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SUPPORTING INFORMATION

Activity-based Protein Profiling Reveals GSTO1 as the Covalent Target of Piperlongumine and a Promising Target for Combination Therapy for Cancer

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General Materials

All chemical reagents were used as supplied by Sigma-Aldrich, J&K and Alfa Aesar Chemicals. DCM, DMF, acetonitrile were distilled from calcium hydride; THF was distilled from sodium/benzophenone ketyl prior to use. N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide were prepared according to the literature reported procedures.[1] S-(4-nitrophenacyl)glutathione (4-NPG) were prepared according to the literature reported procedure.[2] CellTiter-Glo® Luminescent Cell Viability Assay kit (G7570, G7573) was bought from Promega; DCFH-DA (D399) and High Capacity Strepavidin agrose (20361) was bought from Thermo Fisher Scientifc; DMEM Medium, Fetal Bovine Serum (12483-020) and Penicillin-Streptomycin(15140-122) were bought from Life Technology; recombinant GSTO1 protein (enz-434) was bought from Protein Specialists (Prospec); Akt antibody (4691), p-Akt antibody (4060), mTOR (2983), p-mTOR (5536) were bought from Cell Signaling Technology; GSTO1 antibody (GTX105655) were bought from GeneTex; pictilisib (T1994) were bought from TargetMol; siRNA were ordered from Biological Resource Center, National Institute of Biological Sciences, Beijing; Lipofectamine™ RNAiMAX (13778030) and Lipofectamine™ 3000 (L3000015) were bought from Invitrogen, Thermo Fisher Scientific; Ultimate™ ORF Clone plasmid (OH4381) was provided by Biological Resource Center, National Institute of Biological Sciences, Beijing; pcDNA 3.1 plasmid was given by Dr. Xiaodong Wang lab; anti-cancer agents library was provided by Chemistry Center, National Institute of Biological Sciences, Beijing.

^{1}HNMR spectra were recorded on a Varian 400 MHz spectrometer at ambient temperature with stated solvants. ^{13}C NMR spectra were recorded on a Varian 100 MHz spectrometer (with complete proton decoupling) at ambient temperature. Chemical shifts are reported in parts per million relative to chloroform (^{1}H, δ 7.26; ^{13}C, δ77.00). Data for ^{1}H NMR are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration and coupling constants. High-resolution mass spectra were obtained using Agilent Techmologies 6540 UHD Accurate-Mass Q-TOF LC/MS. Mass spectra was obtained by HPLC/MS on a Waters Auto Purification LC/MS system (3100 Mass Detector, 2545 Binary Gradient Module, 2767 Sample Manager, and 2998 Photodiode Array (PDA) Detector). The system was equipped with a Waters C_{18} 5μm SunFire separation column(150*4.6 mm), equilibrated with HPLC grade water (solvent A) and HPLC grade acetonitrile (solvent B) with a flow rate of 0.3 mL/min.

Experimental Protocols

Cell death assay:

Cells were plated at a density of 3000 cells per well in 96-well plates. 24 h later, indicated compounds were added to cells and incubated for another 24 h. Then, cell viability was determined by measuring the ATP levels using a Cell Titer-Glo kit. Cell survival rate was calculated, and IC_{50} of tested compounds was calculated by GraphPad Prism software. IC_{50} values are represented as the mean with the standard error from 3 independent experiments in figures; data points are the mean of duplicates with standard the error from one experiment as a representative in figures.

Testing the reversibility of compound cellular activity:

Cells were plated at a density of 3000 cells per well in 96-well plates. 24 h later, tested compounds were added to cells and incubated for 3h. One subset of these cells was washed free of tested compounds with warmed fresh medium for 3 times; while the other subset of cells were not washed. 24 h after compounds addition, cell viability was determined by measuring the ATP levels using a Cell Titer-Glo kit. Cell survival rate was calculated, and IC_{50} of tested compounds under wash-off/no wash-off conditions was calculated and compared by GraphPad Prism software. IC_{50} values are represented as the mean with the standard error from 3 independent experiments in figures; data points are the mean of duplicates with standard the error from one experiment as a representative in figures.

Reaction of PL-1/PL-2 with GSH/NAC:

NAC/GSH (200μM) was added to PL-1/PL-2 (10μM) in medium and incubated for 3 h at 37 ℃. The reaction was monitored using LC-MS/MS, and the amount of the remaining compounds was analyzed by Lcquan 2.5 Software. Experiments were repeated three times; data points are the mean of duplicates with standard the error from one experiment as a representative in figures.

Activity-based protein profiling:

NCI-H1975 cells were treated with indicated compounds for 3 h and then lysed. The cell lysate was adjusted to a concentration of 1mg/mL protein, and denatured with 2% SDS for 30min. Click reactions were performed with (N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl) pentanamide) (azido-biotin, 500 μM), CuSO4 (200 μM), TBTA (200μM) and sodium L-ascorbate (1 mM) for 1 hr at 37oC. The whole proteome were then precipitated by 5 volumes of methanol, washed 3 times with methanol, and redissolved with 1mL PBS buffer containing 0.2% SDS. Each sample was incubated with 200uL of streptavidin agrose for 2 h at room temperature, and centrifuged at 3000 rpm for 3 min to collect the agrose. The agrose was washed 3 times with PBS buffer, and eluted with 30ul 1× loading buffer. The eluted components were then subjected to western blot detection or SDS-PAGE and protein MS analysis.

Protein MS/MS:

Protein bands on the SDS-PAGE gel were de-stained, and then reduced in 10 mM DTT at 56 °C for 30 min followed by alkylation in 55 mM iodoacetamide at dark for 1 hr. After that the protein bands were in-gel digested with sequencing grade trypsin (10 ng/μL trypsin, 50 mM ammonium bicarbonate, pH 8.0) overnight at 37 °C. Peptides were extracted with 5% formic acid/50% acetonitrile and 0.1% formic acid/75% acetonitrile sequentially and then concentrated to ~ 20 μl. The extracted peptides were separated by an analytical capillary column (50 μm × 15 cm) packed with 5 μm spherical C18 reversed phase material (YMC, Kyoyo, Japan). A Waters nanoAcquity UPLC system (Waters, Milford, USA) was used to generate the following HPLC gradient: 0-30% B in 40 min, 30-70% B in 15 min (A = 0.1% formic acid in water, B = 0.1% formic acid in acetonitrile). The eluted peptides were sprayed into a LTQ Orbitrap Velos mass spectrometer (ThermoFisher Scientific, San Jose, CA, USA) equipped with a nano-ESI ion source. The mass spectrometer was operated in data-dependent mode with one MS scan followed by four CID (Collision Induced Dissociation) and four HCD (High-energy Collisional Dissociation) MS/MS scans for each cycle. Database searches were performed on an in-house Mascot server (Matrix Science Ltd., London, UK) against the IPI human (International Protein Index) protein sequence database. The search parameters are: 7 ppm mass tolerance for precursor ions; 0.5 Da mass tolerance for product ions; three missed cleavage sites were allowed for trypsin digestion and the following variable modifications were included: (1) for pulldown sample, oxidation on methionine, carbamidomethylation on cysteine, PL-N modification (C_5H_7NO) on cysteine, PL-5-biotin modification ($C_{35}H_{47}N_7O_8S$) on cysteine; (2) for protein sample, oxidation on methionine, carbamidomethylation on cysteine, PL-N modification (C_5H_7NO) on cysteine, PL-1 modification ($C_{17}H_{19}NO_5$) on cysteine, PL-5 modification ($C_{17}H_{15}NO_3$) on cysteine. The tandem mass spectra of matched phosphorylated peptides were manually checked for their validity.

Molecular docking simulation:

The X-ray crystal structure of GSTO1 (PDB: 4YQV) was used for docking studies.[3] Before docking simulation, ligands and protein were prepared with the standard protocol using MOE 2015.10 software, including the addition of hydrogens, the assignment of bond order, and assessment of the correct protonation state. All docking calculations were performed using default settings.

GSTO1 enzyme assay:

Cells were incubated with indicated compounds for 3 h, then cells were lysed, and cell lysates were adjusted to protein concentration of 0.5 mg/mL. Enzyme activity tests were performed in 384-well plate. To 50 μL cell lysate was added 1mM S-(4-nitrophenacyl)glutathione (4-NPG) as substrate and 10 mM β-mercaptoethanol as reductant, and the whole enzyme reaction system was incubated at 37^{o}C for 30min[17]. The concentration of 4-NPG was measured and calculated by the absorbance at 305nM. The

relative GSTO1 enzyme activity was calculated based on the mean consumption of 4-NPG during reaction. Experiments were repeated three times; data points are the mean of duplicates with standard the error from one experiment as a representative in figures.

GSTO1 knockdown:

Cells are transfected with *GSTO1* siRNA (siGSTO1-1, siGSTO1-2) or scrambled control siRNA (siSCRAM) using Lipofectamine™ RNAiMAX following the manufacture's instruction. Western blot of GSTO1 was performed 36 h after siRNA treatment. Cell viability was detected 48 h after siRNA treatment using a Cell Titer-Glo kit. Experiments were repeated three times; data points are the mean of duplicates with standard the error from one experiment as a representative in figures.

siGSTO1-1:

Sense: 5'-GCUUGCCAGAAGAUGAUCUUA dTdT-3'

Antisense:5'-UAAGAUCAUCUUCUGGCAAGC dTdT-3'

siGSTO1-2:

Sense: 5'-CAGGCAUGAAGUCAUCAAU UU-3'

Antisense: 5'-AUUGAUGACUUCAUGCCUG UU-3'

siSCRAM:

Sense: 5'- GACCGCUAAUCGUAUGUAAGU dTdT-3'

Antisense: 5'-ACUUACAUACGAUUAGCGGUC dTdT-3'

GSTO1 overexpression:

GSTO1 cDNA was amplified by PCR from the Ultimate™ ORF Clone plasmid (OH4381) using the primers as followes, and then the GSTO1 expression plasmid was constructed in the pcDNA 3.1 vector. Transfections of GSTO1 expression plasmid or empty vector were conducted using Lipofectamine™ 3000 following the manufacture's instruction. Transfected cells were selected using Hygromycin.

GSTO1 primer:

Forward: 5'-AACGCGGATCCATGTCCGGGGAGTCAGCCAG-3'

Reverse: 5'-AATGCTCTAGA TCAGAGCCCATAGTCACAG-3'

Cellular ROS level detection:

NCI-H1975 cells were plated at a density of 10000 cells per well in 96-well optical plate. 24 h later, cells were treated with experimental compounds for 6 h or with indicated siRNA for 24 h. Then cells were washed 3 times with PBS and then incubated in PBS with 2 μM DCFH-DA for 30 min at 37 ℃. Cells were washed twice with PBS and fluorescence was detected with a PerkinElmer EnSpire

Multimode Plate Reader (λex= 485 nm and λem= 525 nm). Immediately after fluorescence detection, cell viability was determined by measuring the ATP levels using a Cell Titer-Glo kit. Mean ROS levels were recorded as ROS fluorescence/cell viability and ROS increases as compared with the negative control group (DMSO or siSCRAM) were calculated. Experiments were repeated three times; data points are the mean of duplicates with standard the error from one experiment as a representative in figures.

Combination effect screen:

For the first round of screen, cells were plated at a density of 1000 cells per well in 384-well plates. 24 h later, tested compounds alone or with PL-1 were added to cells and incubated for 24hr. Tested compounds were added at the final concentration of 10/1/0.1 μM using a 200X stock solution; for each concentration, two concentration of PL-1 (3/1μM) were added using a 200X stock solution for combination test. For the tested compounds alone groups, the same volume of solvent (DMSO) of PL-1 solution was also added. Experiments were carried out in duplicates. 24 hr later, cell viability was determined by measuring the ATP levels using a Cell Titer-Glo kit and cell survival rate was calculated. Combinational index were calculated using CompuSyn software (version 1.0). All compounds with CI<1.0 were chosen for the second round of screen.

In the second round of screen, cells were plated at a density of 3000 cells per well in 96-well plates. 24 h later, compounds were added at the same concentration as in the first round of screen. Experiments were carried out in duplicates. 24 hr later, cell viability and CI were determined and calculated as in the first round of screen. Compounds with CI<0.9 were picked as hits that have synergistic effect.

Supporting Figures and Tables

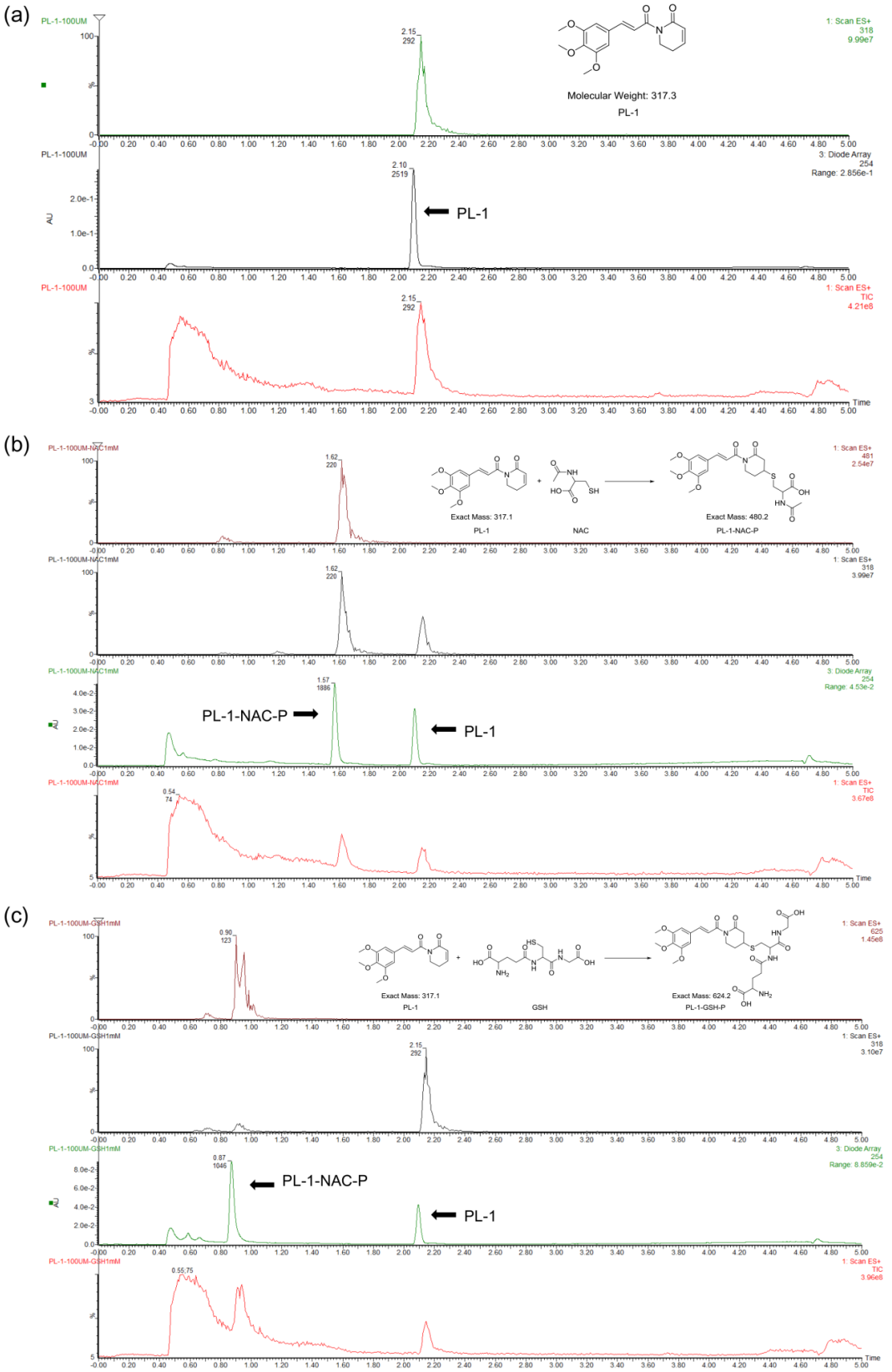


Figure S1. LC-MS detection of PL-1 (a), and reaction of PL-1 with NAC (b) or with GSH (c). PL-1: 100μM. NAC: 1mM. GSH: 1mM.

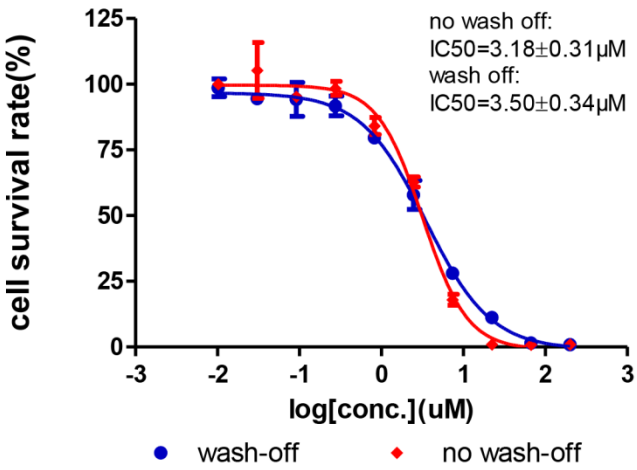


Figure S2. PL-5 functions in an irreversible mode. Cytotoxic activity of PL-5 in wash/no-wash assays was measured: for the wash-off condition, NCI-H1975 cells were incubated with PL-5 for 3 h and then washed with buffer. Cell viability was measured 24hrs after PL-5 administration.

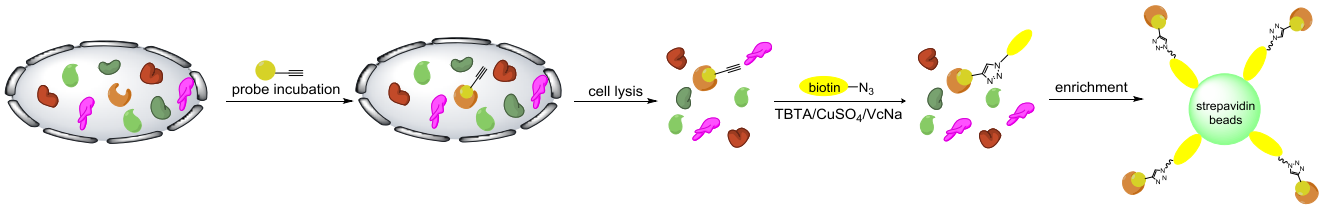
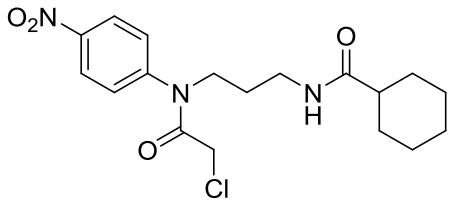


Figure S3. General procedure for probe target identification by click-reaction-assisted ABPP.



KT45,
IC_{50}=1.4±0.08uM on NCI-H1975 cell line.

Figure S4.Structure of the reported GSTO1 inhibitor KT45.

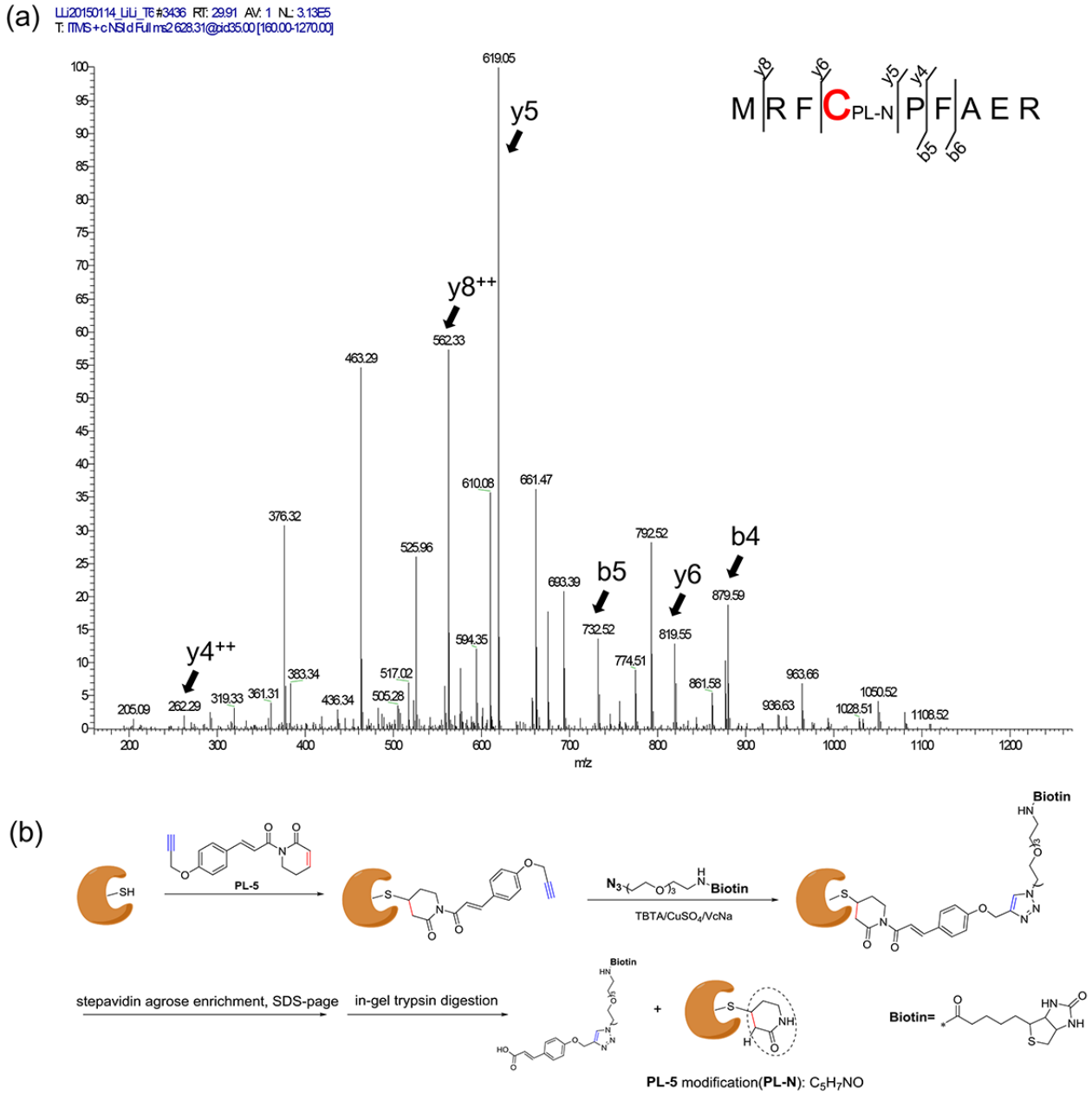


Figure S5. ABPP probe PL-5 covalently midified GSTO1 at Cys32 in the PL-5 enriched samples from pulldown assay. (a) MS/MS spectra of PL-N(C_5H_7NO) modified GSTO1 peptide MRFCPFAER. (b) Detailed model of probe-target binding, click reaction with biotinalyted azide, and speculated amide bond break during in-gel digestion and PL-N modification structure.

