COMBINATORIAL DRUG DESIGN TARGETING A COMPARTMENTALIZED Hsp90 CANCER NETWORK

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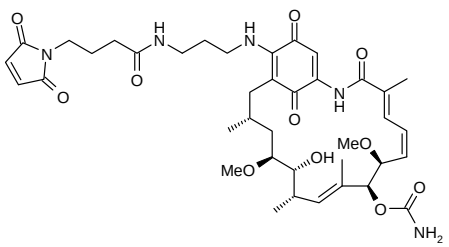
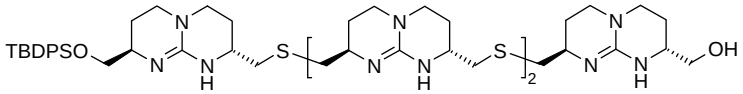
SUPPLEMENTAL INFORMATION

SUPPLEMENTAL METHODS

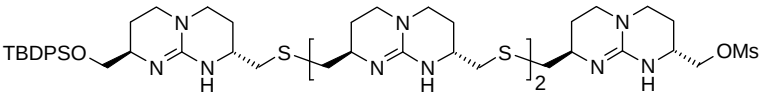
Chemical synthesis and characterization of Gamitrinibs. 1H-NMR spectra were obtained on either Varian Inova 400NB (400 MHz) or Varian Inova 600 (600 MHz) spectrometers. Mass spectra were recorded on a HP1100 series LC/MS spectrometer. The progress of reaction was checked on TLC plates (Macherey-Nagel 0.25 mm silica gel 60 with fluorescent indicator UV_{254}), and the spots were visualized under UV light (254 nm) and/or charring after dipping the TLC plate into nihydrin or Ce-Mo staining solution. Column chromatographies were performed on silica gel (Merck 9385 silica gel 60). The final products were analyzed by HPLC (Waters alliance) equipped with YMC-Pack Pro C18RS column (YMC) and detected at 254 nm. Chemical identity of synthesized compounds was confirmed by high resolution mass spectrometry (HRMS) using Waters Q-TOF Premier mass spectrometer with the $[M+2H]^{2+}$ ion or singly charged product ions from [Glu1]-fibrinopeptide B (CAS 103213-49-6) as the lock mass reference. Theoretical molecular masses were calculated using MassLynx^{TM} software (Waters Corp.) and compared with the measured mass. All measured masses were within measurement error (5 mmu) of the theoretical values and are consistent with the expected elemental compositions. All reagents and solvents (acetonitrile, methanol, diethyl ether and hexanes) were purchased as reagent grade, and used without further purifications. Tetrahydrofuran and dichloromethane were distilled from Na-benzophenone and CaH_2, respectively.



The geldanamycin-maleimide **2** was synthesized as previously reported (1).

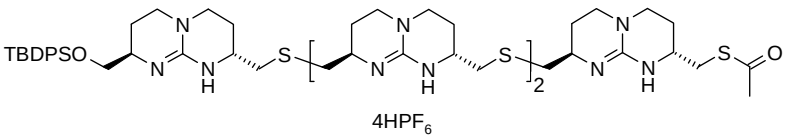


The alcohol **3** was synthesized as previously reported (2).



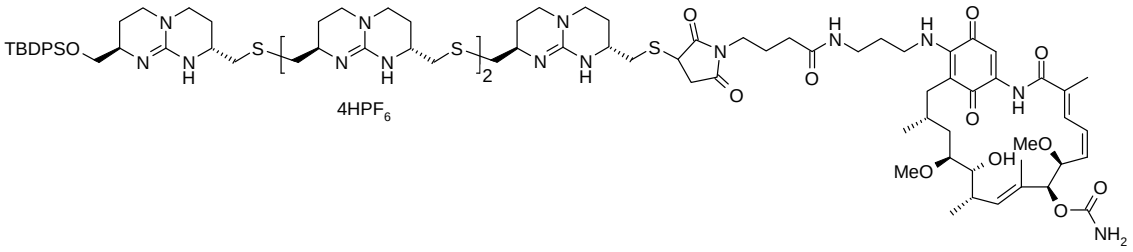
Mesylate 4 : A solution of alcohol **3** (445 mg, 0.276 mmol) in acetonitrile (5 mL) was treated with *N*-methylmorpholine (0.30 mL, 2.76 mmol) and methanesulfonic anhydride (240 mg, 1.38 mmol) at room temperature under N_2. After stirred for 5 h at room temperature, most of the volatiles were removed in vacuo. The residue was diluted with dichloromethane (30 mL) and washed with 0.1 M aq. NH_4PF_6 (20 mL). The aqueous phase was re-extracted with additional dichloromethane (30 mL). The combined organic phase was dried over Na_2SO_4, filtered and concentrated. Purification by column chromatography (2-5% MeOH in CH_2Cl_2) afforded **4** as tetrahexafluorophosphate salt (452 mg, 97 %, pale brown foam).

^{1}H-NMR (600 MHz, acetone-d_6) δ 7.72-7.67 (m, 4H), 7.52-7.48 (m, 2H), 7.48-7.43 (m, 4H), 7.35-7.00 (br, salt protons), 4.46 (dd, 1H, *J* = 4.2 Hz, 10.8 Hz), 4.28 (dd, 1H, *J* = 7.2 Hz, 10.2 Hz), 4.00-3.95 (m, 1H), 3.85-3.68 (m, 9H), 3.60-3.47 (m, 16 H), 3.18 (s, 3H), 3.04-2.95 (m, 6H), 2.76-2.68 (m, 6H), 2.28-2.14 (m, 8H), 2.05-1.89 (m, 8H), 1.06 (s, 9H).



