 Hz, 1H), 2.69 (ds, 2H), 2.36 – 2.07 (m, 8H), 2.10 (s, 3H), 1.97 (td, *J* = 7.2, 2.4 Hz, 2H), 1.59 (m, 4H). ^{13}C NMR (150 MHz, DMSO-*d*$_6$) δ 170.8, 169.1, 166.7, 166.7, 157.7, 140.4, 137.8, 136.8, 132.8, 130.8, 129.9, 129.0, 128.2, 127.3, 124.1, 119.7, 117.6, 108.6, 98.4, 83.6, 72.2, 59.6, 55.0, 52.7, 46.1, 40.5, 37.2, 34.8, 34.7, 32.4, 31.8, 27.8, 13.2. ESI-MS (m/z): calcd. for $C_{37}H_{41}N_8O_3$ $[M+H]^+$ 645.3302; found, 645.3267.

2.7 Synthesis of Probe P4.



6 **P4**

Scheme S6. Synthetic route of P4

Methyl (Z)-3-(((4-((2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)(methyl) amino)phenyl)amino)(phenyl)methylene)-2-oxoindoline-6-carboxylate (Probe P4)

To a solution of compound **6** (160 mg, 0.4 mmol, 1.0 equiv) in anhydrous DMF (3 mL) was added K_2CO_3 (110 mg, 0.8 mmol, 2.0 equiv) followed by 3-(but-3-yn-1-yl)-3-(2-iodoethyl)-3*H*-diazirine (89 µL, 0.6 mmol, 1.5 equiv). The reaction was stirred in dark condition at 70 °C for 20 h. After completion of the reaction, water (30 ml) was added and the resulting mixture was extracted with DCM (3 x 10 mL). The combined organic layers were washed with water (3 x 20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by reversed-phase HPLC (Acetonitrile / H_2O elution) to give product **P4** (167 mg, 80% yield) as an orange solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 12.06 (s, 1H), 7.51 – 7.44 (m, 4H), 7.39 – 7.35 (m, 3H), 6.67 (d, *J* = 8.4 Hz, 2H), 6.36 (d, *J* = 8.4 Hz, 2H), 5.92 (d, *J* = 8.4 Hz, 1H), 3.90 – 3.88 (t, *J* = 7.8 Hz,, 2H), 3.86 (s, 3H), 2.75 (s, 3H), 2.07-2.05 (m, 3H), 1.89 (t, *J* = 7.8 Hz, 2H), 1.75 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.0, 167.5, 159.8, 146.8, 135.9, 132.7, 130.0, 129.2, 128.7, 128.1, 125.3, 123.9, 122.7, 117.5, 112.4, 108.0, 95.6, 82.7, 69.4, 51.9, 34.9, 32.0, 31.8, 30.8, 26.8, 13.3. ESI-MS (m/z): calcd. for $C_{31}H_{30}N_5O_3$ $[M+H]^+$ 520.2349; found, 520.2349.

3 Biological Experimental Procedures

3.1 Methods and General Materials

All reagents were purchased from Sigma-Aldrich unless specified. For click chemistry, TAMRA-azide and biotin-azide were purchased from Click Chemistry Tools (https://www.clickchemistrytools.com). Phosphate-buffered saline (PBS) and streptavidin beads (20349) was purchased from Thermo Scientific. Human umbilical vein endothelial cells (HUVEC) were obtained from National Infrastructure of Cell Line Resource (Beijing). All medium and supplements for cell culture were purchased from Gibco (Thermo Fisher Scientific). The reagents and probes for cell treatment were prepared as 1000x stock solutions in DMSO and stored at -20 °C.

3.2 Cell Culture

HUVEC cells were cultured in high glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (certified), 1% Non-Essential Amino Acid (NEAA), and 100 units/mL penicillin-streptomycin at 37 °C in a humidified atmosphere with 5% CO_2.

3.3 HUVEC Proliferation Inhibition Assay

HUVEC proliferation inhibition was performed based on previously published methods with some modifications,[4,5] 6000 cells per well were seeded in 96-well plates in a volume of 100 μL DMEM (10% FBS, 1% NEAA, 1% penicillin-streptomycin). The cells were grown to 70% confluence after cultured at 37 °C in a humidified atmosphere with 5% CO_2 for 24 h. Then cells were starved in DMEM medium containing 0.1%FBS overnight. The cells were incubated with nintedanib or different probes (P1-P4) at 0.125–8.0 μM (10 μL, 10x) for 30 min, then stimulated with VEGF (293-VE; R&D Systems) at 20 ng/ml (10 μL, 10x) for 72 h. Subsequently, CCK8 reagent (10%) was added and the absorbance at 450 nm was measured by a microplate reader after incubation for 3 h. The GraphPad Prism 8 software was used to calculate inhibition rate and evaluate the activity.

3.4 *In situ* Photoaffinity Labeling of HUVEC Cells

Referring to previously reported procedures,[6] HUVEC cells were seeded in 6 cm cell culture dishes and grown to 90% confluence prior to the labeling experiment. At the time of experiment, the mediums were replaced with 3 mL of fresh medium. For control group, 0.1% DMSO was added. For probe group, 3 μL of 2 mM solution (1000x) of P2 or P3 in DMSO was added, and the cells were incubated for 2 h at 37 °C with 5% CO_2. The mediums were discarded and the dishes were washed twice with pre-cooled PBS (2 x 2 mL). Then 1 mL PBS was added and the cells were irradiated with 365 nm UV light (8 Watt) with ice cooling for 40 min. The cells were harvested with a cell scraper, washed with PBS (2 mL). The cell suspensions were centrifuged at 300 x g for 5 min at 20 °C and the supernatant was removed to yield cell pellets.

3.5 Kinases Inhibition Assay

This assay was performed using the HTRF KinEASE TK kit (Cisbio), a universal tool for assessing kinase activity. First, kinase buffer was prepared by adding 4 mL sterile water into 1 mL enzymatic buffer (HEPES 250 mM (pH7.0), NaN_3 0.1%, BSA 0.05%, Orthovanadate 0.5 mM), following by addition of 5 μL 1 M DTT and 25 μL 1 M $MgCl_2$ with final concentration of 1 mM and 5 mM, respectively. Subsequently, compounds (dissolved in DMSO), FGFR1 kinase, TK substrate-biotin and ATP were prepared with kinase buffer. For the experiment, four groups were set up, including complete activity control of FGFR1 kinase (MAX), non-kinase negative control (NEG), NDNB, probe P2 and P3. At the beginning, 4 μL of compounds (NDNB, P2 or P3) or kinase buffer (for MAX and NEG), 2 μL of kinase (kinase buffer for NEG), 2 μL of TK substrate-biotin and 2 μL of ATP were added to the 96-well plate, with final concentrations of 10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003 μM for each compound. For FGFR1 kinase, 0.0123 ng/μL was used. For ATP, 99.99 μM was used. Shaking for 1 min and the enzymatic reaction was incubated at room temperature for 50 min. Once completing the enzymatic step, 5 μL Streptavidin-XL665 and 5 μL TK Antibody-Cryptate, prepared with detection buffer, were added into the 96-well plate. Shaking for 1 min and the detection step was continued at room temperature for 1 h. Finally, the emission intensities of 665 nm and 620 nm were detected with 330 nm excitation. The BioTek H1 microplate reader automatically calculates the ratio (665 nm / 620 nm * 10000). According to the formula, $(Ratio_{MAX}\text{-}Ratio_{sample}) / (Ratio_{MAX}\text{-}Ratio_{NEG}) * 100$, the inhibition rate of

kinase activity was calculated, following by determination of IC_{50} value of NDNB, probe P2 and P3 for FGFR1 kinase inhibition.

3.6 Concentration-Dependent and Time-Dependent Experiments

In concentration-dependent experiment, control group and probe group were treated with 0.1% DMSO and P3 of different concentrations (1.25, 2.5, 5, 10, 20, 30 μM), respectively, for 2 h. After completing incubation, cells were irradiated at 365 nm on ice for 40 min. Then, cells were harvested and lysed, following copper-catalyzed click chemistry reaction to conjugate TAMRA-azide to achieve fluorescence imaging of labeled proteins. For time-dependent experiment, control group and probe group were treated with 0.1% DMSO and P3 (10 μM) for different time (0.5, 1, 2, 4 h). After completing incubation, cells were irradiated at 365 nm on ice for 40 min. Then, cells were harvested and lysed, following copper-catalyzed click chemistry reaction to conjugate TAMRA-azide to achieve fluorescence imaging of labeled proteins.

3.7 Analysis of In-Gel Fluorescence

Cell pellets were lysed in 80 μL lysis buffer (1 % (v/v) Triton X-100, 0.1 % SDS and 1% protease and phosphatase inhibitor cocktail in PBS; 30 min, 4 °C). Following centrifugation (16000 x g, 15 min, 4 °C), the supernatant was transferred to a new 1.5 mL tube. The concentration of the proteomes was measured by BCA assay (Thermo Fisher Scientific) and adjusted to the same concentration (1-2 mg/ml) for each group to guarantee equal protein amounts. A pre-mixture of TAMRA-azide (100 μM, 0.8 μL/sample, 10 mM in DMSO), TCEP (1 mM, 0.8 μL/sample, 100 mM in ddH_2O), TBTA (100 μM, 0.8 μL/sample, 10 mM in 4:1 DMSO : t-BuOH), $CuSO_4$ (1 mM, 0.8 μL/sample, 100 mM in ddH_2O) were added and each sample was gently rotated at room temperature for 1 h.[7,8] Proteins were precipitated by the addition of a 4-fold volume excess of cold acetone (320 μL) and incubated over night at –20 °C to precipitate proteins completely. The suspension was centrifuged (16000 x g, 10 min, 4 °C). The supernatant was discarded and the protein pellet was washed with 200 μL pre-chilled methanol twice and air dried. Then 20 μL SDS loading buffer (1 x) was add to dissolve the protein and the samples were heated for 10 min at 95 °C. For gel-based analysis, 10 μL samples were loaded to a NuPAGE 4-12% bis-tris gel or SDS−PAGE 10 % acrylamide gel and run at 120 V with MES running buffer until 15 kDa band reached the end. The gels were scanned with a Typhoon FLA 9500 Biomolecular Imager (GE

Healthcare) with 532 nm laser excitation for TAMRA and 635 nm (Cy5) for pre-stained marker. To stain the proteins, the gel was subsequently incubated with Coomassie brilliant blue reagent (CBS Scientific).

