Supporting Information

Discovery of CA-4948, an Orally Bioavailable IRAK4 Inhibitor for Treatment of Hematologic Malignancies

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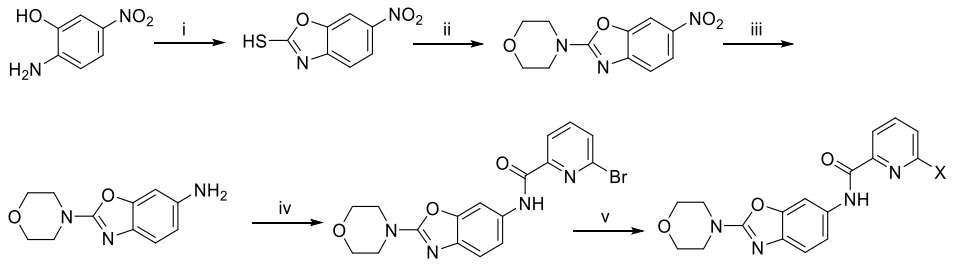
Contents

Synthetic Chemistry

General. Starting materials and solvents of reagent grade were purchased from commercial suppliers, and used without further purification. All non-aqueous reactions were performed under an argon atmosphere in oven dried glassware with magnetic stirring. Column chromatography was performed using silica gel 60-120 mesh and common solvents (hexane/DCM/EtOAc/MeOH/CHCl3) mixture/gradient. The reaction progress was monitored by TLC on SiO_2. TLC was carried out employing silica gel 60 F254 plates (Merck, Darmstadt) and developed chromatograms were visualized by UV (254 nm). NMR spectra were recorded on a Varian 300MHz or 400MHz (1H, 300 or 400 MHz; 13C, 75 or 100MHz) spectrometer. 1H and 13C NMR spectra were recorded using TMS as an internal reference. Chemical shifts are expressed in δ (ppm), and J values are given in Hz. Liquid chromatography mass spectra (LCMS) were obtained from Agilent 1100-LC/MSD VL using mobile phase: 0.1% formic acid in water and acetonitrile; ionization was achieved either by positive or negative mode. Melting points were measured on a DBK programmed melting point apparatus from Servewell Instruments and uncorrected. Purity of final compounds was determined by analytical HPLC, which was carried out on an Agilent 1100 series, conditions: Agilent XDB C18 (150x 4.6mm, 5micron); flow rate 1 mL/min; UV detection at 210nm; linear gradient from 95:5% of 0.01% TFA in water: acetonitrile to 100% acetonitrile in 12 min. All intermediates were characterized by 1H NMR and LCMS.

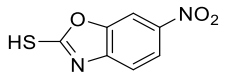
General synthetic scheme-1: Compounds **1**, **2** & **3** (Table 1) can be synthesized by using below scheme.



(i) CS_2, Et_3N, DCM, 40°C, 12h; (ii) Morpholine, 90-100°C, 17h; (iii) 10% Pd/C, H_2, methanol, 20-30°C, 4h; (iv) 6-bromopicolinic acid, EDC-HCl, HOBT, DIPEA, DCM, 20-30°C, 8-14h; (v) corresponding boronic acid or boronate ester, Cs_2CO_3, $Pd(Pph_3)_4$, 1,4-dioxane, H_2O, 100-120°C, 12h;

Synthesis of compound 1

Step-1:

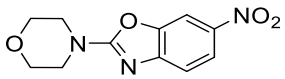


6-nitrobenzo[*d*]oxazole-2-thiol

To a solution of 2-amino-5-nitrophenol (5 g, 0.0308 mol) in DCM (50 mL) were added carbon disulfide (4.93 g, 0.064 mol) and triethylamine (6.46 g, 0.064 mol) at room temperature and the reaction mixture was stirred at 40 °C for 12h. After completion of the reaction (monitored by TLC using 50% EtOAc in hexane), the reaction mixture was extracted using water (50 mL) and DCM (30 mL). The organic layer was separated, dried over Na_2SO_4 and concentrated to get crude product. (4 g, 63%).

Molecular Formula: $C_7H_4N_2O_3S$; Exact Mass: 195.99; LCMS: m/z = 194.9 $(M-1)^-$.

Step-2:

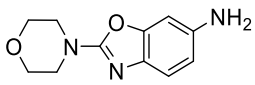


2-morpholino-6-nitrobenzo[*d*]oxazole

6-Nitrobenzo[d]oxazole-2-thiol (4 g, 0.020 mol) was taken with morpholine (40 mL) at room temperature and the mixture was stirred at 100 °C for 12h. After completion of reaction (monitored by TLC using 50% EtOAc in hexane), the reaction was quenched with ice water (40 mL). The precipitated solid was filtered and dried under vacuum to get crude product, which was purified by CombiFlash® chromatography eluted with 20% EtOAc in hexane to afford the pure product which was used for next step. (3 g, 60 %)

Molecular Formula: $C_{11}H_{11}N_3O_4$; Exact Mass: 249.07; LCMS: m/z = 250.1 $(M+1)^+$.

Step-3:

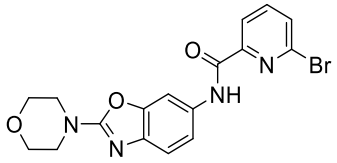


2-morpholinobenzo[*d*]oxazol-6-amine

To a solution of 2-morpholino-6-nitrobenzo[d]oxazole (3 g, 0.012 mol) in methanol (30 mL) was added 10% Pd/C (300 mg). The reaction mixture was stirred under 1.0 kg H_2 pressure at room temperature for 4h. After completion of reaction (monitored by TLC using 10% MeOH in $CHCl_3$), reaction mixture was filtered over Celite® bed and the filtrate was concentrated under vacuum to get desired compound which was used for next step (1.3 g, 50%).

Molecular Formula: $C_{11}H_{13}N_3O_2$; Exact Mass: 219.10; LCMS: m/z = 220.1 $(M+1)^+$.

Step-4:

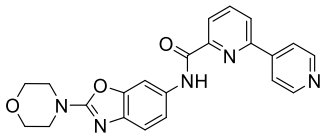


6-bromo-*N*-(2-morpholinobenzo[*d*]oxazol-6-yl)picolinamide

To a solution of 2-morpholinobenzo[d]oxazol-6-amine (1.3 g, 0.0059 mol) in DCM (20 mL) were added 6-bromopicolinic acid (1.19 g, 0.0059 mol), EDCI. HCl (1.13 g, 0.0059 mol), HOBT (0.8 g, 0.0059 mol), and DIPEA (1.53 g, 0.0118 mol). The reaction mixture was stirred at room temperature for 12h. After completion of reaction (monitored by TLC using 10% MeOH in DCM), the reaction mixture was extracted using water (25 mL) and DCM (30 mL). The organic layer was separated, dried over Na_2SO_4 and concentrated to get crude product, which was purified by CombiFlash® chromatography eluted with 100% $CHCl_3$ to afford the title compound (1.2 g, 50%).

Molecular Formula: $C_{17}H_{15}BrN_4O_3$; Exact Mass: 402.03; LCMS: m/z = 403.1 $(M+1)^+$.

Step-5:



N-(2-morpholinobenzo[*d*]oxazol-6-yl)-[2,4'-bipyridine]-6-carboxamide

To a solution of 6-bromo-N-(2-morpholinobenzo[d]oxazol-6-yl) picolinamide (50 mg, 0.124 mmol) in ethanol/toluene mixture (1 mL/2 mL) were added pyridin-4-ylboronic acid (15 mg, 0.124 mmol) and K_3PO_4 (52 mg, 0.248 mmol). The reaction mixture was degassed for 15 mins with argon. Then tetrakis(triphenylphosphine)palladium, (7 mg, 0.006 mmol) was added and heated at 90°C for 16 h. After completion of reaction (monitored by TLC using 20% EtOAc in DCM), the reaction mixture was cooled to room temperature and diluted with DCM/MeOH mixture (20 mL/ 10mL). Then it was filtered over Celite® bed and the filtrate was concentrated under vacuum to get the crude product, which was purified by CombiFlash® chromatography, eluted with 20% EtOAc in DCM to afford the title compound (10 mg, 20%).

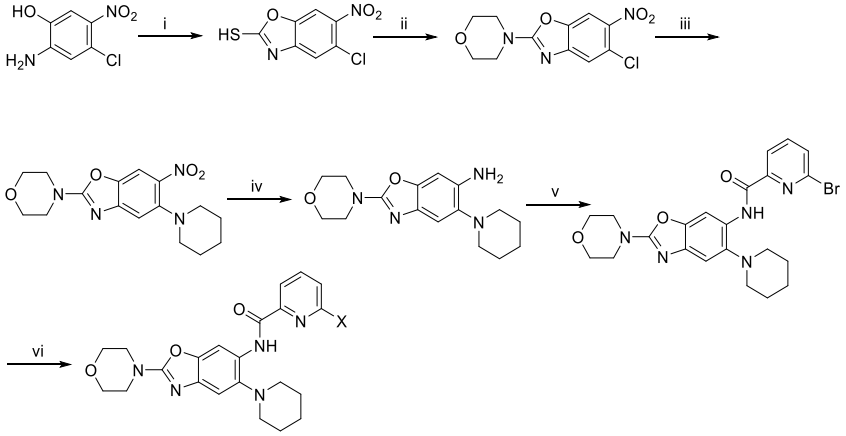
Molecular Formula: $C_{22}H_{19}N_5O_3$; Exact Mass: 401.15; LCMS (M+1): 402.14; ^{1}HNMR (DMSO-d_6, 400MHz): δ 10.61 (s, 1H), 8.76 (d, 2H), 8.41-8.37 (m, 3H), 8.23-8.09 (m, 3H), 7.63 (d, 1H), 7.33 (d, 1H), 3.74-3.59 (m, 8H).

Compound 2 was synthesized by following the above procedure of general synthetic scheme-1 using pyridin-3-ylboronic acid in step-v while compound 3 was synthesized by following the above procedure using N-(4-methoxybenzyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-amine as coupling agent in step-v followed by hydrogenation reaction to deprotect 4-methoxyl benzyl group and the analytical data are shown below.

Compound 2: Molecular Formula: $C_{22}H_{19}N_5O_3$; Exact Mass: 401.15; LCMS (M+1): 402.14; ^{1}HNMR (DMSO-d_6, 400MHz): δ 10.61 (s, 1H), 9.58 (s 1H), 8.77-8.70 (m, 2H), 8.34-8.31 (m, 1H), 8.20-8.09 (m, 3H), 7.64-7.59 (m, 2H), 7.32 (d, 1H), 3.59-3.39 (m, 8H).

Compound 3: Molecular Formula: $C_{22}H_{20}N_6O_3$; Exact Mass: 416.16; LCMS (M+1): 417.1; ^{1}HNMR (DMSO-d_6, 400MHz): δ 10.58 (s, 1H), 8.96 (s, 1H), 8.88 (d, 1H), 8.21-8.08 (m, 6H), 7.59 (d, 1H), 7.33 (d, 1H), 7.04 (d, 1H), 3.74-3.59 (m, 8H).

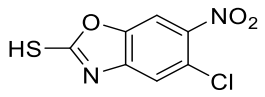
General synthetic scheme-2: Compounds **4** & **5** (Table 1) can be synthesized by using the below scheme.



(i) CS_2, KOH, Ethanol, 70-80°C, 17h; (ii) Morpholine, 90-100°C, 2h; (iii) Piperidine, K_2CO_3, $Pd(OAC)_2$, Xanthophos, 1,4-dioxane, 100-120°C, 50h; (iv) Fe powder, Conc.HCl, Ethanol, H_2O, 70-80°C, 3h; (v) 6-bromopicolinic acid, EDC-HCl, HOBT, DMAP, DMF, 20-30°C, 48h; (vi) boronate ester, Cs_2CO_3, $Pd(dppf)Cl_2$, 1,4-dioxane, H_2O, 100-120°C, 3h;

Synthesis of compound 5

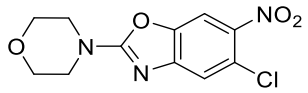
Step-1:



5-chloro-6-nitrobenzo[*d*]oxazole-2-thiol

To a solution of 2-amino-4-chloro-5-nitrophenol (2 g, 0.0106 mol) in ethanol (30 mL) were added carbon disulfide (20 mL) and KOH (0.71 g, 0.0127 mol) at room temperature and the reaction mixture was stirred at 70 °C for 17h. After completion of the reaction (monitored by TLC using 30% EtOAc in hexane), the reaction mixture was extracted using water (50 mL) and EtOAc (150 mL). The organic layer was separated, dried over Na_2SO_4 and concentrated to get the desired product which was used for next step. (2.3 g, 94%).

Step-2:

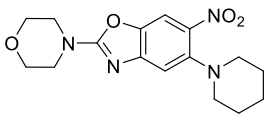


5-chloro-2-morpholino-6-nitrobenzo[*d*]oxazole

5-Chloro-6-nitrobenzo[d]oxazole-2-thiol (4.2 g, 0.018 mol) was taken with morpholine (42 mL) at room temperature and the mixture was stirred at 100 °C for 2h. After completion of reaction (monitored by TLC using 30% EtOAc in hexane), the reaction mixture was quenched with ice water (40 mL). The precipitated solid was filtered and dried under vacuum to get crude product, which was purified by CombiFlash® chromatography, eluted with 30% EtOAc in hexane to afford the pure product. (1.1 g, 21 %).

Molecular Formula: $C_{11}H_{10}ClN_3O_4$; Exact Mass: 283.03; LCMS: m/z = 284.1 $(M+1)^+$.

