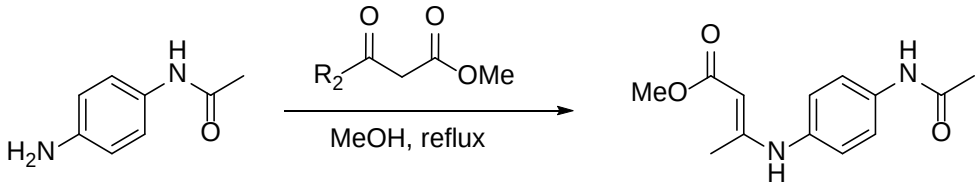
Supplementary Materials and Methods

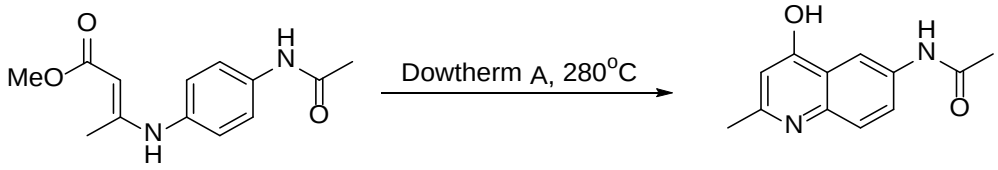
Preparation of JT801 analogues

Compounds were prepared according to the reported synthetic approach [1]. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Thermo Fisher Scientific Inc. (Pittsburgh, PA, USA), unless otherwise specified. ^{1}H NMR spectra were recorded on Bruker DRX 400 MHz spectrometers (Billerica, MA, USA).



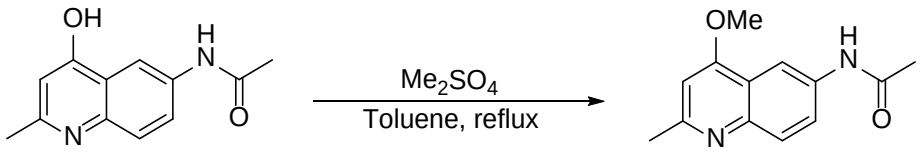
A total of 10 g methyl acetoacetate (88 mM) was added to the solution of 12 g (80 mM) 4-aminoacetanilide in 50 ml methanol, and the reaction mixture was refluxed overnight. 2 ml H_2O was subsequently added and the reaction mixture was cooled to 0℃. Precipitate was then collected to give 15 g product as white crystals.

Yield 76%. ^{1}H NMR (400 MHz, DMSO) δ 10.22 (s, 1H, NH), 9.98 (s, 1H, NH), 7.57 (d, *J* = 6.8 Hz, 2H, CH), 7.12 (d, *J* = 6.8 Hz, 2H, CH), 4.67 (s, 1H, CH), 3.57 (s, 3H, OCH_3), 2.04 (s, 3H, CH_3), 1.95 (s, 3H, CH_3).



6g (24 mM) methyl 3-(4-acetylaminophenylamino)crotonate was slowly added to the refluxed Dowtherm A via the top of the condenser. After 10 min, the reaction mixture was cooled down to room temperature. Precipitate was then filtered and washed with ethyl acetate followed by methanol to give 4 g product.

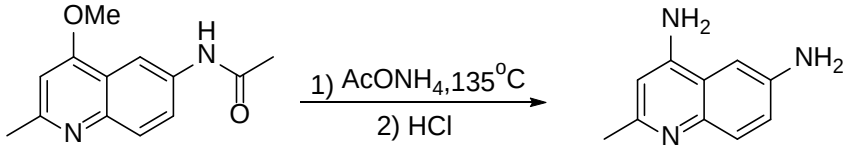
Yield 77%. ^{1}H NMR (400 MHz, DMSO) δ 11.53 (s, 1H, NH), 10.07 (s, 1H, OH), 8.24 (d, *J* = 2.4 Hz, 1H, CH), 7.84 (dd, *J* = 8.8, 2.4 Hz, 1H, CH), 7.44 (d, *J* = 8.8 Hz, 1H, CH), 5.85 (s, 1H, CH), 2.32 (s, 3H, CH_3), 2.06 (s, 3H, CH_3).



1.5 ml (16 mM) dimethyl sulfate dropwise was added to a suspension of 2g (9.3 mM)

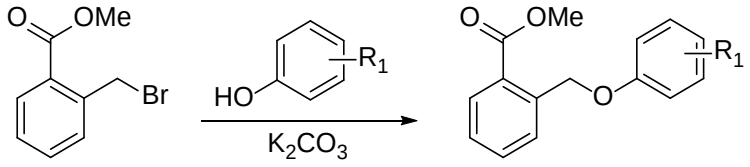
6-acetamide-4-hydroxy-2-methylquinoline in toluene. The reaction mixture was refluxed for eight hours. Precipitate was filtered and redissolved in 40 ml H_2O. The solution was basified with 0.25 ml 10N NaOH, and the precipitate was collected to give 0.8 g product.

Yield 38%. ^{1}H NMR (400 MHz, DMSO) δ 10.17 (s, 1H, NH), 8.46 (d, *J* = 2.4 Hz, 1H, CH), 7.78-7.72 (m, 2H, CH), 6.88 (s, 1H, CH), 4.01 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.09 (s, 3H, CH_3).

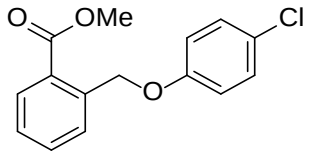


A mixture of 0.5 g (2.2 mM) 6-acetamide-4-methoxy-2-methylquinoline and 2.5 g (32 mM) ammonium acetate was heated at 135℃ for four hours. 3 ml H_2O and 4.5 ml 37% hydrochloric acid were then added to the reaction mixture, which was heated at 90℃ for 5h. Upon cooling to 0℃, the precipitate was filtered and dissolved in 1ml hot water. The solution was basified with 1ml 10N NaOH at 0℃ and the resulting precipitate was collected to give 170mg product.

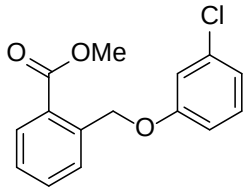
Yield 36%. ^{1}H NMR (400 MHz, DMSO) δ 7.40 (d, *J* = 8.8 Hz, 1H, CH), 6.97 (dd, *J* = 8.8, 2.4 Hz, 1H, CH), 6.91 (d, *J* = 2.4 Hz, 1H, CH), 6.29 (s, 1H, CH), 6.01 (s, 2H, NH_2), 5.03 (s, 2H, NH_2), 2.31 (s, 3H, CH_3).



50 mM potassium carbonate (5equiv) was added to a solution of 10 mM 2-bromo-toluate (1equiv) and 10 mM substituted phenol (1equiv) in 50 ml DMF. The reaction mixture was heated at 100℃ for overnight. Precipitate was removed. Filtrate was then diluted with 200 ml water. The resulting precipitate was collected as the product.

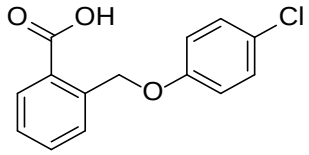


Yield 59%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 10.4 Hz, 1H, CH), 7.72 (d, *J* = 10.4 Hz, 1H, CH), 7.56 (t, *J* = 10 Hz, 1H, CH), 7.39 (t, *J* = 10 Hz, 1H, CH), 7.24 (d, *J* = 12 Hz, 2H, CH), 6.92 (d, *J* = 12 Hz, 2H, CH), 5.48 (s, 2H, CH_2), 3.90 (s, 3H, OCH_3).

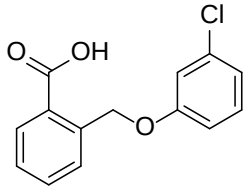


Yield 67%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 6.0 Hz, 1H, CH), 7.72 (d, *J* = 6.0 Hz, 1H, CH), 7.57 (t, *J* = 6.0 Hz, 1H, CH), 7.39 (t, *J* = 6.0 Hz, 1H, CH), 7.20 (t, *J* = 6.4 Hz, 1H, CH), 7.01 (t, *J* = 1.6 Hz, 1H, CH), 6.95 (dd, *J* = 6.4, 1.6 Hz, 1H, CH), 6.88 (dd, *J* = 6.4, 1.6 Hz, 1H, CH), 5.49 (s, 2H, CH_2), 3.91 (s, 3H, OCH_3).

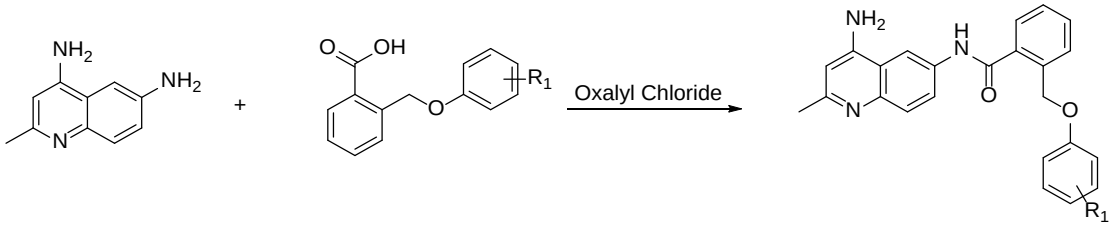
2 ml 4N aqueous sodium hydroxide was added to 3.3mmol substituted methyl 2-(phenoxymethyl) benzoate dissolved in a mixture of tetrahydrofuran (5 ml) and methanol (5 ml). The reaction mixture was refluxed overnight. Organic solvent was then removed and the aqueous portion was acidified with 2N HCl. Precipitate was collected as the product.



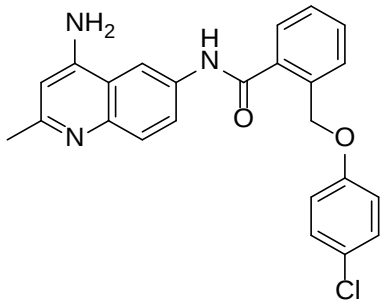
Yield 75%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, *J* = 7.6 Hz, 1H, CH), 7.77 (d, *J* = 7.6 Hz, 1H, CH), 7.83 (t, *J* = 7.6 Hz, 1H, CH), 7.44 (t, *J* = 7.6 Hz, 1H, CH), 7.25 (d, *J* = 8.8 Hz, 2H, CH), 6.93 (d, *J* = 8.8 Hz, 2H, CH), 5.52 (s, 2H, CH_2).



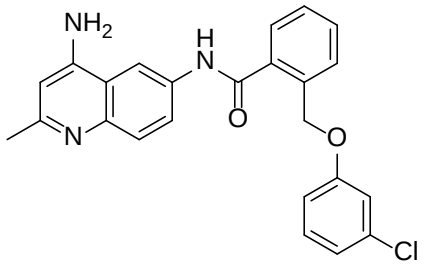
Yield 83%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, *J* = 6.0 Hz, 1H, CH), 7.77 (d, *J* = 6.0 Hz, 1H, CH), 7.63 (t, *J* = 6.0 Hz, 1H, CH), 7.44 (t, *J* = 6.0 Hz, 1H, CH), 7.21 (t, *J* = 6.4 Hz, 1H, CH), 7.02 (t, *J* = 1.6 Hz, 1H, CH), 6.96 (dd, *J* = 6.4, 1.6 Hz, 1H, CH), 6.89 (dd, *J* = 6.4, 1.6 Hz, 1H, CH), 5.53 (s, 2H, CH_2).



1.5mmol oxalyl chloride and a catalytic amount of dimethylformamide were added to 1 mM substituted 2-(phenoxymethyl) benzoic acid dissolved in 10 ml $CHCl_3$. The reaction mixture was stirred at room temperature for 12 hours and concentrated under the vacuum. The residue was subsequently treated with 0.6 mM 2-methylquinoline-4,6 diamine dissolved in 10 ml pyridine and stirred for another three hours. The reaction mixture was then alkalinized with 10 ml 2N potassium hydroxide and extracted with 50 ml ethyl acetate. The organic layer was isolated and washed with 50 ml H_2O followed by 50 ml brine and dried on the Na_2SO_4. Ethyl acetate was removed and the residue was purified by the column chromatography (methanol: dichloromethane = 1 : 10) to obtain the product.

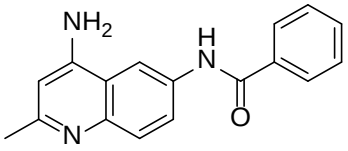


Yield 30%. ^{1}H NMR (400 MHz, DMSO) δ 10.84 (s, 1H, NH), 8.67 (s, 1H, CH), 8.42 (s, 2H, NH_2), 7.92-7.87 (m, 2H, CH), 7.69 – 7.50 (m, 4H, CH), 7.26 (d, *J* = 7.2 Hz, 2H, CH), 6.96 (d, *J* = 7.2 Hz, 2H, CH), 6.58 (s, 1H, CH), 5.33 (s, 2H, CH_2), 2.56 (s, 3H, CH_3).

