Supporting information

Discovery of Hydroxyamidine Derivatives as Highly Potent, Selective Indoleamine-2, 3-dioxygenase 1 Inhibitors

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Biological Assays

IDO1 enzymatic assay

Human IDO1 gene purchased from Origene (SC126221) was transferred to Pet30a plasmids by gene cloning, and transferred to competent Escherichia coli Rosseta. This IDO1 gene was scale-up cultured in liquid LB (Luria-Bertani) medium which was prepared according to "Molecular Cloning A Laboratory Manual" (Sambrook, J.; Russell, D. W.). The bacteria were collected and broken by the ultrasonic wave. The purified IDO1 was obtained through the column by elution.

24 μL of enzyme (IDO1) was diluted 100 times with 50 mM KPB to 2400 μL. The concentration of enzyme solution was 2.6ng/μL. A 96 well reaction plate (AXYGEN, PCR-96-FLT-C, hereinafter referred to as the reaction plate) was added with the enzyme solution at 24 μL/well. The blank well was added with 24 μL of KPB [Preparation of KPB buffer (50mM): 6.805 g of KH2P04 was weighed by an analytical balance, and placed into a 1000 ml of beaker, deionized water was added with a measuring cylinder to 900 ml, the pH was adjusted to 6.5 by 1M KOH, then the mixture was introduced into a 1 L measuring cylinder, and water was added to 1 L. It was stored at 4°C]. 1 μL of a compound or DMSO was added into the corresponding wells in the reaction plate. Preparation of solution A: 200 μL of 500 mM sodium ascorobate was added with 1050 μL of KPB, then the mixture was mixed uniformly for 3 seconds at the maximum speed in a turbine mixer. Solution B: 100 μL of 10 mM tryptophan was added with 100 μL of 100000 unit/ml catalase, 5 μL of 10 mM methylene blue, and 1050 μL of KPB sucessively, then the mixture was mixed uniformly for 3 seconds at the maximum speed in a turbine mixer. 1200 μL of solution A and 1200 μL of solution B were taken and mixed uniformly for 3 seconds at the maximum speed in a turbine mixer. The mixture was added to the reaction plate at 24μL/well. The reaction plate was placed in a plate centrifuge and centrifuged for 15 seconds at the maximum speed, so the reaction liquids were converged to the bottom. The reaction mixture was mixed uniformly for 30 seconds on a shaker, and incubated for 1 hour at 37°C in a constant temperature incubator. In the reaction plate, 30% (W/V) trichloroacetic acid was added at 10 μL/well, then the mixture was incubated for 15 minutes at 65°C in a incubator. The reaction plate was centrifuged in a centrifuge for 5 minutes at 4700RPM at room temperature. 40 μL of the supernatant was transferred from the reaction plate to the corresponding 96 wells test plate (Corning, #3599) by a multi-channel pipette. 2% (W/V) 4 (dimethylamino)benzaldehyde/glacial acetic acid solution was added at 40 μL/well, then the mixture was mixed uniformly for 1 minute on a shaker at the maximum speed. After

incubation for 2 minutes at room temperature, the absorbance at 480 nm was read on Synergy HT (BIOTEK).

TDO enzymatic assay

Human TDO gene purchased from Suzhou Genewiz Biological Technology Co., Ltd. (U32989.1) was transferred to competent Escherichia coli Rosseta. This TDO gene was scale-up cultured in liquid LB (Luria-Bertani) medium which was prepared according to "Molecular Cloning A Laboratory Manual" (Sambrook, J.; Russell, D. W.). The bacteria were collected and broken by the ultrasonic wave. The purified TDO was obtained through the column by elution.

24 μL of enzyme (TDO) was diluted 100 times with 50 mM KPB to 2400 μL. The concentration of enzyme solution was 2.6ng/μL. A 96 well reaction plate (AXYGEN, PCR-96-FLT-C) (hereinafter referred to as the reaction plate) was added with the enzyme solution at 24 μL/well. The blank well was added with 24 μL of KPB [Preparation of KPB buffer (50mM): 6.805 g of KH2PO4 was weighed by an analytical balance, and placed into a 1000 ml of beaker, deionized water was added with a measuring cylinder to 900 ml, the pH was adjusted to 6.5 by 1M KOH, then the mixture was introduced into a 1 L measuring cylinder, and water was added to 1 L. It was stored at 4°C]. 1 μL of a compound or DMSO was added into the corresponding wells in the reaction plate. Preparation of solution A: 200 μL of 500 mM sodium ascorobate was added with 1050 μL of KPB, then the mixture was mixed uniformly for 3 seconds at the maximum speed in a turbine mixer. Solution B: 100 μL of 10 mM tryptophan was added with 100 μL of 100000 unit/ml catalase, 5 μL of 10 mM methylene blue, and 1050 μL of KPB sucessively, then the mixture was mixed uniformly for 3 seconds at the maximum speed in a turbine mixer. 1200 μL of solution A and 1200 μL of solution B were taken and mixed uniformly for 3 seconds at the maximum speed in a turbine mixer. The mixture was added to the reaction plate at 24 μL/well. The reaction plate was placed in a plate centrifuge and centrifuged for 15 seconds at the maximum speed, so the reaction liquids were converged to the bottom. The reaction mixture was mixed uniformly for 30 seconds on a shaker, and incubated for 1 hour at 37°C in a constant temperature incubator. In the reaction plate, 30% (W/V) trichloroacetic acid was added at 10 μL/well, then the mixture was incubated for 15 minutes at 65°C in a incubator. The reaction plate was centrifuged in a centrifuge for 5 minutes at 4700RPM at room temperature. 40 μL of the supernatant was transferred from the reaction plate to the corresponding 96 wells test plate (Corning, #3599) by a multi-channel pipette. 2% (W/V) 4 (dimethylamino)benzaldehyde/glacial acetic acid solution was added at 40 μL/well, then

the mixture was mixed uniformly for 1 minute on a shaker at the maximum speed. After incubation for 2 minutes at room temperature, the absorbance at 480 nm was read on Synergy HT (BIOTEK).

Hela cellular assay

This method is used to determine the inhibition effect of the test compounds on the activity of IDO in Hela cells. (Note: indoleamine 2,3-dioxygenase (IDO) is expressed in the Hela cell line and induced by interferon gamma (INFγ))

Hela cell suspension was prepared with a fresh cell medium, and added into a 96 cell late with 100 μL culture system at 10000 cells/well，then incubated for 24 hours in 5% carbon dioxide at 37°C. The supernatant was removed, serum-free OMEM high glucose medium was added at 90 μL/well, then the test compounds contained in the culture medium with INFγ and tryptophan were added at 10 μL/well (the final concentration: 10000, 1000, 100, 10, 1, 0.1 nM), the mixture was incubated for 48 hours in 5% carbon dioxide at 37°C. 80 μL of the supernate was transferred from the 96-well cell culture plate to a 96 well round-bottomed plate, then 30% *(WN)* trichloroacetic acid was added at 16μL/well, then the mixture was incubated for 25 minutes at 65°C in an incubator. The reaction plate was centrifuged in a centrifuge for 5 minutes at 4700RPM. 50 μL of the supernatant was transferred from the reaction plate to a 96-well flatbottomed transparent plate by a multi-channel pipette. 2% *(WN)* 4-(dimethylamino)benzaldehyde/glacial acetic acid solution was added at 50 μL/well, then the mixture was mixed uniformly for 1 minute on a shaker. After incubation for 2 minutes at room temperature, the absorbance at 480 nm was read on Synergy HT Reader

Table S1 The mean value of all IC_{50} and the standard deviations

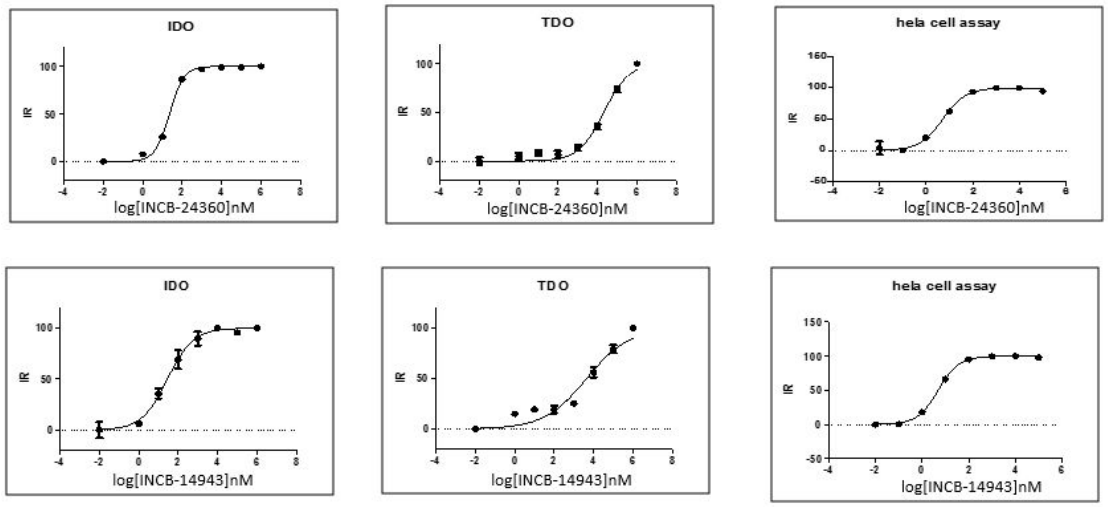
Compound	IDO1 IC_{50} (nM)	IDO1_SD	TDO IC_{50} (nM)	TDO_SD	Hela IC_{50} (nM)	Hela_SD
INCB-24360	26 (3)	11	10000 (3)	7593	5 (3)	1.7
1	51 (2)	4.1	11808 (2)	4391	1772 (2)	1147
2	117 (1)	39.4	63091 (1)	21741	272 (1)	12.8
3	467 (1)	ND	100000 (1)	ND	1677 (1)	ND
4	410 (1)	ND	79985 (2)	ND	1428 (1)	ND

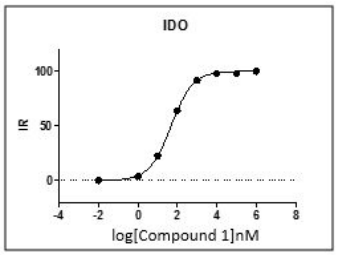
5	132 (2)	47.4	79348 (2)	24338	63 (2)	9.4
6	497 (1)	ND	100000 (1)	ND	578 (1)	ND
7	198 (3)	81	58738 (3)	28188	21 (3)	16.7
8	102 (1)	ND	100000 (1)	ND	291 (1)	ND
9	150 (3)	18	100000 (3)	ND	197 (3)	139.3
10	80 (1)	ND	80960 (1)	ND	204 (1)	ND
11	149 (1)	ND	100000 (1)	ND	1019 (1)	ND
12	106 (1)	ND	100000 (1)	ND	281 (1)	ND
13	40 (2)	8	57970 (2)	59439	54 (2)	17.9
14	14 (7)	4.4	100000 (7)	ND	15 (7)	9.8
15	43 (1)	ND	89770 (1)	ND	28 (1)	ND
16	24 (1)	ND	100000 (1)	ND	262 (1)	ND
17	98 (1)	ND	65721 (1)	ND	150 (1)	ND
18	63 (12)	17.5	62457 (12)	19302	45 (12)	14.7

ND=Not determined

The numbers of the experiments are shown in the brackets behind each IC50 values.

Figure S1 The Dose-response curves for all measured IC_{50}

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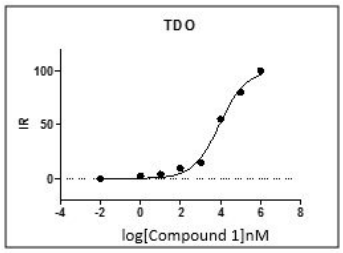
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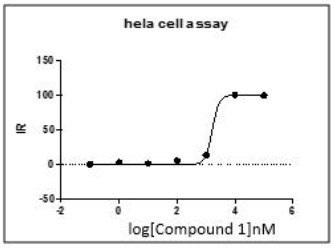
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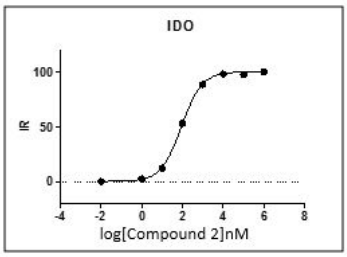
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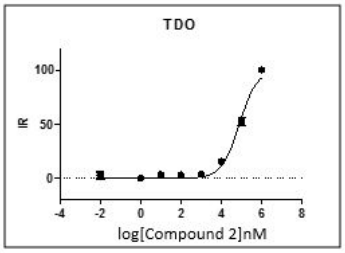
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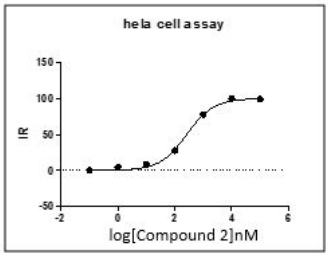
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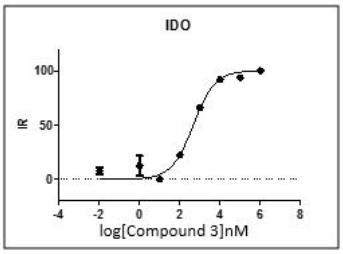
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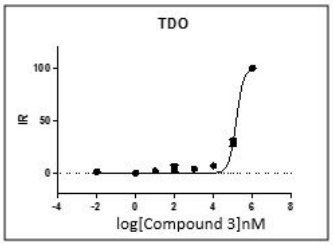
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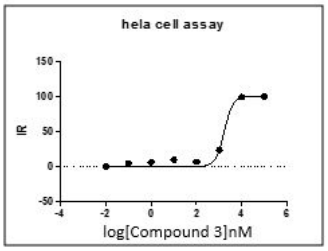
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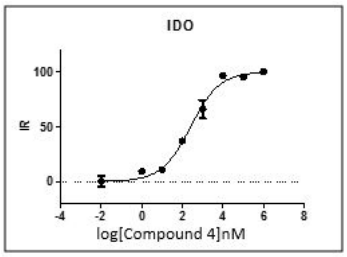
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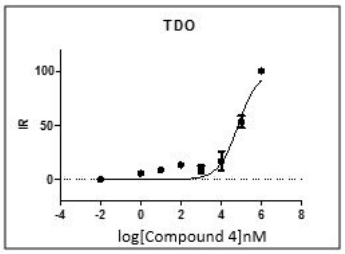
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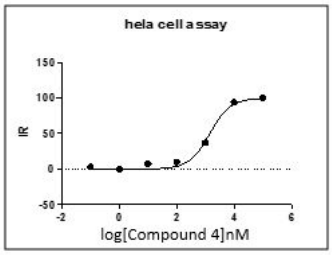
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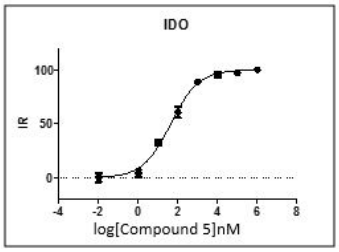
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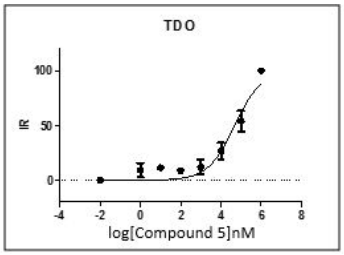
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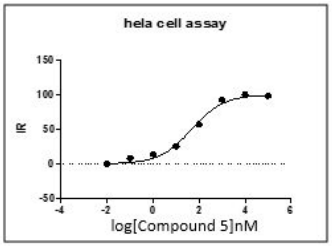
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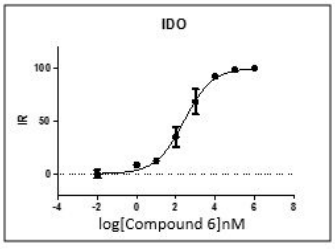
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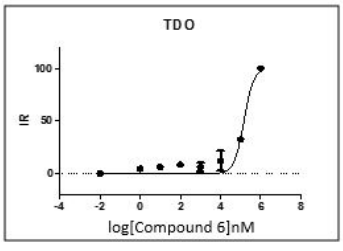
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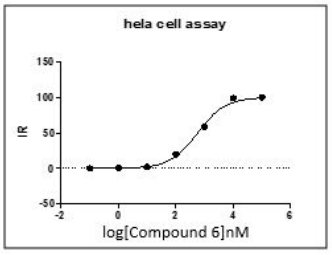
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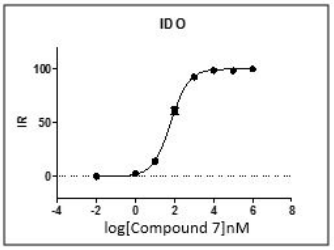
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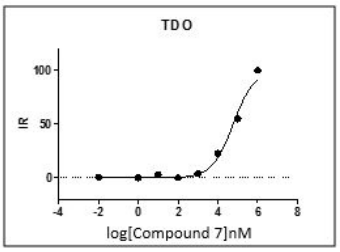
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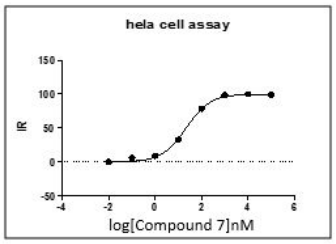
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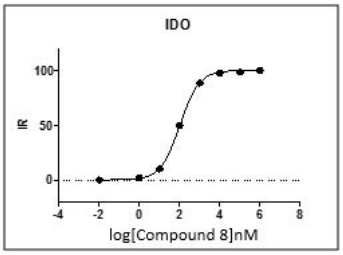
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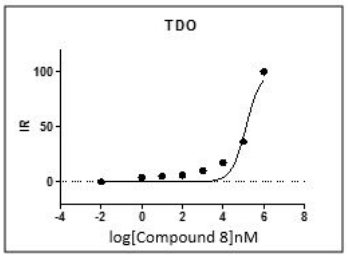
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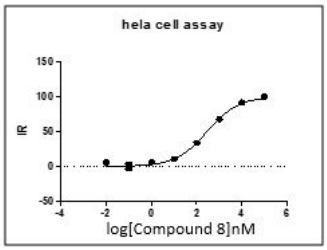
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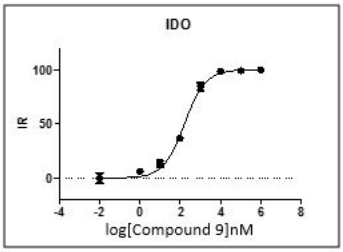
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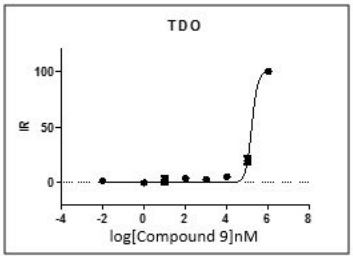
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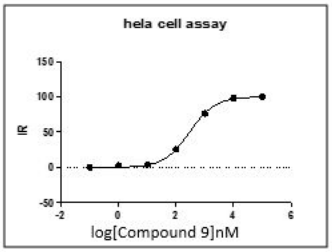
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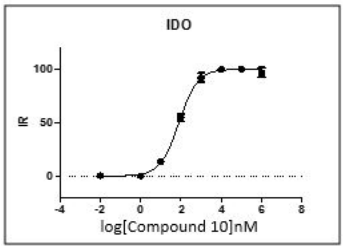
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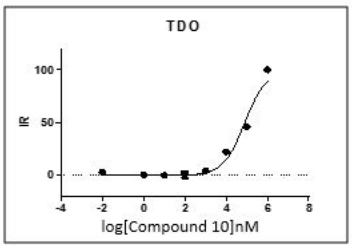
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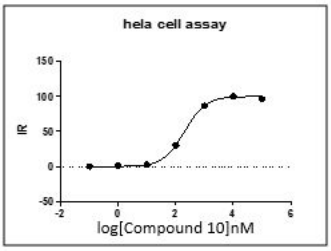
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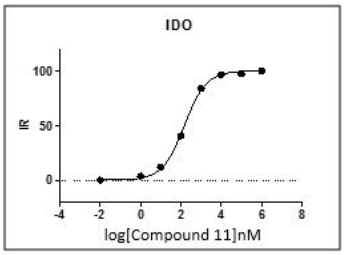
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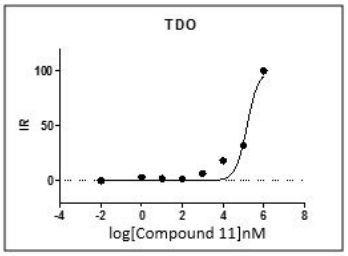
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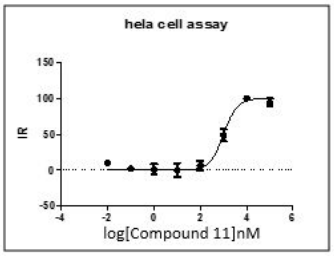
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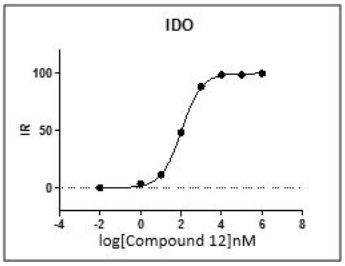
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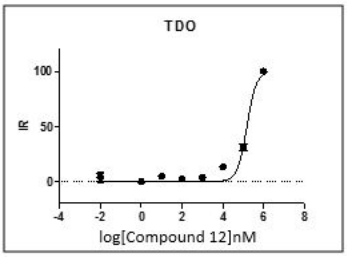
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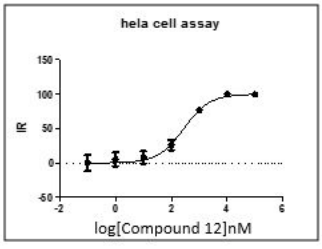
hela cell assay
IR
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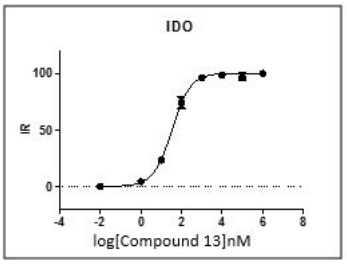
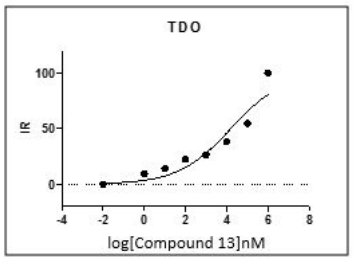
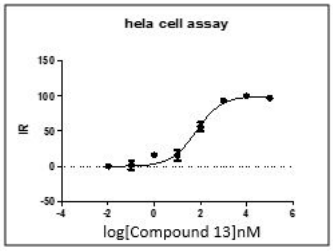
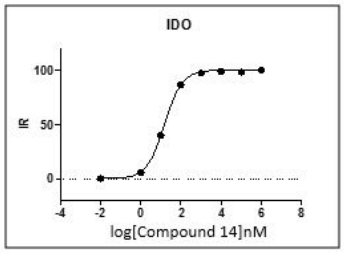
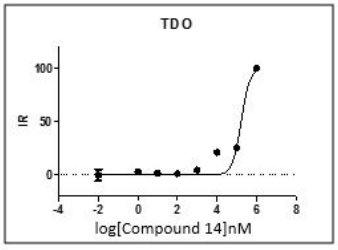
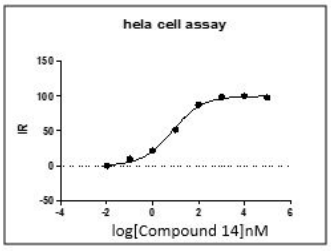


