Cell Active Hydroxylactam Inhibitors of Human Lactate Dehydrogenase with Oral Bioavailability in Mice

Hans E. Purkey[a*], Kirk Robarge[a], Jinhua Chen[b], Zhongguo Chen[b], Laura B. Corson[a], Charles Z. Ding[b], Antonio DiPasquale[c], Peter S. Dragovich[a], Charles Eigenbrot[a], Marie Evangelista[a], Benjamin P. Fauber[a], Zhenting Gao[b], Hongxiu Ge[b], Anna Hitz[a], Qunh Ho[b], Sharada S. Labadie[a], Kwong Wah Lai[b], Wenfeng Liu[b], Yajing Liu[b], Chiho Li[b], Shuguang Ma[a], Shiva Malek[a], Thomas O'Brien[a], Jodie Pang[a], David Peterson[a], Laurent Salphati[a], Steven Sideris[a], Mark Ultsch[a], BinQing Wei[a], Ivana Yen[a], Qin Yue[a], Huihui Zhang[b], Aihe Zhou[a]

[a]Genentech, Inc., 1 DNA Way, South San Francisco, California 94080, United States

[b]WuXi AppTec Co., Ltd. 288 Fute Zhong Road, Waigaoqiao Free Trade Zone, Shanghai 200131, P. R. China

[c]College of Chemistry, University of California, Berkeley, Berkeley, California, 94720, United States

Supporting Information

***In vitro* drug treatment experiments.** All cell lines were obtained from our in-house tissue culture cell bank (original source was ATCC). Lines were authenticated by short tandem repeat (STR) and genotyped upon re-expansion. Cells were maintained in RPMI 1640 media supplemented with 10% FBS (Sigma; F2442). Cells were plated using optimal seeding densities in 384-well plates using RPMI, 5% FBS (Sigma F4135), 100 ug/ml penicillin, 100 units/ml streptomycin (Gibco 15140-122). Optimal seeding densities were established for each cell line in order to reach 75-80% confluence at the end of the assay. The following day, cells were treated with compound **29** using a 6 pt dose titration scheme. After 72 hours, cell viability was assessed using the CellTiter-Glo® Luminescence Cell Viability assay. Absolute inhibitory concentration (IC) values were calculated using four-parameter logistic curve fitting.

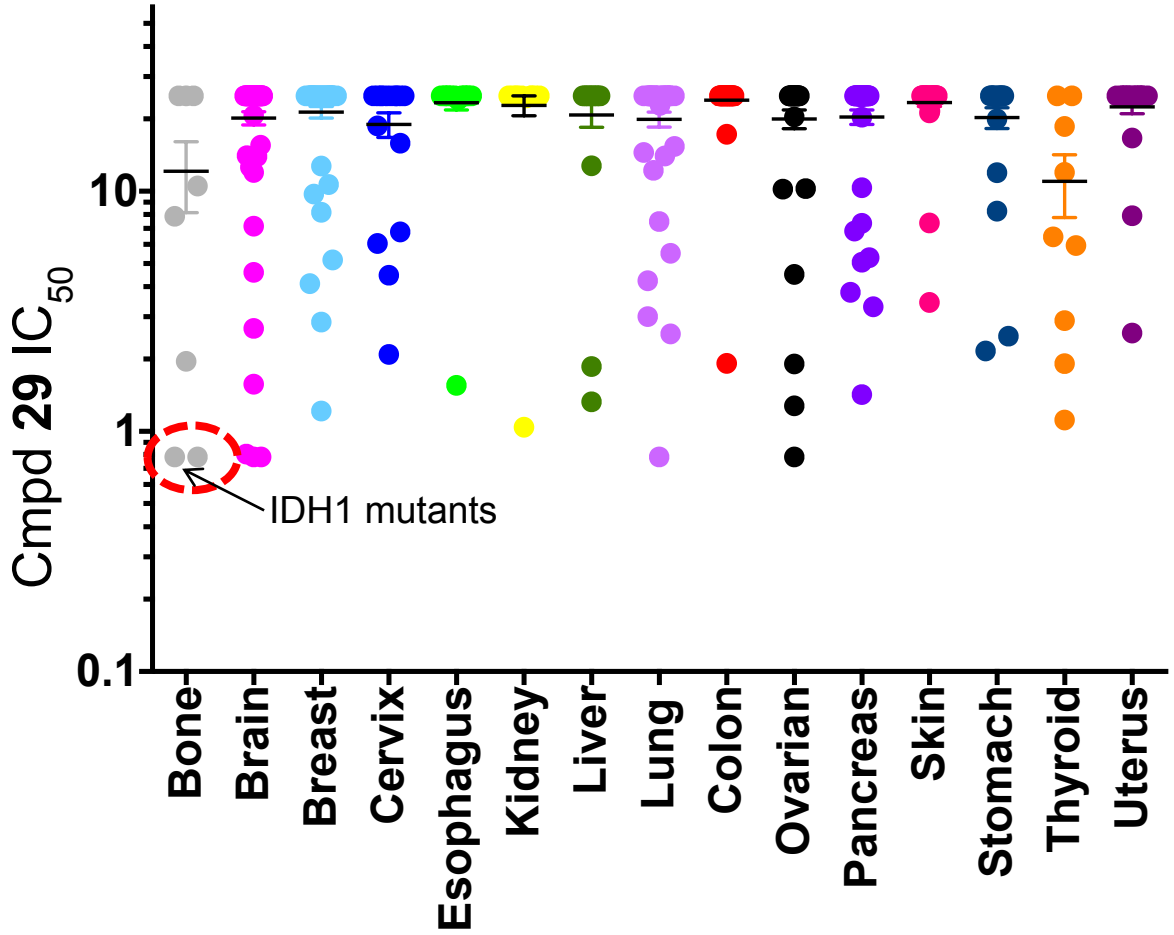


Figure S1. Inhibition of cell proliferation by **29** in 347 cancer cell lines. Cells were grown in the presence of **29** for 72 hours.

