

Supporting Information

Discovery of Lanraplenib (GS-9876), a Once – Daily Spleen Tyrosine Kinase Inhibitor for Autoimmune Diseases

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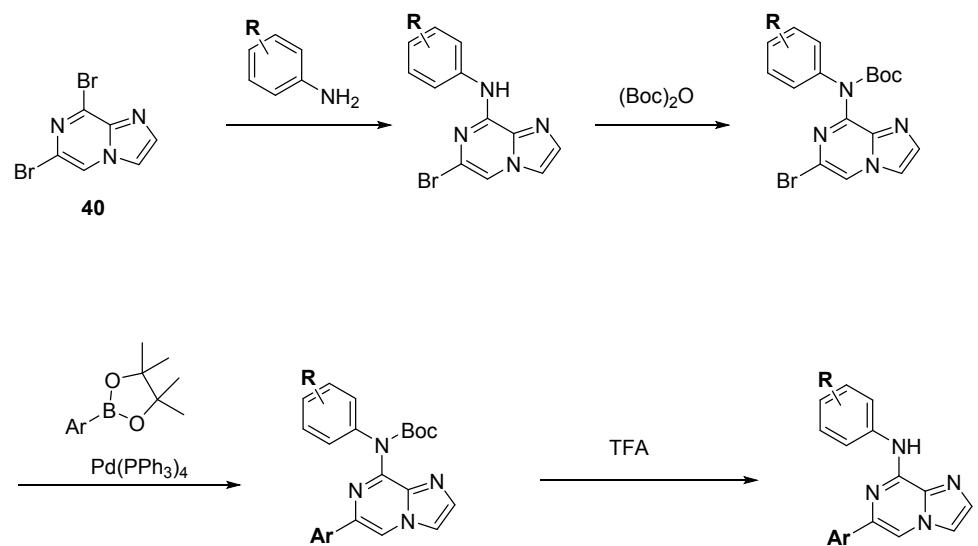
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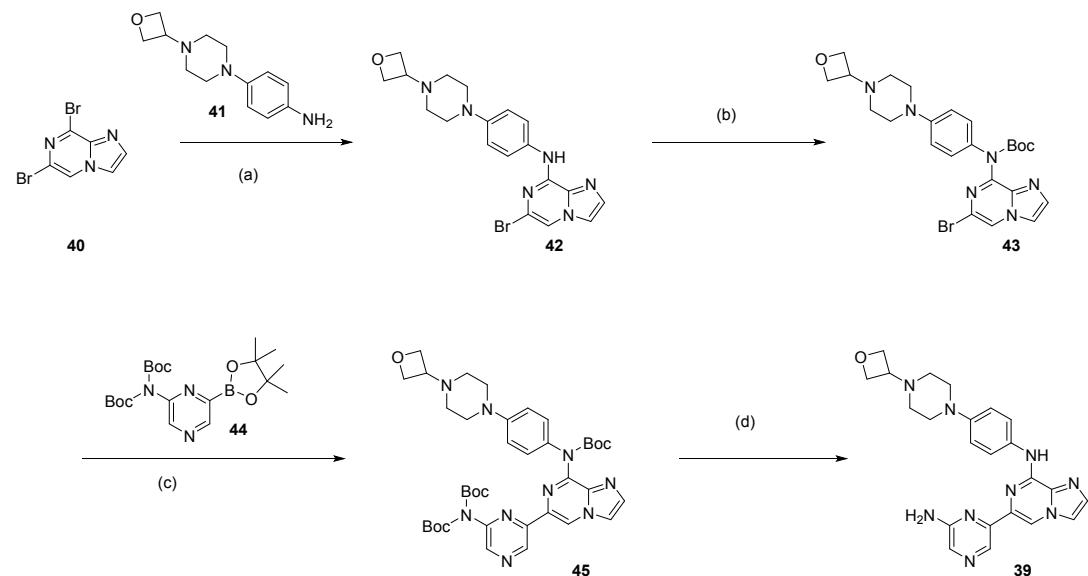
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General Methods All final compounds were synthesized at Gilead Sciences, Inc. Commercial solvents and reagents were used as received without further purification. Air or moisture sensitive reactions were carried out under a nitrogen atmosphere. ¹H NMR and ¹³C NMR spectra were recorded on a Varian Mercury Plus (300 MHz) spectrometer in the specified deuterated solvent. Flash chromatography for the purification of intermediates and final compounds was performed on an ISCO Combiflash Companion purification system with RediSep Rf prepakced silica gel cartridges supplied by Teledyne Isco. The purity of the tested compounds was assessed to be at least 95% by HPLC analysis unless indicated otherwise. A Gemini C18 110 Å column (50 mm × 4.6 mm, 5 µm particle size) was used with gradient elution of acetonitrile in water, 0–30% for 5 min and then 30–98% for 5 min at a flow rate of 2 mL/min with detection at 254 nm wavelength. For all samples 0.1% TFA was added to both eluents. LC/MS analysis was performed on a Waters SQD (Model F085QD294W) with electrospray ionization in the positive mode. The analytical method consisted of an Acquity UPLC BEH C18 column (2.1 x 50 mm, 1.7 µm), 25–75% gradient of 0.1% trifluoroacetic acid in acetonitrile and 0.1% trifluoroacetic acid in water, at a 0.8 mL/min flow rate over 1.75 minutes.

Scheme S1. General synthesis of compounds



Synthesis of Compound 39



Reagents and conditions: (a) Hunig's base, DMF, 85°C, 82%; (b) (Boc)₂O, DMAP, DCM, 65°C, 97%; (c) Pd(PPh₃)₄, Na₂CO₃, DME, 110°, 90%; (d) TFA, DCM, RT, 83%

Step 1. 4-(4-(Oxetan-3-yl)piperazin-1-yl)aniline (41). In a 500 mL round bottom flask 1-(oxetan-3-yl)piperazine (3.02 g, 21.26 mmol), potassium carbonate (5.87 g, 42.52 mmol), 1-fluoro-4-nitrobenzene (3.00 g, 21.26 mmol) were combined in acetonitrile (33 mL) and stirred under nitrogen overnight at 100 °C. The mixture was diluted with water (100 mL) and extracted with DCM (100 mL x 3), dried over anhydrous sodium carbonate, filtered, and the filtrate was concentrated. The residue was dissolved in minimal DCM using a sonicator and the solution was treated with hexane. The precipitate was filtered, washed with hexane and dried to provide the title compound 1-(4-nitrophenyl)-4-(oxetan-3-yl)piperazine as an orange solid (4.70 g, 84%).

In a hydrogenation vessel 1-(4-nitrophenyl)-4-(oxetan-3-yl)piperazine (4.70 g, 17.85 mmol) was dissolved in MeOH (26 mL) and DCM (5 mL). Pd/C (10%) (2.85 g, 2.68 mmol) was added and the reaction was stirred under nitrogen. The reaction was shaken on a Parr

hydrogenator at 45 PSI. After 15 minutes, the reaction was fully recharged to 45 PSI and shaken for an additional hour. The reaction mixture was filtered through celite, washed with 25% MeOH/DCM and the filtrate was concentrated to provide the title compound **41** as a light brown solid (4.16 g, 98%).

Step 2. 6-Bromo-N-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)imidazo[1,2- a]pyrazin-8-amine (42). 4-(4-(oxetan-3-yl)piperazin-1-yl)aniline (2.00 g, 8.57 mmol), Hunig's base (3.29 mL) and 6,8-dibromoimidazo[1,2-a]pyrazine (**40**, 2.37 g, 8.57 mmol) were added to a pressure vessel and dissolved in DMF (43 mL). The reaction was then stirred at 85 °C overnight. The material was quenched with saturated sodium bicarbonate, extracted with DCM (120 mL x 3) and the organic layers were combined and washed with water (120 mL x 3), dried over anhydrous sodium sulfate, and concentrated. The crude material was purified by column chromatography using a gradient of 0-60% (10% MeOH/DCM). The desired fractions were combined and concentrated to provide the title compound **42** as a light yellow solid (3.00 g, 82%).

Step 3. tert-Butyl(6-bromoimidazo[1,2-a]pyrazin-8-yl)(4-(oxetan-3-yl)piperazin-1-yl)phenyl)carbamate (43). 6-bromo-N-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)imidazo[1,2-a]pyrazin-8-amine (1000 mg, 2.33 mmol), di-tert-butyl dicarbonate (1016.72 mg, 4.66 mmol) and N,N-dimethylpyridin-4-amine (21.34 mg, 0.17 mmol) were stirred in DCM (10 ml) and refluxed at 65 °C for 3h. The reaction was diluted with 100 mL of DCM, washed with H₂O (50mL x 3), dried, filtered and concentrated. The crude material was dissolved in minimal DCM, and purified by column chromatography using 0-30% MeOH/DCM over 20 column volumes. The desired fractions were combined and concentrated to provide the title compound **43** (1.2 g, 97%).

Step 4. N,N-bis(tert-butoxycarbonyl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazin-2-amine (44). Step 1: To a mixture of 6-bromopyrazin-2-amine (5 g, 28.7 mmol) and di-tert-butyl dicarbonate (25.09 g, 114.94 mmol) was added DCM (10 ml) followed by DMAP (0.351 g, 29 mmol). The reaction was heated to 55 °C for 1h, cooled to RT, and the reaction was partitioned between water and DCM, and the organic layer was purified on silica gel using DCM-MeOH. The desired fractions were pooled and concentrated to provide 10.75 g (87% yield) of 2- bis(tert-butoxycarbonyl)amino-6-bromopyrazine. LCMS-ESf (m/z): [M+H]⁺: 374.14. ¹H NMR (DMSO) δ: 8.84(d, 2H), 1.39 (s, 18H). Step 2: To a dry 250 mL round-bottomed flask was added 2-bis(tert-butoxycarbonyl)amino-6-bromopyrazine **44** (1.0g, 1.0equiv, 2.67mmol), KOAc (790mg, 8.02mmol, 3.0equiv), 4,4,4',4',5,5,5',5'-octamethyl-2,2'- bi(1,3,2-dioxaborolane) (750mg, 2.94mmol, 1.1equiv), Pd(dba) (171mg, 0.187mmol, 0.07equiv) and X-phos (128mg, 0.267mmol, 0.1equiv) followed by 1,4-dioxane (25mL) and the solution was sonicated for 5 min and then purged with N₂ gas for 5 min. The reaction mixture was then placed under an N₂ atmosphere and heated at 110 °C for 90 min. The reaction was removed from heat and allowed to cool to RT. Once cool, the reaction contents were filtered through Celite and the filter cake was washed with 3 x 20 mL EtOAc. The resultant solution was then

concentrated to provide N,N-bis(tert-butoxycarbonyl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazin-2-amine **44**, as a red-orange syrup which was used directly in the next step.

