Supplementary Information

A Mitochondrial-targeting MET Kinase Inhibitor Kills Erlotinib-resistant Lung Cancer Cells

Contents:

1. Docking of PHA665752 and TM608 in the Met Kinase binding pocket (2WKM)

(A) (B)

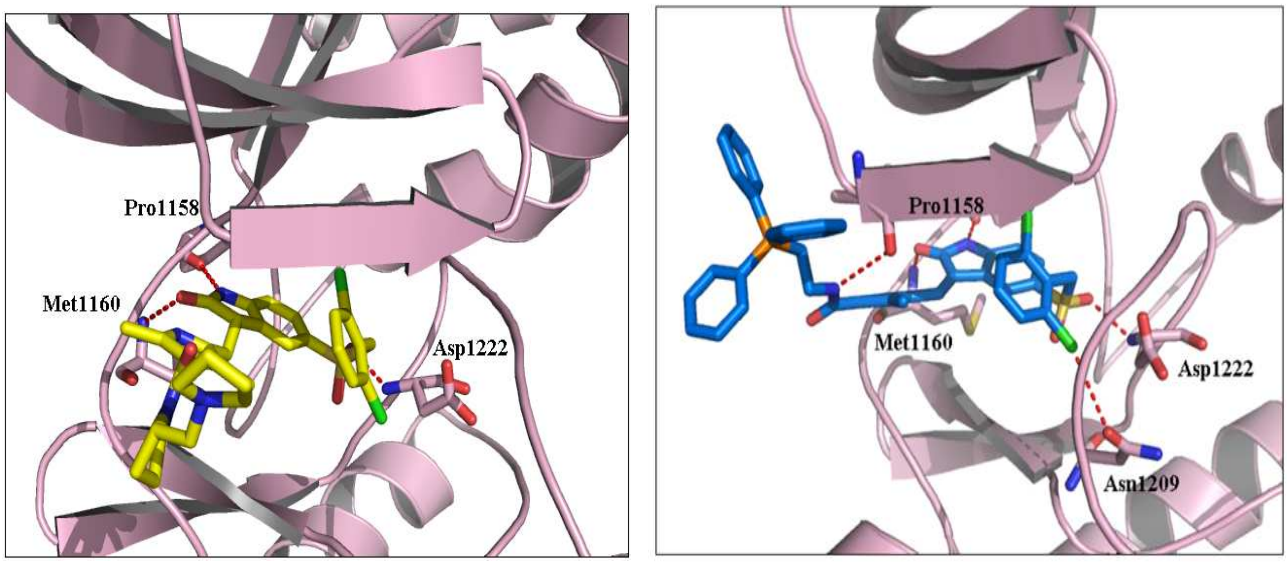


Figure S1. (A) Binding pose of PHA665752in the Met kinase binding pocket (2WKM).[S1] *The indolinone N forms a hydrogen (H) bond with carbonyl O of Pro1158 while the carbonyl O of the indolinone ring forms a H bond to backbone NH of Met 1160. The sulfonyl moiety of the terminal dichlorobenzylsulfonyl ring forms a H bond to Asp1222 (Pymol Schrodinger,OR, USA). (B) Docking pose of TM608 in the Met kinase binding pocket (2WKM). The TPP side chain projects out of the binding pocket into the solvent space. H bonding interactions with the hinge amino acids Met 1160, Pro 1158 are observed and interactions between the terminal dichlorobenzylsulfonyl ring and the binding pocket (Asp1222, Asn1209) are likewise retained.*

The co-crystalized structure of PHA665752 in the kinase domain of c-MET was retrieved from the RCSB protein data bank (PDB 2WKM).[S1] Water molecules were removed and the kinase domain was processed for docking using LigX in the software Molecular Operating Environment (MOE, version 2011, Chemical Computing Group, Montreal, Canada). PHA665752 and TM608 were prepared for docking on MOE. Docking simulation was carried out on GOLD v 5.2 (Cambridge Crystallographic Data Centre Software Ltd, Cambridge, UK) with default genetic algorithm settings. PHA665752 was re-docked into the kinase pocket with a radius setting of 6Å. TM608 was then docked with the same setting. GOLD uses a genetic algorithm (GA) for

docking flexible ligands into the binding pocket to explore the full range of ligand conformational flexibility.[S2] The GOLD Score was used as the fitness function for selection of the best docked conformations of test compounds in the binding pocket.