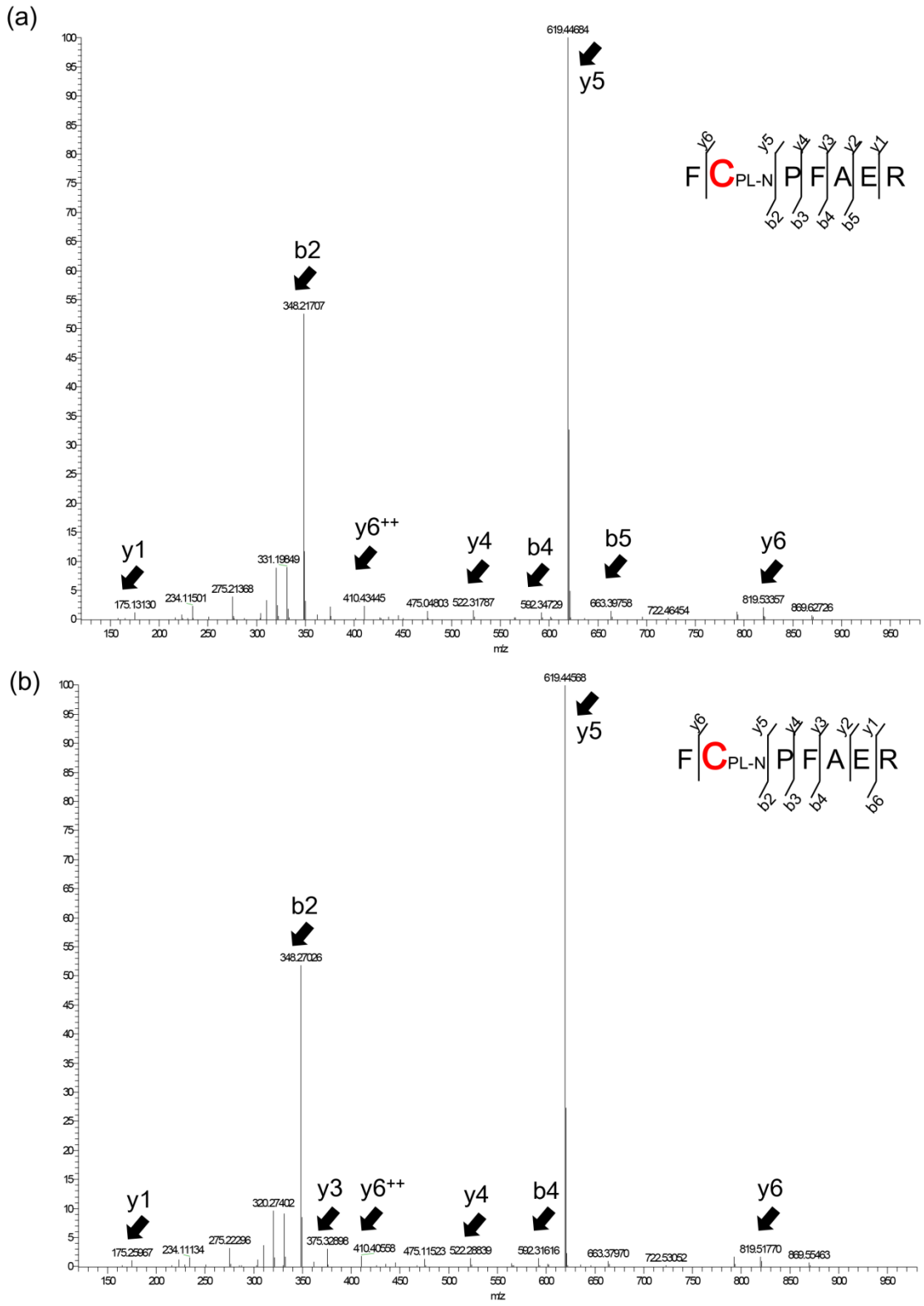


Figure S6. PL-1 (a) and PL-5 (4) covalently midified recomibinant GSTO1 at Cys32 with a PL-N(C_5H_7NO) modification. Recomibinant GSTO1 (1uM) were incubated with β-mercaptoethanol (10uM) for 30min, followed by addition of PL-1 (20uM) or PL-5 (20uM), and incubated for another 2hr. The sulution were then subjected to SDS-PAFE and protein MS/MS detection.

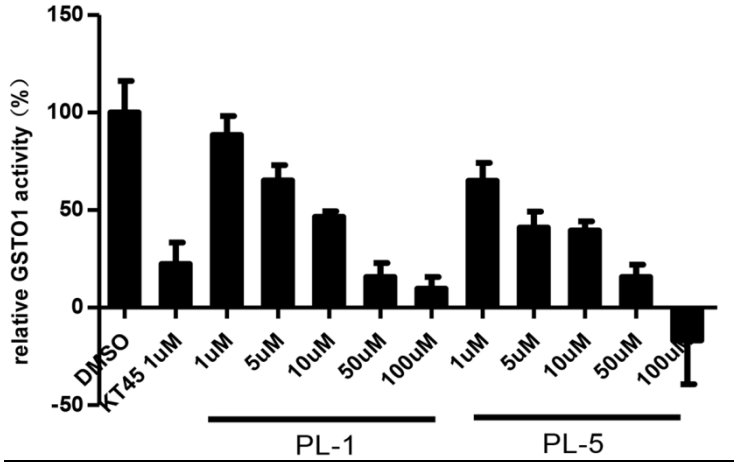


Figure S7. Cellular (NCI-H1975 cells) incubation of PL-1 and PL-5 inhibited GSTO1 catalyzed 4-nitrophenacyl glutathione reduction activity. DMSO and KT45 were applied as negative and positive controls, respectively.

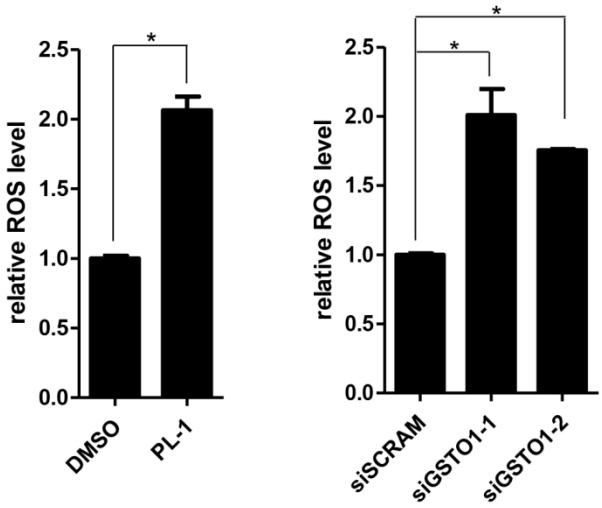


Figure S8. Relative cellular ROS level after PL-1 treatment (left) and GSTO1 knockdown (right) on NCI-H1975 cells. PL-1 treatment: 10μM for 6 h. siRNA treatment: 10 nM for 16 h. *:P value<0.05; **: P value < 0.01; ***: P value < 0.001.

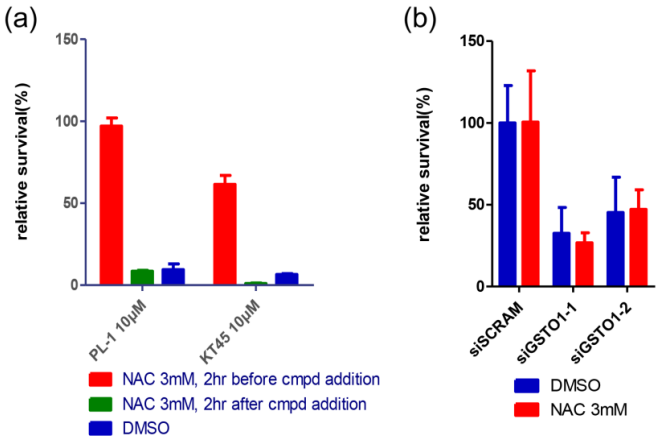


Figure S9. ROS scavenger NAC doesn't rescue cell death caused by PL-1/KT45 (a) or *GSTO1* knockdown (b).

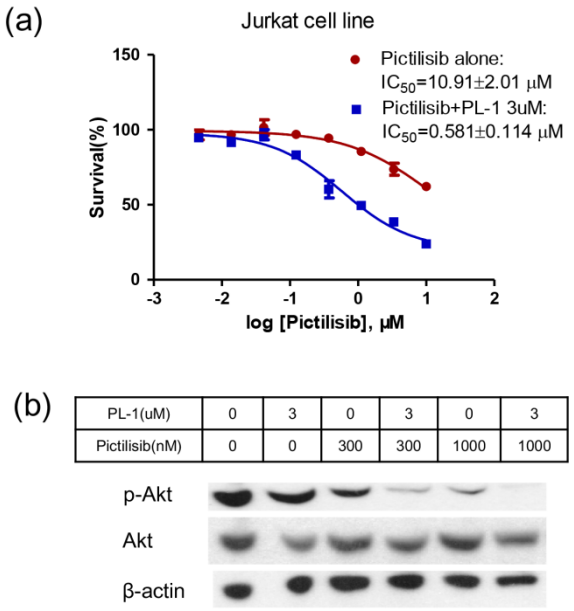


Figure S10. PL-1 synergized with pictilisib in the cell death induction (a) and in the inhibition of Akt phosphorylation (b) of Jurkat cells.

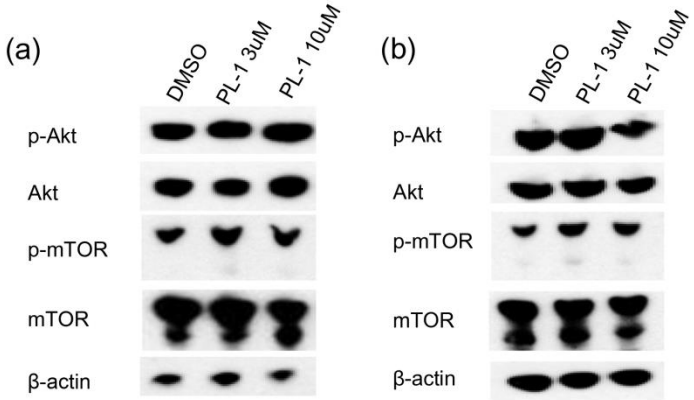


Figure S11. PL-1 has no effect on the phosphorylation of Akt and mTOR on NCI-H1975 (a) and Jurkat (b) cells.

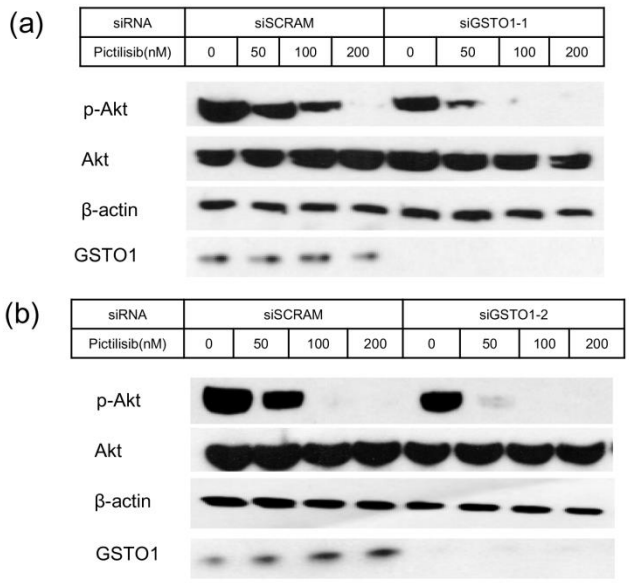


Figure S12. Knockdown of GSTO1 synergized with pictilisib in the inhibition of Akt phosphorylation of NCI-H1975 cells (a) and Jurkat cells (b).

Table S1. IC_{50} of PL-1/2/3 on different cell lines.

Cell line	PL-1 (μM)	PL-2 (μM)	PL-3 (μM)
NCI-H1975	2.9±0.14	>50	>50
BT474	4.1±0.98	>50	>50
PNAC-1	4.0±0.87	>50	>50
Jurkat	3.2±0.43	>50	>50
U2OS	3.1±0.14	>50	>50
MCF-7	3.7±0.62	>50	>50
Hela	1.9±0.24	>50	>50
Molt-4	2.3±0.36	>50	>50
HCT-116	6.0±1.0	>50	>50
A549	4.6±0.3	>50	>50

Table S2. Structure-Activity Relationship of PL-1 analogues.

Cmpd	Structure	IC_{50} on H1975	Cmpd	Structure	IC_{50} on H1975

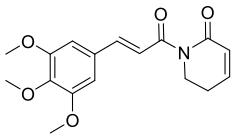
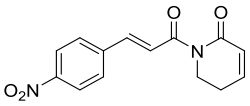
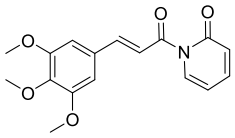
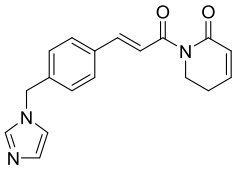
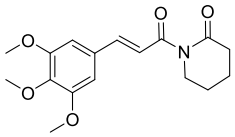
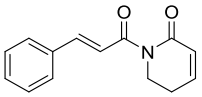
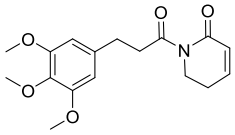
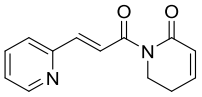
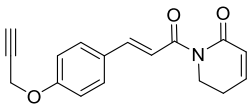
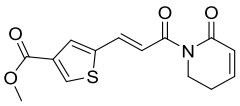
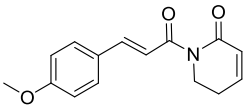
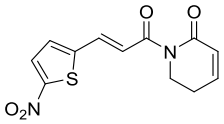
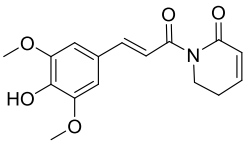
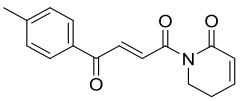
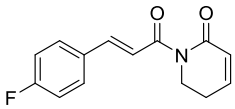
		cell (μM)			cell (μM)
PL-1		2.9 ±0.14	PL-9		1.7 ±0.18
PL-2		>50	PL-10		2.0 ±0.08
PL-3		>50	PL-11		7.6 ±0.38
PL-4		23.0 ±0.39	PL-12		3.6 ±0.25
PL-5		3.1 ±0.31	PL-13		2.6 ±0.23
PL-6		3.8 ±0.74	PL-14		3.6 ±0.35
PL-7		2.5 ±0.42	PL-15		5.2 ±0.33
PL-8		3.8 ±0.08			

Table S3. IC_{50} of PL-5 on different cell lines.

Cell line	IC50 (μM)
NCI-H1975	3.1 ±0.31
Jurkat	1.4 ±0.18
U2OS	4.3 ±0.32

MCF-7	4.3 ±0.54
Hela	3.2 ±0.33
Molt-4	4.2 ±0.21
HCT-116	8.8 ±2.58
A549	5.7 ±0.35

Table S4. Identification of PL-1 covalent targets in four individual tests.

Test1				
Accession number	protein	Mascot score in sample P[a]	Mascot score in sample C[b]	score ratio of P/C
IPI00019755	**GSTO1 Glutathione S-transferase omega-1**	**2730**	**152**	**17.96**
IPI00848226	GNB2L1 Guanine nucleotide-binding protein subunit beta-2-like 1	2324	176	13.20
IPI00025512	HSPB1 Heat shock protein beta-1	1873	459	4.08
IPI00964515	GNB2L1 Protein	1731	152	11.39
IPI00299573	RPL7A;SNORD24 60S ribosomal protein L7a	1697	292	5.81
IPI00025329	RPL19 60S ribosomal protein L19	1607	484	3.32
IPI00018146	YWHAQ 14-3-3 protein theta	1547	179	8.64
IPI00000816	YWHAE Isoform 1 of 14-3-3 protein epsilon	1505	122	12.34
IPI00010896	CLIC1 Chloride intracellular channel protein 1	1142	57	20.04
IPI00895865	ETFA electron transfer flavoprotein subunit alpha, mitochondrial isoform b	1128	321	3.51
IPI00012772	RPL8 60S ribosomal protein L8	1122	287	3.91
IPI01025580	- Possible J 56 gene segment (Fragment)	1038	233	4.45
IPI00291006	MDH2 Malate dehydrogenase, mitochondrial	974	76	12.82
IPI00888712	POTEKP Putative beta-actin-like protein 3	955	54	17.69
IPI00465248	ENO1 Isoform alpha-enolase of Alpha-enolase	954	80	11.93
IPI00219018	GAPDH Glyceraldehyde-3-phosphate dehydrogenase	943	46	20.50
IPI00013485	RPS2 40S ribosomal protein S2	858	78	11.00

IPI00908876	ALB cDNA FLJ50830, highly similar to Serum albumin	553	135	4.10
IPI00218474	ENO3 Isoform 1 of Beta-enolase	399	22	18.14
Test2				
Accession number	protein	Mascot score in sample P[a]	Mascot score in sample C[b]	score ratio of P/C
IPI00010740	SFPQ Isoform Long of Splicing factor, proline- and glutamine-rich	2889	361	8.00
IPI00438229	TRIM28 Isoform 1 of Transcription intermediary factor 1-beta	2714	806	3.37
IPI00302927	CCT4 T-complex protein 1 subunit delta	2483	509	4.88
IPI00019755	**GSTO1 Glutathione S-transferase omega-1**	**2318**	**516**	**4.49**
IPI00215687	GLS Isoform 3 of Glutaminase kidney isoform, mitochondrial	2120	591	3.59
IPI00021812	AHNAK Neuroblast differentiation-associated protein AHNAK	2047	126	16.25
IPI00301154	PABPC3 Polyadenylate-binding protein 3	1722	325	5.30
IPI00644712	XRCC6 X-ray repair cross-complementing protein 6	1711	511	3.35
IPI00179964	PTBP1 Isoform 1 of Polypyrimidine tract-binding protein 1	1686	476	3.54
IPI00031522	HADHA Trifunctional enzyme subunit alpha, mitochondrial	1684	541	3.11
IPI00304925	HSPA1B;HSPA1A Heat shock 70 kDa protein 1A/1B	1663	380	4.38
IPI00026781	FASN Fatty acid synthase	1606	430	3.73
IPI00295400	WARS Isoform 1 of Tryptophanyl-tRNA synthetase, cytoplasmic	1538	74	20.78
IPI00465248	ENO1 Isoform alpha-enolase of Alpha-enolase	1442	216	6.68
IPI00215637	DDX3X ATP-dependent RNA helicase DDX3X	1257	315	3.99
IPI00291510	IMPDH2 Inosine-5'-monophosphate dehydrogenase 2	1247	261	4.78
IPI00012726	PABPC4 Isoform 1 of Polyadenylate-binding protein 4	1077	97	11.10

IPI00015911	DLD Dihydrolipoyl dehydrogenase, mitochondrial	1014	51	19.88
IPI00012442	G3BP1 Ras GTPase-activating protein-binding protein 1	972	236	4.12
IPI00171903	HNRNPM Isoform 1 of Heterogeneous nuclear ribonucleoprotein M	913	46	19.85
IPI00002520	SHMT2 Serine hydroxymethyltransferase, mitochondrial	827	262	3.16
IPI00008943	DDX19B Isoform 1 of ATP-dependent RNA helicase DDX19B	761	92	8.27
IPI00168184	PPP2R1A cDNA FLJ56053, highly similar to Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	672	39	17.23
IPI00026781	FASN Fatty acid synthase	576	47	12.26
IPI00221354	FUS Isoform Short of RNA-binding protein FUS	561	127	4.42
IPI00025874	RPN1 Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 precursor	501	103	4.86
IPI00171199	PSMA3 Isoform 2 of Proteasome subunit alpha type-3	484	161	3.01
IPI00018272	PNPO Pyridoxine-5'-phosphate oxidase	463	76	6.09
IPI00218342	MTHFD1 C-1-tetrahydrofolate synthase, cytoplasmic	302	66	4.58

Test3

Accession number	protein	Mascot score in sample P[a]	Mascot score in sample C[b]	score ratio of P/C
IPI00217966	LDHA Isoform 1 of L-lactate dehydrogenase A chain	1612	0	-
IPI00019755	**GSTO1 Glutathione S-transferase omega-1**	**1131**	**0**	-
IPI00440493	ATP5A1 ATP synthase subunit alpha, mitochondrial	956	270	3.54
IPI00169383	PGK1 Phosphoglycerate kinase 1	488	51	9.57
IPI01026065	AHSA1 23 kDa protein	388	30	12.93
IPI00444262	NCL cDNA FLJ45706 fis, clone FEBRA2028457, highly similar to Nucleolin	334	30	11.13
IPI00014424	EEF1A2 Elongation factor 1-alpha 2	317	0	-

IPI00479217	HNRNPU Isoform Short of Heterogeneous nuclear ribonucleoprotein U	311	0	-
IPI00217469	HIST1H1A Histone H1.1	301	96	3.14
IPI00179964	PTBP1 Isoform 1 of Polypyrimidine tract-binding protein 1	297	62	4.79
IPI00744115	PCCA Isoform 1 of Propionyl-CoA carboxylase alpha chain, mitochondrial	246	49	5.02
Test4				
Accession number	protein	Mascot score in sample P[a]	Mascot score in sample C[b]	score ratio of P/C
IPI00019755	**GSTO1 Glutathione S-transferase omega-1**	**2840**	**493**	**5.76**
IPI00009104	RUVBL2 RuvB-like 2	1222	358	3.41
IPI00420108	DLST;DLSTP1 Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	1108	100	11.08
IPI00250153	YBX2 Y-box-binding protein 2	771	220	3.50
IPI00013933	DSP Isoform DPI of Desmoplakin	588	70	8.40
IPI00023860	NAP1L1 Nucleosome assembly protein 1-like 1	472	150	3.15
IPI00032449	ASPH Isoform Junctate of Aspartyl/asparaginyl beta-hydroxylase	466	150	3.11
IPI01012993	EIF4A1 cDNA FLJ58012, moderately similar to Eukaryotic initiation factor 4A-I	443	122	3.63
IPI00022974	PIP Prolactin-inducible protein	400	30	13.33
IPI00025753	DSG1 Desmoglein-1	306	30	10.20
IPI00014263	EIF4H Isoform Long of Eukaryotic translation initiation factor 4H	301	50	6.02
IPI00411680	PCMT1 Isoform 1 of Protein-L-isoaspartate(D-aspartate) O-methyltransferase	274	27	10.15
Note: a, sample P means the sample in which cells are treated with probe PL-5; b, sample C means the sample in which cells are treated with probe PL-5 and competitor molecule PL-1. All hits that have Mascot score above 200 in sample P and have the P/C score ratio over 3 are listed.				