Step 5. tert-Butyl (6-(6-(bis(tert-butoxycarbonyl)amino)pyrazin-2-yl)imidazo[1,2-a]pyrazin-8-yl)(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)carbamate (45). The freshly formed N,N-bis(tert-butoxycarbonyl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazin-2-amine **44** (2.67 mmol based on 100% conversion, 2.0 equiv based on bromide) was dissolved in 20 mL of 1,2-dimethoxyethane (DME) and to that solution was added tert-butyl (6-bromoimidazo[1,2-a]pyrazin-8-yl)(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)carbamate **43** (707mg, 1.34mmol, 1.0equiv), Na₂CO₃ (283mg, 2.67mmol, 2.0equiv), Pd(PPh₃)₄ (155mg, 0.134mmol, 0.1equiv) and water (10mL) and the solution was degassed for 5 min with N₂. The reaction was then placed under an N₂ atmosphere and heated at 110 °C for 90 min. The reaction was removed from heat and allowed to cool to RT. The reaction mixture was diluted with 100 mL water and 100 mL 20% MeOH/DCM and the organic layer was washed with 1 x saturated NaHCO₃, 1 x saturated brine and then dried over Na₂SO₄. The solution was then filtered and concentrated down to an orange-red solid. The sample was then slurried in warm MeOH, sonicated then filtered, washed with 2 x 20 mL cold MeOH and then the remaining cream-colored solid was dried under vacuum overnight to yield tert-butyl (6-(6-(bis(tert-butoxycarbonyl)amino)pyrazin-2-yl)imidazo[1,2-a]pyrazin-8-yl)(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)carbamate **45** (905mg, 90% yield over 2 steps).

Step 6. 6-(6-Aminopyrazin-2-yl)-N-(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)imidazo[1,2-a]pyrazin-8-amine (39). To a solution of tert-butyl(6-(6-(bis(tert-butoxycarbonyl)amino)pyrazin-2-yl)imidazo[1,2-a]pyrazin-8-yl)(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)carbamate **45** (200 mg, 0.269 mmol) in DCM (2 ml) was added TFA (0.5 ml, 6.578 mmol). The reaction was stirred at room temperature for 16h, treated with saturated sodium bicarbonate, extracted with EtOAc, and purified on silica gel, eluting with 5%MeOH / EtOAc to 20%MeOH / EtOAc. The desired fractions were combined and concentrated to provide 100 mg (83% yield) of the title compound **39**. m/z calcd for C₂₃H₂₅N₉O [M+H]⁺ 444.23, found LCMS-ESI⁺ (m/z): [M+H]⁺ 444.20. 1H NMR (300 MHz d₆-DMSO) δ: 9.5 (s,1H), 8.588 (s, 1H), 8.47 (s, 1H), 8.12 (d, 1H), 7.95-7.92 (d, 2H), 7.88 (s, 1H), 7.62 (s, 1H), 6.99-6.96 (d, 2H), 6.46 (s, 2H), 4.57-4.53 (m, 2H), 4.48-4.44 (m, 2H), 3.43 (m, 1H), 3.15-3.12 (m, 4H), 2.41- 2.38 (m, 4H).

Characterization data for compounds 3-38

6-(1H-indazol-6-yl)-N-(4-morpholinophenyl)imidazo[1,2-a]pyrazin-8-amine, 3: ^1H NMR (300 MHz, DMSO-d₆) δ 13.17 (s, 1H), 9.50 (s, 1H), 8.66 (s, 1H), 8.19 (s, 1H), 8.09 (s, 1H), 8.03 (d, J = 8.7 Hz, 2H), 7.99 (d, J = 0.9 Hz, 1H), 7.84 (d, J = 8.7 Hz, 1H), 7.72 (dd, J = 8.7, 0.9 Hz, 1H), 7.63 (d, J = 0.9 Hz, 1H), 7.00 (d, J = 8.7 Hz, 2H), 3.77 (t, J = 4.6 Hz, 4H), 3.11 (t, J = 4.6 Hz, 4H). m/z calcd for C₂₃H₂₁N₇O [M+H]⁺ 412.19, found ESI MS m/z 412.19 [M + H]⁺.

N-((1R,5S)-8-oxa-3-azabicyclo[3.2.1]octan-3-yl)phenyl)-6-(1H-indazol-6-yl)imidazo[1,2-a]pyrazin-8-amine, 5: ^1H NMR (400 MHz, DMSO-d₆) δ 13.19 – 13.09 (m, 1H), 9.46 (s, 1H), 8.65 (s, 1H), 8.24 – 8.14 (m, 1H), 8.09 (t, J = 1.3 Hz, 1H), 7.99 (dd, J = 9.1, 1.7 Hz, 3H), 7.83 (d, J = 8.5 Hz, 1H), 7.72 (dd, J = 8.5, 1.4 Hz, 1H), 7.63 (d, J = 1.1 Hz, 1H), 6.97 – 6.80 (m, 2H), 4.45 (s, 2H), 3.44 – 3.36 (m, 2H), 2.82 (dd, J = 11.4, 2.5 Hz, 2H), 1.87 (s, 4H). m/z calcd for C₂₅H₂₃N₇O [M+H]⁺ 438.21, found ESI MS m/z 438.36 [M+H]⁺.

N-((1S,4S)-2-oxa-5-azabicyclo[2.2.1]heptan-5-yl)phenyl)-6-(1H-indazol-6-yl)imidazo[1,2-a]pyrazin-8-amine, 6: ^1H NMR (300 MHz, DMSO-d₆) δ 13.17 (s, 1H), 9.40 (s, 1H), 8.61 (s, 1H), 8.16 (s, 1H), 8.07 (s, 1H), 7.94 (d, J = 8.4 Hz, 3H), 7.81 (d, J = 8.6 Hz, 1H), 7.70 (d, J = 8.6 Hz, 1H), 7.61 (s, 1H), 6.67 (d, J = 8.6 Hz, 2H), 4.60 (s, 1H), 4.52 (s, 1H), 3.73 (q, J = 7.4 Hz, 2H), 3.52 (d, J = 9.3 Hz, 1H), 2.98 (d, J = 9.3 Hz, 1H), 2.02 – 1.89 (m, 1H), 1.83 (d, J = 9.7 Hz, 1H). m/z calcd for C₂₄H₂₁N₇O [M+H]⁺ 424.19, found ESI MS m/z 424.5 [M + H]⁺.

N-((1s,5s)-3,7-dioxa-9-azabicyclo[3.3.1]nonan-9-yl)phenyl)-6-(1H-indazol-6-yl)imidazo[1,2-a]pyrazin-8-amine, 7: ^1H NMR (300 MHz, DMSO-d₆) δ 13.15 (s, 1H), 9.47 (s, 1H), 8.63 (s, 1H), 8.15 (d, J = 1.2 Hz, 1H), 8.07 (t, J = 1.3 Hz, 1H), 8.01 (d, J = 9.1 Hz, 2H), 7.96 (d, J = 1.1 Hz, 1H), 7.81 (d, J = 8.5 Hz, 1H), 7.70 (dd, J = 8.6, 1.4 Hz, 1H), 7.62 (d, J = 1.1 Hz, 1H), 6.91 (d, J = 9.2 Hz, 2H), 3.89 (s, 8H), 3.75 (s, 2H). m/z calcd for C₂₅H₂₃N₇O₂ [M+H]⁺ 454.19, found ESI MS m/z 454.04 [M + H]⁺

N-(6-oxa-3-azabicyclo[3.1.1]heptan-3-yl)phenyl)-6-(1H-indazol-6-yl)imidazo[1,2-a]pyrazin-8-amine, 8: ^1H NMR (300 MHz, DMSO-d₆) δ 13.20-13.13 (m, 1H), 9.40 (s, 1H), 8.62 (s, 1H), 8.18 (q, J = 1.0 Hz, 1H), 8.06 (t, J = 1.2 Hz, 1H), 8.04-7.89 (m, 3H), 7.82 (dt, J = 8.5, 0.7 Hz, 1H), 7.70 (dd, J = 8.6, 1.4 Hz, 1H), 7.65-7.54 (m, 1H), 6.85-6.73 (m, 2H), 4.72 (d, J = 6.0 Hz, 2H), 3.58 (d, J = 11.7 Hz, 2H), 3.46-3.36 (m, 2H), 3.20-3.05 (m, 1H), 1.96 (d, J = 8.5 Hz, 1H). m/z calcd for C₂₄H₂₁N₇O [M+H]⁺ 424.19, found ESI MS m/z 424.04 [M + H]⁺.

N-(4-(7-oxa-2-azaspiro[3.5]nonan-2-yl)phenyl)-6-(1H-indazol-6-yl)imidazo[1,2-a]pyrazin-8-amine, 9: ^1H NMR (300 MHz, DMSO- d_6) δ 13.20 (s, 1H), 9.41 (s, 1H), 8.62 (s, 1H), 8.15 (s, 1H), 8.06 (s, 1H), 7.97 – 7.86 (m, 3H), 7.81 (d, J = 8.5 Hz, 1H), 7.68 (d, J = 8.6 Hz, 1H), 7.60 (s, 1H), 6.48 (d, J = 8.2 Hz, 2H), 3.59 (s, 4H), 3.54 (d, J = 6.5 Hz, 4H), 1.74 (s, 4H). m/z calcd for $\text{C}_{26}\text{H}_{25}\text{N}_7\text{O}$ [M+H] $^+$ 452.21, found ESI MS m/z 452.2, [M + H] $^+$.

N-(4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-6-(1H-indazol-6-yl)imidazo[1,2-a]pyrazin-8-amine, 10: ^1H NMR (400 MHz, DMSO- d_6) δ 13.19 (s, 1H), 9.42 (s, 1H), 8.64 (s, 1H), 8.17 (d, J = 1.3 Hz, 1H), 8.08 (t, J = 1.2 Hz, 1H), 7.99 – 7.91 (m, 3H), 7.83 (d, J = 8.5 Hz, 1H), 7.71 (dd, J = 8.5, 1.4 Hz, 1H), 7.62 (d, J = 1.1 Hz, 1H), 6.60 – 6.42 (m, 2H), 4.74 (s, 4H), 3.98 (s, 4H). m/z calcd for $\text{C}_{24}\text{H}_{21}\text{N}_7\text{O}$ [M+H] $^+$ 424.19, found ESI MS m/z 424.19 [M + H] $^+$.

6-(1H-indazol-6-yl)-N-(4-(tetrahydro-1H-furo[3,4-c]pyrrol-5(3H)-yl)phenyl) imidazo[1,2-a]pyrazin-8-amine, 11: ^1H NMR (300 MHz, DMSO- d_6) δ 13.20 (s, 1H), 9.39 (s, 1H), 8.62 (s, 1H), 8.16 (q, J = 1.1 Hz, 1H), 8.06 (t, J = 1.3 Hz, 1H), 7.99 – 7.89 (m, 3H), 7.81 (d, J = 8.5 Hz, 1H), 7.70 (dd, J = 8.6, 1.4 Hz, 1H), 7.61 (d, J = 1.1 Hz, 1H), 6.76 – 6.65 (m, 2H), 3.86 (dd, J = 8.6, 6.6 Hz, 2H), 3.56 (d, J = 3.6 Hz, 1H), 3.53 (d, J = 3.4 Hz, 1H), 3.37 – 3.31 (m, 2H), 3.17 (dd, J = 9.6, 2.7 Hz, 2H), 2.98 (d, J = 5.5 Hz, 2H). m/z calcd for $\text{C}_{25}\text{H}_{23}\text{N}_7\text{O}$ [M+H] $^+$ 438.21, found ESI MS m/z 438.3, [M + H] $^+$.