2. Synthesis of (Z)-(2-(5-((5-(2,6-Dichlorobenzylsulfonyl)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1H-pyrrole-3-carboxamido)ethyl)triphenyl-phosphonium bromide (TM608)

2.1. General Conditions for Organic Synthesis

Reagents were purchased from Sigma-Aldrich Chemical (Singapore) or Alfa Aesar (Ward Hill, MA) and used without further purification. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured on a Bruker Spectrospin 400 Ultrashield magnetic resonance spectrometer. Chemical shifts (δ) were reported in ppm and referenced to residual solvents: $CDCl_3$ (δ7.26), DMSO-d6 (δ2.50), CD_3OD (δ 3.31) (for ^{1}H spectra) or $CDCl_3$ (δ 77.00), DMSO-d6 (δ 39.43), CD_3OD (δ 49.05) (for ^{13}C spectra). Coupling constants (J) were reported in Hz. Reactions were monitored by TLC on Silica Gel 60 F254 (Merck). Column chromatography was carried out on Merck Silica Gel 60 (0.04–0.06 mm). Mass spectra were recorded in positive ion mode using electro-spray ionization (ESI) (Applied Biosystem, Q-Trap 2000 LC/MS) or high-resolution LC-MS (IT TOF: Waters-Micromass QTOF premier mass spectrometer). Accurate mass information was captured on a Bruker microTOFQII mass spectrometer (Bruker, Billerica, MA) run on ESI mode. Purity of TM608 was determined by reverse phase HPLC and found to be ≥ 95%.

2.2. 2-Oxoindoline-5-sulfonyl chloride (**2**)

The method described by Bouchikhi et al was followed.[S3] Indolin-2-one (**1**, 2g) was slowly added to chlorosulfonic acid (4 mL) at 0°C. After the addition, the mixture was stirred at room temperature (25°C) for about 1.5 h, then at 70°C for another 1 h. It was then cooled to room temperature, ice-water was added into the mixture, and stirred for

0.5 h. The mixture was filtered and the solid was washed with water. The solid was dried in the oven (70°C) overnight to give 2.24 g of orange solid. Yield: 64%. ^{1}H NMR (400MHz, DMSO-d6) δ 10.49 (s, 1 H), 7.46- 7.44 (m, 2 H), 6.77 (d, 1 H, J=8.0 Hz), 3.46 (s, 2 H). ^{13}C NMR (100MHz, DMSO-d6) δ 176.95, 144.29, 140.89, 125.33, 125.30, 122.13, 108.20, 35.85. $[M-H]^-$ $C_8H_5ClNO_3S$-, 229.9; found 229.8.

2.3. 5-(2,6-Dichlorobenzylsulfonyl)indolin-2-one (**3**)

The method of Chen et al was followed.[S4] Na_2HPO_4 (368 mg) and Na_2SO_3 (653 mg) were dissolved in 5 mL of water and heated to 30°C. This solution was then added to **2** (600 mg). The mixture was heated at 60^{o}C overnight (≈ 12 h). A solution of 2,6-dichlorobenzyl bromide (622 mg) in 3 mL of acetone was added dropwise to the mixture with vigorous stirring at 60°C followed by an acetone rinse (1.5 mL). It was stirred at 60°C for 2 h and then quenched with 12 mL of water. Stirring at room temperature was continued for 1 h after which the mixture was filtered and the residue washed with water (5 mL) and acetone (2 mL) consecutively. The off-white solid was dried in the oven overnight to give an off-white solid (553 mg). Yield: 60%. ^{1}H NMR (400MHz, DMSO-d6) δ 10.90 (s, 1 H), 7.57-7.49 (m, 4 H), 7.42-7.38 (m, 1 H), 6.97 (d, 1 H, J=8.8 Hz), 4.82 (s, 2 H), 3.58 (s, 2 H). ^{13}C NMR (100MHz, DMSO-d6) δ 176.47, 149.04, 136.41, 131.43, 131.13, 129.01, 128.72, 126.90, 125.75, 124.03, 109.15, 57.69, 35.45. $[M+Na]^+$ $C_{15}H_{11}Cl_2NNaO_3S$, 378.0; found 378.1.

2.4. Ethyl 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylate (**5**)

To the mixture of DCM (10 mL) and DMF (0.5 mL) was added phosphorus oxychloride ($POCl_3$, 690 mg) dropwise at 0°C, and the mixture was stirred at 0°C for 1 h. To the mixture was added a solution of ethyl 2,4-dimethyl-1H-pyrrole-3-carboxylate (**4**, 500 mg) in DCM (5 mL) at 0°C. It was stirred at 0°C for 30 min and RT for 3 h, poured into ice-water and pH adjusted to 10 with 50% NaOH aqueous solution. It was then stirred at RT for ≈ 2 h. The mixture was extracted with DCM (10 mL×3), the DCM layer was washed with water and brine consecutively, after which it was dried with anhydrous Na_2SO_4. The DCM layer was removed under reduced pressure to give a grey solid (250 mg), yield

43%. ^{1}H NMR (400MHz, CDCl3) δ 10.14 (br, 1 H), 9.59 (s, 1 H), 4.29 (q, 2 H, J =7.2 Hz), 2.56 (s, 3 H), 2.54 (s, 3 H), 1.36 (t, 3 H, J =7.2 Hz). ^{13}C NMR (100MHz, CDCl3) δ 177.34, 164.98, 143.47, 136.07, 128.25, 114.14, 59.70, 14.39, 14.31, 10.61. $[M+H]^+$ $C_{10}H_{14}NO_3$, 196.1; found 196.2.