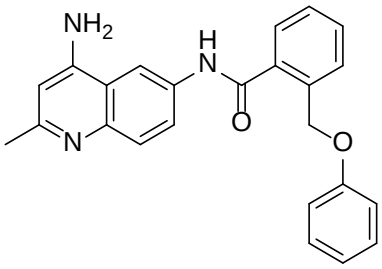


Yield 37%. ^{1}H NMR (400 MHz, DMSO) δ 10.67 (s, 1H, NH), 8.51 (s, 1H, CH), 7.78 – 7.51 (m, 6H, CH), 7.25 (t, *J* = 6.4 Hz, 1H, CH), 7.01 (s, 1H, CH), 6.96-6.91 (m, 2H, CH),

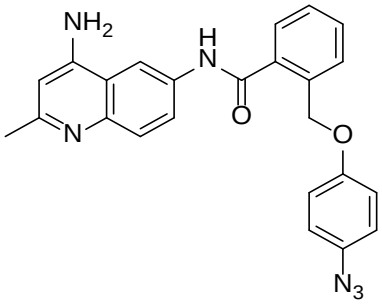
6.50 (s, 1H, CH), 5.36 (s, 2H, CH_2), 2.47 (s, 3H, CH_3).



Yield 51%. ^{1}H NMR (400 MHz, DMSO) δ 10.42 (s, 1H, NH), 8.43 (d, *J* = 1.9 Hz, 1H, CH), 8.06 – 7.99 (m, 2H, CH), 7.77 (dd, *J* = 9.0, 2.1 Hz, 1H, CH), 7.68 (d, *J* = 9.0 Hz, 1H, CH), 7.64 – 7.52 (m, 3H, CH), 6.78 (s, 2H, NH_2), 6.46 (s, 1H, CH), 2.42 (s, 3H, CH_3).



Yield 43%. ^{1}H NMR (400 MHz, DMSO) δ 10.57 (s, 1H, NH), 8.42 (s, 1H, CH), 7.72 – 7.63 (m, 4H, CH), 7.53 (dt, *J* = 15.0, 6.8 Hz, 2H, CH), 7.28 – 7.22 (m, 1H, CH), 6.94 (ddd, *J* = 20.7, 11.7, 4.1 Hz, 4H), 6.64 (s, 2H, NH_2), 6.46 (s, 1H, CH), 5.34 (s, 2H, CH_2), 2.42 (s, 3H, CH_3).



Yield 40%. ^{1}H NMR (400 MHz, DMSO) δ 10.57 (s, 1H, NH), 8.41 (s, 1H, CH), 7.67 (d, *J* = 8.1 Hz, 1H, CH), 7.61 (d, *J* = 8.9 Hz, 1H, CH), 7.52 (dd, *J* = 12.5, 7.6 Hz, 3H, CH), 7.47 – 7.34 (m, 1H, CH), 6.86 (s, 4H, CH), 6.39 (s, 1H, CH), 5.19 (s, 2H, CH_2), 2.36 (s, 3H, CH_3).

pH measurement

Intracellular pH was measured using pHrodo® Green AM Intracellular pH Indicator (Thermo Fisher Scientific Inc., Pittsburgh, PA, #P35373) in accordance with the

manufacturer's guidelines. pHrodo® Green AM Intracellular pH Indicator is a novel fluorogenic probe used to measure intracellular pH in live cells. This pHrodo® Green dye has been modified with AM ester groups, which results in an uncharged molecule that can permeate cell membranes. Once inside the cell, the lipophilic blocking groups are cleaved by nonspecific esterases, resulting in a compound that is retained within the intracellular space. Fluorescence intensity of the probe is then an indicator of intracellular pH. It is weakly fluorescent at neutral pH but increasingly fluorescent as the pH drops. This reagent can quantify cellular cytosolic pH in the range of 9-4 with a pKa of ~6.5 with excitation/emission of 509/533 nm. Subsequent use of the Intracellular pH Calibration Buffer Kit (#P35379) allows this intracellular pH to be quantified. Briefly, cells were washed with Live Cell Imaging Solution (LCIS), then 10 μl of pHrodo™ Red AM or pHrodo™ Green AM was mixed with 100 μl of PowerLoad™ concentrate and was added to 10 mL of LCIS. Cells were incubated with the pHrodo™ AM/PowerLoad™/LCIS at 37°C for 30 minutes and then were analyzed using the Cytation™ 5 Cell Imaging Multi-Mode Reader (BioTek, USA) using the 509/533 maxima. Extracellular pH was measured using pH meter (HI2221 Calibration Check pH/ORP meter, HANNA instrument, USA) in accordance with the manufacturer's guidelines.

RNAi and gene transfection

Human CA9 cDNA (#SC122585) was obtained from OriGene Technologies Inc. (Rockville, MD, USA). Human IKKβ shRNA (#TRCN0000018916 and #TRCN0000018917), human OPRL1 shRNA (#TRCN0000357699), human NRF2 shRNA (#TRCN0000007555), human OPRM1 shRNA (#TRCN0000009274), human CA9 shRNA (#TRCN0000150123 and #TRCN0000183853), and human RelA shRNA (#TRCN0000014683 and #TRCN0000014685) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Transfection with shRNA or cDNA was performed with Lipofectamine 3000 (Invitrogen, Grand Island, NY, USA, #L3000-015) according to the manufacturer's instructions.

Immunoprecipitation

Cells were lysed at 4°C in ice-cold radioimmunoprecipitation assay buffer (Cell Signaling Technology, Danvers, MA, USA, #9806) and cell lysates were cleared by brief centrifugation (13,000 g, 15 min). Concentrations of proteins in the supernatant were determined using BCA assay (Thermo Fisher Scientific Inc., Pittsburgh, PA, #23225). Prior to immunoprecipitation, samples containing equal amounts of proteins were pre-cleared with protein A agarose beads (Santa Cruz Biotechnology, Dallas, Texas, USA, #sc-2027) (4°C, 3 h) and subsequently incubated with various irrelevant IgG or specific antibodies (5 μg/mL) in the presence of protein A agarose beads for 2 h or overnight at 4°C with gentle shaking. Following incubation, agarose beads were washed extensively with phosphate buffered saline and proteins were eluted by boiling in 2 × sodium dodecyl

sulfate sample buffer before sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Cell viability assay

Cells were seeded into 96-well plates and incubated with the indicated treatments. Subsequently, 100 μl fresh medium was added to cells containing 10 μl Cell Counting Kit-8 (CCK-8) solutions (Dojindo Laboratories, Tokyo, Japan, #CK04) and incubated for two hours (37℃, 5% CO_2). Absorbance at 450 nm was measured using a microplate reader (Cytation™ 5 Cell Imaging Multi-Mode Reader, BioTek, USA).

Colony formation assay

Long-term cell survival was monitored in a colony formation assay. In brief, 200 cells were reseeded into 24-well plates after treatment with JTC801 for 24 hours. The cells were allowed to grow for the next 12-14 days to allow colony formation and the colonies were visualized using crystal violet staining.

Q-PCR analysis

Total RNA was extracted and purified from cultured cells using the RNeasy Plus Mini Kit (QIAGEN, Valencia, CA, USA, #74136) according to the manufacturer's instructions. The RNA was quantified by determining absorbance at 260 nm. One μg of total RNA from each sample was reverse transcribed into cDNA using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA, #170-8891) in a volume of 20 μl. cDNA from cell samples was amplified. Quantitative real time PCR was performed using ssoFast EvaGreen Supermix (Bio-Rad, Hercules, CA, USA, #172-5204) on the C1000 Touch Thermocycler CFX96 Real Time System (Bio-Rad, Hercules, CA, USA) according to the manufacturer's protocol. Analysis was performed using the Bio-Rad CFX Manager software (Bio-Rad, Hercules, CA, USA). The primers, which were synthesized and desalted from Sigma-Aldrich, are shown below: mouse CA9: 5'-GGCGAACGATTGAGGCTTCCTT -3' and 5'-GCTGGTGACAGCAAAGAGAAGG -3'; human CA9: 5'-GTGCCTATGAGCAGTTGCTGTC-3' and 5'-AAGTAGCGGCTGAAGTCAGAGG-3'; human CA2: 5'-GTGACCTGGATTGTGCTCAAGG -3' and 5'-GTTGTCCACCATCAGTTCTTCGG -3'; human CA12: 5'-GACCTTTATCCTGACGCCAGCA-3' and 5'-CATAGGACGGATTGAAGGAGCC-3'; human V-ATPaseA: 5'-CTGTTGCTGCTAGAGAAGCCTC -3' and 5'-GCAGGCATTTCAGCTAAACGACC -3'; human V-ATPaseB: 5'-GTCTGCCTCTTCCTGAACTTGG-3' and 5'-CCGTCAGTATGACCAGCACATG-3'; human AE1: 5'-CTGCTGGTGTTTGAGGAAGCCT -3' and 5'-CACCAGCAGGATGAGCCAGAAG -3'; human AE2: 5'-GACTTCCGAGATGCACTTGACC-3' and 5'-CCTATCAGGTCCTGCGTCTTCT-3'; human AE3: 5'-AGCCACACCAGCACTGACTATC-3' and 5'- GAACTCGCTGATGGCACTTAGG -

3’; human NBC1/2: 5’-GGAAAGCCAAGTCCTACCACGA-3’ and 5’-TACCAGCAATCAGGTCGTGCCT-3’; human NDCBE: 5’-CATCGTGACAGCAGAAGTCCAC-3’ and 5’-GCTTGAGAGGATGAAGGTGGTG-3’; human NHE1: 5’-GAACTGGACCTTCGTCATCAGC-3’ and 5’-GGTCAGCTTCACGATACGGAAC-3’; human MCT1: 5’-TTGTTGGTGGCTGCTTGTCAGG-3’ and 5’-TCATGGTCAGAGCTGGATTCAAG -3’; human MCT2: 5’-TGCTGGCTGTTATGTACGCAGG -3’ and 5’-GCCAACACCATTCCAAGACAGC-3’; human MCT3: 5’-TGCAGTTCGAGGTGCTCATGGC -3’ and 5’- GTTCTTCAACACATCCACCAGGC-3’; human MCT4: 5’-GCCATCTTTGCTGGTGGTTACC-3’ and 5’-TGGTCCAGAAAGGACAGCCATC-3’; human MCT4: 5’-GCCATCTTTGCTGGTGGTTACC -3’ and 5’-TGGTCCAGAAAGGACAGCCATC -3’; mouse 18S RNA (5’-GCAATTATTCCCCATGAACG-3’ and 5’-GGCCTCACTAAACCATCCAA-3’); and human 18S RNA (5’-CTACCACATCCAAGGAAGCA-3’ and 5’-TTTTTCGTCACTACCTCCCCG-3’).

Western blot

Cells were lysed with 1X cell lysis buffer (Cell Signaling Technology, #9803) containing protease inhibitor on ice for 10 minutes, homogenized by passing through a 21-gauge needle, and centrifuged at 14,000 x g for 15 minutes at 4°C to pellet the cell debris. Protein was quantified using BCA assay (Thermo Fisher Scientific Inc., #23225) and 20 μg of each sample was resolved on 4%-12% Criterion XT Bis-Tris gels (Bio-Rad, Hercules, CA, USA, #3450124) in XT MES running buffer (Bio-Rad, #1610789) and transferred to PVDF membranes (pore size 0.22 μM, Bio-Rad, #1620233) using the Trans-Blot® Turbo™ Transfer Pack and System (Bio-Rad). Membranes were blocked with TBST containing 5 % skim milk for one hour and incubated overnight at 4°C with various primary antibodies. Following three washes in TBST, membranes were incubated with goat anti-rabbit/Mouse IgG HRP secondary antibody (1:3000, Cell Signaling Technology, #7074 or #7076) at room temperature for one hour and washed. Chemiluminescence substrate was applied using SuperSignal™ West Pico Chemiluminescent Substrate (Thermo Fisher Scientific Inc., #34080) or SuperSignal™ West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific Inc., #34095) and blots were analyzed using the ChemiDoc™ Touch Imaging System (Bio-Rad). Image Lab™ Software (Bio-Rad) was used for relative quantification of bands, normalized to total protein loaded in each lane.