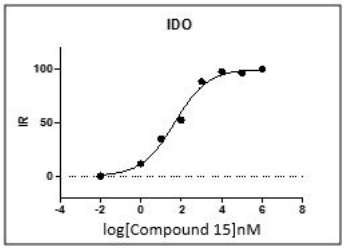


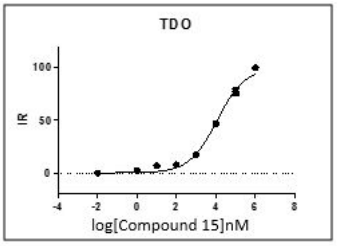


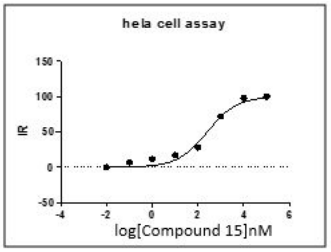


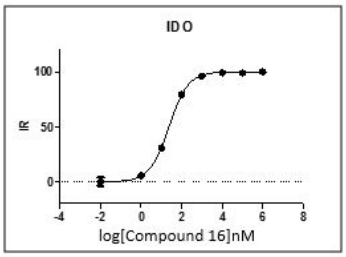


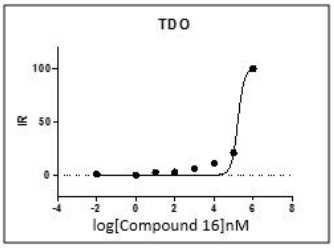


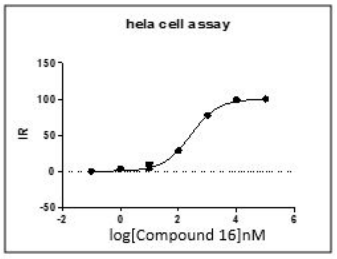


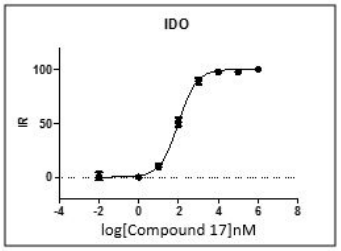


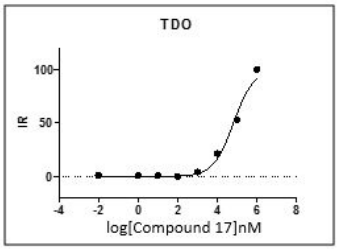


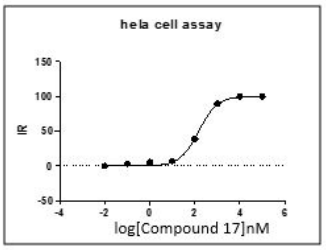


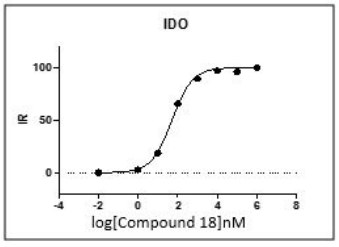


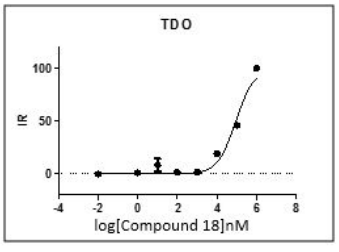


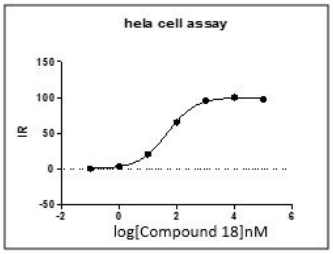












Plasma protein bonding

Mouse, dog and human plasma protein bonding of test compound was determined via

Rapid Equilibrium Dialysis (RED) system according to method described in literatures.

Wan H, Rehngren M. High throughput screening of protein binding by equilibrium dialysis combined with liquid chromatography and mass spectrometry. *J.Chromatogr A*. 2006, 1102, 125-134.

Jones R, Williams G, Sohal B, et al.Validation of a rapid equilibrium dialysis approach for the measurement of plasma protein binding. *J.Pharm.Sci.* 2008, 97, 4586-4595.

Liver microsome stability

Stability of test compound in rat and human liver microsome was determined according to method described in literatures.

Masimirembwa CM, Bredberg U, et al. Metabolic stability for drug discovery and development. Clin Pharmacokinet, 2003, 42(6): 515-528.

Brian Davies, Tim Morris. Physiological Paramaters in Laboratory Animals and Humans. Pharmaceutical Research, 1993,Vol. 10, No. 7:1093-1095.

hERG patch clamp assay

hERG inhibitory activity of test compound was determined by the Automated Patch-Clamp system according to method described in literature.

Kutchinsky, J; Friis, S.; Asmild, M., et al. Characterization of potassium channel modulators with QPatch automated patch-clamp technology: system characteristics and performance. Assay Drug Dev. Technol. 2003, 1, 685-693.

CYP inhibition assay

The inhibitory IC50 values of tested compounds for five major P450 enzymes were determined in human liver microsome (BD Gentest) according to method described in literatures.

Li, G., Huang,K., Nikolic, D., and B. van Breemen, R., High-Throughput Cytochrome P450 Cocktail Inhibition Assay for Assessing Drug-Drug and Drug-Botanical Interactions. Drug Metab Dispos 43:1670–1678, 2015.

9. FDA Guidance for Industry: Drug Interaction Studies-Study Design, Data Analysis, and

Implications for Dosing and Labeling, 2006.

Table S2. CYP, hERG Inhibition and Solubility of Compounds 13-15, 18

Compound	1A2 IC50 (uM)	2C9 IC50 (uM)	2C19 IC50 (uM)	2D6 IC50 (uM)	3A4 IC50 (uM) m/t[a]	hERG (uM)	Solubility (FassIF) (uM)	Solubility (PDS7.4) (uM)
13	>30	>30	>30	>30	>30	>30	ND[b]	ND[b]
14	>30	>30	>30	>30	>30	12.2	0.86	1.87
15	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	5.5	ND[b]	ND[b]
18	>30	>30	>30	23.5	>30	>30	57.33	99.91

[a]midazolam as substrate/testosterone as substrate, ND[b] = not determined.

Pharmacokinetic Assays

Pharmacokinetics in the rats, mice and dogs

Compound **18** was administrated with its suspension in the mixture of 0.5% CMC-Na for p.o and its solution in the mixture of 1% DMSO+99% saline for i.v., respectively.

Sprague-Dawley rats (160–180 g) were obtained from Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China, certificate no. SCXK-2008-0016) and housed with free access to food and water. After a 12 h fast, two males and two females were administrated by oral gavage at a dose of 10 mg/kg compound **18** suspension. The other two males and two females were administrated by intravenous injection at a dose of 1 mg/kg compound **18**. Blood samples (0.1 mL) were collected via the posterior orbital venous plexus at times of 0.083, 0.25, 0.5, 1, 2, 4, 8, 11 and 24 h after administration. Plasma was separated by immediate centrifugation and was kept at -20°C until analyzed.

c57bl/6 mice (20-25 g) were obtained from Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China, certificate no. SCXK-2008-0016) and housed with free access to food and water. After a 12 h fast, nine females were administrated by oral gavage at a dose of 10 mg/kg compound **18** suspension. The other nine females were administrated by intravenous injection at a dose of 2 mg/kg compound **18**. Blood samples (0.2 mL) were collected via the posterior orbital venous plexus at times of 0.083, 0.25, 0.5, 1, 2, 4, 8, 11 and 24 h after administration. Plasma was separated by immediate centrifugation and was kept at -20°C until analyzed.

Beagle dogs were obtained from Suzhou Xishan Zhongke Laboratory Animal Co.,Ltd. After a 12 h fast, two males and two females were administrated by oral gavage at a dose of 2 mg/kg compound **18** suspension. The other two males and two females were administrated by intravenous injection at a dose of 0.5 mg/kg compound **18**. Blood samples (1.0 mL) were collected via the forelimb vein at times of 0.083, 0.25, 0.5, 1, 2, 4, 8, 11 and 24 h after administration. Plasma was separated by immediate centrifugation and was kept at -20°C until analyzed.

In Vivo Pharmacodynamic Study

All animal experimentations described in this study were conducted in accordance with the Guiding Principles in the Care and Use of Animals by the American Physiological Society. All animals used for *in vivo* studies were treated in accordance with Institutional Guide for the Care and Use of Laboratory Animals.

The test compound was administrated (i.g.) to C57BL/6 mice (6 animals, 3 male+3 female/group). Blood samples were collected at 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 11.0, 24.0 h after dosing. The plasma concentrations of test compound and kynurenine were measured. Kynurenine reduction ratio = C0-Ct/C0×100%.

In Vivo Efficacy Study

MC38 Xenograft model in hPD1 transgenic mouse

Xenograft model was developed in hPD1 transgenic mice with human MC38 cancer cell line purchased from American Type Culture Collection (USA). MC38 cells (1×10^5) were implanted s.c. into the hind flank region of each mouse and allowed to grow to the designated size (c.a. 100 mm^3) before administration of test compound. Animals were randomly divided into 6 groups and each group with 8 mice. For the combination group, the test compound was given orally at various dose levels twice daily for 14 days and SHR-1210 (produced by Shanghai Hengrui Pharmaceutical Co., Ltd.) was given intraperitoneally every other day for 8 times.

Tumor response was determined by measurement of tumors with a digital caliper twice weekly. Tumor volume (mm^3) was estimated from the formula: Tumor volume = 1/2 (length × $(width)^2$). Treated animals were checked daily for treatment related toxicity/mortality. Body weights were collected on each group of animals before the

initiation of treatment (Wt1) and then again following the last treatment dose (Wt2). The difference in body weight (Wt2-Wt1) provides a measure of treatment-related toxicity. Additional weights were recorded at each measurement date to monitor toxicity.

Study was terminated when mean tumor volume exceeded 2000mm^3 for any group according to the guidelines of IACUC.

Computational Methods

Small Molecule Preparation

Molecules were constructed in MOE and ionized using MOE's WashMDB function, and hydrogens were added. The small molecule conformation was minimized to a gradient of 0.01 in the MMFF94S force field using distance dependent dielectric constant of 1.

Protein Preparation

Chain A of the IDO-1 crystal structure (PDB: 6E40) was used for all docking runs. Waters greater than 4.5Å from the protein were removed before protons were added and the H-bonding network optimised using MOE's Protonate3D protocol. The Amber10:EHT force field in MOE was used, and iron was parametrized in the Fe^{2+} state. A stepwise minimization followed for residues within 8 Å of the ligand using a quadratic force constant (10) to tether the atoms to their starting geometries.

Docking Calculation

The active site was defined using the ligand in the crystal structure. MOE general docking mode, where the ligand sampling was set flexible, was used for the docking. All other parameters were left at their default values. The top-scored conformation was selected as the best docking result.

General Experimental Procedures and Analytical Characterization Methods

All purchased starting materials were used without further purification. 1HNMR spectra were acquired on a Bruker Avance-400 spectrometer (400 MHz), with tetramethylsilane (TMS) as an internal standard; chemical shifts are expressed in parts per million (ppm, δ units). Mass spectra were obtained on an ACQUITY UPLC-SQD (ESI) from Waters

Corporation (U.S.). Most masses were reported as those of the protonated parent ions. High-resolution mass spectra (HRMS) were recorded on a Thermo Q Exactive instrument (ESI). Where noted, compounds were determined to be >95% pure by analytical reverse-phase HPLC. HPLC conditions: an isocratic program using 60-80% methanol, 40-20% water, and 0.1% aqueous ammonia was employed on a Gemini C18 column (250 mm, 4.6 mm). The flow rate was 1.0 mL/min, and UV detection was at 214 and 254 nm. Chiral separation and chiral analysis were performed on HPLC equipped with Daicel chiral columns. Melting point was measured on a SGW X-4 melting point detector.

Abbreviations used

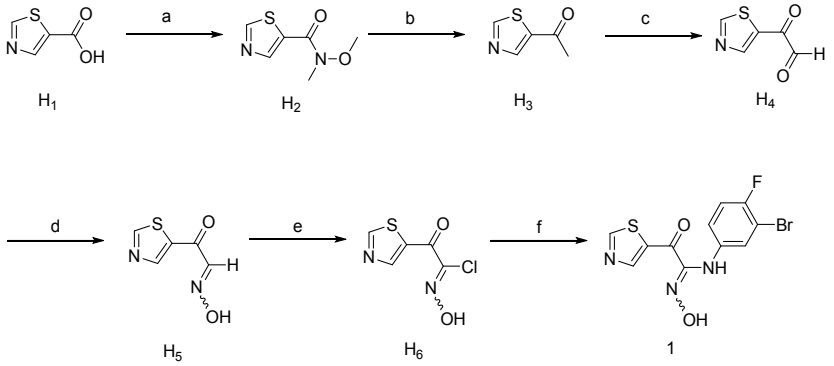
DMSO: dimethyl sulfoxide; DMF: dimethyl formamide; DCM: dichloromethane;

THF: tetrahydrofuran; DME: 1,2-Dimethoxyethane; TEA: triethylamine; MsCl:

Methanesulfonyl chloride; BINAP: 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl; TFA: trifluoroacetic acid; dba: dibenzylideneacetone; DMHH: N,O-dimethylhydroxylamine hydrochloride; DMAP: 4-dimethylaminopyridine; Boc: di-tert-butyl dicarbonate; NCS: N-chlorosuccinimide; EDC: 3-(ethyliminomethylideneamino)-N,N-dimethylpropan-1-amine

Experimental Procedures and Analytical Data for key compounds

Scheme S1. Synthesis of compound 1[a]



[a] Reagents and conditions: (a) DMHH, EDC·HCl, DMAP, DCM, rt, 16h, 93%; (b) CH_3MgBr, THF, rt, 2h, 79%; (c) SeO_2, 1,4-dioxane, 90°C, 6h, >100%; (d) $NH_2OH·HCl$, K_2CO_3, CH_3OH, rt, 2h, 26%; (e) NCS, DMF, rt, 2h, >100%; (f) 3-bromo-4-fluoroaniline, EtOH, rt, 16h, 13%.

***N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-oxo-2-(thiazol-5-yl)acetimidamide (1)**

Step A: *N*-methoxy-*N*-methylthiazole-5-carboxamide (**H2**)

DMAP(1.70 g, 13.9 mmol) was added to a solution of thiazole-5-carboxylic acid(1.80 g,

13.9 mmol) **H1** and N,O-dimethylhydroxylamine hydrochloride(1.62 g, 16.7 mmol) in DCM(30 mL). The resulting mixture was stirred at room temperature for 16h. After the completion of the reaction, the solution was concentrated under pressure. The resulting residue was purified by silica gel column chromatography with ethyl acetate/hexane (1:2) to give the desired product(2.22 g, 93%) as colorless oil.

Step B: 1-(thiazol-5-yl)ethanone (**H3**)

Methylmagnesium bromide(4.7 mL, 14.2 mmol, 3M in diethyl ether) was added to a solution of N-methoxy-N-methylthiazole-5-carboxamide(4.00 g, 23.2 mmol) **H2** in THF(30 mL) . The resulting mixture was stirred at room temperature for 2h. After the completion of the reaction, 25 mL of water was added dropwise to quench the reaction at 0°C, and extracted with ethyl acetate(20 mL×3). The combined ethyl acetate layers were washed with water(20 mL×3), saturated NaCl(20 mL×3), and dried over sodium anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography with ethyl acetate/hexane (1:2) to give the desired product(1.30 g, 79%) as yellow solid.

