Supporting Information

Discovery of 5-Amino-*N*-(1*H*-pyrazol-4-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide Inhibitors of IRAK4

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5-(((1*R*,2*S*)-2-Aminocyclohexyl)amino)-N-(3-carbamoyl-1-methyl-1*H*-pyrazol-4-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**14**).

Step 1**:** Into a 25 mL sealed tube were added ethyl 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carboxylate (500 mg, 2.2 mmol), *tert*-butyl ((1*S*,2*R*)-2-aminocyclohexyl)carbamate (475 mg, 2.2 mmol) and ethanol (5 mL) and the resulting solution was stirred at 90 ^{o}C for 8 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with ethyl acetate in petroleum ether (20-30%) to afford ethyl 5-(((1*R*,2*S*)-2-((*tert*-butoxycarbonyl)amino)cyclohexyl)amino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylate (460 mg, 52% yield) as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.48 (d, J = 7.5 Hz, 1H), 8.12 (s, 1H), 7.48 (d, J = 7.3 Hz, 1H), 6.61 (d, J = 8.3 Hz, 1H), 6.45 (d, J = 7.5 Hz, 1H), 4.31-4.22 (m, 1H), 4.17 (q, J = 6.7 Hz, 2H), 4.03-4.01 (m, 1H), 1.85-1.48 (m, 8H), 1.36 (s, 9H), 1.31 (t, J = 6.7 Hz, 3H). LRMS calc'd $[M+H]^{+}$ 404.2, found 404.4.

Step 2: Into a 25 mL round bottom flask containing a solution of ethyl 5-(((1*R*,2*S*)-2-((*tert*-butoxycarbonyl)amino)cyclohexyl)amino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylate (500 mg, 1.24 mmol) in tetrahydrofuran (2 mL) and water (5 mL) mixture was added lithium hydroxide (156 mg, 3.72 mmol) and the reaction mixture was stirred for 12 h. The mixture was diluted with water and extracted with ethyl acetate. The combined organic fractions were concentrated and purified by flash chromatography eluting with methanol in dichloromethane (2%) to afford 5-(((1*R*,2*S*)-2-((*tert*-butoxycarbonyl)amino)cyclohexyl)amino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (380 mg, 82% yield) as a colorless liquid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.45 (s, 1H), 8.49 (d, J = 7.1 Hz, 1H), 8.09 (s, 1H), 7.48 (brs, 1H), 6.6 (brs, 1H), 6.47 (d, J = 7.2 Hz, 1H), 4.29-4.26 (m, 1H), 3.83-3.80 (m, 1H), 1.79-1.54 (m, 5H), 1.35-1.21 (12 H). LRMS calc'd $[M+H]^{+}$ 376.2, found 376.4.

Step 3**:** Into a 25 mL round bottom flask containing a solution of 5-(((1*R*,2*S*)-2-((*tert*-butoxycarbonyl)amino)cyclohexyl)amino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (140 mg, 0.37 mmol,) in acetonitrile (2 mL) were added *N,N*-diisopropylethylamine (0.28 mL, 0.74 mmol) and 4-amino-1-methyl-1*H*-pyrazole-3-carboxamide (42 mg, 0.3 mmol) follwed by the addition of HATU (215 mg, 0.55 mmol) and the reaction mixture was stirred at 80 ^{o}C for 12 h. The reaction mixture was cooled to room temperature, diluted with water and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by flash chromatography eluting with methanol in chloroform (2-5%) to afford *tert*-butyl ((1*S*,2*R*)-2-((3-((3-carbamoyl-1-methyl-1*H*-pyrazol-4-yl)carbamoyl)pyrazolo[1,5-*a*]pyrimidin-5-yl)amino)cyclohexyl)carbamate (100 mg, 54% yield).