Image analysis

For histological analysis, tissue specimens were fixed with 10% buffered formalin, dehydrated in ethanol, embedded with paraffin, and stained using the standard hematoxylin and eosin method. The fraction of preserved acinar area was calculated as

previously described [2]. Pancreatic ductal dysplasia was graded according to established criteria [3]. Histological images were acquired using an EVOS FL Auto Cell Imaging System (Thermo Fisher Scientific Inc.).

Immunofluorescent staining of mouse tissues was performed using antibodies directed against CA9 (Abcam, #ab184006), p-RelA (Cell Signaling Technology, #3033), and DAPI (Thermo Fisher Scientific Inc., #H3569). Immunofluorescent images were acquired using an AxioObserver Z1 Microscope with Apotome (Carl Zeiss). Quantifications of images were performed by assessing 20X high-power fields per slide and 20 fields per mouse (five mice/group) in a blinded manner.

Immunohistochemistry in human pancreatic cancer tissue array was performed using antibodies directed against CA9 (Cell Signaling Technology, #5649). This tissue microarray (#HPan-Ade180Sur-01) was purchased from US Biomax and included 90 cases of pancreatic cancer tumor. Antigen retrieval was carried out by heating the slides at 95 °C in 10 mM sodium citrate for 20 mins. Endogenous peroxidase was quenched by incubating the slides in 3% hydrogen peroxide solution for 10 min. Slides were stained using a 1:100 dilution of CA9 antibody and detected using the EnVision™+ Dual Link System-HRP (Dako, Carpinteria, CA, USA, #K4063) according to the manufacturer's protocol. Slides were counterstained in hematoxylin (Sigma-Aldrich, #MHS1). Images were captured using an Invitrogen EVOS FL Auto Cell Imaging System (Thermo Fisher Scientific Inc.) via built-in system software.

Secrete-pair luminescence assay

Dual-reporter promoter clones or controls were transfected into two cell lines in duplicates. PANC1 and MiaPaCa2 cells were transfected with pEZX-PG04-CA9-promoter-Gaussia luciferase (GLuc)/secreted alkaline phosphatase (SEAP) (GeneCopoeia, #MPRM23878-PG04). After 48 h, these cells were treated with JTC801 (10 μM) in the absence or presence of IMD0354 at indicated times. The CA9 promoter luciferase activity was measured with secrete-pair dual luminescence assay kit (GeneCopoeia, #SPDA-D010) in accordance with the manufacturer's guidelines.

Biochemical assay

Biochemical measurements of tissue enzymes (AMYL, BUN, and ALT) in serum were performed using the IDEXX Catalyst Dx® Chemistry Analyzer according to the manufacturer's protocol.

Intracellular reactive oxygen species measurement

The intracellular formation of reactive oxygen species was detected using the fluorescent probe CM-H2DCFDA (Molecular Probes, #C6827). In brief, cells (1×10^5/ml) were incubated with CM-H2DCFDDA (10 μM) for 45 min at 37 °C and then measured using a microplate reader (Cytation™ 5 Cell Imaging Multi-Mode Reader,

BioTek, USA).

Glutathione assay

The relative GSH concentration in cell lysates was assessed using a kit from Sigma-Aldrich (#CS0260) according to the manufacturer's instructions. The measurement of GSH used a kinetic assay in which catalytic amounts (nmoles) of GSH caused a continuous reduction of 5,5'-dithiobis (2-nitrobenzoic acid) to 5-thio-2-nitrobenzoic acid and the GSSG formed was recycled by glutathione reductase and NADPH. The reaction rate was proportional to the concentration of glutathione up to 2 mM. The yellow product (5-thio-2-nitrobenzoic acid) was measured spectrophotometrically at 412 nm using a microplate reader (Cytation™ 5 Cell Imaging Multi-Mode Reader, BioTek, USA).

Glutathione disulfide assay

The relative GSSG concentration in cell lysates was assessed using a kit from Cayman (#703002) according to the manufacturer's instructions. The quantification of GSSG, exclusive of GSH, was accomplished by first derivatizing GSH with 2-vinylpyridine. In brief, 1 µl of 1 M 2-vinylpyridine in absolute ethanol were added to 99 µl of cell homogenate. This suspension was incubated at room temperature for 60 minutes to block the thiol group of the GSH already present. NADPH (95 µl of 2 mg/ml) in nanopure water and 5 µl of 2 units/ml glutathione reductase were added to reduce GSSG.

JTC801 metabolism and disposition

Serum samples were prepared by mixing 30 µL of serum with 70 µL of methanol, followed by vortexing for 30s and centrifugation at 15,000 g for 10 min. Liver, kidney, and pancreas samples were homogenized in water (100 mg of liver in 500 µl of water) on ice. Two hundred µl of acetonitrile:methanol (1:1, v/v) was added to 100 µl of each homogenate and followed by vortexing and centrifugation at 15,000 g for 10 min. The supernatant was transferred to a new Eppendorf vial for a second centrifugation (15,000 g for 10 min). Each supernatant was transferred to an autosampler vial and 1.0 µl was injected into time-of-flight mass spectrometry (UPLC-TOFMS) (Waters, Milford, MA) for metabolite analysis.

Statistical analysis

Data are presented as mean ± s.e.m. Unpaired Student's t tests were used to compare the means of two groups. One-way Analysis of Variance (ANOVA) was used for comparison among the different groups. When ANOVA was significant, *post hoc* testing of differences between groups was performed using the Least Significant Difference (LSD) test. The Kaplan-Meier method was used to compare differences in mortality rates between groups. A p-value < 0.05 was considered statistically significant.

References

1. Shinkai H, Ito T, Iida T, et al. 4-Aminoquinolines: Novel Nociceptin Antagonists with Analgesic Activity. Journal of Medicinal Chemistry 2000;43:4667-4677.
2. Ochi A, Nguyen AH, Bedrosian AS, et al. MyD88 inhibition amplifies dendritic cell capacity to promote pancreatic carcinogenesis via Th2 cells. J Exp Med 2012;209:1671-87.
3. Hruban RH, Adsay NV, Albores-Saavedra J, et al. Pancreatic intraepithelial neoplasia: a new nomenclature and classification system for pancreatic duct lesions. Am J Surg Pathol 2001;25:579-86.

Supplementary Figures

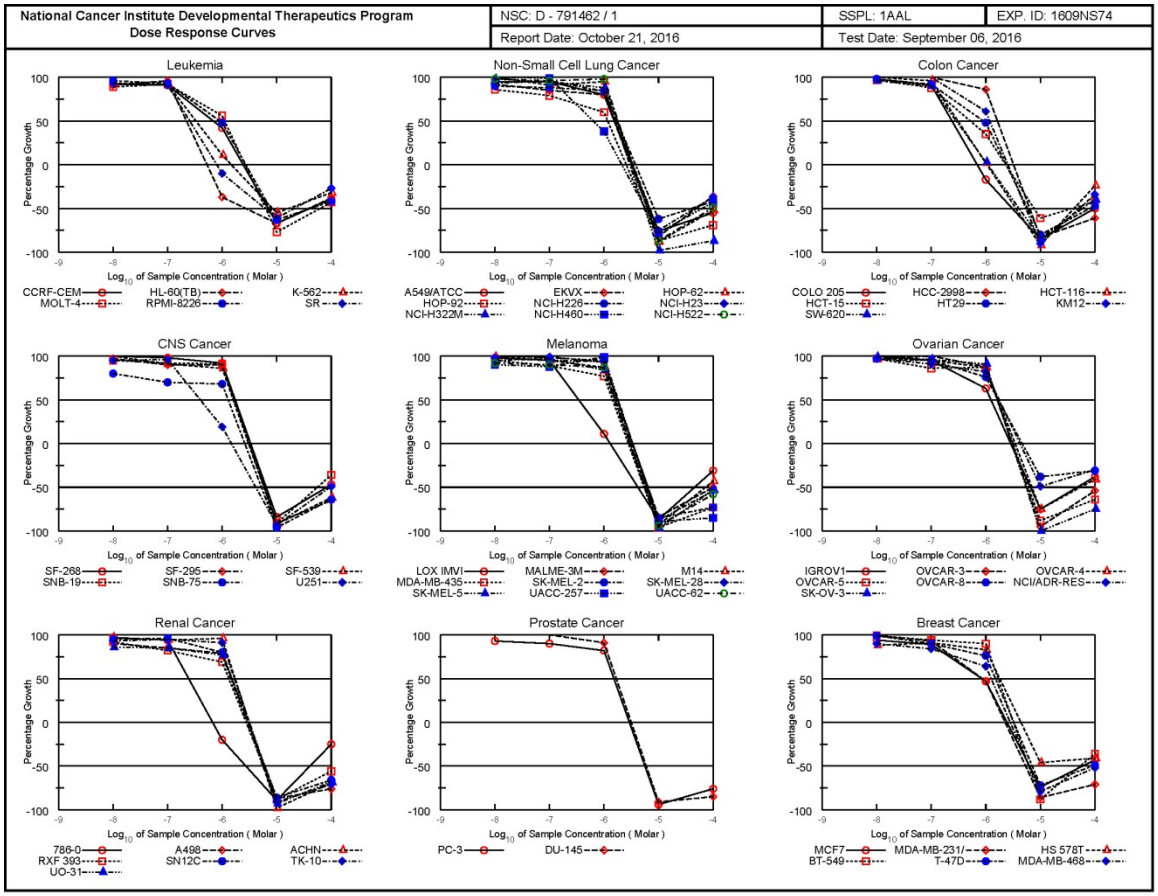


Fig. S1. Dose response curves of NCI-60 cell lines.

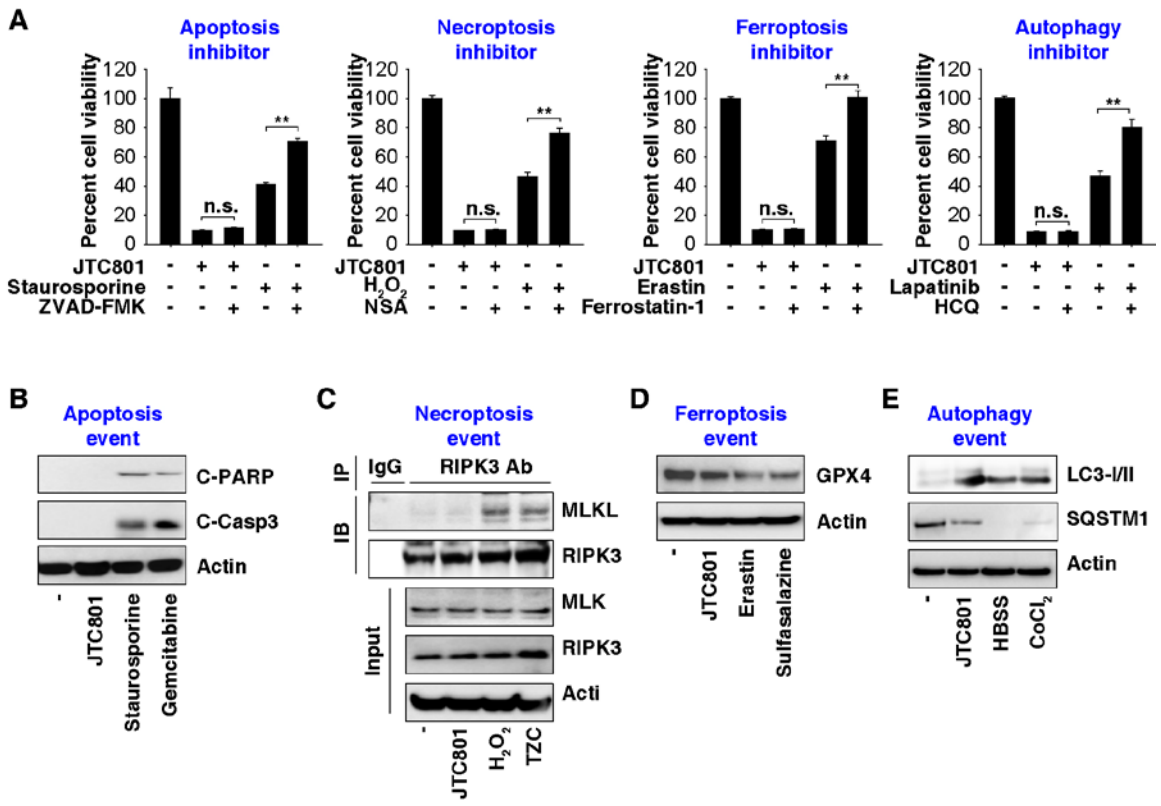


Fig. S2. JTC801 induces alkaliptosis. (A) MiaPaCa2 cells were treated with JTC801 (10 μM), staurosporine (0.5 μM), erastin (20 μM), H_2O_2 (500 μM), or lapatinib (50 μM) in the absence or presence of ZVAD-FMK (20 μM), ferrostatin-1 (500 nM), necrosulfonamide ("NSA", 1 μM), and hydroxychloroquine ("HCQ", 50 μM) for 24 hours. Cell viability was assayed (n=3, **p < 0.01, n.s.=not significant). (B) MiaPaCa2 cells were treated with JTC801 (10 μM), staurosporine (1 μM), or gemcitabine (5 mM) for 24 hours and the levels of cleaved-PARP ("C-PARP"), cleaved-caspase 3 ("C-Casp3"), and actin were assayed using western blot. (C) MiaPaCa2 cells were treated with JTC801 (10 μM), H_2O_2 (500 μM), or TZC (TNF [50 nM]/ZVAD-FMK [20 μM]/cycloheximide [10 μg/ml]) for 24 hours. Cell lysates were immunoprecipitated with anti-RIPK3 antibody or control IgG, and then the levels of MLKL and RIPK3 were assayed using western blot. (D) MiaPaCa2 cells were treated with JTC801 (10 μM), erastin (20 μM), or salazosulfapyridine (1 mM) for 24 hours and then the levels of GPX4 and actin were assayed using western blot. (E) MiaPaCa2 cells were treated with JTC801 (10 μM, 24 hours), $CoCl_2$ (400 μM, 24 hours), or Hank's buffered salt solution (HBSS, six hours), and the levels of LC3-I/II, SQSTM1, and actin were assayed using western blot.

