Discovery of Potent and Selective DDR1 Receptor Tyrosine Kinase Inhibitor

Supplemental Information

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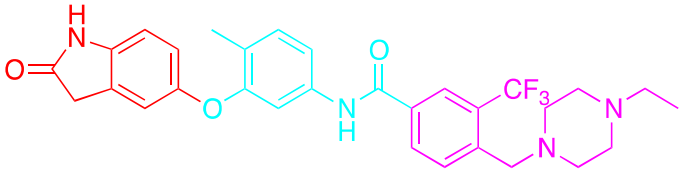
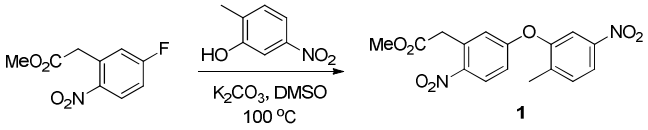
Chemical Synthesis

DDR1-IN-1

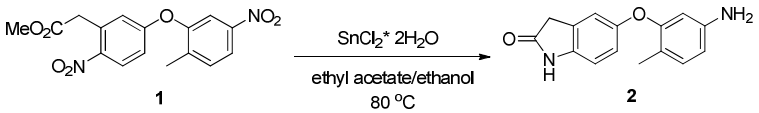


Unless otherwise noted, reagents and solvents were obtained from commercial suppliers and were used without further purification. ^{1}H NMR spectra were recorded on 600 MHz (Varian AS600), and chemical shifts are reported in parts per million (ppm, δ) downfield from tetramethylsilane (TMS). Coupling constants (*J*) are reported in Hz. Spin multiplicities are described as s (singlet), brs (broad singlet), t (triplet), q (quartet), and m (multiplet). Mass spectra were obtained on a Waters Micromass ZQ instrument. Preparative HPLC was performed on a Waters Symmetry C18 column (19 x 50 mm, 5μM) using a gradient of 15-95% methanol in water containing 0.05% trifluoacetic acid (TFA) over 22 min (28 min run time) at a flow rate of 20 mL/min. Purities of assayed compounds were in all cases greater than 95%, as determined by reverse-phase HPLC analysis.



1

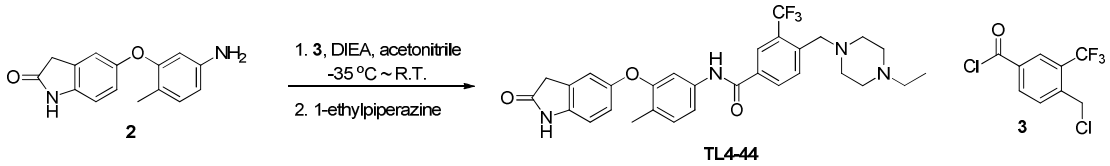
To a stirred solution of methyl 2-(5-fluoro-2-nitrophenyl)acetate(790 mg, 3 mmol) and 2-methyl-5-nitrophenol (700 mg, 4.5 mmol) in 2 mL of DMSO was added K_2CO_3 (830 mg, 6 mmol). The reaction mixture was allowed to stand for 1 hour at 100 °C, and then cooled to RT. The mixture was acidified with 1N HCl solution and extracted with ethyl acetate, the organic phase was washed with water and brine, dried over sodium sulfate, concentrated and purified with column chromatography (hexane : ethyl acetate 3:1). 830 mg (80%) of **1** was obtained. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.18 (d, *J* = 8.4 Hz, 1H), 8.05 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.85 (d, *J* = 2.4 Hz, 1H), 7.48 (d, *J* = 9.0 Hz, 1H), 6.91 (dd, *J* = 9.6, 2.4, 1H), 6.86 (d, *J* = 2.4, 1H), 3.99 (s, 2H), 3.71 (s, 3H), 2.32 (s, 3H).



1 2

To a stirred solution of compound **1**(690 mg, 2 mmol) in 30 mL of ethyl acetate and 3 mL of ethanol was added tin chloride dihydrate (2.26 g, 10 mmol). The reaction mixture was allowed to stand for 4~5 hours at 80 °C, and then cooled to RT. The mixture was eluted with ethyl acetate and added saturated sodium bicarbonate solution, and stirred for 15 min, the organic phase was separated and washed with water and brine, concentrated and purified with column chromatography (dichloromethane : methanol

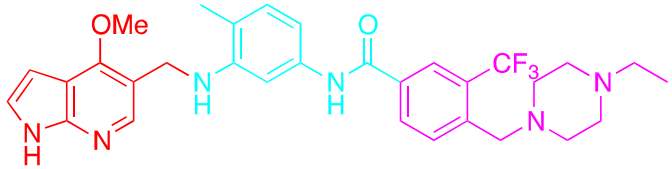
15:1). 380 mg (75%) of **2** was obtained. ^{1}H NMR (600 MHz, CD_3OD) δ 6.97 (d, *J* = 8.4 Hz, 1H), 6.87 (d, *J* = 2.4 Hz, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 6.78 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.44 (dd, *J* = 7.8, 2.4, 1H), 6.24 (d, *J* = 2.4, 1H), 3.50 (s, 2H), 2.09 (s, 3H). MS (ESI) *m/z* 255 $(M+H)^+$.