Step C: 2-oxo-2-(thiazol-5-yl)acetaldehyde (**H4**)

Selenium dioxide(1.20 g, 22.0 mmol) was added to a solution of 1-(thiazol-5-yl)ethanone(1.40 g, 11.0 mmol) **H3** in 1,4-dioxane(20 mL) . The resulting mixture was stirred at 90°C for 6h. After the completion of the reaction, the solution was concentrated under pressure. The resulting residue was purified by silica gel column chromatography with ethyl acetate/hexane (1:3) to give the crude product (1.60 g, >100%) as yellow viscous solid.

Step D: 2-oxo-2-(thiazol-5-yl)acetaldehyde oxime (**H5**)

To a solution of 2-oxo-2-(thiazol-5-yl)acetaldehyde(1.60 g, 11.3 mmol) **H4** in MeOH(10 mL), was added Potassium carbonate(2.34 g, 17.0 mmol), and then hydroxylamine hydrochloride(0.47 g, 6.8 mmol). The resulting mixture was stirred at room temperature for 2h. After the completion of the reaction, the solution was filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography with ethyl acetate/hexane (1:1) to give the desired product (0.46 g, 26%) as yellow solid.

LC-MS (ESI) *m/z*: calcd for $C_5H_4N_2O_2S$ $[M+H]^+$ 157.0; found 157.1.

Step E: *N*-hydroxy-2-oxo-2-(thiazol-5-yl)acetimidoyl chloride (**H6**)

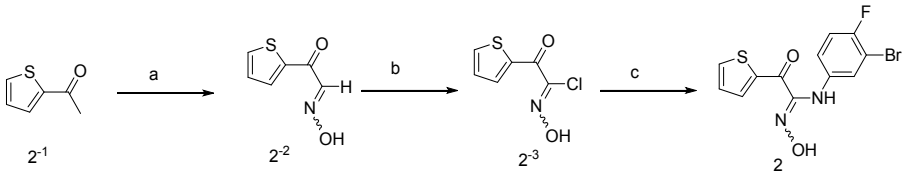
To a solution of 2-oxo-2-(thiazol-5-yl)acetaldehyde oxime (0.46 g, 2.9 mmol) **H5** in

DMF(10 mL), was added NCS(0.39 g, 2.9 mmol). The resulting mixture was stirred at room temperature for 2h. After the completion of the reaction, 25 mL of water was added dropwise to quench the reaction, and extracted with ethyl acetate(20 mL×3). The combined ethyl acetate layers were washed with water(20 mL×3), saturated NaCl(20 mL×3), and dried over sodium anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure to give the desired product (0.84 g, >100%) as brown oil, which was used directly in the next step without further purification.

Step F: *N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-oxo-2-(thiazol-5-yl)acetimidamide (**1**)
To a solution of *N*-hydroxy-2-oxo-2-(thiazol-5-yl)acetimidoyl chloride (0.84 g, 4.4 mmol) **H6** in EtOH(20 mL), was added 3-bromo-4-fluoroaniline(1.26 g, 6.6 mmol). The resulting mixture was stirred at room temperature for 16h. After the completion of the reaction, the mixtrue was filtered, and then the solid was washed with water and ethyl acetate. The solid was recrystallized by MeOH to give the desired product (0.20 g, 13%) as yellow solid.

LC-MS (ESI) *m*/*z*: calcd for $C_{11}H_7BrFN_3O_2S$ $[M+H]^+$ 343.9, 345.9; found 344.1, 346.1. ^{1}HNMR (400 MHz, DMSO-*d*6) δ 11.93 (s, 1H), 9.39 (s, 1H), 8.77 (s, 1H), 8.72 (s, 1H), 7.16-7.20 (m, 1H), 7.10-7.12 (m, 1H), 6.75-6.79 (m, 1H).

Scheme S2. Synthesis of compound 2[a]



[a] Reagents and conditions: (a) *tert*-Butyl nitrite, 4M HCl in 1,4-dioxane, 1,4-dioxane, 0°C, 1h, 49%; (b) NCS, DMF, rt, 1h, >100%; (c) 3-bromo-4-fluoroaniline, EtOH, rt, 16h, 55%.

_N_-(3-bromo-4-fluorophenyl)-_N′_-hydroxy-2-oxo-2-(thiophen-2-yl)acetimidamide (2)

Step A: 2-oxo-2-(thiophen-2-yl)acetaldehyde oxime (**2-2**)
To a solution of 1-(thiophen-2-yl)ethanone(1.00 g, 7.93 mmol) **2-1** in 1,4-dioxane(10 mL) at 0°C, was added 4M HCl in 1,4-dioxane(10 mL), and then *tert*-Butyl nitrite(0.82 g, 7.93 mmol). The resulting mixture was stirred at 0°C for 1h. After the completion of the reaction, 25 mL of water was added dropwise to quench the reaction, and extracted with ethyl acetate(20 mL×3). The combined ethyl acetate layers were washed with water(20 mL×3), saturated NaCl(20 mL×3), and dried over sodium anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure and purified by silica gel column chromatography with ethyl acetate/hexane (1:5) to give the desired product (0.60 g, 49%) as yellow solid.

LC-MS (ESI) *m*/*z*: calcd for $C_5H_4N_2O_2S$ $[M-H]^-$ 154.0; found 154.2.

Step B: *N*-hydroxy-2-oxo-2-(thiophen-2-yl)acetimidoyl chloride (**2-3**)

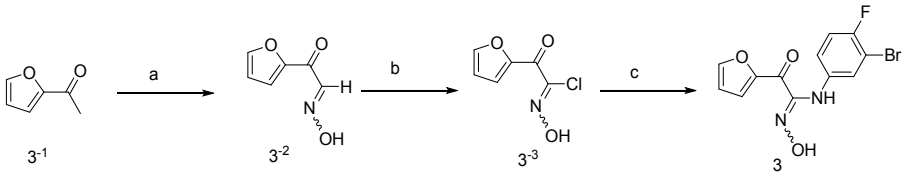
To a solution of 2-oxo-2-(thiophen-2-yl)acetaldehyde oxime (0.10 g, 0.64 mmol) **2-2** in DMF(5 mL), was added NCS(86 mg, 0.64 mmol). The resulting mixture was stirred at room temperature for 1h. After the completion of the reaction, 25 mL of water was added dropwise to quench the reaction, and extracted with ethyl acetate(20 mL×3). The combined ethyl acetate layers were washed with water(20 mL×3), saturated NaCl(20 mL×3), and dried over sodium anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure to give the desired product (0.20 g, >100%) as yellow oil, which was used directly in the next step without further purification.

Step C: *N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-oxo-2-(thiophen-2-yl)acetimidamide **(2)**

To a solution of *N*-hydroxy-2-oxo-2-(thiophen-2-yl)acetimidoyl chloride (0.10 g, 0.53 mmol) **2-3** in EtOH(10 mL), was added 3-bromo-4-fluoroaniline(0.10 g, 0.53 mmol). The resulting mixture was stirred at room temperature for 16h. The resulting mixture was concentrated under reduced pressure and purified by silica gel column chromatography with ethyl acetate/hexane (1:5) to give the desired product (0.10 g, 55%) as yellow solid.

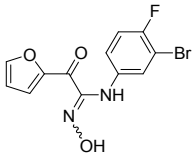
LC-MS (ESI) *m*/*z*: calcd for $C_{12}H_8BrFN_2O_2S$ $[M+H]^+$ 343.0, 345.0; found 343.1, 345.1. ^{1}HNMR (400 MHz, DMSO-*d*6) δ 11.62 (s, 1H), 8.75 (s, 1H), 8.05-8.12 (m, 2H), 7.08-7.28 (m, 3H), 6.71-6.73 (m, 1H).

Scheme S3. Synthesis of compound 3[a]



[a] Reagents and conditions: (a) *tert*-Butyl nitrite, 4M HCl in 1,4-dioxane, 1,4-dioxane, 0°C, 1h, 50%; (b) NCS, DMF, rt, 1h, >100%; (c) 3-bromo-4-fluoroaniline, EtOH, rt, 16h, 3%.

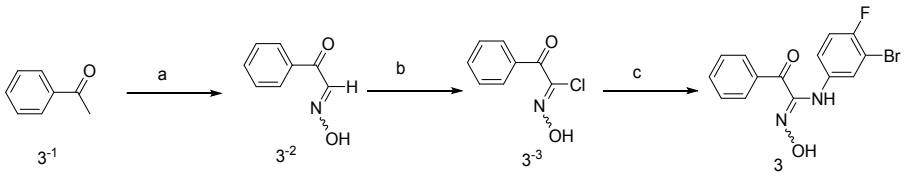
***N*-(3-bromo-4-fluorophenyl)-2-(furan-2-yl)-*N′*-hydroxy-2-oxoacetimidamide (3)**



Prepared in a fashion similar to that used for the synthesis of **2**. LC-MS (ESI) *m*/*z*: calcd for $C_{12}H_8BrFN_2O_3$ $[M+H]^+$ 327.0, 329.0; found 326.9, 328.9. ^{1}HNMR (400 MHz, DMSO-*d*6) δ 11.51 (s, 1H), 8.72 (s, 1H), 8.11 (d, 1H), 7.57 (d, 1H), 7.16-7.19 (m, 1H), 7.07-7.09

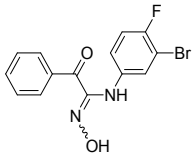
(m, 1H), 6.77-6.78 (m, 1H), 6.68-6.71 (m, 1H).

Scheme S4. Synthesis of compound 4[a]



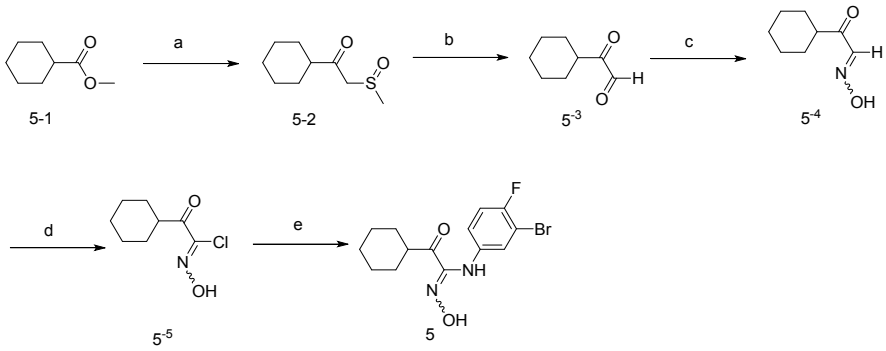
[a] Reagents and conditions: (a) *tert*-Butyl nitrite, 4M HCl in 1,4-dioxane, 1,4-dioxane, 0°C, 1h, 54%; (b) NCS, DMF, rt, 1h, >100%; (c) 3-bromo-4-fluoroaniline, EtOH, rt, 16h, 25%.

_N_-(3-bromo-4-fluorophenyl)-_N′_-hydroxy-2-oxo-2-phenylacetimidamide (4)



Prepared in a fashion similar to that used for the synthesis of **2**. LC-MS (ESI) *m*/*z*: calcd for $C_{14}H_{10}BrFN_2O_2$ $[M+H]^+$ 337.0, 339.0; found 337.3, 339.3. ^{1}HNMR (400 MHz, DMSO-*d*6) δ 11.49 (s, 1H), 8.83 (s, 1H), 7.95-7.97 (m, 2H), 7.52-7.70 (m, 3H), 7.11-7.18 (m, 2H), 6.71-6.75 (m, 1H).

Scheme S5. Synthesis of compound 5[a]



[a] Reagents and conditions: (a) NaH, DMSO, 75°C, 3h, 95%; (b) HCl, DMSO, rt, 40h, 56%; (c) $NH_2OH{\cdot}HCl$, K_2CO_3, CH_3OH, rt, 2h, 63%; (d) NCS, DMF, rt, 2h, >100%; (e) 3-bromo-4-fluoroaniline, EtOH, rt, 16h, 9%.

_N_-(3-bromo-4-fluorophenyl)-2-cyclohexyl-_N′_-hydroxy-2-oxoacetimidamide (5)

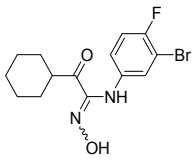
Step A: 1-cyclohexyl-2-(methylsulfinyl)ethanone (**5-2**)

To a solution of methyl cyclohexanecarboxylate(10.10 g, 70.0 mmol) **5-1** in DMSO(70 mL), was added NaH(6.27 g, 15.5 mmol, 60%). The resulting mixture was stirred at 75°C for 3h. After the completion of the reaction, 100 mL of water was added dropwise to quench the reaction, and extracted with ethyl acetate(50 mL×3). The combined ethyl acetate layers were washed with water(50 mL×3), saturated NaCl(50 mL×3), and dried over sodium anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure to give the desired product (12.6 g, 95%) as red oil, which was used directly in the next step without further purification.

Step B: 2-cyclohexyl-2-oxoacetaldehyde (**5-3**)
To a solution of 1-cyclohexyl-2-(methylsulfinyl)ethanone(12.60 g, 67.0 mmol) **5-2** in DMSO(25 mL), was added 1.4M HCl(150ml). The resulting mixture was stirred at rt for 40h. After the completion of the reaction, the mixtrue was filtered, and then the solid was washed with water. The solid was dried in vacuum to give the desired product (7.10 g, 56%) as yellow solid.

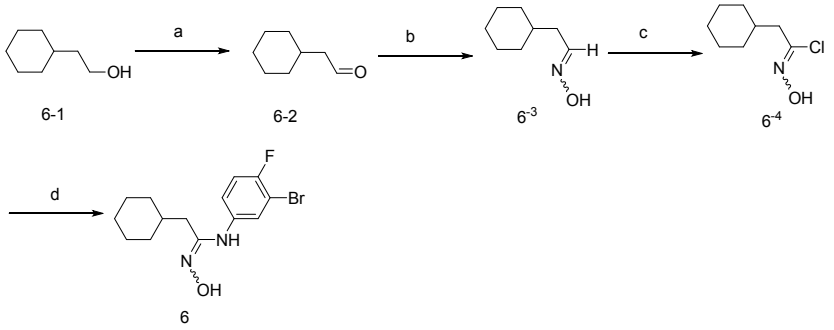
Step C to Step E: Prepared in a fashion similar to that used for the synthesis of **1**.

N-(3-bromo-4-fluorophenyl)-2-cyclohexyl-*N′*-hydroxy-2-oxoacetimidamide (5)



LC-MS (ESI) *m/z*: calcd for $C_{14}H_{16}BrFN_2O_2$ $[M+H]^+$ 343.0, 345.0; found 343.2, 345.2. ^{1}HNMR (400 MHz, $CDCl_3$) δ 7.09-7.11 (m, 1H), 6.98-7.03 (m, 1H), 6.82-6.85 (m, 1H), 3.21-3.26 (m, 1H), 1.79-1.88 (m, 4H), 1.24-1.42 (m, 6H).

Scheme S6. Synthesis of compound 6[a]



[a] Reagents and conditions: (a) PCC, DCM, rt, 3h, 78%; (b) $NH_2OH·HCl$, K_2CO_3, CH_3OH, rt, 2h, 99%; (c) NCS, DMF, rt, 2h, >100%; (d) 3-bromo-4-fluoroaniline, EtOH, rt, 16h, 27%.

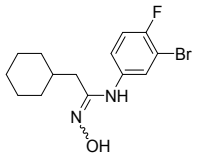
_N_-(3-bromo-4-fluorophenyl)-2-cyclohexyl-_N′_-hydroxyacetimidamide (6)

Step A: 2-cyclohexylacetaldehyde (**6-2**)
To a solution of 2-cyclohexylethanol(3.00 g, 23.4 mmol) **6-1** in DCM(600 mL), was added

PCC(7.74 g, 35.1 mmol). The resulting mixture was stirred at rt for 3h. After the completion of the reaction, 500 mL of diethyl ether was added and stirred for 1h. The resulting mixture was filtered. The filtrate was concentrated under reduced pressure and purified by silica gel column chromatography with ethyl acetate/hexane (1:5) to give the desired product (2.30 g, 78%) as colourless oil.

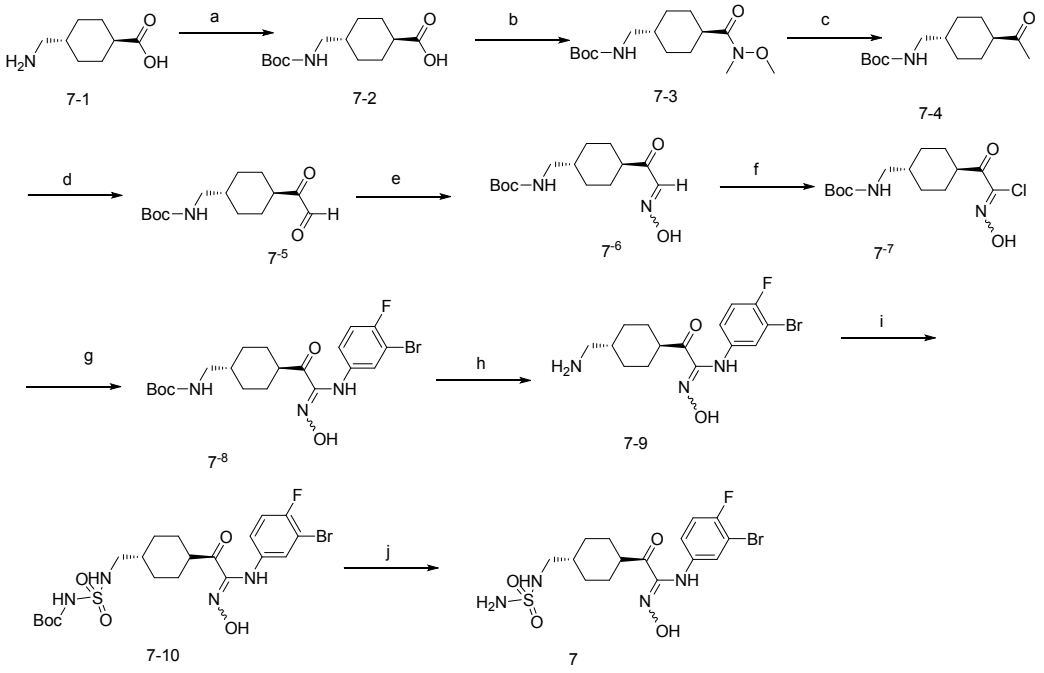
Step B to Step D: Prepared in a fashion similar to that used for the synthesis of **1**.

N-(3-bromo-4-fluorophenyl)-2-cyclohexyl-*N*′-hydroxyacetimidamide (6)



LC-MS (ESI) *m*/*z*: calcd for $C_{14}H_{18}BrFN_2O$ $[M+H]^+$ 329.1, 331.1; found 329.3, 331.3. ^{1}HNMR (400 MHz, DMSO-*d*6) δ 9.25 (s, 1H), 8.09-8.13 (m, 2H), 7.29-7.34 (m, 1H), 7.16-7.21 (m, 1H), 2.28-2.30 (m, 2H), 1.64-1.75 (m, 5H), 0.97-1.23 (m, 6H).