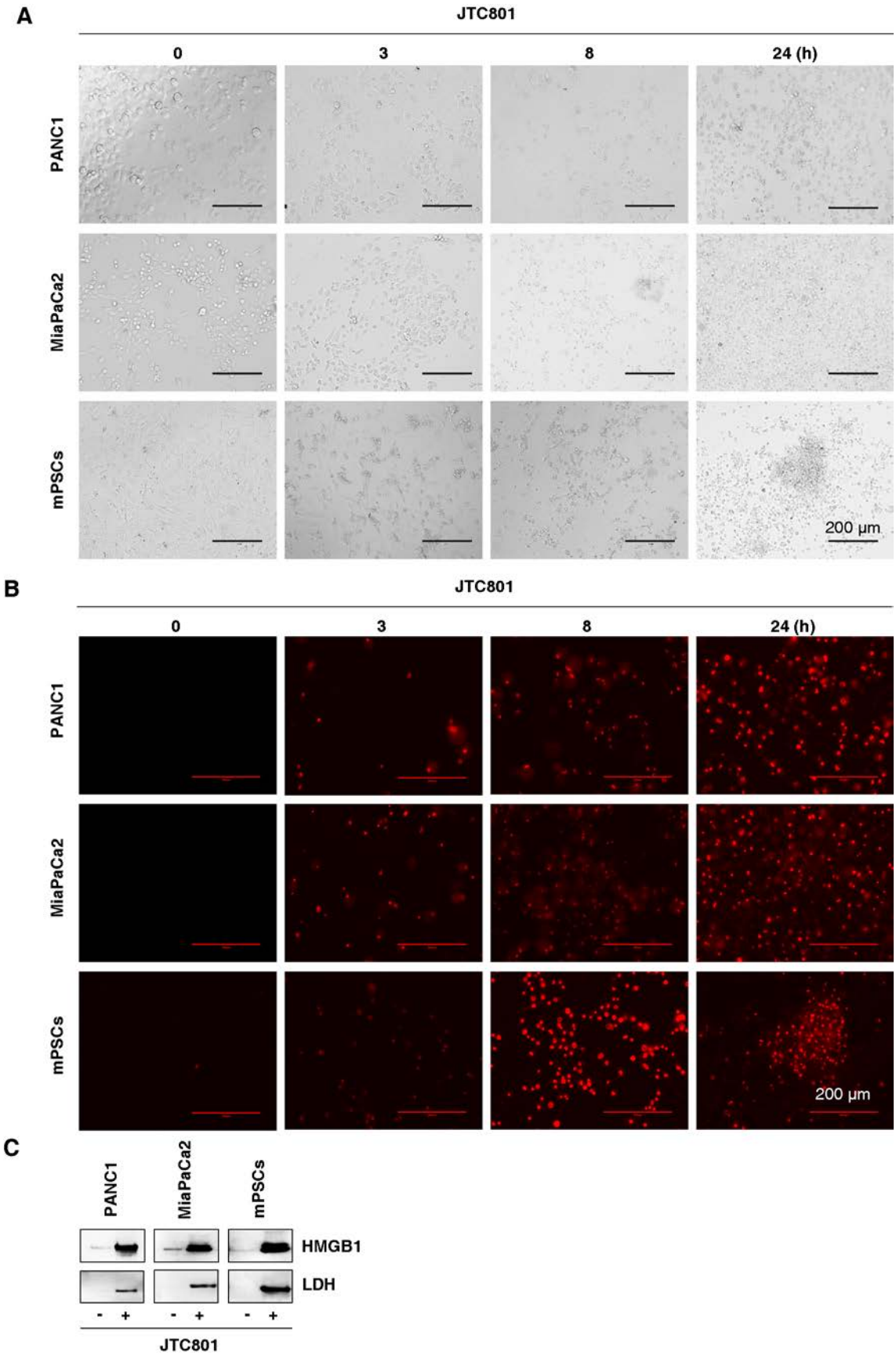

A
JTC801
0
3
8
24 (h)
PANC1
MiaPaCa2
mPSCs
200 μm
B
JTC801
0
3
8
24 (h)
PANC1
MiaPaCa2
mPSCs
200 μm
C
PANC1
MiaPaCa2
mPSCs
HMGB1
LDH
- + - + - +
JTC801

Fig. S3. JTC801-induced alkaliptosis is a necrosis-like form of regulated cell death. (A) Morphological observation of indicated cells following JTC801 (10 μM) treatment for three to 24 hours. (B) Analysis of intact plasma membranes using propidium iodide staining in indicated cells following JTC801 (10 μM) treatment for three to 24 hours. (C) Western blot analysis of LDH and HMGB1 release in cell supernatants in indicated cells following JTC801 (10 μM) treatment for 24 hours.

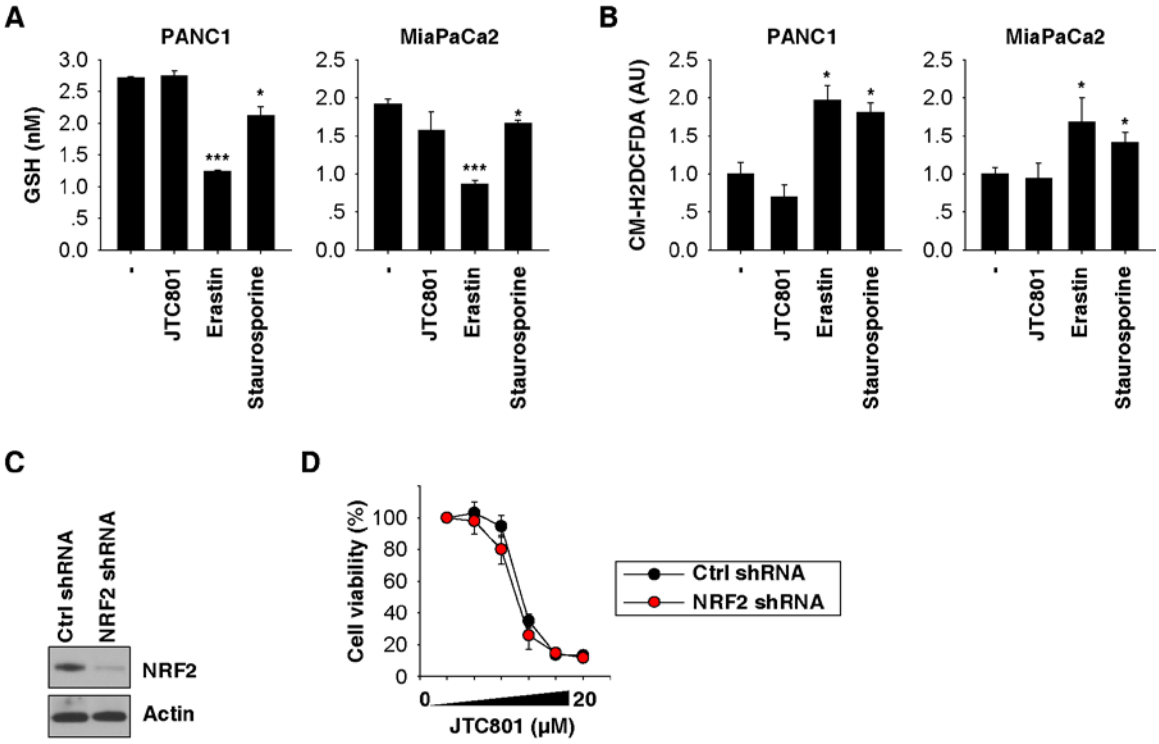


Fig. S4. JTC801 did not cause GSH depletion and reactive oxygen species production. (A, B) PANC1 and MiaPaCa2 cells were treated with JTC801 (10 μM), erastin (20 μM), or staurosporine (0.5 μM) for 24 hours. The levels of GSH (A) and CM-H2DCFDA (an indicator for reactive oxygen species in cells) (B) were assayed (n=3, *p < 0.05, ***p < 0.001 versus untreated group). (C, D) Effects of knockdown of NRF2 by shRNA on the anticancer activity of JTC801 (1.25, 2.5, 5, 10, and 20 μM) for 24 hours in PANC1 cells.

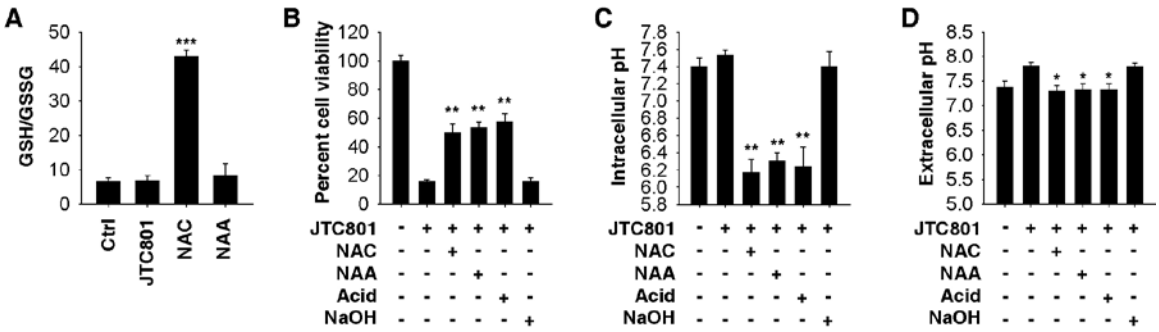


Fig. S5. Increased intracellular pH is required for JTC801-induced cell death. MiaPaCa2 cells were treated with JTC801 (10 μM) in the absence or presence of N-acetylcysteine ("NAC", 100 mM), N-acetyl alanine acid ("NAA", 100 mM), acidic medium (pH=6, adjusted by HCl), and NAC (100 mM, pH=7, adjusted by NaOH) for 24 hours. GSH/GSSG ratio (A), cell viability (B), intracellular pH (C), and extracellular pH (D) were assayed (n=3, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus JTC801 group).

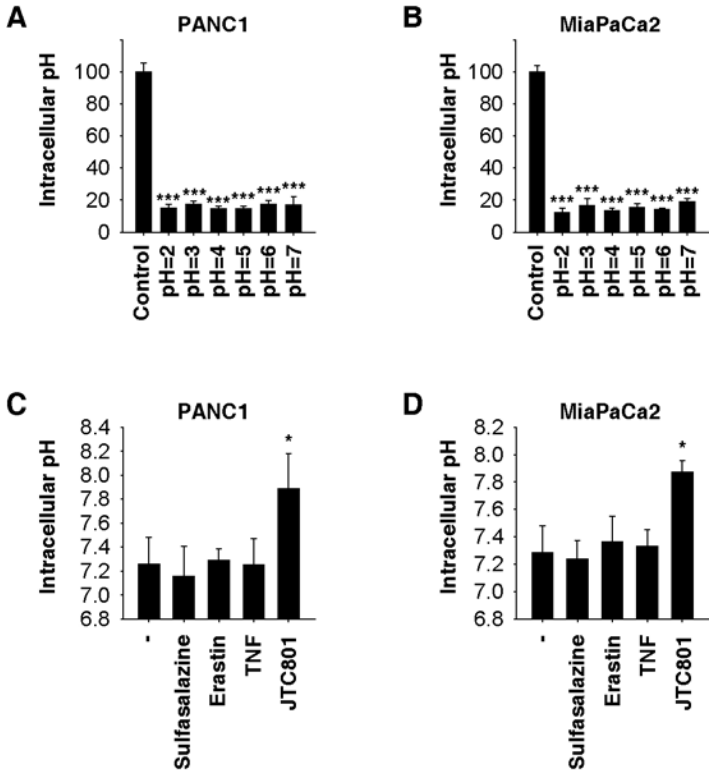


Fig. S6. Relationship between JTC801 and pH. (A, B) The stability of JTC801 in acid stock solution. JTC801 (200 mM) was incubated with acid stock solution (pH=2-6, adjusted by HCl) at 1:20 for six hours. PANC1 and MiaPaCa2 cells were then incubated with JTC801 (10 mM) in acid stock solution at 1:1000 for 24 hours, and then cell viability was assayed (n=3, ***$p < 0.001$ versus control group). (C, D) Effects of cell death inducers on pH. PANC1 and MiaPaCa2 cells were treated with staurosporine (0.5 μM), erastin (20 μM), TNF (50 nM), or JTC801 (10 μM) for 24 hours. Intracellular pH levels were assayed (n=3, *$p < 0.05$ versus untreated group).