3.8 Competitive Experiment

HUVEC cells were seeded in 6 cm cell culture dishes and grown to 90% confluence. At the time of experiment, the mediums were replaced with 3 mL of fresh medium. For control group, 0.1% DMSO was added. For probe group, 0.1% DMSO was added and for competition group, 50 μM or 100 μM nintedanib was added. After 30 min, 10 μM P3 was added to probe group and competition group to label for 2 h. Once completion of probe labeling, the mediums were discarded and the dishes were washed twice with pre-cooled PBS (2 x 2 mL). After irradiation with 365 nm UV light on ice for 40 min, the cells were harvested with a cell scraper, washed with PBS (2 mL). The cell suspensions were centrifuged at 300 x g for 5 min at 20 °C and the supernatant was removed to yield cell pellets. After lysis of cell pellets and preparation of labeling proteomes as before, the click reaction was initiated by addition of pre-mixture of TAMRA-azide (100 μM), TCEP (1 mM), TBTA (100 μM), $CuSO_4$ (1 mM) and completed by mild rotation at room temperature for 1 h. Add a 4-fold volume excess of cold acetone into the mixture and incubate over night at –20 °C to precipitate proteins completely. The suspension was centrifuged (16000 x g, 10 min, 4 °C). Discard the supernatant and wash the protein pellet with pre-chilled methanol twice, followed by air drying. The proteomes of all samples were analyzed by SDS-PAGE and the gel was visualized by in-gel fluorescence scanning, using Typhoon FLA 9500 Biomolecular Imager as before.

3.9 Protein Enrichment by Pull Down Experiment

The general procedures were similar to previously published protocols.[9,10] HUVEC cells were plated out on 10 cm dishes and grown to 90 % confluence prior to the labeling experiment. At the time of experiment, the mediums were replaced with 5 mL of fresh medium. For control group, 0.1% DMSO was added. For probe group, the cells were incubated with 0.1% DMSO for 30 min and then with 10 μM P3 for 2 h. For competition group, the cells were incubated with 100 μM nintedanib (10 x) for 30 min and then with 10 μM P3 for 2 h. The mediums were discarded and the dishes were washed twice with pre-cooled PBS (2 x 3 mL). Then 3 mL PBS was added and the cells were irradiated

with 365 nm UV light (8 Watt) with ice cooling for 40 min. The cells were harvested with a cell scraper and washed with PBS (2 mL). The cell suspensions were centrifuged at 300 x g for 5 min at 20 °C and the supernatant was removed to yield cell pellets. The lysis of cell pellets was performed by addition of 250 µL lysis buffer (1 % (v/v) Triton X-100, 0.1 % SDS and 1% protease and phosphatase inhibitor cocktail in PBS; 30 min, 0 °C). After centrifugation (16000 x g, 15 min, 4 °C), the supernatant was transferred to a new 1.5 mL tube. The concentration of the proteomes was measured by BCA assay (Thermo Fisher Scientific) and adjusted to 2 mg/ml for each group to guarantee equal protein amounts. For click chemistry, a pre-mixture of biotin-azide (100 µM, 2.5 µL/sample, 10 mM in DMSO), TCEP (1 mM, 2.5 µL/sample, 100 mM in ddH_2O), TBTA (100 µM, 2.5 µL/sample, 10 mM in 4:1 DMSO : t-BuOH), $CuSO_4$ (1 mM, 2.5 µL/sample, 100 mM in ddH_2O) were added and each sample was gently rotated at room temperature for 1.5 h. The proteins were precipitated by the addition of a 4-fold volume excess of cold acetone (1 mL) and incubated over night at –20 °C to precipitate proteins completely. The suspension was centrifuged (16000 x g, 10 min, 4 °C). The supernatant was discarded and the protein pellet was washed with pre-chilled methanol twice and air dried. For enrichment, the pellet was dissolved in 0.5 mL of PBS with 0.4 % SDS by sonication on ice (power 20%, 2s on/2s off, total time 1.5min). The protein solution was centrifuged (16000 x g, 15 min, 4 °C) and the supernatant was transferred to 1.5 mL LoBind microcentrifuge tubes. Another 0.5 mL of PBS was added into the tube to dilute SDS to 0.2%. The streptavidin beads (Thermo Fisher Scientific) were pre-washed with PBS (3 x 0.5 mL) and centrifugation was performed at 3000 x g for 3 min each time. 100 µL beads (50% slurry) were added to each sample and incubated under gentle shaking at 20 ℃ for 4 h. The beads were pelleted by centrifugation (3000 x g, 3 min), washed with 1% SDS in PBS (3 x 1 mL), 6M Urea in PBS (3 x 1 mL) and PBS (3 x 1 mL).[11] For the first of each set of washes, the beads were rotated for 5–10 min at room temperature. Finally, the beads were pelleted by centrifugation (3000 x g, 3 min), prepared for subsequent on-bead digestion.

3.10 On-Bead Digestion

The on-bead proteins were reduced with 20 mM dithiothreitol (DTT) in 200 µL ammonium bicarbonate (ABC) at 56 ℃ for 1 h under condition of gentle vortex. The alkylated reaction was completed by addition of 50 mM iodoacetamide (IAA) at room temperature for 45 min in the dark. Once completion of the reaction, the beads were pelleted by centrifugation (3000 x g, 3 min) and washed with 6M urea (200 µL) once

and ABC (200 µL) twice. The on-bead proteins were suspended in 150 µL ABC, vortexed for 1 min. Sequencing grade modified trypsin (Promega) was added at 1:100 enzyme/substrate ratio and the digestion was firstly performed at 37 ℃ for 12 h, followed by 4 h with addition of equal amount of trypsin (1:100). The digestion was stopped by addition of 0.1% formic acid (FA). The beads were vortexed and centrifuged (13000 x g, 4 ℃, 5 min) to collect the supernatant, followed by wash with 200 µL ABC three times and combination of flow-through with the previous fraction. The digested peptides were transferred to new LoBind tubes, desalted and concentrated using Sep-Pak C18 3 cc Vac cartridges (Waters Corp.). For desalting, The C18 column was pre-treated with 3 mL MeOH once, 3 mL 0.1%FA-H_2O twice prior to sample loading. The solution was loaded to the cartridges and washed with 3 mL 0.1%FA-H_2O three times and 3 mL 0.1%FA-2%ACN/H_2O twice. The peptides were eluted with 1mL 80%ACN/0.1%FA, dried in the speed vacuum system and stored at -80 ℃ until LC/MS-MS analysis

3.11 Nano LC-MS/MS Analysis

Before analysis, the peptides were reconstituted in 10 µL of 0.1% formic acid. LC-MS/MS was performed on an Orbitrap Fusion Lumos proteomic mass spectrometer (Thermo Scientific) coupled with EASY-nLC 1100 System. For each sample, 2 µL of volume was loaded onto C18 PepMap100 trapcolumn (300 µm × 5 mm) and eluted on a Thermo Acclaim PepMap RSLC analytical column (75 µm × 15 cm). A procedure of 60 min gradient for each single-shot analysis was performed as followed: 5-30% B in 35 min, 30-90% B in 5 min, then held at 90% B for 20 min (A = 0.1% formic acid in water, B = 0.1% formic acid in 90% acetonitrile). The flow rate was 0.3 µL/min. Data-dependent mode was operated for the mass spectrometer, with a full MS scan (350-1500 m/z) and 3 s cycle time was set. The MS spectra were acquired at a resolution of 60,000 with an automatic gain control (AGC) target value of 4×10^5 ions or a maximum integration time of 50 ms. High energy collision dissociation (HCD) with the energy set at 38 NCE was used to perform peptide fragmentation. The MS/MS spectra were acquired in the top 15 or 20 most intense precursors at a resolution of 15,000 with an AGC target value of 5×10^4 ions or a maximum integration time of 60 ms.

3.12 MS Data Analysis

Proteome Discoverer 2.1 software (Thermo Scientific) was used to process LC-MS/MS

raw files and peptide sequences were identified by the match of a non-redundant human UniProt database and the acquired fragmentation pattern by SEQUEST HT algorithm. The mass tolerance was set to 8 ppm and 0.02 Da for the precursor and the fragment ion respectively, with up to two missed cleavages allowed. Carbamidomethyl (+57.021 Da) was used as a fixed modification and oxidation (M) was used as a variable modification. The results of protein identification were filtered with the criteria of mass tolerance less than 10 ppm for peptides and false positive rate less than 1% at the protein level.[12] The proteins identified in each group were further filtered by at least two unique peptides in each experimental replicate. Protein ratios were calculated as the median of all peptide hits belonging to a protein and p value was determined by two-side one sample t-test over three biological replicates. The difference of protein abundances between different samples is quantified by label-free quantification. Quantitative data were listed in **Supplementary Excel S1**. Potential targets were confirmed by setting fold change more than 2.0 when calculating the ratio of P3/Control or P3/Competition and p-values less than 0.05. The processed data were listed in **Supplementary Excel S2.**

3.13 Analysis of Potential Targets of Nintedanib in Human Proteome

The protein names and gene names were obtained using the UniProt database (https://www.uniprot.org/) according to the accession numbers. Then gene ontology analysis of cellular component was performed using DAVID bioinformatics resources (https://david.ncifcrf.gov/). KEGG pathway was also performed using KEGG Mapper (https://www.kegg.jp/kegg/mapper.html) in order to demonstrate which pathway the potential targets were involved in.

3.14 Western Blot

Before immunoblotting analysis, the experimental procedure was consistent with pull down experiment described above. The HUVEC cells, plated out on 10 cm dishes, were used for each sample in this experiment when grown about 90% confluence. For control group, 0.1% DMSO was added. For probe group, the cells were incubated with 0.1%DMSO for 30 min and then with 10 μM P3 for 2 h. For competition group, the cells were incubated with 25, 50, 100 μM nintedanib for 30 min and then with 10 μM P3 for 2 h. Once completion of incubation, the cells were washed twice with pre-cooled PBS, followed by 365 nm UV irradiation for 40 min on ice. The cells were harvested,

lysed. The supernatant of soluble proteins was obtained by centrifugation (16000 x g, 15 min, 4 °C) and adjusted to 2 mg/mL by BCA assay. To be noted, a small aliquot of each sample should be analyzed by western blot before beads enrichment, indicating equal amount of target protein in each sample. The remaining cell lysates were conjugated to a biotin-azide tag by CuAAC and the biotinylated proteins were enriched with streptavidin beads for 4 h at 20 ℃. The beads were pelleted, washed with 1% SDS in PBS (3 x 1 mL), 6M Urea in PBS (3 x 1 mL), PBS (3 x 1 mL) and boiled with gel loading buffer (1x) to elute off the biotinylated proteins. Samples with equal volume were separated on Blot™ 4-12 % Bis-Tris Plus gel (Life Technology). For immunoblotting, the proteins were transferred onto iBlot 2 PVDF Regular Stacks (Life Technology) using iBlot 2 Dry Blot System (Life Technology). Then membranes were washed in PBS with 0.1% Tween-20 (PBST) and blocked in PBST containing 5% (wt/vol) dried milk for 3 h. After blocking, membranes were incubated at 4 ℃ overnight in PBST containing 5% bovine serum albumin (BSA) (Sigma Aldrich) and primary antibodies of anti-TPP1 (1:1000) (Abcam). Membranes were washed with PBST and incubated at room temperature for 1 h in 5% BSA/PBST containing anti-rabbit IRDye800 conjugated secondary antibody. Immunoblots were imaged on GE Image Quant LAS 500 (General Electric Company).