Scheme S7. Synthesis of compound 7[a]



[a] Reagents and conditions: (a) Boc_2O, NaOH, 1,4-dioxane, H_2O, rt, 4h, >100%; (b) DMHH, EDC·HCl, DMAP, DCM, rt, 16h, 75%; (c) CH_3MgBr, THF, rt, 2h, 71%; (d) SeO_2, 1,4-dioxane, 90°C, 6h, 80%; (e) $NH_2OH·HCl$, K_2CO_3, CH_3OH, rt, 2h, 70%; (f) NCS, DMF, rt, 2h, 69%; (g) 3-bromo-4-fluoroaniline, EtOH, rt, 16h, 8%; (h) 4M HCl in 1,4-dioxane, rt, 15mins, >100%; (i) chlorosulfonyl isocyanate, *tert*-butanol, Et_3N, DCM, rt, 30mins, 87%; (j) 4M HCl in 1,4-dioxane, rt, 2h, 32%.

***N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-oxo-2-(4-((sulfamoylamino)methyl)cyclohexyl)acetimidamide (7)**

Step A: (1r,4r)-4-(((*tert*-butoxycarbonyl)amino)methyl)cyclohexanecarboxylic acid (**7-2**)
To a solution of (1r,4r)-4-(aminomethyl)cyclohexanecarboxylic acid(5.00 g, 31.8 mmol) **7-1** and NaOH(2.54 g, 63.6 mmol) in 1,4-dioxane(30 mL) and H_2O(30 mL), was added Boc_2O (10.45 g, 47.8 mmol) at 0°C. The resulting mixture was stirred at rt for 4h. After the completion of the reaction, the resulting mixture was concentrated under reduced pressure and added H_2O(80 mL). The reaction liquid was adjusted to pH=3 with hydrochloric acid. The mixtrue was filtered, and then the solid was washed with hexane(50 mL). The solid was dried in vacuum to give the desired product (9.00 g, >100%) as white solid.

Step B to Step G: *tert*-butyl (((1r,4r)-4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)cyclohexyl)methyl)carbamate (**7-8**). Prepared in a fashion similar to that used for the synthesis of **1**. LC-MS (ESI) *m*/*z*: calcd for $C_{20}H_{27}BrFN_3O_4$ $[M-H]^-$ 470.1, 472.1; found 470.3, 472.3.

Step H: 2-((1r,4r)-4-(aminomethyl)cyclohexyl)-*N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-oxoacetimidamide hydrochloride (**7-9**)
To a solution of *tert*-butyl (((1r,4r)-4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)cyclohexyl)methyl)carbamate (46 mg, 0.097 mmol) **7-8** in 4M HCl in 1,4-dioxane(1 mL). The resulting mixture was stirred at rt for 15mins. After the completion of the reaction, the resulting mixture was concentrated under reduced pressure to give the desired product (43 mg, >100%) as yellow solid.

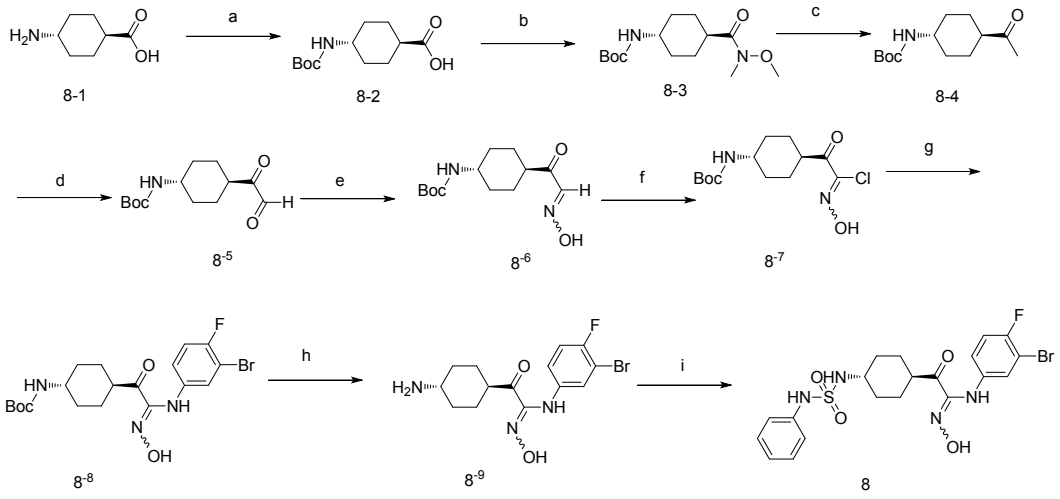
Step I: *tert*-butyl *N*-(((1s,4s)-4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)cyclohexyl)methyl)sulfamoylcarbamate (**7-10**)
To a solution of chlorosulfonyl isocyanate(14 mg, 0.097 mmol) in DCM(1 mL), was added *tert*-butanol (7 mg, 0.097 mmol) at 0°C. The resulting mixture was stirred at 0°C for 15mins. To a solution of 2-((1r,4r)-4-(aminomethyl)cyclohexyl)-*N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-oxoacetimidamide hydrochloride(36 mg, 0.097 mmol) **7-9** in DCM(1 mL), was added above solution and Et_3N(29 mg, 0.29 mmol) at 0°C. The resulting mixture was stirred at rt for 30mins. After the completion of the reaction, 25 mL of water was added dropwise to quench the reaction, and extracted with DCM(20 mL×3). The combined DCM layers were washed with water(20 mL×3), saturated NaCl(20 mL×3), and dried over sodium anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure to give the desired product (46 mg, 87%) as yellow solid, which was used directly in the next step without further purification.

Step J: *N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-oxo-2-(4-((sulfamoylamino)methyl)cyclohexyl)acetimidamide (7)

To a solution of *tert*-butyl *N*-(((1s,4s)-4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)cyclohexyl)methyl)sulfamoylcarbamate (46 mg, 0.083 mmol) **7-10** in 4M HCl in 1,4-dioxane(1 mL). The resulting mixture was stirred at rt for 2h. After the completion of the reaction, 25 mL of saturated $NaHCO_3$(20 mL) was added dropwise to quench the reaction, and extracted with DCM(10 mL×3). The combined DCM layers were washed with water(10 mL×3), saturated NaCl(10 mL×3), and dried over sodium anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure and purified by silica gel column chromatography with DCM/MeOH (10:1) to give the desired product (12 mg, 32%) as yellow solid.

LC-MS (ESI) *m*/*z*: calcd for $C_{15}H_{20}BrFN_4O_4S$ $[M+H]^+$ 451.0, 453.0; found 450.9, 453.3. ^{1}HNMR (400 MHz, DMSO-d6) δ 11.62 (s, 1H), 8.32 (s, 1H), 7.14-7.18 (m, 1H), 6.94-6.96 (m, 1H), 6.67-6.71 (m, 1H), 6.46-6.49 (m, 1H), 6.43 (s, 2H), 3.21-3.31 (m, 1H), 2.70-2.73 (m, 2H), 1.97-2.02 (m, 1H), 1.82-1.88 (m, 4H), 1.36-1.43 (m, 2H), 0.88-0.97 (m, 2H).

Scheme S8. Synthesis of compound 8[a]



[a] Reagents and conditions: (a) Boc_2O, NaOH, 1,4-dioxane, H_2O, rt, 2h, 95%; (b) DMHH, EDC·HCl, DMAP, DCM, rt, 16h, 98%; (c) CH_3MgBr, THF, rt, 2h, 65%; (d) SeO_2, 1,4-dioxane, 90°C, 24h, 75%; (e) $NH_2OH·HCl$, K_2CO_3, CH_3OH, rt, 2h, 60%; (f) NCS, DMF, rt, 2h, 81%; (g) 3-bromo-4-fluoroaniline, EtOH, rt, 16h, 16%; (h) 4M HCl in 1,4-dioxane, rt, 4h, >100%; (i) phenylsulfamoyl chloride, Et_3N, THF, rt, 3h, 18%.

_N_-(3-bromo-4-fluorophenyl)-_N′_-hydroxy-2-oxo-2-(4-((_N_-phenylsulfamoyl)amino)cyclohexyl)acetimidamide (8)

Step A to Step H: 2-((1r,4r)-4-aminocyclohexyl)-*N*-(3-bromo-4-fluorophenyl)-*N*′-

hydroxy-2-oxoacetimidamide hydrochloride (**8-9**). Prepared in a fashion similar to that used for the synthesis of **7**. LC-MS (ESI) *m/z*: calcd for $C_{14}H_{17}BrFN_3O_2$ $[M+H]^+$ 358.1, 360.1; found 358.3, 360.4.

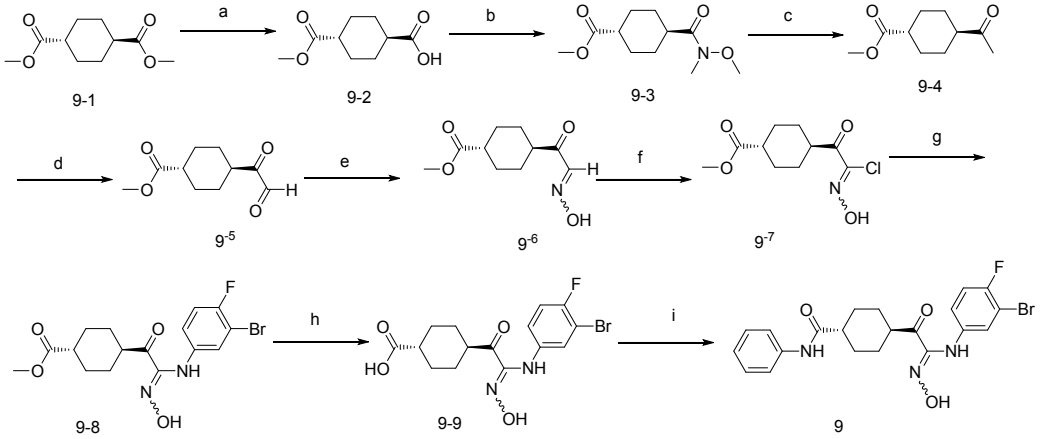
Step I: *N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-oxo-2-(4-((*N*-phenylsulfamoyl)amino)cyclohexyl)acetimidamide (**8**)

To a solution of 2-((1r,4r)-4-aminocyclohexyl)-*N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-oxoacetimidamide hydrochloride (78 mg, 0.218 mmol) **8-9** in THF(3 mL), was added Et_3N(66 mg, 0.097 mmol) and phenylsulfamoyl chloride(42 mg, 0.218 mmol) at 0°C. The resulting mixture was stirred at rt for 3h. The resulting mixture was concentrated under reduced pressure and purified by silica gel column chromatography with DCM/MeOH (10:1) to give the desired product (20 mg, 18%) as yellow solid.

LC-MS (ESI) m/z: calcd for $C_{20}H_{22}BrFN_4O_4S$ [M+H]+ 513.0, 515.0; found 513.0, 515.4.

^{1}HNMR (400 MHz, DMSO-d6) δ 11.58 (s, 1H), 9.61 (s, 1H), 8.30 (s, 1H), 8.10 (s, 1H), 7.45-7.47 (m, 1H), 7.25-7.29 (m, 2H), 7.13-7.17 (m, 2H), 6.93-7.00 (m, 2H), 6.66-6.68 (m, 1H), 3.11-3.18 (m, 1H), 1.97-2.03 (m, 1H), 1.80-1.83 (m, 2H), 1.59-1.63 (m, 2H), 1.34-1.35 (m, 2H), 1.15-1.18 (m, 2H).

Scheme S9. Synthesis of compound 9[a]



[a] Reagents and conditions: (a) KOH, MeOH, reflux, 20h, 47%; (b) DMHH, EDC·HCl, DMAP, DCM, rt, 16h, 58%; (c) CH_3MgBr, THF, rt, 1h, 82%; (d) SeO_2, 1,4-dioxane, 90°C, 16h, 73%; (e) $NH_2OH·HCl$, K_2CO_3, CH_3OH, rt, 1h, 62%; (f) NCS, DMF, rt, 2h, 79%; (g) 3-bromo-4-fluoroaniline, EtOH, rt, 1h, 55%; (h) NaOH, MeOH, H_2O, 60°C, 2h, 33%; (i) aniline, EDC·HCl, HOBt, Et_3N, THF, rt, 16h, 6%.

4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)-*N*-phenylcyclohexanecarboxamide (9)

Step A: (1r,4r)-4-(methoxycarbonyl)cyclohexanecarboxylic acid (**9-2**)

To a solution of (1r,4r)-dimethyl cyclohexane-1,4-dicarboxylate(10.00 g, 50.0 mmol) **9-1** in MeOH(20 mL), was added KOH(10.45 g, 47.8 mmol) in MeOH(80 mL) at 0°C. The mixture was refluxed for 20h. After the completion of the reaction, the resulting mixture was concentrated under reduced pressure and added H_2O(50 mL). The reaction liquid was adjusted to pH=5 with hydrochloric acid. The mixtrue was filtered, and then the solid was washed with water(50 mL). The solid was dried in vacuum to give the desired product (4.10 g, 47%) as white solid.

Step B to Step G: (1r,4r)-methyl 4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)cyclohexanecarboxylate (**9-8**). Prepared in a fashion similar to that used for the synthesis of **7**. LC-MS (ESI) *m/z*: calcd for $C_{16}H_{18}BrFN_2O_4$ $[M-H]^-$ 401.0, 403.0; found 400.8, 402.8.

Step H: (1r,4r)-4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)cyclohexanecarboxylic acid (**9-9**)

To a solution of (1r,4r)-methyl 4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)cyclohexanecarboxylate (450 mg, 1.12 mmol) **9-8** in MeOH(10 mL) and H_2O(2 mL), was added NaOH(67 mg, 1.68 mmol) at rt. The mixture was heated at 60°C for 2h. The resulting mixture was adjusted to pH=4 with hydrochloric acid and extracted with DCM(20 mL×3). The combined DCM layers were washed with water(20 mL×3), saturated NaCl(20 mL×3), and dried over sodium anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure and purified by silica gel column chromatography with DCM/MeOH (10:1) to give the desired product (142 mg, 33%) as white solid.

LC-MS (ESI) m/z: calcd for $C_{15}H_{16}BrFN_2O_4$ $[M+H]+$ 387.0, 389.0; found 387.3, 389.3.

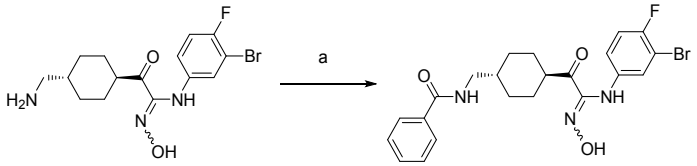
Step I: 4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)-*N*-phenylcyclohexanecarboxamide **(9)**

To a solution of (1r,4r)-4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)cyclohexanecarboxylic acid(140 mg, 0.36 mmol) **9-9** and aniline(50 mg, 0.54 mmol) in THF(15 mL), was added EDC·HCl (83 mg, 0.43 mmol) and HOBt(58 mg, 0.43 mmol) and Et_3N(109 mg, 1.08 mmol)at rt. The resulting mixture was stirred at room temperature for 16h. After the completion of the reaction, the resulting mixture was added water and extracted with DCM(20 mL×3). The combined DCM layers were washed

with water(20 mL×3), saturated NaCl(20 mL×3), and dried over sodium anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure and purified by silica gel column chromatography with DCM/MeOH (10:1) to give the desired product (10 mg, 6%) as white solid.

LC-MS (ESI) *m/z*: calcd for $C_{21}H_{21}BrFN_3O_3$ $[M+H]^+$ 462.1, 464.1; found 462.3, 464.3. ^{1}HNMR (400 MHz, DMSO-d6) δ 11.66 (s, 1H), 9.88 (s, 1H), 8.34 (s, 1H), 7.59-7.61 (m, 2H), 7.26-7.29 (m, 2H), 7.15-7.20 (m, 1H), 6.96-7.03 (m, 2H), 6.70-6.74 (m, 1H), 3.24-3.34 (m, 1H), 2.32-2.38 (m, 1H), 1.91-1.98 (m, 4H), 1.45-1.54 (m, 2H), 1.30-1.40 (m, 2H).

Scheme S10. Synthesis of compound 10[a]



[a] Reagents and conditions: (a) benzoyl chloride, Et_3N, DCM, rt, 0.5h, 21%.

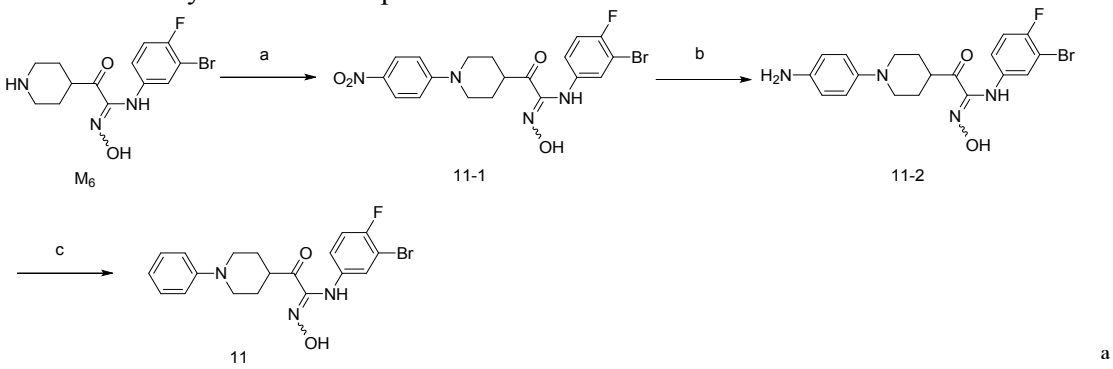
_N_-(((_1r_,_4r_)-4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)cyclohexyl)methyl)benzamide (10)

Step A: _N_-(((_1r_,_4r_)-4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)cyclohexyl)methyl)benzamide (**10**)

To a solution of 2-((1r,4r)-4-(aminomethyl)cyclohexyl)-_N_-(3-bromo-4-fluorophenyl)-_N′_-hydroxy-2-oxoacetimidamide hydrochloride(63 mg, 0.17 mmol) **7-9** in DCM(2 mL), was added Et_3N(51 mg, 0.508 mmol) and benzoyl chloride(24 mg, 0.169 mmol) at 0°C. The resulting mixture was stirred at rt for 0.5h. After the completion of the reaction, the resulting mixture was added saturated $NaHCO_3$ and extracted with DCM(20 mL×3). The combined DCM layers were washed with water(20 mL×3), saturated NaCl(20 mL×3), and dried over sodium anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure and purified by silica gel column chromatography with ethyl acetate/hexane (1:1) to give the desired product (17 mg, 21%) as yellow solid.

LC-MS (ESI) *m/z*: calcd for $C_{22}H_{23}BrFN_3O_3$ $[M+H]^+$ 476.1, 478.1; found 476.3, 478.3. ^{1}HNMR (400 MHz, DMSO-d6) δ 11.60 (s, 1H), 8.45-8.46 (m, 1H), 8.32 (s, 1H), 7.82-7.85 (m, 2H), 7.49-7.51 (m, 1H), 7.44-7.47 (m, 2H), 7.13-7.18 (m, 1H), 6.94-6.96 (m, 1H), 6.67-6.71 (m, 1H), 3.08-3.15 (m, 3H), 2.00-2.02 (m, 1H), 1.82-1.86 (m, 3H), 1.47-1.55 (m, 2H), 1.26-1.27 (m, 1H), 1.00-1.03 (m, 2H).