2.5. 5-Formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (**6**)

To a solution of **5** (250 mg) in methanol (10 mL) was added an aqueous solution of KOH (215 mg). The mixture was refluxed for 4 h after which the organic solvent was removed under reduced pressure. Water (20 mL) was added to the residue and the mixture was extracted with DCM (10 mL×2). The pH of the aqueous layer was adjusted to 3, the precipitated solid was collected by filtration, washed with water and dried to give an off-white powder (85 mg), yield 40%. ^{1}H NMR (400MHz, MeOD) δ 9.58 (s, 1 H), 2.53 (s, 3 H), 2.49(s, 3 H). ^{13}C NMR (100MHz, MeOD) δ 179.20, 168.51, 145.20, 137.42, 129.88, 114.87, 13.92, 10.79. $[M-H]^-$ $C_8H_8NO_3$, 166.1, found 166.1.

2.6. (Z)-5-[{5-(2,6-Dichlorobenzylsulfonyl)-2-oxoindolin-3-ylidene}methyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (**7**).

To a mixture of **3** (82 mg) and **6** (39 mg) in ethanol (6 mL) was added piperidine (20 mg). The mixture was stirred at 80°C overnight, cooled to RT, filtered to remove the solid residue which was then washed with ethanol. On drying, **7** was obtained as a red solid in 87% yield (101 mg). ^{1}H NMR (400MHz, DMSO-d6) δ 13.59 (s, 1 H), 8.20 (d, 1 H, J=1.6 Hz), 7.81 (s, 1 H), 7.49-7.36 (m, 4 H), 7.04 (d, 1 H, J=8.0 Hz), 4.87 (s, 2 H), 2.56 (s, 3 H), 2.54 (s, 3 H). ^{13}C NMR (100MHz, DMSO-d6) δ 169.71, 167.64, 142.30, 141.32, 136.49, 135.06, 131.64, 131.06, 128.74, 126.54, 126.17, 126.13, 126.04, 125.90, 119.49, 118.28, 111.94, 109.38, 57.83, 14.50, 11.52. $[M-H]^-$ $C_{23}H_{17}Cl_2N_2O_5S$, 503.0, found 503.3.

2.7. (2-Aminoethyl)triphenylphosphonium bromide (**9**).

The method of Maryanoff et al was followed.[S5] A solution of (2-bromoethyl) amine hydrobromide (**8**, 4.00 g) and triphenylphosphine (6.10 g, 1.2 equiv) in CH_3CN (20 mL) was refluxed overnight. The precipitate was removed by filtration, dried and dissolved in water, and treated with saturated aqueous K_2CO_3 to a pH of 11. The product was extracted with DCM, dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a white solid (1.2 g, 16%). ^{1}H NMR (400MHz, DMSO-d6) δ 7.97-7.78 (m, 15 H), 3.98-3.90 (m, 2 H), 3.11-3.06 (m, 2 H). ^{13}C NMR (100MHz, DMSO-d6) δ 135.33, 133.62, 130.37, 117.60, 116.73, 33.30, 19.62. ^{31}P NMR (CDCl3) δ 21.69. $[M]^+$ $C_{20}H_{21}NP^+$, 306.1; found 306.3.

2.8. (Z)-(2-(5-((5-(2,6-Dichlorobenzylsulfonyl)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1H-pyrrole-3-carboxamido)ethyl)triphenylphosphonium bromide (**TM608**)

A mixture of **7** (51 mg), EDCI-HCl (24 mg) and DMAP (15 mg) in DMF (8 mL) was stirred at RT for 30 min. **9** (43 mg) was added to the stirred mixture and stirring was continued at RT overnight. After this time, solvent was removed under reduced pressure and the residue purified by silica gel column chromatography (DCM:methanol 20:1). **10** was obtained as a red solid (60 mg, 68% yield). ^{1}H NMR (400MHz, CDCl3) δ 13.22 (s, 1 H), 10.31 (s, 1 H), 8.24 (t, 1 H, J =5.6 Hz), 7.83-7.64 (m, 15 H), 7.38-7.35 (m, 1 H), 7.24-7.13 (m, 5 H), 4.82 (s, 2 H), 3.96-3.85 (m, 4 H), 2.40 (s, 3 H), 2.39 (s, 3 H). ^{13}C NMR (100MHz, CDCl3) δ 169.42, 166.20, 142.51, 139.27, 137.29, 135.27, 133.59, 133.48, 132.31, 131.24, 130.63, 130.50, 130.26, 128.53, 126.46, 126.18, 124.42, 119.08, 118.24, 117.38, 112.75, 110.03, 33.98, 23.09, 22.61, 14.43, 11.52. ^{31}P NMR ($CDCl_3$) δ 21.04. $[M]^+$ $C_{43}H_{37}Cl_2N_3O_4PS^+$, 792.2; found 792.6. High resolution MS (ESI) calcd for $C_{43}H_{37}Cl_2N_3O_4PS^+$[M+] : 792.1616. Found 792.1614.