3.15 Fluorescence Microscopy Experiments

Referring to the previously reported procedures,[13] HUVEC cells were seeded on slide (WHB Scientific) in 24-well cell culture plate and grown to 70% confluence after 24 h. At the time of experiment, the mediums were replaced with 0.5 mL of fresh medium.
In live-cell imaging experiment, HUVEC cells were incubated with 20 μM of nintedanib (NDNB) or P3 for 1 h at 37 °C. 0.1% DMSO treated samples were used as controls. The medium was discarded and the cells were washed twice with PBS (0.5 mL). Then imaging was proceeded immediately with 405 nm and 488 nm excitation as reported.[14] In probe labeling experiment, for control group, 0.1% DMSO was added. For probe group, the cells were incubated with 0.1% DMSO for 30 min and then with 10 μM of P3 for 2 h at 37 °C. For competition group, the cells were incubated with 100 μM of nintedanib (10 x) for 30 min and then with 10 μM of P3 for 2 h. The mediums were discarded and the plate was gently washed once with pre-cooled PBS (0.5 mL). Then, 0.5 mL PBS was added and the cells were irradiated with 365 nm UV light (8 Watt) with ice cooling for 40 min. After finishing photo-labeling, the cells were fixed for 30 min at room temperature with 4% formaldehyde in PBS, washed three times with

cold PBS, and permeabilized with 0.1% Triton X-100 in PBS for 20 min. Cells were then blocked with 5% BSA in PBS for 30 min, washed twice with PBS. For click chemistry, a pre-mixture of TAMRA-azide (100 μM, 2 μL/sample, 10 mM in DMSO), TCEP (1 mM, 2 μL/sample, 100 mM in ddH_2O), TBTA (100 μM, 2 μL/sample, 10 mM in 4:1 DMSO : t-BuOH), $CuSO_4$ (1 mM, 2 μL/sample, 100 mM in ddH_2O) were added to the cells in a 200 μL volume and the cells were gently rotated at room temperature for 2 h. Then cells were washed with PBS three times, followed by staining with DAPI (ZSGB-BIO) at room temperature prior to imaging. In order to perform co-localization studies, cells were further incubated with anti-TPP1 antibody (1:100) at 4 °C overnight, washed three times with PBS, and then incubated with 5% BSA/PBS containing goat anti-rabbit IgG H&L (Alexa Fluor 488) secondary antibody (1:500) at room temperature for 1 h , followed by washing again. Imaging was done with the Leica TCS SP8 confocal microscope system equipped with Leica HCX PL APO 40×/1.20 W CORR CS, 405 nm diode laser, white laser (470-670 nm, with 1 nm increments, with eight channels AOTF for simultaneous control of eight laser lines, each excitation wavelength provides 1.5 mV), and a photomultipliertube (PMT) detector ranging from 410 to 700 nm for steady state fluorescence.

3.16 Cellular Thermal Shift Assay (CETSA)

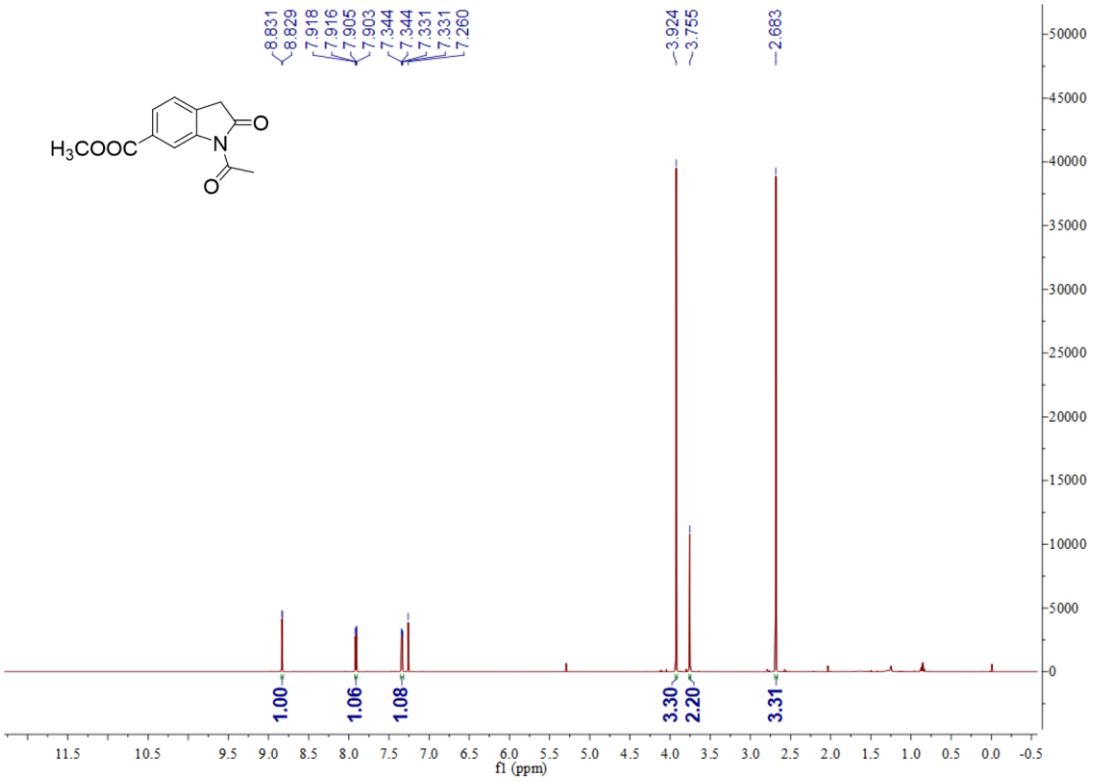
The CETSA assay was performed as previously described.[15] HUVEC cells were seeded in 10 cm cell culture dishes and grown to 90% confluence. At the time of experiment, the mediums were replaced with 5 mL of fresh medium. The cells were treated with nintedanib (10 μM) or DMSO control (0.1%) for 2 h, then washed with PBS twice, harvested with a cell scraper. The cell suspensions were centrifuged at 1000 x g for 5 min at 20 °C and the supernatant was removed to yield cell pellets. The pellets were resuspended in PBS containing protease inhibitor (Thermo), and distributed into each 0.2 mL PCR tubes with 100 μL of cell suspensions. The tubes were heated individually at different temperatures from 43 to 67 °C for 3 min, followed by cooling for 3 min at room temperature. To extract the total proteins, 2 freeze-thawing cycles with liquid nitrogen were performed. After centrifugation (15000 x g, 10 min, 4 °C), the soluble fractions were isolated. 80 μL of supernatant were mixed with 20 μL of 5x loading buffer and the samples were then analyzed by immunoblotting analysis of TPP1.

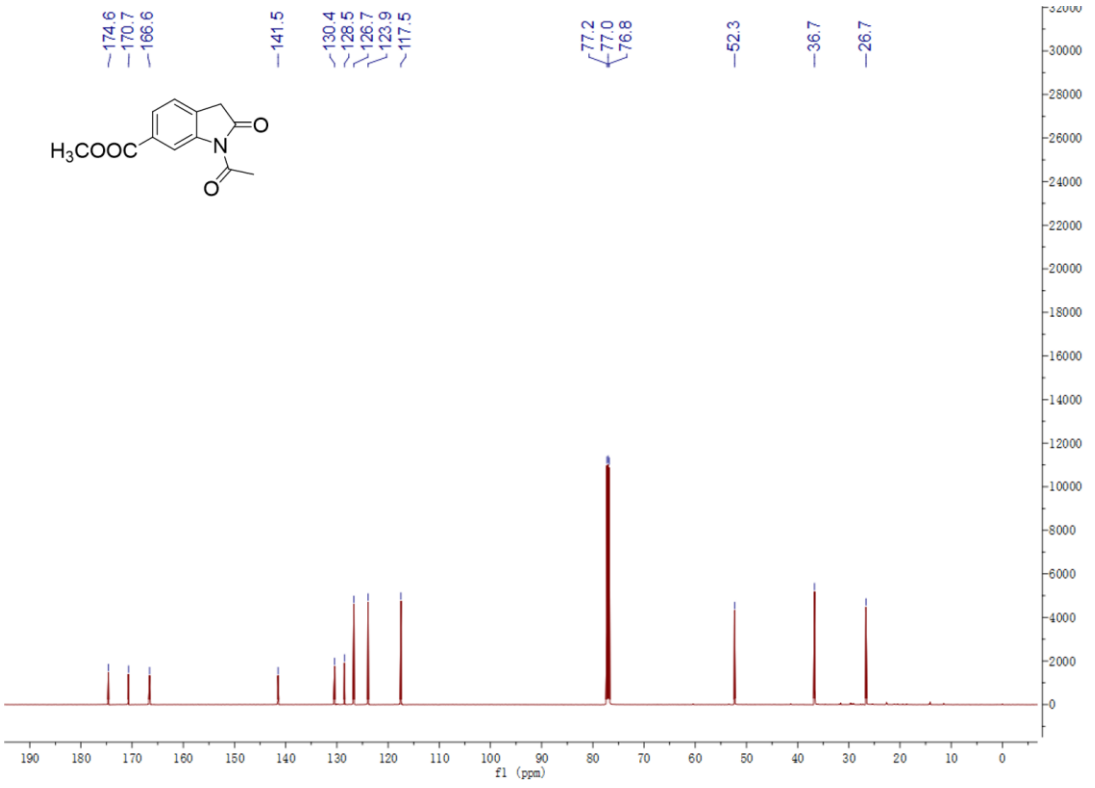
3.17 Telomeric Repeat Amplification Protocol (TRAP) Assay