Table S1. Proliferation IC50s for **29** in 37 sensitive cancer cell lines

Cell Line	Tissue	CELLLINE_ID	IC50
105KC	Bone	586052	0.78
JJ012	Bone	586055	0.78
143B	Bone	584976	2.0
G84	Brain	132488	0.78
1321N1	Brain	586793	0.78
ONS-76	Brain	586962	0.80
G22	Brain	132482	1.6
G140	Brain	132425	2.7
G96	Brain	132516	4.6
HCC1143	Breast	585292	1.2
CAL-120	Breast	130224	2.9
Hs 578T	Breast	130032	4.1
SISO	Cervix	586885	2.1
SKG-II	Cervix	586968	4.5
HCT-15	Colon	132492	1.9
KYSE-520	Esophagus	586703	1.6

KMRC-1	Kidney	586966	1.0
SNU-475	Liver	586253	1.3
SNU-423	Liver	586251	1.9
HT-1080	Lung	585278	0.78
NCI-H1437	Lung	129264	2.5
NCI-H1339	Lung	586367	3.0
LXF-289	Lung	586341	4.2
MCAS	Ovary	586706	0.78
PA-1	Ovary	586195	1.3
Hs 38.T	Ovary	134160	1.9
59M	Ovary	134179	4.5
MIA PaCa-2	Pancreas	584217	1.4
SW 1990	Pancreas	584189	3.3
PSN1	Pancreas	584456	3.8
GR-M	Skin	586575	3.4
Hs 746T	Stomach	587042	2.2
MKN-74	Stomach	586301	2.5
S-117	Thyroid	586883	1.1
FTC-238	Thyroid	586815	1.9
B-CPAP	Thyroid	586852	2.9
HEC-265	Uterus	586958	2.6

Mouse Pharmacokinetics Study

The pharmacokinetics of compound **29** was evaluated following a single intravenous bolus (IV) dose of 1.0 mg/kg and oral administration (PO) of solution/amorphous suspension at a dose of 5 mg/kg in female CD-1 mice (N=3). The vehicle used for IV administration was 10/50/40 EtOH/PEG400/50mM citrate pH3 (v/v, 10/50/40), and for PO, 0.5% methycellulose:0.2% Tween in water (MCT). Blood samples for the IV dose group were collected at 0.033, 0.25, 1, 2, 4, 6 hours post dose. Blood samples for PO dose groups were collected at 0.25, 0.5, 1, 2, 4, and 6 hours post dose. For the high dose oral PK study at 50, 100, and 200 mg/kg, blood samples were collected at 0.25, 0.5, 1, 2, 4, 6, and 8 hours post dose. Blood samples were centrifuged within 29 minutes of collection, and plasma was harvested. Plasma samples were stored at approximately –70°C until the analysis of the compound concentration by a liquid chromatography/tandem mass spectrometry (LC-MS/MS) method. PK parameters were determined by non-compartmental methods using WinNonlin (version 5.2, Pharsight Corporation, Mountain View, CA).

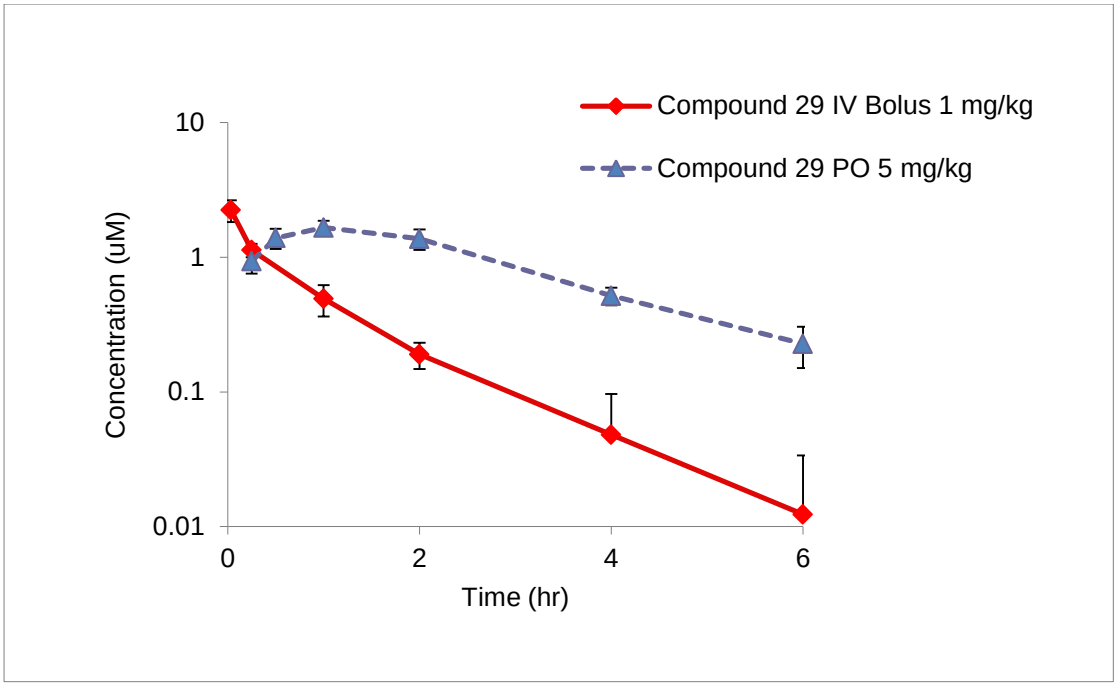


Figure S2. Blood concentration-time profiles of compound **29** in mice following IV (1 mg/kg) and PO (5 mg/kg) administrations.