2 TL4-44 3

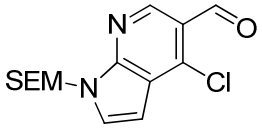
To a stirred solution of compound **2**(254 mg, 1 mmol) in 7 mL of acetonitrile was added DIEA (530 uL, 3 mmol). The reaction mixture was then cooled to -35 °C, and added the acyl chloride **3** (310 mg, 1.2 mmol) dropwise. After the addition was finished, the mixture was allowed to return to room temperature in half an hour, then was added 1-ethylpiperazine (270 uL, 2 mmol) and stirred overnight. The mixture was eluted with ethyl acetate and washed with water and brine, and the organic phase was collected and dried over sodium sulfate, concentrated and purified with column chromatography (dichloromethane : methanol 15:1). 380 mg (69%) of TL4-44 was obtained as white solid**.** ^{1}H NMR (600 MHz, DMSO) δ 10.34 (s, 1H), 10.32 (s, 1H), 8.16 (s, 1H), 8.14 (d, *J* = 7.8 Hz, 1H), 7.87 (d, *J* = 7.8 Hz, 1H), 7.47 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.26 (d, *J* = 1.8 Hz, 1H), 7.24 (d, *J* = 8.4 Hz, 1H), 6.88 (s, 1H), 6.80 (d, *J* = 2.4, 1H), 6.79 (s, 1H), 3.66 (m, 2H), 3.45 (s, 2H), 2.58-2.20 (m, 10H), 2.18 (s, 3H), 1.00 (m, 3H). MS (ESI) *m/z* 553 $(M+H)^+$.

DDR1-IN-2

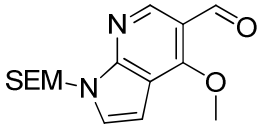


All reactions were monitored by thin layer chromatography (TLC) with 0.25 mm E. Merck pre-coated silica gel plates (60 F_{254}) and Waters LCMS system (Waters 2489 UV/Visible Detector, Waters 3100 Mass, Waters 515 HPLC pump, Waters 2545 Binary Gradient Module, Waters Reagent Manager, Waters 2767 Sample Manager) using SunFire™ C18 column (4.6 x 50 mm, 5 □m particle size): solvent gradient = 100% A at 0 min, 30% A at 5 min; solvent A = 0.035% TFA in Water; solvent B = 0.035% TFA in MeOH; flow rate : 2.5 mL/min. Purification of reaction products was carried out by flash chromatography using CombiFlash®Rf with Teledyne IscoRedi*Sep*®Rf High Performance Gold or SilicycleSilia*Sep*™ High Performance columns (4 g, 12 g, 24 g, 40 g or 80 g). The purity of all compounds was over 95% and was analyzed with Waters LCMS system. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Varian Inova-600 (600 MHz for

^{1}H, and 125 MHz for ^{13}C) spectrometer. Chemical shifts are reported relative to chloroform (δ = 7.24) for ^{1}H NMR or dimethyl sulfoxide (δ = 2.50) for ^{1}H NMR and dimethyl sulfoxide (δ = 39.51) for ^{13}C NMR. Data are reported as (*br* = broad, *s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet, *m* = multiplet).



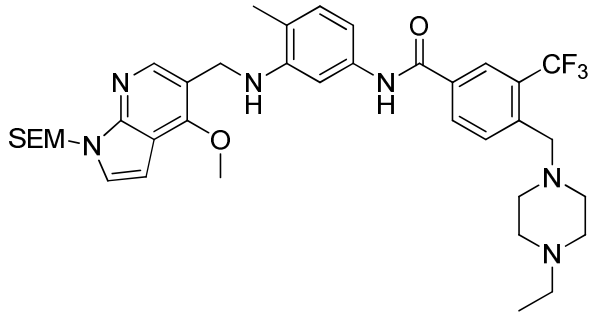
4-chloro-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[2,3-b]pyridine-5-carbaldehyde To a solution of 4-chloro-1H-pyrrolo[2,3-b]pyridine-5-carbaldehyde (500 mg, 2.78 mmol) in THF (9 mL) was added NaH (136 mg, 3.42 mmol) at 0 ^{o}C. After 10 minutes, SEMCl (0.59 mL. 3.33 mmol) was slowly added to the reaction mixture at 0^{o}C. The reaction mixture was warmed to room temperature and stirred for 2 hours after which, it was partitioned between ethyl acetate and water. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered through a pad of celite and concentrated under reduced pressure. The crude product was purified by flash chromatography using (5% to 20% Ethyl acetate/Hexane) as an eluent to afford title compound (810 mg, 94% yield). Rt = 3.58, ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.62 (*s*, 1H), 8.90 (*s*, 1H), 7.52 (*d*, *J* = 3.6 Hz, 1H), 6.84 (*d*, *J* = 4.2 Hz, 1H), 5.76 (*s*, 2H), 3.60 (*t*, *J* = 8.4 Hz, 2H), 0.97 (*t*, *J* = 8.4 Hz, 2H), 0 (*s*, 9H),^{1}H NMR 600 MHz (DMSO-d_6) δ 10.40 (*s*, 1H), 8.73 (*s*, 1H), 7.92 (*d*, *J* = 4.2 Hz, 1H), 6.82 (*d*, *J* = 4.2 Hz, 1H), 5.68 (*s*, 2H), 3.52 (*t*, *J* = 8.4 Hz, 2H), 0.82 (*t*, *J* = 8.4 Hz, 2H), -0.12 (*s*, 9H); ^{13}C NMR 125 MHz (DMSO-d_6) δ 188.80,149.51, 145.10, 138.17, 132.21, 121.29, 119.23, 100.18, 72.89, 65.74, 17.06, -1.53; MS *m/z* : 311.27 [M+1].



4-methoxy-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[2,3-b]pyridine-5-carbaldehyde

Na (370 mg, 16.12 mmol) was dissolved in MeOH (5 mL) and 4-chloro-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[2,3-b]pyridine-5-carbaldehyde (500 mg, 1.61