N-(4-((2S,6R)-2,6-dimethylmorpholino)phenyl)-6-(1H-indazol-6-yl)imidazo[1,2-a]pyrazin-8-amine, 12: ^1H NMR (400 MHz, DMSO- d_6) δ 13.24 – 13.14 (m, 1H), 9.50 (s, 1H), 8.67 (s, 1H), 8.19 (t, J = 1.2 Hz, 1H), 8.09 (t, J = 1.3 Hz, 1H), 8.06 – 8.01 (m, 2H), 7.99 (d, J = 1.1 Hz, 1H), 7.84 (d, J = 8.5 Hz, 1H), 7.72 (dd, J = 8.5, 1.4 Hz, 1H), 7.64 (d, J = 1.1 Hz, 1H), 7.07 – 6.94 (m, 2H), 3.82 – 3.67 (m, 2H), 3.57 (d, J = 11.2 Hz, 2H), 2.26 (dd, J = 11.8, 10.3 Hz, 2H), 1.18 (d, J = 6.2 Hz, 6H). m/z calcd for $\text{C}_{25}\text{H}_{25}\text{N}_7\text{O}$ [M+H] $^+$ 440.22, found ESI MS m/z 440.33 [M + H] $^+$.

4-((6-(1H-indazol-6-yl)imidazo[1,2-a]pyrazin-8-yl)amino)phenylthiomorpholine 1,1-dioxide, 13: ^1H NMR (400 MHz, DMSO- d_6) δ 13.25 – 13.12 (m, 1H), 9.59 (s, 1H), 8.67 (s, 1H), 8.18 (q, J = 1.0 Hz, 1H), 8.10 – 8.05 (m, 3H), 8.00 (d, J = 1.1 Hz, 1H), 7.84 (d, J = 8.5 Hz, 1H), 7.73 (dd, J = 8.6, 1.4 Hz, 1H), 7.65 (d, J = 1.1 Hz, 1H), 7.13 – 7.05 (m, 2H), 3.76 (dd, J = 6.6, 3.6 Hz, 4H), 3.23 – 3.10 (m, 4H). m/z calcd for $\text{C}_{23}\text{H}_{21}\text{N}_7\text{O}_2\text{S}$ [M+H] $^+$ 460.16, found ESI MS m/z 460.25 [M + H] $^+$.

1-((6-(1H-indazol-6-yl)imidazo[1,2-a]pyrazin-8-yl)amino)phenylazetidin-3-ol, 14: ^1H NMR (300 MHz, DMSO- d_6) d 13.18 (s, 1H), 9.38 (s, 1H), 8.63 (s, 1H), 8.17 (s, 1H), 8.08 (s, 1H), 7.97 – 7.93 (m, 3H), 7.83 (d, J = 8.7 Hz, 1H), 7.71 (d, J = 8.7 Hz, 1H), 7.62 (s, 1H), 6.51 (d, J = 8.7 Hz, 2H), 5.58 (d, J = 6.6 Hz, 1H), 4.60 – 4.55 (m, 1H), 4.11 – 4.07 (m, 2H), 3.53 – 3.49 (m, 2H). m/z calcd for $\text{C}_{22}\text{H}_{19}\text{N}_7\text{O}$ [M+H] $^+$ 398.18, found ESI MS m/z 398.3 [M + H] $^+$.

1-(4-((6-(1H-indazol-6-yl)imidazo[1,2-a]pyrazin-8-yl)amino)phenyl)piperidin-4-ol, 15: ^1H NMR (300 MHz, DMSO- d_6) δ 13.19 (s, 1H), 9.48 (s, 1H), 8.66 (s, 1H), 8.20 (m, 1H), 8.09 (m, 1H), 7.99–8.01 (m, 3H), 7.84 (dd, J = 6, 3 Hz, 1H), 7.72 (dd, J = 6, 2 Hz, 1H), 7.64 (d, J = 3 Hz, 1H), 6.99 (d, J = 9 Hz, 2H), 4.68 (d, J = 3 Hz, 1H), 3.63 (m, 1H), 3.52 (m, 2H), 2.83 (dt, J = 6, 2 Hz, 2H), 1.86 (m, 2H), 1.53 (m, 2H). m/z calcd for $\text{C}_{24}\text{H}_{23}\text{N}_7\text{O}$ $[\text{M}+\text{H}]^+$ 426.21, found ESI MS m/z 426.35 $[\text{M} + \text{H}]^+$.

(R)-(4-((6-(1H-indazol-6-yl)imidazo[1,2-a]pyrazin-8-yl)amino)phenyl)morpholin-2-yl)methanol, 16: ^1H NMR (400 MHz, DMSO- d_6) δ 13.19 (s, 1H), 9.52 (s, 1H), 8.67 (s, 1H), 8.19 (s, 1H), 8.08 (d, J = 1.2 Hz, 1H), 8.07 – 8.02 (m, 2H), 7.99 (d, J = 1.1 Hz, 1H), 7.84 (d, J = 8.5 Hz, 1H), 7.72 (dd, J = 8.6, 1.4 Hz, 1H), 7.64 (d, J = 1.1 Hz, 1H), 7.09 – 6.92 (m, 2H), 4.79 (t, J = 5.7 Hz, 1H), 4.01 – 3.91 (m, 1H), 3.67 (td, J = 11.4, 2.6 Hz, 1H), 3.64 – 3.54 (m, 2H), 3.54 – 3.41 (m, 3H), 2.68 (td, J = 11.7, 3.5 Hz, 1H), 2.43 (dd, J = 12.2, 10.6 Hz, 1H). m/z calcd for $\text{C}_{24}\text{H}_{23}\text{N}_7\text{O}_2$ $[\text{M}+\text{H}]^+$ 442.20, found ESI MS m/z 442.71 $[\text{M} + \text{H}]^+$.

(R)-N-(4-(2-((dimethylamino)methyl)morpholino)phenyl)-6-(1H-indazol-6-yl)imidazo[1,2-a]pyrazin-8-amine, 17: ^1H NMR (300 MHz, DMSO- d_6) δ 13.20 (s, 1H), 9.55 (s, 1H), 8.67 (s, 1H), 8.19 (q, J = 1.1 Hz, 1H), 8.10 – 8.01 (m, 3H), 7.99 (d, J = 1.1 Hz, 1H), 7.84 (dd, J = 8.6, 0.7 Hz, 1H), 7.72 (dd, J = 8.5, 1.4 Hz, 1H), 7.64 (d, J = 1.1 Hz, 1H), 7.00 (d, J = 9.2 Hz, 2H), 4.00 – 3.89 (m, 1H), 3.74 – 3.65 (m, 2H), 3.65 – 3.54 (m, 1H), 3.54 – 3.43 (m, 1H), 2.74 – 2.61 (m, 1H), 2.43 – 2.32 (m, 3H), 2.20 (s, 6H). m/z calcd for $\text{C}_{26}\text{H}_{28}\text{N}_8\text{O}$ $[\text{M}+\text{H}]^+$ 469.24, found ESI MS m/z 469.2 $[\text{M} + \text{H}]^+$.

N-(4-(4-ethylpiperazin-1-yl)phenyl)-6-(1H-indazol-6-yl)imidazo[1,2-a]pyrazin-8-amine, 18: ^1H NMR (300 MHz, DMSO- d_6) δ 13.21 (s, 1H), 9.59 (s, 1H), 8.69 (s, 1H), 8.19 (d, J = 3 Hz, 1H), 8.08–8.10 (m, 3H), 8.01 (d, J = 2 Hz, 1H), 7.85 (d, J = 9 Hz, 1H), 7.73 (dd, J = 6, 2 Hz, 1H), 7.65 (d, J = 2 Hz, 1H), 7.09 (d, J = 6 Hz, 2H), 3.81 (m, 2H), 3.6 (m, 2H), 3.21–3.05 (m, 6H), 1.28 (t, J = 6 Hz, 3H). m/z calcd for $\text{C}_{25}\text{H}_{26}\text{N}_8$ $[\text{M}+\text{H}]^+$ 439.24, found ESI MS m/z 439.51 $[\text{M} + \text{H}]^+$.

6-(1H-indazol-6-yl)-N-(4-(4-(2-methoxyethyl)piperazin-1-yl)phenyl)imidazo[1,2-a]pyrazin-8-amine, 19: ^1H NMR (400 MHz, DMSO- d_6) δ 13.25 – 13.03 (m, 1H), 9.50 (s, 1H), 8.66 (s, 1H), 8.19 (d, J = 1.1 Hz, 1H), 8.09 (t, J = 1.3 Hz, 1H), 8.04 – 7.92 (m, 3H), 7.83 (d, J = 8.5 Hz, 1H), 7.72 (dd, J = 8.5, 1.4 Hz, 1H), 7.63 (d, J = 1.1 Hz, 1H), 7.04 – 6.87 (m, 2H), 3.49 (t, J = 5.8 Hz, 2H), 3.26 (s, 3H), 3.12 (t, J = 5.0 Hz, 4H), 2.59 (t, J = 5.0 Hz, 4H), 2.54 (t, J = 5.8 Hz, 2H). m/z calcd for $\text{C}_{26}\text{H}_{28}\text{N}_8\text{O}$ $[\text{M}+\text{H}]^+$ 469.25, found ESI MS m/z 469.46 $[\text{M} + \text{H}]^+$.

6-(1H-indazol-6-yl)-N-(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)imidazo[1,2-a]pyrazin-8-amine, 20: ^1H NMR (300 MHz, DMSO- d_6) δ 13.20 (s, 1H), 9.51 (d, J = 1.6 Hz, 1H), 8.65 (d, J = 2.6 Hz, 1H), 8.16 (s, 1H), 8.06 (s, 1H), 8.03 – 7.93 (m, 3H), 7.81 (d, J = 8.4 Hz, 1H), 7.69 (dd, J = 8.6, 1.4 Hz, 1H), 7.61 (t, J = 0.9 Hz, 1H), 6.98 (d, J = 9.1 Hz, 2H), 4.56 (t, J = 6.5 Hz, 2H), 4.46 (t, J = 6.0 Hz,

2H), 3.52 – 3.40 (m, 1H), 3.13 (d, J = 5.3 Hz, 4H), 2.41 (s, 4H). m/z calcd for $C_{26}H_{26}N_8O$ [M+H]⁺ 467.23, found ESI MS *m/z* 467.2 [M + H]⁺.