2.9. Purity determination of TM608 by reverse phase HPLC.

The purity of TM608 was verified by reverse phase high pressure liquid chromatography (HPLC) on an Agilent 1100 HPLC system or Shimadzu SPD-20AHPLC system. The test compound was dissolved in methanol and injected through a 100 µl loop at a flow rate

of 1mL/min, with UV detection at 254 and 280 nm. Separation was carried out on a Luna 5u C18(2) 100A column (100 ×4.6 mm, 10 µm) from Phenomenex®. Each compound was tested on two mobile phases: Mobile Phase A: 25 mM ammonium formate in acetonitrile + 15% water and Mobile Phase B: 25 mM ammonium formate in methanol + 5% water. The separation on the column was followed for 15 min for the detection of the peak corresponding to the test compound. Purity of compound was assessed from the area of the major peak compared to total area of peaks obtained on the chromatogram.

Table S1: % Purity of TM608 as assessed from peak areas on two mobile phases at two wavelengths by reverse phase HPLC.

Compound	Mobile Phase A[a] (Area%)[c]		Mobile Phase B[b] (Area%)[c]	
	254 nm	280 nm	254 nm	280 nm
10 (TM608)	96.8	95.9	97.3	97.1

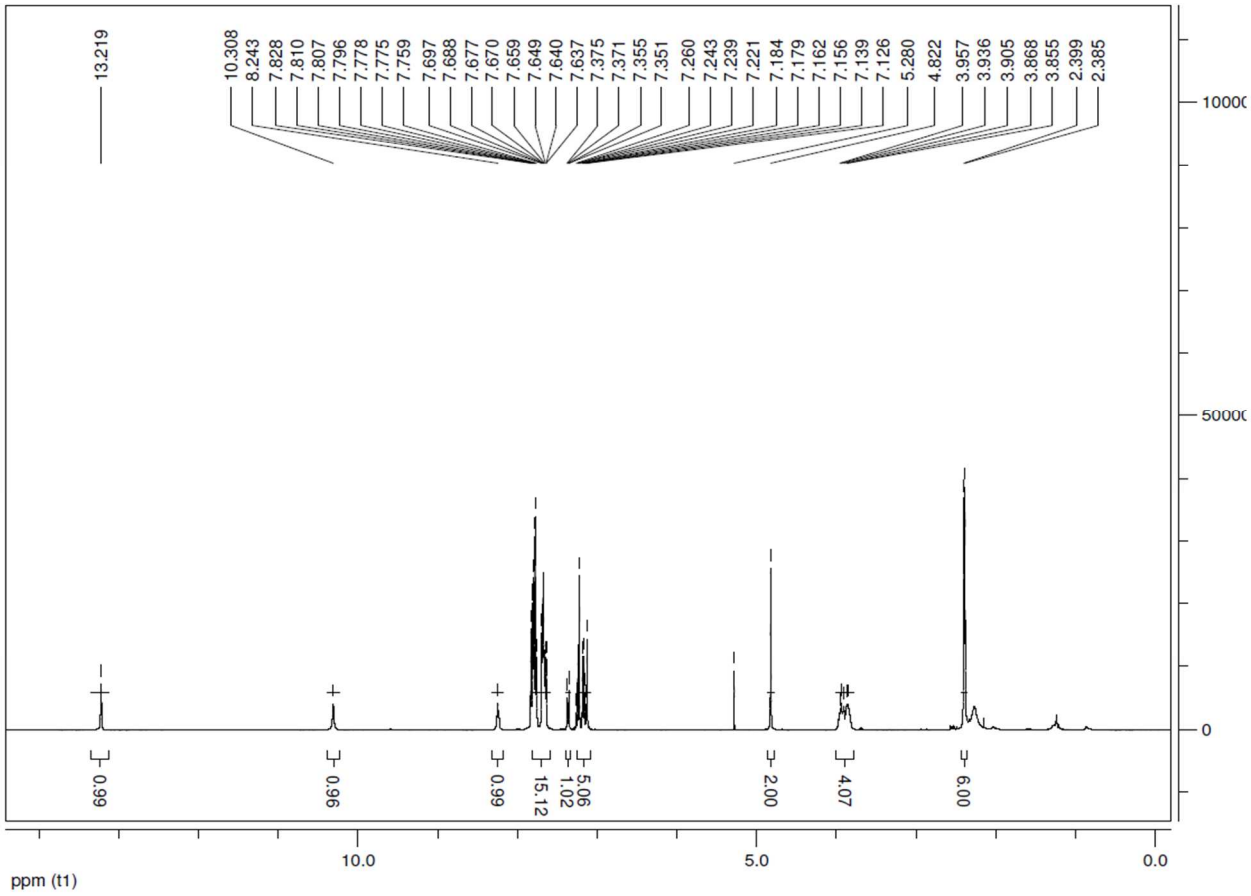
a. Mobile Phase A: 25 mM ammonium formate in acetonitrile + 15% water

