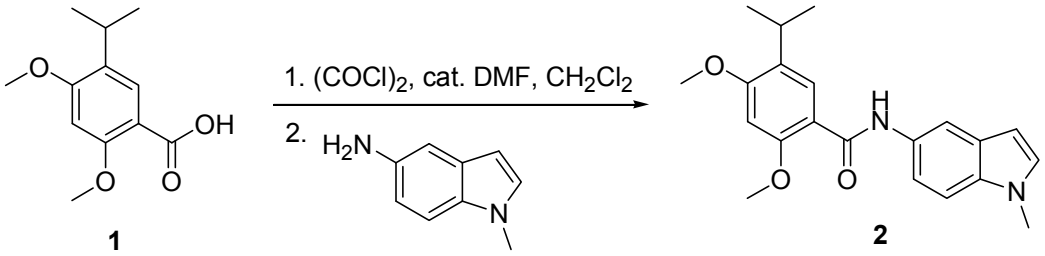
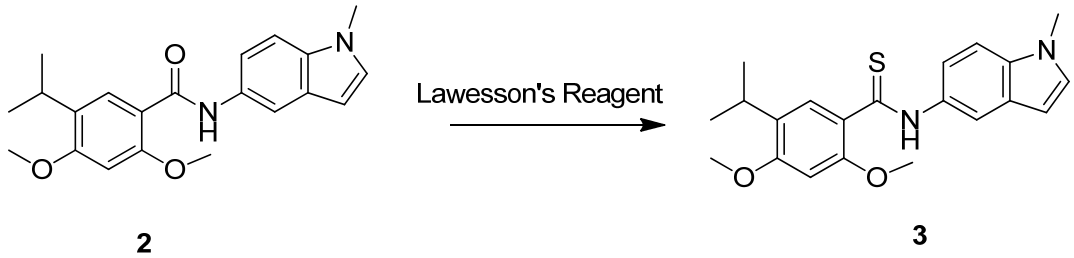
Supplementary Materials and Methods

Ganetespib synthesis

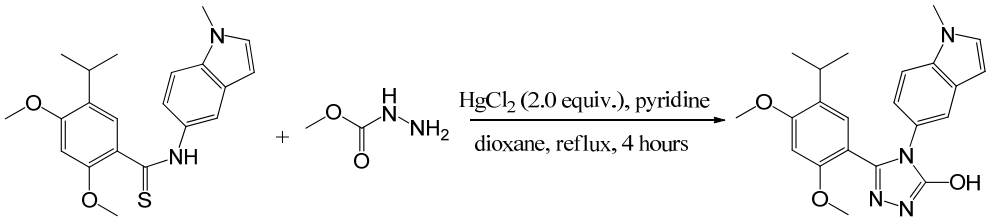


5-isopropyl-2,4-dimethoxybenzoic acid (*1*) (2.24 g, 10.0 mmol, 1.00 equiv.) in 50 ml dichloromethane at room temperature was treated with oxalyl chloride (1.40 g, 11.0 mmol, 1.10 equiv.) and catalytic amount of DMF (0.1 ml) for 1 h. Solvent and excess $(COCl)_2$ were removed on rotary evaporator. The residue was dissolved in 100 ml dichloromethane, and treated with 1-methyl-1H-indol-5-amine (10.0 mmol, 1.00 equiv.) and triethylamine (15.0 mmol, 1.50 equiv.) at 0^oC for 1 h. Normal aqueous workup and removal of solvent gave a light brown solid which was washed with ether to yield off-white solid (6.22 mmol, 62%) of 5-isopropyl-2,4-dimethoxy-N-(1-methyl-1H-indol-5-yl)benzamide (*2*).



To a solution of amide (*2*) (20 g, 56.7 mmol, 1.0 equiv.) in toluene (300ml) was added Lawesson's reagent (13.8 g, 34 mmol, 0.6 equiv.). The reaction was heated to reflux for 3 h (completion confirmed by LCMS) then allowed to cool to room temperature. Hydrazine hydrate (5.5 ml, 113 mmol, 2.0 equiv.) was added and the dark brown solution was stirred at room temperature for 10 min. Water (200 ml) and ethyl acetate (100 ml) were added then the organic layer was washed with water (2x200 ml). The organic layer was then treated with activated

carbon (10 g) and stirred at room temperature for 1 h. Filtration and removal of solvent under reduced pressure produced the desired product 5-Isopropyl-2,4-dimethoxy-N-(1-methyl-1H-indol-5-yl)-thiobenzamide (3) as a bright yellow solid (17.5 g, 84%).