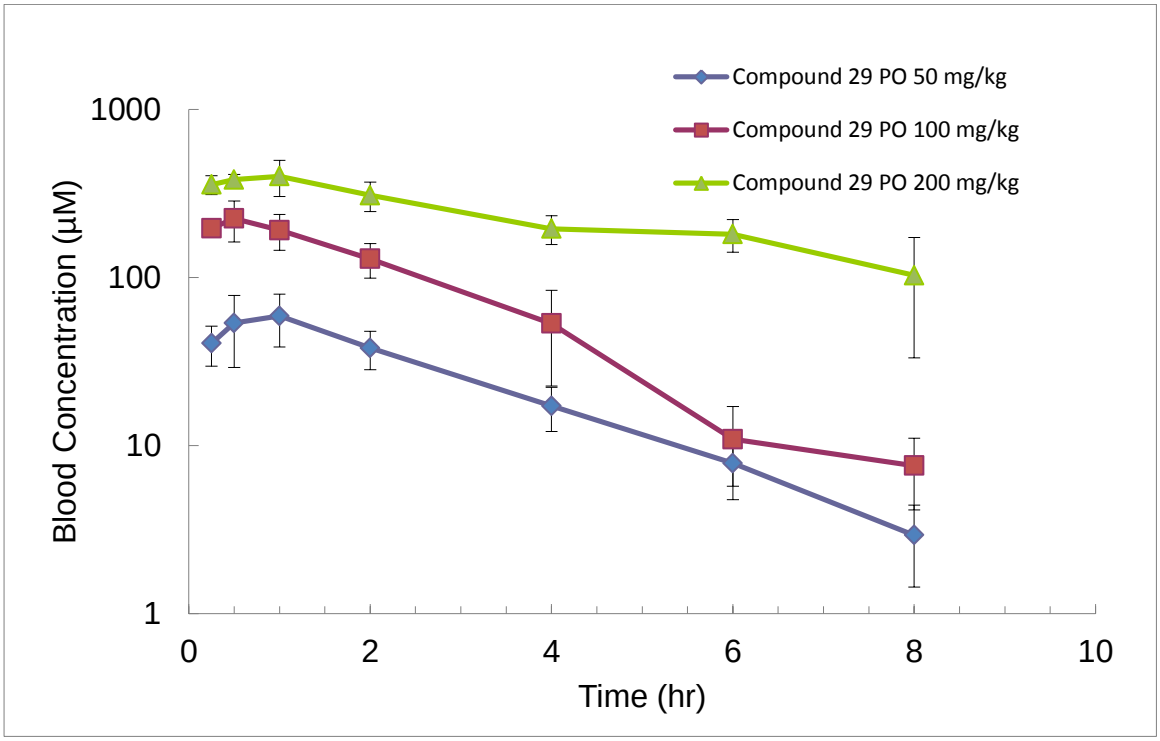


Figure S3. Blood concentration-time profiles of compound 29 in mice following PO (50, 100 and 200 mg/kg) administrations