N-(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)-6-(1H-pyrazolo[3,4-b]pyridin-6-yl)imidazo[1,2-a]pyrazin-8-amine, 21: ¹H NMR (400 MHz, DMSO-d₆) δ 13.61 (s, 1H), 9.55 (s, 1H), 8.91 (s, 1H), 8.44 – 8.34 (m, 1H), 8.25 – 7.93 (m, 5H), 7.67 (d, J = 1.1 Hz, 1H), 7.17 – 6.90 (m, 2H), 4.54 (dt, J = 36.2, 6.3 Hz, 4H), 3.46 (t, J = 6.3 Hz, 1H), 3.17 (t, J = 5.0 Hz, 4H), 2.43 (t, J = 5.0 Hz, 4H). m/z calcd for $C_{25}H_{25}N_9O$ [M+H]⁺ 468.23, found ESI MS *m/z* 468.3 [M + H]⁺.

N-(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)-6-(1H-pyrazolo[4,3-b]pyridin-6-yl)imidazo[1,2-a]pyrazin-8-amine, 22: ¹H NMR (300 MHz, DMSO-d₆) δ 13.2 (s, 1H), 9.60 (d, J = 2.9 Hz, 1H), 9.13 (q, J = 1.9 Hz, 1H), 8.79 (t, J = 2.0 Hz, 1H), 8.47 (s, 1H), 8.30 (s, 1H), 7.96 (dd, J = 9.9, 2.7 Hz, 3H), 7.64 (d, J = 2.7 Hz, 1H), 6.98 (dd, J = 9.0, 2.8 Hz, 2H), 4.55 (q, J = 4.7, 2.7 Hz, 2H), 4.48 (d, J = 6.0 Hz, 2H), 3.49 – 3.39 (m, 1H), 3.14 (d, J = 5.9 Hz, 4H), 2.42 (d, J = 5.6 Hz, 4H). m/z calcd for $C_{25}H_{25}N_9O$ [M+H]⁺ 468.23, found ESI MS *m/z* 468.2 [M + H]⁺.

N-(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)-6-(1H-pyrazolo[3,4-b]pyridin-6-yl)imidazo[1,2-a]pyrazin-8-amine, 23: ¹H NMR (400 MHz, DMSO-d₆) δ 9.65 (s, 1H), 9.42 (s, 1H), 8.96 (s, 1H), 8.47 (s, 1H), 8.21 (d, J = 1.1 Hz, 1H), 8.02 – 7.90 (m, 2H), 7.69 (d, J = 1.1 Hz, 1H), 7.08 – 6.98 (m, 2H), 4.58 (t, J = 6.5 Hz, 2H), 4.49 (t, J = 6.0 Hz, 2H), 3.46 (t, J = 6.3 Hz, 1H), 3.30 (bs, 1H), 3.18 (t, J = 5.0 Hz, 4H), 2.43 (t, J = 5.0 Hz, 4H); m/z calcd for $C_{24}H_{24}N_{10}O$ [M+H]⁺ 469.22, found ESI MS *m/z* 469.21 [M + H]⁺

6-(1H-benzo[d]imidazol-6-yl)-N-(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)imidazo[1,2-a]pyrazin-8-amine, 24: ¹H NMR (400 MHz, DMSO-d₆) δ 12.55 (d, J = 27.7 Hz, 1H), 9.44 (d, J = 13.6 Hz, 1H), 8.58 (d, J = 3.7 Hz, 1H), 8.26 (d, J = 4.3 Hz, 1H), 8.17 (s, 1H), 8.05 – 7.91 (m, 3H), 7.91 – 7.78 (m, 1H), 7.72 (d, J = 8.5 Hz, 1H), 7.61 (d, J = 8.1 Hz, 1H), 7.07 – 6.96 (m, 2H), 4.53 (dt, J = 36.3, 6.3 Hz, 4H), 3.46 (p, J = 6.4 Hz, 1H), 3.16 (t, J = 5.0 Hz, 4H), 2.43 (d, J = 5.3 Hz, 4H). m/z calcd for $C_{26}H_{26}N_8O$ [M+H]⁺ 467.23, found ESI MS *m/z* 467.2 [M + H]⁺.

6-(3H-imidazo[4,5-b]pyridin-5-yl)-N-(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)imidazo[1,2-a]pyrazin-8-amine, 25: ¹H NMR (400 MHz, DMSO-d₆) δ 13.06 (s, 1H), 9.49 (s, 1H), 8.87 (s, 1H), 8.48 (s, 1H), 8.16 (dd, J = 2.6, 1.5 Hz, 3H), 8.01 (d, J = 9.0 Hz, 2H), 7.64 (d, J = 1.1 Hz, 1H), 7.02 (d, J = 9.1 Hz, 2H), 4.58 (t, J = 6.5 Hz, 2H), 4.49 (t, J = 6.1 Hz, 2H), 3.48 (q, J = 6.2 Hz, 1H), 3.17 (t, J = 5.0 Hz, 4H), 2.44 (t, J = 5.0 Hz, 4H). m/z calcd for $C_{25}H_{25}N_9O$ [M+H]⁺ 468.23, found ESI MS *m/z* 468.09 [M + H]⁺.

6-(1H-imidazo[4,5-b]pyridin-6-yl)-N-(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)imidazo[1,2-a]pyrazin-8-amine, 26: ¹H NMR (400 MHz, DMSO-d₆) δ 12.99 (d, *J* = 138.8 Hz, 1H), 9.53 (s, 1H), 9.00 (s, 1H), 8.68 (s, 1H), 8.49 (m, 2H), 8.03 – 7.88 (m, 3H), 7.65 (d, *J* = 1.2 Hz, 1H), 7.05 – 6.90 (m, 2H), 4.58 (t, *J* = 6.5 Hz, 2H), 4.48 (t, *J* = 6.0 Hz, 2H), 3.46 (p, *J* = 6.3 Hz, 1H), 3.16 (t, *J* = 5.0 Hz, 4H), 2.43 (t, *J* = 5.0 Hz, 4H). m/z calcd for C₂₅H₂₅N₉O [M+H]⁺ 468.23, found ESI+ (m/z): 468.36 [M+H]⁺.

6-(1H-imidazo[4,5-b]pyrazin-6-yl)-N-(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)imidazo[1,2-a]pyrazin-8-amine, 27: ¹H NMR (300 MHz, DMSO-d₆) δ 13.2 (s, 1H), 9.58 (s, 1H), 9.20 (s, 1H), 8.82 (s, 1H), 8.76 (s, 1H), 8.15 (s, 1H), 7.99 (d, *J* = 9.1 Hz, 2H), 7.62 (s, 1H), 7.03 (d, *J* = 1.1 Hz, 2H), 4.57 (t, *J* = 6.5 Hz, 2H), 4.48 (t, *J* = 6.1 Hz, 2H), 3.50-3.42 (m, 1H), 3.16 (t, *J* = 5.0 Hz, 4H), 2.42 (t, *J* = 4.9 Hz, 4H). m/z calcd for C₂₄H₂₄N₁₀O [M+H]⁺ 469.22, found ESI MS *m/z* 469.25 [M + H]⁺.

6-(1H-indol-6-yl)-N-(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)imidazo[1,2-a]pyrazin-8-amine, 28: ¹H NMR (400 MHz, DMSO-d₆) δ 11.26 (s, 1H), 9.42 (s, 1H), 8.52 (s, 1H), 8.13 – 8.03 (m, 3H), 7.97 (d, *J* = 1.1 Hz, 1H), 7.61 (m, 3H), 7.41 (dd, *J* = 3.0, 2.4 Hz, 1H), 7.04 – 6.92 (m, 2H), 6.46 (ddd, *J* = 3.0, 1.9, 0.9 Hz, 1H), 4.58 (t, *J* = 6.5 Hz, 2H), 4.49 (t, *J* = 6.0 Hz, 2H), 3.46 (p, *J* = 6.3 Hz, 1H), 3.16 (t, *J* = 5.0 Hz, 4H), 2.43 (t, *J* = 5.0 Hz, 4H). m/z calcd for C₂₇H₂₇N₇O [M+H]⁺ 466.24, found ESI MS *m/z* 466.3 [M + H]⁺.

N-(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)-6-(1H-pyrrolo[2,3-b]pyridin-6-yl)imidazo[1,2-a]pyrazin-8-amine, 29: ¹H NMR (400 MHz, DMSO-d₆) δ 11.60 (s, 1H), 9.46 (s, 1H), 8.79 (s, 1H), 8.26 – 7.91 (m, 5H), 7.64 (d, *J* = 1.1 Hz, 1H), 7.52 (dd, *J* = 3.4, 2.5 Hz, 1H), 7.10 – 6.89 (m, 2H), 6.49 (dd, *J* = 3.4, 1.8 Hz, 1H), 4.54 (dt, *J* = 36.0, 6.3 Hz, 4H), 3.46 (p, *J* = 6.3 Hz, 1H), 3.16 (t, *J* = 4.9 Hz, 4H), 2.43 (t, *J* = 5.0 Hz, 4H). m/z calcd for C₂₆H₂₆N₈O [M+H]⁺ 467.23, found ESI MS *m/z* 467.3 [M + H]⁺.

N-(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)-6-(1H-pyrrolo[3,2-b]pyridin-6-yl)imidazo[1,2-a]pyrazin-8-amine, 30: ¹H NMR (300 MHz, DMSO-d₆) δ 11.53 (s, 1H), 9.50 (s, 1H), 8.96 (d, *J* = 2 Hz, 1H), 8.66 (s, 1H), 8.32 (dd, *J* = 2, 1 Hz, 1H), 7.98-8.02 (m, 3H), 7.70 (t, *J* = 3 Hz, 1H), 7.63 (d, *J* = 3 Hz, 1H), 7.00 (m, 2H), 6.59 (m, 1H), 4.58 (t, *J* = 6 Hz, 2H), 4.49 (t, *J* = 6 Hz, 2H), 3.46 (m, 1H), 3.16 (m, 4H), 2.43 (m, 4H). m/z calcd for C₂₆H₂₆N₈O [M+H]⁺ 467.23, found ESI MS *m/z* 467.2 [M + H]⁺.

N-(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)-6-(5H-pyrrolo[2,3-b]pyrazin-3-yl)imidazo[1,2-a]pyrazin-8-amine, 31: ¹H NMR (400 MHz, DMSO-d₆) δ 12.03 (s, 1H), 9.56 (s, 1H), 9.25 (s, 1H), 8.79 (s, 1H), 8.17 (d, *J* = 1.1 Hz, 1H), 8.04 – 7.85 (m, 3H), 7.66 (d, *J* = 1.1 Hz, 1H), 7.09 – 6.97 (m, 2H), 6.67 (dd, *J* = 3.6, 1.5 Hz, 1H), 4.54 (dt, *J* = 35.4, 6.3 Hz, 4H), 3.46 (t, *J* = 6.3 Hz, 1H), 3.18 (t, *J* = 5.0 Hz, 4H), 2.43 (t, *J* = 5.0 Hz, 4H). m/z calcd for C₂₅H₂₅N₉O [M+H]⁺ 468.23, found ESI MS *m/z* 468.3 [M + H]⁺.