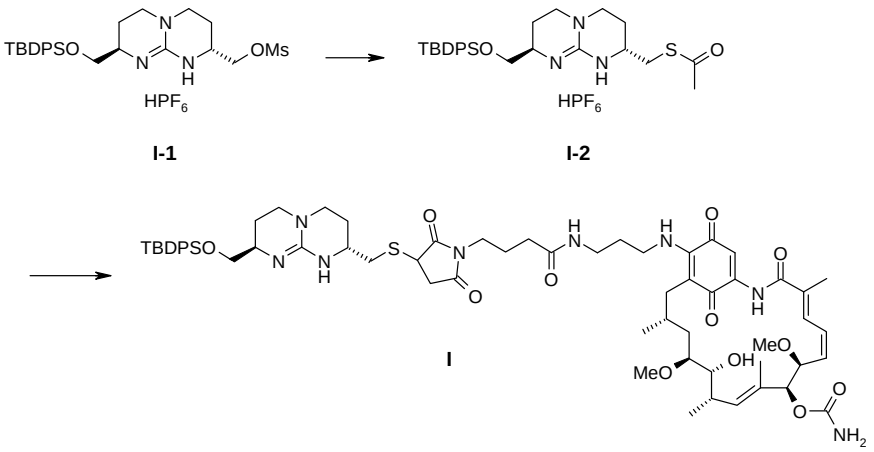
Thioacetate 5: A solution of the mesylate **4** (452 mg, 0.267 mmol) and potassium thioacetate (153 mg, 1.34 mmol) in THF (8 mL)/ H_2O (3 mL) was refluxed for 16 h. After cooling to room temperature, the reaction mixture was diluted with dichloromethane (30 mL) and washed with 0.1 M aq. NH_4PF_6 (20 mL). The aqueous phase was re-extracted with additional dichloromethane (30 mL). The combined organic phase was washed with 0.1 M aq. NH_4PF_6 (20 mL), dried over Na_2SO_4, filtered and concentrated. Trituration from diethyl ether-hexanes (1:1) afforded **5** as tetrahexafluorophosphate salt (420 mg, 94 %, pale brown solid).

^{1}H-NMR (400 MHz, acetone-d_6) δ 7.73-7.66 (m, 4H), 7.53-7.42 (m, 6H), 7.22-6.92 (br, salt protons), 3.85-3.65 (m, 10H), 3.62-3.46 (m, 16H), 3.16 (d, 2H, *J* = 6.4 Hz), 3.04-2.86 (m, 6H), 2.77-2.67 (m, 6 H), 2.37 (s, 3H), 2.29-2.14 (m, 8H), 2.04-1.88 (m, 8H), 1.06 (s, 9H); MS (EI) m/z 1087 (M+1), 1233 (M+HPF_6+1).



Gamitrinib-G4 1: A solution of **5** (118 mg, 0.071 mmol) in degassed MeOH (4 mL) under N_2 at room temperature was treated with potassium *tert*-butoxide (0.21 mL, 0.21 mmol, 1 M in THF). After 30 min, the reaction mixture was neutralized with 1 N aq. HCl (ca. 0.1 mL) and treated with 0.1 N phosphate buffer (pH 6, 3 mL). To the buffered solution under N_2 at room temperature was added a solution of geldanamycin-maleimide **2** (65 mg, 0.085 mmol) in degassed MeOH (2 mL). After 2 h, the reaction was concentrated to ca. 3 mL. The resulting

reaction mixture was diluted with dichloromethane (20 mL) and washed with 0.1 M aq. NH_4PF_6 (30 mL). The aqueous phase was re-extracted with dichloromethane (20 mL). The combined organic phase was dried over Na_2SO_4, filtered and concentrated. Separation by prep-HPLC (5-50% acetonitrile in water, 0.1 % TFA) followed by concentration afforded **1** as TFA salt. The TFA salt was dissolved in dichloromethane (3 mL) and washed successively with 0.1 M aq. NH_4PF_6 (2 mL x 5). Concentration followed by trituration from diethyl ether-hexanes (1:1) afforded **1** as tetrahexafluorophosphate salt (88 mg, 52 %, purple solid). The purity of **1** was more than 99% by HPLC at 254 nm. The measured molecular mass of **1** ($[M+3H]^{3+}$, m/z 604.9698) measured by HRMS was consistent with the theoretical mass (m/z 604.9736). ^{1}H-NMR (400 MHz, CD_3CN) δ 9.25 (s, 1H), 7.70-7.60 (m, 4H), 7.53-7.40 (m, 6H), 7.30 (br s, 1H), 7.11 (d, 1H, *J* = 8.4 Hz), 7.06 (s, 2H), 6.80-6.40 (br, salt protons), 6.74 (dt, 1H, *J* = 18.8 Hz, *J* = 6 Hz), 6.63 (t, 1H, *J* = 11.2 Hz), 5.83 (t, 1H, *J* = 10 Hz), 5.68 (d, 1H, *J* = 9.6 Hz), 5.24 (br s, 2H), 5.08 (s, 1H), 4.43 (d, 1H, *J* = 9.2 Hz), 3.98-3.86 (m, 1H), 3.86-3.78 (m, 1H), 3.75-3.68 (m, 1H), 3.67-3.60 (m, 1H), 3.60-3.42 (m, 14H), 3.42-3.23 (m, 21H), 3.22-3.10 (m, 3H), 3.20 (s, 3H), 3.03 (dd, 1H, *J* = 14 Hz, 5 Hz), 2.92-2.52 (m, 7H), 2.52-2.40 (m, 9H), 2.40-2.30 (m, 1H), 2.25-2.00 (m, 10H), 1.97 (s, 3H), 1.86-1.70 (m, 14H), 1.71 (s, 3H), 1.05 (s, 9H), 0.95 (d, 3H, *J* = 6.4 Hz), 0.92 (d, 3H, *J* = 6.8 Hz) ; MS (EI) m/z 1812.5 (M+1).

