Supplementary Methods

In vitro enzymatic assay

Recombinant EGFR Exon20ins 770_NPG and wild-type EGFR enzyme was purchased from Carna Bio. with catalogue numbers 08-553 and 08-115, respectively. The inhibition potency of compounds against these enzymes was assessed using Homogenous Time Resolved Fluorescence approach (HTRF, CisBio. Cat No. 62TK0PEJ).

In brief, recombinant kinases were pre-incubated in the presence or absence of compound at room temperature for 30 minutes. The reaction was initiated by adding ATP and substrate peptide which could be phosphorylated by kinases in the reaction. After 30 minutes incubation, the reaction was stopped by adding detection reagent mix containing EDTA. The fluorescence was measured at 615 nm and 665 nm, respectively, with excitation wavelength at 320 nm. The calculated signal ratio of 665 nm/615 nm is proportional to the kinase activity. The concentration of compound producing 50% inhibition of the respective kinase (IC_{50}) was calculated using four-parameter logistic fit with Prism GraphPad. The detailed protocols were recorded in notebook.

Kinase panel assay

In vitro profiling of the 117-recombinant kinase panel was performed at Eurofins UK Ltd (Dundee, UK). First, a full panel of 117 kinases was assayed at a single sunvozertinib concentration of 1 μM. Then, a second screen was performed on the kinases that showed >50% inhibition in the first single-point screen to allow determination of IC_{50}. In brief, recombinant kinases were incubated within appropriate buffer containing peptide substrate and radiolabelled γ-^{33}P-ATP together with presence or absence of required compound concentration. The reaction was initiated by adding ATP/Mg^{2+} mix. After incubation for 40 minutes at room temperature, the reaction was stopped by adding 3% phosphoric acid solution. A portion of reaction mix was spotted onto P30 filtermat to trap peptide and washed three times for 5 minutes with phosphoric acid to remove non-specific γ-^{33}P-ATP. The substrate phosphorylation was then measured by scintillation counting, which determined the level of kinase activity inhibition compared to control reactions. Percentage inhibition data for each kinase was mapped to the kinome tree (www.kinhub.org).

Generation of transgenic mouse models

The inducible and lung specific transgenic mouse models of EGFR Exon20ins 769_ASV was developed by Shanghai Model Organisms Center, Inc. The model was generated by homologous recombination in C57BL/129sv embryonic stem (ES) cells and implanted in C57BL/6J blastocysts as standard procedures. Briefly, targeting vector containing the following components were constructed as SA-polyA-2X HS4 chicken beta globin insulators-Tet responsive promoter-EGFR-V769_D770insASV-wpre-bGH poly(A)-Frt-PGK-Neo-bGH poly(A)-Frt. Targeting of the transgene cassettes into the Rosa26 locus was accomplished via standard homologous recombination. The construct was linearized and electroporated into C57BL/129sv embryonic stem cells. Correctly targeted ES cell colonies were screened by PCR, and PCR products were sequenced of essential regions to verify desired recombination. Correctly targeted ES cells were injected into blastocysts to obtain the chimeric mice and subsequent germline transmission. The high-percentage chimeric mice were crossed to FLP-deleter mice to remove the Neo cassette. The resulting tetO-EGFR-V769_D770insASV heterozygous mice were bred to CCSP-rtTA mice to obtain the lung specific

transgenic cohorts. Genotyping of CCSP-rtTA and EGFR-V769_D770insASV was performed by using tail DNA abstracted from mice.

Tumor measurement in EGFR exon20ins transgenic mice

The histological changes in the lungs of mice detected by hematoxylin and eosin (H&E) staining was used to reflect the tumor development after doxycycline induction. Also immunohistochemistry (IHC) with assay condition optimized to specifically detect EGFR protein of engineered human exon20ins transgene, positive EGFR expression were only identified in tumor cells but not in normal cells of the lung (**Supplementary Figure S4A**). Thus, percentage of cells with positive EGFR expression was used as a parameter to quantify tumor burden in the lung of transgenic mice.