Scheme S11. Synthesis of compound 11[a]



Reagents and conditions: (a) 1-fluoro-4-nitrobenzene, DIPEA, DMSO, 60°C, 1h, 45%; (b) sodium hydrosulfite, K_2CO_3, 1,4-dioxane, H_2O, rt, 1h, 64%; (c) *tert*-Butyl nitrite, DMF, 70°C, 10mins, 26%.

_N_-(3-bromo-4-fluorophenyl)-_N′_-hydroxy-2-oxo-2-(1-phenylpiperidin-4-yl)acetimidamide (11)

Step A: *N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-(1-(4-nitrophenyl)piperidin-4-yl)-2-oxoacetimidamide (**11-1**)

To a solution of *N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-oxo-2-(piperidin-4-yl)acetimidamide(500 mg, 1.45 mmol) **M6** and 1-fluoro-4-nitrobenzene(205 mg, 1.45 mmol) in DMSO(30 mL), was added DIPEA(468 mg, 3.63 mmol). The resulting mixture was heated at 60°C for 1h. After the completion of the reaction, the resulting mixture was added water and extracted with ethyl acetate (20 mL×3). The combined ethyl acetate layers were washed with water(20 mL×3), saturated NaCl(20 mL×3), and dried over sodium anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure and purified by silica gel column chromatography with ethyl acetate/hexane (1:2) to give the desired product (300 mg, 45%) as yellow solid.

Step B: 2-(1-(4-aminophenyl)piperidin-4-yl)-*N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-oxoacetimidamide (**11-2**)

To a solution of *N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-(1-(4-nitrophenyl)piperidin-4-yl)-2-oxoacetimidamide(220 mg, 0.47 mmol) **11-1** in 1,4-dioxane(30 mL) and H_2O(10 mL), was added sodium hydrosulfite (823 mg, 4.7 mmol) and K_2CO_3 (324 mg, 2.35 mmol). The mixture was stirred at rt for 1h. After the completion of the reaction, the resulting mixture was added water and extracted with ethyl acetate (20 mL×3). The combined ethyl acetate layers were washed with water(20 mL×3), saturated NaCl(20 mL×3), and dried over sodium anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced

pressure and purified by silica gel column chromatography with DCM/MeOH (30:1) to give the desired product (130 mg, 64%) as white solid.

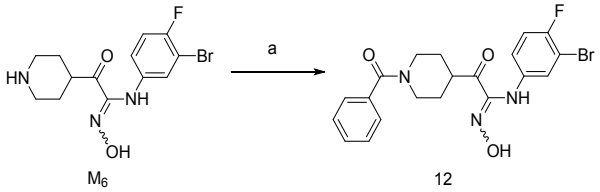
LC-MS (ESI) *m/z*: calcd for $C_{19}H_{20}BrFN_4O_2$ $[M+H]^+$ 435.1, 437.1; found 435.3, 437.3.

Step C: 2-(1-(4-aminophenyl)piperidin-4-yl)-*N*-(3-bromo-4-fluorophenyl)-*N*′-hydroxy-2-oxoacetimidamide (**11**)

To a solution of 2-(1-(4-aminophenyl)piperidin-4-yl)-*N*-(3-bromo-4-fluorophenyl)-*N*′-hydroxy-2-oxoacetimidamide(20 mg, 0.46 mmol) **11-2** in DMF(4 mL), was dropwised *tert*-Butyl nitrite(10 mg, 0.92 mmol) in DMF(0.5 mL). The mixture was stirred at 70°C for 10mins. After the completion of the reaction, the resulting mixture was added water and extracted with ethyl acetate (20 mL×3). The combined ethyl acetate layers were washed with water(20 mL×3), saturated NaCl(20 mL×3), and dried over sodium anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure and purified by silica gel column chromatography with DCM/MeOH (40:1) to give the desired product (5 mg, 26%) as yellow solid.

LC-MS (ESI) *m/z*: calcd for $C_{19}H_{19}BrFN_3O_2$ $[M+H]^+$ 420.1, 422.1; found 420.3, 422.3. ^{1}HNMR (400 MHz, DMSO-*d*6) δ 11.68 (s, 1H), 8.38 (s, 1H), 7.15-7.22 (m, 3H), 6.94-6.99 (m, 3H), 6.69-6.78 (m, 2H), 3.75-3.78 (m, 2H), 3.40-3.49 (m, 1H), 2.69-2.75 (m, 2H), 1.89-1.92 (m, 2H), 1.59-1.67 (m, 2H).

Scheme S12. Synthesis of compound 12[a]



M6 12

[a] Reagents and conditions: (a) benzoyl chloride, Et_3N, THF, rt, 1h, 28%.

2-(1-benzoylpiperidin-4-yl)-*N*-(3-bromo-4-fluorophenyl)-*N*'-hydroxy-2-oxoacetimidamide (12)

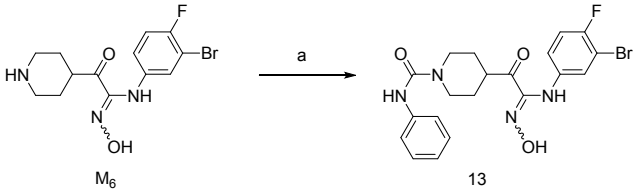
Step A: 2-(1-benzoylpiperidin-4-yl)-*N*-(3-bromo-4-fluorophenyl)-*N*'-hydroxy-2-oxoacetimidamide **(12)**

To a solution of *N*-(3-bromo-4-fluorophenyl)-*N*′-hydroxy-2-oxo-2-(piperidin-4-yl)acetimidamide(30 mg, 0.087 mmol) **M6** in THF(2 mL), was added Et_3N(436 mg, 4.32 mmol) and benzoyl chloride(14 mg, 0.10 mmol) at 0°C. The resulting mixture was stirred

at rt for 1h. After the completion of the reaction, the resulting mixture was added saturated $NaHCO_3$ and extracted with DCM(20 mL×3). The combined DCM layers were washed with water(20 mL×3), saturated NaCl(20 mL×3), and dried over sodium anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure and purified by silica gel column chromatography with ethyl acetate/hexane (1:1) to give the desired product (10 mg, 28%) as white solid.

LC-MS (ESI) *m*/*z*: calcd for $C_{20}H_{19}BrFN_3O_3$ $[M+H]^+$ 448.0, 450.0; found 448.3, 450.3. ^{1}HNMR (400 MHz, DMSO-*d*6) δ 11.67 (s, 1H), 8.37 (s, 1H), 7.42-7.46 (m, 3H), 7.37-7.40 (m, 2H), 7.15-7.18 (m, 1H), 6.98-7.00 (m, 1H), 6.68-6.72 (m, 1H), 4.48-4.52 (m, 1H), 3.56-3.62 (m, 2H), 2.80-3.20 (m, 2H), 1.79-1.99 (m, 2H), 1.42-1.53 (m, 2H).

Scheme S13. Synthesis of compound 13[a]



[a] Reagents and conditions: (a) 4-nitrophenyl chloroformate, aniline, Et_3N, THF, rt, 1h, 20%.

4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)-*N*-phenylpiperidine-1-carboxamide (13)

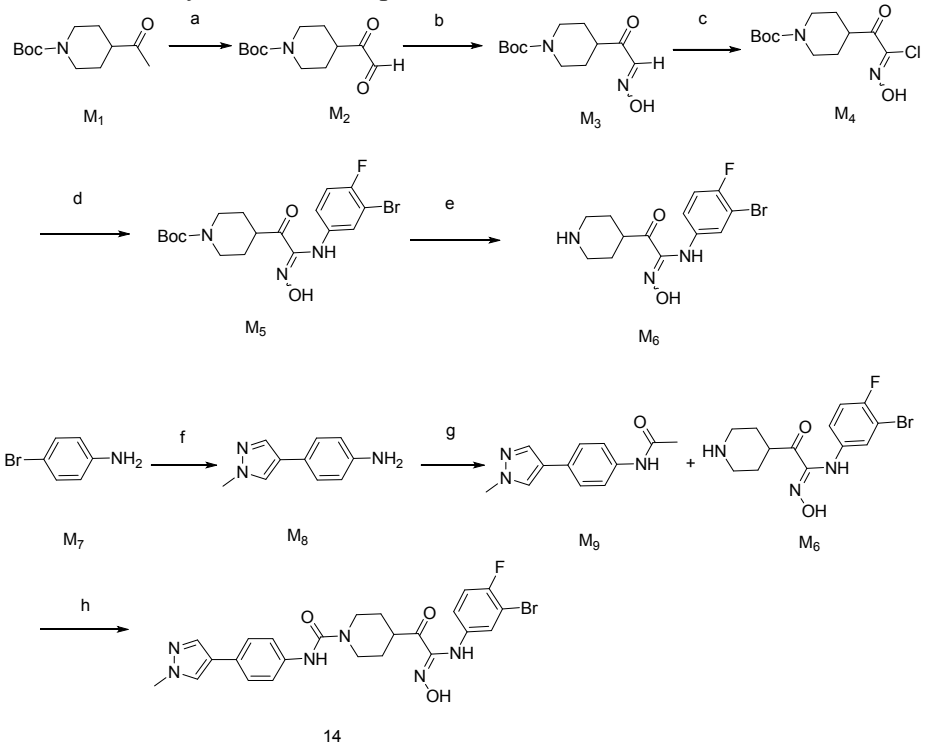
Step A: 4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)-*N*-phenylpiperidine-1-carboxamide **(13)**

To a solution of aniline (15 mg, 0.16 mmol) in THF(10 mL), was added Et_3N(73 mg, 0.72 mmol) and 4-nitrophenyl chloroformate (32 mg, 0.16 mmol) at 0°C. The resulting mixture was stirred at rt for 30mins, and then added *N*-(3-bromo-4-fluorophenyl)-*N*′-hydroxy-2-oxo-2-(piperidin-4-yl)acetimidamide(55 mg, 0.16 mmol) **M6.** The resulting mixture was stirred at rt for 1h. After the completion of the reaction, the resulting mixture was added saturated $NaHCO_3$ and extracted with DCM(20 mL×3). The combined DCM layers were washed with water(20 mL×3), saturated NaCl(20 mL×3), and dried over sodium anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure and purified by silica gel column chromatography with DCM/MeOH (10:1) to give the desired product (15 mg, 20%) as brown solid.

LC-MS (ESI) *m*/*z*: calcd for $C_{20}H_{20}BrFN_4O_3$ $[M+H]^+$ 463.1, 465.1; found 463.3, 465.3. ^{1}HNMR (400 MHz, DMSO-*d*6) δ 11.69 (s, 1H), 8.52 (s, 1H), 8.40 (s, 1H), 7.45 (d, 2H),

7.18-7.24 (m, 3H), 6.98-7.00 (m, 1H), 6.92 (t, 1H), 6.71-6.74 (m, 1H), 4.17-4.20 (m, 2H), 3.52-3.57 (m, 1H), 2.87 (t, 2H), 1.85-1.87 (m, 2H), 1.42-1.50 (m, 2H).

Scheme S14. Synthesis of compound **14**[a]



[a] Reagents and conditions: (a) SeO_2, 1,4-dioxane, 80°C, 16h, 100%; (b) $NH_2OH{\cdot}HCl$, K_2CO_3, CH_3OH, rt, 2h, 73%; (c) NCS, DMF, rt, 16h, 89%; (d) 3-bromo-4-fluoroaniline, EtOH, rt, 3h, 14%; (e) 4M HCl in 1,4-dioxane, 2h, 77%; (f) 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole, $Pd(dppf)Cl_2$, K_2CO_3, 1,4-dioxane, H_2O, 105°C, 16h, 55%; (g) Triphosgene, Et_3N, DCM, 0°C, 30mins, 100%; (h) DCM, rt, 1h, 24%.

4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)-*N*-(4-(1-methyl-1H-pyrazol-4-yl)phenyl)piperidine-1-carboxamide (14)

Step A: *tert*-butyl 4-(2-oxoacetyl)piperidine-1-carboxylate (**M2**)

Selenium dioxide(6.65 g, 59.9 mmol) was added to a solution of *tert*-butyl 4-acetylpiperidine-1-carboxylate(6.80 g, 29.9 mmol) **M1** in 1,4-dioxane(100 mL) . The resulting mixture was stirred at 80°C for 16h. After the completion of the reaction, the solution was filtered and concentrated under reduced pressure. The resulting residue was dissolved in DCM, and washed with water, saturated NaCl, and dried over sodium anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure to give the desired product (7.20 g, 100%) as red oil, which was used directly in the next step without further purification.

Step B: *tert*-butyl 4-(2-(hydroxyimino)acetyl)piperidine-1-carboxylate (**M3**)

To a solution of *tert*-butyl 4-(2-oxoacetyl)piperidine-1-carboxylate(7.20 g, 29.9 mmol) **M2** in MeOH(100 mL), was added potassium carbonate(6.20 g, 44.9 mmol), and then hydroxylamine hydrochloride(1.66 g, 23.9 mmol). The resulting mixture was stirred at room temperature for 2h. After the completion of the reaction, the solution was filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography with MeOH/DCM (1:10) to give the desired product (5.60 g, 73%) as red oil.

LC-MS (ESI) *m*/*z*: calcd for $C_{12}H_{20}N_2O_4$ $[M-H]^-$ 255.1; found 255.1.

Step C: *tert*-butyl 4-(2-chloro-2-(hydroxyimino)acetyl)piperidine-1-carboxylate (**M4**)

To a solution of *tert*-butyl 4-(2-(hydroxyimino)acetyl)piperidine-1-carboxylate (5.60 g, 21.8 mmol) **M3** in DMF(20 mL), was added NCS(2.92 g, 21.9 mmol). The resulting mixture was stirred at room temperature for 16h. After the completion of the reaction, 25 mL of water was added dropwise to quench the reaction, and extracted with ethyl acetate(20 mL×3). The combined ethyl acetate layers were washed with water(20 mL×3), saturated NaCl(20 mL×3), and dried over sodium anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure to give the desired product (5.60 g, 89%) as yellow oil, which was used directly in the next step without further purification.

Step D: *tert*-butyl 4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)piperidine-1-carboxylate (**M5**)

To a solution of *tert*-butyl 4-(2-chloro-2-(hydroxyimino)acetyl)piperidine-1-carboxylate(5.60 g, 19.3 mmol) **M4** in EtOH(50 mL), was added 3-bromo-4-fluoroaniline(7.3 g, 38.4 mmol). The resulting mixture was stirred at room temperature for 3h. After the completion of the reaction, the solution was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography with MeOH/DCM (1:50) to give the desired product (1.20 g, 14%) as yellow oil.

LC-MS (ESI) *m*/*z*: calcd for $C_{18}H_{23}BrFN_3O_4$ $[M+H]^+$ 444.1, 446.1; found 444.0, 446.0.

Step E: *N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-oxo-2-(piperidin-4-yl)acetimidamide (**M6**)

To *tert*-butyl 4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)piperidine-1-carboxylate (1.20 g, 2.7 mmol) **M5** was added 4M HCl in 1,4-dioxane (4 mL). The resulting mixture was stirred at room temperature for 2h. After the completion of the reaction, the solution was concentrated under reduced pressure. The resulting residue was neutralized with aqueous ammonia to give the desired product (0.70 g, 77%) as brown solid, which was used directly in the next step without further purification.

Step F: 4-(1-methyl-1*H*-pyrazol-4-yl)aniline (**M8**)

To a solution of 4-bromoaniline(4.30 g, 25.0 mmol) **M7** and 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole(5.20 g, 25.0 mmol) in 1,4-dioxane(50 mL) and H_2O(5 mL), under an atmosphere of nitrogen, was added potassium carbonate(6.90 g, 50.0 mmol) and [1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(II)(0.91 g, 1.3 mmol). The resulting mixture was stirred at 105°C for 16h. After the completion of the reaction, the solution was filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography with dichloromethane/methanol (50:1) to give the desired product (2.35 g, 55%) as yellow solid.

LC-MS (ESI) *m*/*z*: calcd for $C_{10}H_{11}N_3$ $[M+H]^+$ 174.1; found 174.1.

Step G: 4-(4-isocyanatophenyl)-1-methyl-1*H*-pyrazole (**M9**)

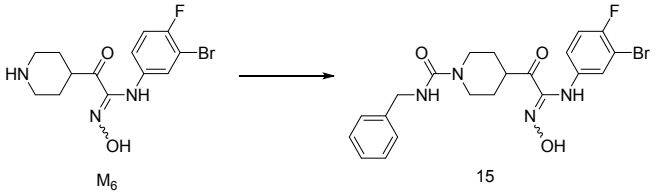
To a solution of 4-(1-methyl-1*H*-pyrazol-4-yl)aniline(24 mg, 0.14 mmol) **M8** and Et_3N(42 mg, 0.42 mmol) in DCM(10 mL), was added triphosgene (14 mg, 0.05 mmol) at 0°C. The resulting mixture was stirred at 0°C for 30mins, which was used directly in the next step without further purification.

Step H: 4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)-*N*-(4-(1-methyl-1H-pyrazol-4-yl)phenyl)piperidine-1-carboxamide (**14**)

To reaction solution of the previous step 4-(4-isocyanatophenyl)-1-methyl-1*H*-pyrazole(28mg, 0.14 mmol) **M9** in DCM(10 mL), was added *N*-(3-bromo-4-fluorophenyl)-*N'*-hydroxy-2-oxo-2-(piperidin-4-yl)acetimidamide(40mg, 0.12 mmol) **M6**. The resulting mixture was stirred at room temperature for 1h. After the completion of the reaction, the solution was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography with dichloromethane/methanol (15:1) to give the desired product (15 mg, 24%) as white solid.

LC-MS (ESI) *m*/*z*: calcd for $C_{24}H_{24}BrFN_6O_3$ $[M+H]^+$ 543.1, 545.1; found 543.4, 545.4. ^{1}HNMR (400 MHz, DMSO-*d*6) δ 11.68 (s, 1H), 8.52 (s, 1H), 8.39 (s, 1H), 8.02 (s, 1H), 7.77 (s, 1H), 7.42-7.45 (m, 4H), 7.16 (t, 1H), 6.98-7.00 (m, 1H), 6.71-6.74 (m, 1H), 4.17-4.20 (m, 2H), 3.84 (s, 3H), 3.52-3.55 (m, 1H), 2.85-2.91 (m, 2H), 1.85-1.88 (m, 2H), 1.45-1.51 (m, 2H).

Scheme S15. Synthesis of compound 15[a]



[a] Reagents and conditions: (a) benzyl isocyanate, Et_3N, THF, rt, 2h, 20%.

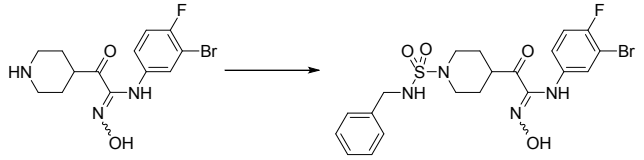
_N_-benzyl-4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)piperidine-1-carboxamide (15)

Step A: *N*-benzyl-4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)piperidine-1-carboxamide **(15)**

To a solution of *N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-oxo-2-(piperidin-4-yl)acetimidamide(30 mg, 0.087 mmol) **M6** in THF(5 mL), was added benzyl isocyanate(40 mg, 0.30 mmol) at 0°C. The resulting mixture was stirred at rt for 1h**.** The resulting mixture was concentrated under reduced pressure and purified by silica gel column chromatography with DCM/MeOH (10:1) to give the desired product (15 mg, 20%) as white solid.