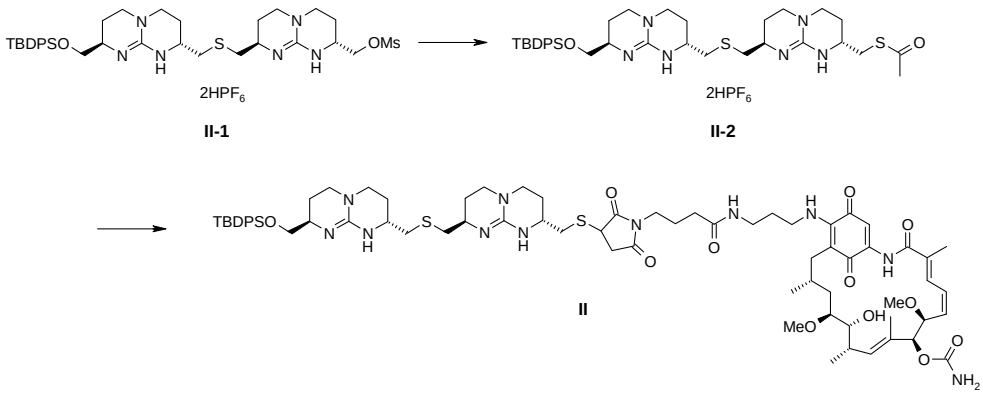


Thioacetate I-2 : The mesylate **I-1** was synthesized as previously reported (2). A stirred solution of **I-1** (2.30 g, 3.48 mmol) and potassium thioacetate (794 mg, 6.95 mmol) in THF (40 mL)/ H_2O (16 mL) was refluxed for 16 h. After cooling to room temperature, the reaction mixture was diluted with dichloromethane (100 mL) and washed with 0.1 M aq. NH_4PF_6 (50 mL). The aqueous phase was re-extracted with additional dichloromethane (50 mL). The combined organic phase was washed with 0.1 M aq. NH_4PF_6 (30 mL), dried over Na_2SO_4, filtered and concentrated. Purification by column chromatography (hexane/ethyl acetate = 1/4 $\rightarrow$ ethyl acetate only) and concentration afforded **I-2** as hexafluorophosphate salt (2.12 g, 95 %, pale yellow solid). ^{1}H-NMR (400 MHz, acetone-d_6) δ 7.72-7.65 (m, 4H), 7.52-7.41 (m, 6H), 6.98 (br, 2H), 3.85-3.73 (m, 3H), 3.73-3.64 (m, 1H), 3.61-3.46 (m, 4H), 3.15 (d, 2H, J = 6.4 Hz), 2.36 (s, 3H), 2.23-2.10 (m, 2H), 2.08-1.91 (m, 2H) 1.06 (s, 9H).

Gamitrinib-G1 I: To a solution of **I-2** (100 mg, 0.156 mmol) in degassed MeOH (3 mL) under N_2 at room temperature was added potassium *tert*-butoxide (0.47 mL, 0.47 mmol, 1 M in THF). After 30 min, the reaction mixture was neutralized with 1 N aq. HCl (ca. 0.5 mL) and treated with 0.1 N phosphate buffer (pH = 6, 2 mL). To the buffered solution under N_2 at room

temperature was added a solution of geldanamycin-maleimide **2** (144 mg, 0.188 mmol) in degassed MeOH (1 mL). After 2 h, the reaction was concentrated to ca. 2 mL. The resulting reaction mixture was diluted with dichloromethane (30 mL) and washed with 0.1 M aq. NH_4PF_6 (30 mL). The aqueous phase was re-extracted with dichloromethane (30 mL). The combined organic phase was dried over Na_2SO_4, filtered and concentrated. Purification by prep. HPLC (5-50% acetonitrile in water, 0.1 % TFA) and concentration afforded **I** as TFA salt. The resulting TFA salt was dissolved in dichloromethane (3 mL) and washed with 0.1 M aq. NH_4PF_6 (2 mL x 5). Concentration followed by trituration from diethyl ether afforded **I** as hexafluorophosphate salt (107 mg, 50 %, purple solid). The purity of **I** was more than 98% by HPLC at 254 nm. The measured molecular mass of **I** (MH+, 1221.6073) measured by HRMS was consistent with the theoretical mass (m/z 1221.6090).

^{1}H-NMR (600 MHz, acetone-d_6) δ 9.40 (d, 1H, *J* = 5.4 Hz), 7.75-7.60 (m, 4H), 7.53-7.40 (m, 6H), 7.40-7.23 (m, 2H), 7.11 (s, 1H), 6.93-6.85 (m, 1H), 6.66 (t, 1H, *J* = 11 Hz), 5.85 (t, 1H, *J* = 10 Hz), 5.78 (d, 1H, *J* = 9.6 Hz), 5.12 (s, 1H), 4.55 (d, 1H, *J* = 9.6 Hz), 4.12-4.05 (br d, 1H), 4.06-3.98 (m, 1H), 3.98-3.93 (m, 1H), 3.85-3.45 (m, 13H), 3.39-3.15 (m, 5H), 3.32 (s, 3H), 3.20 (s, 3H), 3.05-2.95 (m, 1H), 2.87 (s, 3H), 2.77-2.68 (m, 1H), 2.64-2.58 (m, 1H), 2.53-2.39 (m, 2H), 2.30-2.13 (m, 4H), 2.05-1.91 (m, 2H), 2.01 (s, 3H), 1.88-1.76 (m, 4H), 1.75 (s, 3H), 1.75-1.67 (m, 2H), 1.06 (s, 9H), 1.00 (d, 3H), 0.91 (d, 3H) MS (EI) m/z 1221.58 (M+1).