Step 4**:** Into a 10 mL round bottom flask containing a solution of *tert*-butyl ((1*S*,2*R*)-2-((3-((3-carbamoyl-1-methyl-1*H*-pyrazol-4-yl)carbamoyl)pyrazolo[1,5-*a*]pyrimidin-5-yl)amino)cyclohexyl)carbamate (100 mg, 0.2 mmol) in anhydrous 1,4-dioxane (1 mL) was added hydrochloric acid (4.4 M in dioxane, 2 mL) and the reaction mixture was stirred for 2 h at room temperature. The solvent was removed under reduced pressure and the residue was purified by preparative HPLC to afford **14** (25 mg, 31% yield). ^{1}H NMR (400 MHz, CD_3OD): δ 10.79 (s, 1H), 8.45 (d, J = 7.6 Hz, 1H), 8.3 (s, 1H), 8.29 (s, 1H), 6.69 (d, J = 7.6 Hz, 1H), 5.36 (brs, 1H), 3.96 (s, 3H), 3.76-3.74 (m, 1H), 1.98-1.87 (m, 6H), 1.81-1.66 (m, 4H). LRMS calc'd $[M+H]^{+}$ 398.2, found 398.4.

5-(((1*R*,2*S*)-2-Aminocyclohexyl)amino)-*N*-(1-methyl-3-(trifluoromethyl)-1*H*-pyrazol-4-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**18**).

Into a 10 mL round bottom flask containing a solution of 5-(((1*S*, 2*R*)-2-((*tert*-butoxycarbonyl)-amino)-cyclohexyl)amino)pyrazolo[1,5-*a*]-pyrimidine-3-carboxylic acid (50 mg, 0.13 mmol) in acetonitrile (1 mL) were added 1-methyl-3-(trifluoromethyl)-1*H*-pyrazol-4-amine (24 mg, 0.15 mmol), *N*,*N*-diisopropylethylamine (0.05 mL, 0.27 mmol) and HATU (76 mg, 0.20 mmol) and the mixture was stirred at 60 ^{o}C for 8 h. The reaction mixture was treated with water (5 mL) and extracted with ethyl acetate (5 x 10 mL). The combined organics were washed with brine, dried over anhydrous sodium sulfate, and concentrated. The crude mixture was taken to the next step without purification.

Into a 5 mL round bottom flask was added the crude mixture from the previous step. A saturated solution of hydrogen chloride in 1,4-dioxane (3 mL) was added and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated and purified by preparative HPLC purification to afford **18** (14 mg, 25% yield) as a white solid. ^{1}H NMR (400 MHz, CD_3OD): δ 9.46 (s, 1H), 8.51 (d, *J* = 7.6 Hz, 1H), 8.32 (d, *J* = 7.3 Hz, 2H), 7.89 (d, *J* = 8.8 Hz, 1H), 6.64 (d, *J* = 7.6 Hz, 1H), 3.98 (s, 3H), 3.59-3.51 (m, 1H), 2.01-1.67 (m, 8H). LRMS calc'd. $[M+H]^+$ 423.2, found 423.4.

3-(Difluoromethyl)-1-methyl-1*H*-pyrazol-4-amine.

Step 1: Into a 100 mL round bottom flask containing a solution of methyl 1-methyl-4-nitro-1*H*-pyrazole-3-carboxylate (1.5 g, 8 mmol) in dichloromethane (30 mL) was added diisobutylaluminum hydride (12 mL, 1 M in toluene, 12 mmol) at 0 °C and the reaction mixture was stirred at room temperature for 6 h. The reaction mixture was treated with hydrochloric acid (1.5 N, 1 mL) and extracted with ethyl acetate. The organic fraction was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with methanol in dichloromethane (5-10%) to afford (1-methyl-4-nitro-1*H*-pyrazol-3-yl)methanol (800 mg, 63% yield) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.77 (s, 1H), 5.19 (t, *J* = 5.9 Hz, 1H), 4.63 (d, *J* = 5.9 Hz, 2H), 3.85 (s, 3H).

Step 2: Into a 50 mL round bottom flask containing a solution of 2-iodoxybenzoic acid (2.8 g, 10 mmol) in dimethyl sulfoxide (3 mL) was added a solution of (1-methyl-4-nitro-1*H*-pyrazol-3-yl)methanol (800 mg, 5 mmol) in dimethyl sulfoxide (3 mL) and the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with diethyl ether and washed with water and brine. The organic fraction was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with methanol in dichloromethane (7-9%) to afford 1-methyl-4-nitro-1*H*-pyrazole-3-carbaldehyde (500 mg, 87% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.43 (s, 1H), 8.25 (s, 1H), 4.09 (s, 3H).