LC-MS (ESI) *m*/*z*: calcd for $C_{21}H_{22}BrFN_4O_3$ $[M+H]^+$ 477.1, 479.1; found 477.3, 479.3. ^{1}HNMR (400 MHz, DMSO-*d*6) δ 11.66 (s, 1H), 8.37 (s, 1H), 7.17-7.30 (m, 6H), 7.09-7.10 (m, 1H), 6.97-6.98 (m, 1H), 6.72-6.73 (m, 1H), 4.24 (d, 2H), 4.05-4.08 (m, 2H), 3.47-3.50 (m, 1H), 2.74-2.80 (m, 2H), 1.78-1.81 (m, 2H), 1.37-1.43 (m, 2H).

Scheme S16. Synthesis of compound 16[a]



[a] Reagents and conditions: (a) benzylsulfamoyl chloride, Et_3N, THF, rt, 2h, 20%.

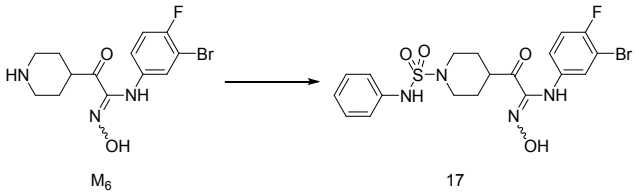
2-(1-(*N*-benzylsulfamoyl)piperidin-4-yl)-*N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-oxoacetimidamide (16)

Step A: 2-(1-(*N*-benzylsulfamoyl)piperidin-4-yl)-*N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-oxoacetimidamide **(16)**

To a solution of *N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-oxo-2-(piperidin-4-yl)acetimidamide(40 mg, 0.116 mmol) **M6** in THF(2 mL), was added Et_3N(35 mg, 0.35 mmol) and benzylsulfamoyl chloride (24 mg, 0.116 mmol) at 0°C. The resulting mixture was stirred at rt for 2h**.** The resulting mixture was concentrated under reduced pressure and purified by silica gel column chromatography with DCM/MeOH (10:1) to give the desired product (20 mg, 20%) as yellow solid.

LC-MS (ESI) *m*/*z*: calcd for $C_{20}H_{22}BrFN_4O_4S$ $[M+H]^+$ 513.1, 515.1; found 513.4, 515.4. ^{1}HNMR (400 MHz, DMSO-d6) δ 11.66 (s, 1H), 8.35 (s, 1H), 7.77-7.80 (m, 1H), 7.30-7.35 (m, 3H), 7.23-7.29 (m, 1H), 7.14-7.18 (m, 1H), 6.95-6.97 (m, 1H), 6.67-6.71 (m, 1H), 4.06-4.08 (m, 2H), 3.53-3.57 (m, 2H), 3.24-3.41 (m, 1H), 3.15-3.16 (m, 1H), 2.60-2.68 (m, 2H), 1.81-1.87 (m, 2H), 1.35-1.48 (m, 2H).

Scheme S17. Synthesis of compound 17[a]



[a] Reagents and conditions: (a) phenylsulfamoyl chloride, Et_3N, THF, rt, 2h, 23%.

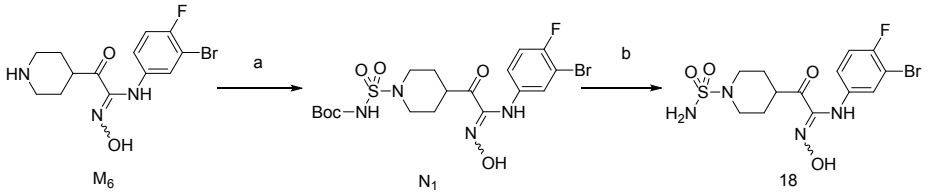
_N_-(3-bromo-4-fluorophenyl)-_N'_-hydroxy-2-oxo-2-(1-(N-phenylsulfamoyl)piperidin-4-yl)acetimidamide (17)

Step A: *N*-(3-bromo-4-fluorophenyl)-*N'*-hydroxy-2-oxo-2-(1-(N-phenylsulfamoyl)piperidin-4-yl)acetimidamide **(17)**

To a solution of *N*-(3-bromo-4-fluorophenyl)-*N'*-hydroxy-2-oxo-2-(piperidin-4-yl)acetimidamide(30 mg, 0.087 mmol) **M6** in THF(5 mL), was added Et_3N(73 mg, 0.72 mmol) and phenylsulfamoyl chloride (17 mg, 0.087 mmol) at 0°C. The resulting mixture was stirred at rt for 2h**.** The resulting mixture was concentrated under reduced pressure and purified by silica gel column chromatography with DCM/MeOH (10:1) to give the desired product (10 mg, 23%) as yellow solid.

LC-MS (ESI) *m*/*z*: calcd for $C_{19}H_{20}BrFN_4O_4S$ $[M+H]^+$ 499.0, 501.0; found 499.3, 501.3. ^{1}HNMR (400 MHz, DMSO-d6) δ 11.65 (s, 1H), 9.92 (s, 1H), 8.33 (s, 1H), 7.28-7.31 (m, 2H), 7.13-7.19 (m, 3H), 7.03-7.06 (m, 1H), 6.93-6.95 (m, 1H), 6.66-6.68 (m, 1H), 3.64-3.67 (m, 2H), 3.31-3.35 (m, 1H), 2.73-2.78 (m, 2H), 1.81-1.85 (m, 2H), 1.33-1.39 (m, 2H).

Scheme S18. Synthesis of compound **18**[a]



[a] Reagents and conditions: (a) *tert*-butyl chlorosulfonylcarbamate, Et_3N, DCM, 0°C, 1h, 78%; (b) 4M HCl in 1,4-dioxane, MeOH, rt, 1h, 21%.

***N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-oxo-2-(1-sulfamoylpiperidin-4-yl)acetimidamide (18)**

Step A: *tert*-butyl (4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)piperidin-1-yl)sulfonylcarbamate (**N1**)

To a solution of *N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-oxo-2-(piperidin-4-yl)acetimidamide (50 mg, 0.15 mmol) **M6** in DCM(10 mL), was added Et_3N (44 mg, 0.44 mmol). Then tert-butyl chlorosulfonylcarbamate (22 mg, 0.10 mmol) in DCM was added dropwise at 0°C. The resulting mixture was stirred at room temperature for 1h. After the completion of the reaction, 10 mL of water was added dropwise to quench the reaction, and extracted with DCM(10 mL×3). The combined ethyl acetate layers were washed with water(10 mL×3), saturated NaCl(10 mL×3), and dried over sodium anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography with MeOH/DCM (1:15) to give the desired product (60 mg, 78%) as colorless oil.

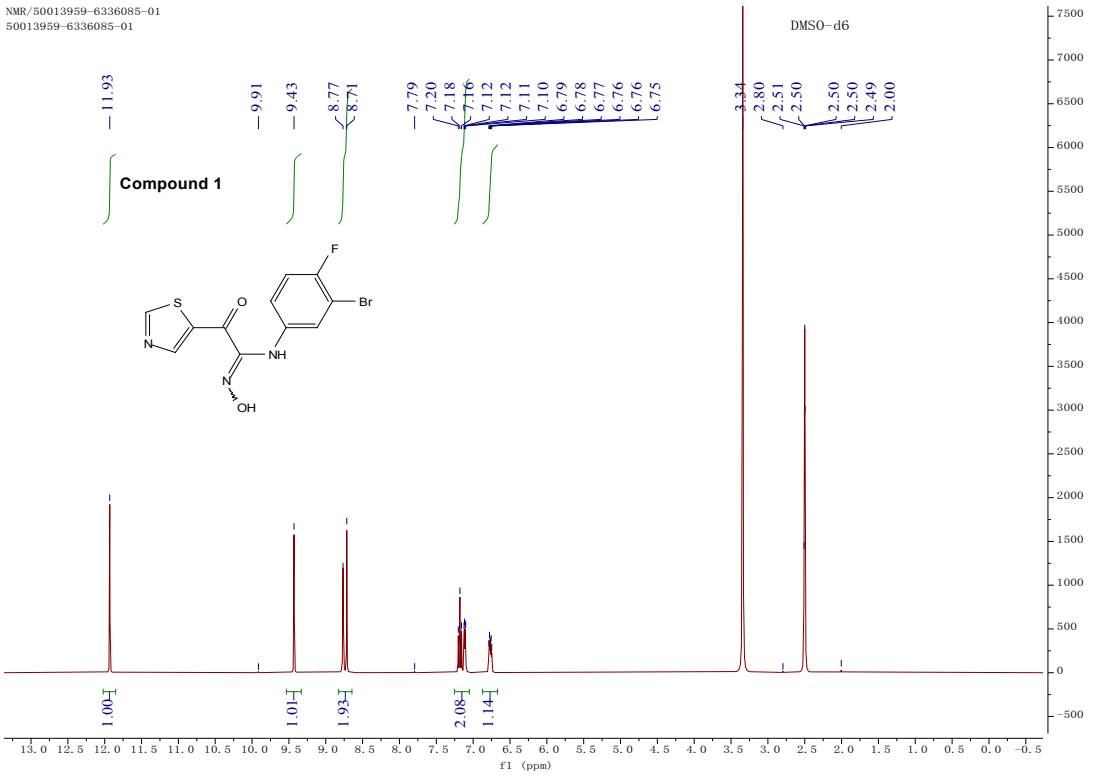
LC-MS (ESI) *m/z*: calcd for $C_{18}H_{24}BrFN_4O_6S$ $[M+H]^+$ 523.1, 525.1; found 523.3, 525.3.

Step B: *N*-(3-bromo-4-fluorophenyl)-*N′*-hydroxy-2-oxo-2-(1-sulfamoylpiperidin-4-yl)acetimidamide (**18**)

To a solution of *tert*-butyl (4-(2-((3-bromo-4-fluorophenyl)amino)-2-(hydroxyimino)acetyl)piperidin-1-yl)sulfonylcarbamate (60 mg, 0.11 mmol) **N1** in DCM(2 mL), was added 4M HCl in 1,4-dioxane (2 mL). The resulting mixture was stirred at room temperature for 1h. After the completion of the reaction, the solution was concentrated under reduced pressure. The resulting residue was neutralized with aqueous ammonia until pH=10, and then extracted with ethyl acetate (10 mL×3). The combined ethyl acetate layers were washed with water(10 mL×3), saturated NaCl(10 mL×3), and dried over sodium anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography with MeOH/DCM (1:10) to give the desired product (10 mg, 21%) as yellow solid.

LC-MS (ESI) *m/z*: calcd for $C_{13}H_{16}BrFN_4O_4S$ $[M+H]^+$ 423.0, 425.0; found 423.3, 425.3.