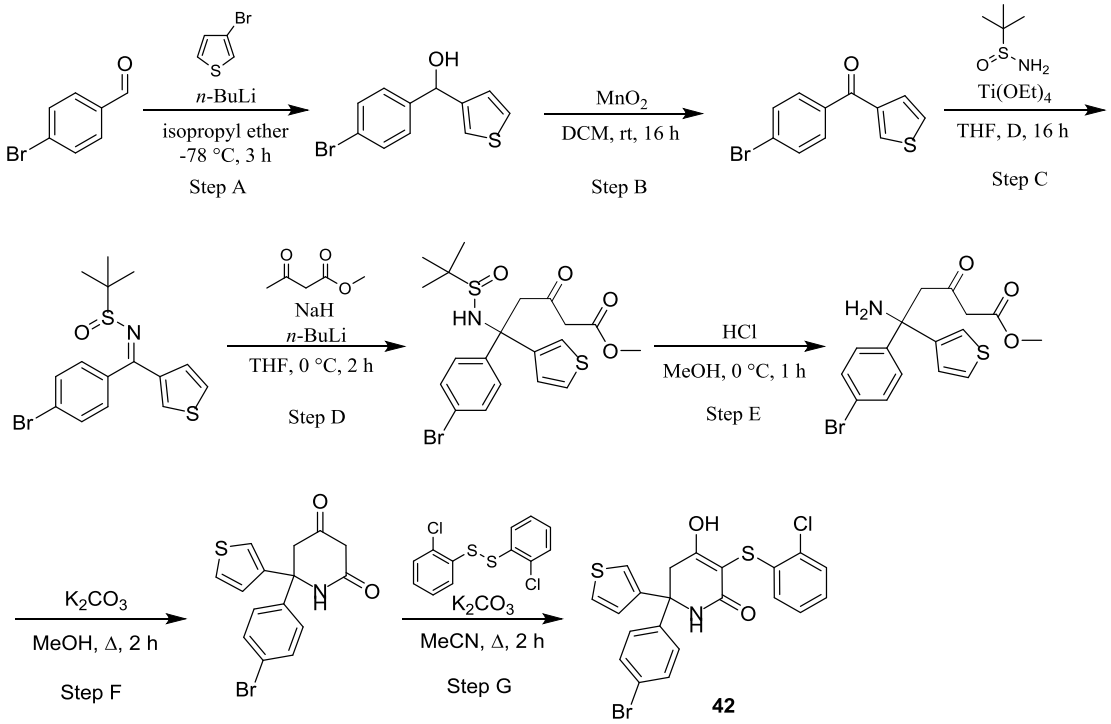
Purity and LCMS Analysis of Compounds

Compound	MW	LC-MS ESI$^+$ [M+1]$^+$	LC-MS Purity (254 nm) [%]
1	331.04	332.1	95
2	407.07	408.1	100
3	414.03	415.0	98
4	413.03	414.1	100
5	414.03	415.1	100
6	431.02	431.7	99
7	490.94	492.0	97
8	428.04	429.1	100
9	429.03	430.1	96
10	438.03	438.9	98
11	453.06	453.8	94
12	443.04	444.1	96
13	456.07	456.8	95
14	429.03	429.7	96
15	498.08	499.0	100
16	512.10	513.2	100
17	510.12	511.2	93
18	522.06	523.1	100
19	513.08	513.9	93
20	523.05	523.8	96
21	495.11	495.8	99
22	497.10	498.2	96
23	553.13	554.2	92
24	526.12	526.9	98
25	496.10	497.1	98
26	482.09	483.1	97
27	539.11	539.9	99
28	498.08	499.1	96
29	498.08	499.1	99
30	498.08	499.1	96
31	526.12	527.0	97
32	512.06	512.8	97
33	526.12	526.9	98
34	546.05	546.8	93
35	538.12	539.0	92
36	521.10	522.0	95

Experiments performed on an Agilent 1200 UHPLC coupled with Agilent MSD (6140) mass spectrometer using ESI as ionization source. The LC separation was using a Agilent ZORBAX SB-C18, 1.8um, 2.1*50mm column with a 0.4 ml / minute flow rate. Solvent A is water with 0.1% FA and solvent B is acetonitrile with 0.1% FA. The gradient consisted with 2 - 98% solvent B over 7 min and hold 98%B for 1.5 min following equilibration for 1.5 min. LC column temperature is 40 ^{0}C. UV absorbance was collected at 220nm and 254nm and mass spec full scan was applied to all experiment.

Synthetic procedures and characterization data for key compounds tested in cellular assays.

Synthesis of **46:**



Step A: *n*-BuLi (650 mL, 2.5M, 1.63 mol) was slowly added to a solution of 3-bromothiophene (265 g, 1.63 mol) in isopropyl ether (3 L) under -78 ^{o}C. After stirring at -78 ^{o}C for 30 min, the reaction mixture was slowly treated with 4-bromobenzaldehyde (250 g, 1.35 mol) in THF (500 mL) and stirred at -78 ^{o}C for 2 hours. The reaction mixture was quenched with sat. NH_4Cl (1.5 L), warmed to ambient temperature. The mixture was extracted with ethyl acetate (800 mL × 3). The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by silica gel chromatography eluting with a gradient of 0% ethyl acetate/petroleum ether to 20% ethyl acetate/petroleum ether to afford (4-bromophenyl)(thiophen-3-yl)methanol (335 g, 92%) as yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.51 (d, *J* = 8.4 Hz, 2H), 7.44 (dd, *J* = 4.8 Hz, 2.8 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 2H), 7.31 (d, *J* = 2.8 Hz, 1H), 6.98 (d, *J* = 4.4 Hz, 1H), 5.96 (d, *J* = 4.0 Hz, 1H), 5.75 (d, *J* = 4.0 Hz, 1H).

Step B: A mixture of (4-bromophenyl)(thiophen-3-yl)methanol (335 g, 1.25 mol), MnO_2 (1083 g, 12.4 mol) in CH_2Cl_2 (3.5 L) was stirred at room temperature for 16 hours. The mixture was filtered over a short pad of silica gel. The filtrate was concentrated to afford (4-bromophenyl)(thiophen-3-yl)methanone (320 g, 96%) as a yellowish solid, which was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (d, *J* = 1.6 Hz, 1H), 7.79 - 7.73 (m, 5H), 7.54 (d, *J* = 4.8 Hz, 1H).

Step C: A mixture of (4-bromophenyl)(thiophen-3-yl)methanone (320 g, 1.20 mol), $Ti(OEt)_4$ (850 g, 3.73 mol), 2-methylpropane-2-sulfinamide (302 g, 2.50 mol) and THF (3.5

L) was heated at 80 ^{o}C for 16 hours. The suspension was allowed to cool to ambient temperature. The mixture was pour into ice water, filtered, washed with ethyl acetate. The filtrate was extracted with ethyl acetate (1.5 L x 2), dried over Na_2SO_4 and concentrated. The crude was purified by silica gel chromatography eluting with a gradient of 0% ethyl acetate/ petroleum ether to 30% ethyl acetate/ petroleum ether to afford (*E*)-*N*-((4-bromophenyl)(thiophen-3-yl)methylene)-2-methylpropane-2- sulfinamide (316 g, 71%) as a yellow solid. LCMS M/Z (M+H) = 370.

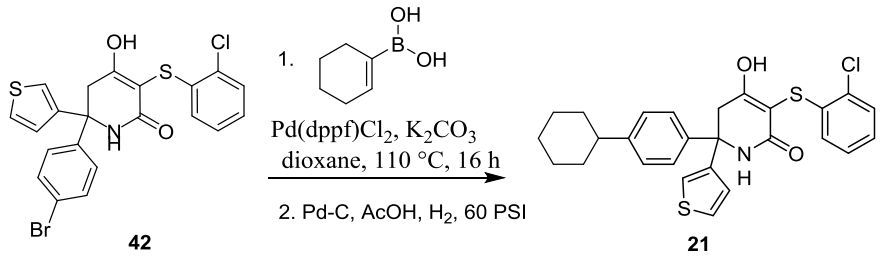
Step D: Methyl 3-oxobutanoate (194 g, 1.67 mol) was added to a suspension of NaH (67.0 g, 1.68 mol) in THF (3 L) under 0 ^{o}C. The reaction mixture was then slowly treated with *n*–BuLi (670 mL, 2.5 M, 1.68 mol) and stirred under 0 ^{o}C for 30 min, (*E*)-N-((4-bromophenyl) (thiophen-3-yl)methylene)-2-methylpropane-2-sulfinamide (310 g, 0.838 mol) in THF (500 mL) was added to the mixture and stirred under 0 ^{o}C for another 2 hours. The reaction mixture was poured into ice-water and warmed to ambient temperature. The mixture was extracted with ethyl acetate (800 mL x 2). The organic layer was dried over Na_2SO_4 and concentrated to afford methyl 5-(4-bromophenyl)-5-(1,1-dimethylethylsulfinamido)-3-oxo-5-(thiophen-3-yl) pentanoate (800 g, crude) as dark oil, which was used in the next step without further purification. LCMS M/Z (M+Na) = 508.