Step-3:



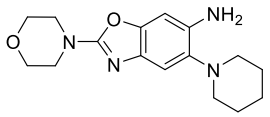
2-morpholino-6-nitro-5-(piperidin-1-yl)benzo[*d*]oxazole

To a solution of 5-chloro-2-morpholino-6-nitrobenzo[d]oxazole (1.36 g, 4.7 mmol) in 1,4-dioxane (13 mL) at room temperature were added piperidine (2.45 g, 28.7 mmol) and K_2CO_3 (1.32 g, 9.54 mmol). The reaction mixture was degassed for 15 mins with argon. Then xantphos (270 mg, 0.47 mmol) and $Pd(OAc)_2$ (54 mg, 0.24 mmol) were added and heated at 110°C for 48 h. After completion of reaction (monitored by TLC using 50% $CHCl_3$ in hexane), the reaction mixture was cooled to room temperature and extracted by using water (50 mL) and EtOAc (150 mL). The organic layer was separated, dried over Na_2SO_4 and

concentrated to get crude product, which was purified by CombiFlash® chromatography, eluted with 1% MeOH in DCM to afford the title compound (780 mg, 49%).

Molecular Formula: $C_{16}H_{20}N_4O_4$; Exact Mass: 332.15; LCMS: m/z = 333.3 $(M+1)^+$.

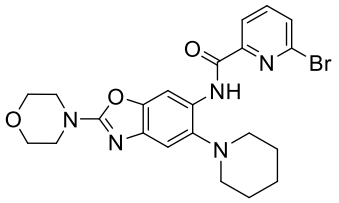
Step-4:



2-morpholino-5-(piperidin-1-yl)benzo[*d*]oxazol-6-amine

To a solution of 2-morpholino-6-nitro-5-(piperidin-1-yl)benzo[d]oxazole (0.78 g, 2.34 mmol) in ethanol/water mixture (7.8 mL/2.4 mL) were added Fe powder (2.34 g, 3 times W/W) and conc. HCl (0.5 mL). The reaction mixture was heated at 80 ^oC for 3 h. After completion of reaction (monitored by TLC using 20% EtOAc in hexane), the reaction mixture was cooled to room temperature and filtered over Celite® bed. The filtrate concentrated to remove ethanol and the residue was basified with satd. $NaHCO_3$, which was extracted with EtOAc (50 mL). The organic layer was separated, dried over Na_2SO_4 and concentrated to get the desired product which was used for next step (655 mg, 92%).

Step-5:

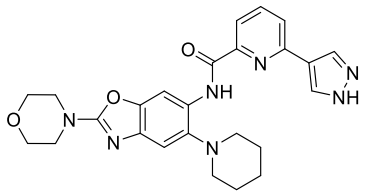


6-bromo-*N*-(2-morpholino-5-(piperidin-1-yl)benzo[*d*]oxazol-6-yl)picolinamide

To a solution of 2-morpholino-5-(piperidin-1-yl)benzo[d]oxazol-6-amine (655 mg, 2.16 mmol) in DMF (10 mL) were added 6-bromopicolinic acid (520 mg, 2.58 mmol), EDCI. HCl (540 mg, 2.82 mmol), HOBT (290 mg, 2.16 mmol), and DMAP (53 mg, 0.434 mmol). Then reaction mixture was stirred at room temperature for 48 h. After completion of reaction (monitored by TLC using 30% EtOAc in Hexane), the reaction mixture was quenched with crushed ice (100 mL). The resulting solid was filtered and dried under vacuum to get crude product, which was purified by CombiFlash® chromatography, eluted with 1.5% MeOH in $CHCl_3$ to afford the pure product (0.9 g, 85%).

Molecular Formula: $C_{22}H_{24}BrN_5O_3$; Exact Mass: 485.10; LCMS: m/z = 486.2 $(M+1)^+$.

Step-6:



N-(2-morpholino-5-(piperidin-1-yl)benzo[*d*]oxazol-6-yl)-6-(1*H*-pyrazol-4-yl)picolinamide

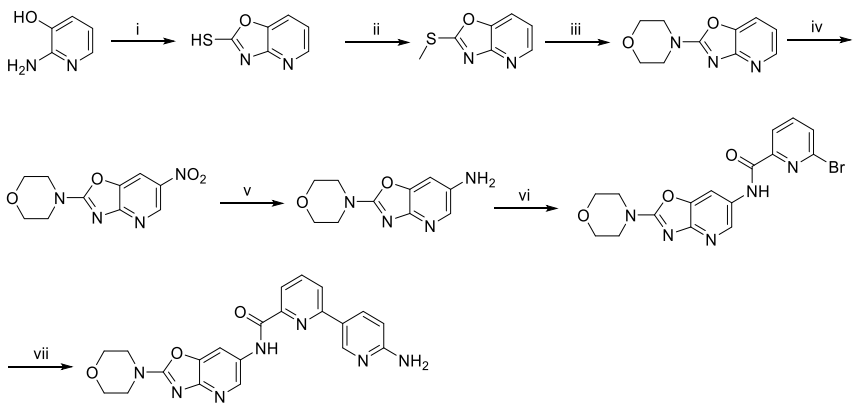
To a solution of 6-bromo-N-(2-morpholino-5-(piperidin-1-yl)benzo[d]oxazol-6-yl)picolinamide (40 mg, 0.08 mmol) in 1,4-dioxane/water mixture (2 mL/0.2 mL) were added 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (19 mg, 0.098 mmol), Cs_2CO_3 (80 mg, 0.246 mmol). The reaction mixture degassed for 15 mins with argon. Then $PdCl_2$ (DPPF) DCM, (7 mg, 0.008 mmol) was added and heated at 120^{o}C for 3 h. After completion of reaction (monitored by TLC using 60% EtOAc in hexane), the reaction mixture was cooled to room temperature and quenched with water (20 mL). The resulting solid was filtered and dried under vacuum to get the crude product. Then it was stirred with EtOAc (5 mL) and the resultant solid filtered to get the pure product (10 mg, 21%).

Molecular Formula: $C_{25}H_{27}N_7O_3$; Exact Mass: 473.22; LCMS (M+1): 474.0; ^{1}HNMR (DMSO-d_6, 400MHz): δ 11.13 (bs, 1H), 8.67 (s, 1H), 8.28 (bs, 1H), 8.18 (d, 2H), 7.90 (t, 1H), 7.66 (d, 1H), 7.26 (s, 1H), 7.25 (s, 1H), 3.84-3.67 (m, 8H), 2.86 (bs, 4H), 1.82-1.79 (m, 6H).

Compound 4 was synthesized by following the above procedure of synthetic scheme-2 using N-(4-methoxybenzyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-amine as coupling agent in step-vi followed by hydrogenation reaction to deprotect 4-methoxyl benzyl group and the analytical data is shown below.

Compound 4: Molecular Formula: $C_{27}H_{29}N_7O_3$; Exact Mass: 499.23; LCMS (M+1): 500.0; ^{1}HNMR ($CDCl_3$, 400MHz): δ 11.30 (s, 1H), 8.80 (d, 1H), 8.72 (s, 1H), 8.43-8.40 (dd, 1H), 8.22 (d, 1H), 7.94 (t, 1H), 7.83-7.81 (m, 1H), 6.62 (d, 1H), 4.73 (bs, 2H), 3.84-3.67 (m, 8H), 2.85 (bs, 4H), 1.77-1.76 (m, 6H).

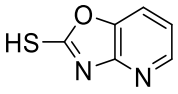
General synthetic scheme-3: Compounds **6** (Table 1) can be synthesized by using the below scheme.



(i) Potassium ethyl xanthate, pyridine, 110°C, 15h; (ii) Ethyl acetate, K_2CO_3, MeI, 20-30°C, 15h; (iii) Morpholine, THF, 75°C, 15h; (iv) Acetic acid, fuming nitric acid, 100°C, 4h; (v) NH_4Cl, THF, H_2O, Zn dust, 50°C, 1h (vi) 6-bromopicolinic acid, EDC-HCl, DIPEA, DMF, 20-30°C, 15h; (vii) tert-butyl (5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolane-2-yl)pyridin2-yl)carbamate, Na_2CO_3, $Pd(Pph_3)_2Cl_2$, 1,2-dimethoxy ethane, H_2O, 95°C, 15h; TFA, DCM, 20-30°C, 1h.

Synthesis of compound 6

Step-1:

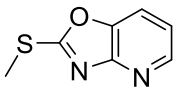


oxazolo[4,5-*b*]pyridine-2-thiol

To a solution of 2-aminopyridin-3-ol (5 g, 0.045 mol) in pyridine (50 mL) was added potassium ethyl xanthate (8 g, 0.05 mol) at room temperature and the reaction mixture was stirred at 110 °C for 16h. After completion of the reaction (monitored by TLC using 30% EtOAc in hexane), the reaction mixture was cooled and quenched with crushed ice (400 mL). Pyridine was removed under vacuum and the reaction mixture was acidified with concentrated HCl by adjusting pH~1. The precipitated solid was filtered and dried under vacuum to get the desired product (6.0 g, 87 %).

Molecular Formula: $C_6H_4N_2OS$; Exact Mass: 152.00; LCMS: m/z = 153.0 $(M+1)^+$.

Step-2:

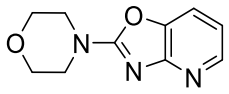


2-(methylthio)oxazolo[4,5-*b*]pyridine

To a solution of oxazolo[4,5-b] pyridine-2-thiol (3 g, 19.73 mmol) in ethyl acetate (30 mL) were added K_2CO_3 (3.81 g, 27.62 mmol) followed by methyl iodide (3.08 g, 21.7 mmol). The resulting mixture was stirred at room temperature for 16 h. After completion of reaction (monitored by TLC using 30% EtOAc in

hexane), the reaction mixture was extracted by using water (50 mL) and ethyl acetate (100 mL). The organic layer was separated, dried over Na_2SO_4 and concentrated to get desired product (3.0 g, 93%).
Molecular Formula: $C_7H_6N_2OS$; Exact Mass: 166.02; LCMS: m/z = 167.0 $(M+1)^+$.

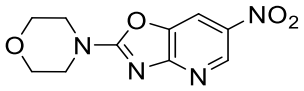
Step-3:



2-morpholinooxazolo[4,5-*b*]pyridine

To a solution of 2-(methylthio)oxazolo[4,5-b]pyridine (2 g, 12.0 mmol) in dry THF (5 mL), were added morpholine (5 mL) at room temperature and the mixture was stirred at 70 °C for 12h. After completion of reaction (monitored by TLC using 50% EtOAc in hexane), THF was removed under vacuum and the reaction mixture was quenched with ice water (50 mL). The precipitated solid was filtered and dried under vacuum to get the desired compound (2.0 g, 81 %).
^{1}HNMR (DMSO-d_6, 300MHz): 8.2 (d, 1H), 7.8 (d, 1H), 7.0 (m, 1H), 3.75 (m, 4H), 3.65 (m, 4H).

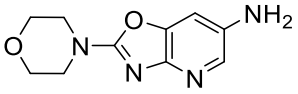
Step-4:



2-morpholino-6-nitrooxazolo[4,5-*b*]pyridine

To a solution of 2-morpholinooxazolo[4,5-b]pyridine (1 g, 4.87 mmol) in acetic acid (10 mL) was added fuming HNO_3 (6 mL) and the resulting mixture was stirred at 100 °C for 4h. After completion of the reaction (monitored by TLC using 50% EtOAc in hexane), the reaction mixture was quenched with crushed ice (100 mL). The resulting solid was filtered and dried under vacuum to afford the product (800 mg, 65%)
Molecular Formula: $C_{10}H_{10}N_4O_4$; Exact Mass: 250.07; LCMS: m/z = 250.9 $(M+1)^+$.

Step-5:



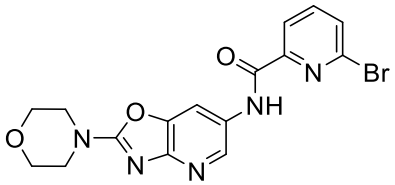
2-morpholinooxazolo[4,5-*b*]pyridin-6-amine

To a solution of 2-morpholino-6-nitrooxazolo[4,5-b]pyridine (700 mg, 2.79 mmol) in THF/water mixture (7 ml /5 ml) were added NH_4Cl (2.37 g, 44.30 mmol) followed by Zn dust(1.82 g, 27.9 mmol). The reaction mixture was stirred at 50 °C for 1h. After completion of reaction (monitored by TLC using 50% EtOAc in

hexane), reaction mixture was filtered over Celite® bed and the filtrate was extracted by using water (20 mL) and ethyl acetate (50 mL). The organic layer was separated, dried over Na_2SO_4 and concentrated to get desired product (600 mg, 97%).

Molecular Formula: $C_{10}H_{12}N_4O_2$; Exact Mass: 220.09; LCMS: m/z = 221.0 $(M+1)^+$.

Step-6:

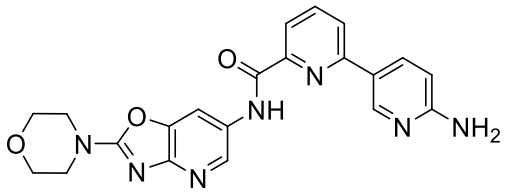


6-bromo-*N*-(2-morpholinooxazolo[4,5-*b*]pyridin-6-yl)picolinamide

To a solution of 2-morpholinooxazolo[4,5-b]pyridin-6-amine (600 mg, 2.72 mmol) in DMF (5 mL) were added 6-bromopicolinic acid (661 mg, 3.27 mmol), EDCI. HCl (783 mg, 4.09 mmol), HOBT (552 mg, 4.09 mmol), and DIPEA (1.05 g, 8.1 mmol). Then reaction mixture was stirred at room temperature for 15h. After completion of reaction (monitored by TLC using 80% EtOAc in hexane), the reaction mixture was quenched with crushed ice (50 mL). The resulting solid was filtered and dried under vacuum to get the crude product, which was purified by CombiFlash® chromatography, eluted with 5% MeOH in $CHCl_3$ to afford the pure product (350 mg, 31.8%).