6-(3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-N-(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)imidazo[1,2-a]pyrazin-8-amine, 32: ¹H NMR (300 MHz, DMSO-d₆) M¹H NMR (300 MHz, DMSO-d₆) δ 9.36 (s, 1H), 8.31 (s, 1H), 8.06 – 7.97 (m, 2H), 7.94 (d, *J* = 1.1 Hz,

1H), 7.58 (d, J = 1.1 Hz, 1H), 7.23 (d, J = 2.1 Hz, 1H), 7.07 (dd, J = 8.3, 2.1 Hz, 1H), 7.01 – 6.92 (m, 2H), 6.72 (d, J = 8.3 Hz, 1H), 5.93 (s, 1H), 4.57 (t, J = 6.5 Hz, 2H), 4.48 (t, J = 6.0 Hz, 2H), 4.16 (dd, J = 5.1, 3.5 Hz, 2H), 3.50 – 3.40 (m, 1H), 3.36 – 3.32 (m, 2H), 3.14 (dd, J = 6.4, 3.6 Hz, 4H), 2.42 (t, J = 5.0 Hz, 4H). m/z calcd for $C_{27}H_{29}N_7O_2$ [M+H]⁺ 484.25, found ESI MS m/z 484.2 [M + H]⁺.

6-(3,4-dihydro-2H-pyrido[3,2-b][1,4]oxazin-6-yl)-N-(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)imidazo[1,2-a]pyrazin-8-amine, 33: 1H NMR (400 MHz, DMSO-*d*₆) δ 9.38 (s, 1H), 8.48 (s, 1H), 8.11 (d, J = 1.0 Hz, 1H), 8.00 – 7.92 (m, 2H), 7.60 (d, J = 1.1 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.06 (d, J = 8.0 Hz, 1H), 7.01 – 6.94 (m, 2H), 6.70 (s, 1H), 4.58 (t, J = 6.5 Hz, 2H), 4.48 (t, J = 6.0 Hz, 2H), 4.16 (t, J = 4.4 Hz, 2H), 3.46 (t, J = 6.2 Hz, 3H), 3.14 (t, J = 5.0 Hz, 4H), 2.42 (t, J = 5.0 Hz, 4H). m/z calcd for $C_{26}H_{28}N_8O_2$ [M+H]⁺ 485.24, found ESI MS m/z 485.32 [M + H]⁺.

6-(2,3-dihydro-1H-pyrido[2,3-b][1,4]oxazin-7-yl)-N-(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)imidazo[1,2-a]pyrazin-8-amine, 34: 1H NMR (300 MHz, DMSO-*d*₆) δ 9.45 (s, 1H), 8.41 (s, 1H), 8.04 – 7.80 (m, 4H), 7.60 (d, J = 1.1 Hz, 1H), 7.45 (d, J = 2.2 Hz, 1H), 7.05 – 6.86 (m, 2H), 6.25 (s, 1H), 4.57 (t, J = 6.5 Hz, 2H), 4.48 (t, J = 6.1 Hz, 2H), 4.36 – 4.23 (m, 2H), 3.51 – 3.40 (m, 1H), 3.37 – 3.29 (m, 2H), 3.14 (t, J = 5.0 Hz, 4H), 2.42 (dd, J = 6.1, 3.8 Hz, 4H). m/z calcd for $C_{26}H_{28}N_8O_2$ [M+H]⁺ 485.24, found ESI MS m/z 485.2 [M + H]⁺.

6-(3,4-dihydro-2H-pyrazino[2,3-b][1,4]oxazin-6-yl)-N-(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)imidazo[1,2-a]pyrazin-8-amine, 35: 1H NMR (400 MHz, DMSO-*d*₆) δ 9.46 (s, 1H), 8.43 (s, 1H), 8.12 (d, J = 1.1 Hz, 1H), 8.09 (s, 1H), 7.94 (d, J = 9.0 Hz, 2H), 7.61 (d, J = 1.0 Hz, 1H), 7.37 (s, 1H), 6.99 (d, J = 9.1 Hz, 2H), 4.57 (t, J = 6.5 Hz, 2H), 4.48 (t, J = 6.1 Hz, 2H), 4.36 (t, J = 4.5 Hz, 2H), 3.46 (dd, J = 12.6, 6.1 Hz, 3H), 3.16 – 3.13 (m, 4H), 2.42 (t, J = 5.0 Hz, 4H). m/z calcd for $C_{25}H_{27}N_9O_2$ [M+H]⁺ 486.24, found ESI MS m/z 486.37 [M + H]⁺.

6-(3-aminophenyl)-N-(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)imidazo[1,2-a]pyrazin-8-amine, 36: 1H NMR (400 MHz, DMSO-*d*₆) δ 9.38 (s, 1H), 8.38 (s, 1H), 8.06 – 7.99 (m, 2H), 7.97 (d, J = 1.1 Hz, 1H), 7.60 (d, J = 1.1 Hz, 1H), 7.27 – 7.18 (m, 1H), 7.14 – 7.07 (m, 2H), 7.03 – 6.94 (m, 2H), 6.58 (dt, J = 5.7, 2.6 Hz, 1H), 5.18 (s, 2H), 4.57 (t, J = 6.5 Hz, 2H), 4.48 (t, J = 6.0 Hz, 2H), 3.46 (p, J = 6.3 Hz, 1H), 3.14 (t, J = 5.0 Hz, 4H), 2.42 (t, J = 5.0 Hz, 4H). m/z calcd for $C_{25}H_{27}N_7O$ [M+H]⁺ 442.24, found ESI MS m/z 442.46 [M + H]⁺.

6-(6-aminopyridin-2-yl)-N-(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)imidazo[1,2-a]pyrazin-8-amine, 37: 1H NMR (300 MHz, DMSO-*d*₆) δ 9.40 (s, 1H), 8.61 (s, 1H), 8.10 (d, J = 1.2 Hz, 1H), 7.96 (d, J = 9.1 Hz, 2H), 7.60 (d, J = 1.2 Hz, 1H), 7.50 (dd, J = 8.1, 7.4 Hz, 1H), 7.37 (dd, J = 7.4, 0.9 Hz, 1H), 6.97 (d, J = 9.1 Hz, 2H), 6.46 (dd, J = 8.2, 0.9 Hz, 1H), 5.91 (s, 2H), 4.56 (t, J = 6.5 Hz,

2H), 4.47 (t, $J = 6.1$ Hz, 2H), 3.48 – 3.39 (m, 1H), 3.18 – 3.09 (m, 4H), 2.41 (t, $J = 5.0$ Hz, 4H). m/z calcd for $C_{24}H_{26}N_8O$ [M+H]⁺ 443.23, found ESI MS *m/z* 443.20 [M + H]⁺.

6-(5-aminopyridin-3-yl)-N-(4-(4-(oxetan-3-yl)piperazin-1-yl)phenyl)imidazo[1,2-a]pyrazin-8-amine, 38: 1H NMR (400 MHz, DMSO-d6) δ 9.48 (s, 1H), 8.50 (s, 1H), 8.33 (d, $J = 1.9$ Hz, 1H), 8.11 – 7.90 (m, 4H), 7.63 (d, $J = 1.1$ Hz, 1H), 7.47 (dd, $J = 2.6, 1.9$ Hz, 1H), 7.08 – 6.84 (m, 2H), 5.47 (s, 2H), 4.53 (dt, $J = 36.4, 6.3$ Hz, 4H), 3.45 (p, $J = 6.3$ Hz, 1H), 3.14 (dd, $J = 6.3, 3.7$ Hz, 4H), 2.42 (t, $J = 5.0$ Hz, 4H). m/z calcd for $C_{24}H_{26}N_8O$ [M+H]⁺ 443.23, found ESI MS *m/z* 443.17 [M + H]⁺.

LogD and Metabolic Stability

Figure S1. LogD values of compounds in Tables 1 and 2

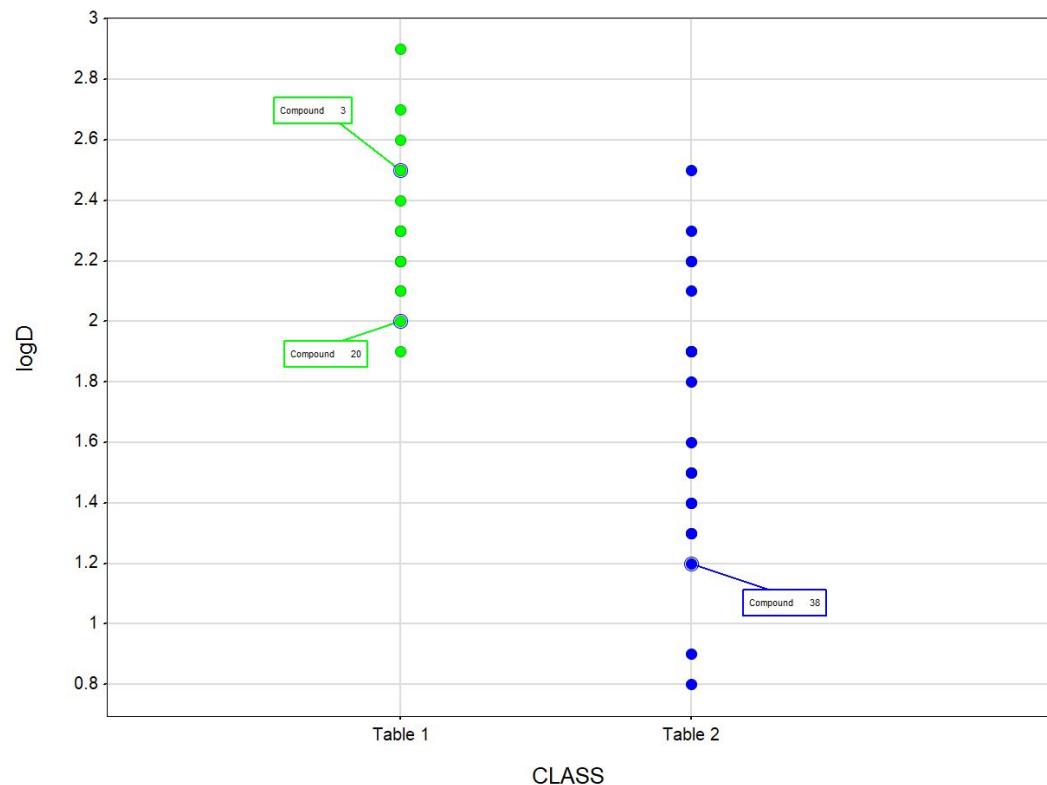


Figure S2. Log D and predicted clearance in human liver microsomes (green = compounds in Table 1, blue = compounds in Table 2)