Step E: HCl/MeOH (4M, 400 mL) was slowly added to a solution of methyl 5-(4-bromophenyl)-5-(1,1-dimethylethylsulfinamido)-3-oxo-5-(thiophen-3-yl)pentanoate (400 g, crude) in MeOH (3 L) under 0 ^{o}C. The mixture was stirred at 0 ^{o}C for 1 hour, and then slowly acidified to pH 7 using 3 N NaOH at 0 °C. The solvent was removed under vacuum. The crude product was extracted with ethyl acetate (1 L x 2), dried over Na_2SO_4 and concentrated to afford methyl 5-amino-5-(4-bromophenyl)-3-oxo-5-(thiophen-3-yl)pentanoate (360 g, crude) as dark oil, which was used in the next step without further purification. LCMS M/Z (M-NH_2) = 365.

Step F: Potassium carbonate (310 g, 2.25 mol) was added to a solution of methyl 5-amino-5-(4-bromophenyl)-3-oxo-5-(thiophen-3-yl)pentanoate (360 g, crude) in MeOH (3.5 L). The mixture was heated at 80 ^{o}C for 2 hours. The suspension was allowed to cool to ambient temperature. The solvent was removed under vacuum, the crude product was dissolved in water (2 L), washed with ethyl acetate (1 L x 2). The aqueous layer was acidified to pH 4 using 3N aqueous hydrochloride and extracted with ethyl acetate (1.5 L x 3). The organic layer was washed with brine, dried over Na_2SO_4 and concentrated to give 6-(4-bromophenyl)-4-hydroxy-6-(thiophen-3-yl)-5,6-dihydropyridin-2(1*H*)-one (160 g, crude) as brown oil, which was used in the next step without further purification. LCMS M/Z (M+H) = 350.

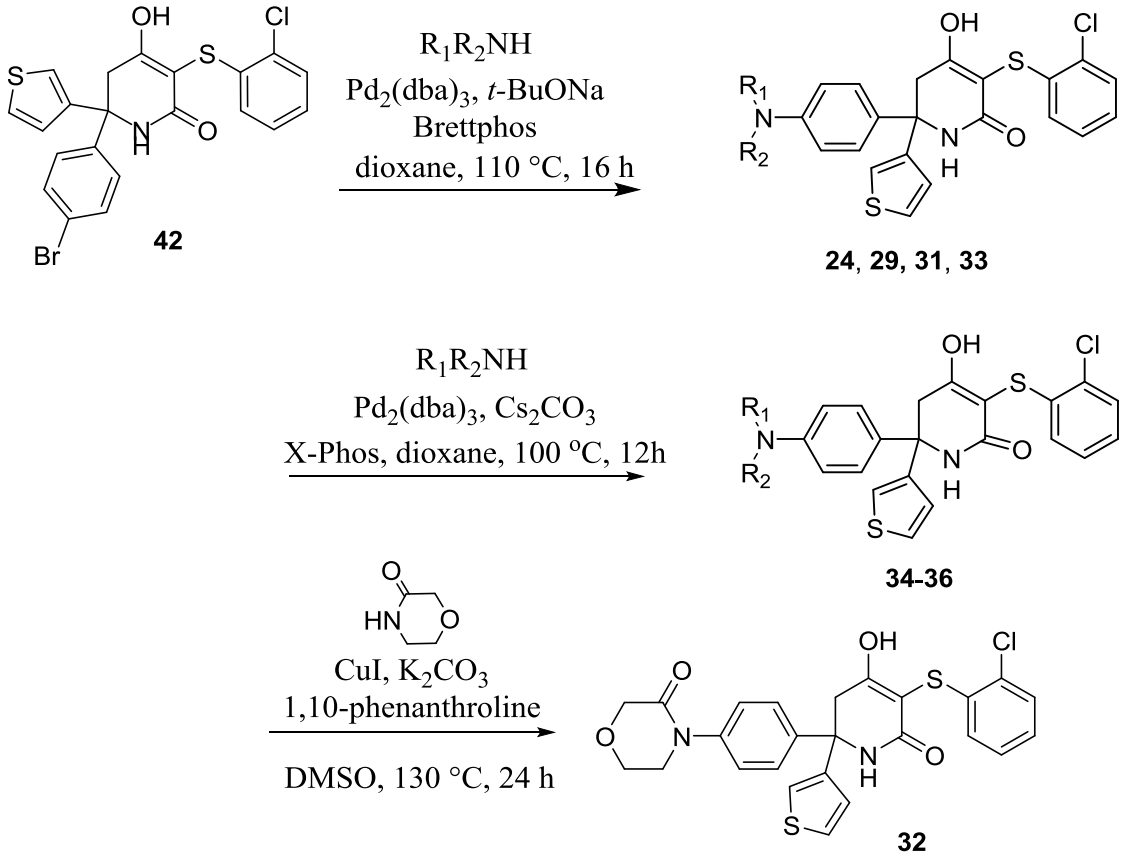
Step G: Potassium carbonate (190 g, 1.38 mol) and 1,2-bis(2-chlorophenyl)disulfane (80.0 g, 0.279 mmol) was added to a solution of 6-(4-bromophenyl)-4-hydroxy-6-(thiophen-3-yl)-5,6- dihydropyridin-2(1*H*)-one in MeCN (2 L). The mixture was heated at 80 ^{o}C for 2 hours. The suspension was allowed to cool to ambient temperature. The solvent was removed under vacuum. The crude product was dissolved in water (2 L), and acidified to pH 4 using 3 N HCl. The aqueous layer was extracted with ethyl acetate (1.5 L x 2). The organic layer was

washed with brine, dried over Na_2SO_4 and concentrated. The crude was purified by silica gel chromatography eluting with a gradient of 0% ethyl acetate/hexanes to 50% ethyl acetate/hexanes to afford 6-(4-bromophenyl)-3-((2-chlorophenyl)thio)-4-hydroxy-6-(thiophen-3-yl)-5,6-dihydropyridin-2(1*H*)-one **42** (190 g, 84%) as a white solid. LCMS M/Z (M+H) = 492.