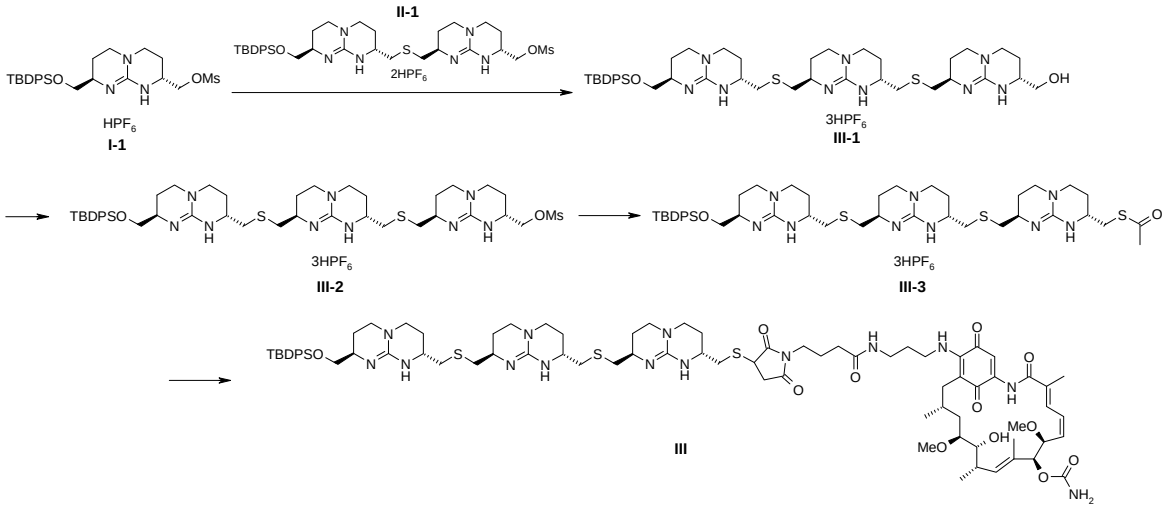


Thioacetate II-2 : The mesylate **II-1** was synthesized as previously reported (2). A stirred solution of **II-1** (1.71 g, 1.70 mmol) and potassium thioacetate (583 mg, 5.10 mmol) in THF (20 mL)/ H_2O (8 mL) was refluxed for 16 h. After cooling to room temperature, the reaction mixture was diluted with dichloromethane (100 mL) and washed with 0.1 M aq. NH_4PF_6 (50 mL). The aqueous phase was re-extracted with additional dichloromethane (50 mL). The combined organic phase was washed with 0.1 M aq. NH_4PF_6 (50 mL), dried over Na_2SO_4, filtered and concentrated. Purification by column chromatography (ethyl acetate only $\rightarrow$ 5 % MeOH in dichloromethane) and concentration afforded **II-2** as dihexafluorophosphate salt (1.45 g, 87 %, pale brown solid). ^{1}H-NMR (400 MHz, acetone-d_6) δ 7.73-7.67 (m, 4H), 7.53-7.42 (m, 6H), 7.27 (br d, 2H), 7.11 (br d, 2H), 3.85-3.63 (m, 6H), 3.62-3.48 (m, 8H), 3.61-3.46 (m, 4H), 3.14 (d, 2H, J = 6 Hz), 2.99 (dd, 2H, J = 14, 4.6 Hz), 2.73 (ddd, 2H, J = 14, 9, 4.6 Hz), 2.36 (s, 3H), 2.28-2.12 (m, 4H), 2.05-1.89 (m, 4H) 1.06 (s, 9H).

Gamitrinib-G2 II: To a solution of **II-2** (110 mg, 0.112 mmol) in degassed MeOH (4 mL) under N_2 at room temperature was added potassium *tert*-butoxide (0.34 mL, 0.34 mmol, 1 M in THF). After 30 min, the reaction mixture was neutralized with 1 N aq. HCl (ca. 0.3 mL) and treated with 0.1 N phosphate buffer (pH 6, 0.5 mL). To the buffered solution under N_2 at room

temperature was added a solution of geldanamycin-maleimide **2** (94 mg, 0.122 mmol) in degassed MeOH (2 mL). After 2 h, the reaction was concentrated to ca. 2 mL. The resulting reaction mixture was diluted with dichloromethane (30 mL) and washed with 0.1 M aq. NH_4PF_6 (30 mL). The aqueous phase was re-extracted with dichloromethane (30 mL). The combined organic phase was dried over Na_2SO_4, filtered and concentrated. Purification by prep. HPLC (5-50% acetonitrile in water, 0.1 % TFA) and concentration afforded **II** as TFA salt. The resulting TFA salt was dissolved in dichloromethane (3 mL) and washed with 0.1 M aq. NH_4PF_6 (2 mL x 5). Concentration followed by trituration from diethyl ether afforded **II** as dihexafluorophosphate salt (55 mg, 29 %, purple solid). The purity of **II** was more than 99% by HPLC at 254 nm. The measured molecular mass of **II** ($[M+2H]^{2+}$, m/z 709.8534) measured by HRMS was consistent with the theoretical mass (m/z 709.8577).

^{1}H-NMR (600 MHz, CD_3CN) δ 9.26 (s, 1H), 7.70-7.65 (m, 4H), 7.52-7.43 (m, 6H), 7.13-7.08 (m, 1H), 7.06 (s, 1H), 6.87 (br, 1H), 6.78-6.68 (m, 1H), 6.64-6.55 (m, 2H), 6.46 (br, 1H), 5.83 (t, 1H, *J* = 10 Hz), 5.72-5.65 (m, 1H), 5.21 (br, 2H), 5.07 (s, 1H), 4.46-4.42 (m, 1H), 3.82-3.78 (m, 1H), 3.73-3.69 (m, 1H), 3.66-3.62 (m, 1H), 3.62-3.41 (m, 9H), 3.41-3.25 (m, 11H), 3.29 (s, 3H), 3.22-3.03 (m, 3H), 3.20 (s, 3H), 2.93-2.71 (m, 3H), 2.70-2.58 (m, 2H), 2.56-2.39 (m, 3H), 2.38-2.32 (m, 1H), 2.25-2.03 (m, 5H), 1.98-1.95 (m, 6H), 1.85-1.70 (m, 8H), 1.71 (s, 3H), 1.05 (s, 9H), 0.97-0.93 (m, 3H), 0.92 (d, 3H) MS (EI) m/z 1418.51 (M+1).



Alcohol III-1 : A stirred solution of the mesylate **I-1** (545 mg, 0.82 mmol) and potassium thioacetate (188 mg, 1.65 mmol) in THF (10 mL)/ H_2O (4 mL) was refluxed for 16 h. Methanesulfonic acid (0.27 mL, 4.12 mmol) was added and the reaction mixture was refluxed for 24 h. After cooling to room temperature, organic and aqueous phases were separated in diethyl ether (50 mL) and water (50 mL). The aqueous phase was re-extracted with additional water (20 mL). The combined aqueous phases were washed with diethyl ether. Then the aqueous phases were neutralized with potassium bicarbonate (495 mg, 4.94 mmol) and the solvent was evaporated to dryness. To this resulting solid was added MeOH (100 mL), and the precipitate was removed by filtration. This procedure was repeated twice with MeOH/ CH_2Cl_2 system ($MeOH/CH_2Cl_2$ = 50/50 → 5/95). Concentration afforded the crude yellow foam. To a solution of this product in MeOH (10 mL) were added cesium carbonate (322 mg, 0.99 mmol) and tributylphosphine (0.12 mL, 0.49 mmol) at room temperature. After stired for 40 min, a solution of the mesylate **II-1** (697 mg, 0.69 mmol) in THF (10 mL) was added and the reaction mixture was stirred for 16 h at room temperature. Then most of the volatiles were removed in vacuo. The residue was diluted with dichloromethane (50 mL) and washed with 0.1 M aq. NH_4PF_6 (30 mL). The aqueous phase was re-extracted with additional dichloromethane (30 mL). The combined

organic phase was dried over Na_2SO_4, filtered and concentrated. Purification by column chromatography (MeOH/CH_2Cl_2: 2 % to 5 %) afforded **III-1** (560 mg, 64 %, white solid) as trihexafluorophosphate salt.

^{1}H-NMR (400 MHz, acetone-d_6) δ 7.74-7.70 (m, 4H), 7.54-7.44 (m, 6H), 4.28 (t, 1H, *J* = 5.2 Hz), 3.84-3.60 (m, 8H), 3.60-3.44 (m, 14 H), 3.07-2.97 (m, 4H), 2.75-2.58 (m, 4H), 2.27-2.14 (m, 5H), 2.14-1.74 (m, 7H), 1.06 (s, 9H).