mmol) was added to the sodium methoxide solution. The reaction mixture was stirred for 8 hours for 60 ^{o}C. The reaction mixture was quenched with water and the organic solvent was removed under reduced pressure. The resulting mixture was partitioned between ethyl acetate and water. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered through a pad of celite and concentrated under reduced pressure. The crude product was purified by flash chromatography using (5% to 20% Ethyl acetate/Hexane) as an eluent to afford title compound (380 mg, 77% yield). Rt = 3.53, ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.51 (*s*, 1H), 8.74 (*s*, 1H), 7.36 (*d*, *J* = 3.6 Hz, 1H), 6.90 (*d*, *J* = 4.2 Hz, 1H), 5.72 (*s*, 2H), 4.48 (*s*, 3H), 3.60 (*t*, *J* = 7.8 Hz, 2H), 0.97 (*t*, *J* = 8.4 Hz, 2H), 0 (*s*, 9H); ^{1}H NMR 600 MHz (DMSO-d_6) δ 10.35 (*s*, 1H), 8.50 (*s*, 1H), 7.65 (*d*, *J* = 3.6 Hz, 1H), 7.06 (*d*, *J* = 4.2 Hz, 1H), 5.62 (*s*, 2H), 4.39 (*s*, 3H), 3.51 (*t*, *J* = 7.8 Hz, 2H), 0.82 (*t*, *J* = 8.4 Hz, 2H), -0.11 (*s*, 9H); ^{13}C NMR 125 MHz (DMSO-d_6) δ 188.18,162.24, 152.53, 144.68, 128.23, 114.51, 107.39, 102.22, 72.59, 65.54, 59.62, 17.10, -1.57; MS *m/z* : 307.32 [M+1].

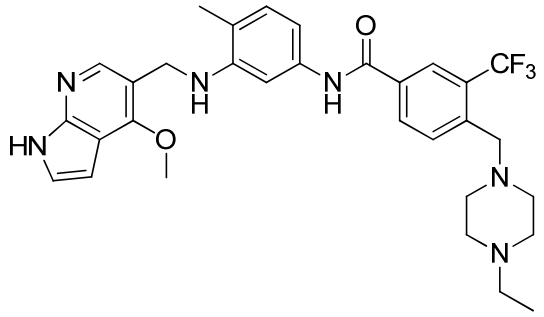


4-((4-ethylpiperazin-1-yl)methyl)-N-(3-((4-methoxy-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[2,3-b]pyridin-5-yl)methylamino)-4-methylphenyl)-3-(trifluoromethyl)benzamide

To a solution of 4-methoxy-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[2,3-b]pyridine-5-carbaldehyde (900 mg, 2.95 mmol) and N-(3-amino-4-methylphenyl)-4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)benzamide (1.36 g, 3.24 mmol) in 1,2-dichloroethane (15 mL) were added $Na(CN)BH_3$ (926 mg, 14.75 mmol) and AcOH (168 □L, 2.95 mmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was neutralized with *sat*. $NaHCO_3$ and the aqueous layer was extracted

with dichloromethane. The organic extracts were washed with brine, dried over $MgSO_4$, filtered through a pad of celite and concentrated under reduced pressure. The crude product was purified by flash chromatography using (1% to 7% MeOH/CH_2Cl_2) as an eluent to afford title compound (43 mg, 61% yield).Rt = 2.58 (Method B), ^{1}H NMR 600 MHz (DMSO-d_6) δ 10.26 (*s*, 1H), 8.20 (*d*, *J* = 1.8 Hz, 1H), 8.06 (*s*, 1H), 8.04 (*dd*, *J* = 1.8 Hz, *J* = 8.4 Hz, 1H), 7.67 (*d*, *J* = 8.4 Hz, 1H), 7.49 (*d*, *J* = 1.8 Hz, 1H), 7.15 (*m*, 3H), 6.84 (*d*, *J* = 4.2 Hz, 1H), 5.33 (*s*, 2H), 5.31 (*m*, 1H), 4.46 (*d*, *J* = 5.4 Hz, 2H), 4.31 (*s*, 3H), 3.64 (*s*, 2H), 3.48 (*t*, *J* = 7.2 Hz, 2H), 3.02 (*br*, 8H), 2.20 (*s*, 3H), 1.18 (*t*, *J* = 7.2 Hz, 3H), 0.78 (*t*, *J* = 8.4 Hz, 2H), -0.16 (*s*, 9H); MS *m/z* : 711.27[M+1].

4-((4-ethylpiperazin-1-yl)methyl)-N-(3-((4-methoxy-1H-pyrrolo[2,3-b]pyridin-5-yl)methylamino)-4-methylphenyl)-3-(trifluoromethyl)benzamide



To a solution of 4-((4-ethylpiperazin-1-yl)methyl)-N-(3-((4-methoxy-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[2,3-b]pyridin-5-yl)methylamino)-4-methylphenyl)-3-(trifluoromethyl)benzamide (40 mg, 0.042 mmol) in CH_2Cl_2 (1 mL) was added TFA (16 µL). The reaction mixture was stirred for 1 hour and the organic solvent was concentrated under reduced pressure. To a solution of the resulting mixture in THF (0.3 mL) and MeOH (0.3 mL) was added LiOH•H_2O (17 mg, 0.42 mmol) in water (0.3 mL). The reaction mixture was stirred for 2 hours at room temperature. The organic solvent was removed under reduced pressure and the aqueous layer was extracted with ethyl acetate. The organic extracts were washed with brine, dried over $MgSO_4$, celite filtered and concentrated under reduced pressure. The crude product was purified by Prep HPLC and acetonitrile was removed under reduced pressure. The remained water was freeze-dried to afford TFA salt formed title compound (21 mg, 71 % yield).Rt = 1.05, ^{1}H

NMR 600 MHz (DMSO-d_6) δ 11.53 (*s*, 1H), 10.24 (*s*, 1H), 8.17 (*d*, *J* = 2.4 Hz, 1H), 8.02 (*s*, 1H), 8.00 (*dd*, *J* = 2.4 Hz, *J* = 9.0 Hz, 1H), 7.66 (*d*, *J* = 7.8 Hz, 1H), 7.31 (*m*, 1H), 7.16 (*dd*, *J* = 1.8 Hz, *J* = 7.2 Hz, 1H), 7.12 (*m*, 2H), 6.73 (*m*, 1H), 5.46 (*m*, 1H), 4.44 (*d*, *J* = 6.0 Hz, 2H), 4.30 (*s*, 3H), 3.54 (*s*, 2H), 3.39 (*br*, 4H), 2.50 (*m*, 2H), 2.40 (*br*, 4H), 2.31 (*s*, 3H), 0.97 (*t*, *J* = 7.2 Hz, 3H); ^{13}C NMR 125 MHz (DMSO-d_6) δ 166.32, 156.96, 151.27, 146.34, 143.89, 138.41, 132.93, 131.60, 131.05, 129.45, 127.38, 127.18, 126.17, 124.03, 123.43, 117.11, 115.03, 113.36, 108.54, 107.98, 98.97, 58.80, 57.43, 52.67, 52.29, 51.52, 17.71, 11.87; MS *m/z* : 581.49 [M+1].