Molecular Formula: $C_{16}H_{14}BrN_5O_3$; Exact Mass: 403.02; LCMS: m/z = 405.6 $(M+2)^+$.

Step-7:



6'-amino-*N*-(2-morpholinooxazolo[4,5-*b*]pyridin-6-yl)-[2,3'-bipyridine]-6-carboxamide

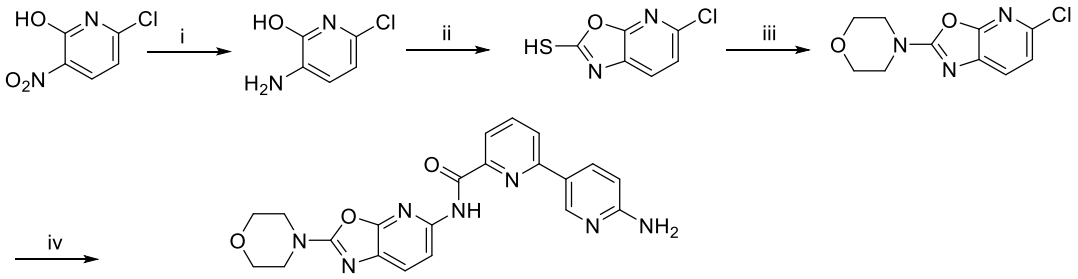
To a solution of 6-bromo-N-(2-morpholinooxazolo[4,5-b]pyridin-6-yl)picolinamide (350 mg, 0.866 mmol) in 1,2-Dimethoxyethane/water mixture (10 mL/1 mL), were added tert-butyl (5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)carbamate (360 mg, 1.125 mmol) and Na_2CO_3 (275 mg, 2.598 mmol). The reaction mixture was degassed for 15 mins with argon. Then bis(triphenylphosphine)palladium chloride (30 mg, 0.043 mmol) was added and heated at 95°C for 15 h. After completion of reaction (monitored by TLC using 50% EtOAc in hexane), reaction mixture was filtered over Celite® bed and the

filtrate was extracted by using water (20 mL) and ethyl acetate (50 mL). The organic layer was separated, dried over Na_2SO_4 and concentrated to get the crude product, which was purified by CombiFlash® chromatography, eluted with 5% MeOH in $CHCl_3$ to afford (6-((2-morpholinooxazolo[4,5-b]pyridin-6-yl)carbamoyl)-[2,3'-bipyridin]-6'-yl)carbamate (300 mg, 67%). LCMS: m/z =517.7 $(M+1)^+$.

To a solution of tert-butyl (6-((2-morpholinooxazolo[4,5-b]pyridin-6-yl)carbamoyl)-[2,3'-bipyridin]-6'-yl)carbamate (300 mg, 0.579 mmol) in DCM (1 mL) was added TFA (5 mL) and stirred at room temperature for 1 h. After completion of reaction (monitored by TLC using 10% MeOH in DCM), the reaction mixture was concentrated to get crude product, then it was purified by preparative HPLC to afford the pure product (34 mg, 14%).

Molecular Formula: $C_{21}H_{19}N_7O_3$; Exact Mass: 417.15; LCMS (M+1): 418.1; ^{1}HNMR (DMSO-d_6, 300MHz): 10.65 (s, 1H), 8.96-8.95 (d, 1H), 8.58-8.58 (d, 1H), 8.44-8.40 (dd, 1H), 8.31-8.30 (d, 1H), 8.11-7.95 (m, 3H), 6.59-6.56 (d, 1H), 6.38 (s, 2H), 3.75-3.74 (t, 4H), 3.68-3.66 (t, 4H); HPLC % purity: 98.32

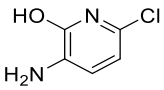
General synthetic scheme-4: Compound **7** (Table1)**.**



(i) NH_4Cl, THF, MeOH, H_2O, Zn dust, 50°C, 1h (ii) Potassium ethyl xanthate, pyridine, 110°C, 15h; (iii) Morpholine, 110°C, 15h; (iv) tert-butyl (6-carbamoyl-[2,3'-bipyridin]-6'-yl)carbamate, Cs_2CO_3, X-phos, toluene, 110°C, 15h;

Synthesis of compound 7

Step-1:

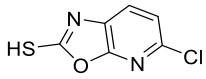


3-amino-6-chloropyridin-2-ol

To a solution of 6-chloro-3-nitropyridin-2-ol (1 gm, 5.547mmol)in THF/water mixture (20 mL/10 mL), were added NH_4Cl (4.92 g, 91.952 mmol) and Zn dust (3 g, 45.977 mmol). The reaction mixture was stirred at 50 °C for 1h. After completion of reaction (monitored by TLC using 50% EtOAc in n-hexane), the reaction mixture was filtered over Celite® bed and the filtrate was concentrated under vacuum. The crude product was purified by CombiFlash® chromatography, eluted with 10% MeOH in DCM to afford the pure product (500 mg, 60.97%).

Molecular Formula: $C_5H_5ClN_2O$; Exact Mass: 144.009; LCMS: 145.1 $(M+1)^+$.

Step-2:

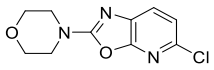


5-chlorooxazolo[5,4-*b*]pyridine-2-thiol

To a solution of 3-amino-6-chloropyridin-2-ol (0.9 g, 6.25mmol) in pyridine (8 mL) was added potassium ethyl xanthate (1.1 g, 6.875 mmol) at room temperature and the reaction mixture was stirred at 110°C for 15 h. After completion of the reaction (monitored by TLC using 50% EtOAc in hexane), the reaction mixture was cooled and quenched with crushed ice (20 mL). Pyridine was removed under vacuum and the reaction mixture was acidified with concentrated HCl by adjusting pH~1. The precipitated solid was filtered and dried under vacuum to get the desired product (1 g, 86.2 %).

Molecular Formula: $C_6H_3ClN_2OS$; Exact Mass: 185.96; LCMS: 185.05 $(M-1)^-$.

Step-3:

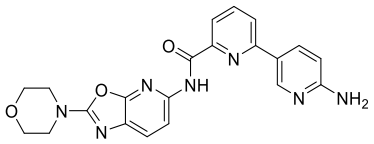


5-chloro-2-morpholinooxazolo[5,4-*b*]pyridine

5-Chlorooxazolo[5,4-b] pyridine-2-thiol (550 mg, 2.956 mmol) was taken in morpholine (5 mL) at room temperature and the mixture was stirred at 110 °C for 15 h. After completion of reaction (monitored by TLC using 50% EtOAc in hexane), solvent was removed under vacuum. The crude product was purified by CombiFlash® chromatography, eluted with 40% EtOAc in hexane to afford the pure product (200 mg, 28.5%).

Molecular Formula: $C_{10}H_{10}ClN_3O_2$; Exact Mass: 239.04; LCMS: 240.0 $(M+1)^+$.

Step-4:

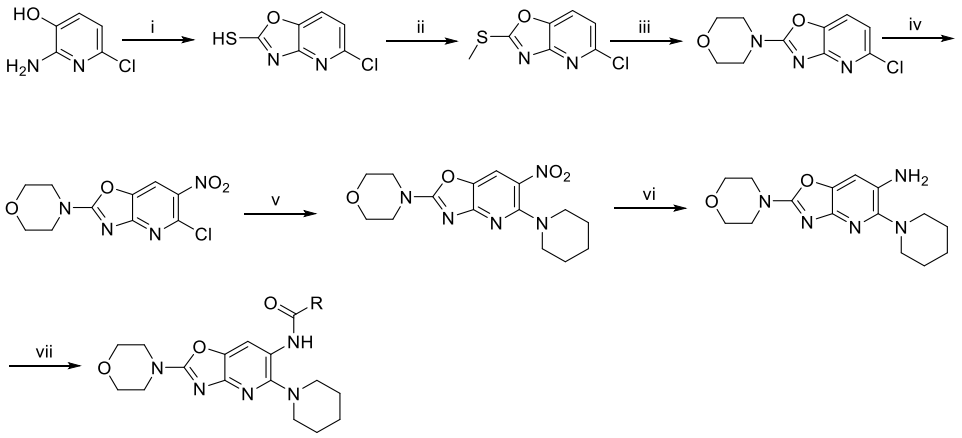


6'-amino-*N*-(2-morpholinooxazolo[5,4-*b*]pyridin-5-yl)-[2,3'-bipyridine]-6-carboxamide

To a solution of 5-chloro-2-morpholinooxazolo[5,4-b]pyridine (76mg , 0.316 mmol) in toluene (5 mL) were added tert-butyl (6-carbamoyl-[2,3'-bipyridin]-6'-yl)carbamate (100 mg, 0.316 mmol), Cs_2CO_3 (257 mg, 0.79 mmol), X-Phos (15 mg, 0.031 mmol) and $Pd_2(dba)_3$ (15 mg, 0.015 mmol) at room temperature and the reaction mixture was stirred at 110 °C for 15 h. After completion of the reaction (monitored by TLC using 10% MeOH in DCM), the reaction mixture was filtered over Celite® bed and the filtrate was concentrated under vacuum. The crude product was purified by prep HPLC by using TFA method to get the desired product (11 mg, 10%).

Molecular Formula: $C_{21}H_{19}N_7O_3$; Exact Mass: 417.15; LCMS (M+1): 418.2; ^{1}HNMR ($CDCl_3$, 300MHz): δ 8.81 (d, 1H), 8.22 (d, 1H), 8.03 (m, 1H), 7.80 (m, 3H), 7.55 (m, 1H), 7.25 (m, 1H), 3.77 (bs, 4H), 3.62 (bs, 5H),3.28 (bs, 2H); HPLC:96.16%.

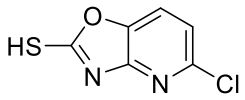
General synthetic scheme-5: Compound **8** - **15** (Tables **1** and **2**) can be synthesized by using the below scheme.



(i) Potassium ethyl xanthate, pyridine, 110°C, 15h; (ii) Ethyl acetate, K_2CO_3, MeI, 20-30°C, 2h; (iii) Morpholine, THF, 75°C, 15h; (iv) Acetic acid, fuming nitric acid, 100°C, 2h; (v) Piperidine,rt, 12h; (vi) NH_4Cl, THF, H_2O, Zn dust, 50°C, 1h (vii) R=corresponding side chains of 8-15, EDC-HCl, TEA, DMF, 20-30°C, 15h; methanolic HCl, 20-30°C, 1h.

Synthesis of compound 8

Step-1:

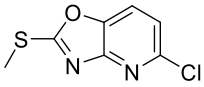


5-chlorooxazolo[4,5-*b*]pyridine-2-thiol

To a solution of 2-amino-6-chloropyridin-3-ol (620 mg, 4.305 mmol) in pyridine (5 mL) was added potassium ethyl xanthate (826 g, 5.16 mmol) at room temperature and the reaction mixture was stirred at 110 °C for 15h. After completion of the reaction (monitored by TLC using 50% EtOAc in hexane), the reaction mixture was cooled and quenched with crushed ice (50 mL). Pyridine was removed under vacuum and the reaction mixture was acidified with concentrated HCl by adjusting pH~1. The precipitated solid was filtered and dried under vacuum to get the desired product (620 mg, 78 %).

Molecular Formula: $C_6H_3ClN_2OS$; Exact Mass: 185.96; LCMS: 187.1(M+1).

Step-2:

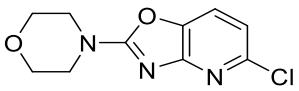


5-chloro-2-(methylthio)oxazolo[4,5-*b*]pyridine

To a solution of 5-chlorooxazolo[4,5-b]pyridine-2-thiol (620 mg, 3.33 mmol) in ethyl acetate (10 mL) were added K_2CO_3 (689 mg, 4.99 mmol) followed by methyl iodide (567 mg, 3.99 mmol). The resulting mixture was stirred at room temperature for 2h. After completion of reaction (monitored by TLC using 50% EtOAc in hexane), the reaction mixture was extracted by using water (25 mL) and ethyl acetate (100 ml). The organic layer was separated, dried over Na_2SO_4 and concentrated to get desired product. (720 mg, 90%).

Molecular Formula: $C_7H_5ClN_2OS$; Exact Mass: 199.98; LCMS: m/z = 201.10 $(M+1)^+$.

Step-3:



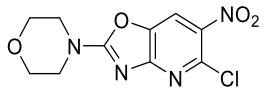
5-chloro-2-morpholinooxazolo[4,5-*b*]pyridine

To a solution of 5-chloro-2-(methylthio)oxazolo[4,5-b]pyridine (720 mg, 38.6 mmol) in dry THF (10 mL), was added morpholine (2 mL) at room temperature and the mixture was stirred at 75 °C for 15 h. After completion of reaction (monitored by TLC using 50% EtOAc in hexane), THF was removed under vacuum

and the reaction was quenched with ice water (50 mL). The precipitated solid was filtered and dried under vacuum to get desired compound (750 mg, 88 %).

Molecular Formula: $C_{10}H_{10}ClN_3O_2$; Exact Mass: 239.04; LCMS: 240.2(M+1)$^+$.