Establishment of brain metastasis (BM) model by intracerebral (ICB) injection

In order to monitor the tumour growth in brain by measuring the bioluminescence signals in tumour cells using the imaging system, the stable cell clone luci-H1975 was generated by transfecting human lung cancer cell line H1975 with pGL4.50[luc2/CMV/Hygro] vector which contains luciferase gene using lipofectamine LTX (Invitrogen™, USA). The clone was selected with 300 μg/ml Hygromycin B by serial dilution and confirmed EGFR T790M mutation positive.

Under anaesthesia, the scalp of mouse was swabbed several times with alcohol-iodine. A sagittal incision (approximately 1 cm long) was made over the parieto-occipital bone using a sterile scalpel. The skull surface was exposed using a cotton swab to make the bregma apparent and a hole was punctured on the skull at 2.5 mm to the right of the bregma and 1 mm anterior to the coronal suture using a sterile 25 gauge sharp drill. Then the syringe perpendicular was placed to the skull through the hole and reach 3 mm deep below the skull surface and cell suspension at 0.3 million/each mouse was slowly injected with infusion pump. The needle was left in place for two minutes after injection and then slowly withdrawn to check if there was any jam in needles. Finally, the sterile bone gel was applied to the hole, the scalp was pulled back to cover the skull and the wound was closed by using wound clips or silk sutures. The mouse was put on heating pad softly until recovered and closely monitored daily. The bioluminescence signals were measured by IVIS spectrum imaging system to monitor tumor growth.

Caco-2 Apical to Basolateral Intrinsic Permeability Assay

The objective of this study is to evaluate the unidirectional intrinsic permeability of sunvozertinib. These samples will be analyzed through LC/MS/MS for estimating the apparent permeability coefficients (P_{app}) of compounds across Caco-2 cell monolayers, where apical chamber is at pH 6.5 and basolateral chamber is at pH 7.4. 3 Efflux transporter of P-gp, BCRP and MRP2 inhibitors, including 50 μM Quinidine, 30 μM benzbromarone, and 20 μM sulfasalazine, are included to block the active efflux transport of compounds. Data will be utilized to apparent permeability (Papp).

The apparent permeability (P_{app}), in units of centimeter per second, can be calculated for Caco-2 drug transport assays using the following equation:

$$P_{app} = \frac{C_R^{120} \times 0.8 - C_R^{45} \times 0.7}{C_D^{45} \times Area \times time}$$

C_R is the concentration of the receiver side at respective time point, C_D is the concentration of the donor side at respective time point. Area is the surface area of the membrane, and time is the duration for equilibrium.

Rat and dog plasma PK

The objective of this study was to determine the pharmacokinetics and the oral bioavailability of sunvozertinib following single intravenous or oral administration in Wistar Han rats and Beagle dogs. Sunvozertinib was freshly formulated as a solution in 5% DMSO, 95% SBE-β-CD (30%, w/v) in water for intravenous PK and an oral suspension in 0.5% HPMC, 0.1% Tween 80 in water for oral PK. Sunvozertinib was administered intravenously (3 mg/kg) and orally (3 mg/kg) to male and female Wistar Han rats. Likewise, sunvozertinib was given intravenously (1 mg/kg) and orally (1 mg/kg) to male and female Beagle dogs. Blood samples were collected from each animal at predefined time points and processed to obtain plasma prior to HPLC/MS analysis. Validated protein precipitation HPLC/MS/MS assays were used to determine sunvozertinib concentrations in samples from rat and dog PK studies. Noncompartmental methods were used to calculate the pharmacokinetics parameters with Phoenix WinNonlin Software version 6.1 (Certara, Princeton, NJ). The linear log trapezoidal algorithm, weighting uniform was used for parameters calculation.

Reversible inhibition of CYP enzymes

The objective of this study is to measure the potentials of sunvozertinib to inhibit a specific cytochrome P450 enzyme in human liver microsomes (HLM). Reversible CYP inhibition of CYPs 1A2, 2C9, 2C19, 2D6, 2A6, 2B6, 2C8, 2E1, 3A4/5 is studied through the incubation of a rising concentration of sunvozertinib with pooled human liver microsomes (0.2 mg/ml) and a substrate which is metabolized specifically by an individual CYP isoform. Known substrates are converted by human CYP isozymes to the corresponding specific metabolites. These substrates are used at concentrations equivalent to their respective K_m values. LC-MS-MS (MRM mode) is used to follow the formation of the CYP specific metabolites. A decrease in the formation of the metabolites in peak area to vehicle control is used to calculate the IC_{50} value. Positive control inhibitors are included as a cocktail to demonstrate the assay is operating within validated limits.