Table S5. IC_{50} of KT45 on different cell lines.

Cell line	IC50 (μM)
NCI-H1975	1.3±0.08
Jurkat	0.62±0.05
U2OS	1.4±0.10
MCF-7	4.0±0.29
Hela	1.0±0.13
Molt-4	0.6±0.15
HCT-116	5.3±0.74
A549	7.06±1.27

Table S6. Screen library for synergism effect.

Cmpd	Target	Cmpd	Target	Cmpd	Target
Finasteride	5-alpha Reductase	Mdivi-1	Dynamin	JSH-23	NF-κB
Dutasteride	5-alpha Reductase	ID-8	DYRK	Andrographolide	NF-κB
Lamotrigine	5-HT Receptor	BAY 11-7082	E2 conjugating,IκB/IKK	Curcumin	NF-κB,Histone Acetyltransferase, Nrf2
Mirabegron	Adrenergic Receptor	Nutlin-3	E3 Ligase ,Mdm2	Oltipraz	Nrf2
Epinephrine bitartrate	Adrenergic Receptor	RITA	E3 Ligase ,p53	Naloxone HCl	Opioid Receptor
StemRegenin 1	AhR	Tenovin-1	E3 Ligase ,p53	(+)-Matrine	Opioid Receptor
Triciribine	Akt	JNJ-26854165	E3 Ligase ,p53	Mesna	Others
Uprosertib	Akt	Thalidomide	E3 Ligase ,TNF-alpha	Procodazole	Others
Afuresertib	Akt	Erlotinib HCl	EGFR	Carbazochrome sodium sulfonate	Others
MK-2206 2HCl	Akt	Gefitinib	EGFR	Uracil	Others
GSK690693	Akt	Pelitinib	EGFR	Mitotane	Others
CCT128930	Akt	Varlitinib	EGFR	Noscapine HCl	Others
TIC10 Analogue	Akt	Olmutinib	EGFR	Pimecrolimus	Others
Ceritinib	ALK	Erlotinib	EGFR	TH-302	Others
ALK-IN-1	ALK	Osimertinib	EGFR	Bindarit	Others
AZD3463	ALK	Rociletinib	EGFR	CB1954	Others
Crizotinib	ALK,c-Met	Dacomitinib	EGFR	Silibinin	Others
Phenformin HCl	AMPK	Poziotinib	EGFR	Urethane	Others
A-769662	AMPK	WZ4002	EGFR	Leucovorin Calcium Pentahydrate	Others

AICAR	AMPK	OSI-420	EGFR	Febuxostat	Others
Flutamide	Androgen Receptor	AG-1478	EGFR	Dimesna	Others
Enzalutamide	Androgen Receptor	CUDC-101	EGFR,HDAC,HER2	Ezetimibe	Others
Bicalutamide	Androgen Receptor	Afatinib	EGFR,HER2	Lonidamine	Others
MK-2866	Androgen Receptor	Lapatinib Ditosylate	EGFR,HER2	Alendronate sodium trihydrate	Others
Andarine	Androgen Receptor	Afatinib Dimaleate	EGFR,HER2	Calcium Levofolinate	Others
Galeterone	Androgen Receptor,P450	Neratinib	EGFR,HER2	PFK15	Others
Moroxydine HCl	Antifection	Genistein	EGFR,Topoisomerase	DDR1-IN-1	Others
Lincomycin HCl	Antifection	Zibotentan	Endothelin Receptor	DASA-58	Others
Clorsulon	Antifection	I-BET-762	Epigenetic Reader Domain	GSK650394	Others
Artemether	Antifection	UNC1215	Epigenetic Reader Domain	Danthron	Others
Famciclovir	Antifection	(+)-JQ1	Epigenetic Reader Domain	4E1RCat	Others
Tolnaftate	Antifection	GSK2801	Epigenetic Reader Domain	10-Deacetylbaccatin-III	Others
Sitafloxacin Hydrate	Antifection	Ulixertinib	ERK	SRPIN340	Others
Methacycline HCl	Antifection	XMD8-92	ERK	RI-1	Others
Sulfabenzamide	Antifection	ERK5-IN-1	ERK	PTC-209	Others
Oleanolic Acid	Antifection	FR 180204	ERK	Malotilate	Others
Formestane	Aromatase	Tamoxifen	Estrogen/progestogen Receptor	CB1954	Others
Anastrozole	Aromatase	Clomifene citrate	Estrogen/progestogen Receptor	Cytidine	Others
Exemestane	Aromatase	Diethylstilbestrol	Estrogen/progestogen Receptor	Formononetin	Others
KU-55933	ATM/ATR	Fulvestrant	Estrogen/progestogen Receptor	Oridonin	Others
KU-60019	ATM/ATR	Toremifene Citrate	Estrogen/progestogen Receptor	Isoliquiritigenin	Others
VE-822	ATM/ATR	Raloxifene HCl	Estrogen/progestogen Receptor	Ursolic Acid	Others
CGK 733	ATM/ATR	Estrone	Estrogen/progestogen Receptor	Hesperidin	Others
AZ20	ATM/ATR	Estradiol	Estrogen/progestogen Receptor	Orotic acid	Others
Esomeprazole sodium	ATPase	Defactinib	FAK	LY2228820	p38 MAPK
BTB06584	ATPase	PF-00562271	FAK	SB203580	p38 MAPK
Tozasertib	Aurora Kinase	TAE226	FAK	SB202190	p38 MAPK
Alisertib	Aurora Kinase	PF-573228	FAK	Doramapimod	p38 MAPK
Barasertib	Aurora Kinase	Erastin	Ferroptosis	Pexmetinib	p38 MAPK,Tie-2
MLN8054	Aurora Kinase	SSR128129E	FGFR	Itraconazole	P450 (e.g. CYP17)
ZM 447439	Aurora Kinase	AZD4547	FGFR	TAK-700	P450 (e.g. CYP17)
Danusertib	Aurora Kinase,Bcr-Abl,c-RET,FGFR	Orantinib	FGFR,PDGFR,VEGFR	Cobicistat	P450 (e.g. CYP17)
Azithromycin	Autophagy	Brivanib	FGFR,VEGFR	Piperine	P450 (e.g. CYP17)
Spautin-1	Autophagy	Quizartinib	FLT3	NSC 319726	p53
ABT-737	Bcl-2	G-749	FLT3	NMS-873	p97
Navitoclax	Bcl-2	TCS 359	FLT3	IPA-3	PAK
Venetoclax	Bcl-2	Dovitinib Lactate	FLT3,c-Kit,FGFR,PDGFR,VEGFR	PF-3758309	PAK
HA14-1	Bcl-2	Pacritinib	FLT3,JAK	Olaparib	PARP

Gambogic Acid	Bcl-2,Caspase	GW4064	FXR	Veliparib	PARP
Nilotinib	Bcr-Abl	Valproic acid sodium salt	GABA Receptor,HDAC,Autophagy	Iniparib	PARP
Radotinib	Bcr-Abl	Cortisone acetate	Glucocorticoid Receptor	Rucaparib phosphate	PARP
Bafetinib	Bcr-Abl	Prednisone	Glucocorticoid Receptor	Anagrelide HCl	PDE
Rebastinib	Bcr-Abl	Triamcinolone Acetonide	Glucocorticoid Receptor	Crenolanib	PDGFR
GNF-2	Bcr-Abl	Meprednisone	Glucocorticoid Receptor	Imatinib	PDGFR
Imatinib Mesylate	Bcr-Abl,c-Kit,PDGFR	STF-31	GLUT1	CP-673451	PDGFR
Dasatinib	Bcr-Abl,c-Kit,Src	SB216763	GSK-3	Sunitinib	PDGFR,c-Kit,VEGFR
Ponatinib	Bcr-Abl,FGFR,PDGFR,VEGFR	Indirubin	GSK-3	Sorafenib Tosylate	PDGFR,Raf,VEGFR
Saracatinib	Bcr-Abl,Src	CHIR-99021 HCl	GSK-3	BX-912	PDK
Avagacestat	Beta Amyloid, Gamma-secretase	TDZD-8	GSK-3	GSK2334470	PDK
Ibrutinib	BTK	Daclatasvir	HCV Protease	Zosuquidar 3HCl	P-gp
CC-292	BTK	Vorinostat	HDAC	Elacridar	P-gp
CNX-774	BTK	Sodium Phenylbutyrate	HDAC	Sal003	Phosphatase
Nilvadipine	Calcium Channel	Pracinostat	HDAC	Quinacrine 2HCl	Phospholipase
Flunarizine 2HCl	Calcium Channel	Belinostat	HDAC	Tanshinone I	Phospholipase
SKF96365	Calcium Channel	Panobinostat	HDAC	LY294002	PI3K
Bithionol	cAMP	Mocetinostat	HDAC	3-Methyladenine	PI3K
ESI-09	cAMP	Ricolinostat	HDAC	Pictilisib	PI3K
Silmitasertib	Casein Kinase	Entinostat	HDAC	Idelalisib	PI3K
D 4476	Casein Kinase	Abexinostat	HDAC	Alpelisib	PI3K
IC261	Casein kinase	Quisinostat 2HCl	HDAC	Duvelisib	PI3K
Tasisulam	Caspase	Resminostat	HDAC	Pilaralisib	PI3K
Apoptosis Activator 2	Caspase	Dacinostat	HDAC	YM201636	PI3K
Maraviroc	CCR	MC1568	HDAC	TG100-115	PI3K
Flavopiridol	CDK	Mocetinostat	HDAC	AZD6482	PI3K
abemaciclib	CDK	Taladegib	Hedgehog,Hedgehog/Smoothened	Idelalisib	PI3K
Palbociclib HCl	CDK	Erismodegib	Hedgehog/Smoothened	GSK2636771	PI3K
Dinaciclib	CDK	Vismodegib	Hedgehog/Smoothened	SGI-1776 free base	Pim
Roscovitine	CDK	BMS-833923	Hedgehog/Smoothened	CX-6258 HCl	Pim
Ribociclib	CDK	Mubritinib	HER2	AZD1208	Pim
Palbociclib Isethionate	CDK	2-Methoxyestradiol	HIF	Enzastaurin	PKC
SNS-032	CDK	Roxadustat	HIF	Sotrastaurin	PKC
Milciclib	CDK	Bepotastine Besilate	Histamine Receptor	Rigosertib	PLK
abemaciclib	CDK	Cimetidine	Histamine Receptor	Volasertib	PLK
Evacetrapib	CETP	C646	Histone Acetyltransferase	BI 2536	PLK
VX-661	CFTR	SP2509	Histone Demethylase	GSK461364	PLK
AZD7762	Chk	Tazemetostat	Histone Methyltransferase	HMN-214	PLK
LY2603618	Chk	Atazanavir Sulfate	HIV Protease	Tolbutamide	Potassium Channel
CHIR-124	Chk	Limonin	HIV Protease	Pioglitazone	PPAR
Dovitinib	c-Kit,FGFR,FLT3,PDGFR,VEGFR	Fluvastatin Sodium	HMG-CoA Reductase	Rosiglitazone	PPAR

Dovitinib Dilactic Acid	c-Kit,FGFR,FLT3,PDGFR,VEGFR	Simvastatin	HMG-CoA Reductase	Ciprofibrate	PPAR
Amuvatinib	c-Kit,FLT3,PDGFR	Mevastatin	HMG-CoA Reductase	FH535	PPAR,Wnt/beta-catenin
Masitinib	c-Kit,PDGFR	Tanespimycin	HSP (e.g. HSP90)	Ixazomib	Proteasome
Axitinib	c-Kit,PDGFR,VEGFR	Ganetespib	HSP (e.g. HSP90)	Delanzomib	Proteasome
Pazopanib HCl	c-Kit,PDGFR,VEGFR	Onalespib	HSP (e.g. HSP90)	Carfilzomib	Proteasome
Sunitinib Malate	c-Kit,PDGFR,VEGFR	Isotretinoin	Hydroxylase	Temocapril HCl	RAAS
Tivozanib	c-Kit,PDGFR,VEGFR	DMOG	Hydroxylase	Sorafenib	Raf
Vatalanib 2HCl	c-Kit,VEGFR	Birinapant	IAP	Dabrafenib	Raf
Tivantinib	c-Met	LCL161	IAP	Vemurafenib	Raf
Tepotinib	c-Met	Birinapant	IAP	GW5074	Raf
PHA-665752	c-Met	Epacadostat	IDO	MLN2480	Raf
SU11274	c-Met	INCB024360 analogue	IDO	Tamibarotene	Retinoid Receptor
Foretinib	c-Met,VEGFR	Linsitinib	IGF-1R	Tretinoin	Retinoid Receptor
Golvatinib	c-Met,VEGFR	BMS-536924	IGF-1R	Bexarotene	Retinoid Receptor
10058-F4	c-Myc	GSK1904529A	IGF-1R	Salirasib	Rho
Sulindac	COX	Dexamethasone	IL Receptor	Azathioprine	Rho
Aspirin	COX	Dexamethasone Acetate	IL Receptor,Autophagy	ZCL278	Rho
Phenylbutazone	COX	Imiquimod	Immunology & Inflammation related	EHop-016	Rho
Celecoxib	COX	Geniposidic acid	Immunology & Inflammation related	Y-27632 2HCl	ROCK
Vitamin E	COX,VEGFR	Cilengitide trifluoroacetate	Integrin	Fasudil HCl	ROCK
TG101209	c-RET,FLT3,JAK	TPCA-1	IκB/IKK	GSK429286A	ROCK
Regorafenib	c-RET,VEGFR	IMD 0354	IκB/IKK	RKI-1447	ROCK
Selinexor	CRM1	SC-514	IκB/IKK	Thiazovivin	ROCK
BLZ945	CSF-1R	BX-795	IκB/IKK,PDK	SKI II	S1P Receptor
Pexidartinib	CSF-1R,c-Kit	Gandotinib	JAK	PF-543	S1P Receptor
Linifanib	CSF-1R,PDGFR,VEGFR	S-Ruxolitinib	JAK	PF-4708671	S6 Kinase
CEP-32496	CSF-1R,Raf	Tofacitinib Citrate	JAK	BI-D1870	S6 Kinase
Disulfiram	Dehydrogenase	Ruxolitinib	JAK	LY2584702 Tosylate	S6 Kinase
Gimeracil	Dehydrogenase	Fedratinib	JAK	Canagliflozin	SGLT
Leflunomide	Dehydrogenase	AZD1480	JAK	Dapagliflozin	SGLT
Mycophenolate Mofetil	Dehydrogenase	Momelotinib	JAK	Phloretin	SGLT
Enasidenib	Dehydrogenase	SP600125	JNK	SRT1720 HCl	Sirtuin
Emodin	Dehydrogenase	JNK-IN-8	JNK	Selisistat	Sirtuin
Methotrexate	DHFR	JNK Inhibitor IX	JNK	Bosutinib	Src
Pemetrexed Disodium Hydrate	DHFR	Ispinesib	Kinesin	KX2-391	Src
Pralatrexate	DHFR	SB743921 HCl	Kinesin	PP2	Src
Pemetrexed	DHFR,DNA/RNA Synthesis	Zileuton	Lipoxygenase	PP1	Src
Altretamine	DNA alkylator	MI-2	MALT	Dasatinib Monohydrate	Src,c-Kit,Bcr-Abl
Cyclophosphamide Monohydrate	DNA alkylator	Idasanutlin	Mdm2	Napabucasin	STAT

Streptozotocin	DNA alkylator	Selumetinib	MEK	S3I-201	STAT
Busulfan	DNA alkylator	Pimasertib	MEK	Aprepitant	Substance P
Azacitidine	DNA Methyltransferase	Trametinib	MEK	YM155	Survivin
Azacitidine	DNA Methyltransferase	Cobimetinib	MEK	Entospletinib	Syk
Decitabine	DNA Methyltransferase	Binimetinib	MEK	R406	Syk
SGI-1027	DNA Methyltransferase	PD184352	MEK	Piceatannol	Syk
Gemcitabine HCl	DNA/RNA Synthesis	PD0325901	MEK	Cabozantinib	TAM Receptor,c-Kit,c-Met,FLT3,Tie-2,VEGFR
Gemcitabine	DNA/RNA Synthesis	SL-327	MEK	BMS-777607	TAM Receptor,c-Met
Mercaptopurine	DNA/RNA Synthesis	Vincristine sulfate	Microtubule Associated	Cabozantinib malate	TAM Receptor,VEGFR
Clofarabine	DNA/RNA Synthesis	Paclitaxel	Microtubule Associated	BIBR 1532	Telomerase
Capecitabine	DNA/RNA Synthesis	Docetaxel Trihydrate	Microtubule Associated	Galunisertib	TGF-beta/Smad
Oxaliplatin	DNA/RNA Synthesis	Docetaxel	Microtubule Associated	GW788388	TGF-beta/Smad
Procarbazine HCl	DNA/RNA Synthesis	Vinorelbine Tartrate	Microtubule Associated	SB431542	TGF-beta/Smad
Cytarabine	DNA/RNA Synthesis	Patupilone	Microtubule Associated	PX-12	Thioredoxin
Temozolomide	DNA/RNA Synthesis	Fosbretabulin Disodium	Microtubule Associated	Eltrombopag Olamine	Thrombin
Lomustine	DNA/RNA Synthesis	ABT-751	Microtubule Associated	Tie2 kinase inhibitor	Tie-2
Floxuridine	DNA/RNA Synthesis	Lexibulin	Microtubule Associated	Motolimod	TLR
Carmofur	DNA/RNA Synthesis	Cabazitaxel	Microtubule Associated	Pomalidomide	TNF-alpha
Ifosfamide	DNA/RNA Synthesis	Vinblastine sulfate	Microtubule Associated,AChR	Lenalidomide	TNF-alpha
Carboplatin	DNA/RNA Synthesis	Batimastat	MMP	Necrostatin-1	TNF-alpha
Hydroxyurea	DNA/RNA Synthesis	Marimastat	MMP	Doxorubicin HCl	Topoisomerase
Fluorouracil	DNA/RNA Synthesis	Ilomastat	MMP	Topotecan HCl	Topoisomerase
Chloroambucil	DNA/RNA Synthesis	SB-3CT	MMP	Etoposide	Topoisomerase
Bleomycin Sulfate	DNA/RNA Synthesis	Nobiletin	MMP	Epirubicin HCl	Topoisomerase
Bendamustine HCl	DNA/RNA Synthesis	Ramelteon	MT Receptor	Daunorubicin HCl	Topoisomerase
Tegafur	DNA/RNA Synthesis	TH588	MTH1	Irinotecan	Topoisomerase
Nelarabine	DNA/RNA Synthesis	TH287	MTH1	Teniposide	Topoisomerase
Fludarabine Phosphate	DNA/RNA Synthesis	Torkinib	mTOR	Amonafide	Topoisomerase
Dacarbazine	DNA/RNA Synthesis	Rapamycin	mTOR	Ellagic acid	Topoisomerase
Raltitrexed	DNA/RNA Synthesis	Temsirolimus	mTOR	Lonafarnib	Transferase
Cladribine	DNA/RNA Synthesis	Everolimus	mTOR	GW441756	Trk receptor
6-Mercaptopurine Monohydrate	DNA/RNA Synthesis	Tacrolimus	mTOR	Entrectinib	Trk receptor,ALK
Nedaplatin	DNA/RNA Synthesis	Ridaforolimus	mTOR	Plinabulin	VDA
Fludarabine	DNA/RNA Synthesis,STAT	KU-0063794	mTOR	Vandetanib	VEGFR
PP121	DNA-PK,mTOR,PDGFR	WYE-354	mTOR	Cediranib	VEGFR
PI-103	DNA-PK,mTOR,PI3K	Voxtalisib Analogue	mTOR,PI3K	Apatinib	VEGFR
PIK-75 HCl	DNA-PK,PI3K	Voxtalisib	mTOR,PI3K	Lenvatinib	VEGFR
NU7441	DNA-PK,PI3K	Omipalisib	mTOR,PI3K	Doxercalciferol	Vitamin
Amisulpride	Dopamine Receptor	Apitolisib	mTOR,PI3K	Vitamin D3	Vitamin
Linagliptin	DPP-4	GMX1778	NAMPT	MK-1775	Wee1
b-AP15	DUB	Triptolide	NF-κB	XAV-939	Wnt/beta-catenin
PR-619	DUB				

Table S7. Synergistic effects of PL-1 on different cell lines.