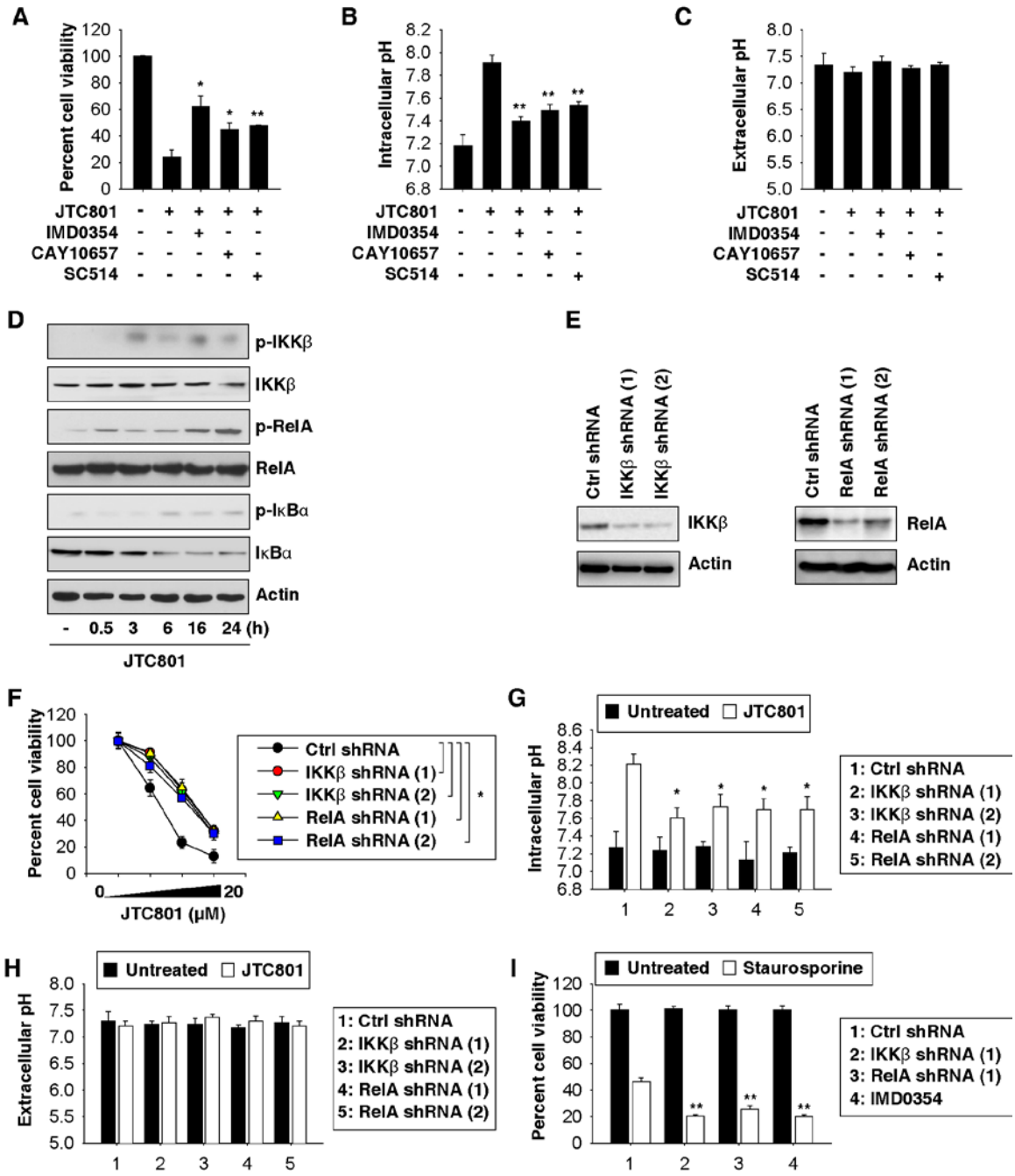


Fig. S7. Activation of the NF-κB pathway contributes to alkaliptosis. (A-C) MiaPaCa2 cells were treated with JTC801 (10 μM) in the absence or presence of IKKβ inhibitors (IMD0354 [10 μM], CAY10657 [10 μM], and SC514 [10 μM]) for 24 hours. Cell viability, intracellular pH, and extracellular pH were measured (n=3, *p < 0.05, **p < 0.01 versus JTC801 group). (D) Western blot analysis of indicated proteins in MiaPaCa2 cells following treatment with JTC801 (10 μM) for 0.5-24 hours. (E) Western

blot analysis of IKKβ and RelA expression in indicated gene-knockdown MiaPaCa2 cells. (F) Knockdown of IKKβ and RelA inhibited JTC801 (1.25, 2.5, 5, 10, and 20 μM)-induced cell death at 24 hours in MiaPaCa2 cells (n=3, *p < 0.05). (G-H) Indicated MiaPaCa2 cells were treated with JTC801 (10 μM) for 24 hours. Intracellular and extracellular pH were assayed (n=3, *p < 0.05 versus untreated group). (I) Indicated MiaPaCa2 cells were treated with staurosporine (1 μM) with or without IMD0354 (10 μM) for 24 hours. Cell viability was measured (n=3, **p < 0.01 versus control shRNA group).

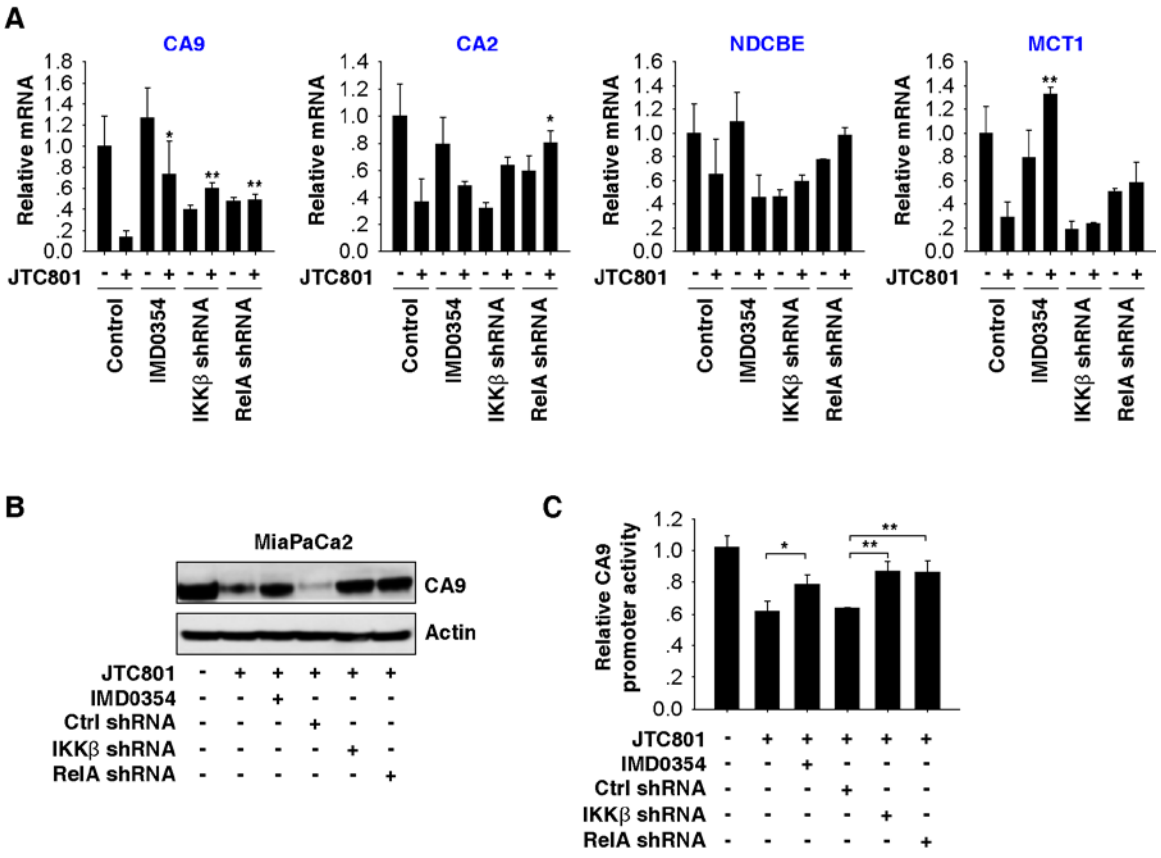


Fig. S8. CA9 is a negative transcriptional target of NF-κB in alkaliptosis. (A) Q-PCR analysis of mRNA levels of CA9, CA2, NDCBE, and MCT1 in indicated MiaPaCa2 cells following treatment with JTC801 (10 μM) in the absence or presence IMD0354 (10 μM) for 24 hours (n=3, *p < 0.05, **p < 0.01 versus control JTC801 group). (B-C) In parallel, the protein expression and promoter luciferase activity of CA9 were measured (n=3, *p < 0.05, **p < 0.01).

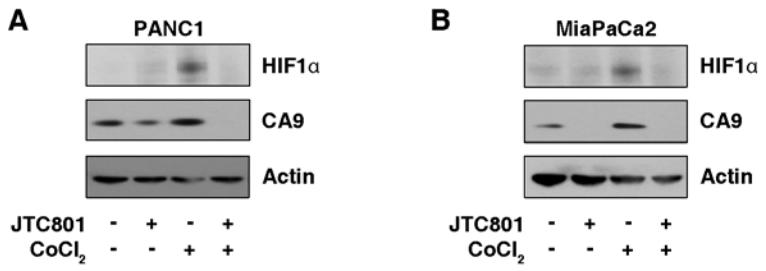


Fig. S9. HIF1α is not required for CA9 expression in alkaliptosis. PANC1 (A) and MiaPaCa2 (B) cells were treated with JTC801 (10 μM) in the absence or presence of $CoCl_2$ (100 uM) for 24 hours. The indicated protein levels were assayed using western blot.