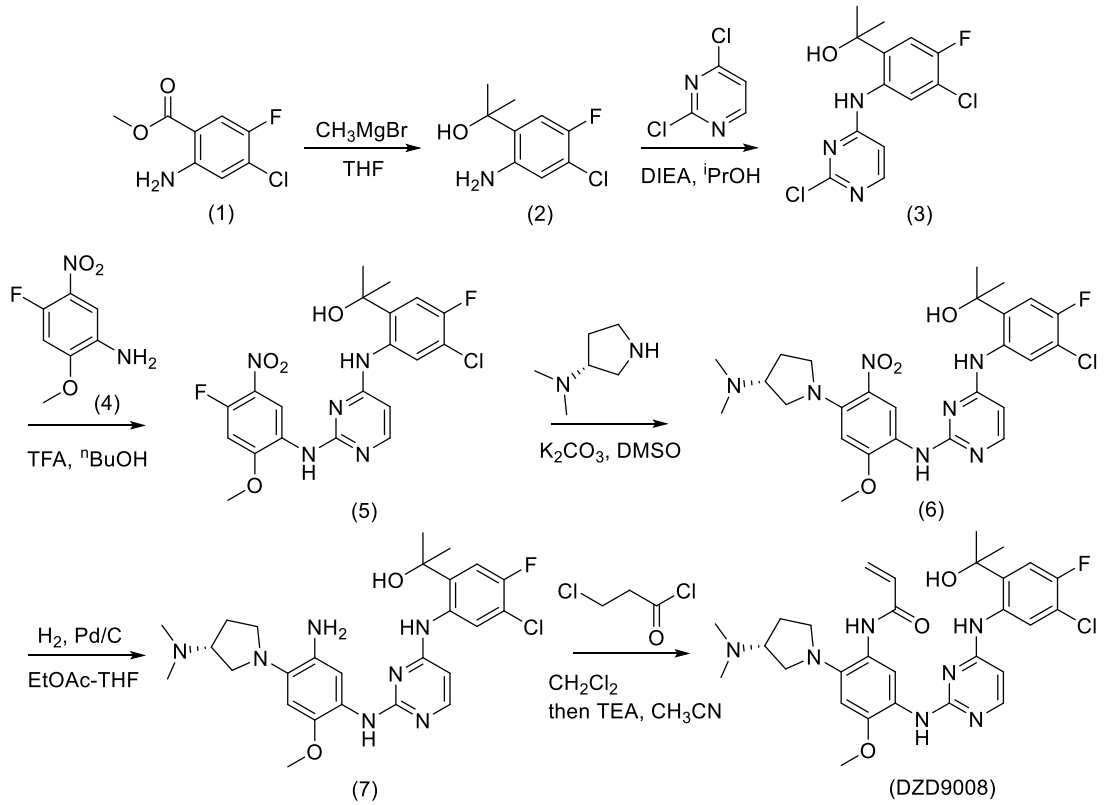
Time dependent inhibition of CYP enzymes

The potential of sunvozertinib to inhibit the Cytochrome P450 (CYP) isoforms CYPs 1A2, 2C9, 2C19, 2D6 and 3A4/5 was assessed by measuring the rate of formation of isoform specific metabolites derived from marker substrates when incubated with metabolically competent pooled human liver microsomes in the presence of NADPH. These activities were measured under linear conditions for time and protein concentration using the following marker substrates at 3 times their K_m concentration.

The potential for time dependent inhibition of CYP enzymes was investigated by a pre-incubation of sunvozertinib at two concentrations (10 μM) with human liver microsomes in the presence and absence of NADPH, followed by a 10-fold dilution and incubation in the presence of the CYP enzyme marker substrates and NADPH. The rate of formation of CYP isoform-specific metabolites was monitored by UPLC-MS/MS followed by determination of the percentage of time dependent inhibition for each test occasion.