Protein expression and purification

The kinase domain of DDR1 (Uniprot Q08345, residues 601-913) was cloned into the transfer vector pFB-LIC-Bse for baculoviral expression in Sf9 insect cells. Some 72 hours post infection, cells were harvested and lysed using a C5 high pressure homogenizer (Emulsiflex). DDR1 protein was purified initially by Ni-affinity chromatography buffered in 50 mM HEPES pH 7.5, 500 mMNaCl, 5% glycerol, 5 mM Imidazole, 1 mM TCEP supplemented with protease inhibitor cocktail set III (Calbiochem) at 1:1000 dilution. Protein was eluted with imidazole and the hexahistidine tag cleaved using TEV protease. DDR1 was further purified by size exclusion chromatography and the final buffer adjusted to 10 mM HEPES pH 7.5, 250 mMNaCl, 5% glycerol, 1 mM TCEP, 2 mM DTT, 5 mM L-arginine, 5 mM L-glutamate.

Crystallization and structure determination

DDR1 was co-crystallized with inhibitor at 20°C in 150 nL sitting drops mixing 50 nL protein solution at 8.5 mg/mL with 100 nL of a reservoir solution containing 0.1 M bis-tris propane pH 7.2, 21%(w/v) PEG 3350 and 0.1 M sodium/potassium phosphate. On mounting crystals were cryo-protected with an additional 25% ethylene glycol. Diffraction data were collected at 100 K on Diamond Light Source beamline I02. Crystals belonged to the tetragonal space group $P4_1\,2_1\,2$ with unit-cell parameters *a*=59 Å *b*=59 Å *c*=178 Å, α=90° β=90° γ=90°. One molecule was present in the asymmetric unit.

Data were indexed and integrated using XDS (*1*) and scaled using AIMLESS (*2, 3*) in the CCP4 suite of programs (*4*). Phases were found using molecular replacement in PHASER (*5*) using PDB entry 3ZOS as a search model. Ligand restraints were generated using PHENIX.ELBOW (*6*) and the complex structure refined and modified using alternate rounds of REFMAC5 (*7*) and COOT (*8, 9*). TLS groups were determined using the TLSMD server (*10*). The refined structure was validated with MolProbity(*11*) and the atomic coordinate files deposited in the Protein Data Bank (PDB) with Autodep(*12*). Structure figures were prepared with PyMOL(*13*).

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General procedure for the EC_{50} test

Tet inducible human DDR1 over-expressing U2OS was used for the EC_{50} test. DDR1 was induced by 2 μg/ml doxycycline for 48 hrs prior to DDR1 activation by rat tail collagen I. The DDR1 over-expressed U2OS was pre-treated by media containing each concentration of the compound for 1 hr and treated by changing the media to the EC_{50} test media containing 10 μg/ml collagen and each concentration of the compound for 2 hrs. Each cells was washed with cold PBS three times and lysed with the lysis buffer (50 mMTris, pH 7.5, 1% Triton X-100, 0.1% SDS, 150 mMNaCl, 5 mM EDTA, 100 mMNaF, 2 mM Na3VO4, 1 mM PMSF, 10 μg/ml aprotinin, and 10 μg/ml leupeptin). The activation of DDR1 was quantified by density using program ImageJ to determine EC_{50} following Western blot using anti-activated human DDR1b (Y513).

General procedure for generating the G707A mutation

Generation of the G707A DDR1 mutant by site-directed mutagenesis using PCR was performed using following primers: sense, 5′ CCTCTGCATGATTACTGACTACATGGAGAACGCCGACCTCAACCA 3′ and antisense, 5′ CTGGTGGGCACTGAGGAACTGGTTGAGGTCGGCGTTCTCCATGT

3′ to substitute Gly707 to Alanine (G707A). The changed DNA bases were confirmed by DNA sequencing.

Combinatorial screening

Kinase Inhibitor Focused Library (LINCS)

A Kinase Inhibitor Focused Library (LINCS) was chosen for screening to identify single agents with little-to-no appreciable efficacy but that are able to synergize with DDR1 inhibitors, DDR1-IN-1 and DDR1-IN-2, respectively, against the human colorectal cancer cell line, SNU1040. The chemical screening concentration was 330 nM. DDR1-IN-1 and DDR1-IN-2 were used at 1micromolar. The LINCS library screening duration was 3 days.The LINCS library is available from Harvard Medical School/NIH LINCS program (https://lincs.hms.harvard.edu/), which contains 202 known selective and potent kinase inhibitors.

Drug combination studies

For drug combination studies performed as validation of results generated in the LINCS library screen, single agents were added simultaneously at fixed ratios to SNU1040 cells. Cell viability was determined using the Cell Titer Glo assay (Promega, Madison, WI) (for proliferation) and carried out according to manufacturer instructions. Data were expressed as the function of growth affected (FA) drug-treated versus control cells; data were analyzed by Calcusyn software (Biosoft, Ferguson, MO and Cambridge, UK), using the Chou-Talalay method (Chou and Talalay, 1984). The combination index=$[D]_1\,[D_x]_1 + [D]_2/[D_x]_2$, where $[D]_1$ and $[D]_2$ are the concentrations required by each drug in combination to achieve the same effect

as concentrations $[D_x]_1$ and $[D_x]_2$ of each drug alone. Values less than one indicate synergy, whereas values greater than one indicate antagonism. Calcusyn combination indices can be interpreted as follows: CI <0.1 indicate very strong synergism; values 0.1-0.3 indicate strong synergism; values 0.3-0.7 indicate synergism; values 0.7-0.85 indicate moderate synergism; values 0.85-0.90 indicate slight synergism; values 0.9-1.1 indicate nearly additive effects; values 1.10-1.20 indicate slight antagonism; values 1.20-1.45 indicate moderate antagonism; values 1.45-3.3 indicate antagonism; values 3.3-10 indicate strong antagonism; values >10 indicate very strong antagonism. Note: For some experiments, namely those in which there was no observed single agent activity for one or more agents, combination indices could not be reliably calculated using the Calcusyn software.