Step-4:

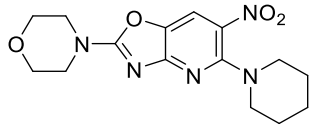


5-chloro-2-morpholino-6-nitrooxazolo[4,5-*b*]pyridine

To a solution of 5-chloro-2-morpholinooxazolo[4,5-b]pyridine (700 mg, 2.928 mmol) in AcOH (8 mL) were added fuming HNO_3 (2mL) and the resulting mixture was stirred at 100 °C for 2 h . After completion of the reaction (monitored by TLC using 50% EtOAc in hexane), the reaction mixture was quenched with crushed ice (50 mL). The resulting solid was filtered and dried under vacuum to afford the product (400 mg, 48%)

^{1}HNMR (DMSO-d_6, 300MHz): δ 8.601 (s, 1H), 3.75 (m, 8H),

Step-5:

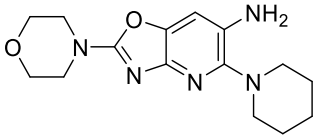


2-morpholino-6-nitro-5-(piperidin-1-yl)oxazolo[4,5-*b*]pyridine

To a solution of 5-chloro-2-morpholino-6-nitrooxazolo[4,5-b]pyridine (300 mg , 1.056 mmol) in dry THF (5 mL) was added piperidine (125 mg, 1.478 mmol). Then reaction mixture was stirred at room temperature for 12 h. After completion of reaction (monitored by TLC using 50% EtOAc in hexane), the reaction mixture was quenched with crushed ice (50 mL). The resulting solid was filtered and dried under vacuum to afford the product (300 mg, 89%)

Molecular Formula: $C_{15}H_{19}N_5O_4$; Exact Mass: 333.14; LCMS: m/z = 334.5 (M+1)$^+$.

Step-6:



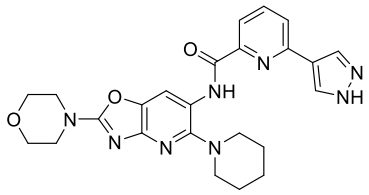
2-morpholino-5-(piperidin-1-yl)oxazolo[4,5-*b*]pyridin-6-amine

A solution of 2-morpholino-6-nitro-5-(piperidin-1-yl)oxazolo[4,5-b]pyridine (300 mg, 0.900 mmol) dissolved in THF/water mixture (20 mL/5 mL), were added NH_4Cl (389 mg, 7.207 mmol), Zn dust(468 mg,

7.207 mmol). The reaction mixture was stirred at 50 °C for 1h. After completion of reaction (monitored by TLC using 50% EtOAc in n-hexane), the reaction mixture was filtered over Celite® bed and the filtrate was concentrated under vacuum to get desired product (260 mg, 96%).

Molecular Formula: $C_{15}H_{21}N_5O_2$; Exact Mass: 303.17; LCMS: m/z = 302.45 $(M-1)^-$.

Step-7:



N-(2-morpholino-5-(piperidin-1-yl)oxazolo[4,5-*b*]pyridin-6-yl)-6-(1*H*-pyrazol-4-yl)picolinamide

To a solution of 2-morpholino-5-(piperidin-1-yl)oxazolo[4,5-b]pyridin-6-amine (90 mg, 0.303 mmol) in DMF (4 ml), were added 6-(1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazol-4-yl)picolinic acid (97 mg, 0.356 mmol), EDC-HCl(85 mg, 0.445 mmol), HOBt (60 mg, 0.445 mmol) and TEA (0.2 ml, 1.188 mmol). Then reaction mixture was stirred at room temperature for 12h. After completion of reaction (monitored by TLC using 5% MeOH in DCM), the reaction mixture was dried and purified by CombiFlash® chromatography eluted with 5% MeOH in $CHCl_3$ to afford N-(2-morpholino-5-(piperidin-1-yl)oxazolo[4,5-b]pyridin-6-yl)-6-(1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazol-4-yl)picolinamide (60 mg, 38%).

Molecular Formula: $C_{29}H_{34}N_8O_4$; Exact Mass: 558.14; LCMS: m/z = 559.60 $(M+1)^+$.

To a solution of N-(2-morpholino-5-(piperidin-1-yl)oxazolo[4,5-b]pyridin-6-yl)-6-(1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazol-4-yl)picolinamide was dissolved in MeOH (2 mL) and added 2 mL of Methanolic HCl at room temperature. Then reaction mixture was stirred at room temperature for 1h. After completion of reaction (monitored by TLC using 10% MeOH in DCM), the reaction mixture was dried under vacuum to get the desired product (50 mg, 90%).

Molecular Formula: $C_{24}H_{26}N_8O_3$; Exact Mass: 474.21; LCMS (M+1): 475.5; ^{1}HNMR (DMSO-d_6, 300MHz): δ 10.78 (s, 1H), 8.65 (s, 1H), 8.43 (s, 2H), 8.05-7.93 (m, 3H), 3.76-3.72 (m, 4H), 3.63-3.62 (m, 4H), 2.98 (bs, 4H), 1.76 (bs, 4H), 1.54 (bs, 2H); HPLC % purity: 90.31

Compounds 9-15 were synthesized by following above procedure and the analytical data are shown below.

Compound 9: Molecular Formula: $C_{26}H_{28}N_8O_3$; Exact Mass: 500.23; LCMS (M+1): 501.1; ^{1}HNMR ($CDCl_3$, 400MHz): δ10.99 (s, 1H), 8.89 (s, 1H), 8.73-8.73 (d, 1H), 8.55-8.52 (dd, 1H), 8.24-8.21 (d, 1H), 8.00-7.96 (t,

1H), 7.86-7.83 (d, 1H), 6.74-6.71 (d, 1H), 5.30 (bs, 2H), 3.84-3.82 (m, 4H), 3.76-3.74 (m, 4H), 3.06-3.03 (m, 4H), 1.78-1.72 (m, 4H), 1.57-1.56(m, 2H); HPLC % purity: 95.00

Compound 10: Molecular Formula: $C_{25}H_{28}N_8O_3$; Exact Mass: 488.23; LCMS (M+1): 489.3; ^{1}HNMR (DMSO-d_6, 400MHz): δ 10.80 (s, 1H), 8.73 (s, 1H), 8.43 (s, 1H), 8.21 (s, 1H), 8.09-8.02 (m, 1H), 8.01-7.92 (m, 2H), 3.92 (s, 3H), 3.75 (bs, 4H), 3.65 (bs, 4H), 2.95 (bs, 4H), 1.75 (bs, 4H), 1.55 (bs, 2H); HPLC % purity: 96.26

Compound 11: Molecular Formula: $C_{25}H_{32}N_8O_3$; Exact Mass: 492.26; LCMS (M+1): 493.3; ^{1}HNMR ($CDCl_3$, 400MHz): δ 10.76 (s, 1H), 8.84 (s, 1H), 7.65-7.61 (t, 1H), 7.57-7.55 (d, 1H), 6.56-6.54 (d, 1H), 3.87-3.63 (m, 10H), 3.39-3.36 (m, 1H), 3.04-3.01 (m, 4H), 2.28-2.25 (m, 1H), 1.90-1.87 (m, 1H), 1.77-1.76 (m, 4H), 1.60-1.56 (m, 6H); HPLC % purity: 97.84

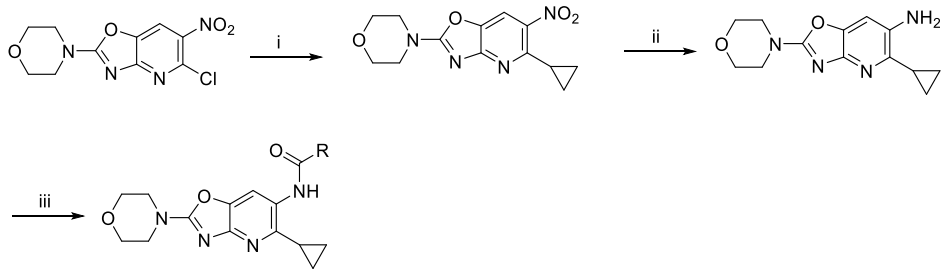
Compound 12: Molecular Formula: $C_{25}H_{31}N_7O_4$; Exact Mass: 493.24; LCMS (M+1): 494.2; ^{1}HNMR ($CDCl_3$, 400MHz): δ 10.66 (s, 1H), 8.83 (s, 1H), 7.64-7.62 (t, 1H), 7.58-7.56 (d, 1H), 6.58-6.56 (d, 1H), 4.70 (bs, 1H), 3.83-3.79 (m, 4H), 3.76-3.72 (m, 7H), 3.04-3.03 (bs, 4H), 2.30-2.10 (m, 2H), 1.77-1.72 (m, 5H), 1.61-1.57(m, 3H); HPLC % purity: 98.603

Compound 13: Molecular Formula: $C_{25}H_{27}N_7O_4$; Exact Mass: 489.21; LCMS (M+1): 490.2; ^{1}HNMR ($CDCl_3$, 400MHz): δ 10.04 (s, 1H), 8.77 (s, 1H), 8.71-8.70 (d, 1H), 8.39 (s, 1H), 7.82 (s, 1H), 7.73-7.71 (d, 1H), 3.83-3.80 (d, 4H), 3.78-3.72 (d, 4H), 3.08-3.05 (t, 4H), 2.67 (s, 3H), 1.93-1.86 (m, 4H), 1.71-1.63 (m, 2H); HPLC % purity: 98.43

Compound 14: Molecular Formula: $C_{25}H_{27}N_7O_5$; Exact Mass: 505.21; LCMS (M+1): 506.2; ^{1}HNMR ($CDCl_3$, 400MHz): δ 10.00 (s, 1H), 8.76 (s, 1H), 8.38-8.33 (d, 2H), 7.51 (s, 1H), 7.38 (s, 1H), 4.01 (s, 3H), 3.82 (s, 4H), 3.74 (s, 4H), 3.06 (s, 4H), 1.89 (s, 4H), 1.69 (s, 2H); HPLC % purity: 95.81

Compound 15: Molecular Formula: $C_{23}H_{29}N_7O_5$; Exact Mass: 483.22; LCMS (M+1): 484.2; ^{1}HNMR ($CDCl_3$, 300MHz): δ 9.78 (s, 1H), 8.76 (s, 1H), 7.82 (s, 1H), 4.65 (bs, 1H), 3.82-3.79 (m, 4H), 3.73-3.67 (m, 7H), 3.60-3.56 (d, 1H), 3.03-3.00 (t, 4H), 2.19-2.11 (m, 2H), 1.81-1.80 (d, 6H), 1.59 (m, 1H); HPLC % purity: 95.55

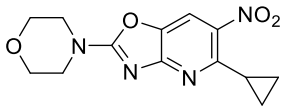
General synthetic scheme-6: Compound **16** & **17** (Table 3) can be synthesized by using below scheme.



(i) Cyclopropyl boronic acid, K_2CO_3, $Pd(Pph_3)_4$, Xylene, 100-120°C, 2h; (ii) NH_4Cl, THF, H_2O, Zn dust, 50°C, 1h (iii) R=16 & 17 side chains, EDC-HCl, TEA, DMAP, DMF, 20-30°C, 15h; methanolic HCl, 20-30°C, 1h.

Synthesis of compound 16

Step:1

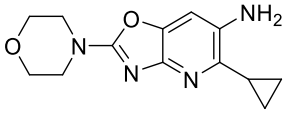


5-cyclopropyl-2-morpholino-6-nitrooxazolo[4,5-*b*]pyridine

To a solution of 5-chloro-2-morpholino-6-nitrooxazolo[4,5-b]pyridine (400 mg, 1.408 mmol) in xylene (10 mL) were added cyclopropyl boronic acid (145 mg,1.690 mmol),K_2CO_3 (388 mg, 2.816 mmol). The reaction mixture was degassed for 15 mins with argon. Then tetrakis(triphenylphosphine)palladium (81 mg, 0.070 mmol) was added and stirred at 120 °C for 2 h. After completion of reaction (monitored by TLC using 50% EtOAc in hexane), the reaction mixture was extracted by using water (25 mL) and ethyl acetate (100 mL). The organic layer was separated, dried over Na_2SO_4 and concentrated to get the desired product. (220 mg, 54%).

Molecular Formula: $C_{13}H_{14}N_4O_4$; Exact Mass: 290.10; LCMS: m/z = 291.1 $(M+1)^+$.

Step:2



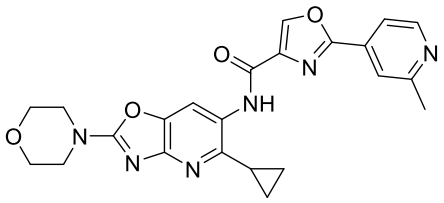
5-cyclopropyl-2-morpholinooxazolo[4,5-*b*]pyridin-6-amine

To a solution 5-cyclopropyl-2-morpholino-6-nitrooxazolo[4,5-b]pyridine (220 mg, 0.758 mmol) in THF/water mixture (10 ml /5 ml) were added NH_4Cl (327 mg, 6.068 mmol) Zn dust(394 mg, 6.068 mmol). The reaction mixture was stirred 50 °C for 1h. After completion of reaction (monitored by TLC using 5% MeOH in DCM), the reaction mixture was filtered over Celite® bed and the filtrate was extracted by using

water (25 mL) and ethyl acetate (100 mL). The organic layer was separated, dried over Na_2SO_4 and concentrated to get the desired product. (160 mg, 84%).

Molecular Formula: $C_{13}H_{16}N_4O_2$; Exact Mass: 260.12; LCMS: m/z = 261.0 $(M+1)^+$.