Step 3: Into a 25 mL round bottom flask containing a solution of 1-methyl-4-nitro-1*H*-pyrazole-3-carbaldehyde (300 mg, 1.9 mmol) in dichloromethane (5 mL) was added diethylaminosulfur trifluoride (0.76 mL, 5.8 mmol) at -20 °C and the reaction was stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by flash chromatography eluting with methanol in dichloromethane (3-5%) to afford 3-(difluoromethyl)-1-methyl-4-nitro-1*H*-pyrazole (120 mg, 35%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.19 (s, 1H), 7.13 (t, *J* = 53.2 Hz, 1H), 4.03 (s, 3H).

Step 4: Into a 25 mL round bottom flask containing a solution of 3-(difluoromethyl)-1-methyl-4-nitro-1*H*-pyrazole (300 mg, 1.93 mmol) in methanol (3 mL) was added palladium on carbon (15 mg, 5% w/w) and the reaction was stirred at room temperature for 8 h under hydrogen bladder pressure. The reaction mixture was filtered through celite and washed with methanol and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography eluting with methanol in dichloromethane (4-7%) to afford 3-(difluoromethyl)-1-methyl-*1*H-pyrazol-4-amine (80 mg, 96%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.09 (s, 1H), 6.86 (t, J = 54.1 Hz, 1H), 3.70 (s, 3H). LRMS calc'd $[M+H]^+$ 148.1, found 148.2.

5-(((1*R*,2*S*)-2-Aminocyclohexyl)amino)-*N*-(3-(difluoromethyl)-1-methyl-1*H*-pyrazol-4-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**19**).

Step 1: A mixture of ethyl 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carboxylate (500 mg, 2.2 mmol) and *tert*-butyl ((1*S*,2*R*)-2-aminocyclohexyl)carbamate (475 mg, 2.2 mmol) in ethanol (5 mL) was taken in a 25 mL sealed tube, and the mixture was heated at 90 °C for 8 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with ethyl acetate in petroleum ether (20-30%) to afford ethyl 5-(((1*R*,2*S*)-2-((*tert*-butoxycarbonyl)amino)cyclohexyl)amino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylate (460 mg, 52% yield) as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.48 (d, *J* = 7.5 Hz, 1H), 8.12 (s, 1H), 7.48 (d, *J* = 7.3 Hz, 1H), 6.61 (d, *J* = 8.3 Hz, 1H), 6.45 (d, *J* = 7.5 Hz, 1H), 4.31-4.22 (m, 1H), 4.17 (q, *J* =

6.7 Hz, 2H), 4.03-4.01 (m, 1H), 1.85-1.48 (m, 8H), 1.36 (s, 9H), 1.31 (t, *J* = 6.7 Hz, 3H). LRMS calc'd $[M+H]^+$ 404.2, found 404.4.

Step 2: Into a 25 mL round bottom flask containing a solution of ethyl 5-(((1*R*,2*S*)-2-((*tert*-butoxycarbonyl)amino)cyclohexyl)amino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylate (500 mg, 1.2 mmol) in tetrahydrofuran (2 mL) and water (5 mL) was added lithium hydroxide (150 mg, 3.7 mmol) and the mixture was stirred for 12 h. The mixture was diluted with water, acidified to pH 2 using hydrochloric acid (1.5 N HCl) and extracted with ethyl acetate (2 x 10 mL). The combined organic fractions were concentrated and the crude was purified by flash chromatography eluting with methanol in dichloromethane (0-2%) to afford 5-(((1*R*,2*S*)-2-((*tert*-butoxycarbonyl)amino)cyclohexyl)amino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (380 mg, 82% yield) as a colorless liquid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.45 (s, 1H), 8.49 (d, *J* = 7.1 Hz, 1H), 8.09 (s, 1H), 7.48 (brs, 1H), 6.60 (brs, 1H), 6.47 (d, *J* = 7.2 Hz, 1H), 4.29-4.26 (m, 1H), 3.83-3.80 (m, 1H), 1.79-1.54 (m, 5H), 1.35-1.21 (12 H). LRMS calc'd $[M+H]^+$ 376.2, found 376.4.