The TRAP assay was performed according to the reported protocol with some modifications.[16] For HUVEC cells, 3 x 10^5 cells were seeded in 6-well plates in a volume of 2 mL DMEM (10% FBS, 1% penicillin-streptomycin, 1%NEAA) and grown to about 70% confluence after cultured at 37 °C in a humidified atmosphere with 5% CO_2 for 24 h. Then the cells were treated with 1 or 2 μM of nintedanib for 24 h. For Hela cells, 0.5 x 10^5 cells were seeded in 12-well plates in a volume of 1 mL DMEM (10% FBS, 1% penicillin-streptomycin) and grown to about 70% confluence after cultured at 37 °C in a humidified atmosphere with 5% CO_2 for 24 h. Then the cells were treated with 5 or 10 μM of nintedanib for 24 h. For control group, 0.1% DMSO were treated. Once completing incubation, the medium was discarded and the cells were harvested with a cell scraper in PBS. The cell suspensions were centrifuged at 5000 rpm for 3 min at 25 °C and the supernatant was removed to yield cell pellets. The pellets were resuspended in 200 μL ice-cold NP-40 lysis buffer at a concentration of 500 cells/μL and lysed on ice for 30 min. After centrifugation (13000 rpm, 10 min, 4 °C), the supernatant was transferred to a new 1.5 mL tube. Subsequently, the control samples were prepared, including negative and positive control. For negative control, cell lysate heat-inactivated at 85 ℃ for 10 min or NP-40 lysis buffer were used. For positive control, equal amount of cell lysate with active telomerase was used. Next, the TRAP master mix was prepared by addition and mixing of 38.6 μL RNase-free H_2O, 5 μL 10x TRAP buffer, 1 μL 50x dNTP mix, 1 μL 50x TRAP primer mix, 1 μL Cy-5 TS primer (100 ng/μL), 1 μL BSA (20 mg/ml), 0.4 μL *Taq* DNA polymerase (5 U/μL) for each sample. Add 2 μL of each sample into 48 μL of the master mix to make a total volume of 50 μL. The samples were incubated at 22-30 ℃ for 30 min to extend the substrate by telomerase. Then the PCR reaction was performed by setting the procedure of 95 ℃ for 5 min, and 24 cycles at 95 ℃ for 30 s, 52 ℃ for 30 s and 72 ℃ for 30 s. Once completion of PCR, 10 μL of 6x DNA loading buffer was added to each TRAP reaction mixture and 25 μL of each sample was separated on 12% nondenaturing acrylamide gel in 0.5x TBE (90 min, 110 V). The gel was fixed in 0.5 M NaCl, 50% (vol/vol) ethanol and 40 mM sodium acetate (pH 4.2) for 15 min. Eventually, the gel was visualized using a Typhoon FLA 9500 Biomolecular Imager (GE Healthcare) with 635 nm (Cy5) channel.

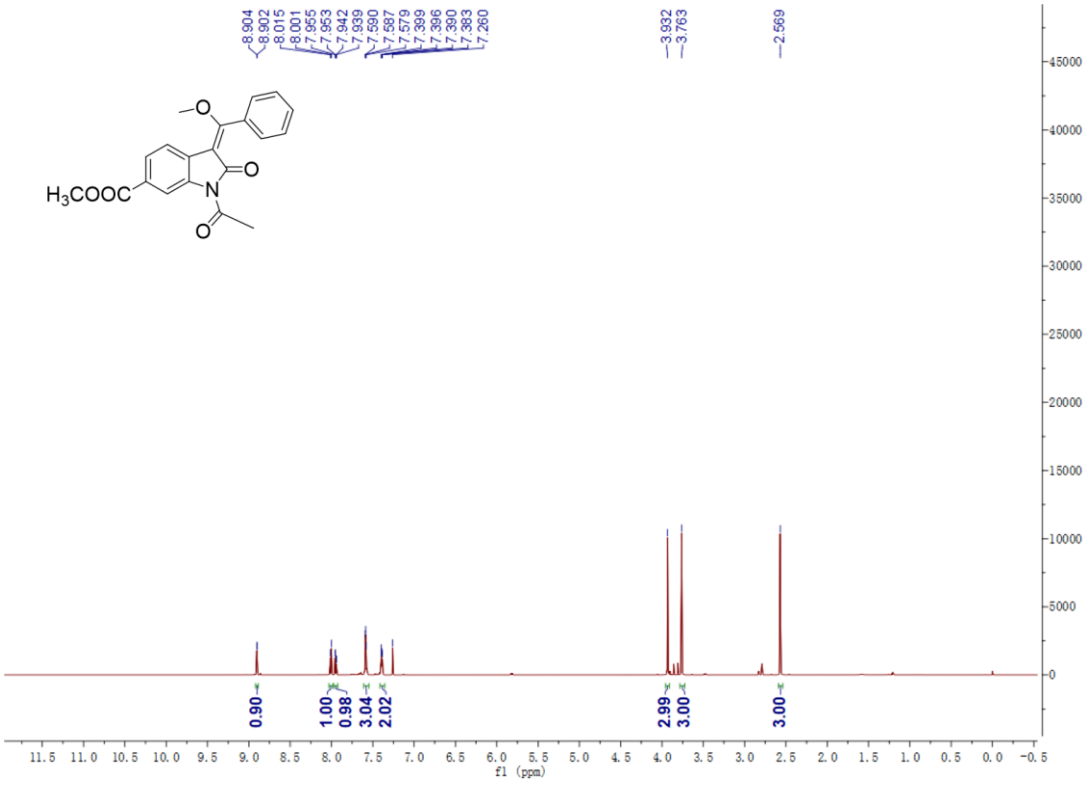
4 Related Spectra of Compounds Characterization

Compound 2





Compound 3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7.396
7.390
7.383
7.260
3.932
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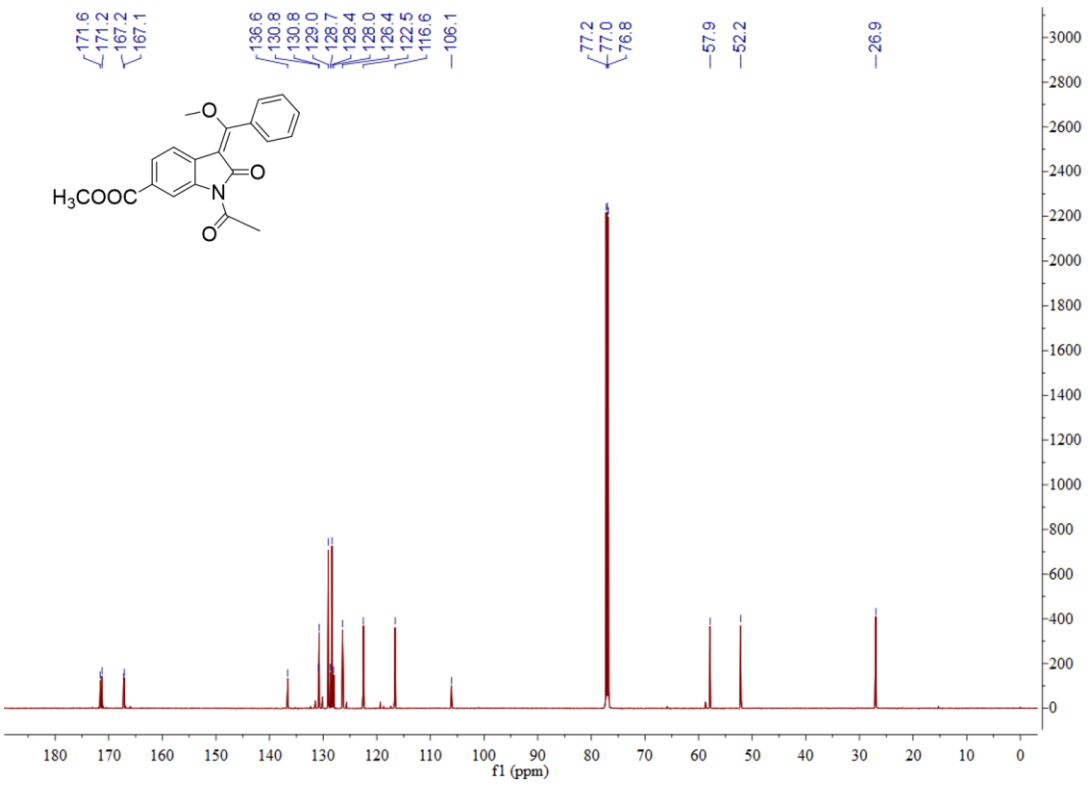
3.04
2.02
2.99
3.00
3.00
f1 (ppm)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167.1
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130.8
130.8
129.0
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128.4
128.0
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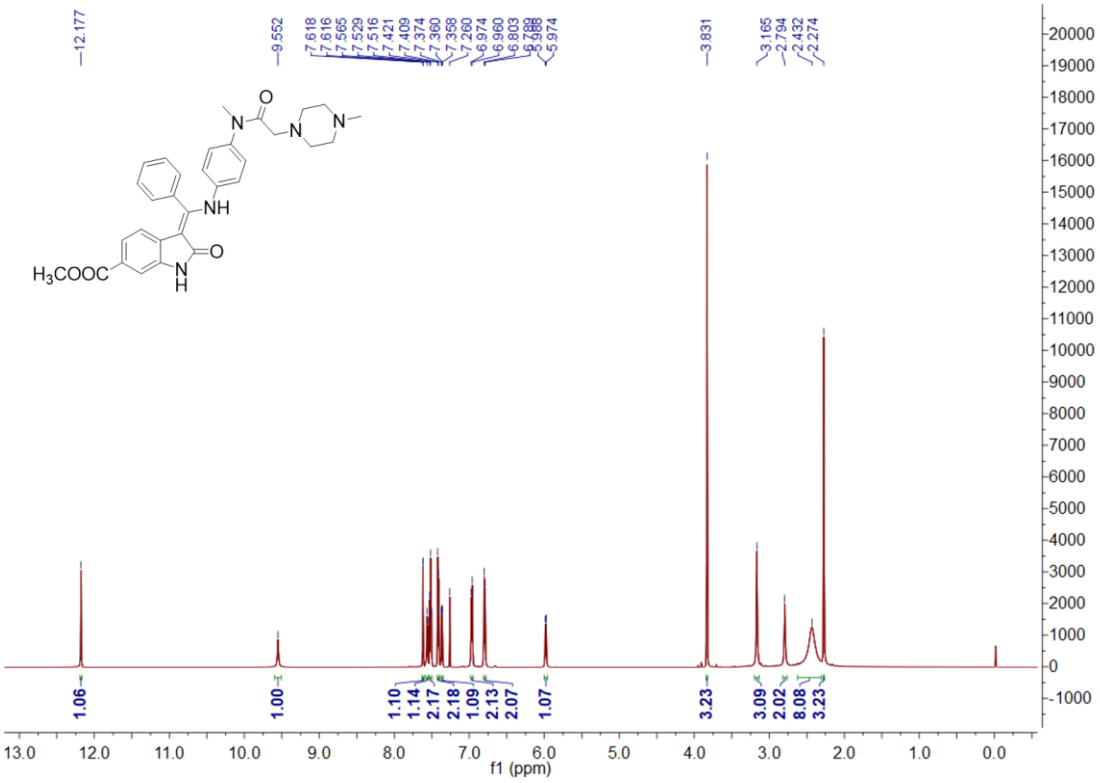
106.1
77.2
77.0
76.8
57.9
52.2
26.9
f1 (ppm)