Step:3



N-(5-cyclopropyl-2-morpholinooxazolo[4,5-*b*]pyridin-6-yl)-2-(2-methylpyridin-4-yl)oxazole-4-carboxamide

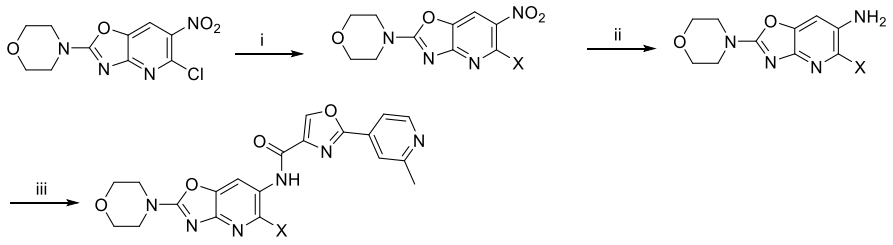
To a solution of 5-cyclopropyl-2-morpholinooxazolo[4,5-b]pyridin-6-amine (60 mg, 0.23 mmol) in DMF(4 mL) were added 2-(2-methylpyridin-4-yl)oxazole-4-carboxylic acid(70.6 mg, 0.396 mmol), EDC-HCl(66 mg, 0.396 mmol), HOBt (46 mg, 0.396 mmol) and TEA (0.13 ml, 0.923 mmol). The reaction mixture was stirred at room temperature for 12 h. After completion of reaction (monitored by TLC using 5% MeOH in DCM), the reaction mixture was concentrated to dryness. The crude product was purified by Prep HPLC to get pure product, which was stirred with 2 ml of methanolic HCl for 30 mins. After concentration to get desired product as a HCl Salt (20 mg, 20%).

Molecular Formula: $C_{23}H_{22}N_6O_4$; Exact Mass: 446.17; LCMS (M+1): 447.1; ^{1}HNMR (DMSO-d_6, 400MHz): δ 10.23 (s, 1H), 9.27 (s, 1H), 8.85-8.83 (d, 1H), 8.25 (s, 1H), 8.14-8.13 (d, 1H), 7.72 (s, 1H), 3.71-3.68 (bs, 4H), 3.61-3.59 (bs, 4H), 2.73 (s, 3H), 2.17-2.14 (m, 1H), 0.89-0.86 (d, 4H); HPLC % purity: 99.0

Compound 17 was synthesized following above procedure and the analytical data is shown below.

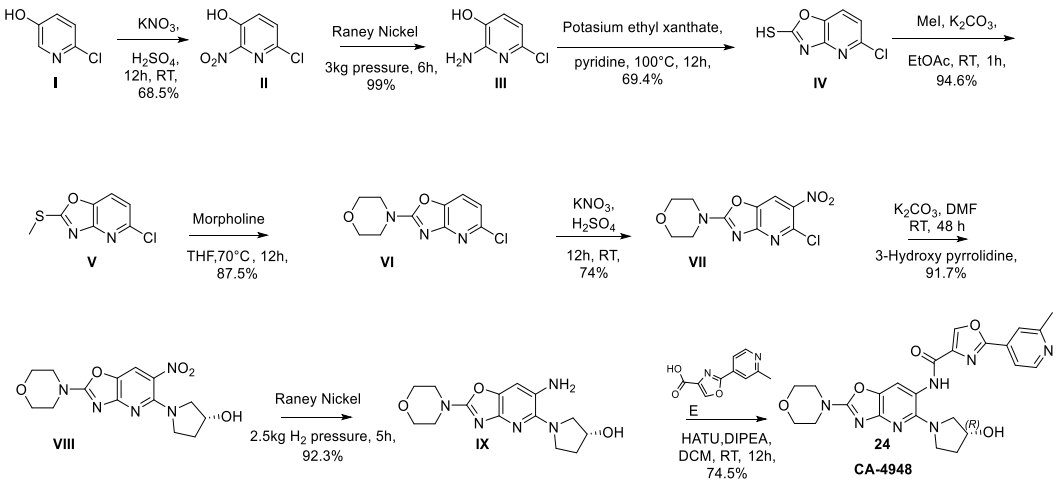
Compound 17: Molecular Formula: $C_{21}H_{24}N_6O_5$; Exact Mass: 440.18; LCMS (M+1): 441.8; ^{1}HNMR ($CDCl_3$, 300MHz): δ9.17 (s, 1H), 8.33 (s, 1H), 7.84 (s, 1H), 4.63 (bs, 1H), 3.82-3.79 (m, 4H), 3.74-3.66 (m, 7H), 3.60-3.56 (d, 1H), 2.13-2.03 (m, 3H), 1.86-1.84 (d, 1H), 1.16-1.14 (m, 2H), 1.04-1.00 (m, 2H); HPLC % purity: 95.51

General synthetic scheme-7: Compound **18** - **25** (Table 3) can be synthesized using below scheme.

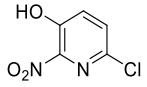


(i) corresponding cyclic amines, K_2CO_3, DMF, 15h; (ii) Pd/C, MeOH, 20-30°C, 2h (iii) 2-(2-methyl pyridin-4-yl)oxazol-4-carboxylic acid, EDC-HCl, HOBT, DIPEA, DMF, 20-30°C, 15h;

Synthesis of compound 24



Step-1:

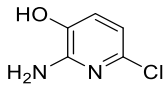


6-chloro-2-nitropyridin-3-ol

Concentrated sulphuric acid (6000 mL) was cooled to 0 °C and was added 6-chloropyridin-3-ol (1000 g, 7.72 mol) in portions over 30 mins (exothermicity observed up to 29°C). The reaction mixture was again cooled to 0°C and added potassium nitrate (1483 g, 14.67 mol) in portions and the resulting mixture was stirred at room temperature for 12 h. After completion of reaction (monitored by TLC using 10% EtOAc in hexane), the reaction mixture was quenched with crushed ice (60 mL). The resulting solid was filtered and dried under vacuum to get the pure product (920 g, 68.5%).

^{1}HNMR (DMSO-d_6, 300MHz): δ 11.90 (s, 1H), 7.68–7.77 (m, 2H) ppm. HPLC: 97.03%

Step-2:

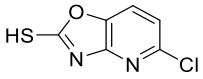


2-amino-6-chloropyridin-3-ol

6-Chloro-2-nitropyridin-3-ol (500 g, 2.87 mol) was dissolved in methanol (5000 mL) in a 10 L autoclave and added raney nickel (150 g). The reaction mixture was stirred at room temperature under 3 kg H_2 pressure for 6 h. After completion of reaction (monitored by TLC using 20% EtOAc in DCM), the reaction mixture was filtered over Celite® bed and the filtrate was concentrated under vacuum to get the desired product (409 g, 99%).

Molecular Formula: $C_5H_5ClN_2O$; Exact Mass: 144.00; LCMS: 145.2 $(M+1)^+$.

Step-3:

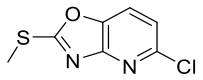


5-chlorooxazolo[4,5-*b*]pyridine-2-thiol

To a solution of 2-amino-6-chloropyridin-3-ol (1000 g, 6.94 mol) in pyridine (10000 mL) was added potassium ethyl xanthate (1447 g, 9.02 mol) at room temperature and the reaction mixture was stirred at 100 °C for 12h. After completion of the reaction (monitored by TLC using 40% EtOAc in hexane), the reaction mixture was cooled and quenched with crushed ice (30 mL). Pyridine was removed under vacuum and the reaction mixture was acidified with concentrated HCl by adjusting pH~1. The precipitated solid was filtered and dried under vacuum to get the desired product (900 g, 69.4 %).

Molecular Formula: $C_6H_3ClN_2OS$; Exact Mass: 185.96; LCMS: 187.1(M+1); ^{1}HNMR (DMSO-d_6, 300MHz): δ 7.94-7.90 (d, 1H), 7.38-7.35 (d, 1H).

Step-4:

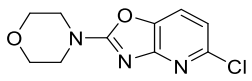


5-chloro-2-(methylthio)oxazolo[4,5-*b*]pyridine

To a solution of 5-chlorooxazolo[4,5-b]pyridine-2-thiol (910 g, 4.89 mol) in ethyl acetate (9100 mL) were added K_2CO_3 (1350 g, 9.78 mol) followed by methyl iodide (630 mL, 9.78 mol). The resulting mixture was stirred at room temperature for 1h. After completion of reaction (monitored by TLC using 30% EtOAc in hexane), the reaction mixture was extracted by using water (5 L) and ethyl acetate (2 L). The organic layer was separated, dried over Na_2SO_4 and concentrated to get the crude product. The crude product was washed with 10% ethyl acetate in hexane (2.5 L) to afford pure compound (650 g, 66%).

Molecular Formula: $C_7H_5ClN_2OS$; Exact Mass: 199.98; LCMS: m/z = 201.10 $(M+1)^+$; ^{1}HNMR ($CDCl_3$, 400MHz): δ 7.66 – 7.64 (d, 1H), 7.21 – 7.19 (d, 1H), 2.79 (s, 3H) ppm; HPLC: 94.66%.

Step-5:

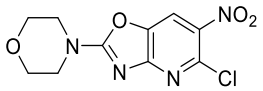


5-chloro-2-morpholinooxazolo[4,5-*b*]pyridine

To a solution of 5-chloro-2-(methylthio)oxazolo[4,5-b]pyridine (1000 g, 5.0 mol) in dry THF (10000 mL), was added morpholine (2000 mL) at room temperature and the mixture was stirred at 100 °C for 12 h. After completion of reaction (monitored by TLC using 50% EtOAc in hexane), THF was removed under vacuum and the reaction was quenched with ice water (10000 mL). The precipitated solid was filtered and dried under vacuum to get the desired compound (1049 g, 87.5 %).

Molecular Formula: $C_{10}H_{10}ClN_3O_2$; Exact Mass: 239.04; LCMS: 240.2$(M+1)^+$; ^{1}HNMR (DMSO-d_6, 400MHz): δ 7.82-7.80 (d, 1H), 7.08-7.06 (d, 1H), 3.74-3.64 (m, 8H).

Step-6:

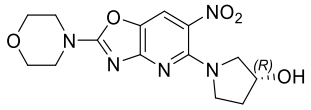


5-chloro-2-morpholino-6-nitrooxazolo[4,5-*b*]pyridine

Concentrated sulphuric acid (6000 mL) was cooled to 0 °C and added 5-chloro-2-morpholinooxazolo[4,5-b]pyridine (1000 g, 4.17 mol) in portions followed by potassium nitrate (800 g, 7.92 mol) in portions and the resulting mixture was stirred at room temperature for 12 h. After completion of the reaction (monitored by TLC using 35% EtOAc in DCM), the reaction mixture was quenched with crushed ice (60 L). The resulting solid was filtered and dried under vacuum to afford the product (875 g, 74%)

Molecular Formula: $C_{10}H_9ClN_4O_4$; Exact Mass: 284.03; LCMS: m/z = 285.20 $(M+1)^+$; ^{1}HNMR (DMSO-d_6, 300MHz): δ 8.60 (s, 1H), 3.72 (s, 8H) .

Step-7:

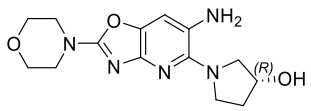


(*R*)-1-(2-morpholino-6-nitrooxazolo[4,5-*b*]pyridin-5-yl)pyrrolidin-3-ol

To a solution of 5-chloro-2-morpholino-6-nitrooxazolo[4,5-b]pyridine (1000 g, 3.51 mol) in dry DMF (8000 mL) at 0 °C was added K_2CO_3 (1450 g, 10.56 mol) followed by (R)-pyrrolidin-3-ol hydrochloride (436 g, 3.514 mol). The reaction mixture was stirred at room temperature for 48 h. After completion of reaction (monitored by TLC using 5% MeOH in DCM), it was quenched with ice water (80 L). The resulting solid was filtered and dried under vacuum to afford the product (1080 g, 91.7%).

Molecular Formula: $C_{14}H_{17}N_5O_5$; Exact Mass: 335.12; LCMS: m/z = 336.1 $(M+1)^+$.

Step-8:

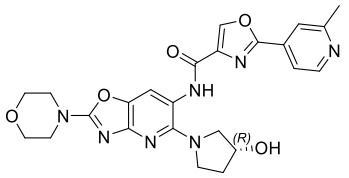


(*R*)-1-(6-amino-2-morpholinooxazolo[4,5-*b*]pyridin-5-yl)pyrrolidin-3-ol

(R)-1-(2-morpholino-6-nitrooxazolo[4,5-b]pyridin-5-yl)pyrrolidin-3-ol (300 g, 0.895 mol) were dissolved in methanol/ethyl acetate mixture (3000 mL/3000 mL) in a 10 L autoclave and was added raney nickel (150 g). The reaction mixture was stirred under 2.5 kg H_2 pressure at room temperature for 5 h. After completion of reaction (monitored by TLC using 50% EtOAc in hexane), the reaction mixture was filtered over Celite® bed and the filtrate was concentrated under vacuum. The crude product was washed with 10% methanol in ether to get the pure compound which was used for next step (252 g, 92.3%).

Molecular Formula: $C_{14}H_{19}N_5O_3$; Exact Mass: 305.14; LCMS: m/z = 306.05 $(M+1)^+$; ^{1}HNMR (DMSO-d6, 300MHz): δ 7.07 (s, 1H), 4.92 (d, 1H), 4.49 (s, 2H), 4.29 (bs, 1H), 3.68 (m, 4H), 3.37 (m, 4H), 3.12 (m, 1H), 3.07 (m, 1H), 2.00 (m, 1H), 1.71 (m, 1H) ppm.