^{1}HNMR (400 MHz, DMSO-*d*6) δ 11.67 (s, 1H), 8.38 (s, 1H), 7.15-7.19 (m, 1H), 6.97-6.99 (m, 1H), 6.77 (br. 2H), 6.70-6.72 (m, 1H), 3.51-3.54 (m, 2H), 3.29-3.37 (m, 1H), 2.54-2.67 (m, 2H), 1.93-1.95 (m, 2H), 1.52-1.59 (m, 2H).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7.18
7.16
7.12
7.12
7.11
7.10
6.79
6.78
6.77
6.76
6.76
6.75
3.34
2.80
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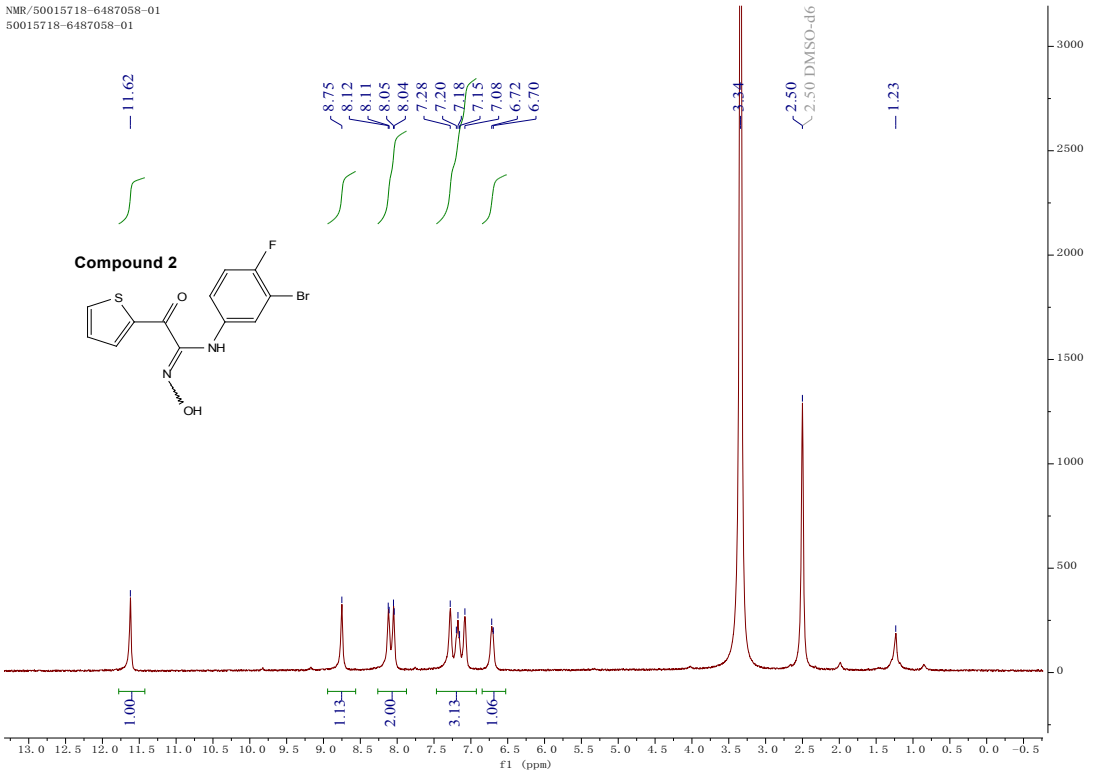
1.01
1.93
2.08
1.14
f1 (ppm)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8.05
8.04
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7.20
7.18
7.15
7.08
6.72
6.70
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2.50 DMSO-d6
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1.00
1.13
2.00
3.13
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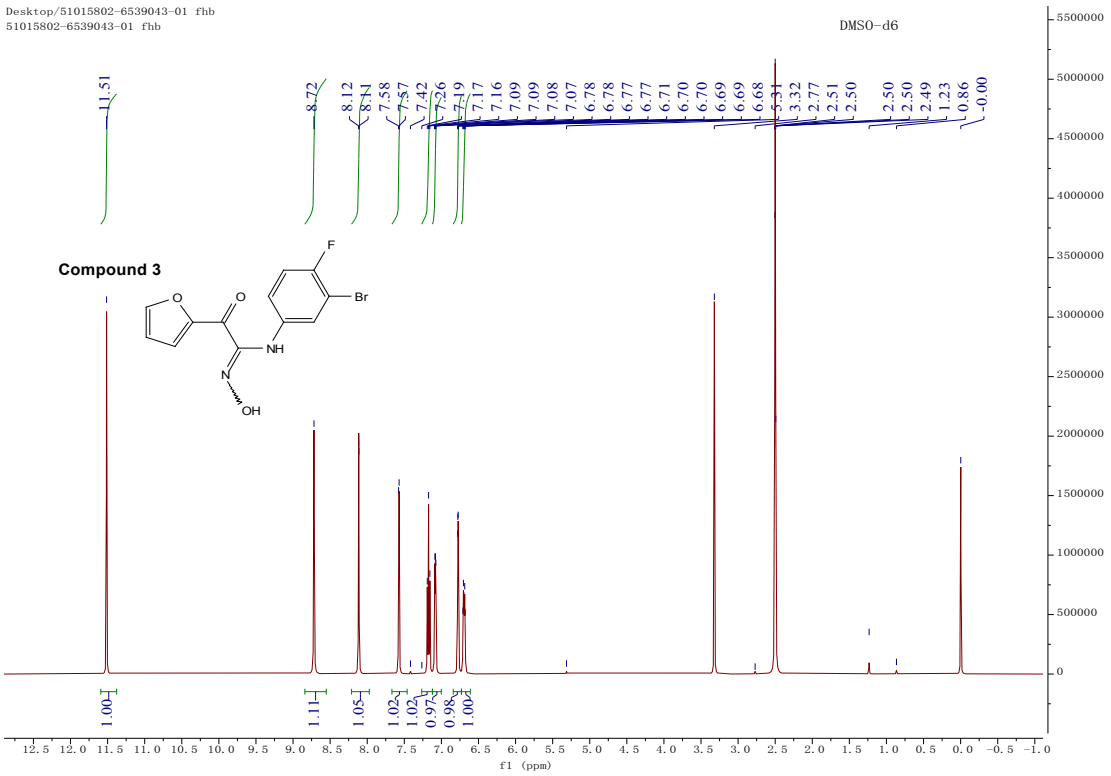
f1 (ppm)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O
O
NH
N
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7.42
7.26
7.19
7.17
7.16
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7.09
7.08
7.07
6.78
6.78
6.77
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6.71
6.70
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2.50
2.50
2.50
2.49
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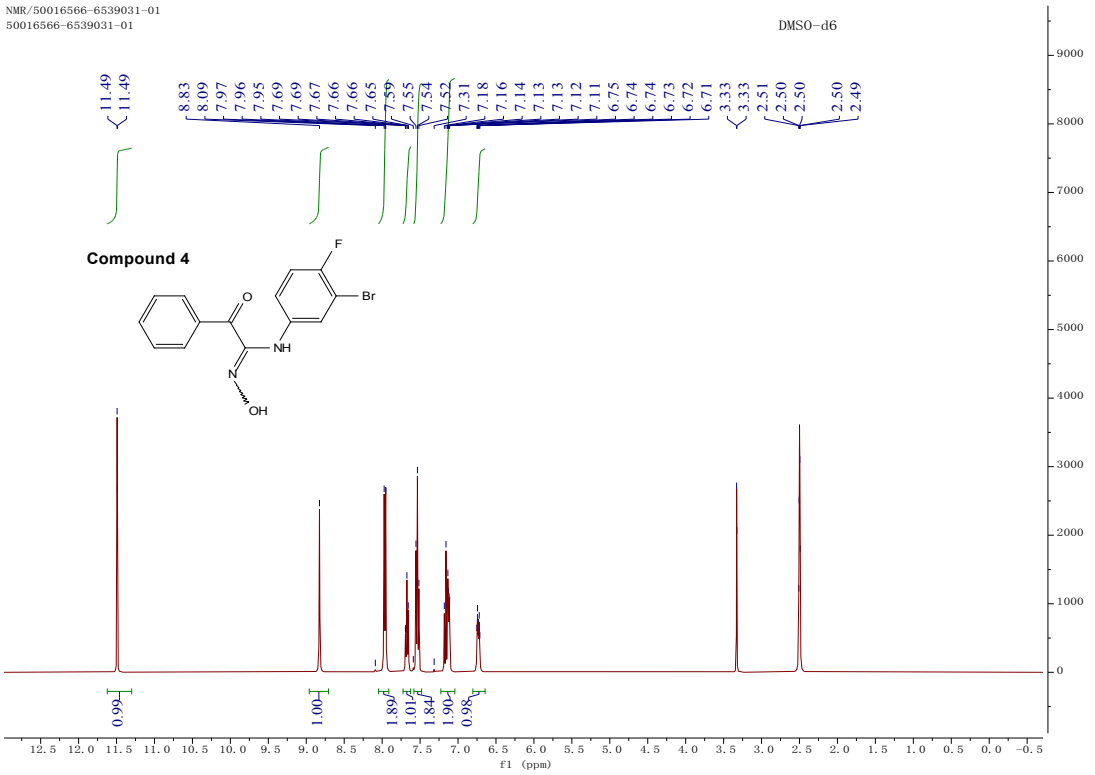
f1 (ppm)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8.09
7.97
7.96
7.95
7.69
7.69
7.67
7.66
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7.55