General procedure of the anti-proliferation assay

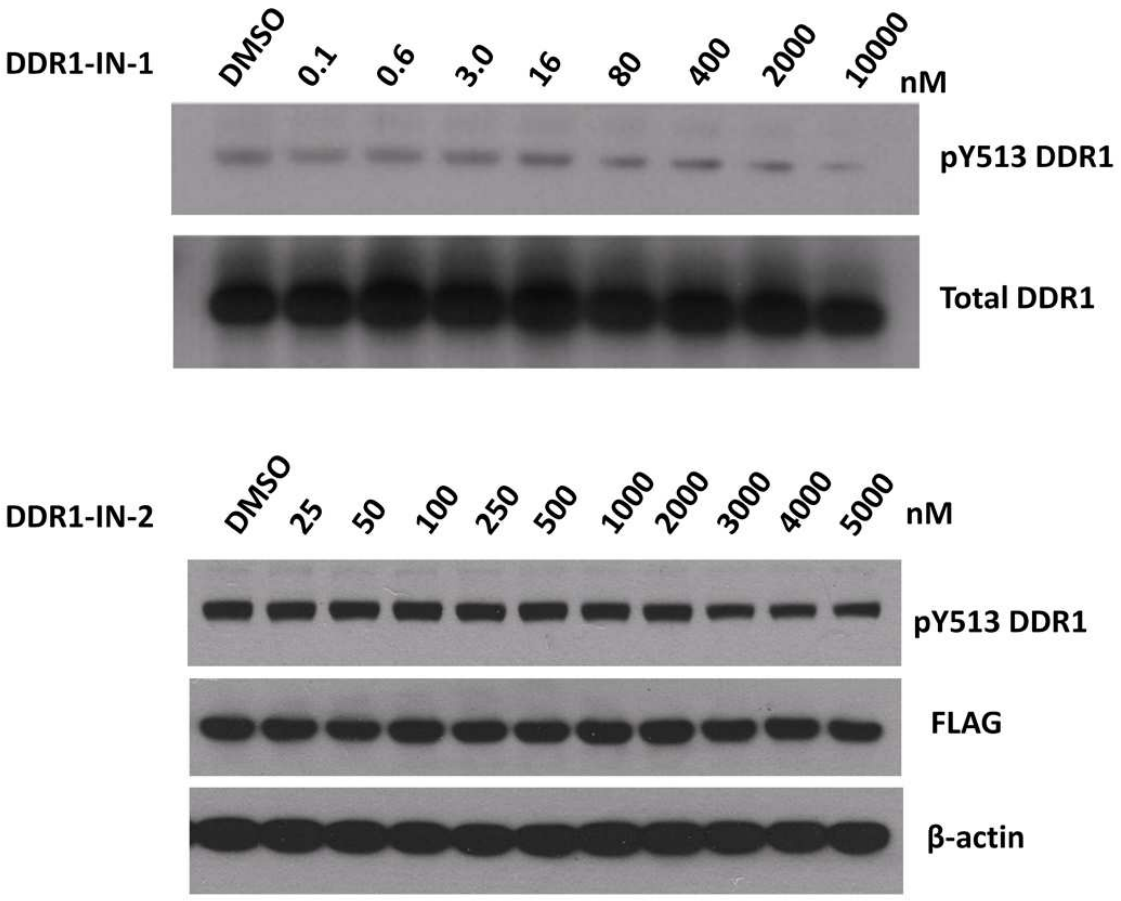
Cells were plated in triplicate at a density of 3000 cells per well in 96-well plates and 1500 cells per well in 384-well plates. Compounds of various concentrations were added into plates for 48 hours. Cell viability was determined using the CellTiter-Glo (Promega, USA) and CCK-8 (Beyotime, China). Both assays were performed according to the manufacturer's instructions. For CellTiter-Glo assay, luminescence was determined in a multi-label reader (Envision, PerkinElmer, USA). For CCK-8 assay, absorbance was measured in a microplate reader (iMARK, Bio-Rad, USA) at 450nm. Data were normalized to control group (DMSO) and represented by the mean of at least two independent measurement with standard error <20%. GI_{50} were calculated using Prism 5.0 (GraphPad Software, San Diego, CA).

General procedure for combinatorial signaling effect

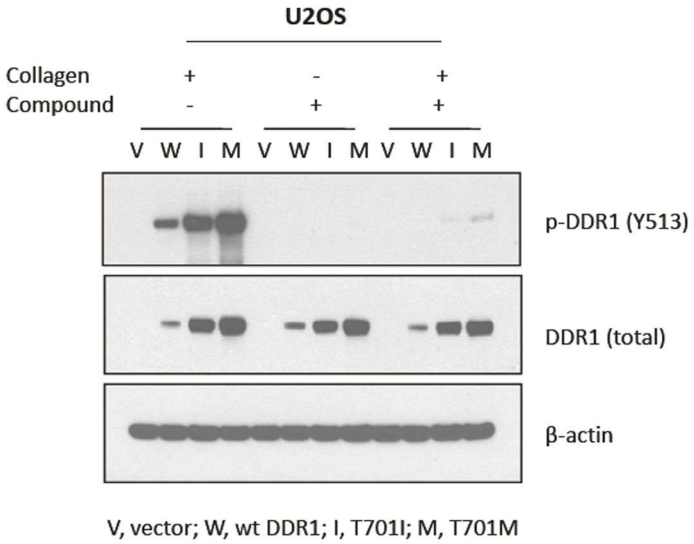
SNU1040 cells were treated with the indicated concentrations of compounds for 24 hours and then lysed (1% Triton X-100, 5mM EDTA pH 8.0, 20mM Tris pH 7.4) and lysates

quantified by Bradford assay. Western blotting was performed with the following antibodies: p-S6, p-AKT S473, and SNAIL (Cell Signaling), and Actin (Sigma).