Step-9:



(*R*)-*N*-(5-(3-hydroxypyrrolidin-1-yl)-2-morpholinooxazolo[4,5-*b*]pyridin-6-yl)-2-(2-methylpyridin-4-yl)oxazole-4-carboxamide

To a solution of (R)-1-(6-amino-2-morpholinooxazolo[4,5-b]pyridin-5-yl)pyrrolidin-3-ol (252 g, 0.826 mol), in DCM (3750 mL) was added 2-(2-methylpyridin-4-yl)oxazole-4-carboxylic acid (170.6 g, 0.836 mol), HATU (392.4 g, 1.032 mol) and DIPEA (213.5 g, 1.652 mol). Then reaction mixture was stirred at room temperature for 12 h. After completion of reaction (monitored by TLC using 7.5% MeOH in DCM), it was

dried and residue was stirred with acetonitrile (7.5 L) and the resultant solid filtered to get the pure product (302 g, 74.5%).

Molecular Formula: $C_{24}H_{25}N_7O_5$; Exact Mass: 491.19; LCMS (M+1): 492.20; ^{1}HNMR (DMSO-d_6, 300MHz): δ 9.82 (s, 1H), 8.96 (s, 1H), 8.68-8.67 (d, 1H), 7.86 (s, 1H), 7.77-7.76 (d, 1H), 7.66 (s, 1H), 4.88-4.87 (d, 1H), 4.27 (bs, 1H), 3.73-3.71 (m, 4H), 3.61-3.55 (m, 6H), 3.44-3.40 (m, 1H), 3.25-3.22 (m, 1H), 2.59 (s, 3H), 1.91-1.88 (m, 1H), 1.77-1.75(m, 1H); ^{13}C NMR (CDCl3, 400MHz): δ (163.6), (159.8), (159.7), (157.9), (152.1), (150.1), (149.8), (142.1), (137.9), (133.8), (119.7), (118.7), (117.3), (110.9), (71.6), (66.1), (59.3), (49.3), (45-47), (34.9), (24.6); FTIR: 882.60(=C-H stretch), 1112.92(C-O stretch), 1646.54(C=C stretch), 1677.54(=C-H stretch), 2861.40(C-H stretch), 3297.68(O-H stretch); HRMS (ESI-TOF) (M^+ + H): calcd, 492.1995; found, 492.1996; HPLC % purity: 95.08%; MP: 230-232°C.

Compounds 18-23 and 25 were synthesized by following general synthetic scheme-7 using corresponding cyclic secondary amine (1 equivalent) in step-i and the analytical data are shown below.

Compound 18: In scheme-7 step-i 3-fluoropiperidine (1 equivalent) used. Molecular Formula: $C_{25}H_{26}FN_7O_4$; Exact Mass: 507.20; LCMS (M+1): 508.0; ^{1}HNMR (DMSO-d_6, 300MHz): δ 9.86 (s, 1H), 9.05 (s, 1H), 8.70-8.68 (d, 1H), 8.62 (s, 1H), 7.85 (bs, 1H), 7.75-7.74 (d, 1H), 5.09-4.87 (m, 1H), 3.73-3.70 (m, 4H), 3.62-3.61 (m, 4H), 3.26-3.07 (m, 2H), 2.90-2.85 (m, 2H), 2.57 (s, 3H), 2.20-1.85 (m, 3H), 1.70-1.69 (m, 1 H); HPLC % purity: 99.27

Compound 19: In scheme-7 step-i piperidin-4-ol (1 equivalent) used. Molecular Formula: $C_{25}H_{27}N_7O_5$; Exact Mass: 505.21; LCMS (M+1): 506.1; ^{1}HNMR (DMSO-d_6, 300MHz): δ9.85 (s, 1H), 9.01 (s, 1H), 8.66-8.64 (d, 1H), 8.58 (s, 1H), 7.83 (bs, 1H), 7.74-7.72 (d, 1H), 4.93-4.80 (bs, 1H), 3.71-3.70 (m, 5H), 3.61-3.59 (m, 4H), 3.12-3.08 (m, 2H), 2.85-2.82 (t, 2H), 2.57 (s, 3H), 1.99-1.96 (m, 2H), 1.79-1.76 (m, 2H); HPLC % purity: 98.00

Compound 20: In scheme-7 step-i (R)-pyrrolidin-3-amine (1 equivalent) used. Molecular Formula: $C_{24}H_{26}N_8O_4$; Exact Mass: 490.21; LCMS (M+1): 491.2; ^{1}HNMR (CD_3OD, 400MHz): δ 8.72 (s, 1H), 8.65-8.63 (d, 1H), 8.00 (bs, 1H), 7.93-7.90 (m, 2H), 3.84-3.81 (m, 4H), 3.73-3.64 (m, 7H), 3.51-3.49 (m, 2H), 2.67 (s, 3H), 2.30-2.21 (m, 1H), 1.90-1.80 (m, 1H); HPLC % purity: 95.80

Compound 21: In scheme-7 step-i 3-(hydroxymethyl) piperidin-3-ol (1 equivalent) used. Molecular Formula: $C_{26}H_{29}N_7O_6$; Exact Mass: 535.22; LCMS (M+1): 536.3; ^{1}HNMR (DMSO-d_6, 400MHz): δ9.93 (s, 1H), 9.18 (s, 1H), 8.84-8.83 (d, 1H), 8.60 (s, 1H), 8.22 (bs, 1H), 8.06 (d, 1H), 3.74-3.72 (m, 4H), 3.64-3.54 (m, 7H),

2.93-2.84 (m, 5H), 2.71(s, 3H), 2.02-1.92 (m, 1H), 1.85-1.72 (m, 2H), 1.51-1.43 (m, 1H); HPLC % purity: 97.87

Compound 22: In scheme-7 step-i 4-fluoropiperidine (1 equivalent) used. Molecular Formula: $C_{25}H_{26}FN_7O_4$; Exact Mass: 507.20; LCMS (M+1): 508.3; ^{1}HNMR ($CDCl_3$, 300MHz): δ9.97 (s, 1H), 8.76 (s, 1H), 8.70-8.69 (d, 1H), 8.39 (s, 1H), 7.82 (bs, 1H), 7.71-7.69 (dd, 1H), 4.97-4.72 (m, 1H), 3.84-3.81 (m, 4H), 3.76-3.73 (m, 4H), 3.29-3.27 (m, 2H), 3.10-3.05 (m, 2H), 2.67 (s, 3H), 2.27-2.18 (m, 4H); HPLC % purity: 90.17

Compound 23: In scheme-7 step-i (R)-3-fluoropyrrolidine (1 equivalent) used. Molecular Formula: $C_{24}H_{24}FN_7O_4$; Exact Mass: 493.19; LCMS (M+1): 494.1; ^{1}HNMR (CD_3OD, 400MHz): δ 8.92 (s, 1H), 8.89-8.87 (d, 1H), 8.56 (bs, 1H), 8.49-8.47 (d, 1H), 8.00 (s, 1H), 5.40-5.26 (d, 1H), 3.94-3.73 (m, 12H), 2.89 (s, 3H), 2.30-2.27 (m, 2H); HPLC % purity: 95.72

Compound 25: In scheme-7 step-i (S)-pyrrolidin-3-ol (1 equivalent) used. Molecular Formula: $C_{24}H_{25}N_7O_5$; Exact Mass: 491.19; LCMS (M+1): 492.1; ^{1}HNMR ($CDCl_3$, 300MHz): δ 9.45 (s, 1H), 8.69-8.67 (d, 1H), 8.51 (s, 1H), 8.40 (s, 1H), 7.81 (s, 1H), 7.74-7.72 (d, 1H), 4.57 (bs, 1H), 3.83-3.80 (m, 4H), 3.75-3.72 (m, 4H), 3.59-3.41 (m, 4H), 2.83 (bs, 1H), 2.67 (s, 3H), 2.29 (m, 1H), 2.03-1.61 (m, 1H); HPLC % purity: 98.03

Biochemical Assays and Broad Kinome Profiling

The potency of compounds to inhibit IRAK4 enzyme was tested in a TR-FRET assay using recombinant IRAK4 kinase obtained from Millipore, USA. The assay buffer was 50 mM Tris-HCl pH 7.5, 20 mM $MgCl_2$, 1 mM EGTA, 3 mM $MnCl_2$, 0.01% Tween 20 and 2 mM DTT. Test compounds were pre-incubated with 5 ng of IRAK4 enzyme at room temperature and after 30 minutes, kinase reaction was initiated by adding the substrate mixture containing 100 nM Biotin labelled Histone H3 (Millipore, USA) and 20 µM ATP (Sigma, USA). After 30 min incubation, the reaction was stopped by the addition of stop mix containing 40 mM EDTA, 1 nM of Europium-Anti-Phospho-Histone H3(Ser10) antibody (Perkin Elmer, USA) and 20 nM sure-Light Allophycocyanin - Streptavidin (Perkin Elmer, USA). Fluorescence emission of the samples at 665 and 615 nm were measured at an excitation of 340 nm and their ratio was plotted against the compound concentrations to generate dose-response curve. IC_{50} values were determined by fitting the dose-response data to sigmoidal curve fitting equation using Graph pad prism software V7. Staurosporine was used as a standard compound in each assay run with measured IC_{50} value of 7.3 ± 1.6 nM (*N*=25).

Screening of the compounds in the in-house kinome panel was done using Time resolved Fluorescence (TR-FRET) assay and Kinase Glo assay formats. Out of 5 kinases, 4 kinase assays (Flt3, CDK2, Aurora A and MUSK) were performed using TR-FRET assay and 1 (GSK3-b) was performed using Kinase Glo assay format. All the kinase assays were performed at their respective ATP Km concentration. The screening of the compounds was done at 0.1 μM and 1 μM and the percent inhibition were calculated.

Compound **24** was also screened in a broad kinome panel using the Eurofins standard Kinase Profiler assays and following relevant standard operating procedures. The inhibitions recorded are shown in Table S1.

Protein Expression and Purification

The protein was essentially cloned, expressed and purified as described elsewhere.[1] To determine the most suitable boundary of the IRAK4 kinase domain for molecular structure determination, sequential 1-aa N-terminal truncations (residues 150–165) and 3-aa C-terminal truncations (residues 417–453) were made from IRAK4 cDNA. Truncated IRAK4 constructs were generated by overlap-extension PCR in which (from the N terminus) a 6-His tag, TEV protease spacer and TEV cleavage site were encoded. PCR products were subcloned into the baculovirus transfer vector pFastBacHT, and DNA sequence was verified. Correct constructs were cotransfected into *Sf9* cells, and the produced virus was plaque-purified to generate clonal populations. Titered, plaque-purified virus was used to determine that a multiplicity of infection of 0.3 with a harvest time of 72 h was optimal for protein expression. An N-terminal truncation in which amino acid residues 160 through 460 of IRAK4 are expressed was one candidate identified to possess good expression and purification properties.

For large-scale purification of IRAK4 kinase domain, pellets from 10 L of infected *Sf9* cell were resuspended in 600 ml of lysis buffer (50 mM HEPES (pH 7.5), 300 mM NaCl, 10 mM 2-ME, 12 Complete protease inhibitor tablets (Roche Applied Science), 5 mg of DNase per 100 mg of cells. Cells were then lysed with a microfluidizer (Microfluidics) at <9,000 psi on ice. The lysate was centrifuged at 25,000 × *g* and the supernatant was applied to a 50-ml Talon Superflow metal affinity column (BD Biosciences) pre-equilibrated in column buffer A (50 mM HEPES (pH 7.5), 300 mM NaCl, 10% (v/v) glycerol, 10 mM 2-ME, and 20 mM imidazole. The bound protein was eluted with buffer B (buffer A with 100 mM imidazole (pH 7.5)) and concentrated to 5 mg/ml. The 6-His tag was cleaved overnight at 4°C with AcTEV protease (Invitrogen Life Technologies) in 0.3% (w/v) *n*-octyl-β-D-glucopyranoside, <20 mM imidazole, 1× stock buffer, 1 mM DTT and 2,000 U AcTEV. The reaction mixture was passed over a 5-ml Ni-HP column (GE

Healthcare) pre-equilibrated in buffer A and the flow-through was concentrated to 5–10 ml with an Amicon Ultra-15 concentrator (Millipore). The last step of the purification was size-exclusion chromatography using a HiLoad Superdex 75 16/60 column equilibrated with 250 mM NaCl, 50 mM Hepes pH 7.5 and 2 mM DTT after which the protein was purified to 16 mg/mL for crystallization. For compound **1**, pFastBacHTB IRAK4 (154-460) construct was used for crystallization. The expression and purification followed similar protocol described above. SF9 cells were infected with 1% of IRAK4 P3 virus for 68hrs at 27^{o}C. The protein was purified using Ni beads followed by size exclusion chromatography using S-75 column.

Crystallization

Crystals of IRAK4 in complex with CA-4948 were obtained using hanging drop vapour diffusion set-ups. IRAK4 at a concentration of 16 mg/ml (50 mM HEPES, 250 mM NaCl, 2 mM DTT, pH 7.5) was pre-incubated with 2.5 mM (5.3-fold molar excess) of CA-4948 (150 mM in DMSO) for 1 h. 1 µl of the protein solution was then mixed with 1 µl of reservoir solution (0.1 M sodium acetate pH 5.4, 2.3 M sodium malonate) and equilibrated at 20 °C over 0.4 ml of reservoir solution. Well diffracting crystals appeared within 4 days and grew to full size over 13 days. For compound **1**-IRAK4 crystallization, the protein at 4mg/ml was incubated with 10 fold molar excess of compound and incubated over night at 4^{0}C. Crystals obtained in most of the conditions within 24hrs -3 days of set up.