Cell line	NCI-H1975		Jurkat		HCT-116	
Tested cmpds[a]	540		540		540	
Active cmpds	86		164		52	
Combination mode[b]	number	% of active cmpds	number	% of active cmpds	number	% of active cmpds
strong synergism	10	12.2	25	15.2	0	0
synergism	32	36.6	34	21.7	13	25
Moderate synergism	2	2.4	7	4.3	2	3.5
Slight synergism	1	1.2	2	1.2	5	9.6
Total synergism	45	52.3	68	41.5	20	38.4

[a] cmpds is short for compounds. [b] Combination mode was based on CI values, which were calculated using CompuSyn software. Strong synergism: CI<0.3; synergism: 0.3<CI<0.7; moderate synergism: 0.7<CI<0.8; slight synergism: 0.8<CI<0.9.

Table S8. Detailed list of screen hits that have synergism effect with PL-1 in NCI-H1975 cell line.

Synergism mode	Target	Compound	Best CI
strong synergism	EGFR,HER2	Lapatinib Ditosylate	0.16
	STAT	Napabucasin	0.16
	Microtubule Associated	Vinorelbine Tartrate	0.2
	Histone Demethylase	SP2509	0.2
	CRM1	Selinexor	0.21
	Autophagy,Bcl-2	ABT-737	0.24
	Microtubule Associated	Lexibulin	0.25
	PI3K	Pictilisib	0.26
	c-Kit,FGFR,FLT3,PDGFR,VEGFR	Dovitinib	0.27
	Casein Kinase	D 4476	0.3
Synergism	EGFR	Poziotinib	0.33
	PI3K	Duvelisib	0.34

	Akt	MK-2206 2HCl	0.34
	HER2	Mubritinib	0.37
	Autophagy,DNA-PK,mTOR,PI3K	PI-103	0.38
	Akt	Uprosertib	0.4
	Autophagy,mTOR	Rapamycin	0.4
	c-Met	SU11274	0.42
	DNA-PK,PI3K	NU7441	0.42
	EGFR,Topoisomerase	Genistein	0.44
	Bcl-2	Venetoclax	0.45
	Casein Kinase	Silmitasertib	0.47
	DNA alkylator	Busulfan	0.48
	IGF-1R	BMS-536924	0.48
	Phospholipase (e.g. PLA)	Tanshinone I	0.49
	Autophagy,HDAC	Vorinostat	0.5
	PI3K	Alpelisib	0.5
	Bcr-Abl,Src	Saracatinib	0.53
	PI3K	YM201636	0.53
	E3 Ligase ,Mdm2	Nutlin-3	0.55
	HDAC	Quisinostat 2HCl	0.55
	EGFR	Erlotinib	0.58
	p97	NMS-873	0.59
	Microtubule Associated	Vinblastine sulfate	0.6
	mTOR	Tacrolimus	0.6
	Estrogen/progestogen Receptor	Fulvestrant	0.61
	c-Met	PHA-665752	0.61
	HMG-CoA Reductase	Mevastatin	0.64
	c-Met,VEGFR	Golvatinib	0.65
	DUB	b-AP15	0.65
	PLK	BI 2536	0.66
	Kinesin	Ispinesib	0.69

moderate synergism	E3 Ligase ,p53	RITA	0.73
	mTOR	WYE-354	0.78
slight synergism	Hedgehog/Smoothened	BMS-833923	0.86

Note: compounds synergism effect are tested with PL concentration at 3 and 1 μM and compound concentration at 10, 1 and 0.1μM.

Table S9. Detailed list of screen hits that have synergism effect with PL-1 in Jurkat cell line.

Synergism mode	Target	Compound	Best CI
strong synergism	mTOR,PI3K	Omipalisib	0.05
	DNA-PK,PI3K	NU7441	0.06
	Akt	GSK690693	0.08
	Autophagy,mTOR	Rapamycin	0.09
	Epigenetic Reader Domain	(+)-JQ1	0.12
	Pim	AZD1208	0.13
	Autophagy,DNA-PK,mTOR,PI3K	PI-103	0.14
	Ferroptosis	Erastin	0.14
	ATM/ATR	AZ20	0.16
	mTOR	WYE-354	0.16
	Autophagy,Bcl-2	ABT-737	0.17
	PI3K	YM201636	0.17
	Raf	GW5074	0.17
	MALT	MI-2	0.18
	PI3K	Pictilisib	0.18
	Autophagy,ROCK	Y-27632 2HCl	0.19
	DNA/RNA Synthesis	Lomustine	0.2
	p97	NMS-873	0.22
	PAK	PF-3758309	0.24
	p38 MAPK,Tie-2	Pexmetinib	0.25
	CDK	Milciclib	0.26
	CDK	Roscovitine	0.28
	mTOR	Tacrolimus	0.28

	Autophagy,mTOR	Torkinib	0.29
	Topoisomerase	Teniposide	0.29
Synergism	DNA/RNA Synthesis	Fludarabine Phosphate	0.32
	Dehydrogenase	Enasidenib	0.34
	PDK	GSK2334470	0.34
	Akt	Uprosertib	0.36
	P-gp	Zosuquidar 3HCl	0.36
	Survivin	YM155	0.36
	ERK	XMD8-92	0.38
	Chk	LY2603618	0.39
	PPAR	Pioglitazone	0.39
	CDK	SNS-032	0.4
	CDK	Flavopiridol	0.4
	Topoisomerase	Etoposide	0.41
	Bcl-2	Navitoclax	0.42
	Autophagy,Topoisomerase	Doxorubicin HCl	0.44
	Akt	CCT128930	0.45
	Bcl-2	Venetoclax	0.46
	Bcr-Abl	Rebastinib	0.46
	Aurora Kinase,Bcr-Abl,c-RET,FGFR	Danusertib	0.47
	Nrf2	Oltipraz	0.47
	Casein Kinase	Silmitasertib	0.49
	STAT	Napabucasin	0.51
	mTOR	Everolimus	0.52
	Autophagy,ROCK	Fasudil HCl	0.54
	FAK	PF-00562271	0.54
	HDAC	Abexinostat	0.54
	JAK	Gandotinib	0.55
	JAK	Fedratinib	0.55
	ATM/ATR	KU-60019	0.57

	Proteasome	Ixazomib	0.57
	BTK	CC-292	0.6
	S6 Kinase	BI-D1870	0.61
	EGFR,HER2	Neratinib	0.63
	HDAC	Resminostat	0.64
	Hedgehog,Hedgehog/Smoothened	Taladegib	0.7
moderate synergism	EGFR,HDAC,HER2	CUDC-101	0.71
	GSK-3	CHIR-99021 HCl	0.74
	PLK	Volasertib	0.74
	EGFR	Pelitinib	0.79
	Estrogen/progestogen Receptor	Tamoxifen	0.79
	HDAC	Mocetinostat	0.79
	ATM/ATR	KU-55933	0.8
slight synergism	S6 Kinase	LY2584702 Tosylate	0.85
	DNA/RNA Synthesis	Cladribine	0.9

note: compounds synergism effect are tested with PL concentration at 3 and 1 μM and compound concentration at 10, 1 and 0.1μM.

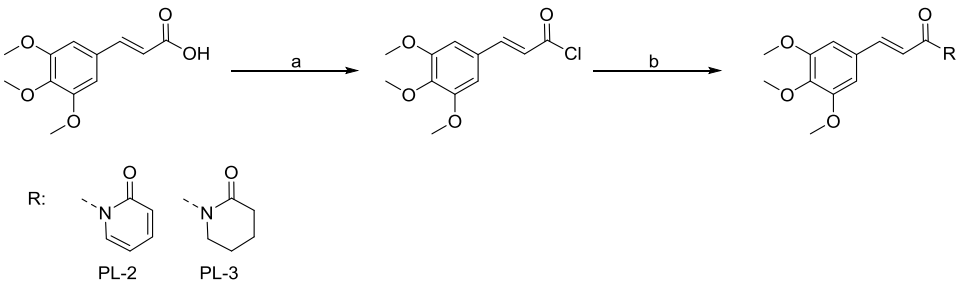
Table S10. Detailed list of screen hits that have synergism effect with PL-1 in HCT-116 cell line.

Synergism mode	Target	compound	best CI
Synergism	Proteasome	Delanzomib	0.41
	Epigenetic Reader Domain	I-BET-762	0.48
	HSP (e.g. HSP90)	Ganetespib	0.49
	PI3K	Pictilisib	0.49
	Casein Kinase	Silmitasertib	0.5
	p38 MAPK	SB202190	0.53
	Autophagy,EGFR	Erlotinib HCl	0.56
	Bcl-2	Venetoclax	0.56
	TNF-alpha	Lenalidomide	0.56
	Akt	Triciribine	0.62

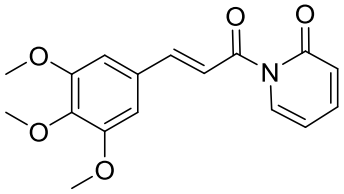
	HSP (e.g. HSP90)	Tanespimycin	0.62
	PI3K	YM201636	0.66
	p53	NSC 319726	0.67
moderate synergism	CRM1	Selinexor	0.75
	DNA Methyltransferase	Decitabine	0.78
slight synergism	Histone Demethylase	SP2509	0.83
	Autophagy,Bcl-2	ABT-737	0.86
	Proteasome	Carfilzomib	0.88
	Autophagy,mTOR	Torkinib	0.89
	HDAC	Dacinostat	0.89

note: compounds synergism effect are tested with PL concentration at 3 and 1 μM and compound concentration at 10, 1 and 0.1μM.

Synthetic Procedures

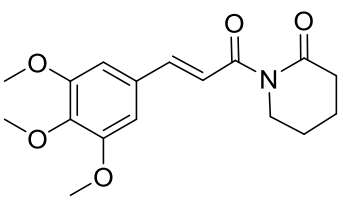


Scheme S1. Synthesis route for **PL-2** and **PL-3**. Reagents and conditions: (a) $(COCl)_2$, DMF, DCM, 0℃ to room temperature (rt), 0.5 hr; (b) TEA, THF, R-H, 0℃ to rt, 3 hr.



(E)-1-(3-(3,4,5-trimethoxyphenyl)acryloyl)pyridin-2(1H)-one (**PL-2**)

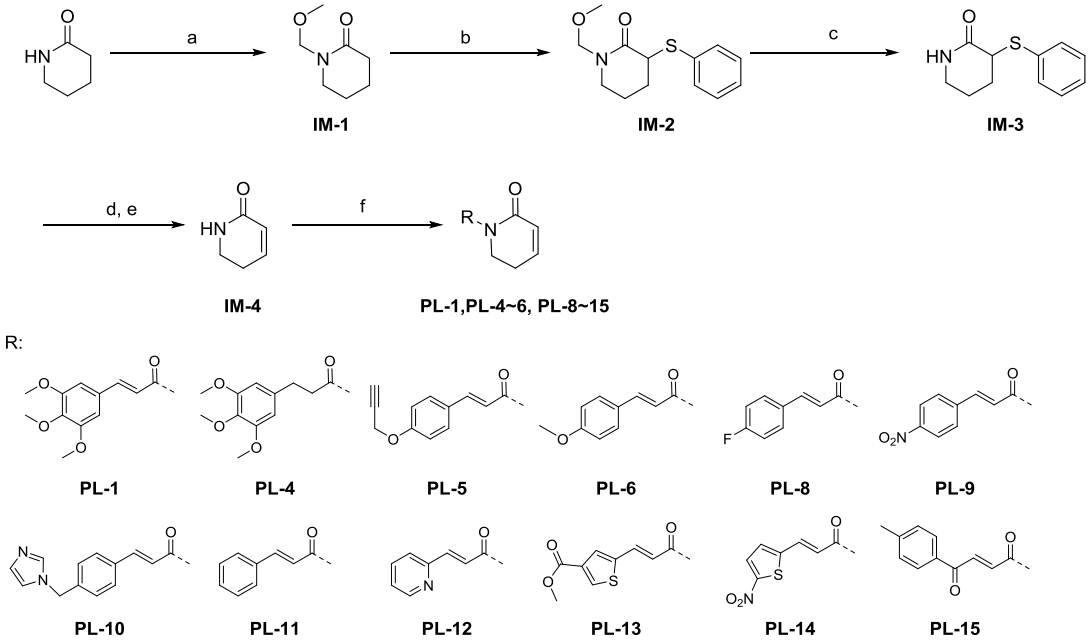
To a solution of (E)-3-(3,4,5-trimethoxyphenyl)acrylic acid (100mg, 0.42mmol) in anhydrous DCM was added $(COCl)_2$ (212mg, 1.68mmol) dropwise and catalytic amount of DMF (0.3mg, 0.004mmol), and the reaction mixture was stirred at 0℃ to rt for 30min. The organic solvent was evaporated in vacuo, and the residue was redissolved in anhydrous THF for further usage without purification. Then TEA (78mg, 0.76mmol) and pyridin-2(1H)-one (38mg, 0.38mmol) were added to the THF solution at 0℃ and the reaction mixture was stirred for another 3hr. The reaction mixture was quenched by NH_4Cl (aq), extracted by EtOAc/H_2O (30mL/30mL) for three times. The EtOAc layer was combined, washed with brine, dried over Na_2SO_4, concentrated and further purified by silica gel column chromatography (PE/EA=2/1), to give (E)-1-(3-(3,4,5-trimethoxyphenyl)acryloyl)pyridin-2(1H)-one (PL-2) as a white solid in 53% yield. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 8.45(d, J=4.04Hz, 1H), 7.84(d, J=15.88Hz, 1H), 7.81-7.86(m, 1H), 7.24-7.28(m, 1H), 7.21(d, J=4.34Hz, 1H), 6.82(s, 2H), 6.55(d, J=15.88Hz, 1H), 3.91(s, 6H), 3.90(s, 3H). ^{13}C NMR (400 MHz, CDCl3-d_6): δ 164.89, 158.20, 153.69, 148.78, 147.39, 140.73, 139.70, 129.61, 122.21, 116.64, 116.24, 105.61, 61.14, 56.37.



(E)-1-(3-(3,4,5-trimethoxyphenyl)acryloyl)piperidin-2-one (**PL-3**)

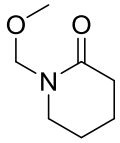
The titled compound was prepared in 59% yield as a white solid from (E)-3-(3,4,5-trimethoxyphenyl)acrylic acid (100mg, 0.42mmol) and piperidin-2-one (38mg, 0.38mmol) according to the procedure for **PL-2**. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 7.64(d, J=15.52Hz, 1H), 7.36(d, J=15.52Hz, 1H),

6.79(s, 2H), 3.89(s, 6H), 3.88(s, 3H), 3.79-3.83(m, 2H), 2.59-2.61(m, 2H), 1.87-1.91(m, 4H). ^{13}C NMR (400 MHz, CDCl3-d_6): δ 174.06, 169.83, 153.40, 143.68, 140.13, 130.87, 121.48, 105.58, 61.19, 56.36, 44.89, 35.14, 22.78, 20.85. HRMS (ESI): calculated for [$C_{17}H_{22}NO_5$+], 320.14925, found 320.15094.



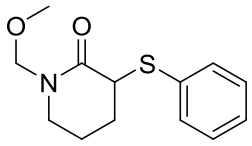
Scheme S2. General synthesis route for **PL-1**, **PL-4~6**, and **PL-8~15**. Reagents and conditions: (a) n-BuLi, CH_3OCH_2Cl, THF, 0℃, 3 hr. (b) LDA, PhSSPh, HMPA, THF, -78℃, overnight. (c) HCl, EtOH, 80℃, 6 hr. (d) mCPBA, DCM, 0℃ to rt, 2 hr. (e) toluene, 120℃, 1 hr. (f) R-OH, $(COCl)_2$, DMF, DCM, 0℃ to room temperature (rt), 0.5 hr; TEA, THF, 0℃ to rt, 3 hr.

1-(methoxymethyl)piperidin-2-one (**IM-1**)



To a solution of piperidin-2-one (2g, 20.1mmol) in anhydrous THF was added n-BuLi (DCM solution, 2.4M, 9.2mL) dropwise at 0℃. The reaction mixture was stirred at 0℃ for 30 min, followed by the addition of CH_3OCH_2Cl (1.9g, 22.1mmol) dropwise. The reaction mixture was stirred for another 3hr, and then extracted by hexane/H_2O (300mL/300mL) for three times. The hexane layer was combined, washed with brine, dried over Na_2SO_4, concentrated and further purified by silica gel column chromatography (DCM/MeOH=20/1), to give 1-(methoxymethyl)piperidin-2-one as a light yellow oil in 95% yield. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 4.18(s, 2H), 3.82-3.85(m, 2H), 3.27(s, 3H), 2.39-2.42(m, 2H), 1.77-1.81(m, 4H).

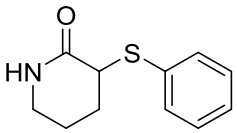
1-(methoxymethyl)-3-(phenylthio)piperidin-2-one (**IM-2**)



To a solution of **IM-1** (3g, 20mmol) in anhydrous THF (50mL) under N_2 atmosphere at -78℃ was added LDA (THF solution, 2M, 20mmol) dropwise and the reaction mixture was stirred for 45min, followed by the addition of PhSSPh

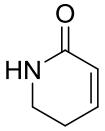
(4.6g, 21mmol) and HMPA (3.8g, 21mmol) portion-wise. The reaction mixture was stirred at -78℃ to rt overnight. The reaction mixture was extracted by Et_2O/H_2O (300mL/300mL) for three times. The Et_2O layer was combined, washed with 3M NaOH, brine, dried over Na_2SO_4, concentrated and further purified by silica gel column chromatography (PE/EA=4/1), to give 1-(methoxymethyl)-3-(phenylthio)piperidin-2-one as a light yellow oil in 40% yield. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 7.54-7.57 (m, 2H), 7.28-7.38 (m, 3H), 4.86(d, J=9.92Hz, 1H), 4.81(d, J=9.92Hz, 1H), 3.89-3.92(m, 1H), 3.35-3.44(m, 2H), 3.31(s, 3H), 2.07-2.20(m, 2H), 1.98-2.04(m, 1H), 1.78-1.85(m, 1H).

3-(phenylthio)piperidin-2-one (**IM-3**)



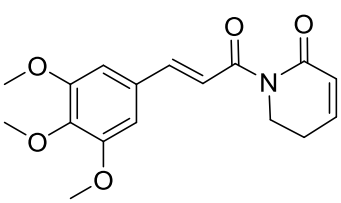
To a solution of **IM-2** (2.1g, 8.37mmol) in EtOH (50mL) was added HCl (10mL, conc.) dropwise and the reaction mixture was stirred and refluxed for 6 hr. The organic solvent was evaporated in vacuo, and extracted by DCM/$NaHCO_3$ (aq) (50mL/50mL) for three times. The DCM layer was combined, washed with brine, dried over Na_2SO_4, concentrated and further purified by silica gel column chromatography (PE/EA=1/1), to give 3-(phenylthio)piperidin-2-one as a light yellow oil in 50% yield. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 7.54-7.57(m, 2H), 7.27-7.34(m, 3H), 3.83(t, J=6Hz, 1H), 3.31-3.35(m, 2H), 1.94-2.18(m, 3H), 1.72-1.81(m, 1H).

5,6-dihydropyridin-2(1H)-one (**IM-4**)



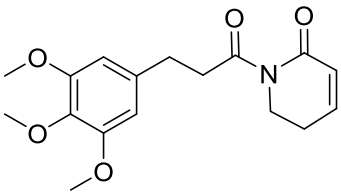
To a solution of **IM-3** (700mg, 3.38mmol) in DCM (20mL) was added mCPBA (723mg, 3.38mmol) at 0℃ and the reaction mixture was stirred for 2 hr at 0℃ to rt. The organic solvent was evaporated in vacuo, and extracted by DCM/$NaHCO_3$ (aq) (20mL/20mL) for three times. The DCM layer was combined, washed with brine, dried over Na_2SO_4, and concentrated.

The residue was redissolved in toluene, stirred and refluxed for another 1 hr. The reaction mixture was concentrated and purified by (PE/EA=1/1), to give 5,6-dihydropyridin-2(1H)-one as a yellow oil in 80% yield. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 6.69-6.73(m, 1H), 5.93(d, J=10.28Hz, 1H), 3.45-3.54(m, 2H), 2.36-2.44(m, 2H).



(E)-1-(3-(3,4,5-trimethoxyphenyl)acryloyl)-5,6-dihydropyridin-2(1H)-one (**PL-1**, also known as piperlongumine)

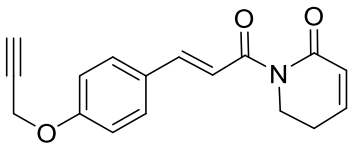
The titled compound was prepared in 30% yield as a colorless oil from (E)-3-(3,4,5-trimethoxyphenyl)acrylic acid (150mg, 0.74mmol) and **IM-4** (60mg, 0.61mmol) according to the procedure for **PL-2**. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 7.68(d, J=15.56Hz, 1H), 7.42(d, J=15.56Hz, 1H), 6.92-6.98(m, 1H), 6.81(s, 2H), 6.05(dt, J=9.72, 1.8Hz, 1H), 4.05(t, J=8.56Hz, 2H), 3.89(s, 6H), 3.88(s, 3H), 2.46-2.51(m, 2H). ^{13}C NMR (400 MHz, CDCl3-d_6): 169.01, 166.03, 153.51, 145.77, 143.99, 139.98, 130.85, 125.92, 121.17,



105.60, 61.17, 56.34, 41.84, 25.00. HRMS (ESI): calculated for [$C_{17}H_{20}NO_5$+], 318.13360, found 318.13565.