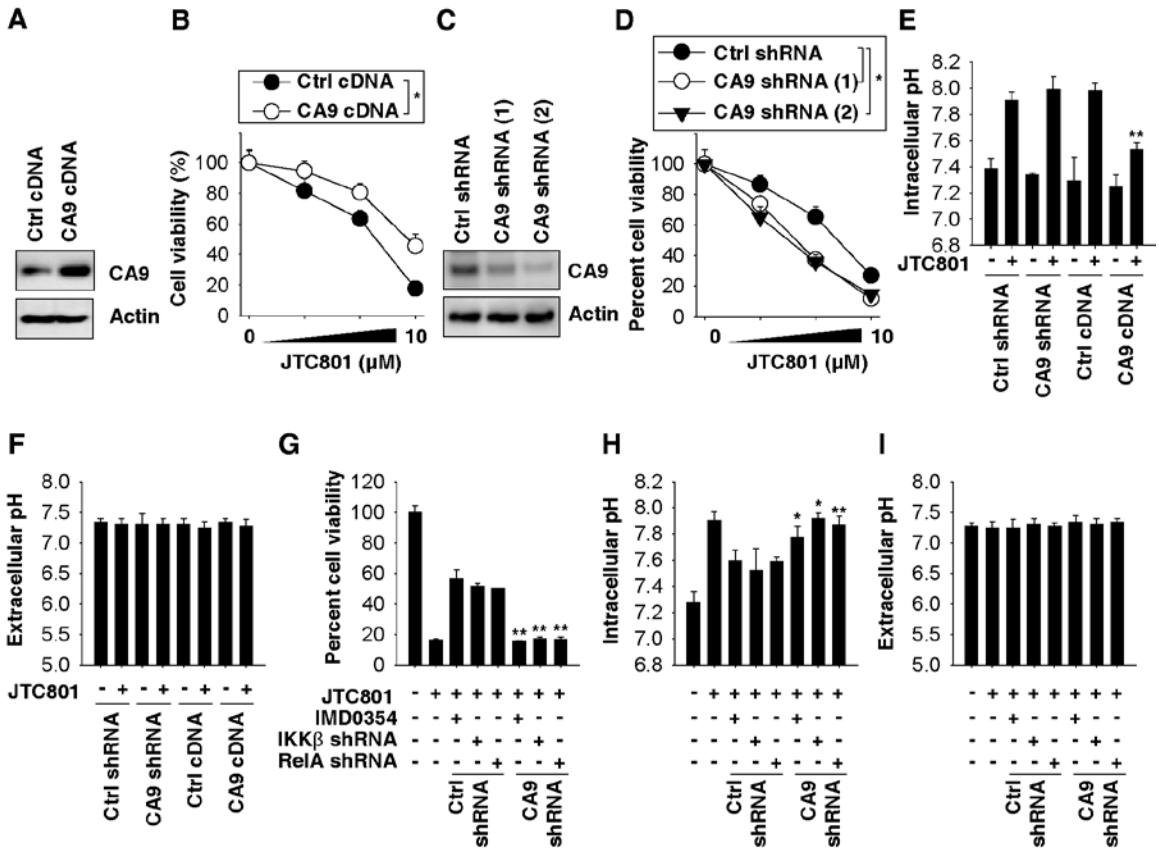


Fig. S10. CA9 downregulation contributes to alkaliptosis. (A) Western blot analysis of CA9 expression in CA9-overexpressed MiaPaCa2 cells. (B) Overexpression of CA9 inhibited JTC801-induced cell death at 24 hours in MiaPaCa2 cells (n=3, *p < 0.05). (C) Western blot analysis of CA9 expression in CA9-knockdown MiaPaCa2 cells. (D) Knockdown of CA9 increased JTC801-induced cell death at 24 hours in MiaPaCa2 cells (n=3, *p < 0.05 versus control shRNA group). (E, F) Intracellular and extracellular pH were assayed in indicated MiaPaCa2 cells following treatment with JTC801 (10 μM) for 24 hours (n=3, *p < 0.05 versus control group). (G-I) Knockdown of CA9 restored JTC801-induced cell death in NF-κB pathway-inhibition (IMD0354 [10 μM]) or -knockdown (IKKβ shRNA or RelA shRNA) MiaPaCa2 cells. In parallel, intracellular and extracellular pH was assayed (n=3, *p < 0.05, **p < 0.01 versus control shRNA group).

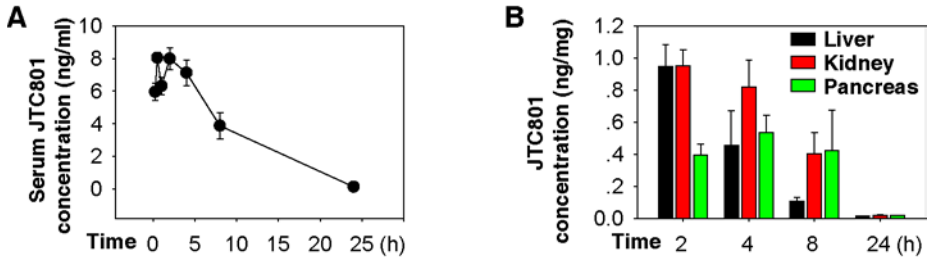


Fig. S11. JTC801 concentration-time courses in serum and tissues resulting from oral injection of JTC801 (20 mg/kg at a single dose) in C57BL/6 mice (n=4 mice/time).

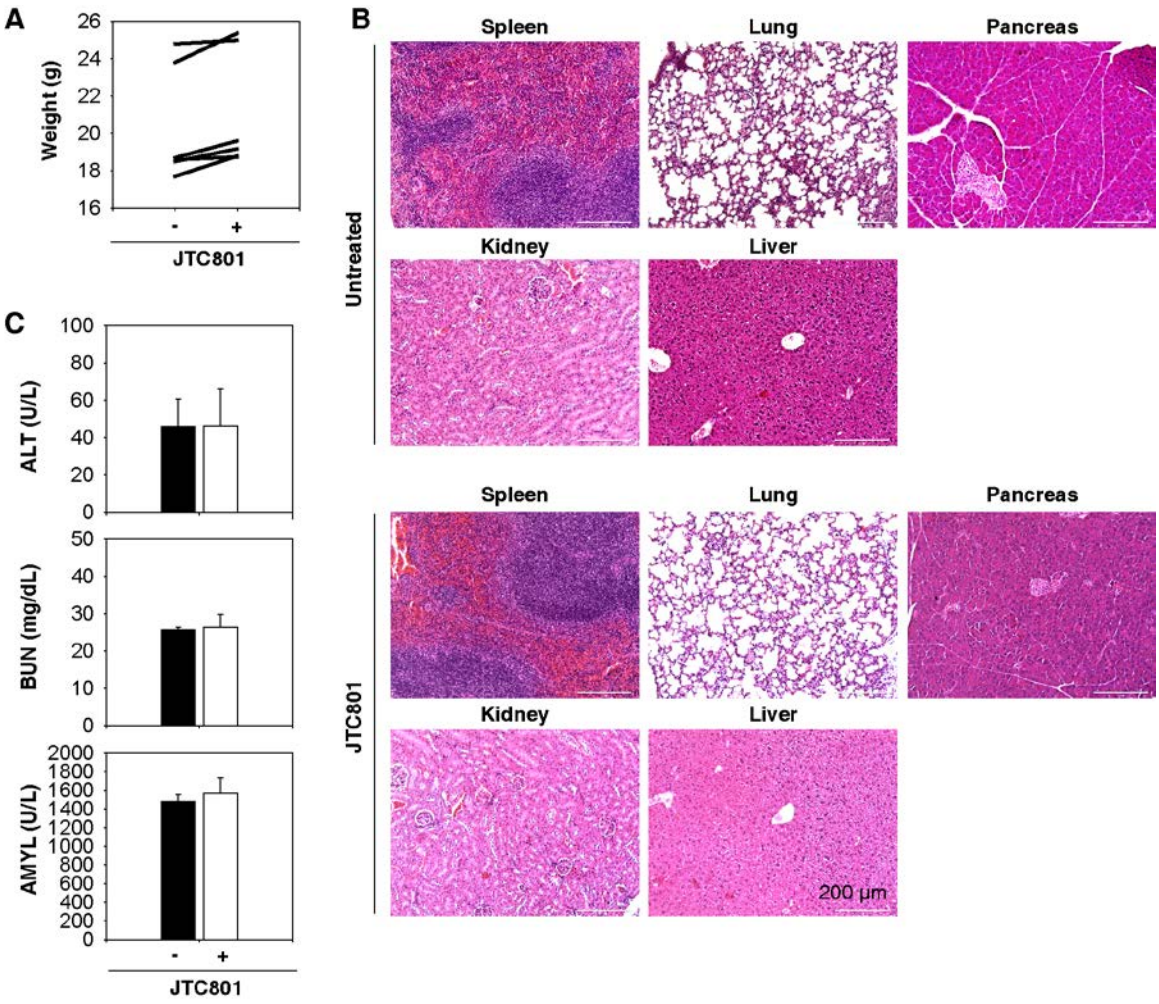


Fig. S12. JTC801 is safe *in vivo.* C57BL/6 mice at six to eight weeks were orally injected with JTC801 (20 mg/kg) for seven days and then sacrificed on day 8. The body weights of mice (A), histopathology (B), and enzyme activities in the serum (C) were assayed. The control group was age-matched C57BL/6 mice without JTC801 treatment (n=6 mice/group).

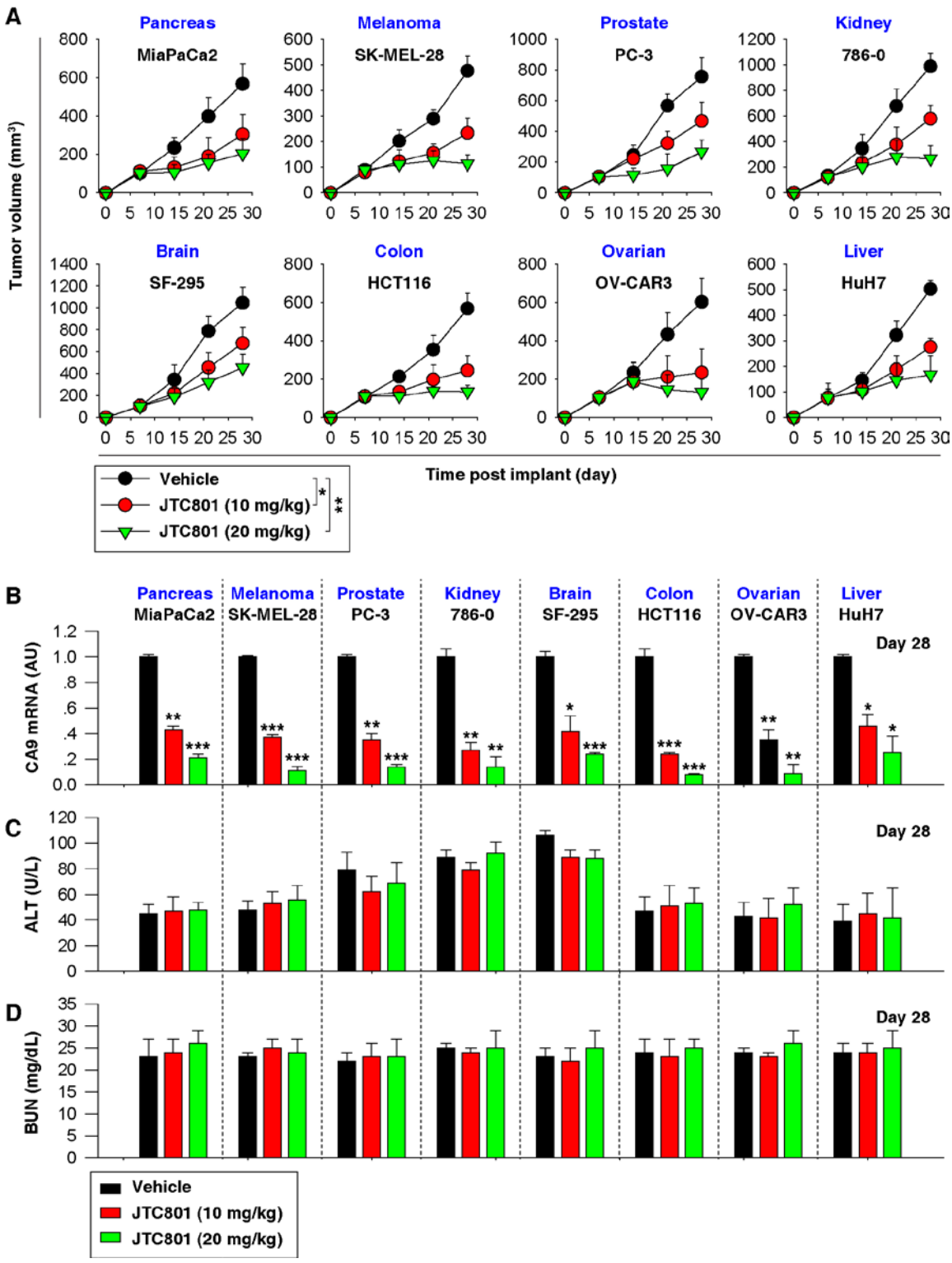


Fig. S13. Anticancer activity of JTC801 *in vivo*. (A) Oral injection of JTC801 (10 or 20 mg/kg, once every day, at day 7 for two weeks) inhibited tumor growth in nude mouse

xenograft models with multiple human tumor cell lines (n=8 mice/group, *p < 0.05, **p < 0.01). (B-D) In parallel, CA9 mRNA in tumor (B) and enzyme-activities (C, D) in the serum were assayed at day 28 (n=3 mice/group, *p < 0.05, **p < 0.01, ***p < 0.001 versus vehicle group).