b. Mobile Phase B: 25 mM ammonium formate in methanol + 5% water

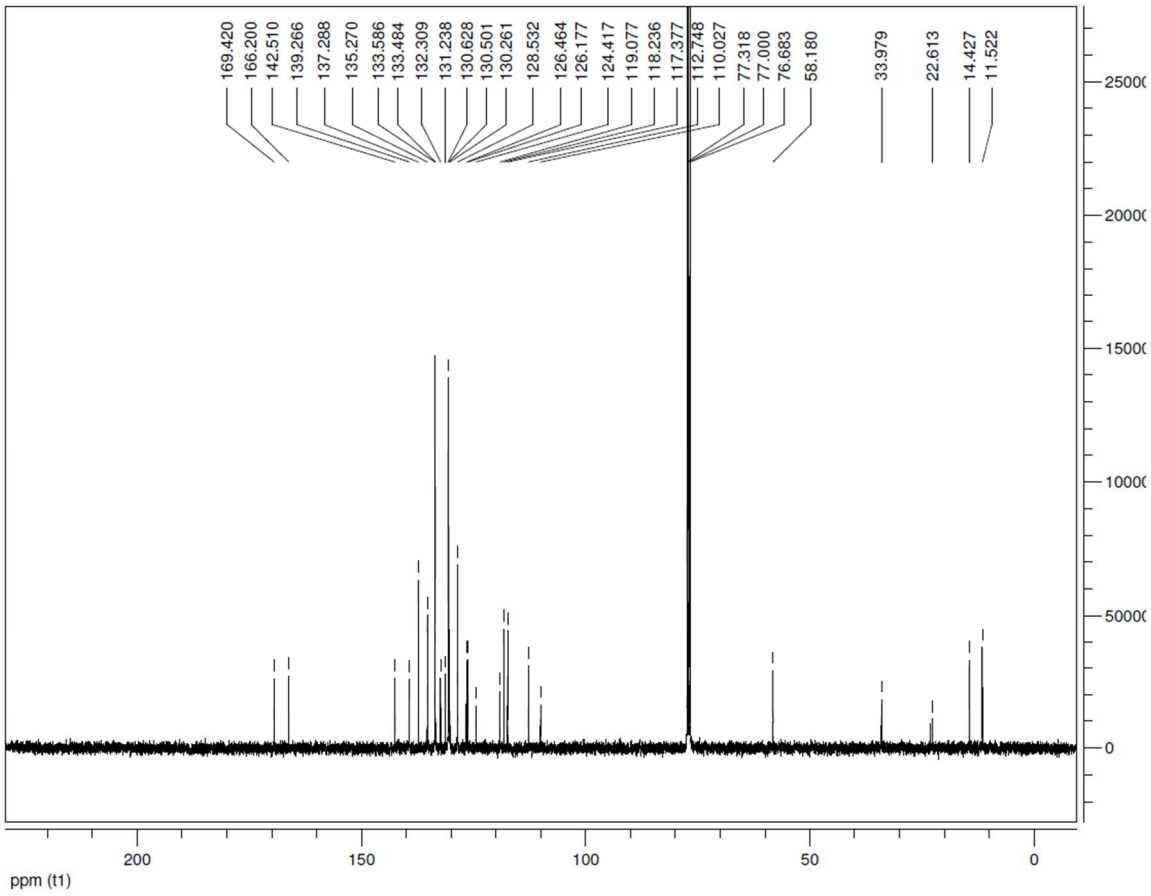
c. Area (%) of Major Peak = [Area of Major Peak / Total Area of All Peaks] ×100