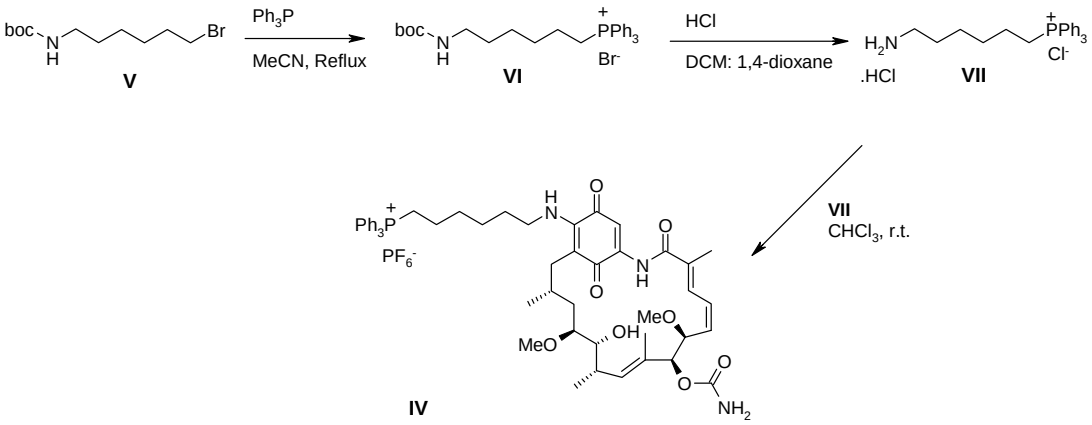
Mesylate III-2 : A solution of alcohol **III-1** (433 mg, 0.34 mmol) in THF (5 mL) was treated with *N*-methylmorpholine (0.19 mL, 1.70 mmol) and methanesulfonic anhydride (178 mg, 1.02 mmol) at room temperature under N_2. After stirred for 2 h at room temperature, the reaction mixture was diluted with dichloromethane (30 mL) and washed with 0.1 M aq. NH_4PF_6 (20 mL). The aqueous phase was re-extracted with additional dichloromethane (30 mL). The combined organic phase was dried over Na_2SO_4, filtered and concentrated. Purification by column chromatography (MeOH/CH_2Cl_2 : 2 % to 5 %) afforded **III-2** as trihexafluorophosphate salt (359 mg, 78 %, pale brown foam). ^{1}H-NMR (400 MHz, acetone-d_6) δ 7.80-7.65 (m, 4H), 7.55-7.40 (m, 6H), 4.41 (dd, 1H, *J* = 10.4 Hz, 4.4 Hz), 4.25 (dd, 1H, *J* = 10.4 Hz, 7.6 Hz), 3.95-3.87 (m, 1H), 3.84-3.58 (m, 7H), 3.58-3.40 (m, 12H), 3.20 (s, 3H), 3.07-2.94 (m, 4H), 2.76-2.57 (m, 4H), 2.28-2.10 (m, 6H), 2.05-1.86 (m, 6H), 1.06 (s, 9H).

Thioacetate III-3 : A stirred solution of the mesylate **III-2** (359 mg, 0.27 mmol) and potassium thioacetate (97 mg, 0.85 mmol) in THF (8 mL)/ H_2O (3 mL) was refluxed for 16 h. After cooling to room temperature, the reaction mixture was diluted with dichloromethane (50 mL) and

washed with 0.1 M aq. NH_4PF_6 (30 mL). The aqueous phase was re-extracted with additional dichloromethane (30 mL). The combined organic phase was washed with 0.1 M aq. NH_4PF_6 (20 mL), dried over Na_2SO_4, filtered and concentrated. Trituration from diethyl ether-hexanes (1:1) afforded **III-3** as trihexafluorophosphate salt (294 mg, 83 %, pale yellow solid). ^{1}H-NMR (600 MHz, acetone-d_6) δ 8.20-7.74 (br, salt protons), 7.74-7.68 (m, 4H), 7.53-7.44 (m, 6H), 3.86-3.75 (m, 3H), 3.73-3.56 (m, 5H), 3.56-3.44 (m, 12H), 3.21 (dd, 1H, *J* = 13.8, 6 Hz), 3.08-2.95 (m, 5H), 2.80-2.61 (m, 4H), 2.37 (s, 3H), 2.24-2.10 (m, 6H), 2.00-1.82 (m, 6H) 1.06 (s, 9H).

Gamitrinib-G3 III: To a solution of **III-1** (209 mg, 0.157 mmol) in degassed MeOH (4 mL) under N_2 at room temperature was added potassium *tert*-butoxide (0.47 mL, 0.47 mmol, 1 M in THF). After 30 min, the reaction mixture was neutralized with 1 N aq. HCl (ca. 0.1 mL) and treated with 0.1 N phosphate buffer (pH 6, 3 mL). To the buffered solution under N_2 at room temperature was added a solution of geldanamycin-maleimide **2** (133 mg, 0.173 mmol) in degassed MeOH (2 mL). After 2 h, the reaction was concentrated to ca. 3 mL. The resulting reaction mixture was diluted with dichloromethane (20 mL) and washed with 0.1 M aq. NH_4PF_6 (30 mL). The aqueous phase was re-extracted with dichloromethane (20 mL). The combined organic phase was dried over Na_2SO_4, filtered and concentrated. Purification by prep. HPLC (5-50% acetonitrile in water, 0.1 % TFA) and concentration afforded **III** as TFA salt. The resulting TFA salt was dissolved in dichloromethane (3 mL) and washed with 0.1 M aq. NH_4PF_6 (2 mL x 5). Concentration followed by trituration from diethyl ether-hexanes (1:1) afforded **III** as trihexafluorophosphate salt (110 mg, 34 %, purple solid). The purity of **III** was more than 96% by HPLC at 254 nm. The measured molecular mass of **III** ($[M+3H]^{3+}$, m/z 539.2695) measured by HRMS was consistent with the theoretical mass (m/z 539.2740).

^{1}H-NMR (400 MHz, CD_3CN) δ 9.26 (s, 1H), 7.70-7.63 (m, 4H), 7.52-7.40 (m, 6H), 7.15-7.08 (br, 1H), 7.06 (s, 1H), 6.81-6.68 (m, 2H), 6.65-6.57 (m, 2H), 6.45-6.15 (br, 6H), 5.83 (t, 1H, *J* = 10.2 Hz), 5.69 (d, 1H, *J* = 7.2 Hz), 5.21 (br s, 2H), 5.08 (s, 1H), 4.44 (dd, 1H, *J* = 9.6, 4.8 Hz), 3.83-3.78 (m, 1H), 3.73-3.70 (m, 1H), 3.66-3.61 (m, 1H), 3.61-3.42 (m, 10H), 3.42-3.25 (m, 18H), 3.22-3.14 (m, 3H), 3.20 (s, 3H), 2.85-2.71 (m, 7H), 2.71-2.41 (m, 7H), 2.38-2.32 (m, 1H), 2.25-2.18 (m, 1H), 2.18-2.04 (m, 8H), 1.97 (s, 3H), 1.85-1.70 (m, 10H), 1.72 (s, 3H), 1.06 (s, 9H), 1.01 (d, 3H), 0.95 (dd, 3H) MS (EI) m/z 1615.51 (M+1).