Extended Data Table 1

The ranking of the relative anticancer activity of 254 GPCR compounds

JTC-801	0.151494183
BMS-833923	0.382131923
PNU-120596	0.664884334
Amfebutamone HCl	0.745468952
Almotriptan Malate	0.750857589
FLI-06	0.752435291
Phenoxybenzamine HCl	0.757838324
Synephrine HCl	0.782454598
SB271046	0.783311881
Amantadine HCl	0.789925208
Hesperetin	0.792007182
Ketanserin	0.799600261
Prucalopride	0.800212606
Isoprenaline HCl	0.80229458
Ciproxifan	0.81478642
NPS-2143	0.814908889
(-)-MK 801 Maleate	0.817970615
Ritodrine HCl	0.819317774
Cisatracurium Besylate	0.823726659
WAY-100635 Maleate	0.825318756
Equol	0.826788385
PF-5274857	0.828380482
Benserazide HCl	0.829972579
Asenapine	0.834995346
IEM 1754 dihydrobroMide	0.835238747
LDE225 (NVP-LDE225,Erismodegib)	0.840872322
L-Adrenaline	0.843811579
GW842166X	0.844423924
Levosulpiride	0.852384411
BML-190	0.855323667
Vismodegib (GDC-0449)	0.858529451
VU 0364770	0.859120207
VUF 10166	0.860589835
Macitentan	0.860979683
ADX-47273	0.865366127
BRL-54443	0.867080694
VU 0357121	0.878715251
Ethynodiol diacetate	0.882688561
Almorexant HCl	0.883001667
Phenylephrine HCl	0.886185861
BRL-15572	0.888390304
Suvorexant (MK-4305)	0.889646535
Canagliflozin	0.890717215
Famotidine	0.896157426
Timolol Maleate	0.90147509
Loratadine	0.902007712
Dopamine HCl	0.902964118
MetoclopraMide HCl	0.908293905

Hydroxyzine 2HCl	0.910381297
Carvedilol	0.915436777
Estriol	0.915578427
MK-801 (Dizocilpine)	0.916925587
Clonidine HCl	0.919742374
Clomifene citrate	0.921701879
Biperiden HCl	0.922350751
Olanzapine	0.924886073
MRS 2578	0.927947799
Naphazoline HCl	0.92905002
SB269970 HCl	0.929784834
ADL5859 HCl	0.934051323
5-hydroxymethyl Tolterodine (PNU 200	0.935418409
Decamethonium Bromide	0.944892847
BMY 7378	0.946440622
Medroxyprogesterone acetate	0.947420374
Bethanechol chloride	0.94791025
Loperamide HCl	0.950849506
Clomipramine HCl	0.951829259
Epinephrine HCl	0.953242416
Lidocaine	0.954660285
Naftopidil	0.955135922
Lafutidine	0.955458051
Naltrexone HCl	0.955748267
Fluvoxamine maleate	0.956255817
Mifepristone	0.956360612
Rotundine	0.965300851
Pancuronium dibromide	0.968852453
Fesoterodine Fumarate	0.97803763
Zolmitriptan	0.978726233
Doxazosin Mesylate	0.989230156
Bisoprolol fumarate	0.993218987
Sotalol	0.994448479
CTEP (RO4956371)	0.995305762
Azatadine dimaleate	0.995825216
Aripiprazole	0.996410052
SB408124	0.997606701
Bazedoxifene HCl	0.99812255
Org 27569	0.998271506
Rivastigmine Tartrate	0.998936312
Naftopidil DiHCl	1.002393299
Estradiol valerate	1.004313944
Tianeptine sodium	1.009307273
Rimonabant	1.009602004
Prasugrel	1.013694987
Toremifene Citrate	1.014226832
Atropine	1.01441093
Bupivacaine HCl	1.015513151
Mianserin HCl	1.01781678
Pramipexole	1.020411912

Cyproheptadine HCl	1.022869299
Clozapine	1.023841045
Sertraline HCl	1.024074589
Zibotentan (ZD4054)	1.02552852
Sitaxentan sodium	1.025605344
Tamoxifen Citrate	1.032841377
Dapagliflozin	1.03696317
RS-127445	1.038904735
Fluoxetine HCl	1.040420157
PRX-08066 Maleic acid	1.041052046
Silodosin	1.041483845
Escitalopram Oxalate	1.046200946
Nefiracetam	1.05105704
Forskolin	1.056172867
Ondansetron HCl	1.056508443
Duloxetine HCl	1.057971014
Roxatidine Acetate HCl	1.060231352
Cimetidine	1.062225768
Raloxifene HCl	1.063289456
Drospirenone	1.06501795
Naratriptan	1.067278287
Orphenadrine Citrate	1.070735275
Scopolamine HBr	1.071603965
Maprotiline HCl	1.074053345
Rocuronium Bromide	1.080441431
Aniracetam	1.081505119
Arecoline	1.082871115
PF-543	1.095185082
Irsogladine	1.096662678
Ramelteon	1.097726366
Agomelatine	1.097726366
Rotigotine	1.099220707
Etomidate	1.099720782
Tropicamide	1.102380003
JNJ-7777120	1.103395491
Trimebutine	1.106368834
Niflumic acid	1.108822711
Ivabradine HCl	1.109825821
SANT-1	1.111049262
Quetiapine Fumarate	1.112618003
Sumatriptan Succinate	1.113681691
Risperidone	1.11474538
WZ811	1.119955469
Ketotifen Fumarate	1.120329743
Domperidone	1.120958981
(+)-Matrine	1.123163424
Azelastine HCl	1.124510583
Estrone	1.126047068
Scopine	1.126470088
Olopatadine HCl	1.134430574

Benztropine mesylate	1.139716115
AM251	1.141533778
VU 0364439	1.141664347
Cinacalcet HCl	1.142534237
Desvenlafaxine	1.144586696
Ranitidine	1.148517484
Hexestrol	1.149126857
Metoprolol Tartrate	1.151176705
Xylazine HCl	1.151698707
Racecadotril	1.156474999
Clemastine Fumarate	1.160882861
Fulvestrant	1.16327616
Betaxolol	1.165403537
Vortioxetine (Lu AA21004) HBr	1.167826329
Bambuterol HCl	1.169774562
Ginkgolide A	1.170589017
Desloratadine	1.170609519
Pregnenolone	1.172982316
Detomidine HCl	1.173115277
VU 0361737	1.17561926
Mosapride Citrate	1.176173381
Terazosin HCl	1.177768914
Desvenlafaxine Succinate	1.179237406
Nebivolol	1.180827018
Methscopolamine	1.180827018
Phentolamine Mesylate	1.181491823
Nizatidine	1.187608031
Labetalol HCl	1.189535207
Brompheniramine hydrogen maleate	1.197991998
Cetirizine DiHCl	1.203962239
Oxymetazoline HCl	1.205952484
Urapidil HCl	1.20662146
Pyridostigmine Bromide	1.209014759
(+)-Bicuculline	1.209852491
Megestrol Acetate	1.211009174
PD128907 HCl	1.213423095
MPEP	1.214157909
Medetomidine HCl	1.217227943
Fexofenadine HCl	1.217645422
AM1241	1.218454993
Propranolol HCl	1.218897857
Memantine HCl	1.21938572
Dapoxetine HCl	1.220050525
Synephrine	1.229589006
Clopidogrel	1.235872889
Trospium chloride	1.239508997
Empagliflozin (BI 10773)	1.243807403
SB742457	1.246312274
Alfuzosin HCl	1.249833799
Paroxetine HCl	1.250208739

Acetylcholine Chloride	1.25249302
Tiotropium Bromide hydrate	1.259104041
Flavoxate HCl	1.26593376
Tizanidine HCl	1.266055046
Guanabenz Acetate	1.283607014
Gallamine Triethiodide	1.286292165
Tolazoline HCl	1.290286669
Epinephrine Bitartrate	1.29559981
Cyclizine 2HCl	1.310743112
Carteolol HCl	1.31658781
Solifenacin succinate	1.317283607
Flumazenil	1.326286398
Eletriptan HBr	1.329529641
Amisulpride	1.33931658
Tropisetron	1.343704295
Homatropine Bromide	1.347620373
Bepotastine Besilate	1.352351795
Otilonium Bromide	1.354578347
Hyoscyamine	1.35917061
Histamine 2HCl	1.368355135
Tripelennamine HCl	1.375173949
Venlafaxine	1.375349023
NMDA (N-Methyl-D-aspartic acid)	1.397300306
Gestodene	1.401010504
Bosentan Hydrate	1.405093237
Acebutolol HCl	1.410659616
Fingolimod (FTY720) HCl	1.430281102
Tolterodine tartrate	1.432887587
Amitriptyline HCl	1.433064292
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Ifenprodil Tartrate	1.511967715
Oxybutynin chloride	1.515725021
Tetrahydrozoline HCl	1.53993877
Allopurinol	1.551788326
Lorcaserin HCl	1.576676872
Naloxone HCl	1.581408294
Diphemanil Methylsulfate	1.592819371
Betahistine 2HCl	1.600473142
Indacaterol Maleate	1.658780963

Mirabegron	1.691344281
Pergolide mesylate	1.691900918
Darifenacin HBr	1.706790982
Levodropropizine	1.801836905
Dexmedetomidine	1.835930977
Atomoxetine HCl	2.210269969

Extended Data Table 2

The relative effects of 416 inhibitors on anticancer activity of JTC801

IMD 0354	2.916986196
BYL719	2.505368098
Torin 2	2.495207055
AZ 628	2.437308282
Torin 1	2.273964724
VX-745	2.16583589
CCT129202	2.10877919
INK 128 (MLN0128)	2.089915644
Tofacitinib citrate (CP-690550 citrate)	2.087423313
Quizartinib (AC220)	2.06621342
Piceatannol	2.007285276
Skepinone-L	1.954371166
TG 100713	1.953412577
Tideglusib	1.938075153
Tofacitinib (CP-690550, Tasocitinib)	1.929447853
NVP-BVU972	1.920437117
SAR131675	1.861004601
Vandetanib (Zactima)	1.841123012
Tyrphostin AG 879 (AG 879)	1.833013804
XL765	1.814809341
SB203580	1.798696319
PD98059	1.777032209
Sorafenib Tosylate	1.761503067
JNJ-7706621	1.741797221
SL-327	1.73006135
Semaxanib (SU5416)	1.728719325
AZD4547	1.708780675
TWS119	1.699822643
GDC-0068	1.698619632
Vemurafenib (PLX4032, RG7204)	1.667753067
AZD8330	1.658934049
CI-1033 (Canertinib)	1.64621876
Nilotinib (AMN-107)	1.646056475
SB 431542	1.64492048
PD0325901	1.633435583
ON-01910	1.630357671
Empty	1.616947853
SB202190 (FHPI)	1.614263804
Empty	1.597776074
HMN-214	1.584540349
Amuvatinib (MP-470)	1.572568726
PIK-90	1.568430387
VX-702	1.54773773
KRN 633	1.535471475
AMG-208	1.527047
WP1066	1.506710123
AS703026 (pimasertib)	1.487732782
PHT-427	1.484776825

PHA-793887	1.479456104
Thiazovivin	1.47635235
Vemurafenib (PLX4032)	1.46689329
Tandutinib (MLN518)	1.462836741
Baricitinib (LY3009104,incb28050)	1.458588957
Dinaciclib (SCH727965)	1.457630368
PLX-4720	1.455329755
Tivozanib (AV-951)	1.451522317
CI-1040 (PD184352)	1.446689387
CYC116	1.446349394
R406 (free base)	1.427135678
INCB28060	1.419095092
SP600125	1.413535276
AST-1306	1.402157848
PD0325901	1.392973061
MGCD-265	1.390186225
BIX 02189	1.37327454
Temsirolimus (Torisel)	1.371957157
PD318088	1.369056748
3-Methyladenine	1.337998466
Motesanib Diphosphate (AMG-706)	1.335929893
AT7519	1.334023056
SB 525334	1.333431865
E7080 (Lenvatinib)	1.326041975
Deforolimus (Ridaforolimus)	1.317185979
Golvatinib (E7050)	1.30732362
SGX-523	1.302499189
Apoptosis Activator 2	1.29765131
TG100-115	1.279337866
Dabrafenib (GSK2118436)	1.259202454
Hesperadin	1.255690216
Pazopanib HCl	1.23823434
AZ628	1.235050167
Vatalanib 2HCl (PTK787)	1.226144109
Desmethyl Erlotinib (CP-473420)	1.223734663
Dasatinib (BMS-354825)	1.220707562
PD98059	1.216523795
Telatinib (BAY 57-9352)	1.214715719
Y-27632 2HCl	1.212917884
ZM 336372	1.212615031
VX-680 (MK-0457, Tozasertib)	1.20464135
Trametinib (GSK1120212)	1.203796012
KU-60019	1.202926397
AEE788 (NVP-AEE788)	1.201152823
PD184352 (CI-1040)	1.196319018
Sunitinib Malate (Sutent)	1.1938494
U0126-EtOH	1.1906037
CP 673451	1.178983151
SB 202190	1.170723791
LY2811376	1.170280036