3-(2-Chlorophenyl)sulfanyl-6-(4-cyclohexylphenyl)-6-(3-thienyl)piperidine-2,4-dione (**21**): To a solution of 6-(4-bromophenyl)-3-((2-chlorophenyl)thio)-6-(thiophen-3-yl) piperidine-2,4-dione **42** (0.25 g, 0.5 mmol) in dioxane (6 mL) and water (2 mL) was added cyclohex-1-en-1-ylboronic acid (126 mg, 1 mmol), $Pd(dppf)Cl_2$ (36 mg, 0.05 mmol) and K_2CO_3 (0.27 g, 2 mmol). The reaction mixture was microwaved at 100 ºC for 1 hour under nitrogen atmosphere. After cooling to room temperature, the reaction mixture was filtered through a short pad of silica gel. The filtrate was concentrated under vacuum and the crude residue was purified by preparative HPLC (formic acid) to afford 3-(2-chlorophenyl)sulfanyl-6-[4-(cyclohexen-1-yl)phenyl]-6-(3-thienyl)piperidine-2,4-dione (11.7 mg, 5% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.47 (s, 1H), 7.56 - 7.55 (m, 1H), 7.54 - 7.39(m, 2H), 7.32 - 7.20 (m, 3H), 7.27 (d, *J* = 8 Hz, 1H), 7.14 (dd, *J* = 5.2, 4.8 Hz, 1H), 6.93 (dd, *J* = 7.6, 4.8Hz, 1H), 6.15 (s, 1H), 5.85 (d, *J* = 8.0 Hz, 1H), 3.39 (s, 2H), 2.47 (s, 2H), 2.33 (s, 2H), 1.71 - 1.68 (m, 2H), 1.58 - 1.56 (m, 2H). LCMS M+1 = 493.9.

To a solution of 3-(2-chlorophenyl)sulfanyl-6-[4-(cyclohexen-1-yl)phenyl]-6-(3-thienyl)piperidine-2,4-dione (0.8 g,1.6 mmol) in acetic acid (20 mL) was added Pd/C (0.1 g). The reaction mixture was stirred at room temperature for 24 hours under hydrogen atmosphere (60 PSI). After relieving the pressure, the reaction mixture was filtered over Celite and the filtrate was concentrated under vacuum. The crude residue was purified by preparative HPLC (formic acid) to afford the product **21** (10 mg, 1.2% yield) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.49 (s, 1H), 7.35-7.32 (m, 2H), 7.26-7.25 (m, 4H), 7.19 (d, *J* = 8.0 Hz, 1H), 6.93 (dd, *J* = 6.8, 6.8 Hz, 1H), 6.72 (dd, *J* = 6.8, 6.8 Hz, 1H), 5.98 (d, *J* = 6.8 Hz, 1H), 3.45 (s, 2H), 1.96 - 1.74 (m, 5H), 1.48 - 1.27 (m, 5H). LCMS M+1 = 495.8.



(*6R*)-3-(2-Chlorophenoxy)-6-(4-morpholinophenyl) -6-(thiophen-3-yl)piperidine-2,4-dione (**29**): To a solution of 6-(4-bromophenyl)-3-(2-chlorophenoxy)-6-(thiophen-3-yl) piperidine-2,4-dione (600 mg, 1.26 mmol) in dioxane (10 mL) was added morpholine (328 mg, 3.77 mmol), Brettphos (65 mg, 0.13mmol), $Pd_2(dba)_3$ (64 mg, 0.07 mmol) and t-BuONa (362 mg, 3.77 mmol). The solution was stirred for 8 h at 110 oC under nitrogen. The solvent was removed under vacuum and the residue was purified by Prep-HPLC (FA) and chiral superfluid chromtography (stationary phase: Welko, MeOH w/ 0.1% NH_4OH) to afford (*6R*)-3-(2-chlorophenoxy)-6-(4-morpholinophenyl) -6-(thiophen-3-yl)piperidine-2,4-dione (35 mg, 6%) as white solid: ^{1}H NMR (400MHz, DMSO-d_6) δ 8.11 (s, 1H), 7.54 (dd, J=5.0, 2.9Hz, 1H), 7.35-7.22 (m, 1H), 7.25 (dd, J=8.2, 5.5 Hz, 3H), 7.14 (dd, J=5.1, 1.4 Hz, 1H), 6.93 (t, J = 10.1 Hz, 3H), 6.74 (t, J = 7.7 Hz, 1H), 5.95 (d, J = 8.0 Hz, 1H), 3.80 – 3.66 (m, 4H), 3.33 (s, 2H), 3.16– 3.05 (m, 4H); ^{13}C NMR (101 MHz, DMSO-d6) δ 173.52, 166.20, 149.94, 146.66, 137.45, 134.92, 128.87, 128.62, 127.29, 126.87, 126.75, 126.46, 125.00, 124.79, 121.44, 114.45, 93.20, 65.99, 59.06, 48.15, 42.37; HRMS (ESI^+) calcd for $C_{25}H_{24}O_3N_2ClS_2$ $(M + H)^+$ 499.0911, found 499.0910.

Compounds **24**, **31** and **33** were prepared using the conditions described for compound **29**.