Data collection, Structure Determination and Refinement A complete 2.4 Å data set of an IRAK4/CA-4948 crystal was collected at Diamond synchrotron radiation source (Didcot, UK, beamline i041). IRAK4/compound **1** crystals X-ray diffraction data were collected using in-house RU-300 rotating anode Rigaku machine mounted with R-IV++ image plate detector. The diffraction data sets were processed using programs under CCP4 suite.[2] Molecular replacement was done using published structures of IRAK4 (pdb accession codes 2OIB, 2NRU) as the starting model.[1] Clear electron density in the FoFc omit map of the initial model at the compound binding site revealed the binding of the entire compound and allowed an unambiguous placement of the ligand. Further refinement cycles confirmed the initial placement. Several rounds of alternating manual re-building and refinement with REFMAC5[2,3] resulted in the final model. The final model has excellent stereochemistry with one outlier in a Ramachandran plot. The protein modeling and ligand building into clear electron density was modeled using COOT[4] and Pymol[5] for drawing structural figures. Data collection and Structure Refinement Statistics are shown in Table S2.

Molecular Modeling

Atomic coordinates of IRAK4 co-crystal structures were employed for setting up molecular docking protocols. The protein was initially prepared through appropriate bond-order assignment, exclusion of water molecules, addition of hydrogen atoms, refinement of internal hydrogen bondings and restrained minimization using OPLS 2005 force-field. Few side-chain hydrogen bonding constraints were specified during receptor grid generation so that they could be used in docking if needed. All designed molecules were energy minimized through LigPrep and further docked in to generated receptor grid (IRAK4 active-site) through standard precision mode using Glide software. The structural images were prepared using PyMOL. All software names mentioned in this section are products of Schrodinger Inc.

Cell Based Mechanistic Assays[6-9]

Human Whole Blood Assay Blood was collected from healthy human volunteers in sodium Heparin coated vacutainer tubes (BD, Cat # 367874) and diluted 1:1 in serum free RPMI-1640 media (Sigma, Cat # R6504). 160 µL of diluted blood was added per well in a 96 well U-bottom plate (Corning, Cat # 3799). On a separate U-bottom plate a 200X stock of the required test concentration of the compound was made in 100% DMSO (Sigma, Cat # D5879). 10 µL of this solution was diluted into 190 µL of serum free RPMI-1640 to get a 20X concentration. 20 µL of the 20X solution was added to 160 µL of diluted blood that was already plated in U-bottom plates. The plates were incubated for 1 hour at 37^0C in a CO_2 incubator with 5% CO_2. After pretreatment with compound 20 µl of 10X IL-1β (R&D system Cat # 201-LB-005, 0.01 µg /ml final concentration) or 10X LTA (Invivogen Cat # tlrl-pslta ,1 µg/ml final concentration) or 10X FLA-ST (Invivogen Cat # tlrl-epstfla , 0.1 µg/ml final concentration) was added to the wells and incubated in an incubator with 5% CO_2 at 37^0C for 16 hours. Wells treated with 0.5% DMSO alone was used as unstimulated and untreated control. Wells treated with 0.5% DMSO and TLR agonist were used as stimulation control. The plates were then centrifuged at 1500 x g for 10 min and plasma was collected for cytokine analysis. The samples were analyzed for IL-6 levels using the Human IL-6 ELISA kit from R&D systems (Cat # DY206) following the kit manufacturer's protocol. Absorbance was read at 450 & 570 nM on Spectramax Gemini M3 (Molecular Devices). Absorbance at 570nM was subtracted from the absorbance at 450nM as background control for all the wells. Blank subtracted absorbance values from unstimulated wells were subtracted from blank subtracted absorbance values of experimental wells. Cytokine levels in experimental wells were extrapolated from the standard curve for IL-6. % inhibition was calculated by normalizing the IL-6 inhibition in the stimulation control wells to 0%. The % inhibition values were plotted against compound concentration in GraphPad prism to calculate the IC_{50} for the compound.

Inhibition of LTA induced TNFα in THP-1 Cells THP-1 cells were cultured in RPMI-1640 (Sigma cat # R6504) + 10% FBS (Gibco cat # 10437-028) + 1% penicillin streptomycin (Sigma cat # P0781). 10^5 cells were seeded per well of a 96 well plate (Corning cat # 3596) in 160 µl of serum free RPMI-1640. Compounds were dissolved in DMSO (Sigma cat # D5879) to make a 2mM stock solution. It was then serially diluted in DMSO in a round bottom 96 well plate (Corning cat # 3799) to prepare 8 different concentrations for a dose response study. A 200X of the required final concentration was prepared in DMSO and 10 µl of each concentration was then diluted in 190 µl of plain RPMI to make an intermediate concentration of 20X. 20µl of each intermediate dilution was then added to 160µl of cells already seeded in appropriate wells in triplicates. Cells were treated with compound for 60 minutes. Cells treated with 0.5% DMSO served as unstimulated control. After 60 minutes, 20 µl of 10X LTA (10µg/ml) was added to compound treated and DMSO treated control wells and the plates were incubated at 37°C for 5 hours in a CO_2 incubator. After 5 hours, the plates were centrifuged at 300 x g for 5 min and supernatants were collected and analyzed for TNFα levels using the human TNF-alpha ELISA Kit (R&D systems cat # DY210) following the manufacturers protocol. Absorbance at 570nM was subtracted from the absorbance at 450nM as background control for all the wells. Blank subtracted absorbance values from unstimulated wells were subtracted from blank subtracted absorbance values of experimental wells. Cytokine levels in experimental wells were extrapolated from the standard curve for TNFα. % inhibition was calculated by normalizing the TNFα inhibition in the stimulation control wells to 0%. The % inhibition was plotted against compound concentration using Graph Pad Prism to calculate the IC_{50}.

DMPK assays

Aqueous solubility assay A high throughput solubility assay was carried out by shake flask method in 96-well format using PBS at pH 7.4 with theoretical test concentration of 200 µM. After 24 h of incubation, the supernatant was subjected for analysis by HPLC-UV/VIS. The solubility was calculated using formula Analyte peak area in buffer versus DMSO multiplied by 200 µM.

Transport assay Caco-2 assay - Briefly, Caco-2 cells (ATCC, USA) were grown in DMEM supplemented with 10% fetal bovine serum, 1 mM non-essential amino acids, 1 mM sodium pyruvate, and gentamicin sulfate (50 µg/ml) to 70% to 80% confluency prior to seeding in 24-well plates loaded with polycarbonate Millicell inserts (12-mm diameter, 0.4 µm, 40,000 cells/insert; Millipore Co., MA, USA) at 37°C, 5% CO_2 for 21 days. Cell monolayer integrity was assessed by measuring TEER. Compounds were applied at 10 µM in Hank's buffered salt solution to the apical or basal chamber, and transport assay was carried out for 2 h

at 37°C. At the end of the assay, samples from both apical and basal chambers were collected for analysis, and the monolayer integrity was re-assessed by dye rejection using Lucifer yellow. Apparent permeability (P_{app}) was calculated using the equation:

$$P_{app}\left(\mathrm{cm/sec}\right)=\mathrm{dQ/dt}\times 1/\mathrm{C_o}\times 1/\mathrm{A},$$

where dQ/dt is the amount of drug transported within a given time period (μmol/sec); C_0 is the initial concentration in the donor solution (μM); A is the surface area of insert filter membrane (cm^2); and t is the incubation time (sec).

$$\mathrm{Efflux\ ratio}\left(\mathrm{ER}\right)=P_{\mathrm{app\,B\,to\,A}}/P_{\mathrm{app\,A\,to\,B}},$$

where $P_{app\ B\ to\ A}$ is the P_{app} value measured in the B to A direction; $P_{app\ A\ to\ B}$ is the P_{app} value measured in the A to B direction. Note that efflux ratios greater than 2 (or 3) are generally considered to be evidence for transport. Follow-up studies using inhibitors of drug transporters (e.g., cyclosporin A) was done to develop further evidence.

Microsomal stability Liver microsomes (at 0.3mg/ml) from mouse, rat and human (Xenotech, USA) were incubated with 1 μM test compounds at 37°C for 0, 5, 15, 30, 45- and 60-minutes using NADPH. The reaction was stopped by the addition of cold acetonitrile, precipitated protein was removed, and the supernatants were analyzed using LC-MS/MS. The percent parent remaining was calculated using area ration obtained from analyte peak area to the internal standard peak area in comparison to time 0 min.

Pharmacokinetics of new chemical entities in Mice Animal experimental procedures used in this study were approved by the Institutional Animal Ethical Committee based on the Committee for the Purpose of Control and Supervision on Experiments on Animals guidelines. Animals were used for the experiment after one-week acclimatization to standard laboratory conditions. Animals were fed with standard diet and water *ad libitum*.

Compound(s) were administered intravenous and oral to the male CD-1 mice and/or male wistar rats at a dose of 3 mg/kg for IV and 10 mg/kg for oral arm. 50 parts of 1% tween 20 in water and 50 parts of 0.5% hydroxy ethyl cellulose was used for formulating the compound. After administration, blood samples were collected at 5 (for IV only), 10, 15, 30 min, 1, 3, 6, 8, and 24 h and centrifuged to obtain the plasma fraction. For dogs, compound 24 was administered intravenous as slow infusion at 1 mg/kg dose and oral at 10 mg/kg dose. The plasma samples were deproteinized with acetonitrile, followed by centrifugation, and the supernatants were analyzed by LC-MS/MS to determine the plasma concentration of the

compounds. The pharmacokinetics parameters were calculated by using WinNonlin software. The plasma concentration after injection (C_0 min), the area under the concentration−time curve from time zero to 24 h (AUC_{0-t}), Vdss, and CL_{total} for after iv administration were obtained. The maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}), AUC_{0-t}, and %F after po administration were also obtained.

CYP inhibition and PPB assays

CYP inhibition assays were performed as reported by Hickman et. al.[10]. PPB determination was performed by equilibrium dialysis method using RED device inserts.[11, 12]

Genotoxicity, mutagenesis and micronucleus assays

The assays were performed following standard protocols reported elsewhere[13, 14]

In vivo studies

All the procedures related to animal handling, care, and the treatments were performed according to the guidelines approved by Institutional Animal Ethics Committee (IAEC). 8-9 weeks old male Scid beige were supplied by Harlan laboratories.

OCI-Ly3 xenograft study OCI-Ly3 cells were harvested in vitro, and cells were suspended in a mixture of Iscove's Modified Dulbecco's Medium (IMDM) medium and Matrigel in a total volume of 200 ml (IMDM/Matrigel, 1:1). Male Scid beige mice were injected s.c. with the 10 x 10^6 OCI-Ly3 tumor cell suspensions in the left flank region. Mice were randomized 22 days after tumor cell injection into five groups of nine mice each with a mean tumor volume of 222 mm^3. The animals were randomized according to tumor volume. Tumor sizes were measured three times weekly with use of digital calipers. Tumor volumes were calculated using the formula Tumor Volume (TV) = (D x d2)/2, Where D represents the “largest tumor diameter (mm)”; d represents the “smallest tumor diameter (mm)”. Compound 24 was dissolved in 50 parts of 1% tween 20 in water and 50 parts of 0.5% hydroxy ethyl cellulose and administered orally for 14 days at indicated doses.

Pharmacokinetic and pharmacodynamic study in OCI-Ly3 xenograft tumors To determine the relationship between pharmacokinetic and pharmacodynamic profile of Compound **24** in the OCI-Ly3 xenograft model, 10 × 10^6 OCI-Ly3 tumor cells in 200 µL of 1:1 Hank's balanced salt solution and Matrigel were injected subcutaneously in the left flank of 7-8 week old male SCID beige mice. OCI-Ly3 tumors in mice were allowed to reach 300-400 mm3 before being treated with Compound **24**. At 4, 8, 12 and 24

hours after oral gavage administration of the single dose of Compound **24** (200 mg/kg), tumors and plasma samples were collected for pharmacokinetic and pIRAK1modulation analysis. Tumor samples were analyzed for inhibition of pIRAK1 by immunoblot at the same time points.

Analysis of tumor tissues by Immunoblot Resected tumors were collected and immediately dipped into liquid nitrogen. Tumor powder was later crushed in a liquid nitrogen-prechilled pestle and mortar. Powdered tissues were immediately stored at −80°C. CST lysis buffer was diluted 2X; protease and phosphatase inhibitor cocktails were added to 1X, and 300 µL of cell lysis buffer was added to tumor samples weighing 50 mg or less. The tumor powder was homogenously resuspended in cell lysis buffer and incubated on ice for 1 hour. During incubation, the tumor samples were sonicated three times for 10 s each. After incubation, the samples were centrifuged at 12,000 rpm for 15 minutes at 4°C. Lysate protein concentrations were determined by BCA Assay and 50 to 60 mg of each lysate were separated by 10% SDS-PAGE gel, transferred to nitrocellulose membrane and probed with specific antibodies. Protein signals were quantitated using Odyssey Infrared Imaging (Li-Cor Biosciences). Results were normalized to vehicle control.

Determination of plasma and tumor drug concentration Plasma samples were deproteinized with acetonitrile and centrifuged. The supernatant was evaporated and reconstituted with the mobile phase. The samples were analyzed for drug concentration by liquid chromatography–tandem mass spectrometry (LC/MS-MS) in the multiple reaction monitoring mode. Tumor samples were homogenized and subjected to the same procedure as plasma. A set of calibration standards and quality control samples were used for both plasma and tumor samples.