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6.74
6.74
6.73
6.72
6.71
3.33
3.33
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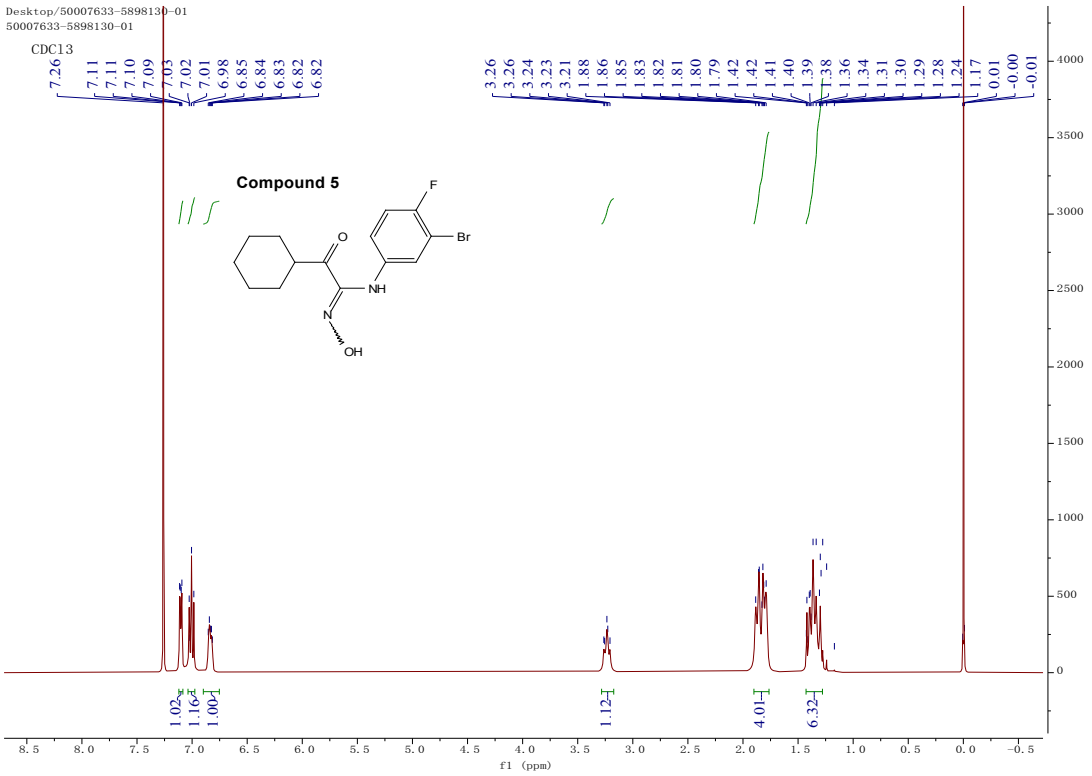
f1 (ppm)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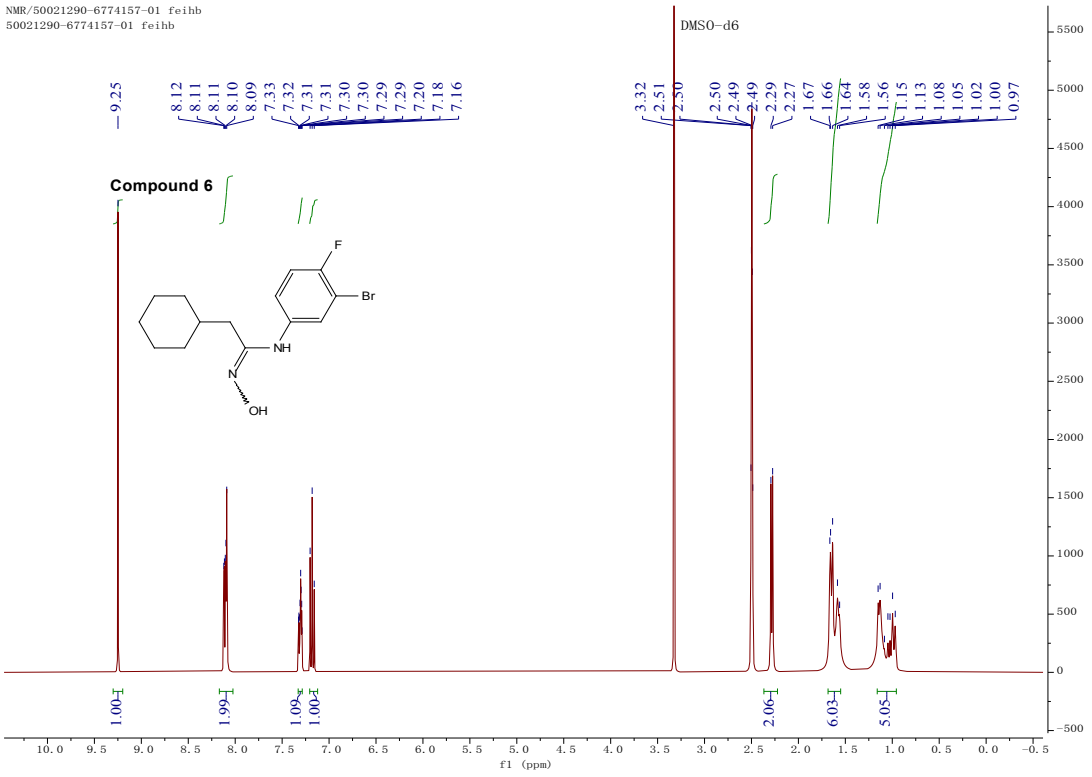
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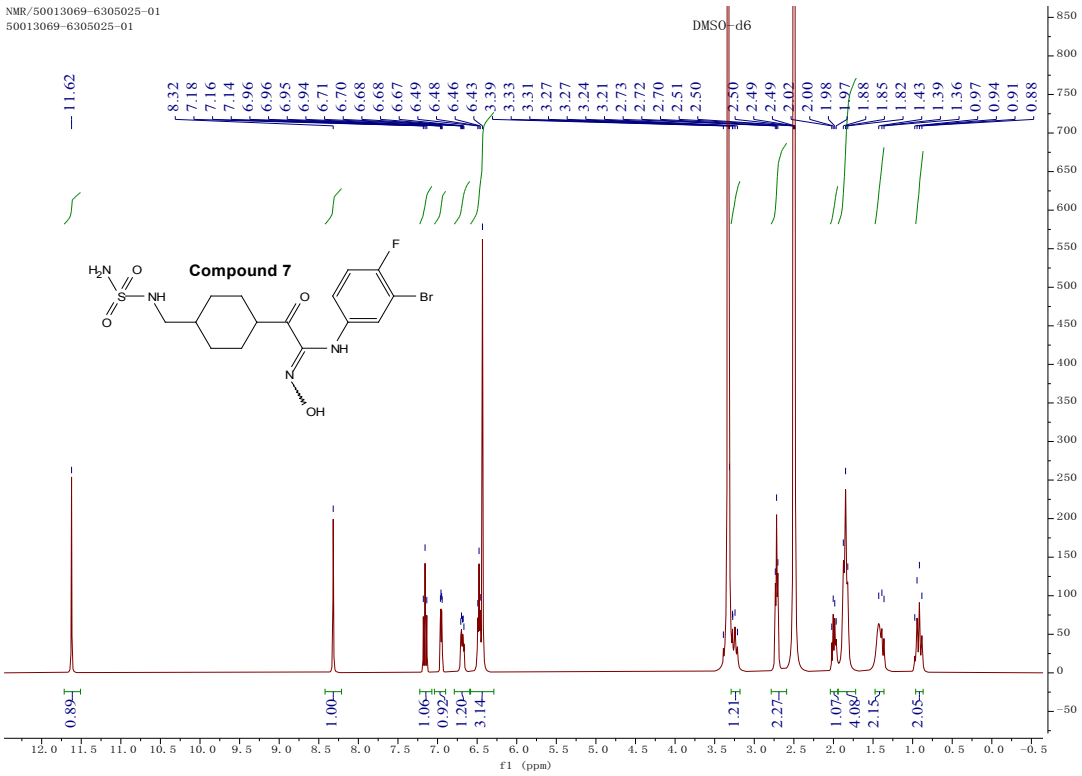
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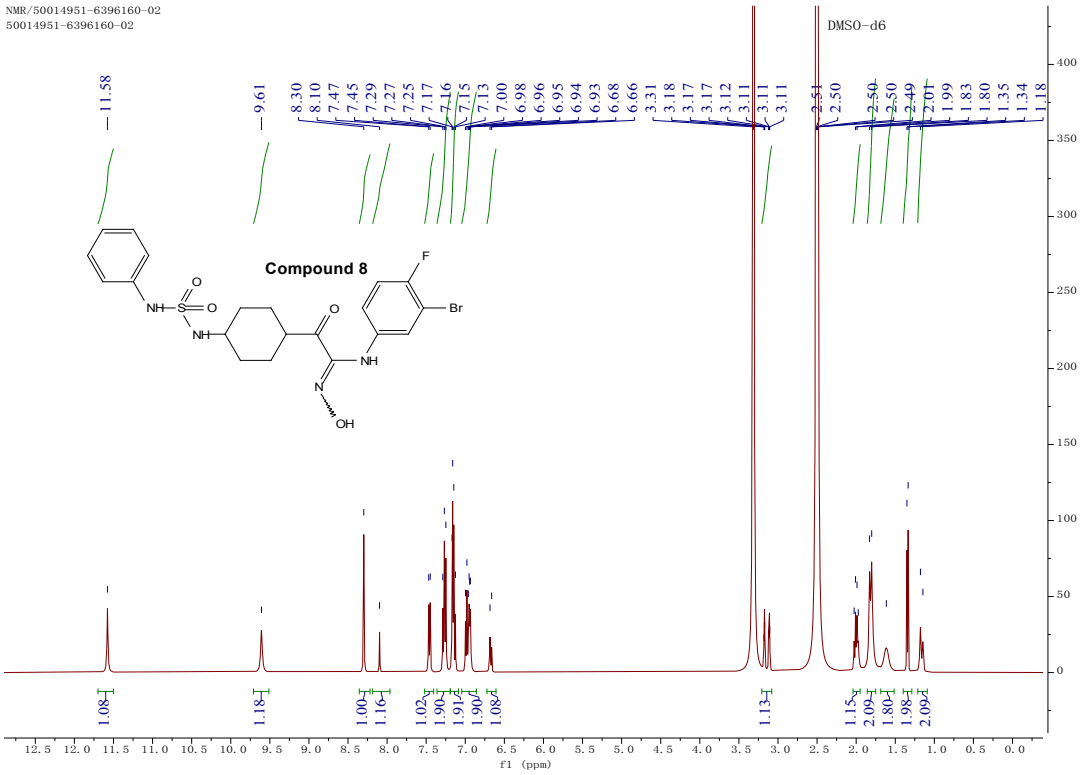
f1 (ppm)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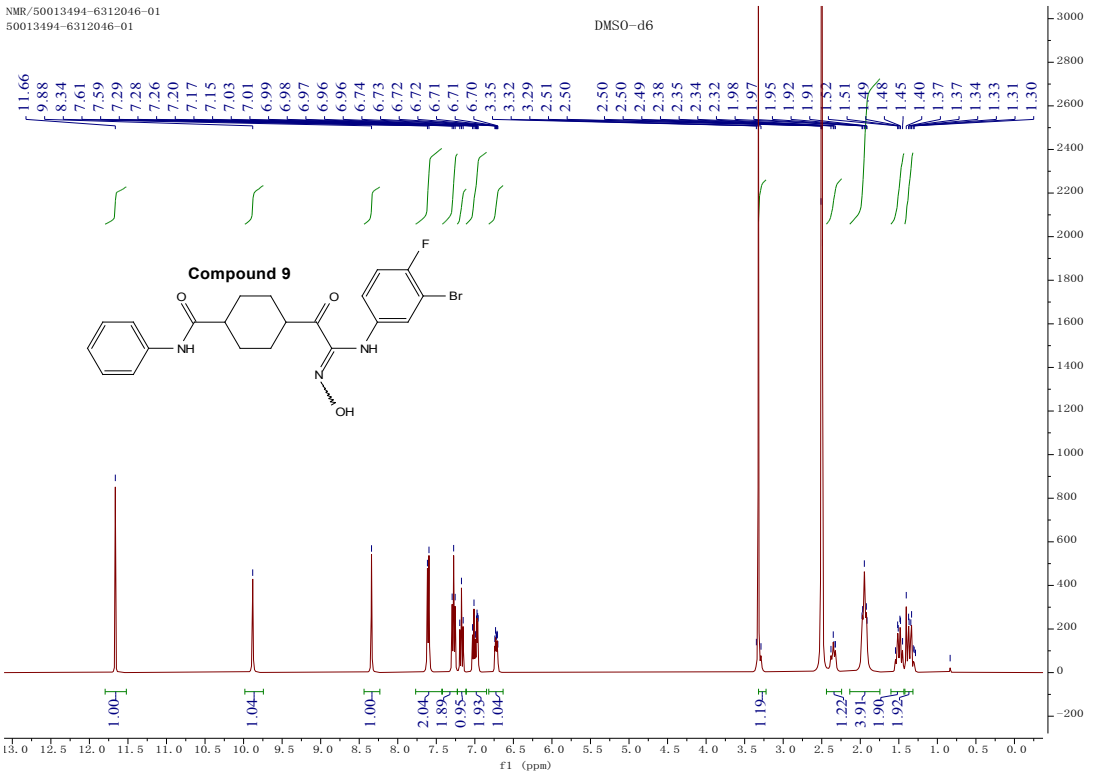
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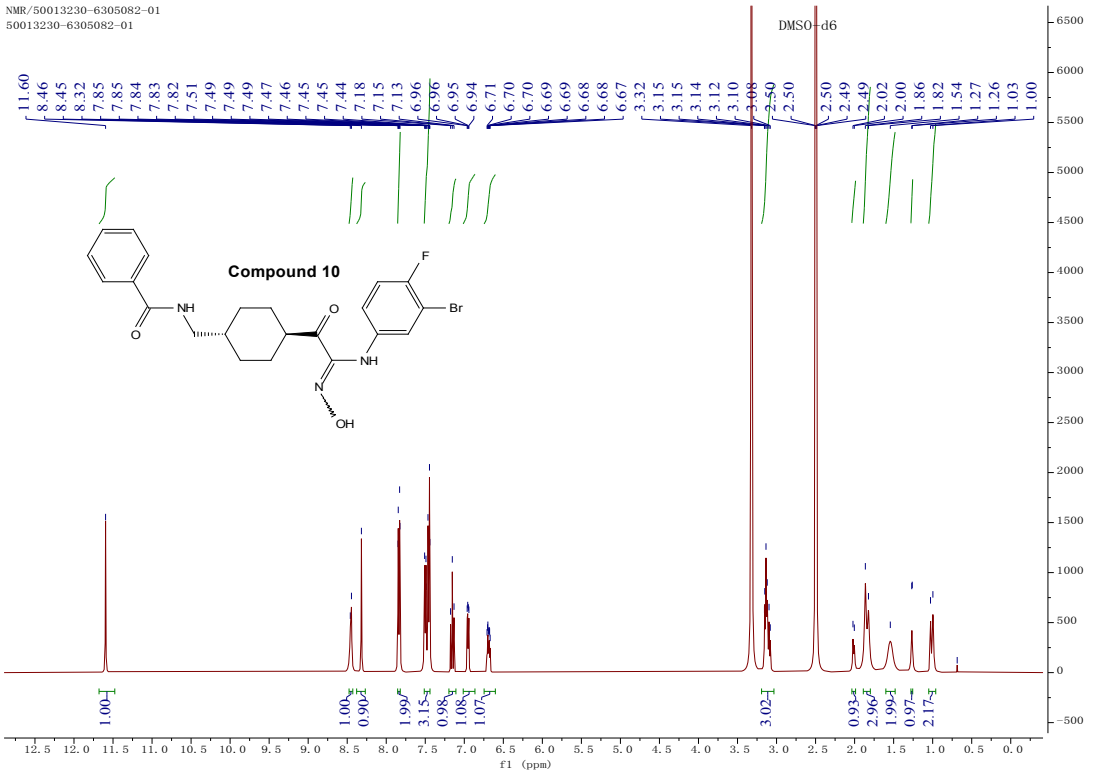
f1 (ppm)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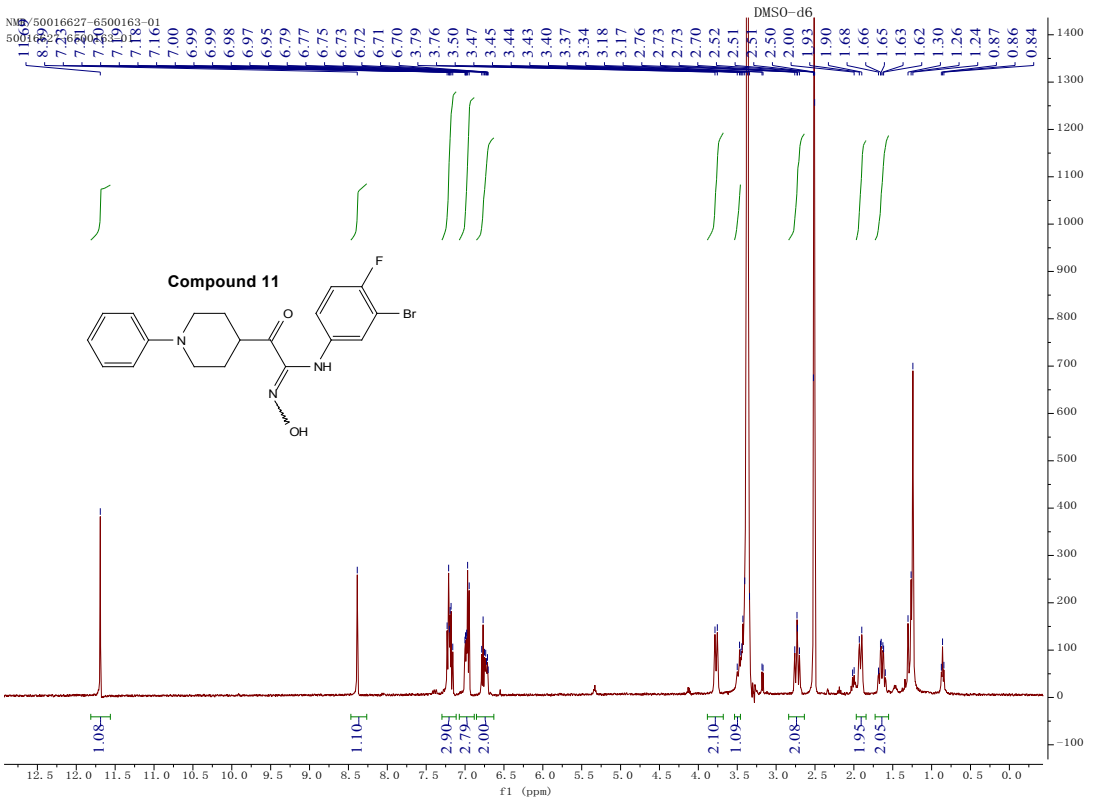
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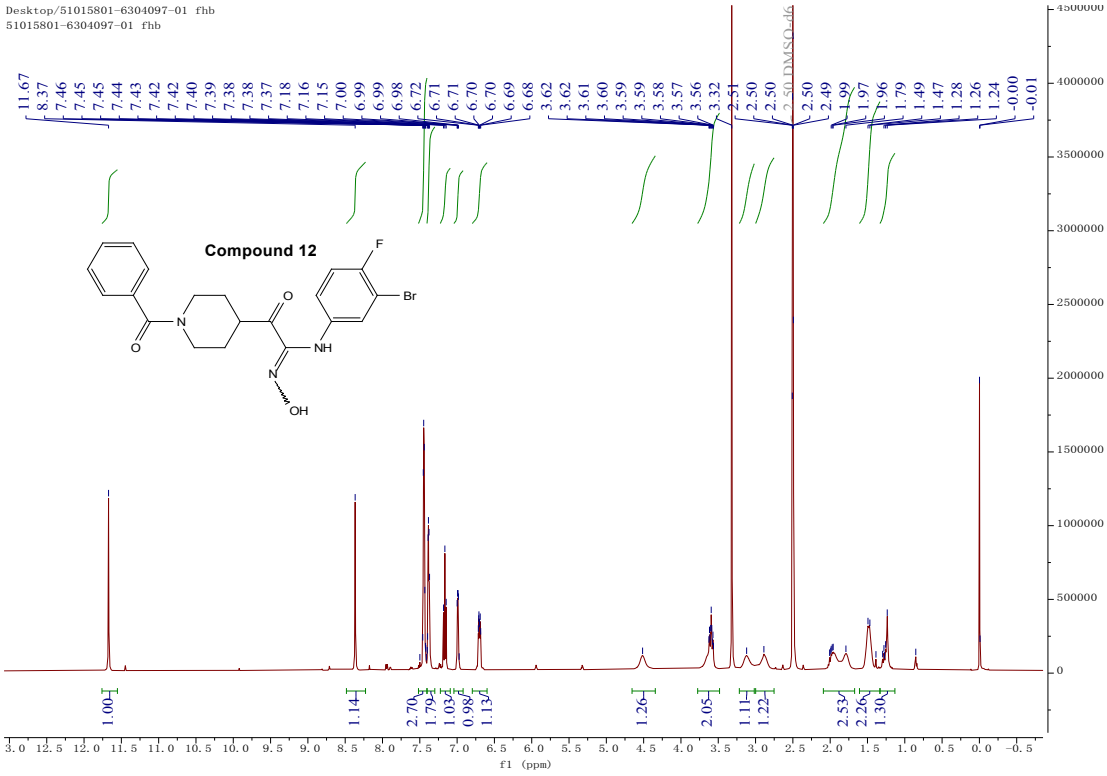
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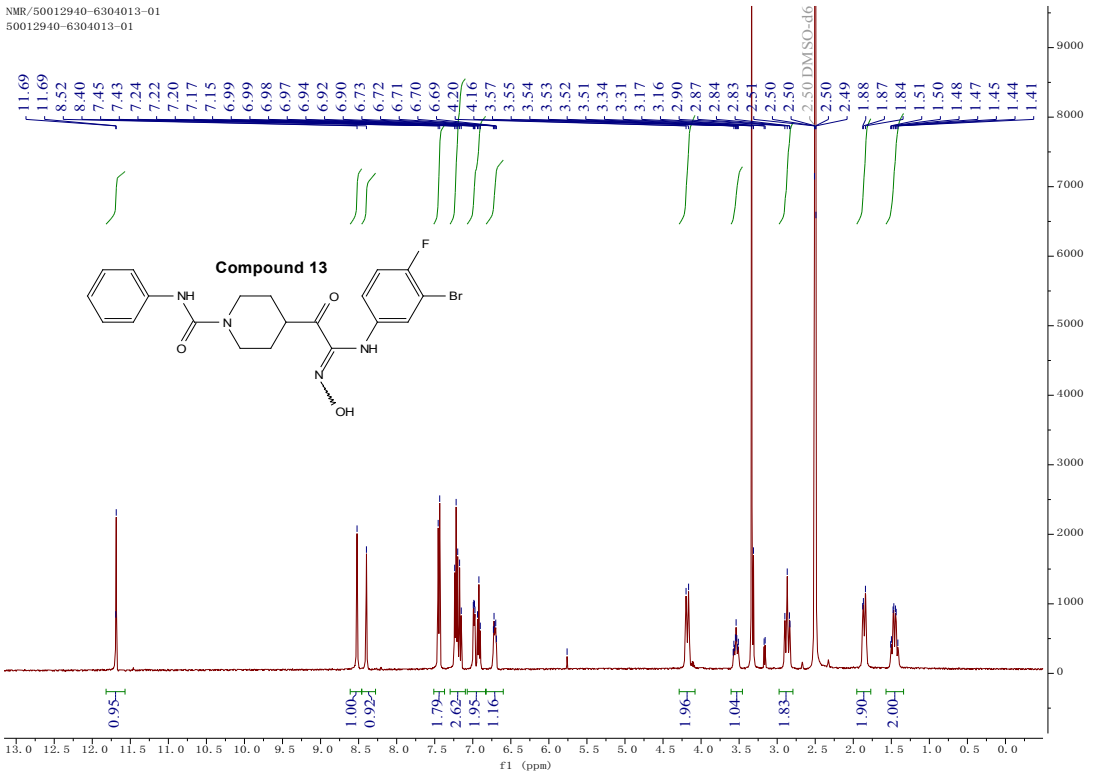
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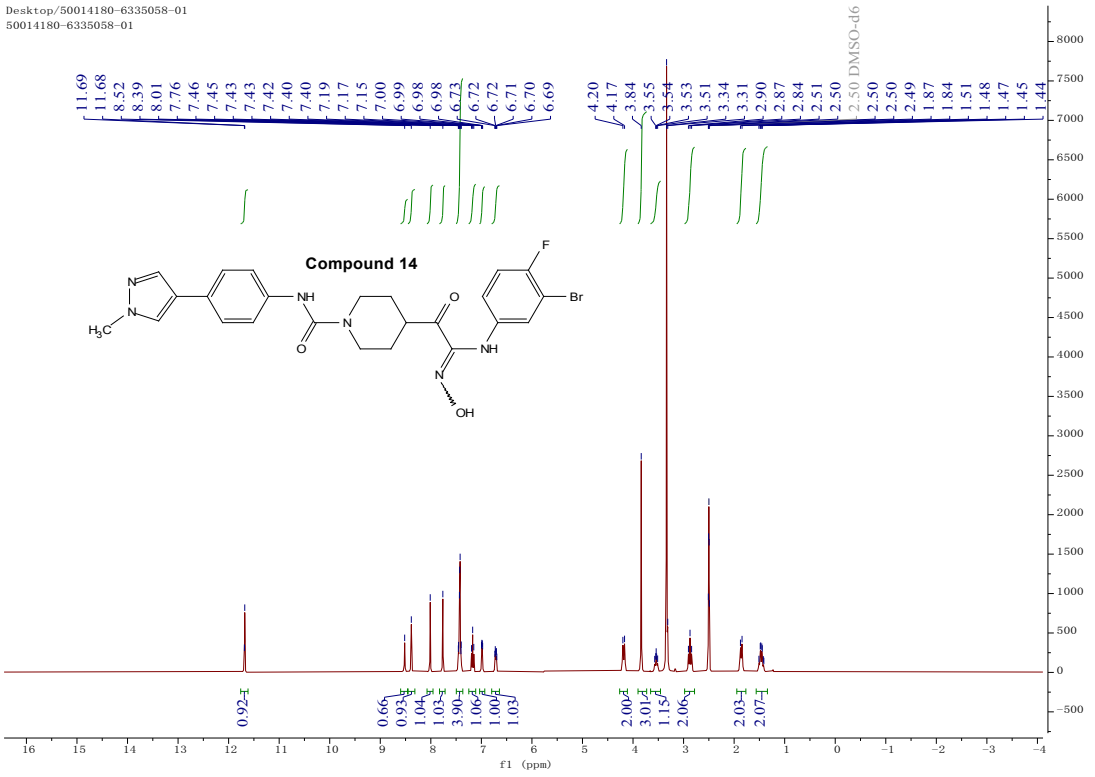
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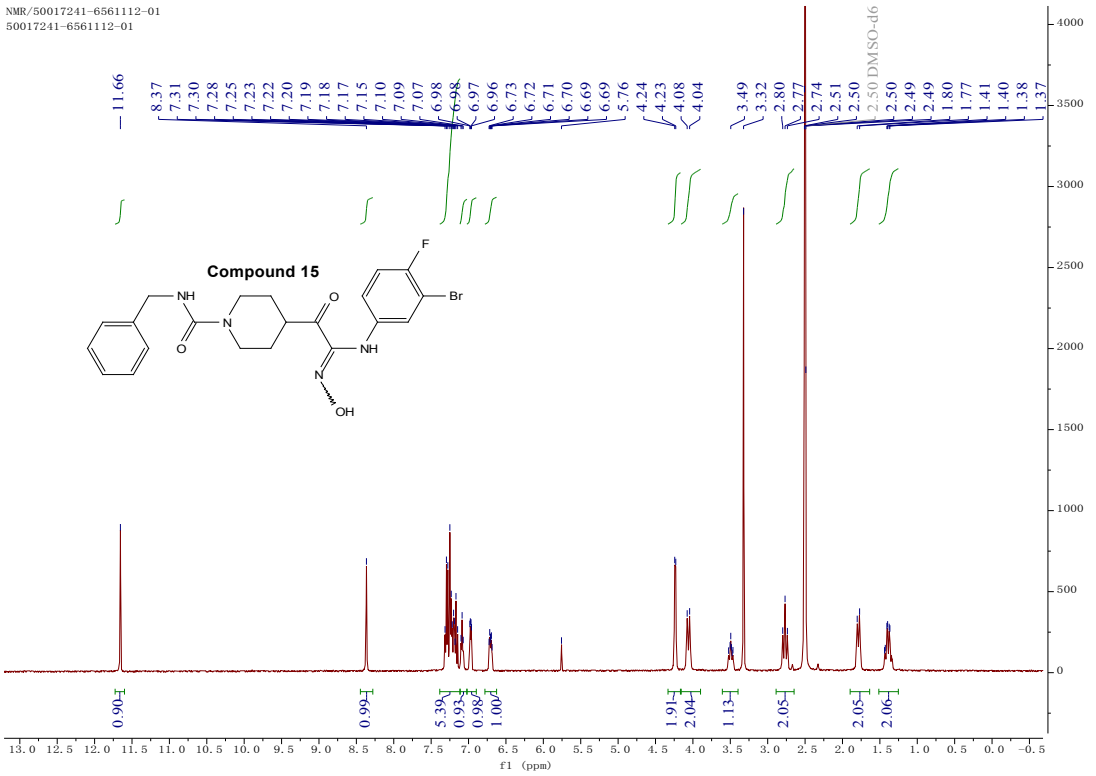
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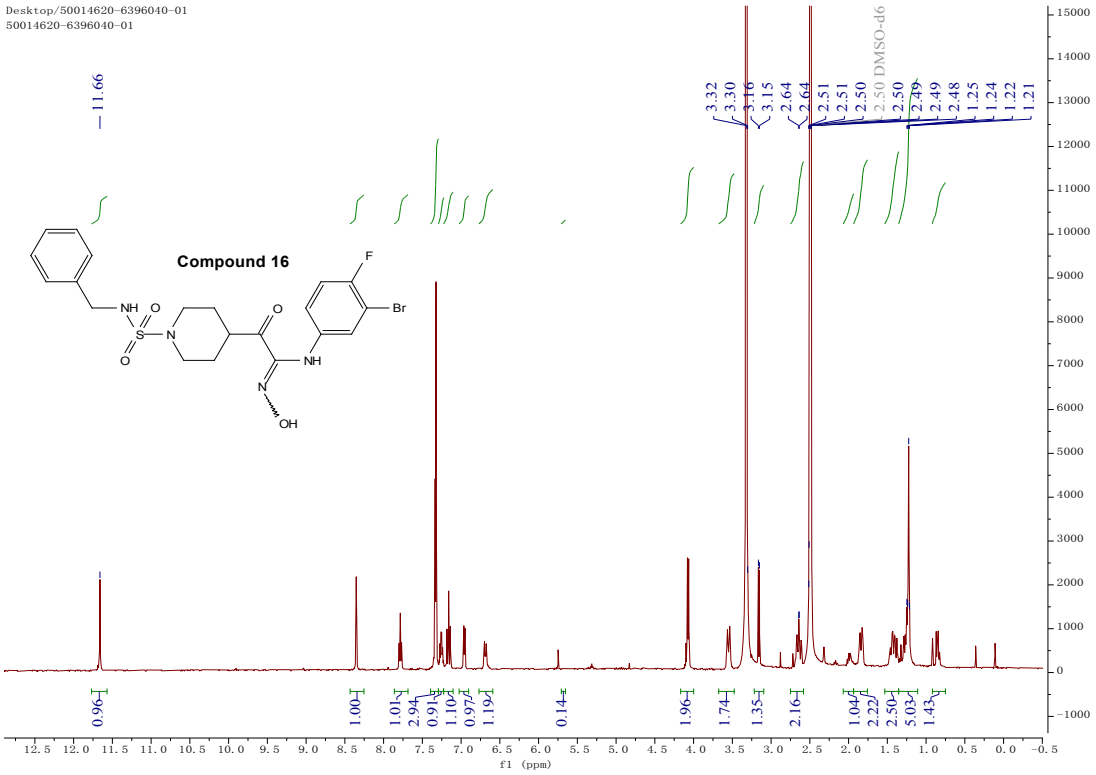
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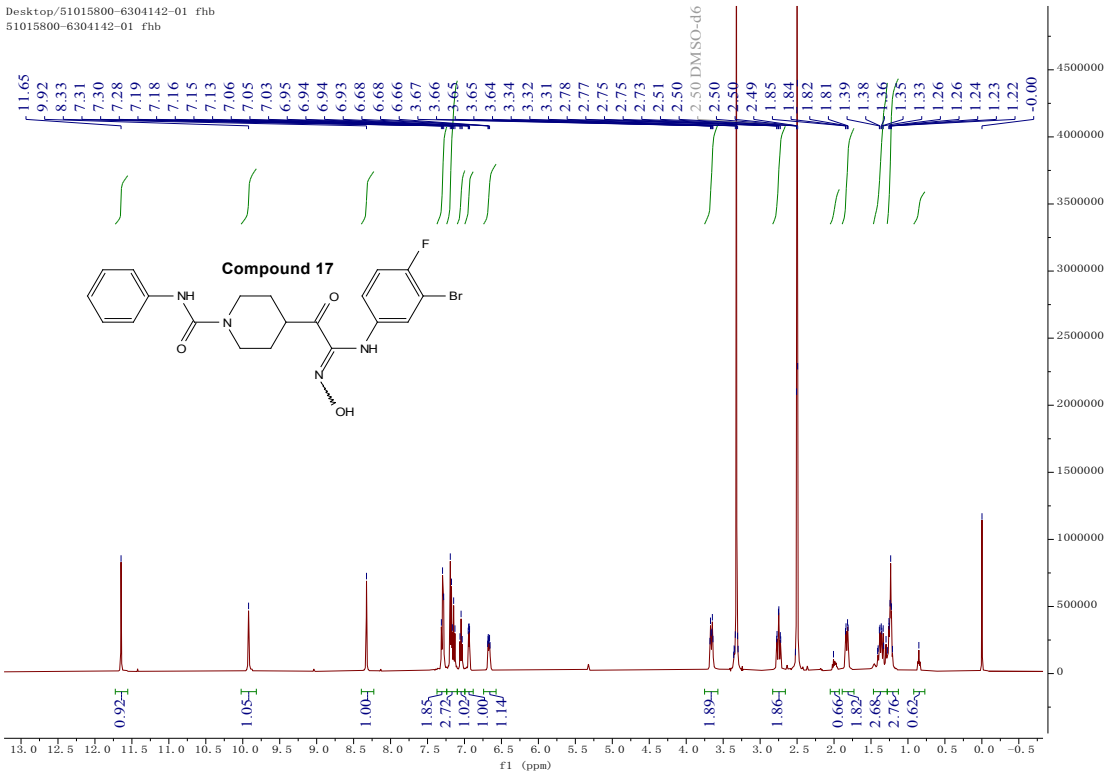
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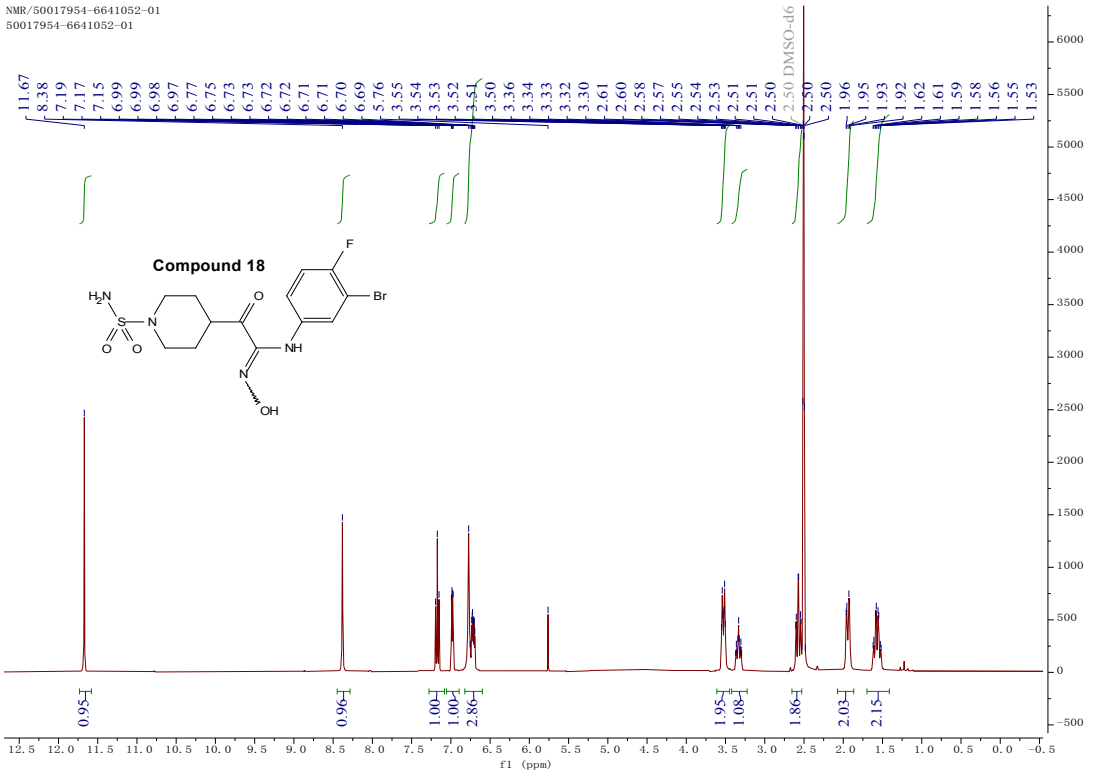
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