The metabolism of marker substrates was determined by UPLC-MS/MS methods. Relevant control incubations were performed and CYP isoform-selective time dependent inhibitors were used as positive controls.

Preparation, purification, and characterization of (*R*)-N-(5-(4-(5-chloro-4-fluoro-2-(2-hydroxypropan-2-yl)phenylamino)pyrimidin-2-ylamino)-2-(3-(dimethylamino)pyrrolidin-1-yl)-4-methoxyphenyl)acrylamide (sunvozertinib)



- ***Procedure for the preparation of compound (2)***

To a solution of methyl 2-amino-4-chloro-5-fluorobenzoate (1) (12.0 g, 58.9 mmol) in THF (200 mL) was added CH_3MgBr (99 mL, 3M in ether, 294.7 mmol) at 0~5°C. The mixture was stirred at 12-17°C for 1.5 h. The reaction mixture was quenched by the addition of aq. NH_4Cl (100 mL), then extracted with EtOAc (3×100 mL). The organic layers were washed with brine (3×100 mL), and concentrated under reduced pressure to afford **compound (2)** (11.5 g, 96%) as light yellow oil.

LCMS: R_t = 3.283 min in 10-80CD_7MIN_220&254 chromatography (XBrige Shield RP18 2.1*50 mm), MS (ESI) *m/z* 186.1 $[M\text{-}OH]^+$.

^{1}H NMR ($CDCl_3$, 400MHz): δ (ppm) 6.90 (d, *J*=10.8 Hz, 1H), 6.62 (d, *J*=6.8 Hz, 1H), 1.63 (s, 6H).

^{13}C NMR (*d_6*-DMSO, 101 MHz) δ (ppm) 149.7, 147.4, 144.3, 131.3, 131.2, 116.9, 116.7, 115.7, 113.6, 113.4, 71.6, 28.7.

- ***Procedure for the preparation of compound (3)***

To a solution of **compound (2)** (11.5 g, 56.5 mmol) and DIEA (14.6 g, 112.9 mmol) in isopropanol (200 mL) was added 2,4-dichloropyrimidine (10.1 g, 67.8 mmol). The resulting yellow mixture was heated at 90°C for 60 h. The reaction mixture was concentrated in vacuum to give the crude product, which was purified by column chromatography on silica gel (30-43% EtOAc in petroleum ether) to

give **compound (3)** (12.0 g, 67%) as a white solid.

LCMS: t_R = 0.850 min in 5-95AB_220&254.lcm chromatography (Xtimate C18 2.1*30 mm), MS (ESI) *m/z* = 315.9 $[M+H]^+$.

^{1}H NMR ($CDCl_3$, 400MHz): δ (ppm) 9.17 (br s, 1H), 8.15 (d, *J*=5.6 Hz, 1H), 7.95 (d, *J*=6.8 Hz, 1H), 7.12 (d, *J*=10.0 Hz, 1H), 6.58 (d, *J*=6.0 Hz, 1H), 2.35 (s, 1H), 1.65 (s, 6H).

^{13}C NMR (*d_6*-DMSO, 101 MHz) δ (ppm) 161.9, 159.4, 157.8, 155.2, 152.8, 142.7, 132.8, 132.7, 126.6, 117.4, 117.2, 114.6, 114.4, 105.3, 71.7, 29.7.

- ***Procedure for the preparation of compound (5)***

To a solution of **compound (3)** (12.0 g, 38.0 mmol) and 4-fluoro-2-methoxy-5-nitroaniline (4) (7.44 g, 40.0 mmol) in ^{n}BuOH (160 mL) was added TFA (16 mL). The resulting orange mixture was heated at 50°C for 15 h. The reaction mixture changed from orange to pale yellow and solid precipitated out, additional 300 mg of 4-fluoro-2-methoxy-5-nitroaniline was added and the reaction mixture was heated at 50°C for another 4 h. The reaction mixture was filtered, the filter cake was washed with EtOAc/petroleum ether=1/1 (25 mL × 3) and EtOAc (25 mL × 3), then dried in vacuum to give **compound (5)** (15.2 g, 86%) as a grey solid.