Step 3: Into a 25 mL round bottom flask containing a solution of 3-(difluoromethyl)-1-methyl-1*H*-pyrazol-4-amine (20 mg, 1.3 mmol) in acetonitrile (5 mL) were added 5-(((1*R*,2*S*)-2-((*tert*-butoxycarbonyl)amino)cyclohexyl)amino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (50 mg, 0.1 mmol), HATU (76 mg, 0.2 mmol) and diisopropylethylamine (0.05 mL, 0.3 mmol) and the reaction mixture was heated at 60 ^{o}C for 6 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate (15 mL), washed with water and brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography eluting with methanol in dichloromethane (0-5%) to furnish *tert*-butyl ((1*S*,2*R*)-2-((3-((3-(difluoromethyl)-1-methyl-1*H*-pyrazol-4-yl)carbamoyl)pyrazolo[1,5-*a*]pyrimidin-5-yl)amino)cyclohexyl)carbamate (40 mg, 59% yield). ^{1}H NMR (CD_3OD, 300 MHz): δ 8.36 (d, *J* = 7.7 Hz, 1H), 8.25 (s, 1H), 8.24 (s, 1H), 7.01 (t, *J* = 54.0 Hz, 1H), 6.50 (d, *J* = 7.7 Hz, 1H), 4.81-4.79 (m, 1H), 3.94 (s, 3H), 3.91-2.89 (m, 1H), 1.75-1.63 (m, 8H), 1.36 (s, 9H). LRMS calc'd $[M+H]^+$ 505.2, found 505.4.

Step 4: Into a 10 mL round bottom flask containing a solution of *tert*-butyl ((1*S*,2*R*)-2-((3-((3-(difluoromethyl)-1-methyl-1*H*-pyrazol-4-yl)carbamoyl)pyrazolo[1,5-*a*]pyrimidin-5-yl)amino)cyclohexyl)carbamate (40 mg, 0.07 mmol) in dichloromethane (2 mL) was added trifluoroacetic acid (0.5 mL) and the reaction was stirred at room temperature for 2 h. The solvent was removed under reduced pressure and the residue was purified by trituration with dichloromethane and hexanes to afford **19** (30 mg, 94% yield) as a white solid. ^{1}H NMR (CD_3OD, 400 MHz): δ 8.50 (d, *J* = 7.6 Hz, 1H), 8.29 (s, 2H), 6.95 (t, *J* = 54.1 Hz, 1H), 6.64 (d, *J* = 7.6 Hz, 1H), 5.08 (brs, 1H), 3.93 (s, 3H), 3.58-3.53 (m, 1H), 1.96-1.64 (m, 8H). LRMS calc'd $[M+H]^+$ 405.2, found 405.2.

5-(5-amino-3,3-difluoropiperidin-1-yl)-*N*-(3-(difluoromethyl)-1-methyl-1*H*-pyrazol-4-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**34**).

Step 1 & 2: Into a 50 mL sealed tube were added 5-hydroxypyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (2.50 g, 14.0 mmol) and phosphorous oxychloride (50 mL) and the resulting solution was heated to 120 ^{o}C for 12 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was dissolved in dichloromethane (100 mL), washed with water and brine, and dried over anhydrous sodium sulfate. The mixture was filtered and concentrated to afford crude 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbonyl chloride (**2**) as a gummy solid.

To a stirred solution of the crude 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbonyl chloride in dichloromethane (60 mL) were added 3-(difluoromethyl)-1-methyl-1*H*-pyrazol-4-amine (2.46 g, 16.7 mmol) and *N*,*N*-diisopropylethylamine (7.17 mL, 42 mmol) and the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was triturated with methanol and filtered to afford 5-chloro-*N*-(3-(difluoromethyl)-1-methyl-1*H*-pyrazol-4-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (3.5 g, 81% yield) as a white solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.70 (s, 1H), 9.39 (d, *J* = 7.3 Hz, 1H), 8.71 (s, 1H), 8.32 (s, 1H), 7.43 (d, *J* = 7.2 Hz, 1H), 7.13 (t, *J* = 54.0 Hz, 1H), 3.88 (s, 3H). LRMS calc'd. $[M+H]^+$ 327.1, found 327.0.