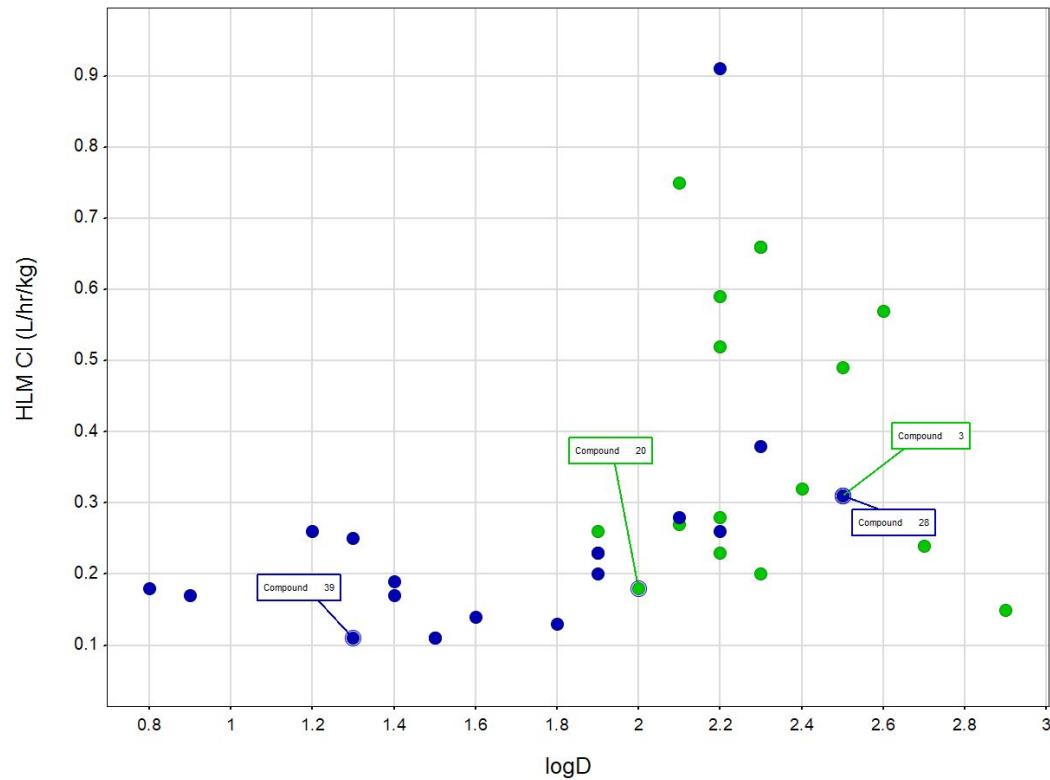
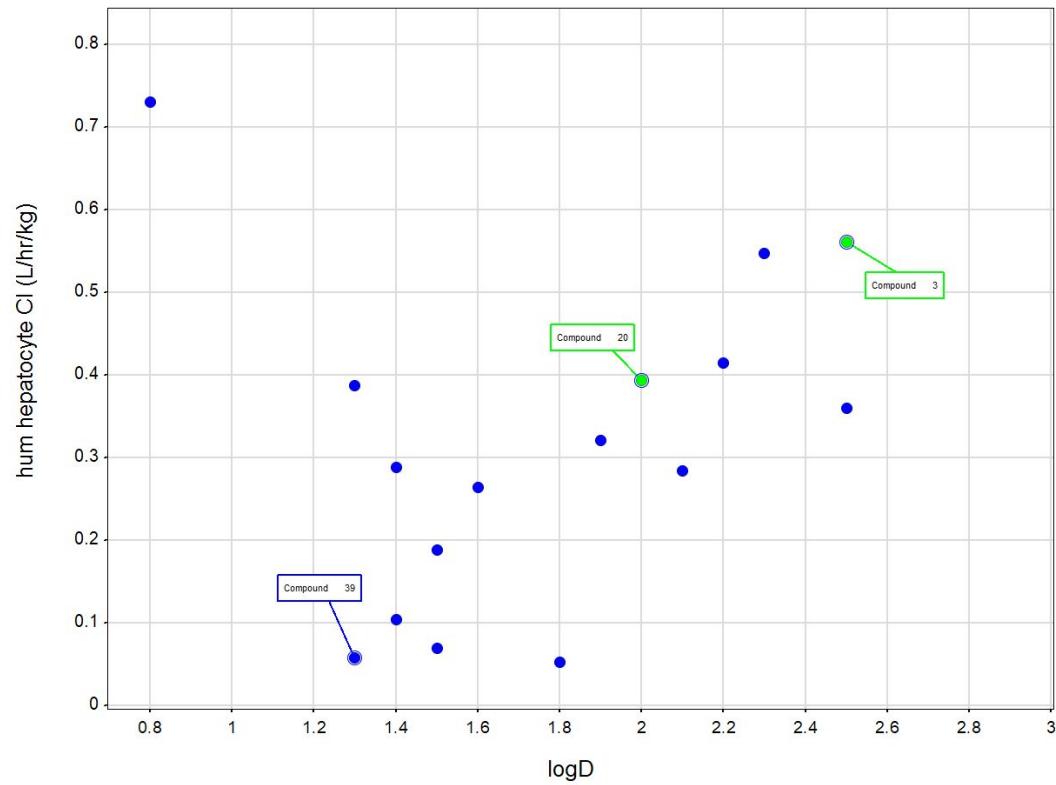


Figure S3. LogD and predicted clearance in human hepatocytes (green = compounds in Table 1, blue = compounds in Table 2)



Crystallography

SYK protein expression and purification

The SYK kinase domain (residues 363-637) was expressed and purified in a similar method as reported¹. In short, the protein was expressed in the Hi5 protein expression system according to standard protocols. Harvested cell pellet (50g) was homogenized and lysed in lysis buffer (50 mM Tris pH 8.0, 150mM NaCl, 100mM L-arginine, 10% glycerol, 0.1% NP40, 1ul/50ml benzonase). The lysate was loaded onto a 5 ml Ni-HP column (GE lifescience) and then washed with buffer A (50mM Tris, pH 8.0,150mM NaCl, 10% glycerol, and 10mM methionine). Bound proteins were then eluted from the column using 0-100% gradient of buffer B (50 mM Tris pH 8.0, 150mM NaCl, 10% glycerol, 500mM imidazole, and 10mM methionine). The fractions containing SYK protein were pooled and dialyzed against buffer C (50mM Tris pH 8.0, 150 mM NaCl, 10% glycerol, 10mM methionine) overnight at 4°C. The dialysate was then centrifuged at 20,000 rpm for 30 minutes and the supernatant was injected into a size exclusion chromatography column (S200) pre-equilibrated with a buffer containing 10mM HEPES pH 8.0, 150mM NaCl, 5mM DTT, 10mM methionine, and 10% glycerol. Fractions containing SYK protein were pooled and concentrated to 11 mg/ml for crystallization trials. The final sample purity was determined as 98% by capillary electrophoresis technique (Caliper Bioscience).

Crystallization, data collection, structure determination

Compound **39** was added to the purified SYK protein at a final concentration of 0.6mM. Crystals were grown by hanging drop vapor diffusion by mixing equal parts of protein and reservoir solution containing 20% PEG 3350 and 100mM Tris pH 8.5 at 20°C. Data was collected at a temperature of 100K at The Advanced Light Source and processed with HKL2000².

Molecular replacement of SYK was performed with the refinement software package Phenix³ using the starting model PDB code 1XBA. Rigid body refinement, simulated annealing, energy minimization, and B-factor refinement were likewise carried out with Phenix. Bulk solvent correction and anisotropic B-factor scaling were used during refinement. Model building was performed with the molecular graphics program Coot⁴. The final model statistics are listed in Table S1.

Table S1. Data collection and refinement statistics for X-ray structure of **Compound 39**.

Syk with Compound 39	
X-ray source	ALS BL 5.0.2
Space Group	<i>P</i> 1
Unit Cell (a, b, c in Å and α, β, γ in °)	40.0, 42.1, 87.6, 81.0, 90.3, 79.3
Resolution (Å)	50-1.95 (1.99-1.95)
No. of reflections	85,949
No. unique	38,088
<i>I</i> / <i>σ</i>	13.8 (1.5)
<i>R</i> _{merge} ^a (%)	5.7 (47.9)
Completeness (%)	94.6 (81.3)
Refinement Statistics	
Resolution (Å)	35-1.95
No. reflections (<i>F</i> ≥0)	30,215
<i>R</i> -factor ^b	21.9
<i>R</i> -free ^b	28.7
RMS bond lengths (Å)	0.007
RMS bond angles (°)	1.06

^a $R_{\text{merge}} = [\sum h \sum i |I_h - \bar{I}_h| / \sum h \sum i \bar{I}_h]$ where I_h is the mean of I_{hi} observations of reflection h . Numbers in parenthesis represent highest resolution shell.

^b R -factor and R -free = $\sum |F_{\text{obs}}| - |F_{\text{calc}}| / \sum |F_{\text{obs}}| \times 100$ for 90% of recorded data (R -factor) or 10% of data (R -free).

Kinase selectivity

Table S2. Kinase selectivity of compound **39** using KINOMEscan™ profiling services by DiscoveRx. Data is presented as % of control binding at 1 uM, where lower values indicate stronger binding.

DiscoveRx Gene Symbol	Entrez Gene Symbol	% Control
AAK1	AAK1	37
ABL1(E255K)-phosphorylated	ABL1	29
ABL1(F317I)-nonphosphorylated	ABL1	72
ABL1(F317I)-phosphorylated	ABL1	60
ABL1(F317L)-nonphosphorylated	ABL1	77
ABL1(F317L)-phosphorylated	ABL1	39
ABL1(H396P)-nonphosphorylated	ABL1	6.4
ABL1(H396P)-phosphorylated	ABL1	53
ABL1(M351T)-phosphorylated	ABL1	35
ABL1(Q252H)-nonphosphorylated	ABL1	26
ABL1(Q252H)-phosphorylated	ABL1	24
ABL1(T315I)-nonphosphorylated	ABL1	74
ABL1(T315I)-phosphorylated	ABL1	57
ABL1(Y253F)-phosphorylated	ABL1	31
ABL1-nonphosphorylated	ABL1	40
ABL1-phosphorylated	ABL1	33
ABL2	ABL2	74
ACVR1	ACVR1	97
ACVR1B	ACVR1B	67
ACVR2A	ACVR2A	100
ACVR2B	ACVR2B	76

ACVRL1	ACVRL1	100
ADCK3	CABC1	100
ADCK4	ADCK4	66
AKT1	AKT1	79
AKT2	AKT2	93
AKT3	AKT3	94
ALK	ALK	78
ALK(C1156Y)	ALK	43
ALK(L1196M)	ALK	100
AMPK-alpha1	PRKAA1	85
AMPK-alpha2	PRKAA2	100
ANKK1	ANKK1	61
ARK5	NUAK1	100
ASK1	MAP3K5	100
ASK2	MAP3K6	62
AURKA	AURKA	28
AURKB	AURKB	76
AURKC	AURKC	74
AXL	AXL	63
BIKE	BMP2K	71
BLK	BLK	18
BMPR1A	BMPR1A	74
BMPR1B	BMPR1B	51
BMPR2	BMPR2	100
BMX	BMX	97
BRAF	BRAF	88
BRAF(V600E)	BRAF	75
BRK	PTK6	61
BRSK1	BRSK1	98
BRSK2	BRSK2	98