2.10. Spectroscopic data of TM608

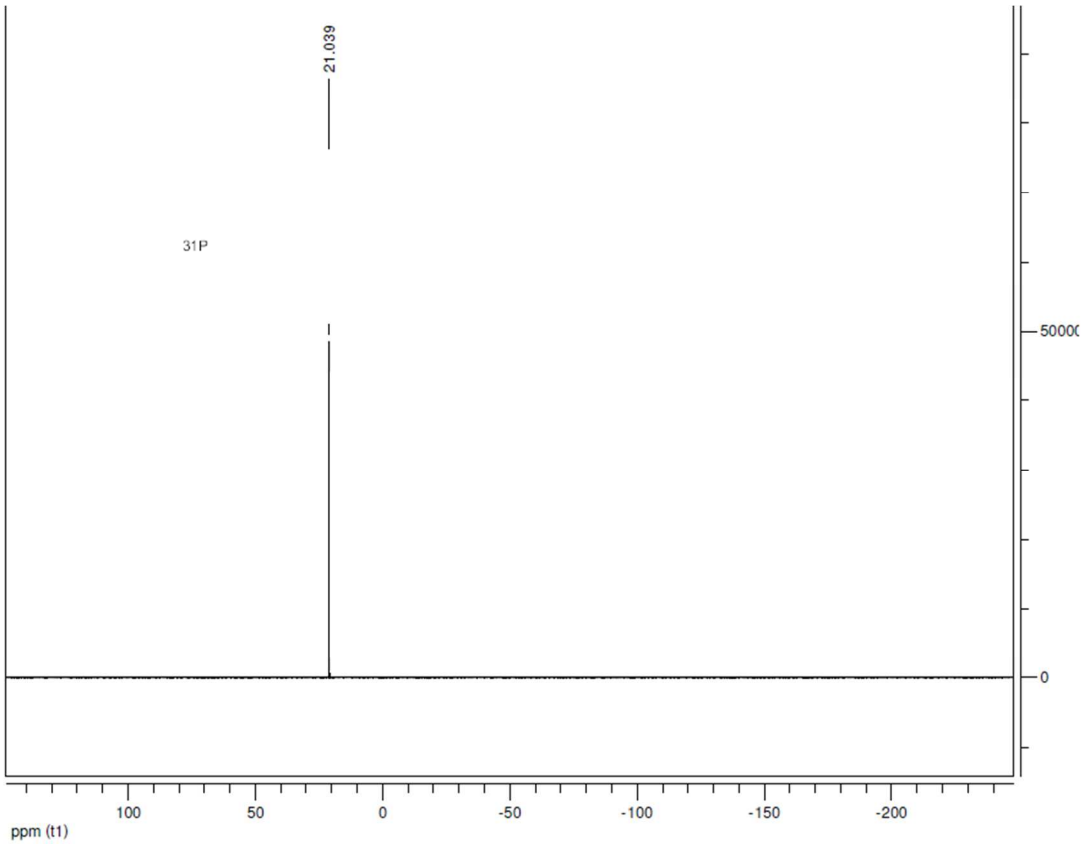
(A) ^{1}H NMR (400MHz, CDCl3)



(B) ^{13}C NMR (100MHz, CDCl3)



(C) ^{31}P NMR ($CDCl_3$)



3. Cell lines and cell culture conditions

HeLa cells were cultured in Dulbecco's Modified Eagle Medium High Glucose media supplemented with 1% penicillin and 10% fetal bovine serum (all from Hyclone Laboratories, Logan UT). The non-small cell lung cancer isogenic pair of HCC827 cells were a gift from Professor Tetsuya Mitsudomi and Professor Kenichi Suda (Kinki University, Japan) and comprise parental cells (HCC827A) which are sensitive to the

TKIs gefinitib and erlotinib and the resistant derivative (HCC827B) which is resistant to erlotinib and MET-amplified. Resistance to erlotinib was induced by chronic exposure to the drug. Once induced, the resistant phenotype was stable and persisted even when HCC827B cells were cultured in the absence of erlotinib. Cells were cultured in Hyclone™ Roswell Park Memorial Institute (RPMI) 1640 media with 25 mM HEPES and 2.05 mM L-glutamine (GE Life Sciences, Buckinghamshire, UK) supplemented with 10% fetal bovine serum (Thermo Scientific, Waltham, MA, USA), 100 U/mL penicillin (GE Life Sciences, Buckinghamshire, UK) and 100 mg/mL streptomycin (GE Life Sciences, Buckinghamshire, UK).Ttransforming growth factor-alpha mouse hepatocytes (TAMH, a gift from Prof Nelson Fausto, Department of Pathology, University of Washington) were cultured in DMEM-F12 (Dulbecco's Modified Eagle's Medium/ Nutrient Mixture F12) with ITS premix (5 mg insulin, 5 mg human transferrin, and 5 mg selenous acid), 100 nM dexamethasone, 10 mM nicotiamide, and 10 mg/L gentamicin. HL-1 (American Type Culture Collection, VA, USA) were cultured in Clay Comb Medium with 0.1 mM Norpinephrine, 2 mM L- Glutamine, 100 U/ml penicillin, 100 µg/ml Streptomycin, and 10% v/v Fetal bovine serum.

4. Cell viability assay

Cells were seeded at 6 × 10^3 cells per well in 96 well plates containing 100 µL media per well and incubated for 24 h (37°C, 5% CO_2). Media was then removed from each well by aspiration and replaced with fresh media containing test compound at various concentrations. Incubation was continued for 72 h (37°C, 5% CO_2), after which media was removed and an aliquot (100 µL) of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Alfa Aesar Inc, Lancashire, UK) solution (0.5 mg/mL MTT in PBS) was added to each well and incubated for 3 hours. The MTT-containing solution was replaced with 100 µL DMSO to dissolve the purple formazan crystals. Plates were agitated at 600 rpm for 3 minutes on a plate shaker before absorbance readings were collected at 570 nm on a microplate reader (Tecan Infinite™ M200 Pro, Tecan, Maennedorf, Switzerland). Cell viability was determined by the following expression:

$$\text{Percentage viability} = [(Ab_{compound} - Ab_{blank})/ (Ab_{control} - Ab_{blank})] \times 100\%,$$