Nintedanib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7.529
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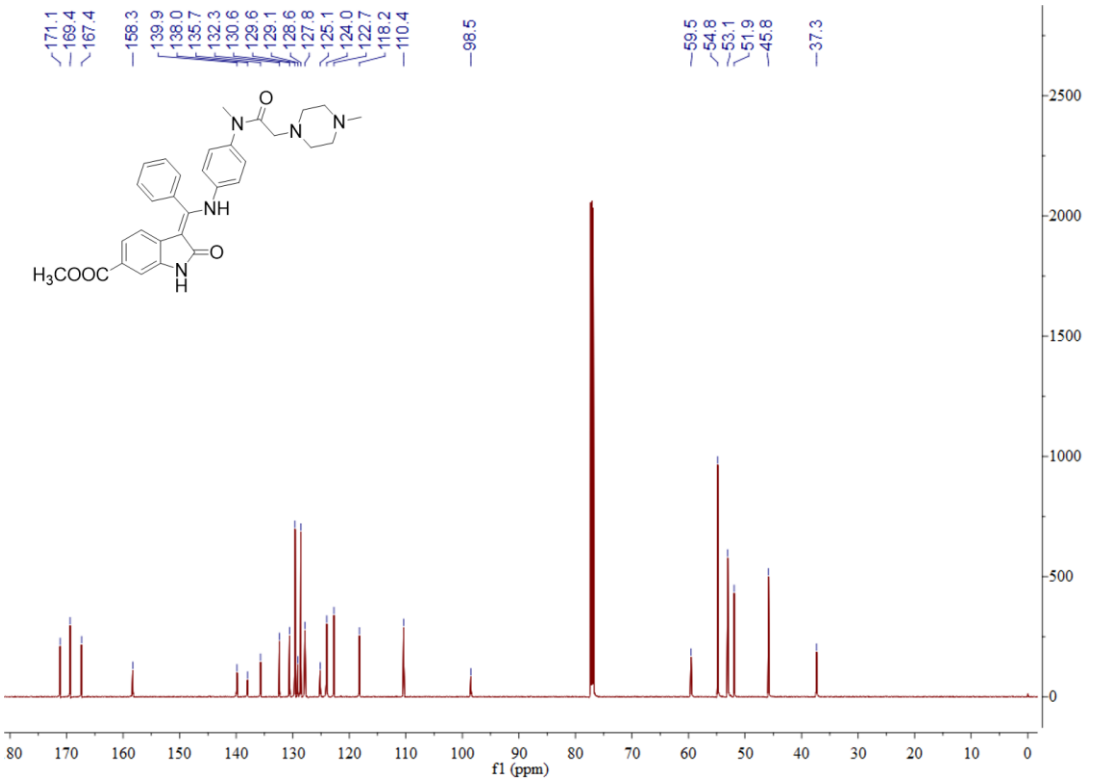
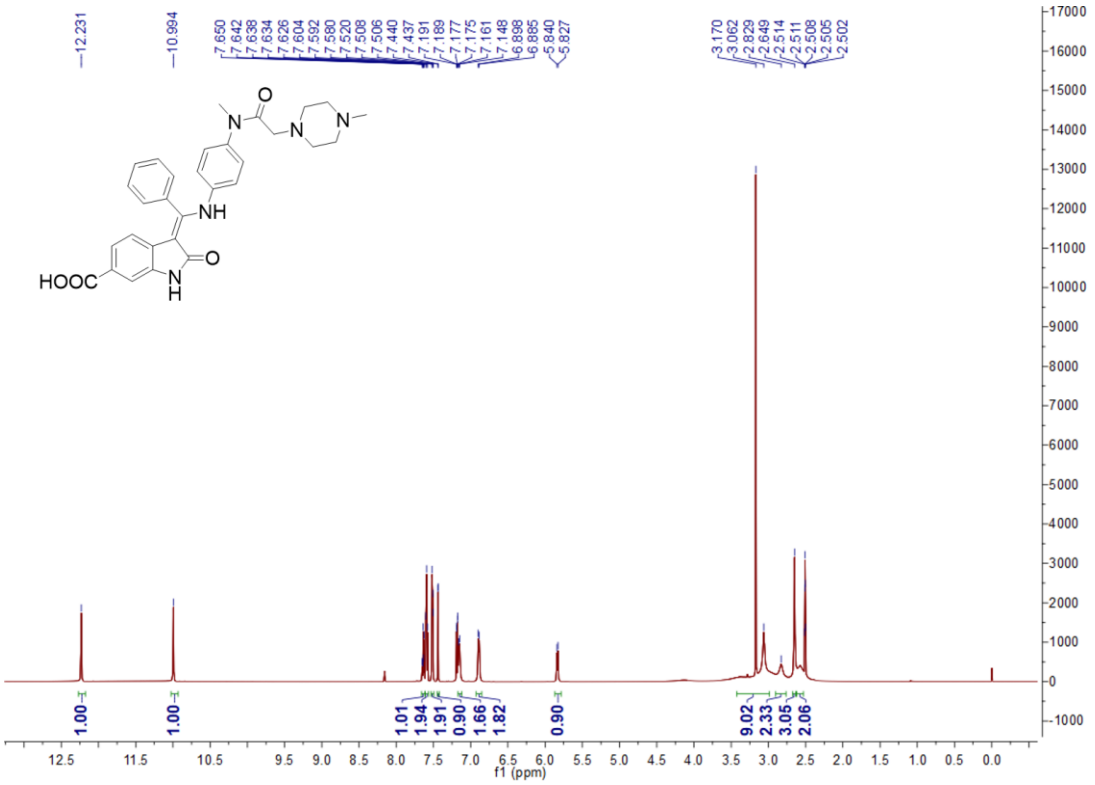
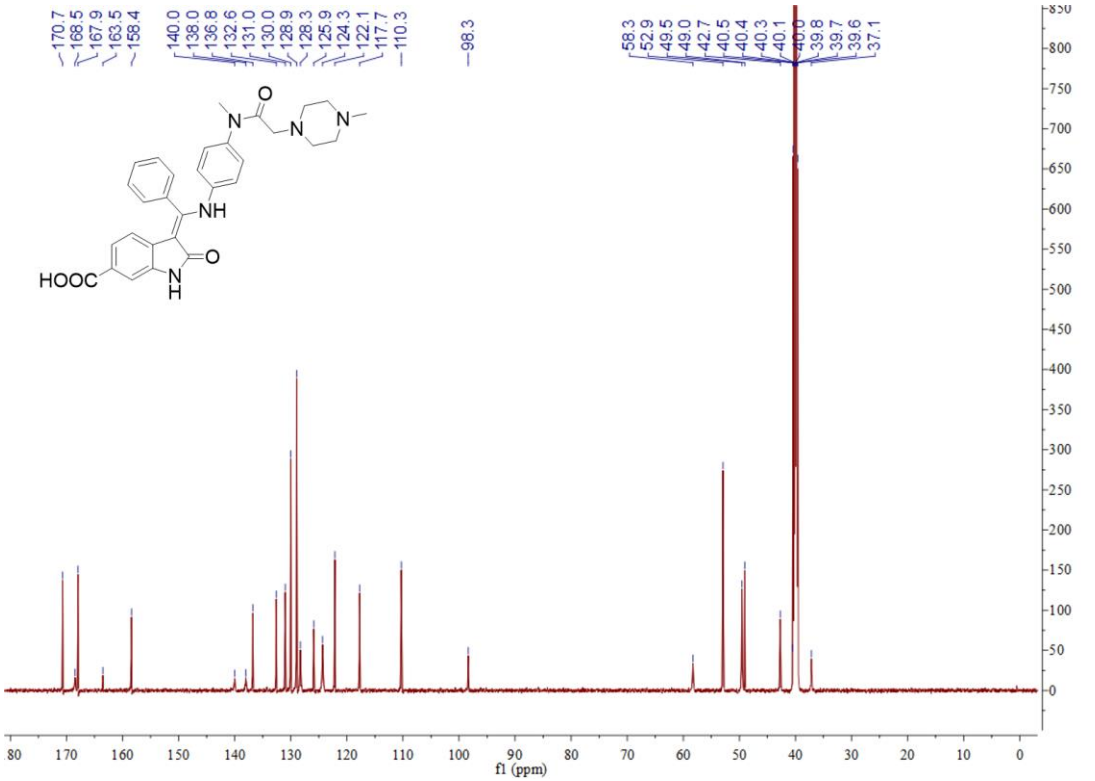
5.974
3.831
3.165
2.794
2.432
2.274
1.06
1.00
1.10
1.14
2.17
2.18
1.09
2.13
2.07
1.07
3.23
3.09
2.02
8.08
3.23
f1 (ppm)