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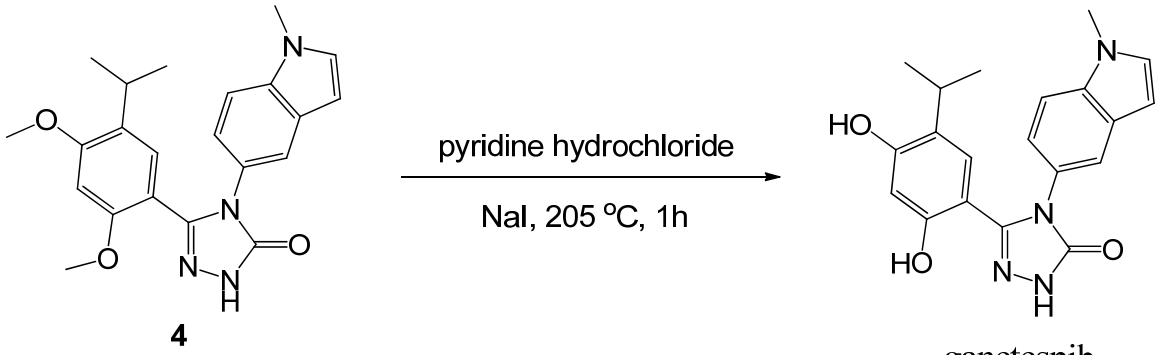
Thioamide (*3*) (3.68 g, 10.00 mmol, 1.0 equiv.), methyl hydrazino carboxylate (1.80 g, 20.0 mmol, 2.0 equiv.), pyridine (2.37 ml, around 30.0 mmol, 3.0 equiv.) and 40 ml dioxane were mixed in a 100 ml round bottom flask. Mercury (II) chloride (5.43 g, 20.0 mmol, 2.0 equiv.) was added to the flask, and stirred at room temperature for 0.5 h. The mixture was refluxed for 4 h. Enough Na_2S was added to the mixture after it was cooled to room temperature and stirred for 30 min to quench excess mercury chloride. Solid was removed by filtration through celite, and the solution was subjected to EtOAc/aqueous workup. Flash chromatography purification gave an off-white solid 3-(5-isopropyl-2,4-dimethoxyphenyl)-4-(1-methyl-1H-indol-5-yl)-1H-1,2,4-triazol-5(4H)-one (*4*) (3.10 g, 79%).



4 ganetespib

3-(5-isopropyl-2,4-dimethoxyphenyl)-4-(1-methyl-1H-indol-5-yl)-1H-1,2,4-triazol-5(4H)-one (*4*) (2.13 g, 5.42 mmol, 1.0 equiv.) was treated with pyridine hydrochloride (12.53 g, 108.3 mmol, 20.0 equiv.), NaI (0.81 g, 5.42 mmol, 1.0 equiv.) and 0.5 ml of water at 205°C under nitrogen

protection for 1 h. The reaction mixture was treated with 200 ml of water. The solid was collected by filtration, washed with 3 x 20 ml of water, and dissolved in 50 ml of 2M NaOH solution. The aqueous solution was extracted with 100 ml of EtOAc, and the EtOAc layer was extracted with 2 x 20 ml of 0.5M NaOH. EtOAc layer was discarded. The aqueous layer were combined, neutralized with HCl to pH around 5, and extracted with 3 x 100 ml of EtOAc. The combined EtOAc layer was diluted with 50 ml of THF, dried over $MgSO_4$, and filtered through silica gel plug. Most of solvents were removed to form slurry with around 2 ml of solvent left. Solid was collected by filtration, washed with 2 ml of EtOAc, and dried. The desired product 3-(2,4-dihydroxy-5-isopropylphenyl)-4-(1-methyl-1H-indol-5-yl)-1H-1,2,4-triazol-5(4H)-one (ganetespib) was obtained as an off-white solid (1.69 g, 4.63 mmol, 85%).

Co-crystal structure

An untagged N-terminal domain construct comprising residues 1–232 of human Hsp90 (26mg/ml) with 0.2M MgOAc and 20% PEG 3350 was co-crystallized with a saturated solution of compound ganetespib. Final freezing conditions were obtained by adding 20% ethylene glycol to the reservoir. The structures were refined using REFMAC 5.2. No. of protein chains 2; amino acids 417; 720 water molecules were located. Amino acids 16-224 for each monomer are visible in the structure. Number reflections = 48595 (46137 working set, 2458 test set); % completeness = 94; No. of protein chains:2; R_{work} = 0.172; R_{free} (5%) = 0.213; resolution (Å) = 1.80–47.22; space group = $P2_1$; unit cell: a = 56.36 Å; b= 88.88 Å; c = 56.50 Å; a = 90º; b = 99º; c = 90º.

Cell cycle analysis

Ganetespib-treated NCI-H1975 cells were incubated with 250 nM compound for 24, 48 and 72 h prior to harvest. Cells were fixed, washed and stained with propidium iodide before being analyzed by flow cyotmetry. The percentage of cells in each phase of the cell cycle (sub-G_0/G_1, G_1, S and G_2/M) was determined from the FL2-A histogram.

Langendorff assay

Hearts from male New Zealand white rabbits were surgically removed, placed on the perfusion apparatus, and control measurements of all physiological variables (PQ, QRS, RR, QT, and dLVP/dt) collected for 15 min during the perfusion with Krebs solution (equilibration period). The hearts were then perfused with the vehicle for 15 min followed by perfusion with escalating doses of ganetespib (10^{-8} – 10^{-4} M), with measurements taken the last 60 sec of each 15 min epoch. Data was collected for the entire period of exposure. The heart from one animal was perfused with vehicle solution (negative control) and another heart was perfused with escalating concentrations of the positive control (quinidine). For each 15 min epoch the last 60 sec period of collection was analyzed and presented. Data was acquired on the EMKA IOX Data Acquisition System. Measurements for all physiological parameters (PQ, QRS, RR, QT, LVP_{dev}) were made manually using EMKA ECG Auto software [QTc(Fridericia[2]) was calculated] from beats manifesting sinus rhythm. All physiological parameters were analyzed for each collection interval. Mean values for each parameter were calculated for each concentration, and mean values (±SEM) were plotted against concentration for all parameters assessed, both for ganetespib-exposed and vehicle-treated hearts.

Supplementary Figure Legends

Figure 1. Ganetespib induces cell cycle arrest in NSCLC tumor cells *in vitro*. NCI-H1975 cells were treated with 250nM ganetespib for 24, 48 and 72 h. Cells were harvested, stained with propidium iodide staining and analyzed by flow cytometry to determine DNA content.

Figure 2. Chemical structures of erlotinib and 17-DMAG