3-((2-Chlorophenyl)thio)-6-(4-(4-methoxypiperidin-1-yl)phenyl)-6-(thiophen-3-yl)piperidine-2,4-dione (**24**): ^{1}H NMR (400MHz, DMSO-d_6) δ 7.60-7.55 (m, 5H), 7.37 (dd, J=4.2, 4.2Hz, 1H) 7.24 (d, J=8.0Hz, 1H), 7.17 (d, J=4 .0Hz, 1H), 6.96 (dd, J=8.0, 8.0 Hz,

1H), 6.10 (d, J=8.0Hz, 1H), 3.76-3.74 (m, 2H), 3.57-3.55 (m,1H), 3.52-3.45 (m, 4H), 3.42 (s, 3H), 2.24-2.06 (m, 4H).

3-((2-Chlorophenyl)thio)-6-(4-(2,6-dimethylmorpholino)phenyl)-6-(thiophen-3-yl)piperidine-2,4-dione (**31**): ^{1}H NMR (400MHz, Methanol-d_4) δ 7.48 (dd, *J* = 5.2, 3.2 Hz, 1H), 7.34 - 7.32 (m, 2H), 7.31 (d, *J* = 3.2Hz, 1H), 7.28 (d, *J* = 2.4 Hz, 1H), 7.27 (d, *J* = 2.4 Hz, 1H), 6.99 - 6.97 (m, 2H), 6.90 (dd, *J* = 5.2, 2.4 Hz, 1H), 6.73 (dd, *J* = 5.2, 2.4 Hz, 1H), 6.06 (dd, *J* = 8.0, 1.2 Hz, 1H), 3.85 - 3.78 (m, 2H), 3.36 (d, *J* = 16.0 Hz, 2H), 3.32 (t, *J* = 5.2 Hz, 2H), 3.38 (t, *J* = 5.2Hz, 2H), 1.38 (s, 6H).

3-((2-Chlorophenyl)thio)-6-(4-(2,2-dimethylmorpholino)phenyl)-6-(thiophen-3-yl)piperidine-2,4-dione (**33**): ^{1}H NMR (400 MHz, Methanol-d4) δ 7.48 (dd, *J* = 5.2 Hz, 5.2 Hz, 1H), 7.33 (d, *J* = 8.8 Hz, 2H), 7.24 (d, *J* = 3.2 Hz, 1H), 7.18 (d, *J* = 8.8 Hz 1H), 7.15 (d, *J* = 3.2 Hz, 1H), 7.14(d, *J* = 8.8 Hz, 2H), 7.01 (dd, *J* = 7.6 Hz, 7.6 Hz, 1H), 6.75(dd, *J* = 7.6 Hz, 7.6 Hz 1H), 5.94(dd, *J* = 7.6 Hz, 7.6 Hz 1H), 3.88 (t, *J* = 4.8 Hz, 2H), 3.40 (s, 2H), 3.16 (t, *J* = 4.8 Hz, 2H), 3.03 (s, 2H), 1.32 (s, 6H).

Compounds **34** – **36** were prepared as described for compound **29** using X-phos as ligand and cesium carbonate as base.

3-((2-Chlorophenyl)thio)-6-(4-(1,1-dioxidothiomorpholino)phenyl)-6-(thiophen-3-yl)piperidine-2,4-dione (**34**): ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.43 (s, 1H), 8.39 (s, 1H), 7.57 - 7.51 (m, 1H), 7.31 - 7.21 (m, 4H), 7.14 (d, J = 4.0 Hz, 1H), 7.00 (d, J = 8.8 Hz, 2H), 6.97 - 6.91 (m, 1H), 6.74 - 6.67 (m, 1H), 3.81 - 3.72 (m, 4H), 3.37 (s, 2H), 3.13 - 3.04 (m, 4H).

6-(4-(2-Oxa-7-azaspiro[3.5]nonan-7-yl)phenyl)-3-((2-chlorophenyl)thio)-6-(thiophen-3-yl)piperidine-2,4-dione (**35**): ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.47(dd, J=5.2, 3.2 Hz, 1H), 7.29-7.25 (m, 3 H), 7.19 (d, J=8.0 Hz, 1 H), 7.13 (d, J=8.0 Hz, 1 H), 6.98(d, J=8.8 Hz, 2 H), 6.90 (dd, J = 8.0,8.0 Hz, 1 H), 6.73 (dd, J=8.0 ,8.0 Hz, 1 H), 5.98 (d, J = 7.6 Hz, 1 H), 4.48 (s, 4 H), 3.40 (s, 2 H), 3.14 (t, J=5.6 Hz, 4 H), 1.99 (t, J=5.6 Hz, 4 H).

1-(4-(5-((2-Chlorophenyl)thio)-4,6-dioxo-2-(thiophen-3-yl)piperidin-2-yl)phenyl)piperidine-4-carbonitrile (**36**): ^{1}H NMR (400MHz, Methanol-d_4) δ 7.43 (dd, J=7.6, 2.4 Hz, 1H), 7.30 (d, J=8.8 Hz, 2H), 7.23 (d, J=3.2 Hz, 1H), 7.13-7.10 (m, 2H), 6.97 (d, J=8.8 Hz, 2H), 6.82 (dd, J=7.6, 7.6Hz, 1H), 6.70 (dd, J=7.6, 7.6Hz, 1H), 6.05 (d, J=7.6 Hz, 1H), 3.42 (t, J=5.2 Hz, 2H), 3.28 (s, 2H), 3.09 (t, J=5.2 Hz, 2H), 3.01-2.94 (m, 1H), 2.03 (t, J=5.2 Hz, 2H), 1.92 (t, J=5.2 Hz, 2H).