BTK	BTK	45
BUB1	BUB1	93
CAMK1	CAMK1	76
CAMK1D	CAMK1D	87
CAMK1G	CAMK1G	70
CAMK2A	CAMK2A	75
CAMK2B	CAMK2B	81
CAMK2D	CAMK2D	76
CAMK2G	CAMK2G	76
CAMK4	CAMK4	100
CAMKK1	CAMKK1	86
CAMKK2	CAMKK2	88
CASK	CASK	74
CDC2L1	CDK11B	100
CDC2L2	CDC2L2	96
CDC2L5	CDK13	81
CDK11	CDK19	100
CDK2	CDK2	100
CDK3	CDK3	89
CDK4-cyclinD1	CDK4	91
CDK4-cyclinD3	CDK4	92
CDK5	CDK5	92
CDK7	CDK7	99
CDK8	CDK8	83
CDK9	CDK9	99
CDKL1	CDKL1	99
CDKL2	CDKL2	76
CDKL3	CDKL3	96
CDKL5	CDKL5	96
CHEK1	CHEK1	100

CHEK2	CHEK2	82
CIT	CIT	100
CLK1	CLK1	74
CLK2	CLK2	62
CLK3	CLK3	67
CLK4	CLK4	88
CSF1R	CSF1R	43
CSF1R-autoinhibited	CSF1R	29
CSK	CSK	74
CSNK1A1	CSNK1A1	82
CSNK1A1L	CSNK1A1L	92
CSNK1D	CSNK1D	100
CSNK1E	CSNK1E	84
CSNK1G1	CSNK1G1	85
CSNK1G2	CSNK1G2	90
CSNK1G3	CSNK1G3	72
CSNK2A1	CSNK2A1	9.6
CSNK2A2	CSNK2A2	1.6
CTK	MATK	62
DAPK1	DAPK1	48
DAPK2	DAPK2	57
DAPK3	DAPK3	55
DCAMKL1	DCLK1	76
DCAMKL2	DCLK2	94
DCAMKL3	DCLK3	100
DDR1	DDR1	72
DDR2	DDR2	78
DLK	MAP3K12	74
DMPK	DMPK	99
DMPK2	CDC42BPG	87

DRAK1	STK17A	92
DRAK2	STK17B	90
DYRK1A	DYRK1A	98
DYRK1B	DYRK1B	99
DYRK2	DYRK2	74
EGFR	EGFR	75
EGFR(E746-A750del)	EGFR	54
EGFR(G719C)	EGFR	88
EGFR(G719S)	EGFR	83
EGFR(L747-E749del, A750P)	EGFR	58
EGFR(L747-S752del, P753S)	EGFR	57
EGFR(L747-T751del,Sins)	EGFR	69
EGFR(L858R)	EGFR	70
EGFR(L858R,T790M)	EGFR	29
EGFR(L861Q)	EGFR	82
EGFR(S752-I759del)	EGFR	74
EGFR(T790M)	EGFR	47
EIF2AK1	EIF2AK1	83
EPHA1	EPHA1	26
EPHA2	EPHA2	100
EPHA3	EPHA3	55
EPHA4	EPHA4	100
EPHA5	EPHA5	82
EPHA6	EPHA6	94
EPHA7	EPHA7	75
EPHA8	EPHA8	100
EPHB1	EPHB1	59
EPHB2	EPHB2	100
EPHB3	EPHB3	96
EPHB4	EPHB4	79

EPHB6	EPHB6	3.8
ERBB2	ERBB2	88
ERBB3	ERBB3	100
ERBB4	ERBB4	81
ERK1	MAPK3	91
ERK2	MAPK1	89
ERK3	MAPK6	76
ERK4	MAPK4	100
ERK5	MAPK7	93
ERK8	MAPK15	74
ERN1	ERN1	71
FAK	PTK2	50
FER	FER	45
FES	FES	68
FGFR1	FGFR1	62
FGFR2	FGFR2	87
FGFR3	FGFR3	100
FGFR3(G697C)	FGFR3	80
FGFR4	FGFR4	93
FGR	FGR	53
FLT1	FLT1	90
FLT3	FLT3	57
FLT3(D835H)	FLT3	35
FLT3(D835Y)	FLT3	27
FLT3(ITD)	FLT3	52
FLT3(K663Q)	FLT3	56
FLT3(N841I)	FLT3	44
FLT3(R834Q)	FLT3	74
FLT3-autoinhibited	FLT3	92
FLT4	FLT4	62

FRK	FRK	92
FYN	FYN	74
GAK	GAK	47
GCN2(Kin.Dom.2,S808G)	EIF2AK4	94
GRK1	GRK1	92
GRK4	GRK4	100
GRK7	GRK7	96
GSK3A	GSK3A	97
GSK3B	GSK3B	94
HASPIN	GSG2	94
HCK	HCK	33
HIPK1	HIPK1	81
HIPK2	HIPK2	79
HIPK3	HIPK3	82
HIPK4	HIPK4	64
HPK1	MAP4K1	86
HUNK	HUNK	76
ICK	ICK	78
IGF1R	IGF1R	79
IKK-alpha	CHUK	78
IKK-beta	IKBKB	92
IKK-epsilon	IKBKE	81
INSR	INSR	52
INSRR	INSRR	78
IRAK1	IRAK1	2.2
IRAK3	IRAK3	13
IRAK4	IRAK4	83
ITK	ITK	65
JAK1(JH1domain-catalytic)	JAK1	60
JAK1(JH2domain-pseudokinase)	JAK1	0.25

JAK2(JH1domain-catalytic)	JAK2	1.2
JAK3(JH1domain-catalytic)	JAK3	17
JNK1	MAPK8	29
JNK2	MAPK9	66
JNK3	MAPK10	35
KIT	KIT	24
KIT(A829P)	KIT	54
KIT(D816H)	KIT	71
KIT(D816V)	KIT	59
KIT(L576P)	KIT	7.8
KIT(V559D)	KIT	15
KIT(V559D,T670I)	KIT	62
KIT(V559D,V654A)	KIT	70
KIT-autoinhibited	KIT	71
LATS1	LATS1	91
LATS2	LATS2	100
LCK	LCK	60
LIMK1	LIMK1	92
LIMK2	LIMK2	100
LKB1	STK11	100
LOK	STK10	68
LRRK2	LRRK2	63
LRRK2(G2019S)	LRRK2	62
LTK	LTK	45
LYN	LYN	94
LZK	MAP3K13	69
MAK	MAK	91
MAP3K1	MAP3K1	93
MAP3K15	MAP3K15	88
MAP3K2	MAP3K2	69

MAP3K3	MAP3K3	70
MAP3K4	MAP3K4	87
MAP4K2	MAP4K2	54
MAP4K3	MAP4K3	88
MAP4K4	MAP4K4	82
MAP4K5	MAP4K5	80
MAPKAPK2	MAPKAPK2	92
MAPKAPK5	MAPKAPK5	83
MARK1	MARK1	95
MARK2	MARK2	76
MARK3	MARK3	96
MARK4	MARK4	96
MAST1	MAST1	88
MEK1	MAP2K1	83
MEK2	MAP2K2	74
MEK3	MAP2K3	69
MEK4	MAP2K4	99
MEK5	MAP2K5	12
MEK6	MAP2K6	85
MELK	MELK	47
MERTK	MERTK	39
MET	MET	94
MET(M1250T)	MET	79
MET(Y1235D)	MET	85
MINK	MINK1	69
MKK7	MAP2K7	85
MKNK1	MKNK1	70
MKNK2	MKNK2	72
MLCK	MYLK3	100
MLK1	MAP3K9	55

MLK2	MAP3K10	48
MLK3	MAP3K11	41
MRCKA	CDC42BPA	98
MRCKB	CDC42BPB	100
MST1	STK4	84
MST1R	MST1R	83
MST2	STK3	84
MST3	STK24	100
MST4	MST4	93
MTOR	MTOR	100
MUSK	MUSK	96
MYLK	MYLK	74
MYLK2	MYLK2	94
MYLK4	MYLK4	81
MYO3A	MYO3A	82
MYO3B	MYO3B	98
NDR1	STK38	98
NDR2	STK38L	97
NEK1	NEK1	79
NEK10	NEK10	100
NEK11	NEK11	86
NEK2	NEK2	82
NEK3	NEK3	63
NEK4	NEK4	64
NEK5	NEK5	52
NEK6	NEK6	91
NEK7	NEK7	100
NEK9	NEK9	100
NIK	MAP3K14	72
NIM1	MGC42105	89

NLK	NLK	100
OSR1	OXSR1	99
p38-alpha	MAPK14	99
p38-beta	MAPK11	82
p38-delta	MAPK13	84
p38-gamma	MAPK12	76
PAK1	PAK1	78
PAK2	PAK2	69
PAK3	PAK3	61
PAK4	PAK4	66
PAK6	PAK6	96
PAK7	PAK7	61
PCTK1	CDK16	99
PCTK2	CDK17	94
PCTK3	CDK18	86
PDGFRA	PDGFRA	88
PDGFRB	PDGFRB	63
PDPK1	PDPK1	72
PFCDPK1(<i>P.falciparum</i>)	CDPK1	76
PFPK5(<i>P.falciparum</i>)	MAL13P1.279	84
PFTAIRE2	CDK15	100
PFTK1	CDK14	79
PHKG1	PHKG1	97
PHKG2	PHKG2	93
PIK3C2B	PIK3C2B	83
PIK3C2G	PIK3C2G	82
PIK3CA	PIK3CA	81
PIK3CA(C420R)	PIK3CA	88
PIK3CA(E542K)	PIK3CA	98
PIK3CA(E545A)	PIK3CA	81

PIK3CA(E545K)	PIK3CA	93
PIK3CA(H1047L)	PIK3CA	80
PIK3CA(H1047Y)	PIK3CA	76
PIK3CA(I800L)	PIK3CA	89
PIK3CA(M1043I)	PIK3CA	69
PIK3CA(Q546K)	PIK3CA	92
PIK3CB	PIK3CB	93
PIK3CD	PIK3CD	92
PIK3CG	PIK3CG	78
PIK4CB	PI4KB	12
PIM1	PIM1	84
PIM2	PIM2	63
PIM3	PIM3	66
PIP5K1A	PIP5K1A	52
PIP5K1C	PIP5K1C	56
PIP5K2B	PIP4K2B	87
PIP5K2C	PIP4K2C	49
PKAC-alpha	PRKACA	96
PKAC-beta	PRKACB	94
PKMYT1	PKMYT1	97
PKN1	PKN1	74
PKN2	PKN2	95
PKNB(M.tuberculosis)	pknB	100
PLK1	PLK1	93
PLK2	PLK2	91
PLK3	PLK3	76
PLK4	PLK4	48
PRKCD	PRKCD	64
PRKCE	PRKCE	91
PRKCH	PRKCH	100