Supplemental Fig. 1 Determination of DDR1-IN-1/2 effect on DDR1Y513 auto-phosphorylation without collagen activation



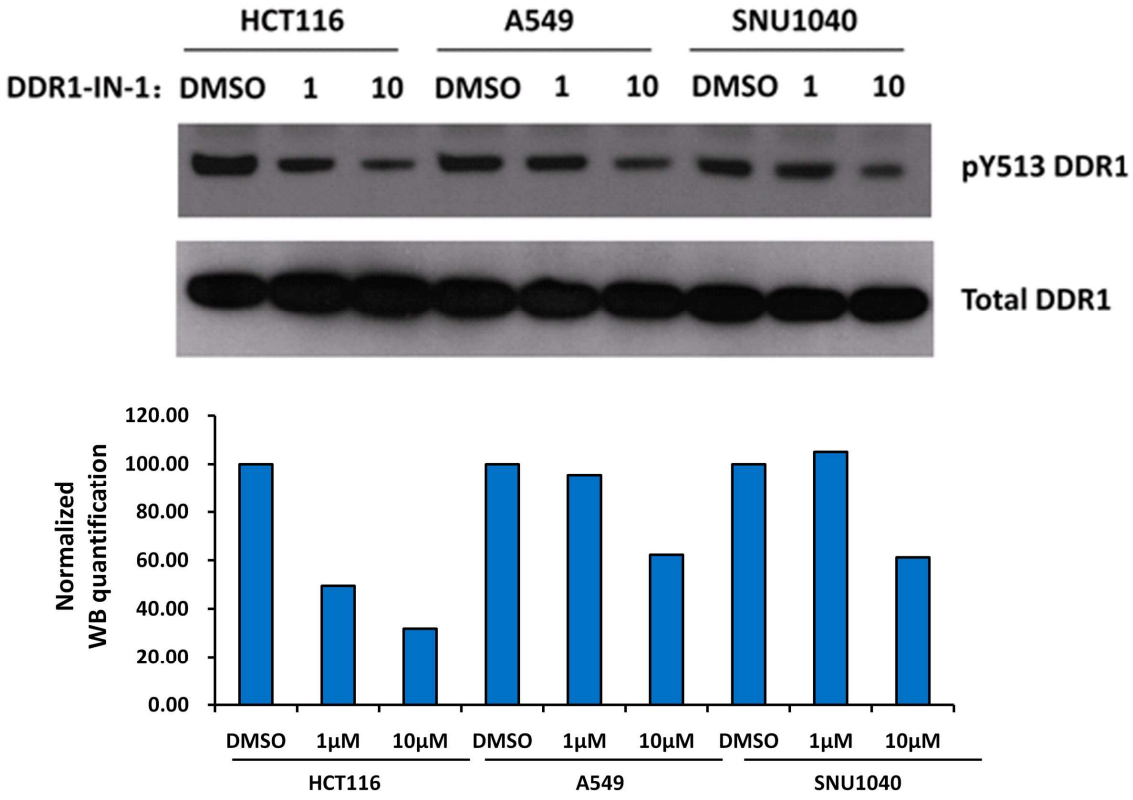
Supplemental Fig. 2 Drug resistance mutation studies on gatekeeper residue with U2OS cell lines.



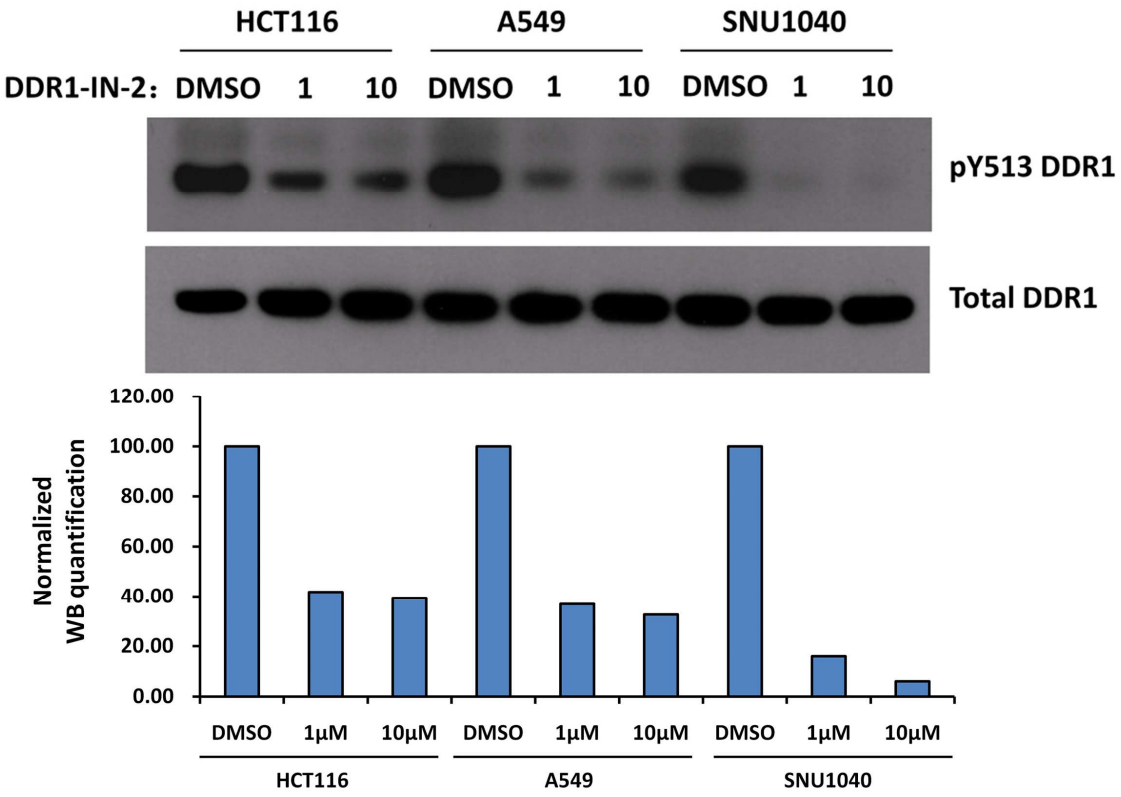
V, vector; W, wt DDR1; I, T701I; M, T701M