3-((2-Chlorophenyl)thio)-6-(4-(3-oxomorpholino)phenyl)-6-(thiophen-3-yl)piperidine-2,4-dione (**32**):To a solution of 6-(4-bromophenyl)-3-((2-chlorophenyl)thio)-6-(thiophen-3-yl)piperidine-2,4-dione (300 mg, 0.6 mmol) in DMSO (5 mL) was added copper(I) iodide (23 mg, 0.12 mmol), 1,10-phenanthroline (10 mg, 0.06 mmol), K_2CO_3 (168 mg, 1.2 mmol) and 4-iodo- morpholin-3-one (123 mg, 1.2 mmol). The reaction mixture was heated to 130 °C for 24 h under a nitrogen atmosphere. After cooling to room temperature, the mixture was filtered and concentrated in vacuo. The crude residue was purified by reverse

phase chromatography (formic acid) to afford 3-((2-chlorophenyl)thio)-6-(4-(3-oxomorpholino)phenyl)-6-(thiophen-3-yl)piperidine-2,4-dione (56 mg, 18%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.57 - 7.52 (m, 3H), 7.46 - 7.41 (m, 2H), 7.37 (s, 1H), 7.26 - 7.19 (m, 2H), 6.98 - 6.93 (m, 1H), 6.91 - 6.84 (m, 1H), 6.10 (d, *J* = 7.6 Hz, 1H), 4.31 (s, 2H), 4.07 (t, *J* = 4.8 Hz, 1H), 3.82 (t, *J* = 4.8 Hz, 1H), 3.52 (s, 2H).

Small molecule X-ray crystallography. X-ray quality crystals were grown from a saturated acetonitrile solution with the addition of 1 equivalent of tetramethylammonium hydroxide (25% solution in MeOH) followed by the slow vapor diffusion of diisopropyl ether to deposit the crystal diffracted. A colorless prism 0.050 x 0.030 x 0.030 mm in size was mounted on a Cryoloop with Paratone oil. Data were collected in a nitrogen gas stream at 100° K using phi and omega scans. Crystal-to-detector distance was 40 mm and exposure time was 10 seconds per frame using a scan width of 1.0°. Data collection was 99.8% complete to 25.000° in □. A total of 55134 reflections were collected covering the indices, -10<=*h*<=10, -21<=*k*<=21, -21<=*l*<=22. 5481 reflections were found to be symmetry independent, with an R_{int} of 0.0793. Indexing and unit cell refinement indicated a primitive, orthorhombic lattice. The space group was found to be $P2_12_12_1$ (No. 19). The data were integrated using the Bruker SAINT software program and scaled using the SADABS software program. Solution by iterative methods (SHELXT-2014) produced a complete heavy-atom phasing model consistent with the proposed structure All non-hydrogen atoms were refined anisotropically by full-matrix least-squares (SHELXL-2014). All hydrogen atoms were placed using a riding model. Their positions were constrained relative to their parent atom using the appropriate HFIX command in SHELXL-2014. Absolute stereochemistry was unambiguously determined to be *R* at C1.

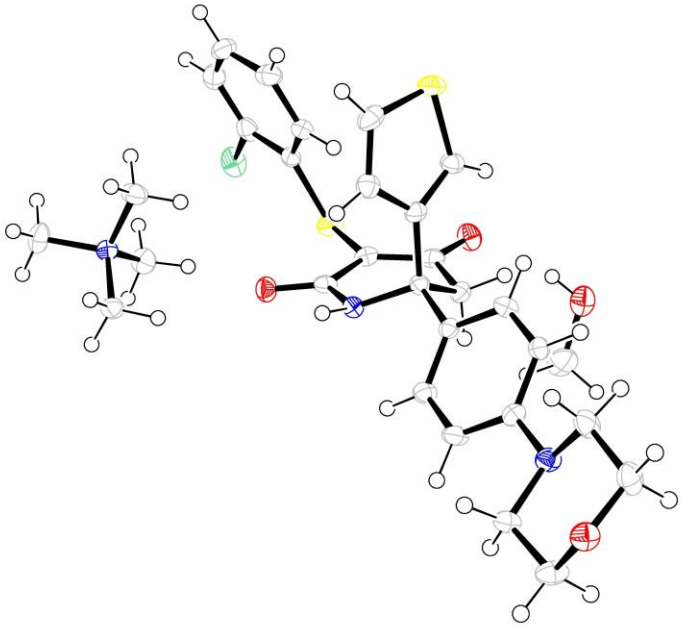


Figure S4. Small molecule crystal structure of compound **29**.

Table S2. X-ray Structure Determination Data and Statistics

	Compound **9**/LDHA	Compound **30**/LDHA
PDB code	5IXS	5IXY
Data collection/reduction		
X-ray source	SSRL 11-1	SSRF 17U
Wavelength (Å)	0.9795	0.9791
Resolution range (Å)	37.8 -2.05 (2.128 -2.05)	33.1-3.0 (3.11 - 3.0)
Space group	$P2_1$	$P2_1$
Unit cell edges (Å)	74.62 81.23 104.80	78.45 80.70 102.24
Unit cell angles (˚)	90, 97.56, 90	90, 97.90, 90
Total reflections	265849	75308
Unique reflections	764670	22595
Multiplicity	3.5 (3.3)	3.1(3.3)
Completeness (%)	98.2	95.2
Mean I/σ (I)	16.6(2.1)	10.4(3.4)

Wilson B-factor (Å^2)	32.7	49.2
R-symm	0.058(0.599)	0.103(0.402)
Refinement		
Reflections used for R-free	1571	506
R-work	0.174	0.236
R-free	0.221	0.279
Number of non-H atoms	11112	10593
macromolecules	10190	10250
ligands	353	343
water	569	0
Protein residues	1305	1321
RMS(bonds) (Å)	0.007	0.008
RMS(angles) (°)	1.4	1.1
Ramachandran favored (%)	97	96
Average B-factor (Å^2)	38.4	55.38
macromolecules	38.34	55.34
ligands	43.6	56.35
solvent	35.8	-