Phosphonium bromide VI : The bromide **V** was synthesized as previously reported (3). To a solution of **V** (1.60 g, 5.71 mmol) in acetonitrile (10 mL) was added triphenylphosphine (1.57 g, 5.99 mmol) and the reaction was refluxed for 16 h. After the reaction was cooled to room temperature, excess triphenylphosphine was removed by extraction with n-hexane (100 mL x 3). Concentration and drying under vacuum gave the phosphonium salt **VI** (3.09 g, 99 %, white solid). ^{1}H-NMR (600 MHz, DMSO-d_6) δ 7.89-7.84 (m, 3H), 7.80-7.71 (m, 12H), 6.72 (br t, 1H), 3.57-3.50 (m, 2H), 2.85-2.79 (m, 2H), 1.50-1.38 (m, 4H), 1.30-1.16 (m, 4H).

Amine VII : A solution of the phosphonium salt **VI** (1.5 g, 0.765 mmol) in dichloromethane (100 mL) was treated with HCl solution (4 N in 1,4-dioxane, 171 mL) at room temperature. After stirred for 3 h, the reaction was concentrated. Drying under vacuum afforded the amine **VII** (1.31 g, 99%, white solid). The amine **VII** was used without further purification.

^{1}H-NMR (600 MHz, DMSO-d_6) δ 8.12 (br s, 3H), 7.94-7.88 (m, 3H), 7.86-7.75 (m, 12H), 3.70-3.60 (m, 2H), 2.75-2.68 (m, 2H), 1.58-1.44 (m, 6H), 1.38-1.30 (m, 2H).

Gamitrinib-TPP IV: To a solution of geldanamycin (150 mg, 0.27 mmol) in chloroform (25 mL) under N_2 at room temperature was added amine **VII** (390 mg, 0.81 mmol) and *N*,*N*-diisopropylethylamine (0.47 mL, 2.70 mmol). After stirred for 3 h, additional amine **VII** (390 mg, 0.81 mmol) was added. After 10 h, the reaction was concentrated and purified by column chromatography (2-10% methanol in dichloromethane). The resulting salt was dissolved in dichloromethane (3 mL) and washed with 0.1 M aq. NH_4PF_6 (2 mL x 5). Concentration followed by trituration from diethyl ether afforded **IV** as hexafluorophosphate salt (173 mg, 62 %, purple solid). The purity of **IV** was more than 98% by HPLC at 254 nm. The measured molecular mass of **IV** (M+, m/z 890.4559) measured by HRMS was consistent with the theoretical mass (m/z 890.4509).

^{1}H-NMR (600 MHz, acetone-d_6) δ 9.39 (s, 1H), 8.00-7.87 (m, 9H), 7.87-7.75 (m, 6H), 7.30 (d, 1H, *J* = 11.4 Hz), 7.10 (s, 1H), 6.66 (t, 1H, *J* = 11 Hz), 6.58 (br, 1H), 5.85 (t, 1H, *J* = 10 Hz), 5.78 (d, 1H, *J* = 9.6 Hz), 5.11 (s, 1H), 4.55 (d, 1H, *J* = 9.6 Hz), 4.06 (d, 1H, *J* = 6 Hz), 3.66-3.55 (m, 4H), 3.54-3.47 (m, 1H), 3.37-3.33 (m, 1H), 3.31 (s, 3H), 3.21 (s, 3H), 2.78-2.68 (m, 1H), 2.59 (dd, 1H, *J* = 13.8, 4.2 Hz), 2.40 (dd, 1H, *J* = 13.8, 9.6 Hz), 2.01 (s, 3H), 1.90-1.77 (m, 3H),

1.74 (s, 3H), 1.74-1.62 (m, 6H), 1.54-1.45 (m, 3H), 0.97 (d, 3H, *J* = 7.2 Hz), 0.91 (d, 3H, *J* = 7.2 Hz) MS (EI) m/z 890.08 (M+).

Computational methods. The crystal structure of Hsp90 used for all docking calculations was taken from the protein data bank with coordinates corresponding to the pdb code 1YET.pdb (4). The original X-ray structure contained the ligand Geldanamycin (GA), which was removed from the active site to yield the apo-open form of Hsp90. Gamitrinib was docked into the active site of Hsp90 using different docking procedures, different computational approaches programs, and energy functions to define a consensus structure representative of the free energy minimum of the Gamitrinib-Hsp90 complex. First, the structure of Gamitrinib was minimized using the Macromodel program (5), the AMBER force field (6) and the GB/SA approach (7) to take into account the effects of the water solvent.

In a first set of docking calculations, the energy minimized structure of Gamitrinib was subjected to blind docking experiments on the putative N-terminal Hsp90 receptor using the program AutoDock (8). Mass-centered grid maps were generated with 0.35 Å spacing by the program Autogrid around the ATPase pocket of Hsp90. Lennard–Jones parameters 12-10 and 12-6 (default parameters in the program package) were used for modeling H-bonding and Van der Waals interactions, respectively. The distance dependent dielectric permittivity of Mehler and Solmajer (9) was used for the calculation of the electrostatic grid maps. The Lamarckian genetic algorithm (LGA) and the pseudo-Solis and West methods were applied for minimization using default parameters. The number of generations was set to 25 million in all runs, and the stopping criterion was therefore defined by the total number of energy evaluations. Random starting positions on the grid, random orientations, and torsions (flexible ligand only) were used for the ligand. A total of 310 runs were performed. At the end of the docking runs,

conformations of the ligand were listed in increasing energy order. Subsequently, the ligand conformation with lowest energy was used as a reference, and all conformations with a center of mass to center of mass distance of <1.5 Å from the reference were taken to belong to the first cluster. Once a conformation was assigned to a cluster, it was not used again for other (energetically less favorable) clusters. Then the process was repeated for all hitherto unclassified conformations until all conformations were put in a cluster. Most of the docked structures shared common conformational characteristics which are prototypically represented by the structure of the global minimum of the complex (Fig. 1B). The 17-AAG region of free energy minimum structure obtained from the Autodock runs is well superimposible to the benzoquinone ansamicyin backbone of GA with a root mean square deviation (rmsd) of all heavy atoms of 0.56 Å.