First column shows vector control, wide type DDR1, gatekeeper T701I mutation and T701M mutation under collagen stimulation still remain the enzymatic activities. Second column shows without collagen stimulation but DDR1-IN-2 treatment(100nM) no resistance occurred. Third column shows with collagen stimulation and DDR1-IN-2(100nM) treatment no apparent drug resistance occurred, which indicated that gatekeeper residue is not critical for the drug resistance.

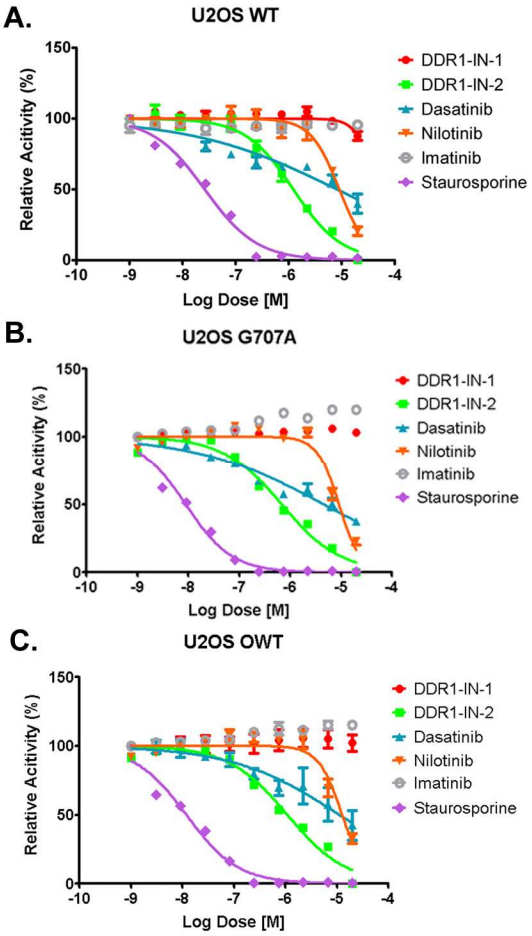
Supplemental Fig. 3A DDR1-IN-1 treatment effect on DDR1 Y513 phosphorylation with variety of cell lines



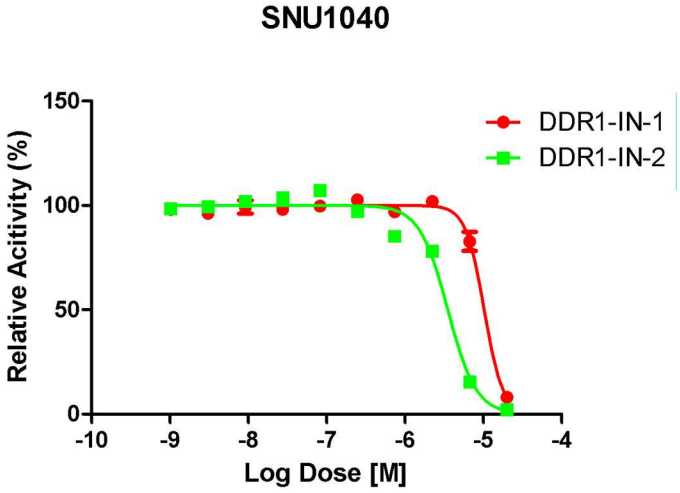
Supplemental Fig. 3B DDR1-IN-2 treatment effect on DDR1 Y513 phosphorylation with variety of cell lines



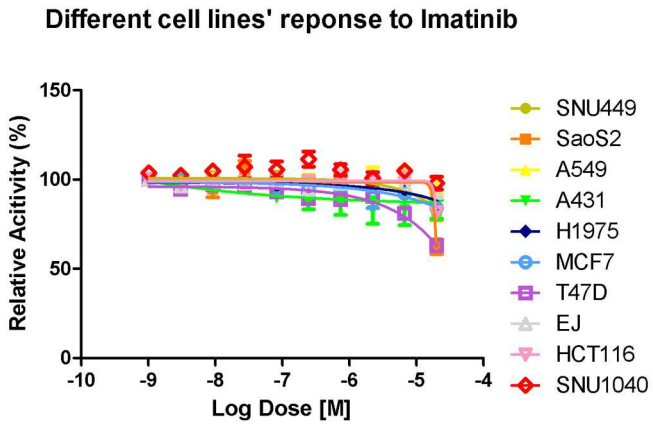
Supplemental Fig. 4 Anti-proliferative effects of DDR1 inhibitors on U2OSWT, U2OSOWT and U2OSG707A cell line



Supplemental Fig.5 Antiproliferation dose response curve for DDR1-IN-1/2 against SNU1040 cell line



Supplemental Fig.6. Imatinib antiproliferation efficacy against variety of cell lines



Supplemental Table 2. Strong hits(less than 1%) for DDR1-IN-1 on DiscvoeRx KinomeScan™ profiling and Invitrogen SelectScreen® biochemical IC_{50} confirmation

DDR1-IN-1	Ambit(% control)	Invitrogen IC_{50}(nM)	
	1uM		
ABL1(F317I)-nonphosphorylated	1	1810	ABL1
ABL1(H396P)-nonphosphorylated	0.35		
DDR1	0.1	105	DDR1
KIT	0.85	>10000	KIT
KIT(V559D)	0.4		
PDGFRB	0.85	>10000	PDGFRB