Step 3: Into a 10 mL sealed tube containing a solution of 5-chloro-*N*-(1-methyl-3-(trifluoromethyl)-1*H*-pyrazol-4-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (50 mg, 0.15 mmol) and *tert*-butyl (5,5-difluoropiperidin-3-yl)carbamate (39 mg, 0.17 mmol) in ethanol (5 mL) was added *N*,*N*-diisopropylethylamine (0.08 mL, 0.46 mmol) and the mixture was heated to 90 ^{o}C for 4 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography eluting with 50% ethyl acetate in petroleum ether to afford *tert*-butyl (1-(3-((3-(difluoromethyl)-1-methyl-1*H*-pyrazol-4-yl)carbamoyl)pyrazolo[1,5-*a*]pyrimidin-5-yl)-5,5-difluoropiperidin-3-yl)carbamate (30 mg, 37% yield) as a white solid. LRMS calc'd. $[M+H]^+$ 527.2, found 527.4.

Step 4: Into a 10 mL round bottom flask containing a solution of *tert*-butyl (1-(3-((3-(difluoromethyl)-1-methyl-1*H*-pyrazol-4-yl)carbamoyl)pyrazolo[1,5-*a*]pyrimidin-5-yl)-5,5-difluoropiperidin-3-yl)carbamate (30 mg, 0.06 mmol) in dichloromethane (2 mL) was added trifluoroacetic acid (1 mL) and the mixture was stirred for 2 h. The solvent was removed under reduced pressure and the residue was triturated with diethyl ether to afford 5-(5-amino-3,3-difluoropiperidin-1-yl)-*N*-(3-(difluoromethyl)-1-methyl-1*H*-pyrazol-4-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide trifluoroacetate (17 mg, 70% yield) as a white solid. The racemic mixture was purified by chiral column (Phenomenex Lux Cellulose-4; mobile phase A, 0.1% TFA in hexanes (95%); mobile phase B, EtOH (5%)) to afford **34** (second eluting isomer, retention time 26.4 min, 8 mg). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.71 (d, J = 7.6 Hz, 1H), 8.41 (s, 1H), 8.31 (s, 1H), 6.95 (d, J = 8.0 Hz, 1H), 6.91 (t, J = 54.0 Hz, 1H), 4.37-4.18 (m, 3H), 4.10-4.05 (m, 1H), 3.93 (s, 3H), 3.81 (brs, 1H), 2.75-2.63 (m, 1H), 2.48-2.36 (m, 1H). LRMS calc'd. $[M+H]^+$ 427.2, found 427.4.

Conditions for IRAK4 kinase assay, hPBMC assay, and rat whole blood assay

IRAK4 kinase assay

The kinase activity of IRAK4 is determined by its ability to catalyze the phosphorylation of a fluorescent polypeptide substrate. The extent of phosphorylation is measured using the IMAP technology (Molecular Devices) where the phosphorylated fluorescent substrate binds to the large M(III)-based nanoparticles which reduces the rotational speed of the substrate and thus increases its fluorescent polarization (FP).

20 μL reaction mixture contains 10 mM TriHCl, pH 7.2, 0.5 nM GST tagged IRAK4 (SignalChem), 100 nM fluorescent peptide substrate (RP7030, Molecular Devices), 100 μM ATP, 1 mM DDT, 1 mM $MgCl_2$, and 0.01% Tween 20. The reaction is initiated by the addition of ATP. After incubation for 30 minutes at 25 °C, 60 μL of Progressive IMAP Reagent (Molecular Devices) is added to stop the reaction. Change in RP7030's FP is determined by a FP reader (Analyst HT, LJL BioSystems).