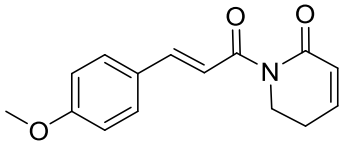
1-(3-(3,4,5-trimethoxyphenyl)propanoyl)-5,6-dihydropyridin-2(1H)-one (**PL-4**)

The titled compound was prepared in 25% yield as a colorless oil from 3-(3,4,5-trimethoxyphenyl)propanoic acid (88mg, 0.37mmol) and **IM-4** (30mg, 0.31mmol) according to the procedure for **PL-2**. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 6.89(dt, J=9.72, 4.16Hz, 1H), 6.47(s, 2H), 5.99(dt, J=9.72, 1.84Hz, 1H), 3.97(t, J=6.52Hz, 2H), 3.85(s, 6H), 3.81(s, 3H), 3.25(t, J=7.72Hz, 2H), 2.93(t, J=7.72Hz, 2H), 2.36-2.42(m, 2H). ^{13}C NMR (400 MHz, CDCl3-d_6): δ 175.67, 165.56, 153.23, 145.48, 137.12, 136.33, 125.90, 105.43, 60.89, 56.21, 41.19, 41.10, 31.73, 24.80.



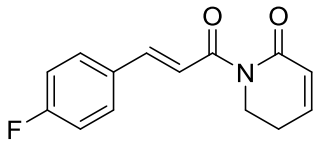
(E)-1-(3-(4-(prop-2-yn-1-yloxy)phenyl)acryloyl)-5,6-dihydropyridin-2(1H)-one (**PL-5**)

The titled compound was prepared in 37% yield as a colorless oil from (E)-3-(4-(prop-2-yn-1-yloxy)phenyl)acrylic acid (75mg, 0.37mmol) and **IM-4** (30mg, 0.31mmol) according to the procedure for **PL-2**. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 7.73(d, J=15.6Hz, 1H), 7.53-7.57(m,2H), 7.42(d, J=15.6Hz, 1H), 6.96-7.04(m, 3H), 6.04(dt, J=1.84, 9.72Hz, 1H), 4.72(d, J=2.4Hz, 2H), 4.04(t, J=6.48Hz, 2H), 2.54(t, J=2.4Hz, 1H), 2.43-2.55(m, 2H). ^{13}C NMR (400 MHz, CDCl3-d_6): δ 169.13, 166.00, 159.21, 145.59, 143.44, 131.10, 130.12, 128.84, 126.04, 120.11, 115.26, 78.26, 75.95, 55.96, 41.79, 24.98. HRMS (ESI): calculated for [$C_{17}H_{16}NO_3$+], 282.11247, found 282.11383.



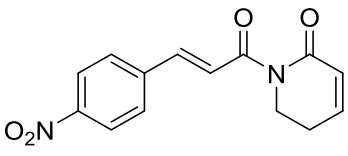
(E)-1-(3-(4-methoxyphenyl)acryloyl)-5,6-dihydropyridin-2(1H)-one (**PL-6**)

The titled compound was prepared in 38% yield as a yellow solid from (E)-3-(4-methoxyphenyl)acrylic acid (66mg, 0.37mmol) and **IM-4** (30mg, 0.31mmol) according to the procedure for **PL-2**. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 7.73(d, J=15.6Hz, 1H), 7.51-7.56(m, 2H), 7.42(d, J=15.6Hz, 1H), 6.91-6.95(m, 1H), 6.86-6.91(m, 2H), 6.04(dt, J=9.72, 1.88Hz, 1H), 4.04(t, J=6.4Hz, 2H), 3.34(s, 3H), 2.44-2.49(m, 2H). HRMS (ESI): calculated for [$C_{15}H_{16}NO_3$+], 258.11247, found 258.11388.



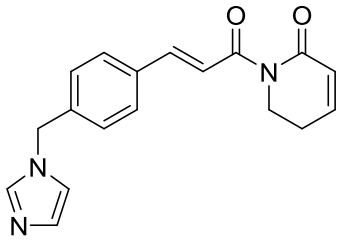
(E)-1-(3-(4-fluorophenyl)acryloyl)-5,6-dihydropyridin-2(1H)-one (**PL-8**)

The titled compound was prepared in 33% yield as a white solid from (E)-3-(4-fluorophenyl)acrylic acid (31mg, 0.186mmol) and **IM-4** (15mg, 0.155mmol) according to the procedure for **PL-2**. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 7.70(d, J=5.68Hz, 1H), 7.54-7.59(m, 2H), 7.43(d, J=15.68Hz, 1H), 7.03-7.09(m, 2H), 6.95(dt, J=9.56, 4.12Hz, 1H), 6.05(dt, J=9.56, 1.84Hz), 4.04(t, J=6.68Hz, 2H), 2.45-2.51(m, 2H). HRMS (ESI): calculated for [$C_{14}H_{13}FNO_2$+], 246.09248, found 246.09309.



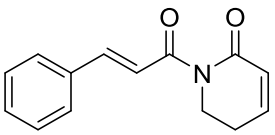
(E)-1-(3-(4-nitrophenyl)acryloyl)-5,6-dihydropyridin-2(1H)-one (**PL-9**)

The titled compound was prepared in 33% yield as a white solid from (E)-3-(4-nitrophenyl)acrylic acid (31mg, 0.186mmol) and **IM-4** (15mg, 0.155mmol) according to the procedure for **PL-2**. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 8.21-8.25(m, 2H), 7.69-7.73(m, 3H), 7.59(d, J=15.92Hz, 1H), 6.98(dt, J=9.72, 4.24Hz, 1H), 6.06(dt, J=9.72, 1.84Hz), 4.06(t, J=6.52Hz, 2H), 2.48-2.53(m, 2H). HRMS (ESI): calculated for [$C_{14}H_{13}N_2O_4$+], 273.08698, found 273.08788.



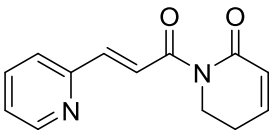
(E)-1-(3-(4-((1H-imidazol-1-yl)methyl)phenyl)acryloyl)-5,6-dihydropyridin-2(1H)-one (**PL-10**)

The titled compound was prepared in 12% yield as a white solid from (E)-3-(4-((1H-imidazol-1-yl)methyl)phenyl)acrylic acid (52mg, 0.227mmol) and **IM-4** (20mg, 0.206mmol) according to the procedure for **PL-2**. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 7.89(s,1H), 7.70(d, J=15.6Hz, 1H), 7.59(s, 1H), 7.57(s, 1H), 7.49(dd, J=1.24, 15.6Hz, 1H), 7.16-7.20(m, 3H), 6.93-6.99(m, 2H), 6.03-6.06(m, 1H), 5.19(s, 2H), 4.04(t, J=6.36Hzz, 2H), 2.46-2.51(m, 2H). HRMS (ESI): calculated for [$C_{18}H_{18}N_3O_3$+], 308.13935, found 308.13986.



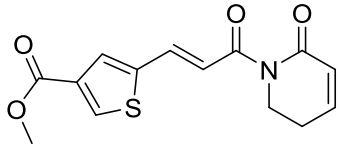
1-cinnamoyl-5,6-dihydropyridin-2(1H)-one (**PL-11**)

The titled compound was prepared in 19% yield as a white solid from cinnamic acid (31mg, 0.206mmol) and **IM-4** (20mg, 0.206mmol) according to the procedure for **PL-2**. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 7.75(d, J=15.64Hz, 1H), 7.57-7.60(m, 2H), 7.51(d, J=15.64Hz, 1H), 7.34-7.39(m, 3H), 6.94(dt, J=9.72, 4.2Hz, 1H), 6.05(dt, J=9.72, 1.84Hz, 1H), 4.05(t, J=6.48Hz, 2H), 2.45-2.51(m, 2H). HRMS (ESI): calculated for [$C_{14}H_{14}NO_2$+], 228.10191, found 228.10233.



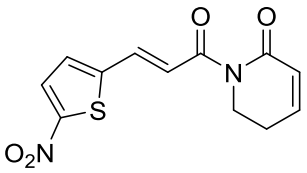
(E)-1-(3-(pyridin-2-yl)acryloyl)-5,6-dihydropyridin-2(1H)-one (**PL-12**)

The titled compound was prepared in 17% yield as a light yellow solid from (E)-3-(pyridin-2-yl)acrylic acid (31mg, 0.206mmol) and **IM-4** (20mg, 0.206mmol) according to the procedure for **PL-2**. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 8.80(s, 1H), 8.63(s, 1H), 8.04(d, J=7.64Hz, 1H), 7.69(d, J=15.72Hz, 1H), 7.58(d, J=15.72Hz, 1H), 7.42-7.48(m, 1H), 6.95-7.00(m, 1H), 6.06(dt, J=9.72, 1.84Hz, 1H), 4.058(t, J=6.44Hz, 2H), 2.48-2.53(m, 2H). HRMS (ESI): calculated for [$C_{13}H_{13}N_2O_2$+], 229.09715, found 229.09867.



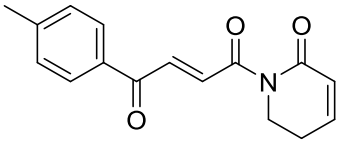
Methyl E)-5-(3-oxo-3-(6-oxo-3,6-dihydropyridin-1(2H)-yl)prop-1-en-1-yl)thiophene-3-carboxylate (**PL-13**)

The titled compound was prepared in 12% yield as a light yellow oil from (E)-3-(4-(methoxycarbonyl)thiophen-2-yl)acrylic acid (53mg, 0.25mmol) and **IM-4** (20mg, 0.206mmol) according to the procedure for **PL-2**. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 8.06(t, J=1.04Hz, 1H), 7.80(dt, J=0.56, 15.44Hz, 1H), 7.66(t, J=0.56Hz, 1H), 7.36(d, J=15.44Hz, 1H), 6.94(dt, J=9.72, 4.16Hz, 1H), 6.05(dt, J=9.72, 1.88Hz, 1H), 4.03(t, J=6.48Hz, 2H), 3.87(s, 3H), 2.45-2.51(m, 2H). HRMS (ESI): calculated for [$C_{14}H_{14}NO_4S$+], 292.06381, found 292.06476.



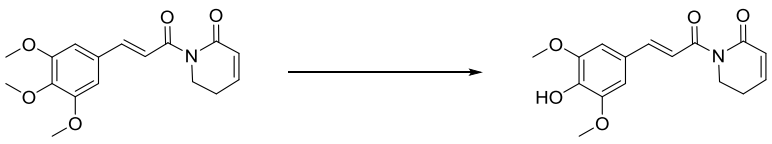
(E)-1-(3-(5-nitrothiophen-2-yl)acryloyl)-5,6-dihydropyridin-2(1H)-one (**PL-14**)

The titled compound was prepared in 4% yield as a light yellow oil from (E)-3-(5-nitrothiophen-2-yl)acrylic acid (28mg, 0.14mmol) and **IM-4** (14mg, 0.14mmol) according to the procedure for **PL-2**. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 7.83-7.85(m, 1H), 7.67(dd, J=0.56, 15.44Hz, 1H), 7.47(d, J=15.44Hz, 1H), 7.19(d, J=4.28Hz, 1H), 6.98(dt, J=9.72, 4.28Hz, 1H), 6.06(dt, J=9.72, 1.84Hz, 1H), 4.04(t, J=6.48Hz, 2H), 2.47-2.53(m, 2H). HRMS (ESI): calculated for [$C_{12}H_{11}N_2O_4S$+], 279.04340, found 279.04400.



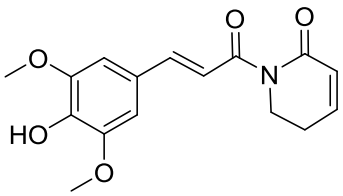
(E)-1-(6-oxo-3,6-dihydropyridin-1(2H)-yl)-4-(p-tolyl)but-2-ene-1,4-dione (**PL-15**)

The titled compound was prepared in 4% yield as a white solid from (E)-4-oxo-4-(p-tolyl)but-2-enoic acid (47mg, 0.247mmol) and **IM-4** (20mg, 0.21mmol) according to the procedure for **PL-2**. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 7.91-7.95(m, 2H), 7.74(d, J=15.28Hz, 1H), 7.66(m, J=15.28Hz, 1H), 7.28-7.32(m, 2H), 6.98(dt, J=9.8, 4.16Hz, 1H), 6.05(dt, J=9.8, 1.88Hz, 1H), 4.04(t, J=6.52Hz, 2H), 2.48-2.54(m, 2H), 2.43(s, 3H). HRMS (ESI): calculated for [$C_{16}H_{16}NO_3$+], 270.11247, found 270.11263.



PL-1 PL-7

Scheme S3. Synthesis route for **PL-7**. Reagents and conditions: $AlCl_3$, DCM, rt, 30min.

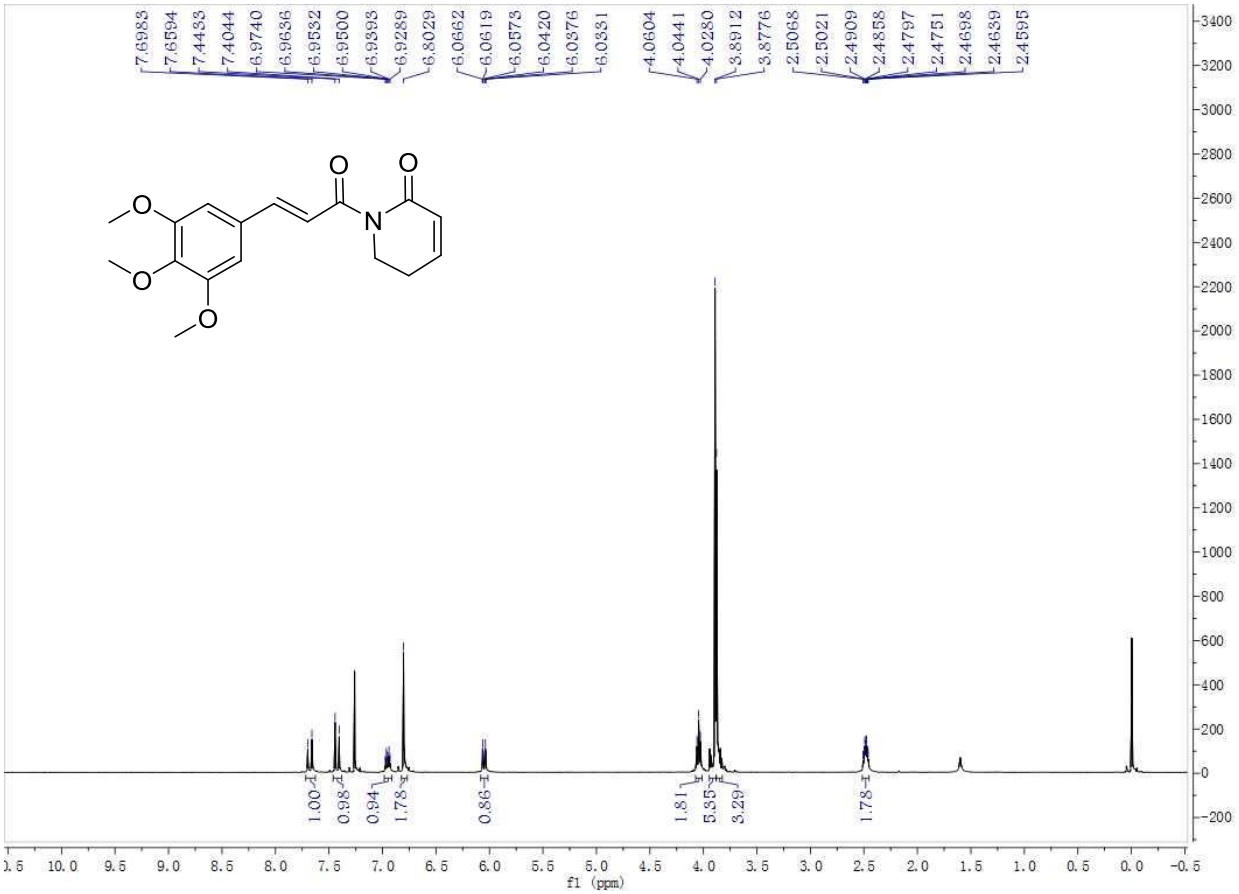


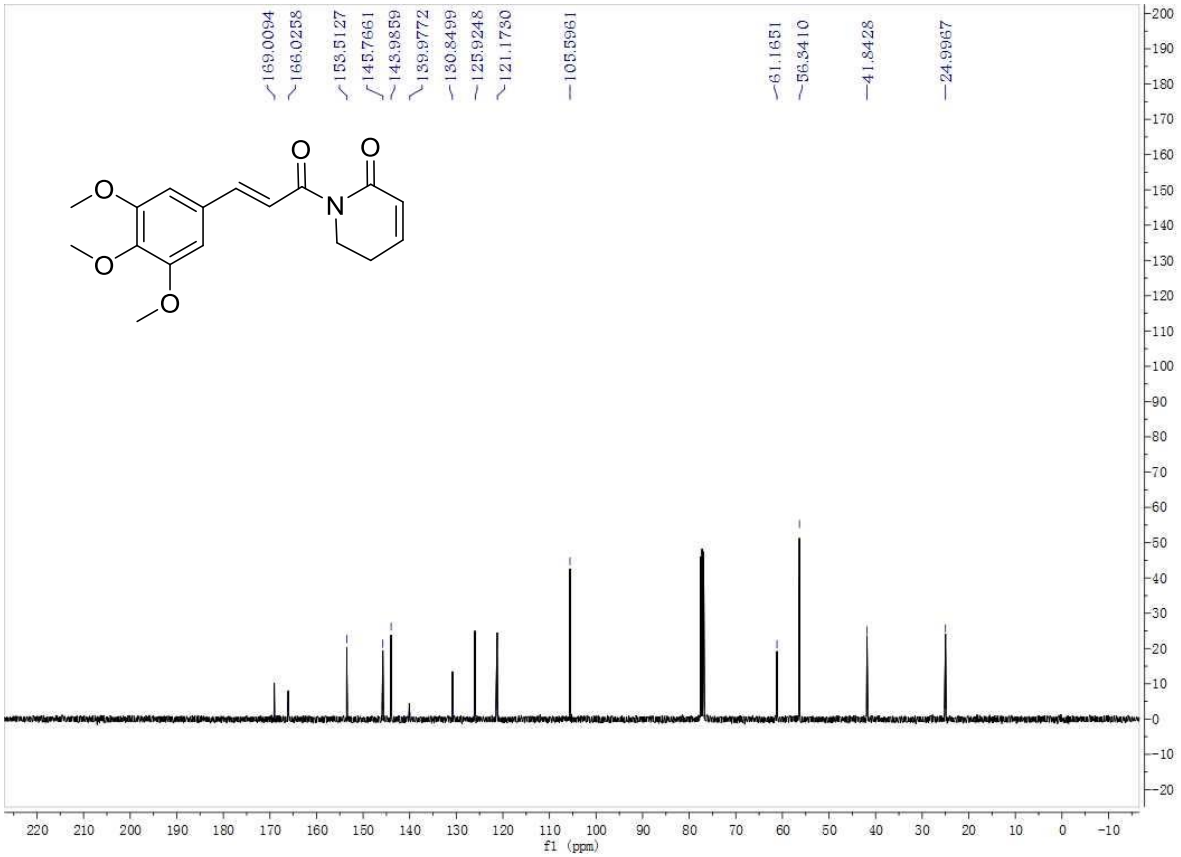
(E)-1-(3-(4-hydroxy-3,5-dimethoxyphenyl)acryloyl)-5,6-dihydropyridin-2(1H)-one (**PL-7**)

To a solution of PL-1 (30mg, 0.11mmol) in anhydrous DCM was added $AlCl_3$ (117mg, 0.88mmol) portion-wise. The reaction mixture was stirred at rt for 30 min, and then quenched by icy NH_4Cl solution. The quenched mixture was extracted by DCM/ NH_4Cl(aq) (20mL/20mL) 3 times. The DCM layer was combined, washed with brine, dried over Na_2SO_4, concentrated and further purified by silica gel column chromatography (PE/EA=2/1), to give

(E)-1-(3-(4-hydroxy-3,5-dimethoxyphenyl)acryloyl)-5,6-dihydropyridin-2(1H)-one as a yellow solid in 42% yield. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 7.69(d, J=15.52Hz, 1H), 7.40(d, J=15.52Hz, 1H), 6.94(dt, J=9.64, 4.16Hz, 1H), 6.82(s, 2H), 6.05(dt, J=9.64, 1.84Hz, 1H), 5.74(s, 1H), 4.04(t, J=6.4Hz, 2H), 3.93(s, 6H), 2.45-2.50(m, 2H). HRMS (ESI): calculated for [$C_{16}H_{18}NO_5$+], 304.11795, found 304.11912.