LCMS: t_R = 0.776min in 5-95AB_220&254.lcm chromatography (Xtimate C18 2.1*30 mm), MS (ESI) *m/z* = 466.0 $[M+H]^+$.

^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm) 8.52 (d, *J*=8.0 Hz, 1H), 7.96 (d, *J*=6.8 Hz, 1H), 7.84 (d, *J*=7.2 Hz, 1H), 7.32 (d, *J*=10.8 Hz, 1H), 7.20 (d, *J*=12.8 Hz, 1H), 6.47 (d, *J*=6.8 Hz, 1H), 4.00 (s, 3H), 1.59 (s, 6H).

^{13}C NMR (*d_6*-DMSO, 101 MHz) δ (ppm) 161.6, 156.2, 153.7, 152.6, 143.9, 143.5, 131.0, 128.6, 128.1, 121.9, 117.3, 117.1, 114.6, 114.4, 102.1, 101.9, 100.0, 71.5, 57.5, 29.9.

- ***Procedure for the preparation of compound (6)***

To a solution of **compound (5)** (5.0 g, 10.7 mmol) and K_2CO_3 (5.9 g, 42.9 mmol) in DMSO (50 mL) was added (*R*)-*N*,*N*-dimethylpyrrolidin-3-amine (2.6 g, HCl salt, 14.0 mmol). The resulting mixture was stirred at 50°C for 12 h while the color was changed from pale yellow to deep yellow. The reaction mixture was poured into ice water (500 mL) with stirring and yellow solid was precipitated. The precipitated solid was collected by filtration and then dissolved into CH_2Cl_2 (500 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **compound (6)** (5.6 g, 93%) as yellow solid.

LCMS: R_t = 0.676 min in 5-95AB_220&254.lcm chromatography (MK RP-18e 25-2mm), MS (ESI) *m/z* = 560.1 $[M+H]^+$.

^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm) 9.00 (s, 1H), 8.91 (s, 1H), 8.09 (d, *J*=5.8 Hz, 1H), 7.93 (d, *J*=7.0 Hz, 1H), 7.19 (s, 1H), 7.11 (d, *J*=10.5 Hz, 1H), 6.31 (s, 1H), 6.18 (d, *J*=5.8 Hz, 1H), 5.31 (s, 1H), 3.94 (s, 3H), 3.55 (td, *J*=10.1, 6.4 Hz, 1H), 3.31-3.39 (m, 1H), 3.10-3.22 (m, 2H), 2.81 (br s, 1H), 2.30 (s, 6H), 2.15-2.25 (m, 1H), 1.83-1.98 (m, 1H), 1.67 (s, 6H).

^{13}C NMR (*d_6*-DMSO, 101 MHz) δ (ppm) 159.0, 158.9, 155.7, 154.9, 152.5, 150.1, 140.4, 137.7, 133.7, 127.4, 122.8, 119.8, 117.6, 116.0, 115.8, 112.9, 112.7, 97.0, 96.5, 71.0, 63.7, 55.0, 48.4, 42.8, 28.5.

- ***Procedure for the preparation of compound (7)***

To a solution of **compound (6)** (5.6 g, 10.0 mmol) in EtOAc (100 mL) and THF (50 mL) was added Pd/C (1.2 g). The resulting mixture was purged and degassed with H_2 for 3 times, then stirred at 11-18 ºC under H_2 (hydrogen balloon, 15 psi) for 16 h. The reaction mixture was filtered and concentrated under reduced pressure to give **compound (7)** (5.0 g, 94%) as light yellow solid.

LCMS: R_t = 0.660 min in 5-95AB_1.5 min_220&254 chromatography (MK RP18e 25-2mm), MS (ESI) *m/z* = 530.1 $[M+H]^+$.

^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm) 8.80 (s, 1H)**,** 8.15 (d, *J*=7.3 Hz, 1H), 8.04 (d, *J*=5.5 Hz, 1H), 7.87 (s, 1H), 7.43 (s, 1H), 7.09 (d, *J*=10.5 Hz, 1H), 6.67 (s, 1H), 6.06 (d, *J*=5.5 Hz, 1H), 3.82 (s, 3H), 3.24 - 3.13 (m, 2H), 3.07 - 2.96 (m, 2H), 2.91 - 2.83 (m, 1H), 2.28 (s, 6H), 2.18 - 2.08 (m, 1H), 1.90 - 1.85 (m, 1H), 1.66 (s, 6H).