Human PBMC assay

Frozen human PBMCs were thawed and incubated in culture medium with 0.5% FBS (RPMI+GlutMax +amino acid+NaPyruvate) overnight. 36 μl of PBMC cells (20K) were plated into 384-well with compounds and incubated at 37 ^{o}C for 0.5 hour. 4 μl of R848 (final concentration 1.5 μg/ml; R&D #4536) was added into each well and incubated at 37 ^{o}C for 5 hours. Additional 50 μl of medium were added and the plate was spin at 1000 x g for 5 min. 15 μl of supernatant was transferred to human TNFα assay plate (Meso Scale Discovery #K211BHB) and TNFα was measured according to MSD manufacture's protocol.

Rat whole blood assay

Freshly isolated rat whole blood (Lewis female rat) were filter through 100 μm mesh. 90 μl of the rat blood were plated into 96-well with compounds. 10 μl of R848 (final concentration 0.5 μg/ml; R&D #4536) was added into each well and incubated at 37 ^{o}C for 4 hours. 150 μl of RPMI medium was added into each well and the plate was spin at 1000 x g for 5 min. 60 μl of supernatant was transferred into 384-well plate and 15 μl of the supernatant was further transferred to rat TNFα assay plate (Meso Scale Discovery #K213BHB) and TNFα was measured according to MSD manufacture's protocol.

Table S1. PK profile of compounds 13 and 14 in rats

compd	PSA (Å)	cLogD[a]	P_{app} (x 10^{-6} cm/s)	Rat Cl_p (mL/min/kg)[b]	Rat %F[c]
13	153	-4.3	< 2[d]	79	0
14	152	-4.3	2[e]	56	0

[a] at pH 7.4 from ACD Labs v10. [b] IV dosed at 0.5 mg/kg as a solution in DMSO:PEG400:H_2O (20:40:20 (v/v/v)). [c] PO dosed at 1 mg/kg as a solution in DMSO:PEG400:H_2O (20:40:20 (v/v/v)). [d] from MDCK cells. [e] from LLC-PK1 cells.

Crystallization conditions for compound 18

Crystals of the IRAK4 kinase domain containing residues Val-160 to Ser-460 were grown using the vapor diffusion method. Briefly, a frozen aliquot of purified protein was thawed rapidly and lightly centrifuged prior to dispensing. In a VDX plate containing 1 mL reservoir solution comprised of 2.0 M sodium malonate pH 6.7, 0.05% (w/v) pluronic F-68, 200 mM sodium acetate, and 25 mM hexamine cobalt(III) chloride, 1 uL of protein was added to 1 uL of a reservoir solution on a siliconized glass cover slip, inverted over the reservoir solution the edge of which was lined with vacuum grease. The tray was incubated at 14 ^{o}C. Crystals appeared within 3 days and continued to grow for one week. Compound **18** was obtained as a solid and dissolved in DMSO to a final concentration of 100 mM to form a compound stock solution. 2 uL of the compound stock solution was added to 50 uL of a soaking/cryoprotection buffer containing 2.4 M sodium malonate pH 7.0, 10% (v/v) glycerol, 10 mM haxammine cobalt(III) chloride, and 180 mM L-arginine. A crystal was transferred to this soaking/cryoprotection solution and incubated at 14 ^{o}C for 3 days prior to flash cooling directly into liquid nitrogen and synchrotron data collection.

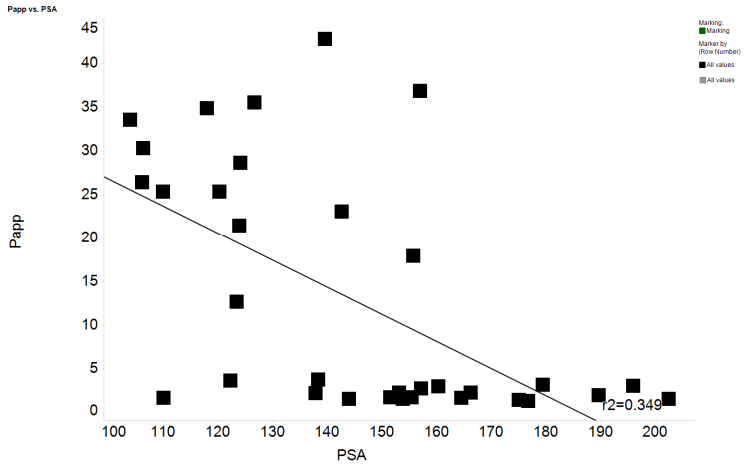
The carbon atoms of **18** are shown in yellow; the hinge in cyan; the gatekeeper residue in pink; the remainder of the protein in green. Modeled water molecules are depicted as small red spheres. Polar interactions between **18** and neighboring atoms are depicted as gray dashes.