In a second set of docking calculations, the minimized structure of Gamitrinib was docked onto the Hsp90 receptor using the Glide software (10, 11). A cubic bounding box of 14 Å length on for each side was build for the ligand around the ATPase binding pocket. Full flexibility was allowed for the ligand and the docking poses were scored using the Glide standard-precision (SP) mode. The 17-AAG region of Gamitrinib in best docking pose obtained from this procedure is once again superimposible to the benzoquinone ansamicyin backbone of GA in the X-ray structure (4), and in previous docking calculation (rmsd of 0.51 Å).

Finally, in order to evaluate the possibility that different conformations of the flexible ligand may determine a different complex geometry, Gamitrinib was subjected to a preliminary conformational analysis in isolation in solution, with an implicit representation of water through the GB/SA method. To explore the conformational space of Gamitrinib, a torsion-based conformational search was run using 10000 steps of Monte Carlo Multiple Minimum method

(12) and the AMBER force field, as implemented in Macromodel. 4223 unique conformations were identified and saved for the ligand. All the conformations obtained from this calculation were then used as ligands for docking calculations on the Hsp90 receptor using the same procedures as described in the simple Glide docking approach. Each the conformations docked into the receptor with a different score. Importantly, the top-ranked 226 poses are once more fully overlapping in their 17-AAG region with the to the benzoquinone ansamicyin backbone of GA, with an average rmsd of 0.6 Å.

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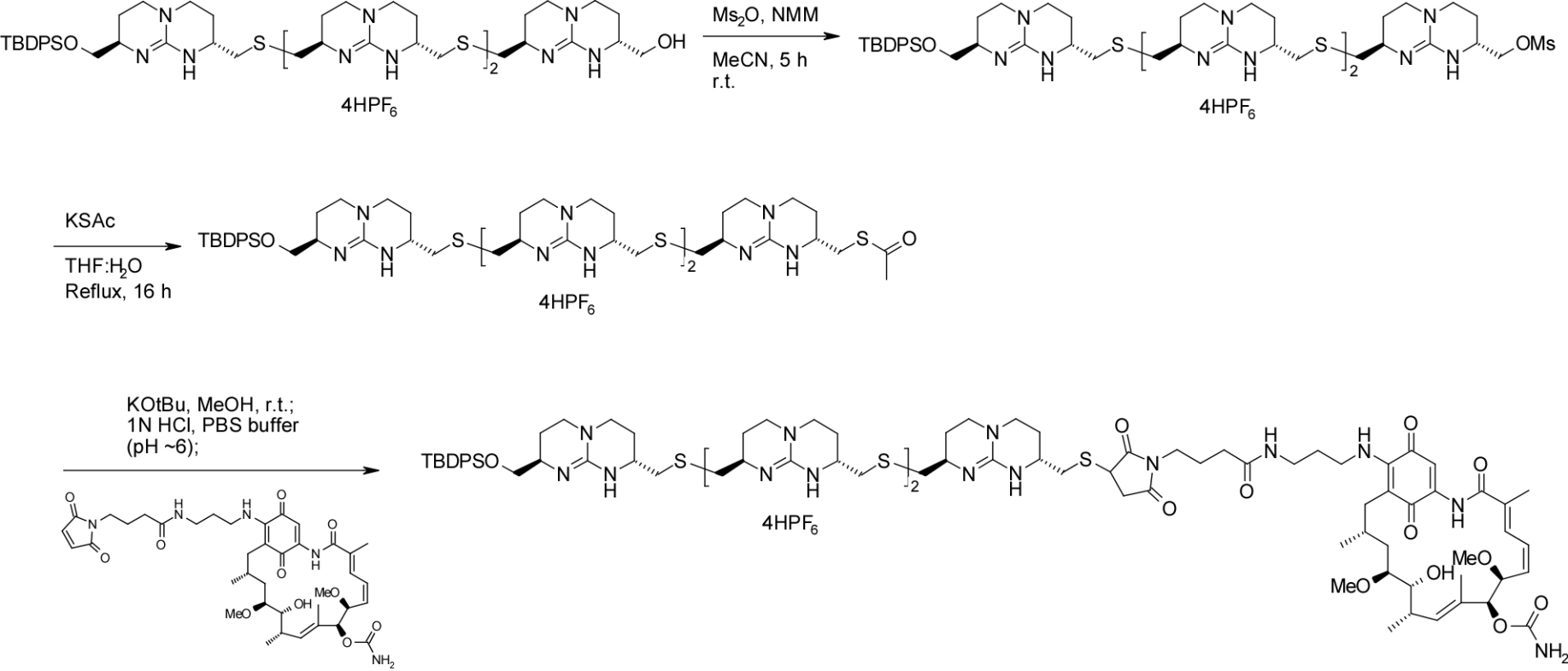
SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure. 1. Chemical synthesis of Gamitrinib-G4. See text for details.

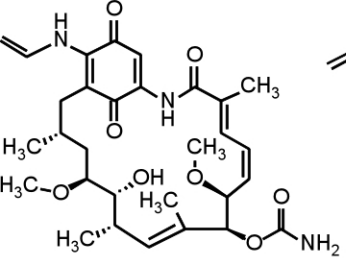
Supplemental Figure 2. Chemical structure of GA (17-AAG), IPI-504, and non-GA based (BIIB021 and NVP-AUY922) Hsp90 inhibitors used in these studies.

Supplemental Figure 3. Gamitrinib anticancer activity, in vivo. Established (100-150 mm^3) subcutaneous acute leukemia HL-60 (**A**) or breast adenocarcinoma MDA-MB-231 (**B**) xenograft tumors (2/mouse, 6 tumors/group) were treated with vehicle or Gamitrinib-G4 at 2 mg/kg twice daily i.p. (HL-60) or with a dose escalation regimen (MDA-MB-231) starting at 2 mg/kg twice daily (day 0-2), 2.5 mg/kg twice daily (day 3-5), and 3 mg/kg twice daily for the duration of treatment. *Arrow*, start of treatment.

Supplemental Figure 4. Tissue histology. Animals carrying H460 xenograft tumors in the vehicle or Gamitrinib group were sacrificed at the end of treatment, and the indicated organs were harvested, paraffin-embedded, and sections were stained with hematoxylin-eosin. Slides were analyzed by light microscopy. Magnification, x100.