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Table S1. Eurofins 329 panel kinase inhibition profiling data

Kinase	% Inhibition		Kinase	% Inhibition		Kinase	% Inhibition	
	1 μM	10 μM		1 μM	10 μM		1 μM	10 μM
Abl (H396P) (h)	6	37	CK2α2(h)	13	53	Fms(Y969C)(h)	18	21
Abl (M351T)(h)	5	37	CLK2(h)	100	99	Fyn(h)	14	29
Abl (Q252H) (h)	20	48	CLK3(h)	1	19	GCK(h)	-5	12
Abl(T315I)(h)	-7	13	CLK4(h)	100	99	GCN2(h)	-24	14
Abl(Y253F)(h)	14	36	cKit(D816V)(h)	17	1	GRK1(h)	-17	0
ACK1(h)	-11	2	cKit(D816H)(h)	55	43	GRK5(h)	0	-5
ALK(h)	-25	12	cKit(V560G)(h)	11	24	GRK6(h)	-1	-6
ALK1(h)	0	-8	cKit(V654A)(h)	-2	0	GRK7(h)	14	16
ALK2(h)	5	14	CSK(h)	3	-4	GSK3α(h)	9	42
ALK4(h)	-5	8	c-RAF(h)	10	-11	GSK3β(h)	5	9
ALK6(h)	13	-4	DAPK1(h)	-15	7	Hck(h)	2	18
Arg(h)	1	6	DAPK2(h)	20	17	HIPK1(h)	5	26
AMPKα1(h)	-15	-5	DCAMKL2(h)	-2	-6	HIPK3(h)	9	23
AMPKα2(h)	-4	-2	DCAMKL3(h)	9	0	IGF-1R(h)	-16	-24
A-Raf(h)	9	-6	DDR1(h)	6	29	IGF-1R(h), activated	-1	7
Arg(m)	2	17	DDR2(h)	-4	-5	IKKα(h)	4	12
ARK5(h)	-3	1	DMPK(h)	7	4	IKKε(h)	4	5
ASK1(h)	-8	-16	DRAK1(h)	13	10	IR(h)	-1	-4
Aurora-B(h)	-12	-29	DYRK1A(h)	84	98	IR(h), activated	-9	-23
Aurora-C(h)	27	9	DYRK1B(h)	72	95	IRE1(h)	5	-1
Axl(h)	-2	0	eEF-2K(h)	-8	-2	IRR(h)	11	21
Blk(h)	-1	10	EGFR(L858R)(h)	-9	-18	Itk(h)	31	61
Blk(m)	26	18	EGFR(L861Q)(h)	25	3	JAK1(h)	-4	2
Bmx(h)	11	38	EGFR(T790M)(h)	-16	-14	JAK3(h)	1	-3
BrSK1(h)	-2	-7	EGFR(T790M,L858R)(h)	-4	-2	JNK1α1(h)	-3	0
BrSK2(h)	2	-1	EphA1(h)	-15	-8	JNK2α2(h)	-5	-5
BTK(R28H)(h)	9	12	EphA2(h)	3	5	JNK3(h)	-29	7
B-Raf(h)	6	-9	EphA3(h)	-1	-3	LIMK1(h)	-11	7
B-Raf(V599E)(h)	-8	-4	EphA4(h)	30	3	LKB1(h)	-7	-6
CaMKI(h)	-10	-4	EphA5(h)	-8	-16	LOK(h)	5	2
CaMKIIβ(h)	12	7	EphA7(h)	0	6	Lyn(m)	14	19
CaMKIδ(h)	11	-1	EphA8(h)	-6	-9	MAPK2(h)	6	2
CaMKIIδ(h)	3	31	EphB2(h)	-18	1	MAPK2(m)	-3	-7
CaMKIV(h)	10	18	EphB1(h)	-4	-3	MAPKAP-K2(h)	-12	4
CaMKK2(h)	4	4	EphB3(h)	-20	-10	MAPKAP-K3(h)	-6	5
CDK1/cyclinB(h)	-3	27	EphB4(h)	0	8	MARK1(h)	-4	19
CDK2/cyclinE(h)	1	22	ErbB2(h)	4	5	Met(h)	-1	9
CDK3/cyclinE(h)	12	20	ErbB4(h)	3	-6	Met(D1246H)(h)	7	2
CDK5/p25(h)	-3	14	FAK(h)	-8	1	Met(D1246N)(h)	2	-3
CDK5/p35(h)	-8	19	Fer(h)	2	-23	Met(M1268T)(h)	7	11
CDK6/cyclinD3(h)	-4	-9	Fes(h)	-3	12	Met(Y1248C)(h)	13	8
CDK7/cyclinH/MAT1(h)	7	28	FGFR1(h)	15	31	Met(Y1248D)(h)	-13	-16
CDK9/cyclin T1(h)	-8	-5	FGFR1(V561M)(h)	9	23	Met(Y1248H)(h)	-4	1
CHK1(h)	14	5	FGFR2(h)	-10	-4	MINK(h)	-11	7
CHK2(I157T)(h)	11	-1	FGFR2(N549H)(h)	12	45	MKK4(m)	1	-10
CHK2(R145W)(h)	-9	-2	FGFR3(h)	-4	1	MKK6(h)	19	24
CK1γ1(h)	2	1	FGFR4(h)	5	5	MKK7β(h)	-17	-2
CK1γ2(h)	4	1	Flt1(h)	-9	45	MRCKα(h)	-1	3
CK1γ3(h)	6	8	Flt3(D835Y)(h)	99	101	MRCKβ(h)	-1	-1
CK1δ(h)	-6	2	Fms(h)	-31	-2	MSK1(h)	-7	-1
MSK2(h)	6	10	PKG1α(h)	-4	-2	TLK1(h)	4	-1

Kinase	% Inhibition		Kinase	% Inhibition		Kinase	% Inhibition	
	1 μM	10 μM		1 μM	10 μM		1 μM	10 μM
MST1(h)	-18	6	PKG1β(h)	-3	-12	TLK2(h)	6	7
MST2(h)	-8	2	PKR(h)	15	16	TrkB(h)	48	79
MST3(h)	14	4	Plk1(h)	-11	-7	TrkC(h)	41	83
MST4(h)	8	-2	Plk3(h)	-1	0	TSSK2(h)	1	1
mTOR/FKBP12(h)	-1	-13	PRAK(h)	-18	0	Txk(h)	-11	11
NEK2(h)	-6	23	PRK2(h)	-6	22	TYK2(h)	-6	5
NEK3(h)	-5	-9	PrKX(h)	-11	-7	ULK2(h)	-11	6
NEK6(h)	-2	10	Pyk2(h)	-7	-1	ULK3(h)	-1	-2
NEK7(h)	1	6	Ret(h)	27	75	Wee1(h)	-14	6
NEK9(h)	7	4	Ret (V804L)(h)	94	96	WNK2(h)	15	10
NIM1(h)	-1	0	Ret(V804M)(h)	24	79	WNK3(h)	2	-2
NEK11(h)	46	39	ROCK-I(h)	0	8	VRK2(h)	0	-5
NLK(h)	-6	-4	ROCK-II(h)	-3	5	Yes(h)	4	42
p70S6K(h)	5	17	ROCK-II(r)	-9	-7	ZAP-70(h)	15	13
PAK1(h)	0	-3	Ron(h)	-16	-8	ZIPK(h)	-15	-5
PAK2(h)	-8	-4	Ros(h)	-11	-10	ATM(h)	-2	-3
PAK4(h)	1	14	Rse(h)	-8	0	DNA-PK(h)	-2	-4
PAK5(h)	-7	-4	Rsk1(r)	-14	13	PI3 Kinase (p120γ)(h)	2	1
PAK6(h)	-7	-6	Rsk2(h)	25	19	PI3 Kinase (p110δ/p85α)(h)	-1	-1
PAR-1Bα(h)	-1	7	Rsk3(h)	3	40	PI3 Kinase (p110α/p85α)(m)	6	5
PASK(h)	3	18	Rsk4(h)	7	27	PI3 Kinase (p110α/p65α)(m)	5	5
PEK(h)	13	-6	SAPK2a(h)	-16	-14	PI3 Kinase (p110α(E545K)/p85α)(m)	6	6
PDGFRα(h)	1	8	SAPK2a(T106M)(h)	0	-5	PI3 Kinase (p110α(H1047R)/p85α)(m)	4	4
PDGFRα(D842V)(h)	38	61	SAPK2b(h)	2	-11	PI3 Kinase (p110ß/p85ß)(m)	3	4
PDGFRα(V561D)(h)	-21	41	SAPK3(h)	-17	9	PI3 Kinase (p110ß/p85α)(m)	8	7
PDK1(h)	-10	-6	SAPK4(h)	-24	1	PI3 Kinase (p110δ/p85α)(m)	4	3
PhKγ2(h)	-1	8	SGK(h)	-13	-4	PI3 Kinase (p110α(E542K)/p85α)(m)	4	2
Pim-1(h)	-3	7	SGK2(h)	-1	-4	PI3 Kinase (p110α/p85α)(h)	4	4
Pim-2(h)	-2	21	SGK3(h)	-19	-1	PI3 Kinase (p110α(E542K)/p85α)(h)	5	5
Pim-3(h)	-18	-17	Snk(h)	7	4	PI3 Kinase (p110α(H1047R)/p85α)(h)	2	8
PKA(h)	-13	-3	SNRK(h)	21	20	PI3 Kinase (p110α(E545K)/p85α)(h)	3	4
PKBα(h)	5	-7	Src(1-530)(h)	2	22	PI3 Kinase (p110α/p65α)(h)	4	4
PKBβ(h)	-9	-11	Src(T341M)(h)	4	47	PI3KC2α(h)	-7	-5
PKBγ(h)	-5	0	SRPK2(h)	-7	6	PI3KC2γ(h)	0	4
PKCα(h)	3	2	STK25(h)	-4	3	PIP4K2α(h)	5	-4
PKCβI(h)	-6	-2	STK33(h)	-1	3	PIP5K1α(h)	-10	-12
PKCβII(h)	-11	0	TAK1(h)	-7	13	PIP5K1γ(h)	0	4
PKCγ(h)	-3	6	TAO1(h)	-10	-7	Abl(h)	15	40
PKCδ(h)	13	-8	TAO2(h)	-3	4	Abl(m)	11	42
PKCε(h)	6	-4	TAO3(h)	-10	-10	Aurora-A(h)	9	11
PKCη(h)	-2	-8	TBK1(h)	-8	4	BRK(h)	-2	-2
PKCι(h)	0	0	TGFBR1(h)	-1	6	BTK(h)	11	18
PKCμ(h)	2	1	Tie2 (h)	-6	8	CaMKIIγ(h)	10	47
PKCζ(h)	-3	4	Tie2(R849W)(h)	-8	-10	CDK2/cyclinA(h)	14	33
PKD2(h)	-6	2	Tie2(Y897S)(h)	-10	-17	CHK2(h)	-8	-2

Kinase	% Inhibition		Kinase	% Inhibition		Kinase	% Inhibition	
	1 µM	10 µM		1 µM	10 µM		1 µM	10 µM
CK1(y)	18	34	IRAK4(h)	82	96	mTOR(h)	4	-8
CK2(h)	8	19	JAK2(h)	8	20	MuSK(h)	-9	6
CLK1(h)	97	100	KDR(h)	10	57	PDGFRβ(h)	40	44
cKit(h)	8	10	Lck(h)	8	21	PKCθ(h)	6	14
cSRC(h)	11	27	Lck(h) activated	9	12	PTK5(h)	16	29
DYRK2(h)	20	71	Lyn(h)	12	26	RIPK2(h)	14	34
EGFR(h)	6	5	LRRK2(h)	18	65	Rsk1(h)	15	31
Fgr(h)	18	27	MAPK1(h)	2	10	SIK(h)	11	31
Flt3(h)	95	101	MEK1(h)	13	24	SRPK1(h)	12	15
Flt4(h)	30	78	MELK(h)	28	75	Syk(h)	2	6
Haspin(h)	56	91	Mer(h)	9	35	Tec(h) activated	21	47
Hck(h) activated	5	34	MLCK(h)	10	15	TrkA(h)	68	95
HIPK2(h)	2	30	MLK1(h)	-1	29	TSSK1(h)	25	21
IKKβ(h)	14	6	Mnk2(h)	31	43	PI3 Kinase (p110b/p85a)(h)	5	6
IRAK1(h)	9	30	MSSK1(h)	32	43			

Table S2. Diffraction data collection and Structure Refinement Statistics

Parameter	CA-4948	1
PDB Code	7C2V	7C2W
Space Group	C2	C2
Cell Parameters (Å)	a=142.8, b=138.6, c=87.4 β= 124.5^o	a=137.6, b=140.8, c=86.4 β= 127.6^o
Resolution range(Å)	30.00-2.44 (2.57-2.44)	100.00-3.14 (3.21-3.10)
Unique reflections	51404 (7467) a*	21131 (2073) a*
Completeness (%)	98.9 (98.7)	89.9 (86.8)
Rsym	0.133 (0.703)	0.095 (0.381)
I/σI	6.2 (1.9)	4.5 (1.7)
Multiplicity	2.8 (2.8)	2.4 (2.3)
Refinement		
Resolution (Å)	30.00-2.44 (2.50-2.44)	30.00-3.24 (3.28-3.20)
Data Completeness (%)	98.8 (98.8)	89.2
Rwork/Rfree	0.198 (0.306) / 0.264 (0.348)	0.266(0.29) / 0.35(0.42)
r.m.s. deviations		
Bond lengths (Å)	0.008	0.006
Bond angles (°)	1.267	1.134
Ramachandran plot		
Residues in the most favored region (%)	96.9	90.0
Residues in the allowed region (%)	3.0	7.2

a*: Values corresponding to the outermost shell are given within parentheses