^{1}H and ^{13}C NMR spectra

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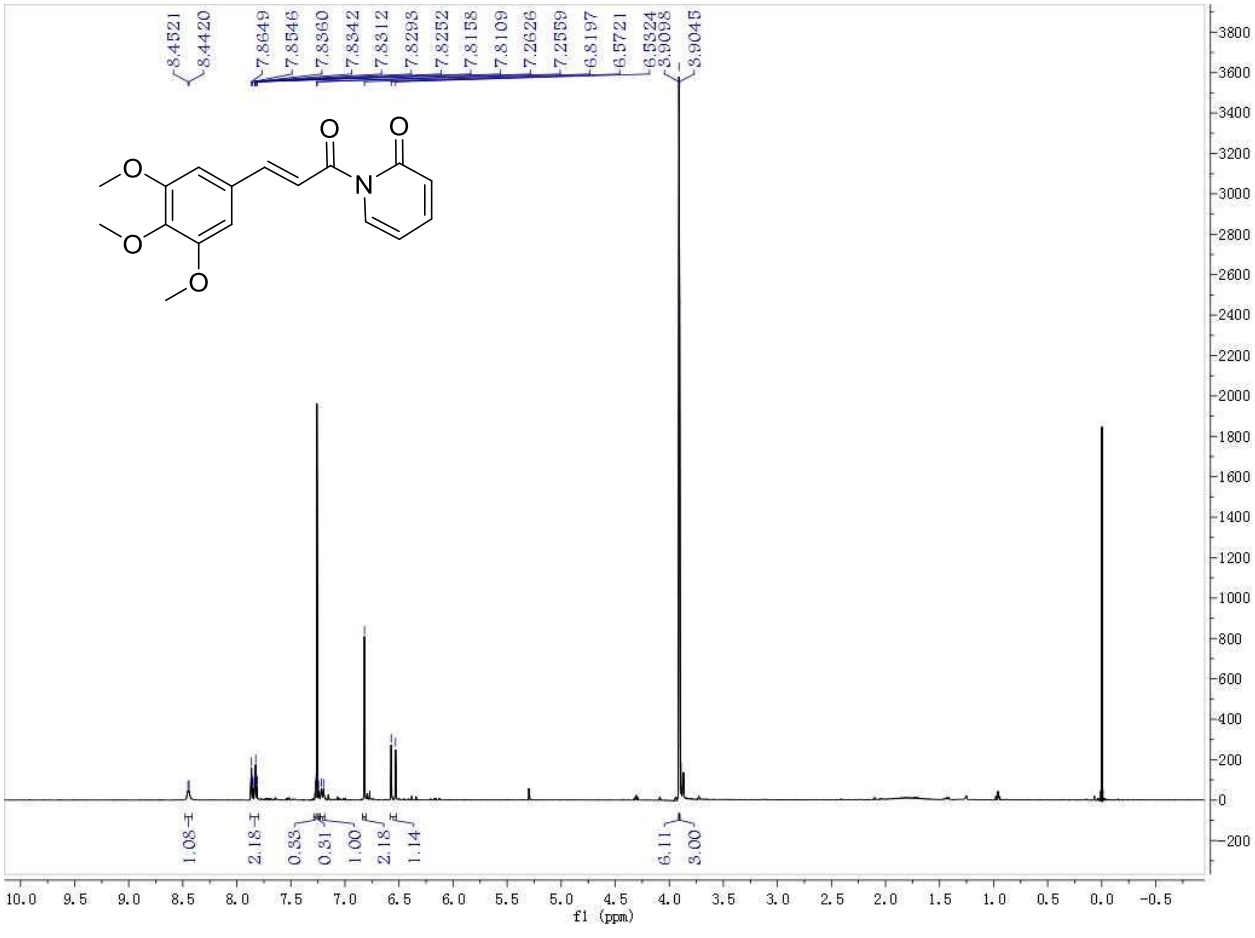
f1 (ppm)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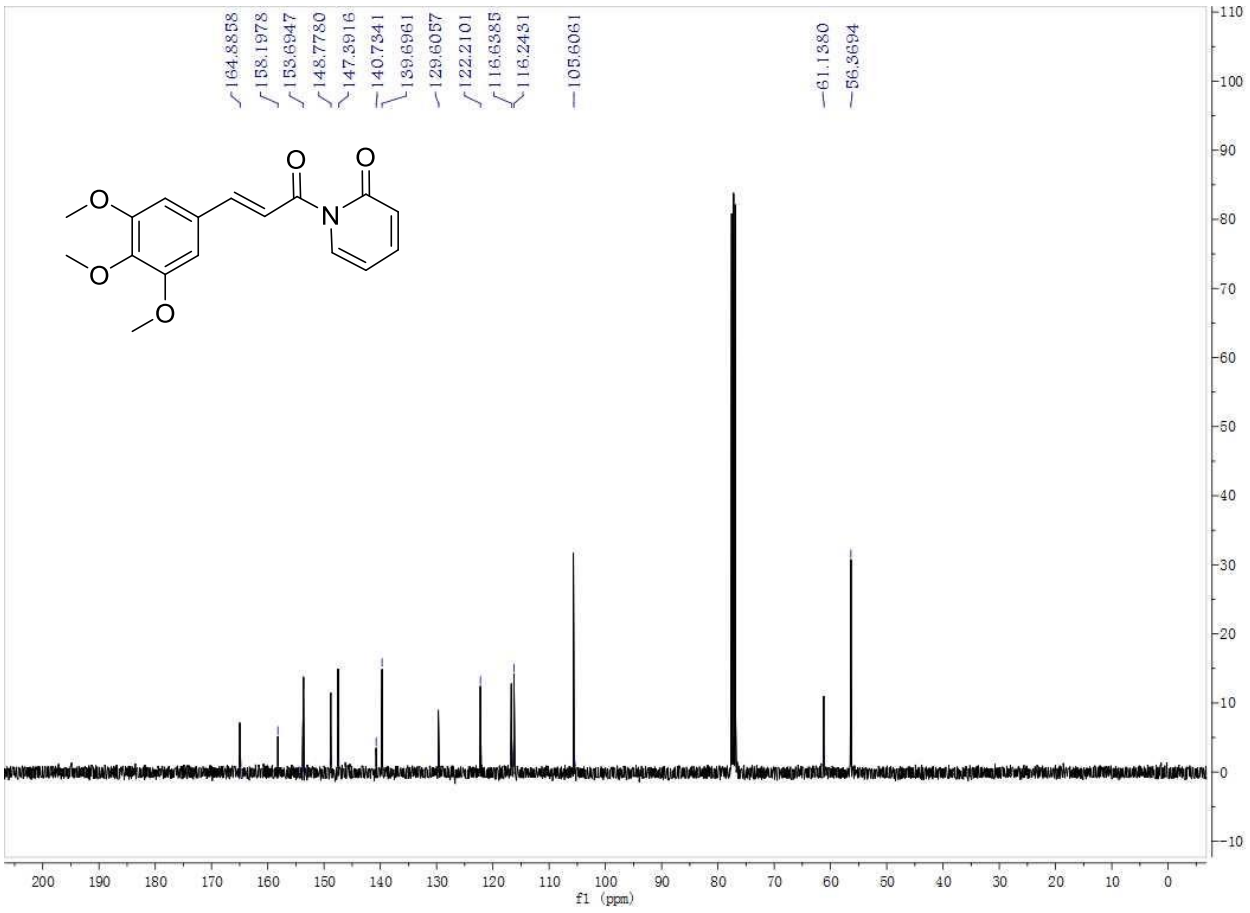
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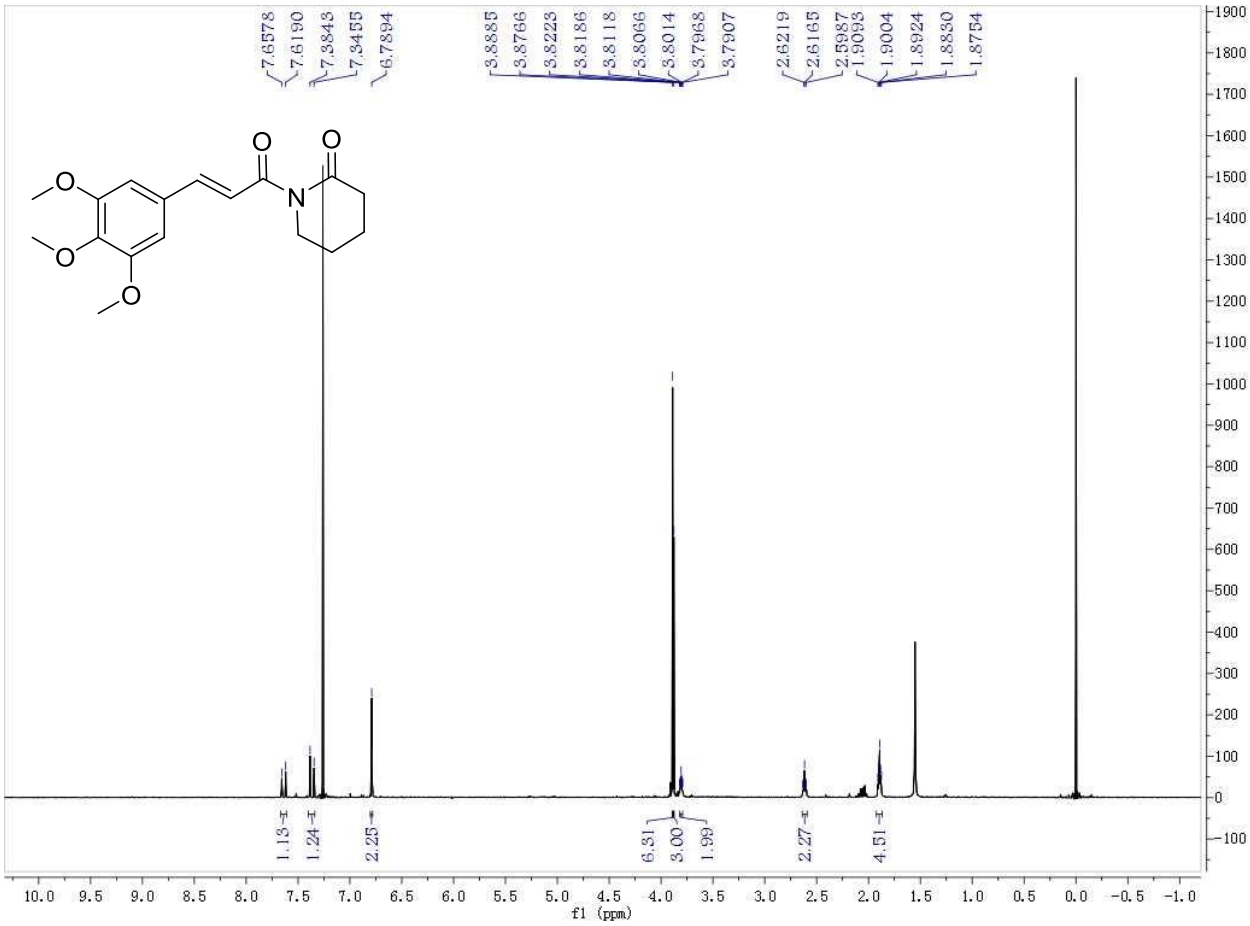
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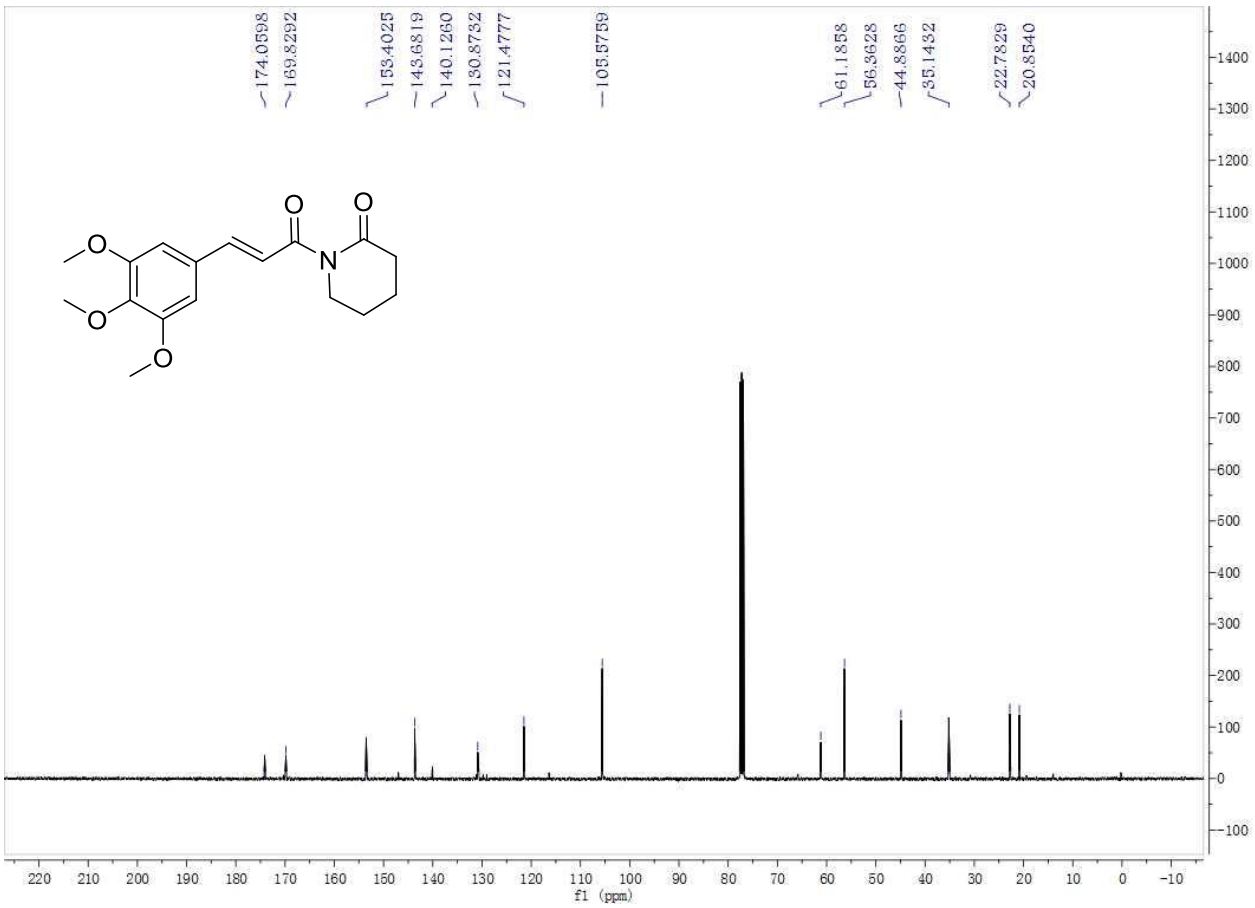
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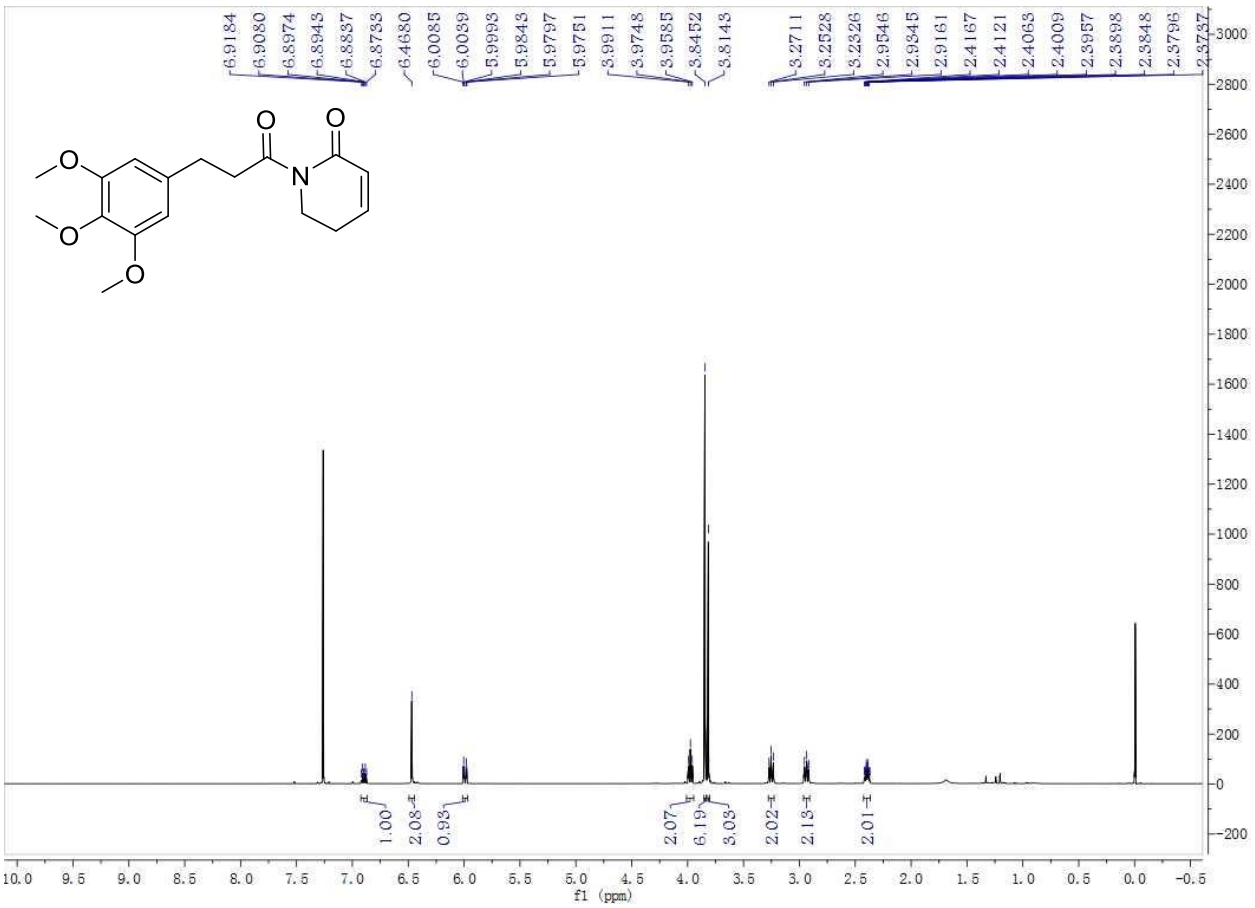
f1 (ppm)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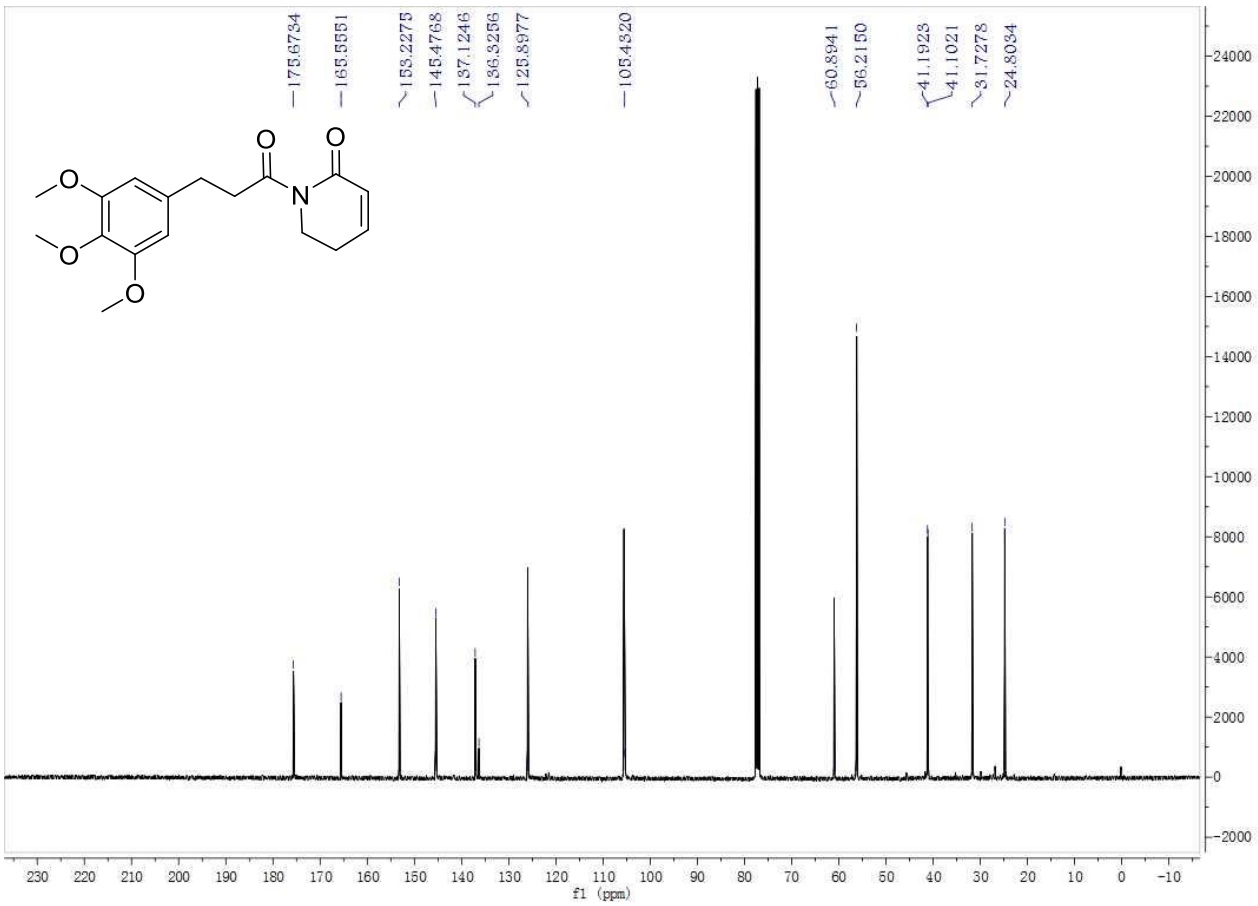
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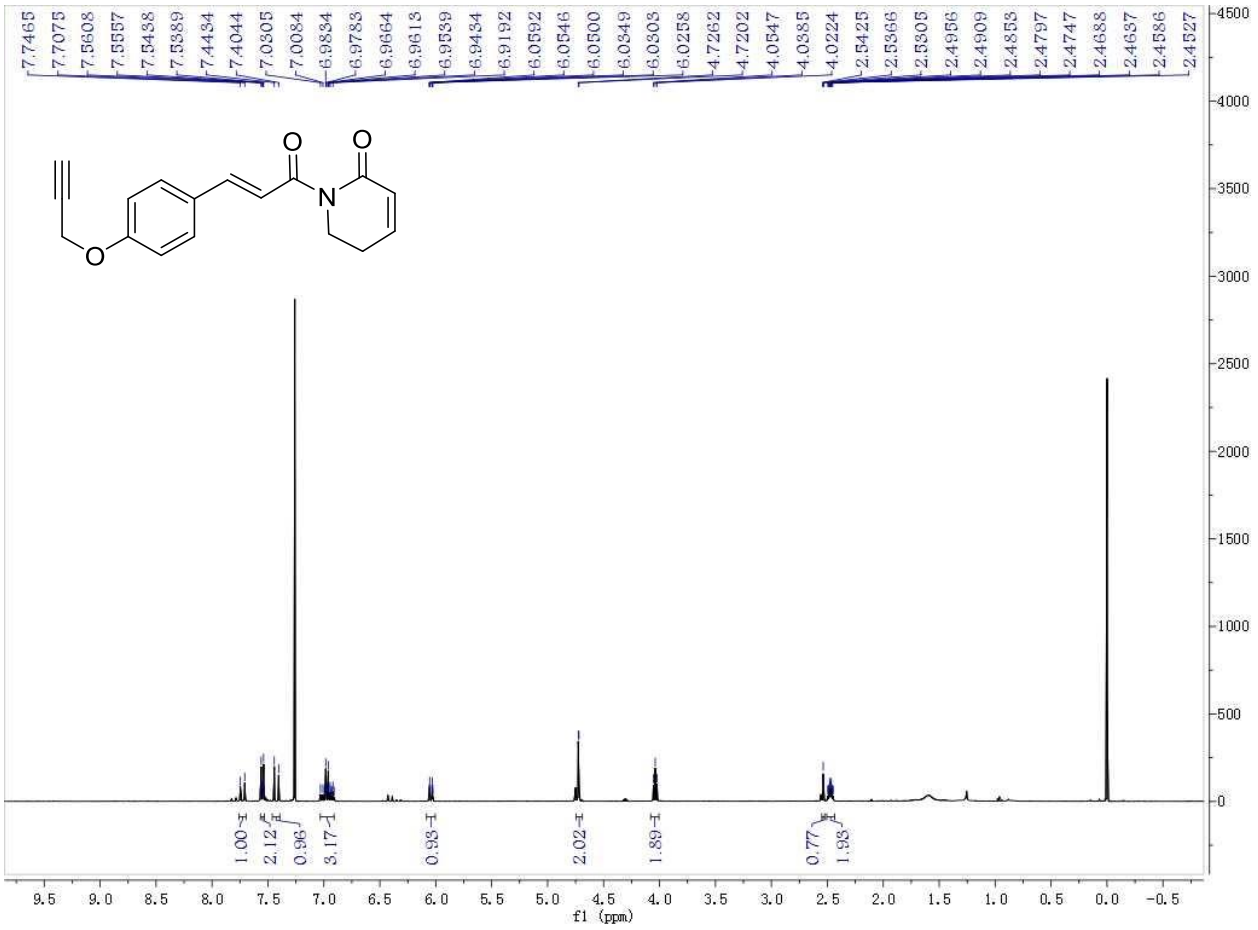
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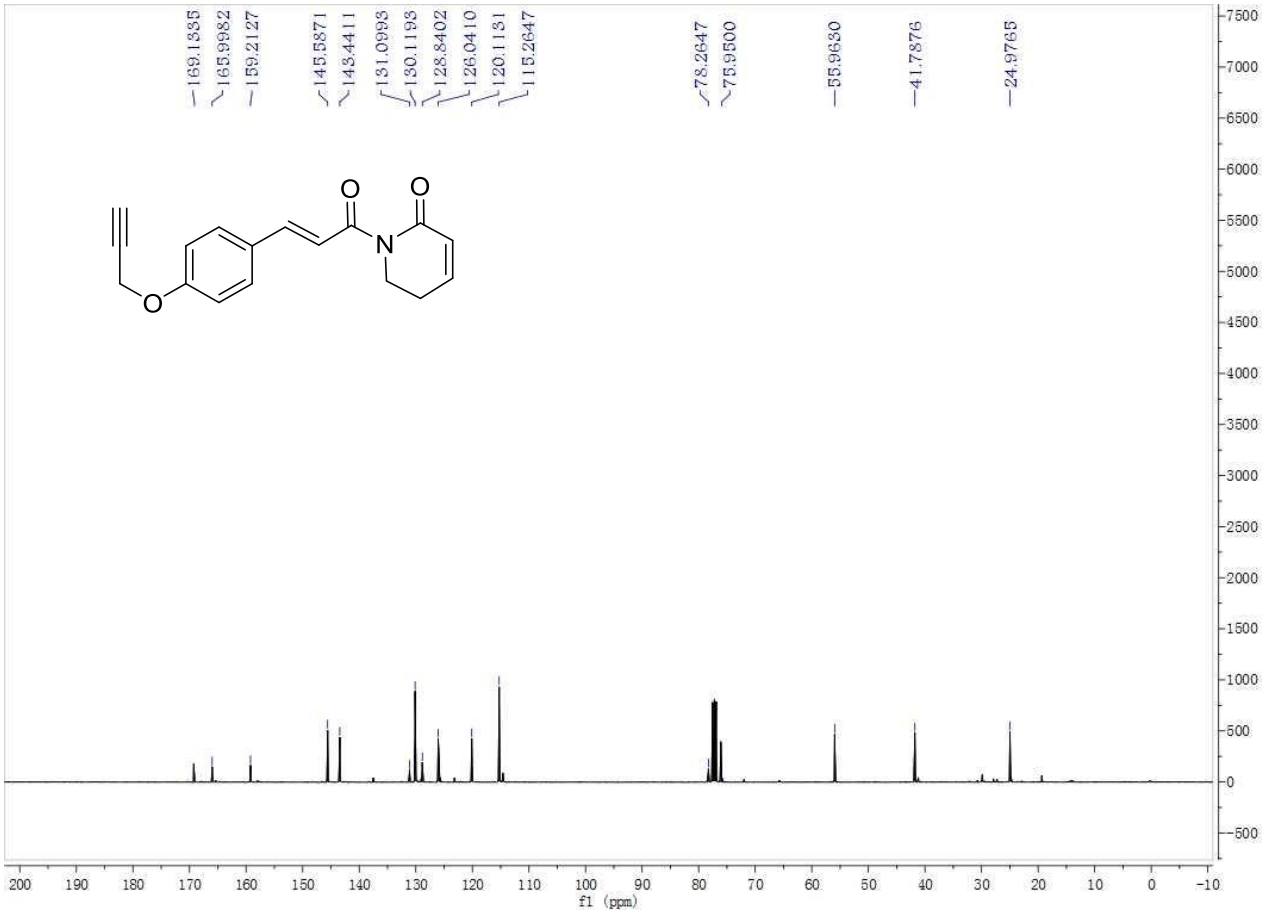
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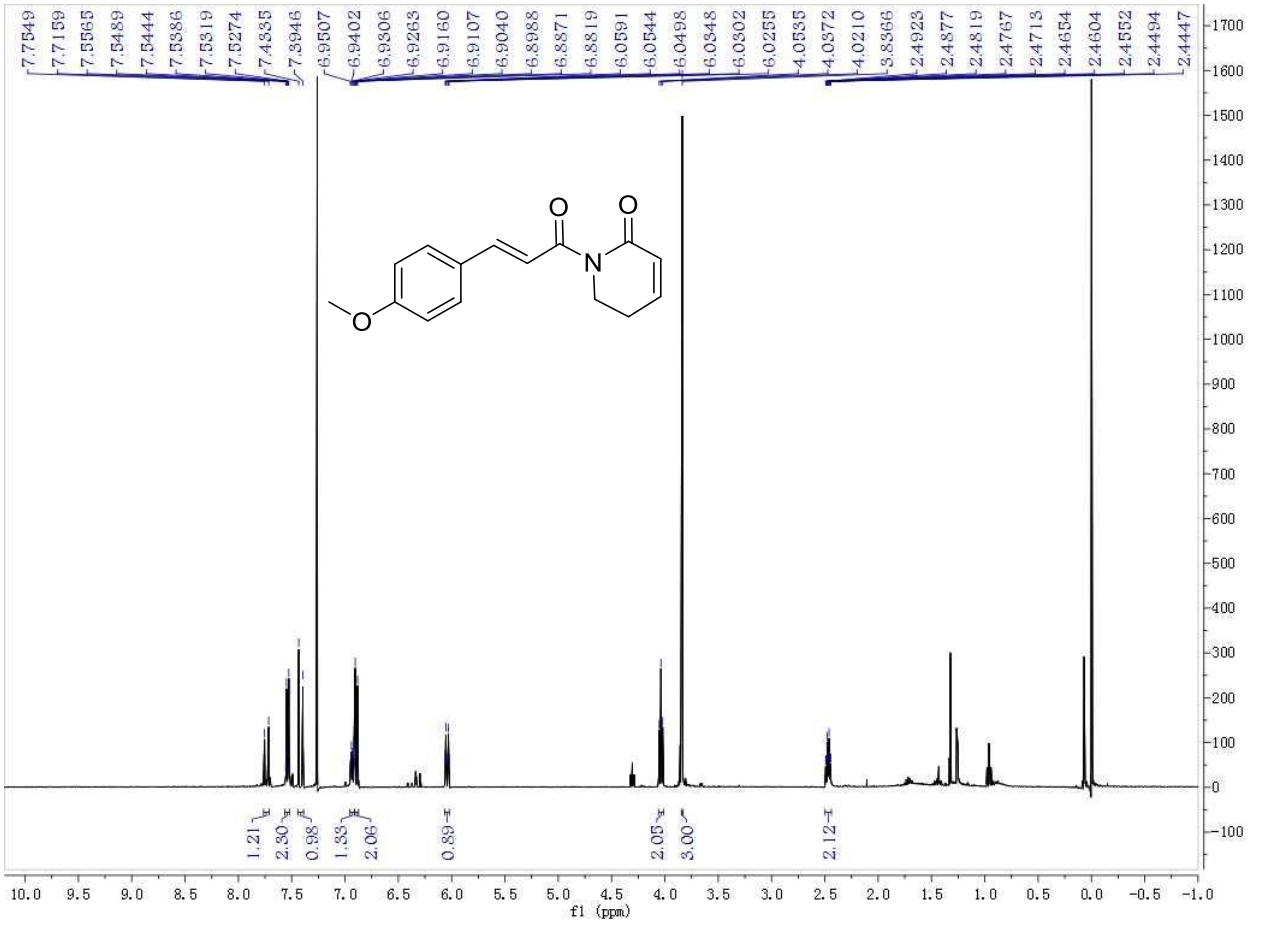
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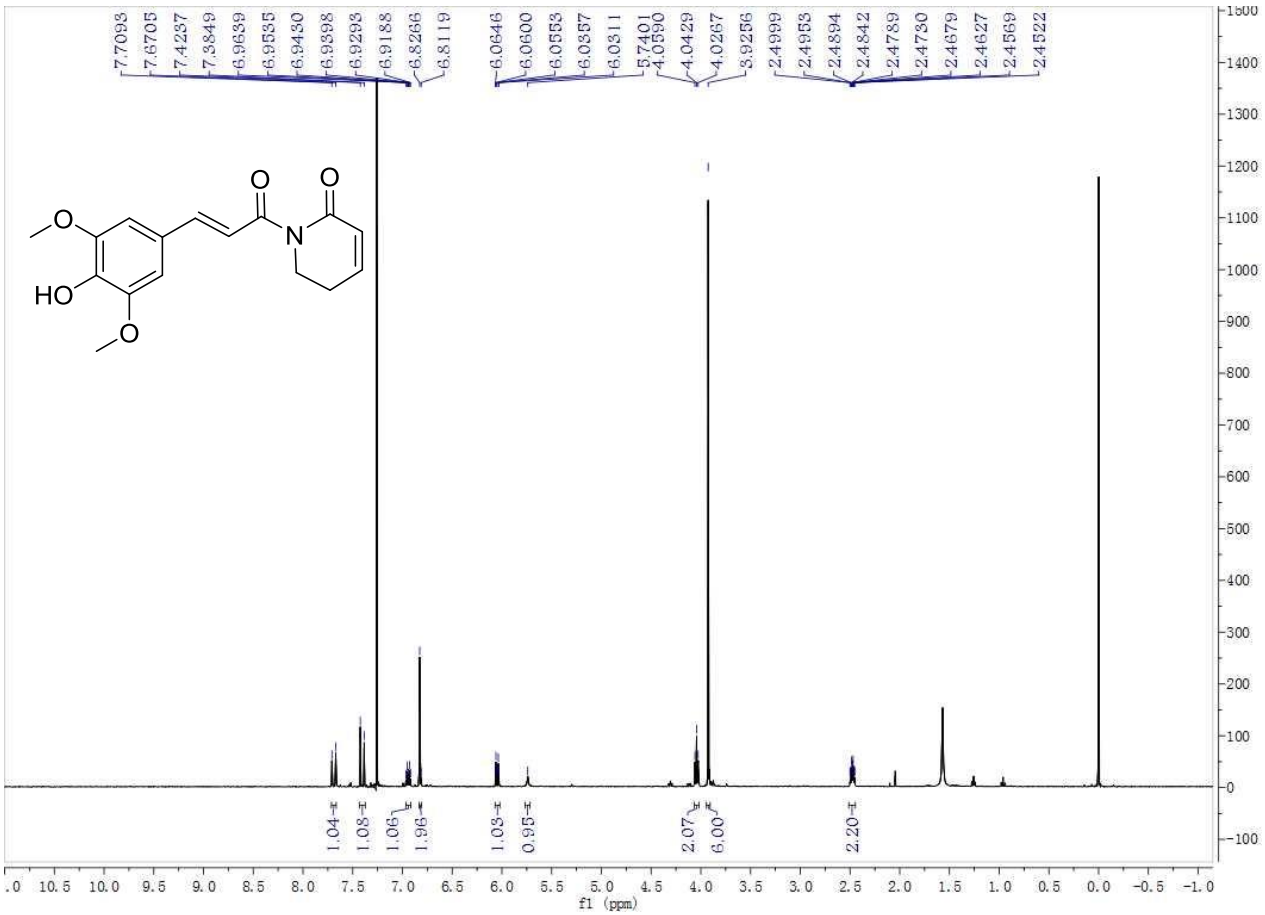
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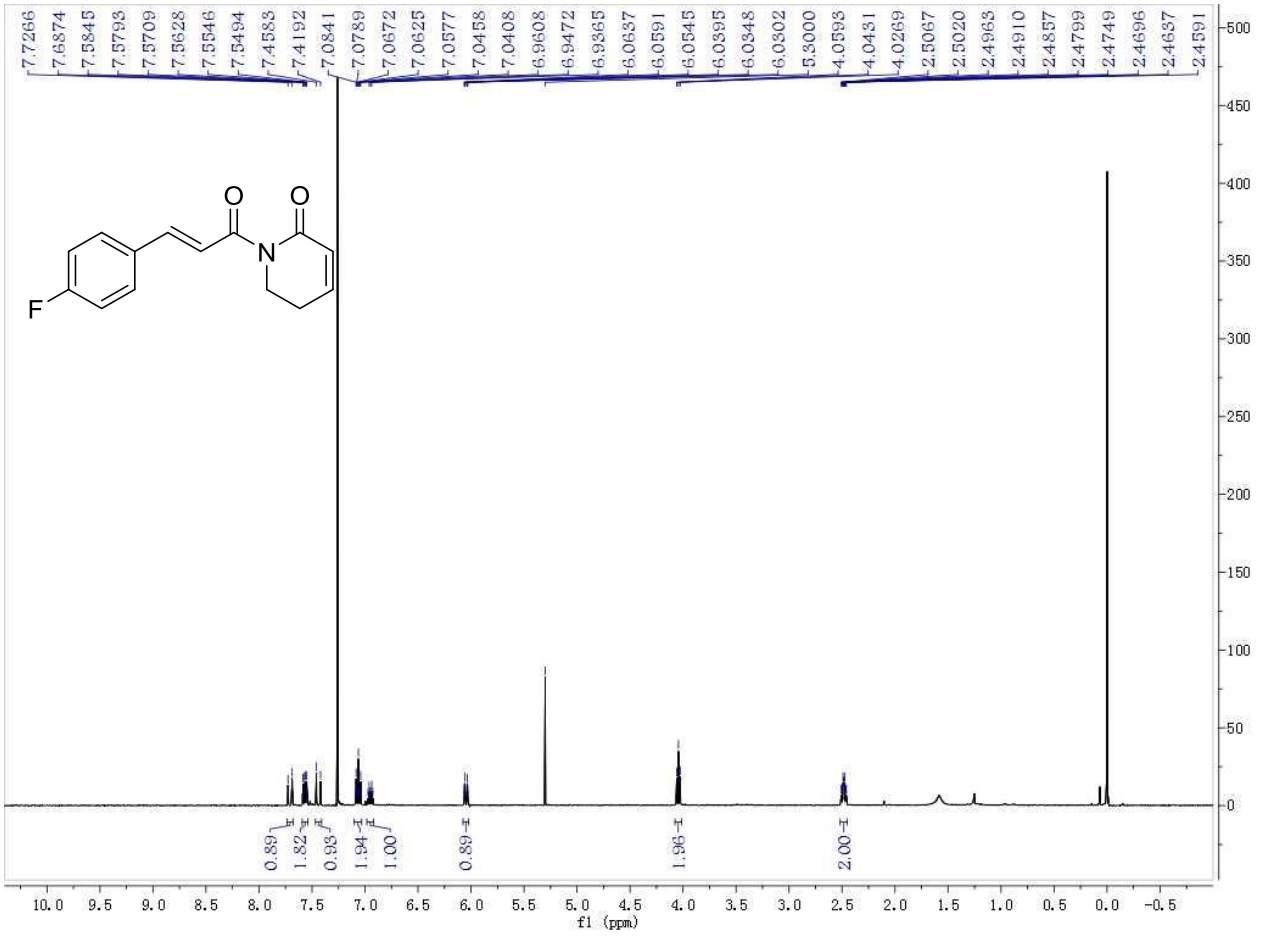
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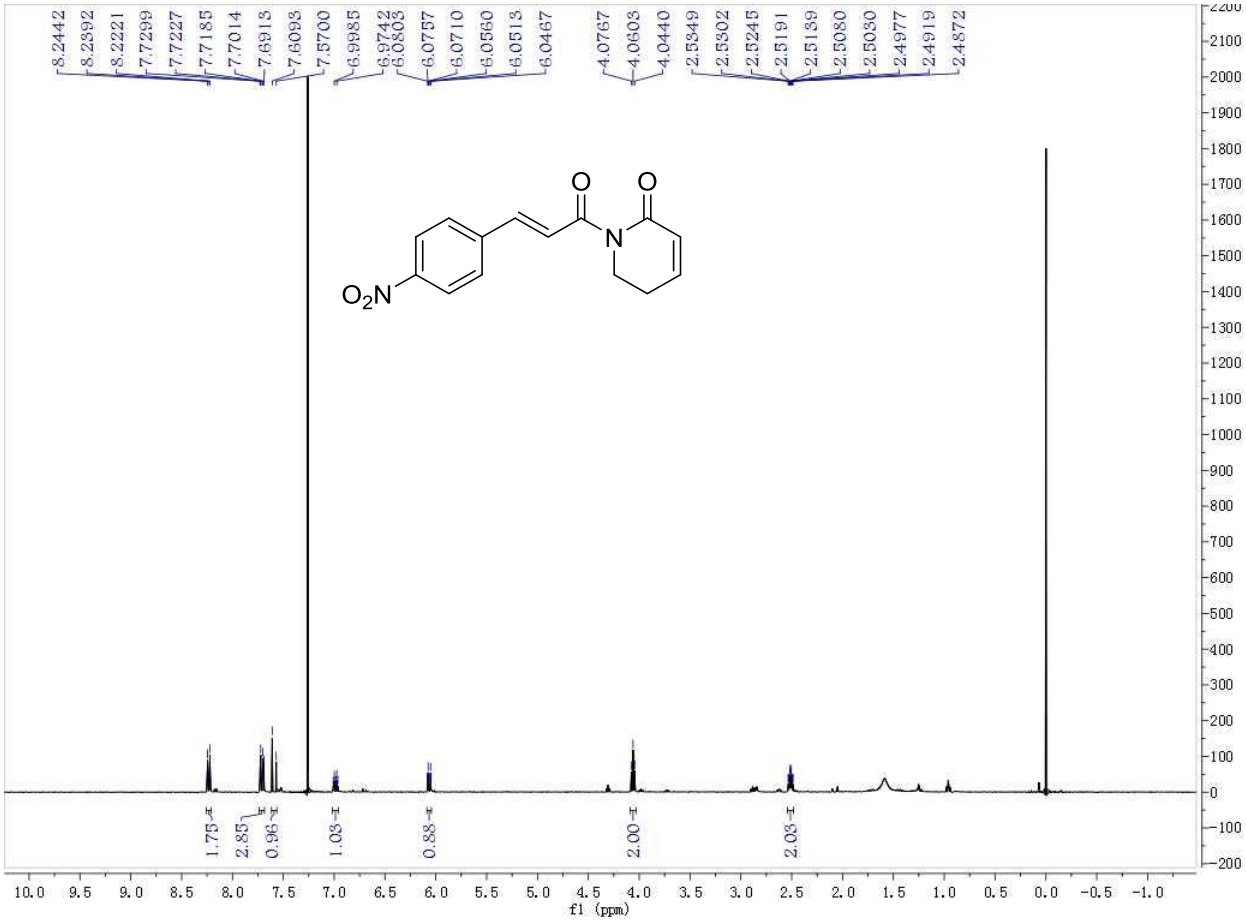
2.00