Supplemental Table 4.Strongs hits (less than 1%) for DDR1-IN-2 on DiscoverRxKinomeScan™

Assay Label	% Ctrl	Assay Label	% Ctrl
ABL1(F317L)-phosphorylated	0.8	HCK	0.45
ABL1(H396P)-nonphosphorylated	0.8	IKKa	0.6
ABL1(H396P)-phosphorylated	0.45	IKKb	1
ABL1(M351T)-phosphorylated	0.4	KIT	0.15
ABL	0.45	KIT(WildType)*	0.15
ABL1-phosphorylated(WildType)*	0.45	KIT(L576P)	0.8
ARG	0.55	KIT(V559D)	0.1
BLK	0	LCK	0.3
FMS/CSFR	0.55	LOK	0
CSK	1	LYN	0.4
DDR1	0.15	HGK/ZC1	0.75
EGFR(L747-T751del,Sins)	0.85	p38a	0.9
EGFR(L861Q)	0	PDGFRb	0
HER4	0.75	PIP5K1C	0.9
FGFR1	0.95	RET	0.25
FGR	1	RET(WildType)*	0.25
FLT3	0.2	RET(M918T)	0.2
FLT3(WildType)*	0.2	SRC	0.3
FLT3(K663Q)	0.55	Fused	0.85
FLT3(N841I)	0.75	TIE2	0.35
		ZAK	0.6

Supplemental Table 5: Data processing and refinement statistics. Values in parentheses refer to the highest resolution shell.

	DDR1 with DDR1-IN-1
Data	
Wavelength (Å)	0.9795
Resolution range (Å)	49.44 - 2.2 (2.278 - 2.199)
Space group	P 4_1 2_1 2
Unit cell (Å)	59.3 59.3 178.5
Unit cell (°)	90 90 90
Total reflections	183798 (14101)
Unique reflections	17062 (1526)
Multiplicity	11 (9.2)
Completeness (%)	100 (100.00)
$I/\sigma(I)$	17.5 (2.4)
Wilson B-factor (Å^2)	36.2
R-merge	0.1 (0.956)
R-meas	0.109 (1.071)
$CC_{1/2}$	0.999 (0.720)
Refinement	
R-work / R-free	0.1985 / 0.2437
Number of atoms	2422
macromolecules	2324
ligands	60
water	38
Protein residues	301
RMS(bonds) (Å)	0.01
RMS(angles) (°)	1.4
Ramachandran favored (%)	96.2
Ramachandran outliers (%)	0.34
Clashscore	1.93
Average B-factor (Å^2)	42.8
macromolecules	42.9
ligands	40.9
solvent	41
PDB ID	4BKI

Supplemental Table 6: Anti-proliferative effects of DDR1-IN-2 on U2OS wide type and G707A mutations

Cell lines	U2OS (GI_{50}:μM)	U2OS(OWT) (GI_{50}:μM)	U2OS(OMT-G707A) (GI_{50}:μM)
DDR1-IN-2	1.2	1.1	0.68
Staunosparine	0.026	0.012	0.009

*****OWT- overexpressed wide type , OMT-overexpressed mutant type

Supplemental Table 7: Positive hits from combinatorial screening of DDR1-IN-1 on LINCS library

SNU1040-DMSO vehicle	SNU-1040-DDR1-IN-1	SNU-1040-LINCS compound only	SNU-1040-DDR1-IN-1+LINCS compound	Drug	Targets
100%	95%	4J (82%)	4J+TL (63.4%)	WZ-4-145	CSF1R/DDR1/EGFR/TIE1/PDGFR2
100%	95%	6F (75.5%)	6F+TL (62.7%)	WYE-125132	mTOR
100%	95%	7D (47%)	7D+TL (37.4%)	CGP60474	Cdk1/cyclinB
100%	95%	8H (97.5%)	8H+TL (68.1%)	PF477736	Chk1
100%	95%	8I (100%)	8I+TL (68.2%)	PI103	PI3K
100%	95%	8J (63.1%)	8J+TL (31.7%)	GSK2126458	PI3K/mTOR
100%	95%	12J (88%)	12J+TL (70.1%)	AP24534	Src-bcr-Abl
100%	95%	21J (83.3%)	21J+TL (66.7%)	EKB-569	EGFR
100%	95%	22F (75.6%)	22F+TL (65.3%)	Torin2	mTOR

Supplemental Table 8: Positive hits from combinatorial Screening of DDR1-IN-2 on LINCS library

SNU1040-DMSO vehicle	SNU-1040-DDR1-IN-2	SNU-1040-LINCS compound only	SNU-1040-DDR1-IN-2+LINCS compound	Drug	Targets
100%	85%	4J (82%)	4J+HG (49%)	WZ-4-145	CSF1R/DDR1/EGFR/TIE1/PDGFR2
100%	85%	6F (75.5%)	6F+HG (42%)	WYE-125132	mTOR
100%	85%	6G (104%)	6G+HG (38%)	WZ3105	CLK2/CNSK1E/FLT3/ULK1
100%	85%	7E (78.6%)	7E+HG (34%)	A443644	Akt1
100%	85%	8J (63%)	8J+HG (27%)	XMD11-50	LRRK2
100%	85%	9C (79.6%)	9C+HG (30.7%)	AZD8055	mTOR
100%	85%	13I (89.7%)	13I+HG (56.9%)	ARQ197	c-MET
100%	85%	14I (90%)	14I+HG (40%)	BMS-387032	CDK
100%	85%	14K (91%)	14K+HG (44.6%)	GSK1059615	PI3K
100%	85%	16C (112%)	16C+HG (54.7%)	GW843682	PLK1
100%	85%	18E (102%)	18E+HG (56.3%)	AZD6244	MEK
100%	85%	22F (75.6%)	22F+HG (32.5%)	Torin2	mTOR