Supplemental Figure 1



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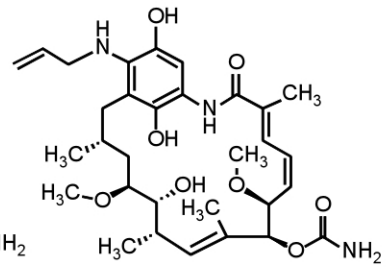

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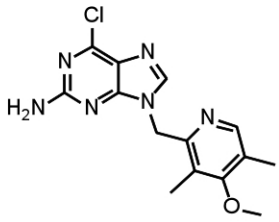

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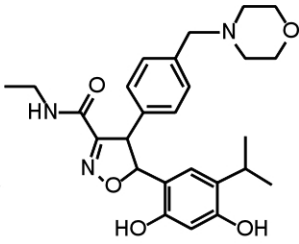
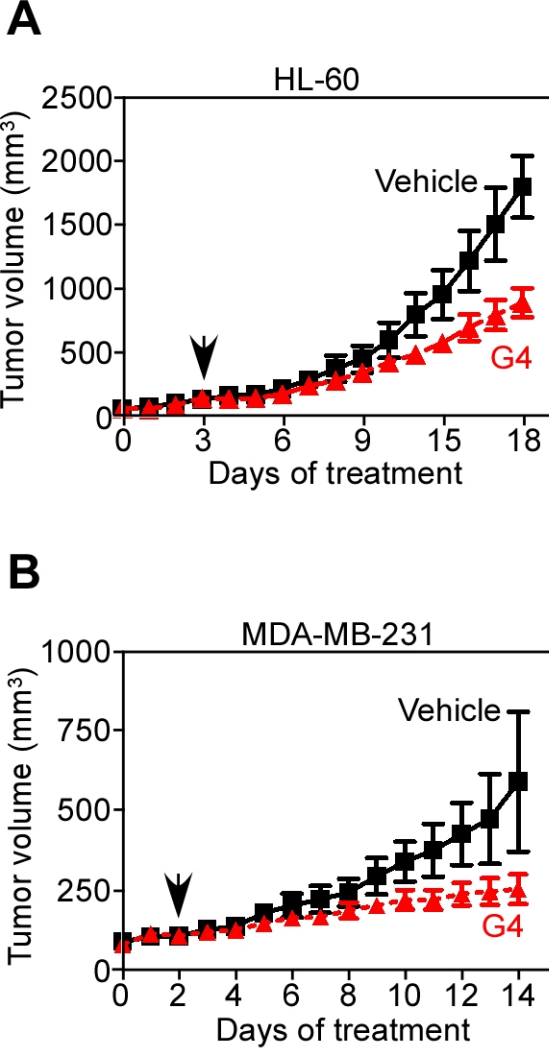
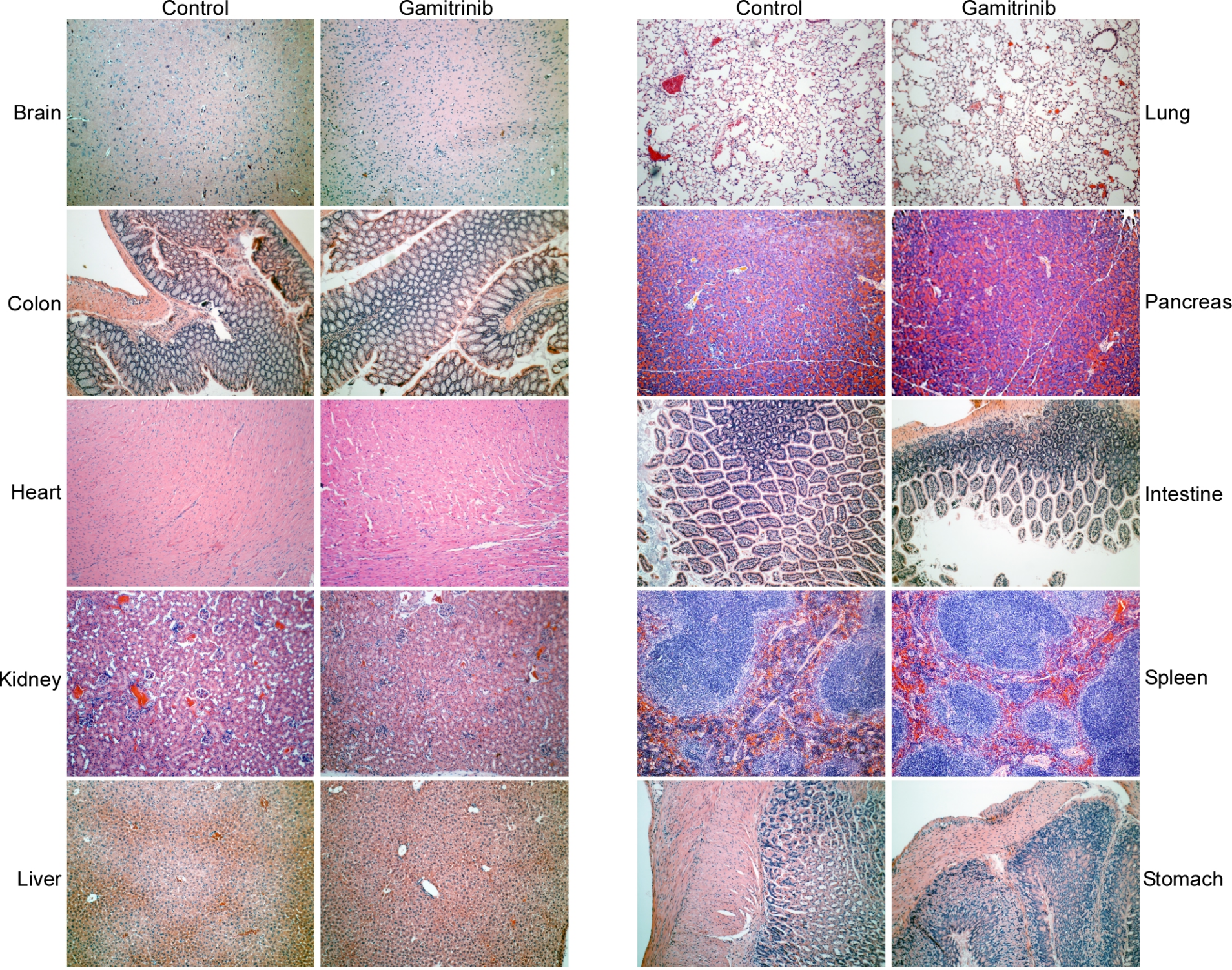


NVP-AUY922

Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4