f1 (ppm)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8.2221
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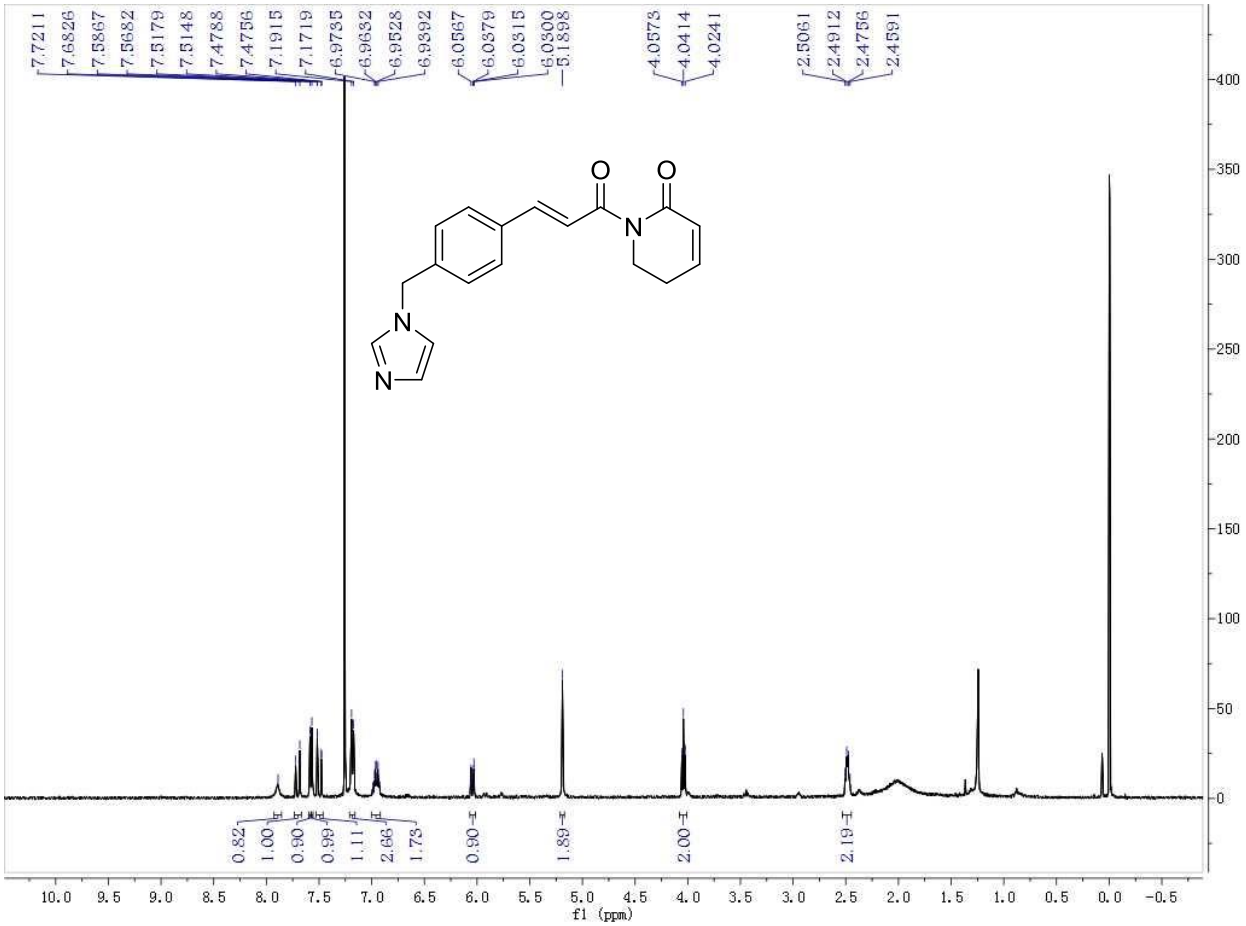
7.7227
7.7185
7.7014
7.6913
7.6093
7.5700
6.9985
6.9742
6.9603
6.0757
6.0710
6.0560
6.0513
6.0467
4.0767
4.0603
4.0440
2.5849
2.5302
2.5245
2.5191
2.5139
2.5080
2.5030
2.4977
2.4919
2.4872
O
O
N
O2N
1.75
2.85
0.96
1.03
0.88
2.00
2.03
f1 (ppm)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7.5867
7.5682
7.5179
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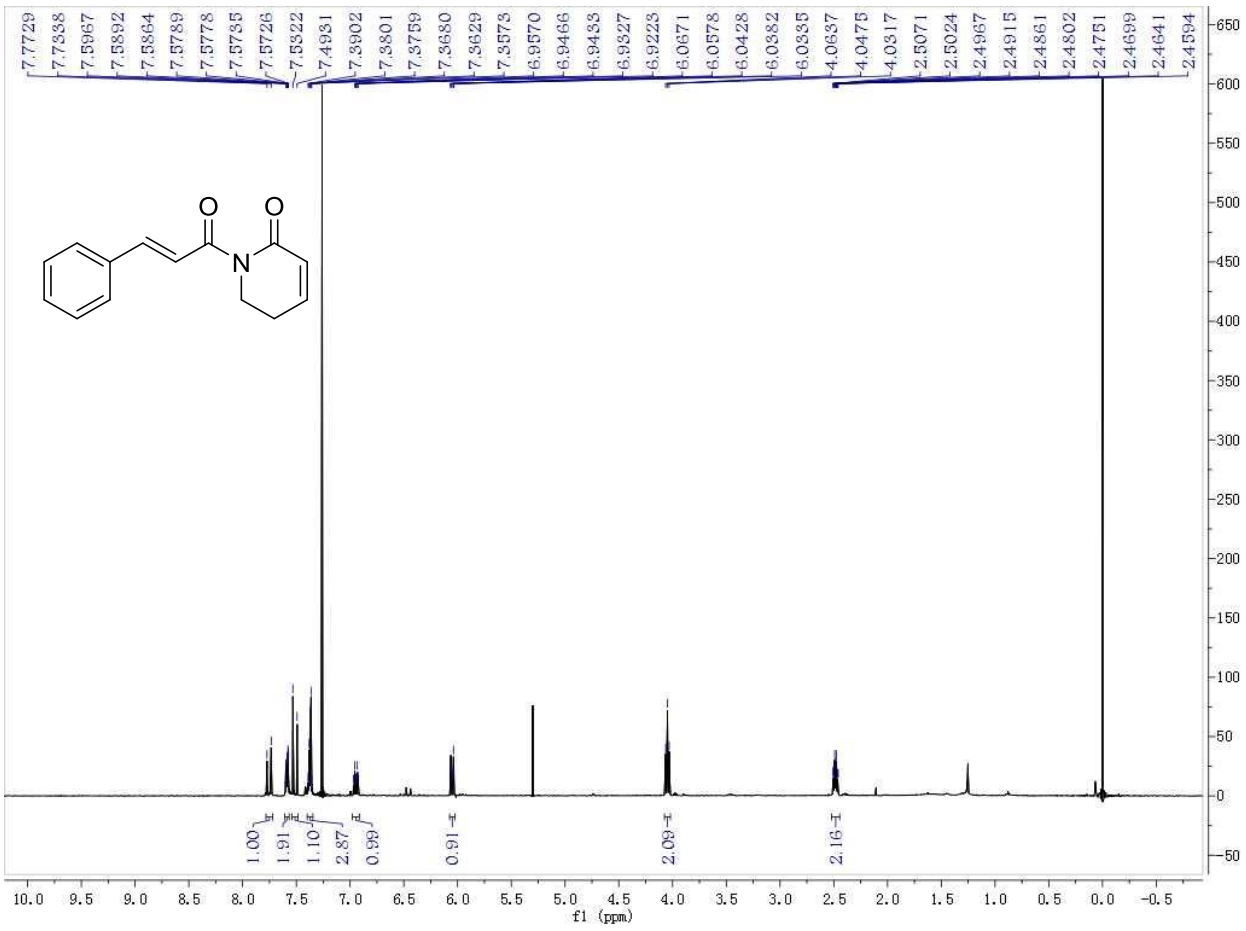
7.4756
7.1915
7.1719
6.9735
6.9632
6.9528
6.9392
6.0567
6.0379
6.0315
6.0300
5.1898
4.0573
4.0414
4.0241
2.5061
2.4912
2.4756
2.4591
O
O
N
N
N
0.82
1.00
0.90
0.99
1.11
2.66
1.75
0.90
1.89
2.00
2.19
f1 (ppm)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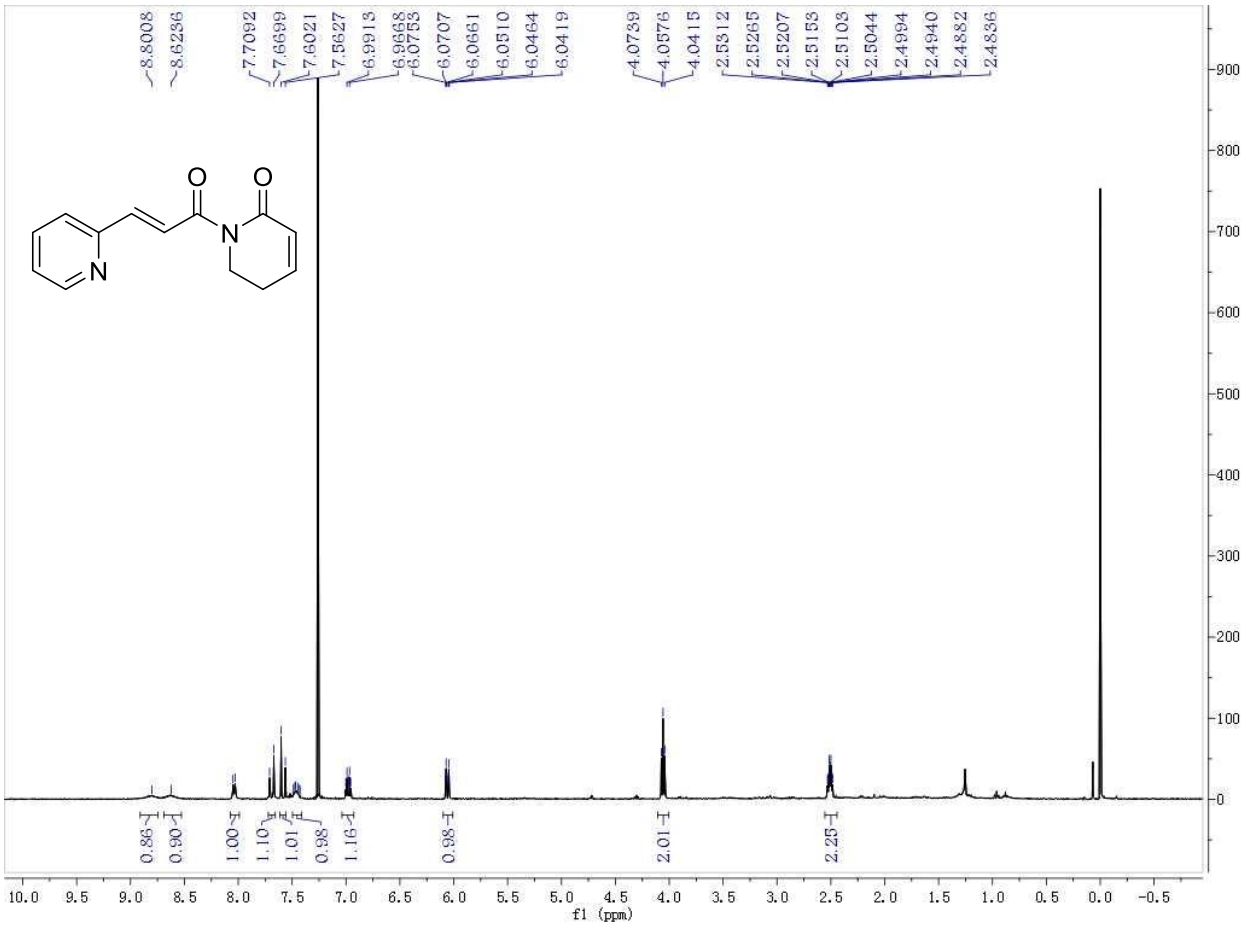
7.5322
7.4931
7.3902
7.3801
7.3759
7.3680
7.3629
7.3573
6.9570
6.9466
6.9433
6.9327
6.9223
6.0671
6.0578
6.0428
6.0382
6.0335
4.0637
4.0475
4.0317
2.5071
2.5024
2.4967
2.4915
2.4861
2.4802
2.4751
2.4699
2.4641
2.4594
O
O
N
1.00
1.91
1.10
2.87
0.99
0.91
2.09
2.16
f1 (ppm)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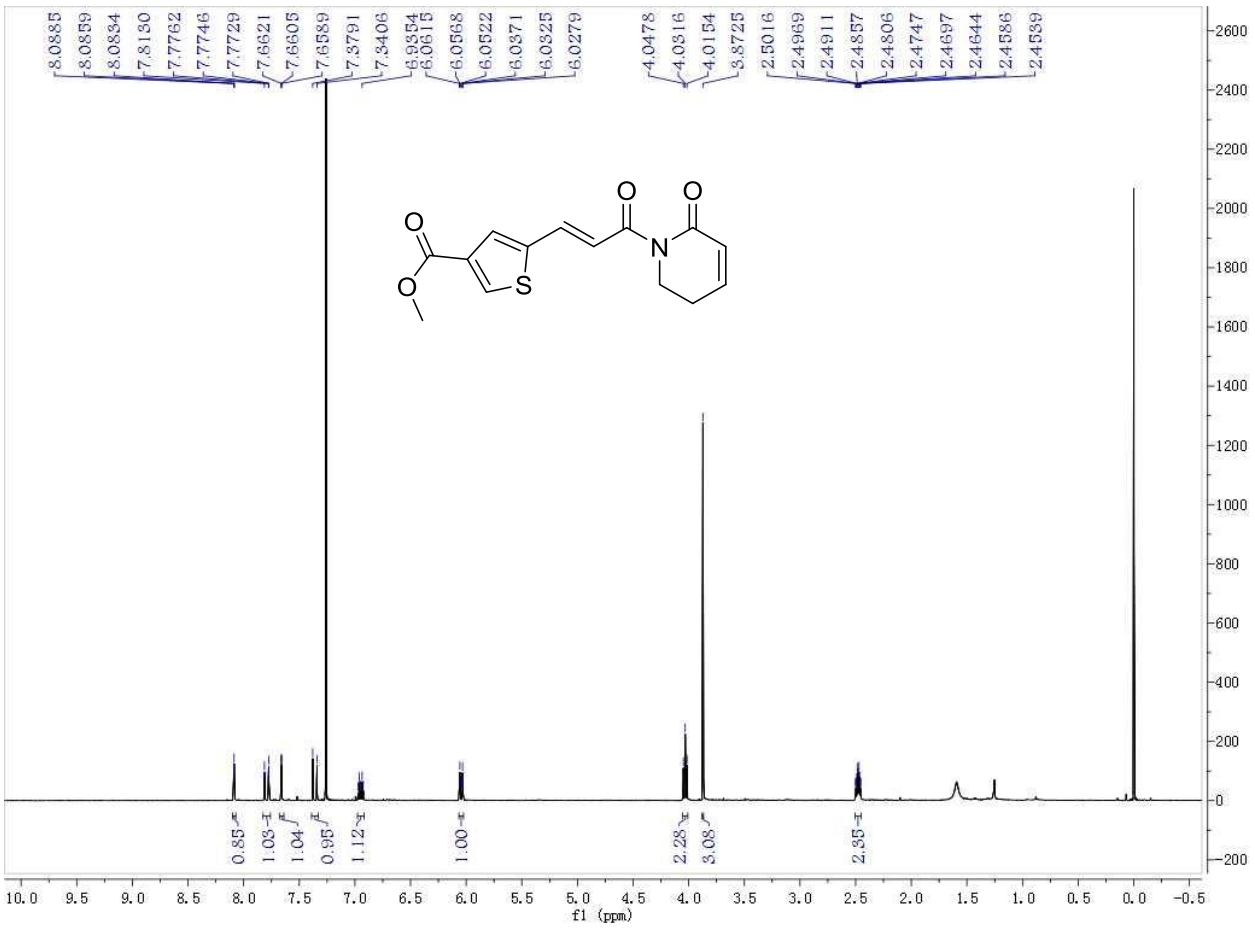