Table S2. X-ray data collection and refinement statistics of 18

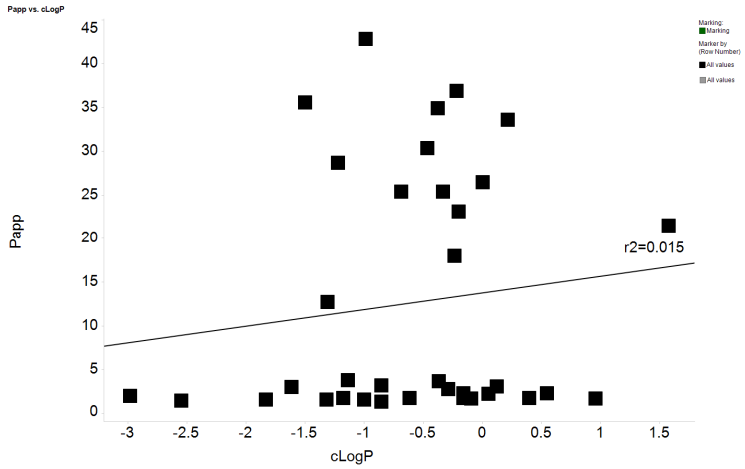
Data collection	
Space group	*C*2
Cell dimensions	
a, *b*, *c* (Å)	142.9,138.8, 88.0
α, β, γ (°)	90.0, 124.6, 90.0
Wavelength	1.0 Å
Resolution (Å)	74–2.14 (2.31–2.14)[a]
R_{merge}	0.055 (0.416)
I / σ(I)	12.9 (2.5)
Completeness (%)	99.4 (99.6)
Redundancy	3.4 (3.3)
CC(1/2)	0.998 (0.894)
Refinement	
Resolution (Å)	74–2.14
No. reflections	76,998
R_{work} / R_{free}	0.194 / 0.215
# Atoms / *B* factors	
Protein Atoms	9,756 / 55.7 Å^2
Solvent Atoms	407 / 51.8 Å^2
Ligand Atoms	120 / 37.8 Å^2
r.m.s. deviations	
Bond lengths (Å)	0.010
Bond angles (°)	1.12

[a] number in parentheses represents outer resolution shell

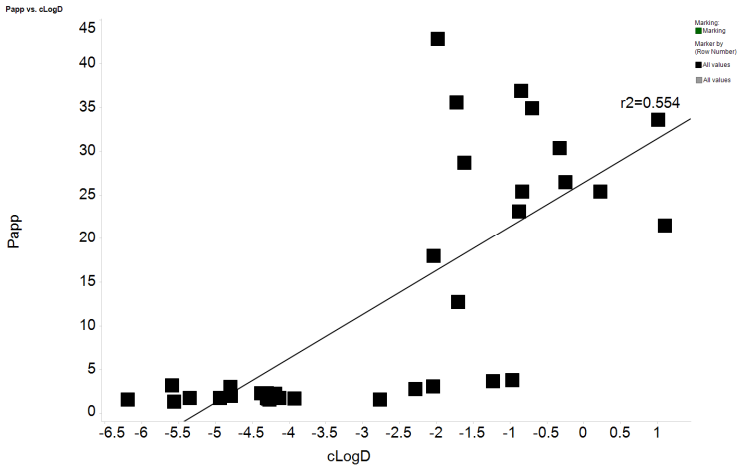
Figure S1. P_{app} plots versus PSA, cLogP, and cLogD



(a) P_{app} versus PSA



(b) P_{app} versus cLogP



(c) P_{app} versus cLogD