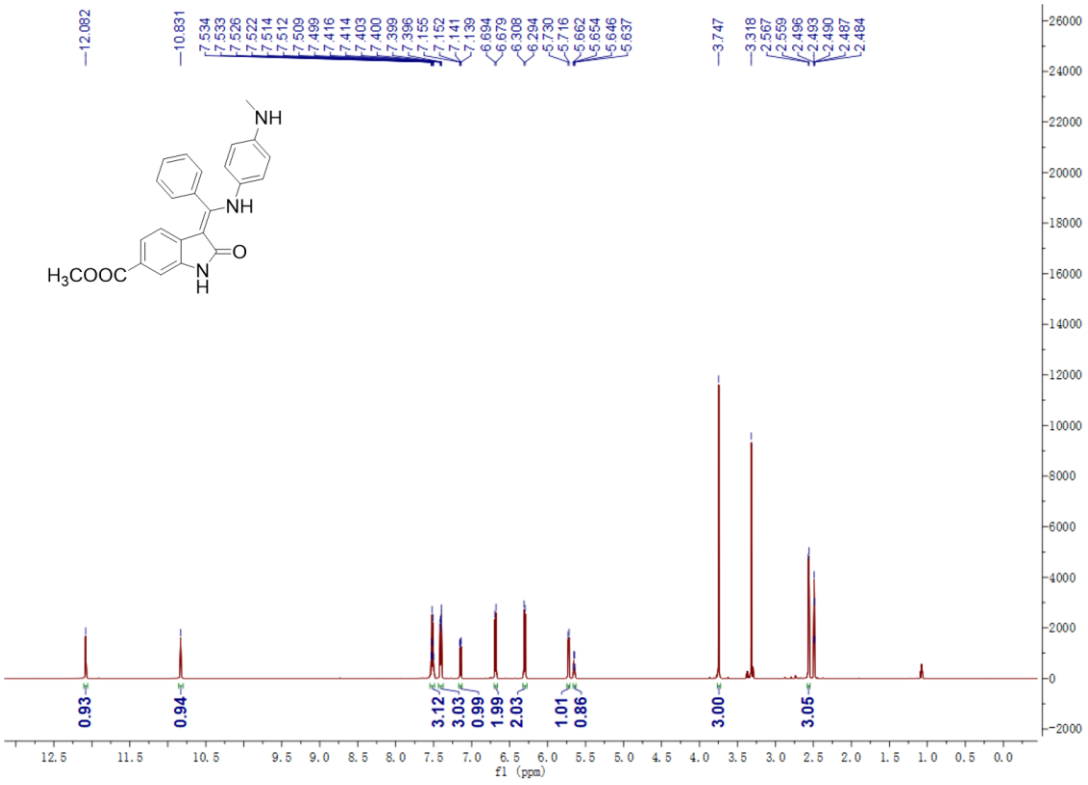

171.1
169.4
167.4
158.3
139.9
138.0
135.7
132.3
130.6
129.6
129.1
128.6
127.8
125.1
124.0
122.7
118.2
110.4
98.5
59.5
54.8
53.1
51.9
45.8
37.3
f1 (ppm)

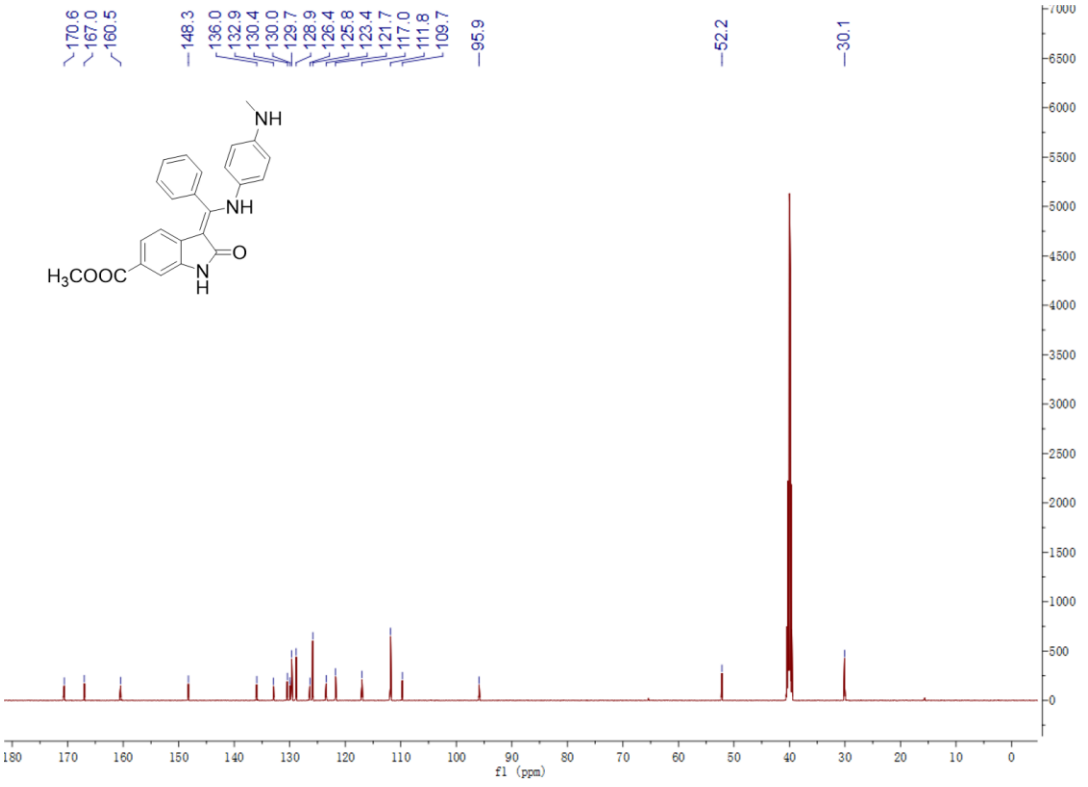
Nintedanib-COOH



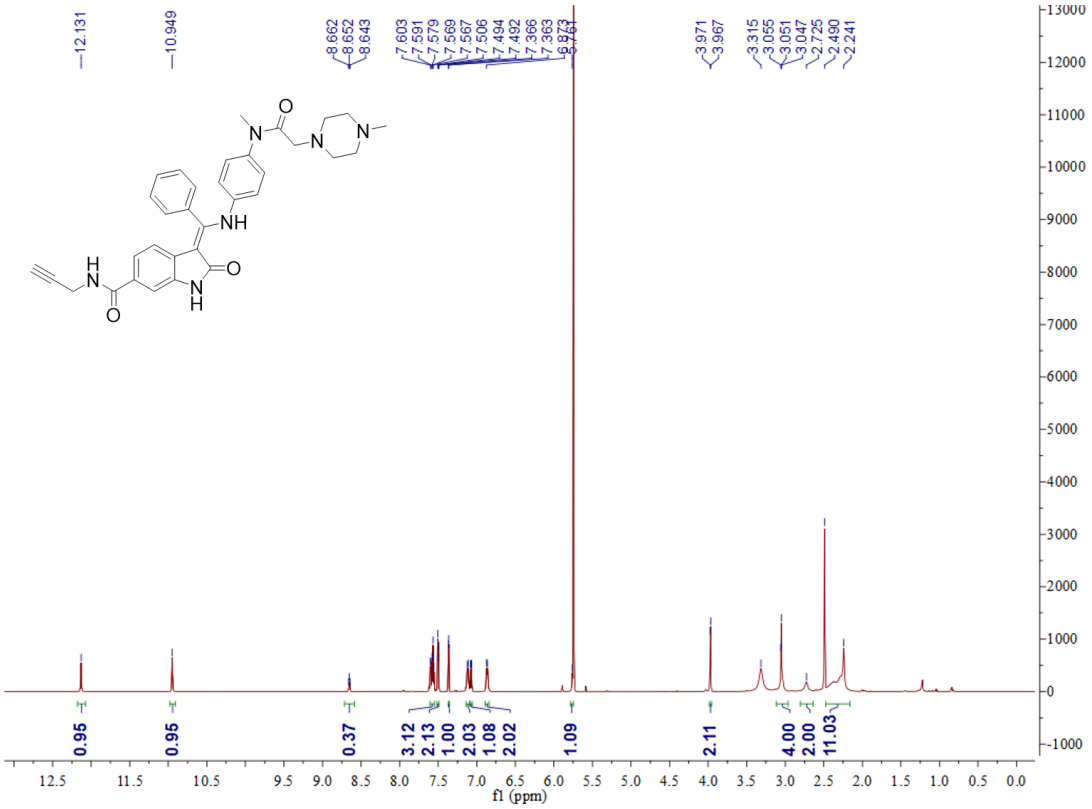


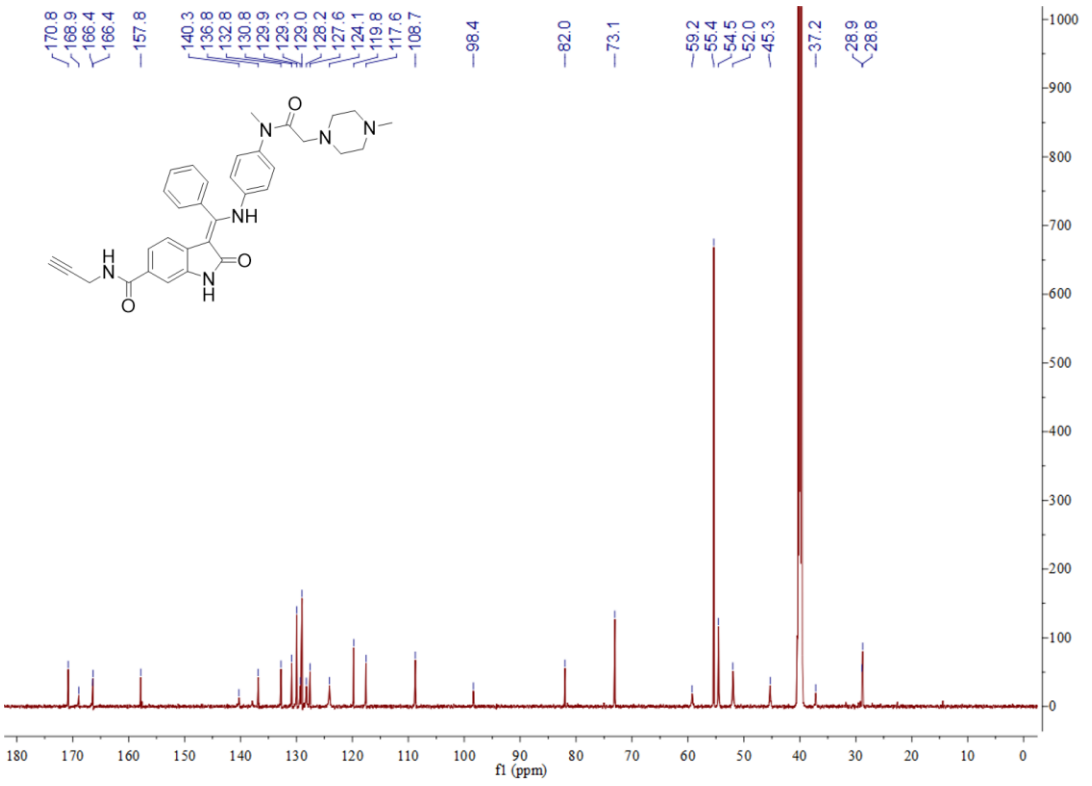
Compound 6


12.082
10.831
7.534
7.533
7.526
7.522
7.514
7.512
7.509
7.499
7.416
7.414
7.403
7.400
7.399
7.396
7.155
7.152
7.141
7.139
6.694
6.679
6.308
6.294
5.730
5.716
5.662
5.654
5.646
5.637
3.747
3.318
2.567
2.559
2.496
2.493
2.490
2.487
2.484
NH
NH
O
N
H
H3COOC
0.93
0.94
3.12
3.03
0.99
1.99
2.03
1.01
0.86
3.00
3.05
f1 (ppm)