Where $Ab_{compound}$ = Absorbance of compound-treated cells corrected for DMSO (Ab_{blank}) and,
$Ab_{control}$ = Absorbance of untreated/control cells, corrected for DMSO (Ab_{blank})

The IC_{50} (concentration required to inhibit cell growth by 50%) of PHA665752 (Sigma Aldrich, Cat. No: PZ0147) and TM608 were determined by plotting percentage viability versus logarithmic concentration of test compound using GraphPad Prism™ version 6.0 (GraphPad Software, Inc., San Diego, CA, USA).

5. Determination of cytotoxicities of PHA665752 and TM608

Cytotoxicities of the compounds were determined by the CellTiter-Glo® Cell Viability Assay (Promega Corporation, Wisconsin, USA). An aliquot (200 µL, 6 ×10^4 cells/mL of TAMH, 7.5 ×10^4 cells/mL of HL-1) of medium was added to each well of a 96 well microtitre plate. After incubation for 24 h at 37 deg C, 5% CO_2, media was removed from the well and replaced with fresh media (200 µL) containing a known concentration of test compound. The final concentration of DMSO in each well was maintained at 0.5 v/v. Incubation was continued for another 24 h at 37^o C, 5% CO_2, after which cell viability was determined with the Cell Titer-Glo® Cell Viability Assay Kit (Promega, Singapore) following manufacturer's instructions. The cell-reagent mixture was then transferred to a solid white flat-bottom 96-well plate (Greiner, Wemmel, Belgium) for the measurement of luminescence on a microplate reader (Tecan, Infinite 200). The viability of cells at a given concentration of test compound was determined from the equation (3):

$$\text{Percentage Cell Viability} = \frac{< lum\_compound > - < lum\_blank >}{< lum\_vehcontrol > - < lum\_blank >} \times 100\%$$

where lum_compound = luminescence of wells containing cells and test compound in media, lum_vehicle = luminescence of wells containing cells in media only and lum_blank = luminescence of wells containing media only.

Each concentration of test compound was evaluated at least 3 times on separate occasions, and two different stock solutions were used. The highest concentration of test compound used in the assay was 100 μM. The IC_{50} value (concentration that inhibited 50% of cell growth) was determined from the sigmoidal curve obtained by plotting percentage viability versus logarithmic concentration of test compound using GraphPad Prism 5 (San Diego, USA).

6. Protein immunoblotting

Whole cell pellets were prepared by centrifuging a suspension of about 10^6 cells at 500g for 5 min. The supernatant was discarded and the cell pellet was frozen immediately in liquid nitrogen. Pure mitochondria were isolated using Miltenyi Biotec's Human Mitochondria Kit (See Mitochondria Isolation in main manuscript). Isolated mitochondria were pelleted by centrifugation at 12,000g for 2 min. The supernatant was removed and the mitochondrial pellet was frozen in liquid nitrogen immediately.

Pellets of whole cells and isolated mitochondria treated with the test compound (TM608 or PHA665752) were lysed in M-PER (Mammalian Protein Extraction Reagent, Thermo Scientific Inc.) supplemented with Pierce™ Protease and Phosphatase Inhibitor (Thermo Scientific Inc.) for 1 h on ice. The lysates were centrifuged at 14,000g for 20 min at 4°C to obtain clear supernatants. Protein concentrations were determined using the Bradford protein assay reagent (Bio-Rad). Ten μg protein of each lysate were denatured (65°C for 5 min) and resolved in 10% polyacrylamide gels in Tris/glycine/SDS running buffer (24.76 mM Tris, 191.83 mM glycine and 0.1% SDS) and then transferred to 0.45 μm nitrocellulose membrane (Bio-Rad) in Tris/glycine/methanol transfer buffer (24.76 mM Tris, 191.83 mM glycine and 20% methanol). The membranes were blocked with 5% non-fat milk in 0.1% Tween 20 in phosphate-buffered saline (PBST) for 1 h at room temperature before blotting with primary antibodies for 1.5 h. Dilutions of the primary antibodies were: 1:1000 α-Met (Abcam; ab51067); 1:1000 β-Met (Abcam; ab14571); 1:1000 phospho-Met Y1234/1235 (Cell Signaling, 3077); 1:1000 TOM20 (Abcam; ab56783); 1:20000 SDHA (Abcam; ab14715); and 1:50000 actin (Sigma-Aldrich; A1978). After 4 washes (5 min per wash) in PBST, the blots were incubated

with anti-rabbit or anti-mouse horseradish perioxidase (HRP)-linked secondary antibody (GE Healthcare Life Sciences; NA934 or NA931) for 30 min at room temperature. After another 4 washes in PBST, Pierce™ SuperSignal West Dura Extended Duration Substrate (Thermo Scientific Inc.) was added to the blots and incubated for 5 min at room temperature. Excess liquid was dripped off and the blots were wrapped in polyethylene for exposure to UltraCruz® Autoradiography Film (Santa Cruz Biotechnology, CA). Images were scanned using GS-800™ calibrated densitometer (Bio-Rad).