^{13}C NMR (*d_6*-DMSO, 101 MHz) δ (ppm) 160.1, 156.8, 153.7, 151.3, 142.3, 139.1, 135.4, 134.9, 131.4, 124.2, 123.9, 117.2, 117.0, 114.1, 113.9, 110.0, 103.5, 97.5, 72.1, 64.9, 56.3, 54.5, 49.8, 29.6, 28.6.

- ***Procedure for the preparation of sunvozertinib***

Step 1: To a solution of compound (7) (5.0 g, 9.43 mmol) in CH_2Cl_2 (150 mL) was added 3-chloropropanoyl chloride (1.3 g, 10.37 mmol) in ice water bath. The resulting mixture was stirred at 0-5 °C for 30 min (little un-dissolved oil was precipitated out). The reaction mixture was poured into saturated $NaHCO_3$ (50 mL) and stirred at 12-17 °C for 2 h, and extracted with CH_2Cl_2 (150 mL × 2). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to give the crude residue, which was purified by column chromatography on silica gel (3% MeOH in CH_2Cl_2) to give a light yellow solid (3.4 g, 58% yield).

LCMS: R_t = 1.547 min in 10-80AB_4min_220&254 chromatography (Xtimate C18 2.1*30mm), MS (ESI) *m/z* = 620.0 $[M+H]^+$.

^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm) 9.58 (s, 1H), 9.31 (s, 1H), 8.56 (br s, 1H), 8.10 (d, J=5.8 Hz, 1H), 7.62 - 7.45 (m, 2H), 7.15 (d, J=10.5 Hz, 1H), 6.76 (s, 1H), 6.34 (d, J=5.8 Hz, 1H), 3.90 (t, J=6.3 Hz, 2H), 3.86 (s, 3H), 3.16 - 3.03 (m, 4H), 2.90 (br s, 3H), 2.32 (br s, 6H), 2.19 (br dd, J=6.3, 12.3 Hz, 1H), 1.98 (br s, 1H), 1.75 - 1.68 (m, 6H).

Step 2: To a solution of the yellow solid from step 1 (3.4 g, 5.48 mmol) in CH_3CN (70 mL) was added TEA (2.2 g, 21.92 mmol). The resulting mixture was stirred at 80°C for 12 h. The reaction mixture was concentrated under reduced pressure to remove about 35 mL CH_3CN, and then poured onto 500 mL H_2O and stirred for additional 30 min. The mixture was filtered, the filter cake was collected and then lyophilized to give **sunvozertinib** (2.64 g, 82%) as a white solid.

LCMS: R_t = 1.471 min in 10-80AB_4min_220&254 chromatography (Xtimate C18 2.1*30mm), MS (ESI) *m/z* = 584.0 $[M+H]^+$.

^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm) 9.67 (s, 1H), 9.44 (s, 1H), 8.55 (br s, 1H), 8.10 (d, *J*=6.0 Hz, 1H), 7.52 (br d, *J*=7.0 Hz, 1H), 7.48 (s, 1H), 7.15 (d, *J*=10.8 Hz, 1H), 6.76 (s, 1H), 6.42 - 6.28 (m, 3H), 5.82 - 5.75 (m, 1H), 5.66 (br s, 1H), 3.86 (s, 3H), 3.14 - 3.02 (m, 4H), 2.96 - 2.86 (m, 1H), 2.30 (s, 6H), 2.23 - 2.12 (m, 1H), 2.00 - 1.90 (m, 1H), 1.73 (s, 6H).

^{13}C NMR (d_6-DMSO, 101 MHz) δ (ppm) 163.5, 160.5, 159.9, 156.8, 153.5, 151.1, 149.9, 141.5, 138.5, 135.0, 132.0, 125.7, 123.7, 123.4, 119.9, 117.9, 117.2, 117.0, 114.0, 113.8, 99.5, 97.5, 72.2, 65.2, 55.6, 49.3, 43.9, 29.6.