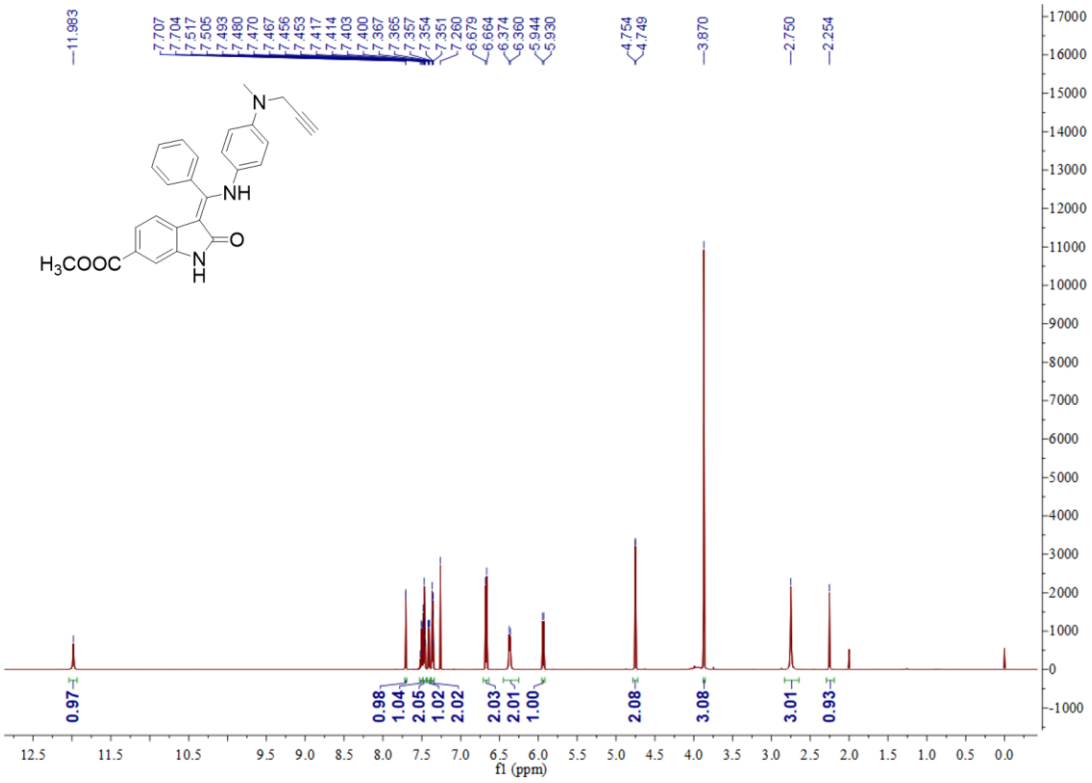

170.6
167.0
160.5
148.3
136.0
132.9
130.4
130.0
129.7
128.9
126.4
125.8
123.4
121.7
117.0
111.8
109.7
95.9
52.2
30.1
NH
NH
O
N
H
H3COOC
f1 (ppm)

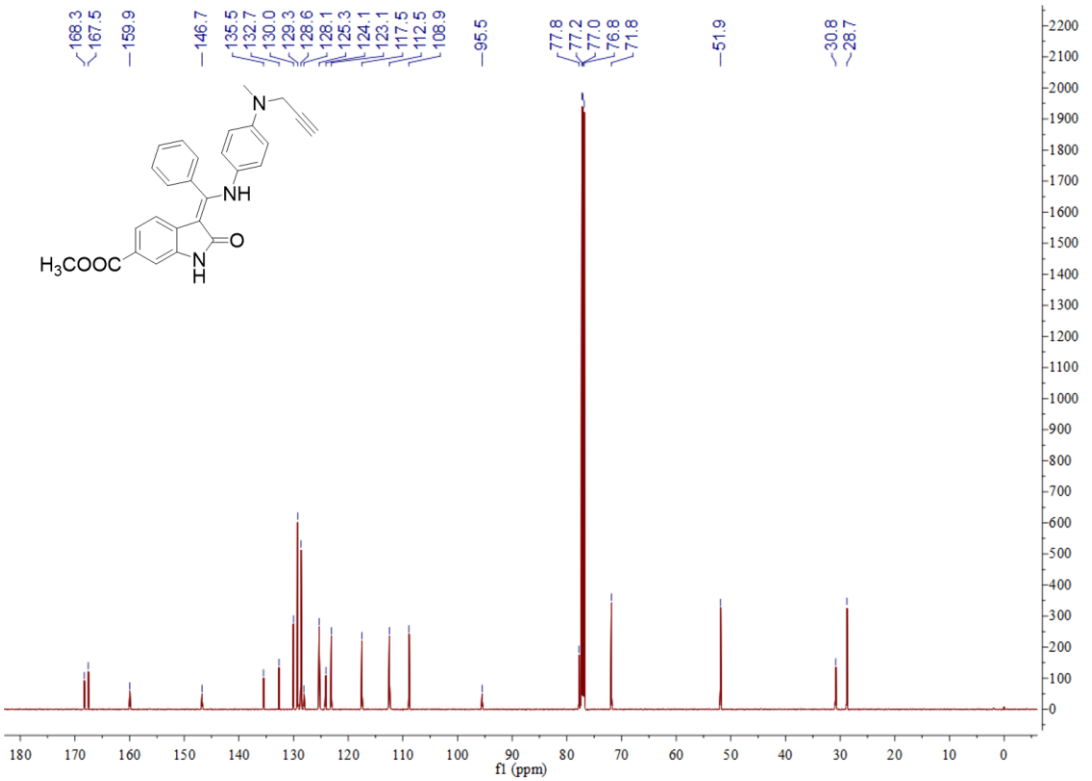
P1


12.131
10.949
8.662
8.652
8.643
7.603
7.591
7.579
7.569
7.567
7.506
7.494
7.492
7.366
7.363
3.971
3.967
3.315
3.055
3.051
3.047
2.725
2.490
2.241
0.95
0.95
0.37
3.12
2.13
1.00
2.03
1.08
2.02
1.09
2.11
4.00
2.00
11.03
f1 (ppm)

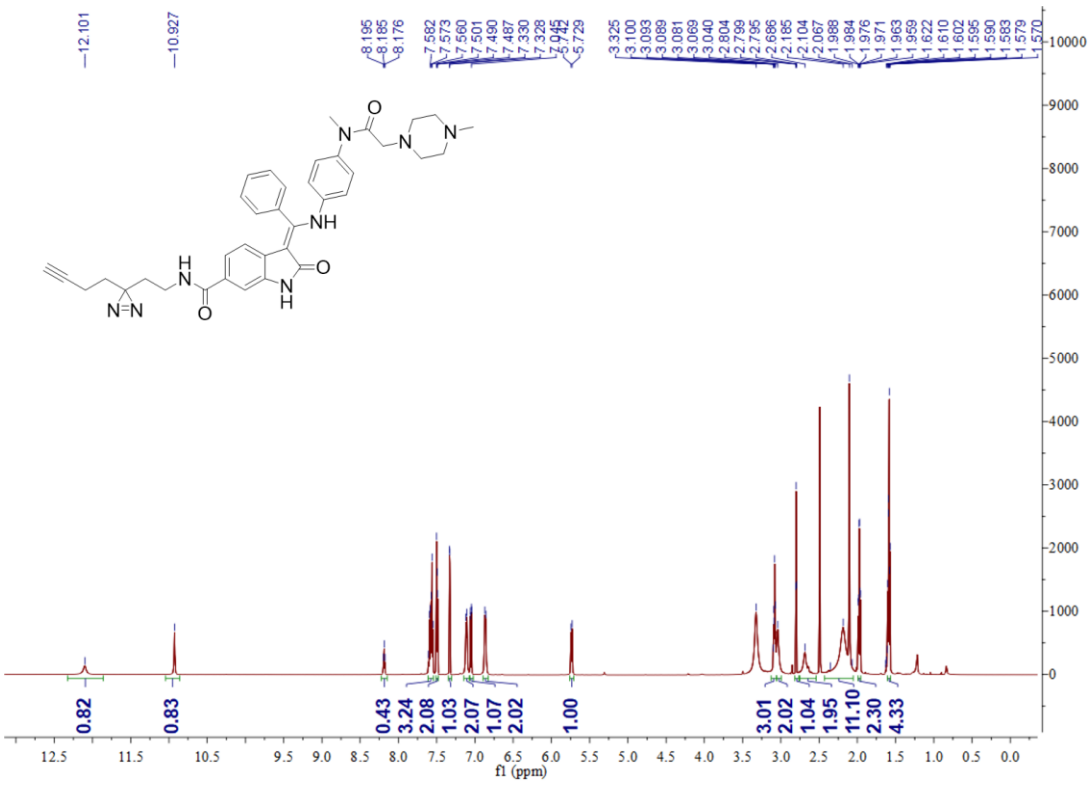

170.8
168.9
166.4
166.4
157.8
140.3
136.8
132.8
130.8
129.9
129.3
129.0
128.2
127.6
124.1
119.8
117.6
108.7
98.4
82.0
73.1
59.2
55.4
54.5
52.0
45.3
37.2
28.9
28.8
f1 (ppm)