7. Live cell imaging

HeLa cells were seeded at a density of 1 x 10^4 cells per well in Nunc™ Lab-Tek ™ Chambered Coverglass (Cat No. 155383, Thermo Scientific Inc Waltham MA) containing DMEM High Glucose media (SI). Cells were incubated for 24 h at 37°C, following which media was removed and replaced with fresh media containing TM608 or PHA665752 at 4 μM and incubated for 3 h. After this time, media was removed and fresh media (0.5 mL) containing MitoTracker® Red CMXRos (MTR, Life Technologies, Carlsbad, CA) at 200 nM was added to each well for 30 min. The process was repeated with Hoechst 33342 (Cell Signaling Technologies, Danvers, MA) at 10 ug/mL for 20 min. After this time, the cell layer was rinsed with media, incubated for another 10 min and then treated with 0.4% paraformaldehyde (0.3 mL, 10 min) to fix the cells onto the slide. Fluorescence images were taken on a Fluoview FV1000 Confocal Microscope (Olympus Melville NY) with a 64 x oil-immersion lens. The images corresponding to PHA/TM608, Hoechst 33382 and MTR were obtained using excitation (emission) wavelengths of 460 nm (520 nm), 405 nm (510 nm) and 579 nm (599 nm), respectively.

8. Mitochondria isolation

Mitochondria of HCC827A and HCC827B cells were isolated using Human Mitochondria Isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) following the manufacturer's instructions. Briefly, 10^7 cells were harvested and washed with phosphate-buffered saline. Cells were lysed in 1 mL of the provided lysis buffer

supplemented with Pierce™ Protease and Phosphatase Inhibitor (Thermo Scientific Inc.). The cell lysate was incubated with 50 µL of monoclonal anti-TOM22 MicroBeads for 1 h with gentle shaking at 4°C. The suspension was loaded on a pre-equilibrated MACS column placed in the magnetic field of a MACS Separator. The column was washed three times with 3 mL of separation buffer before removing the column from the magnetic field and eluting the mitochondria with 2 ml of separation buffer. The eluted mitochondria were pelleted by centrifugation (3000g, 10 min) and resuspended in the storage buffer provided.

9. Flow cytometry

Mitochondria freshly isolated from 10^7 HCC827A and HCC827B cells were stained with either 100 nM MitoTracker™ Red CMXRos (MTR), 4 µM TM608 or 4 µM PHA665752 in Miltenyi Biotec storage buffer for 1h. Double staining of samples was performed by combining MTR + TM608 or MTR + PHA665752 at the same concentrations and conditions mentioned above. Control sample was incubated with Miltenyi Biotec storage buffer containing 0.05% (v/v) DMSO. After incubation, the stained samples were centrifuged at 500g for 5 min, washed once with phosphate-buffered saline and analyzed on a FACSCalibur flow cytometer (BD Biosciences) equipped with air-cooled argon and red diode lasers to detect MTR (FL4 channel), TM608 (FL1 channel) and PHA665752 (FL1 channel). Data were analyzed using FlowJo7.6.5 software (Treestar). Experiments were repeated twice.

10. Determination of activated MET protein in HCC827A and HCC827B cells

Activated MET (MET phosphorylated at Y1234/1235) was assessed by probing protein immunoblots of 10^5 HCC827A and 10^5 HCC827B whole cells with an antibody specific for MET phosphorylated at Y1234/1235. The subcellular fractions (heavy membranes, flow-through fraction and pure mitochondria) were derived at each sequential fractionation step leading to pure mitochondria (Human Mitochondria Isolation kit, Miltenyi Biotec) Heavy membranes were obtained by centrifuging whole cell lysates (500g, 5 min) and were enriched in plasma membranes. Flow-through was the fraction which did not bind to anti-TOM22 beads. Pure mitochondria were obtained

by capture on anti-TOM22 beads. Protein bands were quantified by imaging with GS-800™ Calibrated Imaging Densitometer, Bio-Rad.

11. Localization of MET in mitochondria of HCC827B cells

Purified and intact mitochondria from 10^7 HCC827B cells were digested with trypsin (20 μg/mL) for 1, 2 and 3 minutes. Untrypsinized whole cell lysates and pure mitochondria were concurrently obtained using procedures described above and in SI and served as controls. Protein (10μg) from each control and trypsinization treatment were analyzed by immunoblotting with antibodies against α- and β-subunits of MET, TOM20, SDHA and actin. Trypsin cleavage of unprocessed MET was observed to expose β-subunit epitopes.

12. References

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