7.6021
7.5627
6.9913
6.9668
6.0755
6.0707
6.0661
6.0510
6.0464
6.0419
4.0739
4.0576
4.0415
2.5312
2.5265
2.5207
2.5153
2.5103
2.5044
2.4994
2.4940
2.4882
2.4836
O
O
N
N
0.86
0.90
1.00
1.10
1.01
0.98
1.16
0.98
2.01
2.25
f1 (ppm)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7.7762
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7.7729
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7.6605
7.6589
7.3791
7.3406
6.0568
6.0522
6.0371
6.0325
6.0279
4.0478
4.0316
4.0154
3.8725
2.5016
2.4969
2.4911
2.4857
2.4806
2.4747
2.4697
2.4644
2.4586
2.4539
O
O
O
N
S
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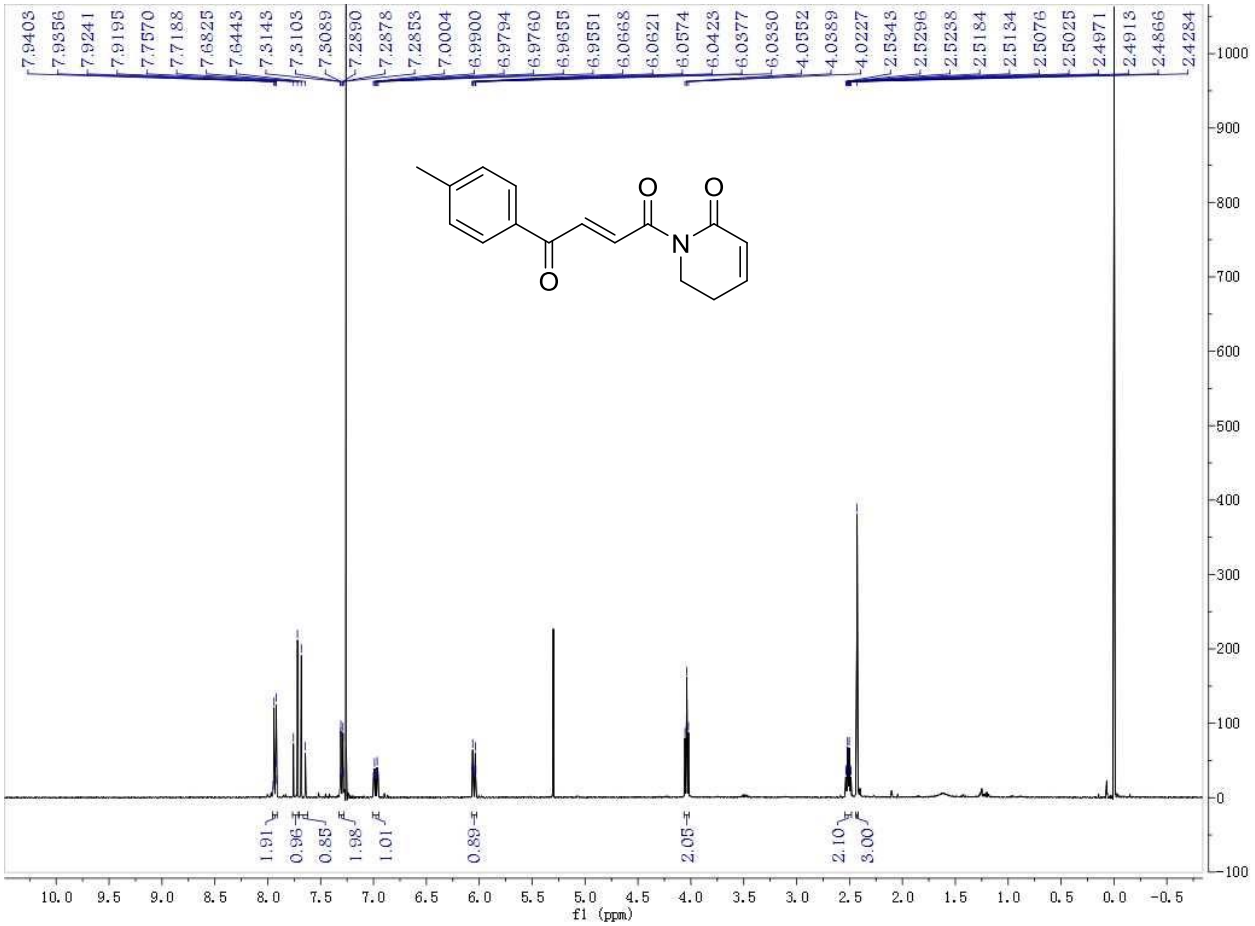
1.03
1.04
0.95
1.12
1.00
2.28
3.08
2.35
f1 (ppm)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7.7570
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7.6825
7.6443
7.3143
7.3103
7.3089
7.2890
7.2878
7.2853
7.0004
6.9900
6.9794
6.9760
6.9655
6.9551
6.0668
6.0621
6.0574
6.0423
6.0377
6.0330
4.0552
4.0389
4.0227
2.5343
2.5296
2.5238
2.5184
2.5134
2.5076
2.5025
2.4971
2.4913
2.4866
2.4284
O
O
O
N
1.91
0.96
0.85
1.98
1.01
0.89
2.05
2.10
3.00
f1 (ppm)

References

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