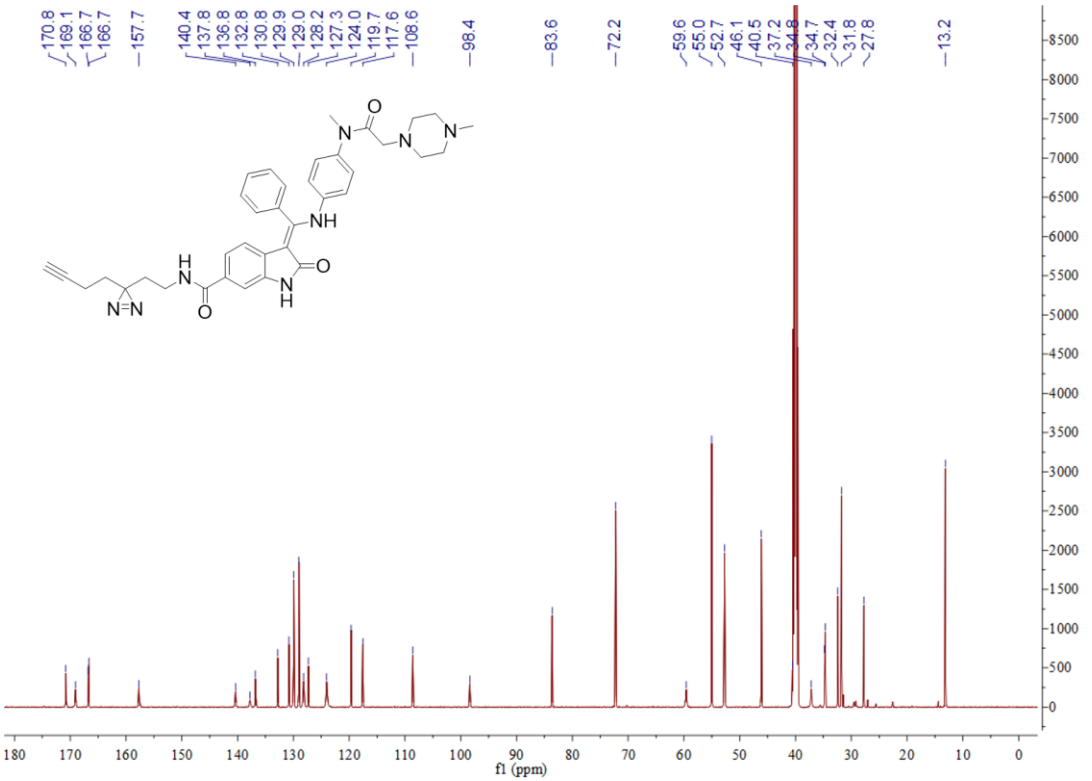
P2


11.983
7.707
7.704
7.517
7.505
7.493
7.480
7.470
7.467
7.456
7.453
7.417
7.414
7.403
7.400
7.367
7.365
7.357
7.354
7.351
7.260
6.679
6.664
6.374
6.360
5.944
5.930
4.754
4.749
3.870
2.750
2.254
NH
O
N
H
H3COOC
0.97
0.98
1.04
2.05
1.02
2.02
2.03
2.01
1.00
2.08
3.08
3.01
0.93
f1 (ppm)

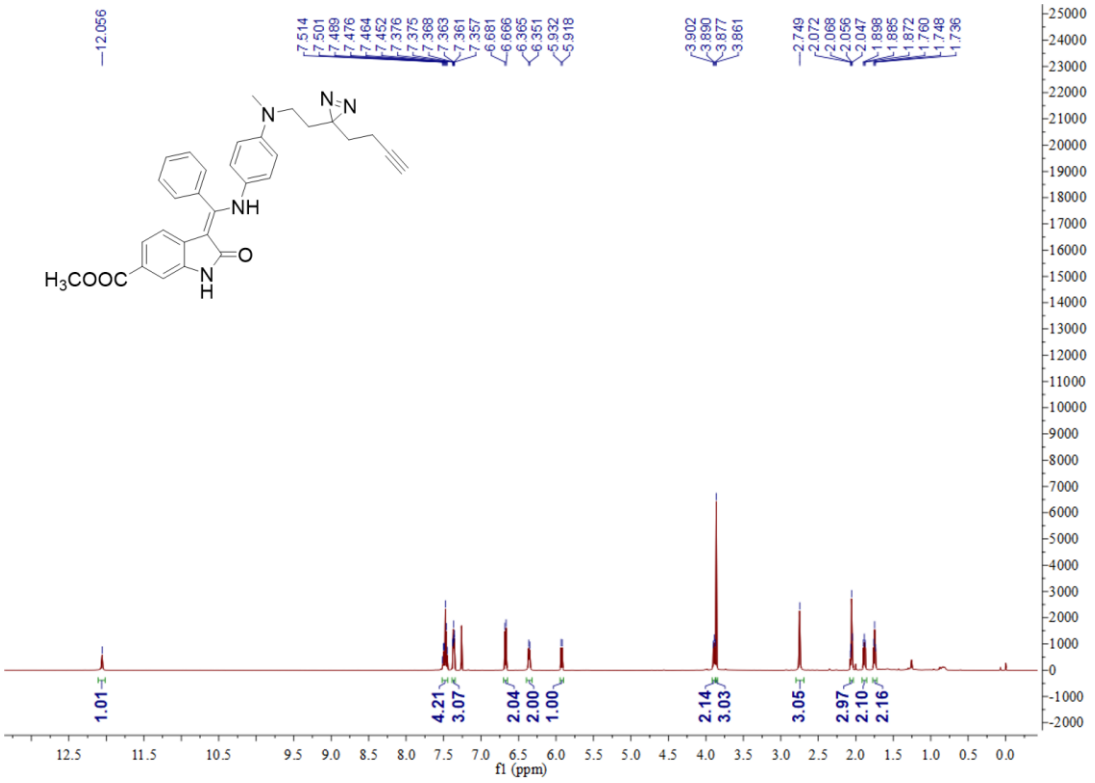

168.3
167.5
159.9
146.7
135.5
132.7
130.0
129.3
128.6
128.1
125.3
124.1
123.1
117.5
112.5
108.9
95.5
77.8
77.2
77.0
76.8
71.8
51.9
30.8
28.7
NH
O
N
H
H3COOC
f1 (ppm)

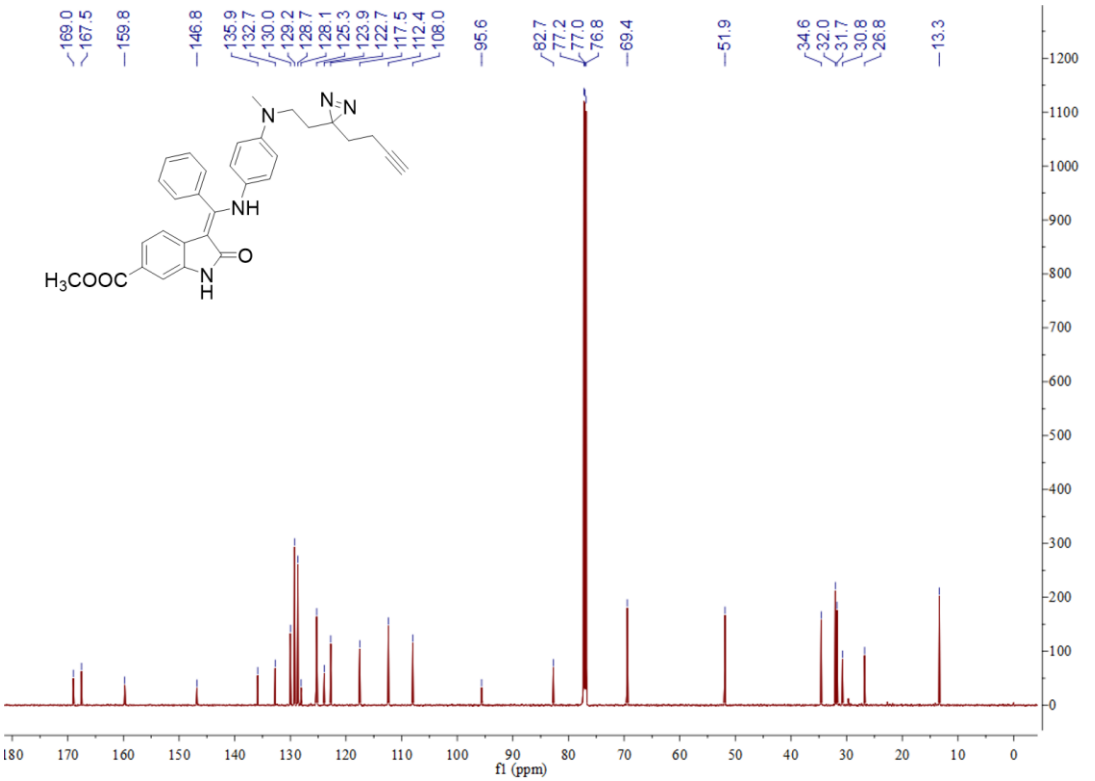
P3


12.101
10.927
8.195
8.185
8.176
7.582
7.573
7.560
7.501
7.490
7.487
7.330
7.328
5.945
5.942
5.729
3.325
3.100
3.093
3.089
3.081
3.069
3.040
2.804
2.799
2.795
2.686
2.185
2.104
2.067
1.988
1.984
1.976
1.971
1.963
1.959
1.622
1.610
1.602
1.595
1.590
1.583
1.579
1.570
0.82
0.83
0.43
3.24
2.08
1.03
2.07
1.07
2.02
1.00
3.01
2.02
1.04
1.95
11.10
2.30
4.33
f1 (ppm)


170.8
169.1
166.7
166.7
157.7
140.4
137.8
136.8
132.8
130.8
129.9
129.0
128.2
127.3
124.0
119.7
117.6
108.6
98.4
83.6
72.2
59.6
55.0
52.7
46.1
40.5
37.2
34.8
34.7
32.4
31.8
27.8
13.2
f1 (ppm)

P4


12.056
7.514
7.501
7.489
7.476
7.464
7.452
7.376
7.375
7.368
7.363
7.361
7.357
6.681
6.666
6.365
6.351
5.932
5.918
3.902
3.890
3.877
3.861
2.749
2.072
2.068
2.056
2.047
1.898
1.885
1.872
1.760
1.748
1.736
1.01
4.21
3.07
2.04
2.00
1.00
2.14
3.03
3.05
2.97
2.10
2.16
f1 (ppm)


169.0
167.5
159.8
146.8
135.9
132.7
130.0
129.2
128.7
128.1
125.3
123.9
122.7
117.5
112.4
108.0
95.6
82.7
77.2
77.0
76.8
69.4
51.9
34.6
32.0
31.7
30.8
26.8
13.3
f1 (ppm)

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