IC-87114	1.16553355
ZSTK474	1.164556962
PI-103	1.163096397
AZD6244 (Selumetinib)	1.154089581
Carfilzomib (PR-171)	1.152213189
SU11274	1.150843882
Roscovitine (Seliciclib, CYC202)	1.149302175
KW 2449	1.144546261
TAK-632	1.144171779
XL-184 (Cabozantinib)	1.137698799
Bosutinib (SKI-606)	1.130963973
PIK-93	1.124445758
VX-702	1.108128834
Saracatinib (AZD0530)	1.106945797
AMG458	1.106622074
GSK1120212 (Trametinib)	1.100869565
Ki8751	1.087200709
Axitinib	1.085361895
PD 0332991 (Palbociclib) HCl	1.084631613
Cyt387	1.082809365
GDC-0980 (RG7422)	1.079866221
AG-490	1.07603051
MLN8054	1.074813372
SB 203580	1.069782538
CHIR-98014	1.06367893
Oprozomib (ONX 0912)	1.061878952
Nicorandil (Ikorel)	1.056698445
Amiodarone HCl	1.055193176
Nateglinide (Starlix)	1.05469142
AZD8931	1.053207213
XL147	1.04633236
PF-04217903	1.04211295
Leupeptin Hemisulfate	1.038392051
Vinpocetine (Cavinton)	1.037631711
(+)-Bicuculline	1.036126443
PHA-767491	1.028227425
YM201636	1.024534437
Nimodipine (Nimotop)	1.023080783
Gliclazide (Diamicron)	1.02207727
Amlodipine (Norvasc)	1.020070246
VX-222	1.017163505
Phenytoin sodium (Dilantin)	1.017059709
A-803467	1.017059709
AG-1024	1.016848951
Lacidipine (Lacipil, Motens)	1.01304566
PD318088	1.010493645
TAK-438	1.009533367
TSU-68 (SU6668)	1.008276678
Repaglinide	1.007526342
BIRB 796 (Doramapimod)	1.007093558

Nefiracetam (Translon)	1.006021074
GDC-0941	1.005517689
GSK1059615	1.005025126
YO-01027	1.004065041
Glimepiride	1.003613369
Azelnidipine	1.003512293
Cleviprex (Clevidipine)	1.001003512
BS-181 HCl	1.000295596
ZM 336372	1
SB590885	0.999063545
Alogliptin (SYR-322)	0.998193315
Triamterene	0.995985951
Saxagliptin (BMS-477118,Onglyza)	0.994579946
AEBSF HCl	0.990966576
RAF265 (CHIR-265)	0.990030675
Gabapentin HCl	0.987957852
Gabapentin (Neurontin)	0.987957852
Tetracaine HCl (Pontocaine)	0.984445559
Valproic acid sodium salt (Sodium valproate)	0.983943803
Brivanib alaninate (BMS-582664)	0.98336579
ML133 HCl	0.981936779
AZD5438	0.981003344
Ambroxol HCl	0.979929754
DCC-2036 (Rebastinib)	0.979531773
Z-VAD-FMK	0.97696477
Ibutilide fumarate	0.976417461
Phenytoin (Lepitoin)	0.975413949
Ginkgolide A	0.974410437
PHA-680632	0.974283181
Amiloride HCl dihydrate	0.97390868
MK-0752	0.973803071
Strontium ranelate (Protelos)	0.972905168
Tolbutamide	0.972905168
Lamotrigine	0.972905168
Amlodipine besylate (Norvasc)	0.972403412
TRAM-34	0.9713999
Linagliptin (BI-1356)	0.970189702
Lopinavir (ABT-378)	0.96928636
WZ3146	0.96689329
(R)-baclofen	0.966884094
Oxcarbazepine	0.965378826
Manidipine 2HCl (CV-4093)	0.965378826
Propafenone (Rytmonorm) HCl	0.965378826
TGX-221	0.964824121
Cilnidipine	0.963873557
Danoprevir	0.963414634
GDC-0879	0.962615031
PF-03814735	0.961471572
MLN8237 (Alisertib)	0.961376177
Ouabain	0.961364777

Flunarizine 2HCl	0.960863021
BI 2536	0.959996754
Rufinamide (Banzel)	0.959357752
Everolimus (RAD001)	0.95658877
Daclatasvir (BMS-790052)	0.956187895
Carbamazepine (Carbatrol)	0.955845459
Mitiglinide calcium	0.955343703
Mubritinib (TAK 165)	0.954916388
Procaine (Novocaine) HCl	0.954841947
Dibucaine (Cinchocaine) HCl	0.954340191
Z-FA-FMK	0.953929539
Nilvadipine (ARC029)	0.95183141
CP-724714	0.951078924
Brivanib (BMS-540215)	0.950503083
SNS-032 (BMS-387032)	0.950340798
Riluzole (Rilutek)	0.949322629
Proparacaine HCl	0.949322629
BIBF1120 (Vargatef)	0.948474521
Manidipine (Manyper)	0.947315605
BKM120 (NVP-BKM120)	0.944749164
Rotundine	0.944305068
Nitrendipine	0.943803312
JNJ-38877605	0.941415125
MK-5108 (VX-689)	0.941142638
Etomidate	0.940291019
Semagacestat (LY450139)	0.939476061
Tetrandrine (Fanchinine)	0.939287506
Gliquidone	0.939287506
TAK-733	0.938996656
Felodipine (Plendil)	0.936778726
Aspirin (Acetylsalicylic acid)	0.934507678
Sorafenib (Nexavar)	0.934193444
MLN2238	0.933604336
Ritonavir	0.933152665
AZD7762	0.933047591
Neratinib (HKI-272)	0.932751995
DAPT (GSI-IX)	0.932700994
Aurora A Inhibitor I	0.929796039
Nafamostat mesylate	0.928635953
Isradipine (Dynacirc)	0.928248871
SP600125	0.928022465
A66	0.920668896
Nelfinavir Mesylate	0.920505872
Ranolazine 2HCl	0.915203211
PAC-1	0.914634146
Masitinib (AB1010)	0.914556962
Sitagliptin phosphate monohydrate	0.913279133
Camostat Mesilate (FOY-305)	0.91237579
Chlorpromazine (Sonazine)	0.912192674
BGJ398 (NVP-BGJ398)	0.911616908

BIRB 796 (Doramapimod)	0.911173515
Flumazenil	0.91018565
Losmapimod (GW856553X)	0.90970092
Vildagliptin (LAF-237)	0.906052394
Atazanavir sulfate	0.906052394
Niflumic acid	0.90366282
SB-3CT	0.902439024
Amprenavir (Agenerase)	0.901535682
AZ 960	0.89993311
Telaprevir (VX-950)	0.898373984
RO4929097	0.897922313
Gabexate mesylate	0.897470641
PF-00562271	0.897391304
Doxycycline HCl	0.895663957
AS-605240	0.895359149
PI-1840	0.889792231
Tie2 kinase inhibitor	0.889742832
MLN9708	0.888888889
PLX-4720	0.888185654
Alvelestat (AZD9668)	0.886178862
Ruxolitinib (INCB018424)	0.882648537
WP1130	0.881471572
PD 151746	0.873532069
CEP33779	0.866564417
GW5074	0.865989264
LY411575	0.864498645
AMG 900	0.858595318
A-769662	0.858461538
Refametinib (RDEA119, Bay 86-9766)	0.856595092
Bortezomib (Velcade)	0.855916893
Odanacatib (MK 0822)	0.853658537
Pimasertib (AS-703026)	0.851610429
SB590885	0.851418712
PCI-32765 (Ibrutinib)	0.848561873
TAK-901	0.839063545
PD153035 HCl	0.832765336
KU-55933	0.826598507
Apatinib (YN968D1)	0.82541806
MK-2461	0.810966258
Phenformin HCl	0.810167224
Flavopiridol HCl	0.807625418
BIX 02188	0.806173313
CX-4945 (Silmitasertib)	0.803210702
GSK2126458	0.802408027
Barasertib (AZD1152-HQPA)	0.799902629
Palomid 529	0.799866221
BI6727 (Volasertib)	0.799464883
WYE-354	0.793822051
PH-797804	0.784125767
CAL-101 (GS-1101)	0.783143813

Triciribine (Triciribine phosphate)	0.782781564
BEZ235 (NVP-BEZ235)	0.763794223
Erlotinib HCl	0.757951964
BX-912	0.756577003
BMS-265246	0.755690216
BMS 777607	0.742979604
LY294002	0.739208049
ZM-447439	0.73726063
R406	0.732842809
LGX818	0.732361963
AZD8055	0.724209282
SB 216763	0.72062642
TAE684 (NVP-TAE684)	0.72030185
Indirubin	0.711839465
JNK Inhibitor IX	0.709739264
CCT128930	0.707959866
Crizotinib (PF-02341066)	0.704397923
PF-04691502	0.688160535
AT9283	0.686222006
A-674563	0.673311037
OSI-930	0.669819687
PIK-75	0.669671889
WZ4002	0.660360627
PIK-294	0.65993311
Dabrafenib (GSK2118436)	0.659317485
Quercetin (Sophoretin)	0.655652174
CAY10505	0.64
NVP-TAE226	0.639953988
Raf265 derivative	0.636120401
AZD8330	0.633313627
MEK162 (ARRY-162, ARRY-438162)	0.627875767
TAK-733	0.619248466
Danusertib (PHA-739358)	0.618873742
Gefitinib (Iressa)	0.618630315
TAK-715	0.612154908
WZ8040	0.605675436
PF-05212384 (PKI-587)	0.602943144
SNS-314	0.589175592
TG101348 (SAR302503)	0.575117057
MK-2206 2HCl	0.553716326
TAK-285	0.544670245
CH5424802	0.537960123
OSI-027	0.529899666
BIX 02188	0.52837718
PIK-293	0.526287625
Sotrastaurin (AEB071)	0.523773006
AS-604850	0.521337793
KX2-391	0.519732441
AS-252424	0.502207358
WHI-P154	0.49827454

R935788 (Fostamatinib disodium, R788 disoc	0.496722408
NU7441 (KU-57788)	0.488294314
XMD8-92	0.487921779
JNK-IN-8	0.484279141
Afatinib (BIBW2992)	0.48369036
TG101209	0.482541806
Rapamycin (Sirolimus)	0.470707562
Arry-380	0.469431438
BIX 02189	0.466449897
OSI-420	0.461672241
ARQ 197 (Tivantinib)	0.457123746
PP242	0.450167224
BMS-599626 (AC480)	0.449123661
Imatinib Mesylate	0.44287569
Ku-0063794	0.43718593
Foretinib (GSK1363089, XL880)	0.416423239
AT7867	0.410730121
PD173074	0.407774165
Selumetinib (AZD6244)	0.40625
Asiatic Acid	0.40625
GDC-0879	0.404495294
BMS 794833	0.4
Dovitinib Dilactic acid (TKI258 Dilactic acid)	0.399731595
Pelitinib (EKB-569)	0.396245936
LY2603618 (IC-83)	0.395585284
TPCA-1	0.395513804
Wortmannin	0.39493865
Milciclib (PHA-848125)	0.393177258
SB 415286	0.387023411
LY2228820	0.38649128
GSK1904529A	0.38477767
Regorafenib (BAY 73-4506)	0.376884422
Sorafenib	0.375
Lapatinib Ditosylate (Tykerb)	0.374472574
U0126-EtOH	0.374424847
GSK461364	0.373189477
GSK690693	0.372444012
ARRY334543	0.367759197
Crenolanib (CP-868596)	0.366956522
Dovitinib (TKI-258)	0.366764038
Imatinib (Gleevec)	0.359331104
LY2784544	0.355158144
PKI-402	0.353578595
PH-797804	0.35277592
BX-795	0.343777712
AZD2014	0.333397239
LY2228820	0.331096626
Dacomitinib (PF299804,PF-00299804)	0.330301003
NVP-ADW742	0.309964297
PHA-665752	0.309802012

CEP-32496	0.305981595
ENMD-2076	0.304611292
R788 (Fostamatinib)	0.298595318
CHIR-124	0.288695652
WYE-687	0.288294314
Ponatinib (AP24534)	0.286284363
CCT137690	0.283879599
Staurosporine	0.274608336
PP-121	0.266354515
AG-1478 (Tyrphostin AG-1478)	0.256588629
GSK1070916	0.256187291
WYE-125132	0.256187291
NVP-BHG712	0.249364548
NVP-BSK805	0.242006689
GSK1838705A	0.240133779
Enzastaurin (LY317615)	0.238964622
Cediranib (AZD2171)	0.228334956
LDN193189	0.226755853