PRKCI	PRKCI	100
PRKCQ	PRKCQ	84
PRKD1	PRKD1	100
PRKD2	PRKD2	98
PRKD3	PRKD3	100
PRKG1	PRKG1	89
PRKG2	PRKG2	97
PRKR	EIF2AK2	97
PRKX	PRKX	72
PRP4	PRPF4B	100
PYK2	PTK2B	71
QSK	KIAA0999	74
RAF1	RAF1	100
RET	RET	77
RET(M918T)	RET	69
RET(V804L)	RET	86
RET(V804M)	RET	98
RIOK1	RIOK1	83
RIOK2	RIOK2	63
RIOK3	RIOK3	80
RIPK1	RIPK1	55
RIPK2	RIPK2	97
RIPK4	RIPK4	58
RIPK5	DSTYK	92
ROCK1	ROCK1	89
ROCK2	ROCK2	78
ROS1	ROS1	29
RPS6KA4(Kin.Dom.1-N-terminal)	RPS6KA4	99
RPS6KA4(Kin.Dom.2-C-terminal)	RPS6KA4	92
RPS6KA5(Kin.Dom.1-N-terminal)	RPS6KA5	94

RPS6KA5(Kin.Dom.2-C-terminal)	RPS6KA5	100
RSK1(Kin.Dom.1-N-terminal)	RPS6KA1	81
RSK1(Kin.Dom.2-C-terminal)	RPS6KA1	84
RSK2(Kin.Dom.1-N-terminal)	RPS6KA3	82
RSK2(Kin.Dom.2-C-terminal)	RPS6KA3	100
RSK3(Kin.Dom.1-N-terminal)	RPS6KA2	96
RSK3(Kin.Dom.2-C-terminal)	RPS6KA2	91
RSK4(Kin.Dom.1-N-terminal)	RPS6KA6	87
RSK4(Kin.Dom.2-C-terminal)	RPS6KA6	97
S6K1	RPS6KB1	83
SBK1	SBK1	98
SGK	SGK1	96
SgK110	SgK110	100
SGK2	SGK2	92
SGK3	SGK3	93
SIK	SIK1	85
SIK2	SIK2	98
SLK	SLK	22
SNARK	NUAK2	100
SNRK	SNRK	85
SRC	SRC	4.6
SRMS	SRMS	66
SRPK1	SRPK1	62
SRPK2	SRPK2	98
SRPK3	SRPK3	88
STK16	STK16	32
STK33	STK33	93
STK35	STK35	87
STK36	STK36	93
STK39	STK39	79

SYK	SYK	0.8
TAK1	MAP3K7	85
TAOK1	TAOK1	92
TAOK2	TAOK2	64
TAOK3	TAOK3	87
TBK1	TBK1	75
TEC	TEC	96
TESK1	TESK1	75
TGFBR1	TGFBR1	84
TGFBR2	TGFBR2	100
TIE1	TIE1	93
TIE2	TEK	94
TLK1	TLK1	82
TLK2	TLK2	93
TNIK	TNIK	65
TNK1	TNK1	54
TNK2	TNK2	48
TNNI3K	TNNI3K	85
TRKA	NTRK1	61
TRKB	NTRK2	36
TRKC	NTRK3	50
TRPM6	TRPM6	76
TSSK1B	TSSK1B	86
TTK	TTK	100
TXK	TXK	67
TYK2(JH1domain-catalytic)	TYK2	4.2
TYK2(JH2domain-pseudokinase)	TYK2	1.2
TYRO3	TYRO3	91
ULK1	ULK1	100
ULK2	ULK2	98

ULK3	ULK3	66
VEGFR2	KDR	70
VRK2	VRK2	94
WEE1	WEE1	90
WEE2	WEE2	91
WNK1	WNK1	100
WNK3	WNK3	100
YANK1	STK32A	78
YANK2	STK32B	99
YANK3	STK32C	86
YES	YES1	77
YSK1	STK25	75
YSK4	YSK4	0.4
ZAK	ZAK	79
ZAP70	ZAP70	6.8

SYK Biochemical Assay. Syk activity was measured using KinEASE (Cisbio), a time-resolved fluorescence energy transfer (TR-FRET) immunoassay. In this assay Syk catalyzes the phosphorylation of a XL665-labeled peptide substrate. Europium conjugated phospho-tyrosine specific antibody binds the resulting phosphorylated peptide. Formation of phosphorylated peptide is quantified by TR-FRET with Europium as the donor and XL665 the acceptor in a 2-step endpoint assay. Test compounds were serially diluted in DMSO and delivered into 384 well plates using the Echo550 acoustic liquid dispenser. Syk enzyme and substrates were dispensed into assay plates using a Multi-Flo. The standard 5 μ L reaction mixture contained 20 μ M ATP, 1 μ M biotinylated peptide, 0.015 nM of Syk in reaction buffer (50 mM Hepes, pH 7.0, 0.02% NaN₃, 0.1% BSA, 0.1mM orthovanadate, 5 nM MgCl₂, 1 mM DTT, 0.025% NP-40). After 30 minutes incubation at room temperature, 5 μ L of Stop and Detect solution (1:200 Europium Cryptate labeled anti-phosphorylated peptide antibody solution and 125 nM streptavidin-XL665 tracer in a 50 mM Hepes pH 7.0 detection buffer containing sufficient EDTA) was added. The plate was then further incubated for 120 minutes at room temperature and read using an Envision 2013 Multilabeled reader with excitation/emission/FRET emission at 340 nm / 615 nm / 665 nm respectively.. IC50 values for test compounds were determined using a four-parameter linear regression algorithm.

pBLNK assay Ramos cells were serum starved at 2×10^6 cells/mL in serum-free RPMI for 1 h in an upright T175 Falcon TC flask. The cells were centrifuged (1100 rpm for 5 min) and incubated at a density of 5×10^6 cells/mL in the presence of 3 \times serial dilutions of test compound or DMSO controls for 1 h at 37 °C. The cells were stimulated by incubation with 3 μ g/mL antihuman IgM F(ab)2 (Southern Biotech, Birmingham, AL) for 5 min at 37 °C. The cells were pelleted and lysed in 50 μ L of cell lysis buffer. Phospho-BLNK was detected using an MSD high bind plate coated for 1 h with 30 ng/ well total BLNK capture antibody (Santa Cruz Biotechnology). Lysate was added, and the cells were washed in TBS–1% Tween-20 and probed with an antiphospho-Blnk-Y96 antibody (Santa Cruz Biotechnology). Inhibition of the pBLNK was quantitated versus the control well (Meso Scale Discovery).

CD63 whole blood assay. Syk activity was assessed in relation to reduced activation of basophils as measured by reduced activation of basophils measured by the expression of CD63 in a human whole blood basophil assay (25% blood). Basophil activation was measured using the FlowCAST kit following the protocol provided by the manufacturer with minor modifications. Fresh human whole blood I heparin was collected and delivered the same day. Whole blood samples were incubated in either DMSO (1% final) or serially diluted compounds in DMSO for 60 minutes at 37C. Basophils were activated using the anti-Fc ϵ R1 mAb and stained with anti-CD63-FITC and anti-CCR3-PE for 20 minutes at 37C. (per well: 50 μ L of whole blood was mixed with 113 μ L of stimulation buffer, 8.5 μ L anti-Fc ϵ R1 mAb, 8.5 μ L CCR3-PE/CD63-FITC). Cells were centrifuged at 1000x g for 18 minutes and 150 μ L/well of supernatant removed. Red blood cells were lysed and cells fixed by two rounds of cell lysing: resuspending pellets with 150 μ L/well 1x lysis buffer, incubating at room temperature for 10 minutes, and collecting cell pellets by centrifuging at 1200 rpms for 5 minutes. Cells were washed twice with 150 μ L/well wash buffer and resuspended in a final volume of 75 μ L/well of fresh wash buffer for either immediate flow cytometry analysis or overnight incubation at 4C followed by flow cytometry analysis. Degranulation (basophil activation) was detected by CD63 surface expression on CCR3 positive cells. CD63 expression (%) versus DMSO controls was used to determine the EC₅₀ in whole blood.

B cell proliferation assay. Isolated human B-cells (Stem Cell Technologies) were thawed in a 37 °C water bath and rested in RPMI 1640 medium supplemented with 10% FBS, 100 units/mL penicillin–streptomycin, 0.01 M HEPES, 2 mM GlutaMAX, 5 mM sodium pyruvate, and 10 mM β -mercaptoethanol for 5 h in a 37 °C incubator with 5% CO₂ and subsequently loaded with 5 μ M CFSE per the

manufacturer's instructions (Life Technologies, Carlsbad, CA). The cells (3×10^5 cells/200 μ L per well) in a round-bottom 96-well plate were incubated with compound for 1 h in a 37 °C incubator, then stimulated with 20 μ g/mL goat F(ab')2 antihuman IgM and 20 μ g/mL mouse anti-CD40, and incubated for 90 h in a 37 °C incubator. The cells were rinsed once in PBS + 4% FBS and incubated with 7AAD for 30 min on ice. The cells were pelleted at 300g for 10 min, rinsed twice, and analyzed by flow cytometry on the 7AAD⁻ population, and proliferation was estimated on the basis of the reduction of fluorescein staining.

T cell proliferation assay Isolated human T-cells (Stem Cell Technologies) were thawed in a 37 °C water bath and rested RPMI-1640 containing 10% FBS, 100 U/mL Penicillin, 100 μ g/mL Streptomycin, 2 mM GlutaMaxTM, 1 mM sodium pyruvate, 10 mM HEPES pH 7.4, and 55 μ M β -mercaptoethanol at a density of $2.0 - 4.0 \times 10^6$ cells/mL and acclimated for 5 hours at 37°C. Subsequently, cells were incubated with 10 μ M CFSE (Life Technologies, Carlsbad, CA) in 2.5 mL of DPBS for 10 min and quenched by dilution to 50 mL with growth media above. Cells (3×10^5 cells/100 μ L per well) were added to 100 μ L of growth media in a prepared flat-bottom 96-well plate precoated with 500 ng/well OKT3 (anti-CD3e antibody) in PBS, 2 μ L of 100X diluted stock compounds, and 5 μ g/mL anti-CD28 and incubated for 90 h in a 37 °C incubator. The cells were rinsed once in PBS + 4% FBS and incubated with 7AAD for 30 min on ice. The cells were pelleted at 300g for 10 min, rinsed twice, and analyzed by flow cytometry on the 7AAD⁻ viable cell population, and proliferation was estimated on the basis of the reduction of fluorescein staining.

Mouse NZB/W SLE model Female NZBWF1/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME) at 20 weeks and enrollment in the study at 28 weeks. Mice were dosed orally with compound **39** (0.25%) or vehicle administered ad libitum in chow. Positive control mice were treated with cyclophosphamide (5 mg/kg) administered daily intraperitoneally. Efficacy evaluation was based on animal survival and weekly urine protein analysis. For mice that died or were euthanized prior to study termination, terminal clinical scores were carried through to study termination for the purpose of analysis.

Electrostatic potential calculations The coordinates of the SYK protein and the ligand from Copy A of the crystal structure (pdb:4PUZ) were extracted and processed through the Protein Preparation Wizard protocol of Maestro software⁵ to add hydrogen atoms, and also to assign tautomer and protonation states for all residues. Gasteiger partial charges⁶ were assigned to all the atoms using a Pipeline Pilot protocol⁷. The contribution of the electrostatic potential due to each atom on the protein to a specific site on the ligand is q/r , where q is the partial charge on the protein atom and r is the distance to the ligand site. The values were summed over all protein atoms to derive the total electrostatic potential.

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